Project no. 506820

HEATOX

Heat-generated food toxicants:
identification, characterisation and risk minimisation

Final report

Instrument: Specific Targeted Research Project

Thematic Priority: 5. Food Quality and Safety

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Duration: 40 months

Project coordinator name: Kerstin Skog
Project coordinator organisation name: Lunds Universitet

FOOD QUALITY AND SAFETY

Content

Final report – Contract FOOD-CT-2003-506820

Heat-generated food toxicants:
Identification, Characterisation and Risk Minimisation (HEATOX)

1. Summary

Heating food gives many advantages – it adds taste, colour and texture and minimises harmful germs. Flavour and aroma compounds are produced via the Maillard reaction but at the same time various health hazardous compounds may form. The main route for acrylamide formation goes via the Maillard reaction between reducing sugars (glucose and fructose) but also sucrose, and the amino acid asparagine. The HEATOX project involved 24 partners in 14 countries. The focus of the HEATOX project was health risks associated with hazardous compounds, for example acrylamide, in heat-treated carbohydrate-rich foods. The main objectives were to estimate health risks that may be associated with hazardous compounds in heat-treated food and to find cooking/processing methods which minimise the amounts of these compounds, thereby providing safe, nutritious and high-quality foodstuffs. Among the important results are recommendations to consumers, restaurants and the food industry on how to minimise the amounts of heat-generated toxicants in foods, while ensuring product quality from a nutritional and sensory point of view.

The HEATOX project started on the 1st November 2003 and had a duration of 40 months.

Project web-site: www.heatox.org

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The HEATOX project has dealt with questions regarding heat-generated food toxicants e.g.
- In which foods are these compounds mainly found?
- How are they formed?
- Do they constitute a health risk?
- How can we measure/control the amounts produced?
- How much is consumed?
- What are the effects on the human body?
- How can they be avoided?
- Is there a cooking method to be recommended?

Modern science has showed that heating of meat and other protein rich foods can generate various kinds of potentially hazardous compounds, some of which are genotoxic and carcinogenic. The focus of the HEATOX project was health risks associated with hazardous compounds in heat treated carbohydrate-rich foods where substantial amounts of acrylamide and similar compounds can be formed. HEATOX explored their mechanisms of formation, impact of raw material composition, inhibiting factors, cooking and processing methods in industry and households with the to aim control and minimise the formation of hazardous compounds.

Acrylamide was given particular emphasis in the HEATOX project. Other compounds representing potential health hazards, such furans, are also formed during heating, thus it is important that efforts in reducing acrylamide exposure at the same time do not increase the formation of other hazardous compounds. Validated methods for food analysis and exposure biomarkers have been developed. Different hazards have been explored and characterised in various toxicological models, for example genotoxicity, carcinogenicity, neuro-developmental and reproductive toxicity. Molecular characterisation by toxicogenomics has been performed. Experimental animal and cell systems and also humans were studied. Particularly the relevance of low dose exposure, bioavailability and extrapolation from experimental systems to humans was addressed using biomarkers of exposure and effect. The exposure assessment and data on hazard characterisation, including data generated outside HEATOX, were combined in a risk characterisation of intake of heat-treated carbohydrate rich foods. To support and strengthen the project profile, a risk communication strategy was developed early in the project. A tailored action plan was implemented successively during the whole project period. The ultimate aim was to ensure that project deliverables were as useful and focused as possible and that the dialogue within HEATOX and between HEATOX and the outside world was effective. The HEATOX project co-operated with other research activities carried out by other groups around the world, e.g. the project was linked with the COST 927 action. Dissemination of the project results was a key issue, which involved for example publications, conferences, and the HEATOX workshops.

Project overview
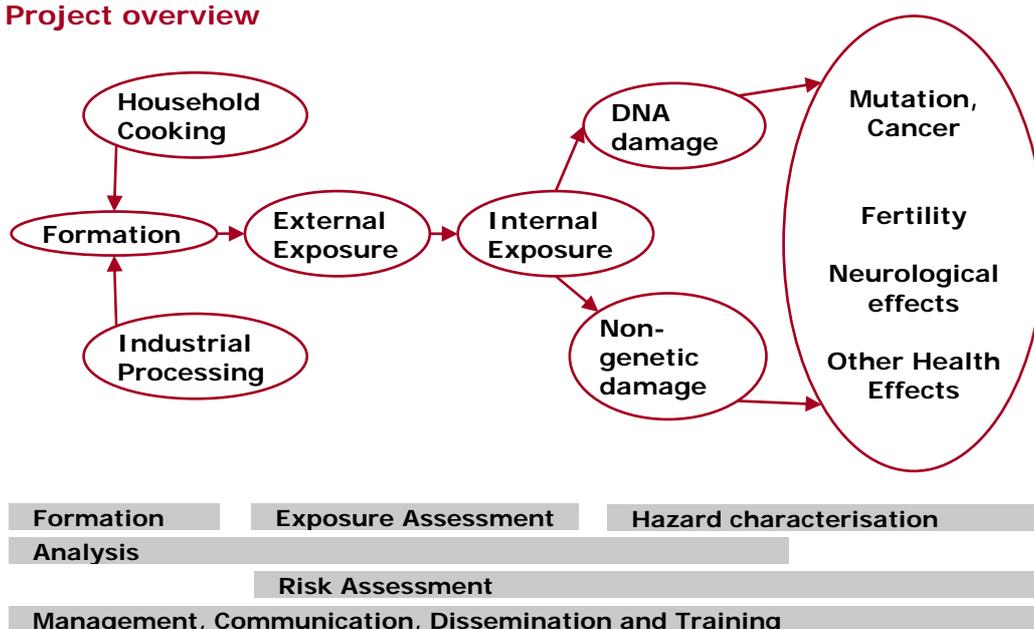
1.1 Detailed objectives:

I. To elucidate the chemical reaction mechanisms involved and to develop and validate new/improved production methods and technological minimisation strategies

♦ Effects of variation in the composition of the raw material
♦ Reaction pathways and mechanisms, inhibiting/enhancing compounds, and kinetics
♦ Cooking and processing methods for the food industry, catering facilities and households

Expected results: Recommendations on how to limit the formation of heat-induced toxicants in order to minimise the formation of heat-induced toxicants, while ensuring product quality from safety, nutritional and sensory points of view.

II. To provide validated analytical methods (i.e. screening and confirmatory) and quality-controlled analytical data needed for the execution of the other tasks

• Quantitative analytical methods for biomarkers and for toxicants in foods
• Identification methods and data on new toxicants or known toxicants in new food matrices
• Quality-controlled data on toxicant levels in foods

Expected results: Validated analytical methods for a wide range of heat-induced toxicants and for biomarkers of exposure.

III. To perform hazard characterisation

♦ Neurodevelopmental toxicity, mechanisms of tumourgenicity, and mechanisms of reproductive toxicity
♦ Bioavailability in animals and humans determined by different biomarkers for internal dose and effect
♦ Dose-response relationships in the low-dose region

Expected results: Characterisation of toxic effects including their mechanisms and dose-response relationships.

IV. To assess the exposure of heat-induced toxicants

♦ Improved estimation of the intake of heat-induced toxicants based on analysis of cooked foods and food intake data, especially concerning variability and risk groups
♦ Comparison of dietary intake data regarding heat-induced toxicants with data on the internal dose (heat-induced toxicants adduct levels, e.g. DNA-adduct levels)
♦ Elucidation of the role of possible endogenous formation of heat-induced toxicants
♦ Assessment of the possibility of using statistics for conclusive cancer epidemiology studies

Expected results: Validated data on heat-induced toxicants exposure and intra- and inter-individual variation in exposure.

V. To perform risk assessment and communicate the results of the project

♦ Assessment of the health risk associated with the current exposure to known heat-induced toxicants (risk characterisation) and possibly new compounds, also taking into account existing information on hazards from sources outside the project
♦ Comparative risk/benefit assessments of heat-treated foods by linking results from animal and human studies to human health risk
♦ Targeted recommendations and guidelines to all important user groups
♦ Broad information to the general public, the food industry and its different relevant associations/federations, national food agencies, consumer groups/associations, EFSA, different Commission services (e.g. DG Research, DG Health and Consumer Protection, DG Agriculture, DG Enterprise, etc.) on the project, its results and their implications including also future aspects.

Expected results: Increased knowledge and harmonised agreement on the possible risks of heat-induced food toxicants and recommendations on how the toxicant levels can be controlled.

1.2 HEATOX Partners

P 1 Lund University (Sweden)
- Kerstin Skog, Co-ordinator

P 2 Graz University of Technology (Austria)
- Michael Murkovic

P 3 The University of Reading (UK)
- Don Mottram

P 4 Swedish University of Agricultural Sciences (Sweden)
- Per Åman

P 5 University of Bologna (Italy)
- Marco Dalla Rosa

P 6 Swedish Institute for Food and Biotechnology (Sweden)
- Hans Lingnert

P 7 Wageningen University (The Netherlands)
- Tiny van Boekel

P 8 Central Science Laboratory, York (UK)
- Laurence Castle

P 9 Swedish National Food Administration (Sweden)
- Karl-Erik Hellenäs

P10 Institute of Chemical Technology Prague (Czech Republic)
- Jana Hajslova

P11 Agrotechnology and Food Innovations (The Netherlands)
- Charon Zondervan

P12 University of Barcelona (Spain)
- Maria Teresa Galceran

P13 TÜBÝTAK-Marmara Research Center (Turkey)
- Hülya Ölmez

P14 Stockholm University (Sweden)
- Margareta Törnqvist

P15 National Food Institute (Denmark)
- Henrik Frandsen

P16 Norwegian Institute of Public Health (Norway)
- Jan Alexander

P17 RIKILT Institute of Food Safety (The Netherlands)
- Jacob van Klaveren

P18 German Institute for Human Nutrition (Germany)
- Hansruedi Glatt

P19 University of Leeds (UK)
- Bronek Wedzicha

P20 BEUC, European Consumers' Organisation (Belgium)
- Barbara Gallani

P21 National Veterinary Institute (Norway)
- Helga Odden Reksnes

P22 University of Zürich (Switzerland)
- Hanspeter Naegeli

P23 University of Chile (Chile)
- Lilia Masson

P24 Queens' University Belfast (UK)
- Chris Elliott

1.3 PhD's

Exchange of young scientist has been encouraged within the HEATOX project; several young researchers have visited other HEATOX partners for 1-4 weeks to learn new methods, plan collaborative work, discuss results etc. The successful integration of the young researchers with senior researchers and cooperation between the different groups has contributed to interdisciplinary relations and building of networks. Several partners participate in Cost Action 927 "Thermally Processed Foods: Possible Health Implications", co-ordinated by V Fogliano (University of Naples, Italy), which has provided additional opportunities for student exchange.

Special emphasis has been put on communication training for PhDs and young researchers. At the semi annual meetings, seminars have been arranged on the topic of risk communication and dissemination in relation to their HEATOX work, for example short oral presentations, posters and also participation in the HEATOX workshop.

PhD students/Young scientists working in the HEATOX project:

German Institute of Human Nutrition (Germany)
- Yasmine Sommer

Graz University of Technology (Austria)
- Kristina Bagdonaite

Institute of Chemical Technology Prague (Czech Republic)
- Lenka Dunovska

Leeds University
- Jonas Mojica Lazaro

Lund University (Sweden)
- Peter Viberg, Gunilla Viklund

National Food Institute (Denmark)
- Pelle T Olesen

Norwegian Institute of Public Health (Norway)
- Thomas Bjellaas, Siri Helland Hansen, Hege Benedikte Ölstörn

RIKILT Institute of Food Safety (The Netherlands)
- Anika de Mul

Stockholm University (Sweden)
- Anna Vikström

Swedish National Food Administration (Sweden)
- Louise Durling, Erik Petersson

Swedish University of Agricultural Sciences (Sweden)
- Arwa Mustafa

The University of Reading (UK)
- Mei Yin Low

University of Barcelona (Spain)
- Soubhi Altaki, Erika Teixido

University of Bologna (Spain)
- Alessandro Angioloni, Mara Bacchiocca, Andrea Gasparri, Pietro Rocculi

University of Zürich (Switzerland)
- Flurina Clement, Ramiro Dip

Wageningen University (The Netherlands)
- Jeroen Knol

Queen's University Belfast (UK)
- Andrew Preston

1.4 External Panel

The External Panel constituted the core risk communication network and provided valuable discussion with HEATOX' stakeholders. In charge: Karl-Erik Hellenäs.

Members:

Consumer
- Barbara Gallani (from June 2005)/Beate Kettlitz (until March 2005), BEUC, BE

Industry
- Beate Kettlitz (from March 2005)/Domenique Teaymans (until March 2005), CIAA, BE
- Sam Lalljie, ILSI, BE
- Julia Gelbert, BLL/ZUTECH, GE

Research
- David Lineback, JIFSAN, USA
- Erland Bråthen (until 2005), MATFORSK, NO
- Vincenzo Fogliano, COST-927, IT
- Elke Anklam (until July 2006), JRC irmm, BE

Authorities
- Almut Bitterhof (from spring 2006)/Martin Slayne (until spring 2006), DG Sanco, BE
- Claudia Heppner, EFSA, Contam panel, IT
- Lutz Dehne, BfR, DE
- Wendy Matthews (from 2004)/Karen Goonan (until 2004), FSA, UK
- Sara Henry, FDA, USA
- Lauren Jackson, FDA, USA

Management & Information Flow
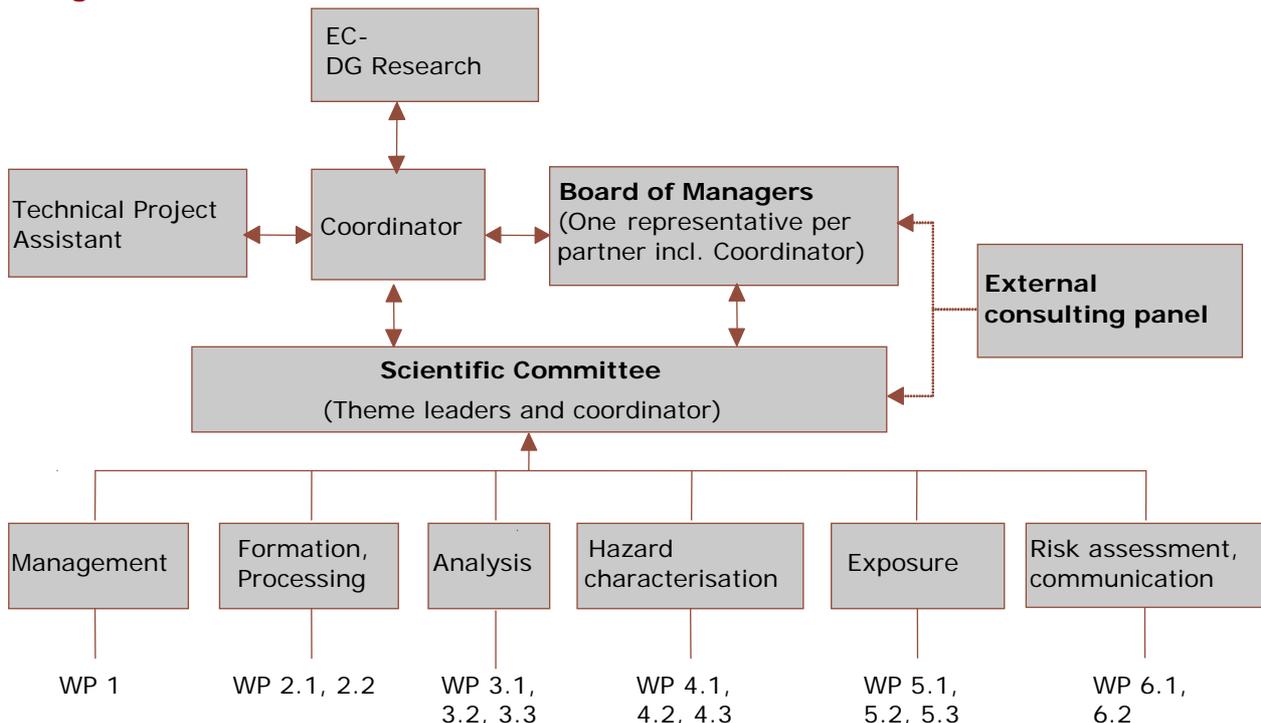
2. Formation and Processing

Wageningen University (The Netherlands), University of Leeds (United Kingdom),
The University of Reading (United Kingdom), Graz University of Technology (Austria), Lund University (Sweden),
Agrotechnology & Food Innovations B.V. (The Netherlands), University of Chile, University of Bologna (Italy),
Swedish Institute for Food and Biotechnology (Sweden), Swedish University of Agricultural Sciences (Sweden)

2.1. Chemical mechanisms and kinetics of formation of hazardous compounds

The Maillard reaction between amino acids and sugars is essential for the development of desirable colour and flavour in baked and fried foods. However, the Maillard reaction has been shown to generate acrylamide at the same time, through the reaction of the amino acid asparagine with reducing sugars. Thus, acrylamide is formed by the thermal reaction of natural food components, when moisture levels become low. A proposed mechanism for acrylamide formation (see Figure 1) involves the formation of a Schiff base from the reaction of a carbonyl compound with asparagine. Decarboxylation of the Schiff base, in a Strecker-type reaction, gives an unstable intermediate that can hydrolyse to 3-aminopropamide, which on elimination of ammonia yields acrylamide. Alternatively the decarboxylated Schiff base could form acrylamide via elimination of an imine.
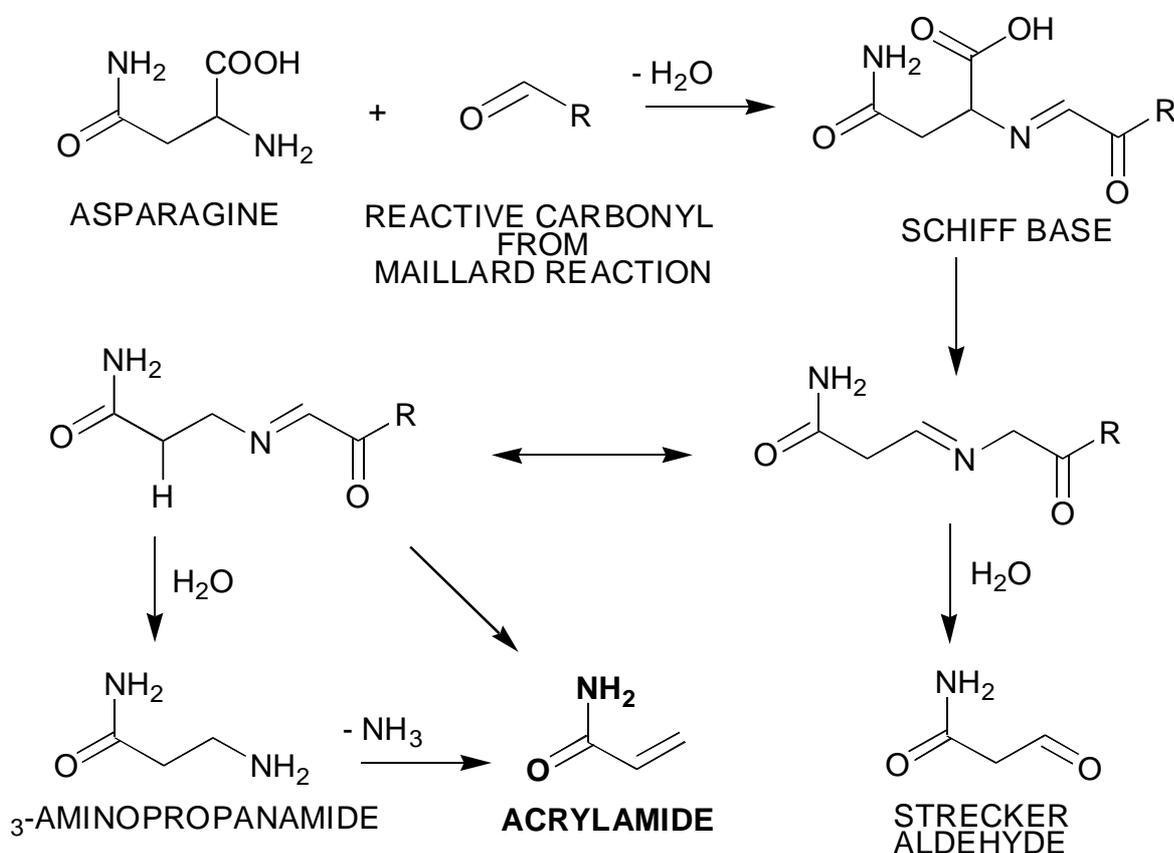
Figure 1. Proposed mechanism for the formation of acrylamide in heat-treated foods.

The formation of acrylamide in food is under kinetic control; this means that the amount which is formed depends on the rate of reaction and the time for which it has been allowed to proceed. Reaction kinetics relates to the rate limiting steps in chemical reactions; these are the control points. For this reason, an understanding of the factors which control the rate limiting steps in acrylamide formation offers opportunity to control the extent of its production.

Chemical mechanisms and kinetics of formation were studied, based on reactions between sugars and amino acids via the Maillard reaction. Equimolar solutions of glucose and asparagine (0.1 and 0.2 M) were prepared in phosphate buffer (0.1 M) with different pH (4.5, 5.5, 6.0 and 6.8). Samples were heated at 120-200°C for 0-128 min. A mechanistic kinetic model has been derived for the aqueous model systems from the proposed reaction network (Figure 2). For each reaction step a differential equation was set up, and translated into a mathematical model.
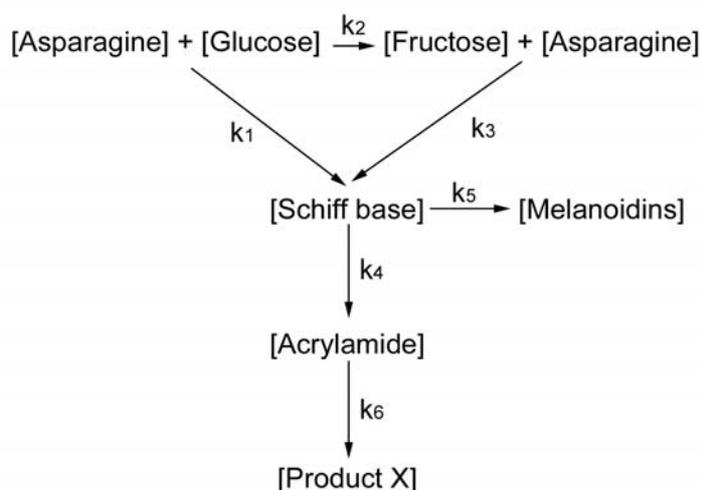
Figure 2. *Proposed reaction network for the formation of acrylamide from asparagine and glucose/ fructose through early Maillard reaction. Product X: product(s) formed from acrylamide breakdown.*

Multiresponse modelling using non-linear regression with the determinant criterion was used to estimate model parameters, i.e. the rate constants and activation energies based on the experimental data obtained from the experiments with the aqueous model systems (Table 1).

Table 1. *Estimates of rate constants (k) at 120, 140, 160, 180 and 200 °C and activation energies (E_a) ± 95% Highest Posterior Density (HPD) interval as found by kinetic modelling for the proposed kinetic model for pH 6.8.*

k (10^{-3} min^{-1} or 10^{-3} mol·min^{-1})	120 °C	140 °C	160 °C	180 °C	200 °C	E_a (kJ/mol)
k_1	0.131 ± 0.025	0.308 ± 0.049	0.668 ± 0.13	1.35 ± 0.36	2.58 ± 0.89	57.6 ± 8.0
k_2	4.98 ± 1.2	16.7 ± 3.0	50.1 ± 9.6	136 ± 35	341 ± 114	81.7 ± 8.6
k_3	0.0819 ± 0.045	0.369 ± 0.14	1.45 ± 0.44	5.04 ± 1.6	15.8 ± 6.4	102 ± 14
k_4	0.176 ± 0.081	0.712 ± 0.23	2.53 ± 0.51	8.05 ± 1.2	23.2 ± 4.3	94.4 ± 11
k_5	15.7 ± 3.5	28.4 ± 4.6	48.7 ± 5.9	79.6 ± 8.9	125 ± 16	40.1 ± 5.0
k_6	7.96 ± 5.1	28.1 ± 13	88.1 ± 25	250 ± 45	650 ± 136	85.1 ± 14

Figure 3 shows how the model fits the experimental data for the formation of acrylamide in the aqueous reaction system at pH 6.8. The proposed model gives a reasonable estimation for the formation of acrylamide in an aqueous model system. The mechanistic kinetic model derived in this study is an important step into the realization of a tool that can be used to predict how acrylamide reduction in foods containing asparagine and reducing sugars can be accomplished.
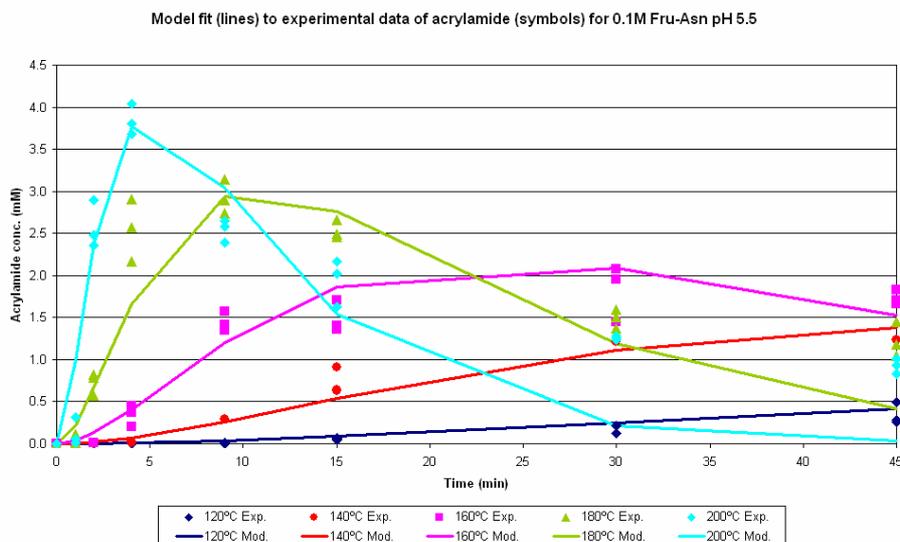
Figure 3. *Model fit (lines) to experimental data (symbols) of acrylamide concentration for glucose/ asparagine (0.2 mol/l, pH 6.8) aqueous reaction system heated at 120 (blue), 140 (red), 160 (purple), 180 (green) and 200 °C (light blue).*

Another kinetic model for the formation of acrylamide in foods (potato, wheat and rye products) involves steps which lead to the destruction, binding or physical 'loss' of acrylamide. Our ability to formulate correctly the kinetic model for acrylamide levels in foods thus depends critically on us being able to formulate the kinetics of these subsequent processes. This is particularly important with regard to the control (*i.e.*, reduction) of acrylamide levels because such 'losses' could be maximised through adjustment of the reaction conditions. Furthermore, there are views that perhaps much more acrylamide is actually formed in foods than is measured, and an understanding of the fate of acrylamide is important so that we might be able to identify the chemical nature of any 'bound' acrylamide and the stability of any reaction products which are formed. If acrylamide is being formed in much greater yield than so far observed, there is also a danger that a change in the reaction conditions could alter the balance between the rates of formation and of loss, so that much higher yields are obtained. We believe, therefore, that consideration of the kinetics of acrylamide loss is fundamental to the derivation of the kinetic model.

The kinetics of the reaction of acrylamide with glycine and proline were determined in aqueous systems as a function of pH and temperature, and the relative reactivities of all 20 amino acids measured at pH 5.5. Maltodextrin gels were prepared containing acrylamide and the amino acids, and were freeze-dried before being heated to 180 °C. The experiments in aqueous systems have shown the relevance of Michael addition type reactions of amino acids with acrylamide.

Acrylamide reacted more readily with glycine than with Maillard precursors in solution and this reaction presented second order kinetics, *i.e.*,

$$-\frac{d[\text{acryl}]}{dt} = k[\text{acryl}][\text{gly}]$$

However, glycine was not the most reactive amino acid towards acrylamide; proline and cysteine showed much higher rates of reaction in comparison with other amino acids. pH was a significant factor in the reaction; glycine and proline react by a common mechanism in which there exists an acid-base catalytic effect by a species with pKa=5.2-5.5.

$$k = k_0[\text{A}^-] + k_1[(\text{glyNH}_2)^-]$$

where k is the apparent first order rate constant and A$^-$ is the suggested catalyst.

The maltodextrin dry solid system demonstrated reactivity of amino acids towards acrylamide when heated in the absence of water at 180 °C. The rate of loss of acrylamide in this system was very similar to that in starchy foods. In this model, most of the amino acids showed similar reactivity towards acrylamide, with the exception of lysine, which was higher. Lysine is also one of the most reactive amino acids in the Maillard reaction. The effect of temperature on the amino acid-acrylamide reaction followed the Arrhenius relationship, showing the highest E_a (83 kJ mol^{-1}) for the reaction of acrylamide alone in the matrix. The reaction of glycine and proline with acrylamide showed lower but similar E_a of approximately 70 kJ mol^{-1}.

Our data indicates that acrylamide is lost from food by reaction with amino acids. This augments the already established effect of amino acids (e.g., glycine) on acrylamide formation by competing with asparagine for a key precursor of acrylamide, and explains published reports describing the effects of amino-containing food ingredients on acrylamide residues. Maltodextrin proved to be a good model system in which to study reactions of acrylamide with amino acids in the dry state, as evidenced by rates of reaction of acrylamide which are similar to those observed in foods.

The relationships between the formation (and loss) of flavour compounds and acrylamide were measured in potato and rye. Simple cakes were prepared from flour and water and were heated at 180 C, from 10 to 60 min. The flavour compounds studied were Strecker aldehydes, which are formed relatively early in the Maillard reaction and may react further, and alkylpyrazines, which may be considered as end-products. The data obtained was used to create a kinetic model, which was then used to predict the effect of glycine on flavour and acrylamide formation in potato cakes.

The relationships between the formation (and loss) of flavour compounds and acrylamide were different in potato and rye. In both potato and rye, Strecker aldehydes formed and decomposed in a similar way. However, in rye the rate of formation of the Strecker aldehydes was similar to that for acrylamide, whereas, in potato, acrylamide formed at a slower rate than in rye. Pyrazine formation in rye and potato mirrored acrylamide formation. However, acrylamide levels decreased on prolonged heating whilst pyrazine levels were maintained in both rye and potato. It appeared that moisture levels below 5% were needed for pyrazines and acrylamide to form in both foods. Potato cakes contained higher initial moisture levels and hence formation of both acrylamide and pyrazines occurred later in the cooking process for potato than for rye.

The Maillard reaction could be responsible for potential toxicants other than acrylamide. Two databases, each containing chemical and toxicity data, have been compiled in Microsoft Access for Maillard compounds and for lipid-derived compounds. The Maillard database lists approximately 570 volatile compounds, while approximately 200 volatile compounds are listed as products of heated lipid systems. A molecular modelling approach has been used to assess the potential toxicity of each compound. Both databases are fully searchable and contain the following information:

- Chemical name, Chemical structure, CAS number
- Other names
- Foods in which the compound is present
- Foods in which the compound is present at levels above 1 ppm
- Reaction mixtures in which the compound is present
- EU Approved Flavourings Register FL No.
- Predicted carcinogenicity probability
- Predicted mutagenicity probability
- Predicted rat oral LD50 (mg/kg)
- Predicted chronic lowest observed adverse effect level (LOAEL) (mg/kg)
- Predicted skin sensitisation probability

The databases provide a useful source of information for chemists and toxicologists. They are an important starting point for future research into toxic compounds formed during cooking.
The data bases can be found at www.heatox.org, Achievements.

The formation of acrylamide during the roasting process of coffee was investigated in detail in lab scale using a roasting device that employs convection roasting. The model system was a mixture of asparagine with glucose or sucrose using a heating device that allowed the constant heating of small samples (max 300 mg) up to 300 °C with an efficient heat transfer. Studies using ^{13}C-labelled asparagine showed that this amino acid is directly converted to acrylamide. Intermediates were synthesised (N-glucosyl-asparagine and the asparagine Amadori compound) that were converted to acrylamide by heating. At high temperatures – which are used during roasting of coffee – these two compounds could be identified as intermediate reaction products. A statistical analysis suggests that asparagine is the limiting compound in reactions leading to acrylamide in coffee. Sucrose is present in green coffee in concentrations up to 9%. A statistical analysis suggests that asparagine is the limiting compound in reactions leading to acrylamide in coffee. In coffee shorter roasting times and lower temperatures are leading to higher acrylamide concentrations. This means that darker roasted coffee – higher degree of roasting – has lower acrylamide concentrations.

HMF (hydroxymethylfurfural) is formed in coffee very quickly which means that within 2 min a maximum concentration is achieved. After this time a quick degradation occurs to levels significantly lower (ca 100 μg/g). This means that HMF is not heat stable and reacts further during the roasting process to substances that are not known now. HMF is formed from sucrose with and without the participation of amino acids.

2.2. New and improved processing technologies to minimise the formation of hazardous compounds

2.2.1. Potato

Potato has been a staple food for many decades and mainly consumed as boiled or fried potatoes. Our dietary habits have changed and today we are used to many different potato dishes and there are a number of potato products on the market. Potatoes are rich in starch, and the aim of heating potato is to make them palatable and to gelatinise the starch to make it available for digestion.

At the beginning of the project a **mindmap** was made, se Figure 4 below. Such a mindmap serves to find relationships between the numerous variables that – in this case – potentially influence various quality parameters of French fries. With a special focus on acrylamide, the mindmap gave clusters of variables that were somehow related. These clusters of variables formed the basis of our three-year research programme.

Figure 4. Mindmap for potato processing and acrylamide formation

2.2.1.1. Heating model: Fry Simulator – French fries

A computational model was made to estimate the impact of various processing parameters on the formation of acrylamide in French fries. The model uses a cross section of a French fry of infinite length and is made up from a 40*40 grid. The model was validated by measuring temperature and water profiles in time and at various positions inside the French fries. At a later stage also acrylamide measurement were performed on parts of the fries. The latter results showed that although the trends are correctly predicted in the Fry Simulator, its value in absolute sense is limited. Moreover, since the model only calculates acrylamide in the middle, it leaves out the acrylamide that is formed on both ends of the fries. From experimental data it is well known that the highest levels are found at both ends.

2.2.1.2. French fries

Experiments were performed with potatoes grown in the Netherlands. Batches of three varieties of 1000 kg each (Asterix, Bintje, Lady Olympia) were supplied by a potato processor, shortly after the harvest and were stored at 8°C until the end of June. In the varieties Asterix and Bintje the sugar concentrations increased during a 6 month storage period. The sugar contents of the Lady Olympia potatoes decreased during the storage. We also found that the acrylamide levels were positively correlated to the levels of reducing sugars. We found that acidic blanching prior to industrial frying is an efficient way to reduce acrylamide during final product preparation at home or in restaurants and caterers. When using 0.5% of citric or lactic acid, acrylamide levels are reduced some 80% while expert panels do not detect any acidic aftertaste. Other methods of introducing small amounts of acid onto the surface have been considered, such as spraying acid solutions on frozen fries. Such methods were expected to be no more efficient than acidic blanching but much more difficult to implement.

2.2.1.3. Potato crisps

Panda Potatoes variety, crops 2004, 2005, 2006 were used for experiments and were pre-treated before frying at 120, 150 and 180 ºC: washing with distilled water, blanching at 90 ºC per 1 minute and immersion in dilute citric acid solution 1-0.25% for 1 hour. Experiments were performed in vacuum and at atmospheric pressure. Vacuum frying with and without pre-treatments was shown to produce low level acrylamide crisps, see Table 2. It is possible to improve their quality characteristics. The pre-treatments represent a practical industrial alternative to reduce acrylamide level in potatoes crisps.

Table 2: Effect of pre-treatments, frying methods and frying temperatures on acrylamide content and acceptability score (1-7) of crisps.

Pre-treatment		Frying method			
Blanching	Immersion citric acid 0,25%	Atmospheric	Vacuum (50 mm Hg)	Acrylamide	Acceptability
90ºC/1 min	1h	Temp.ºC	Temp.ºC	ppb	Score*
+	+	*150*	-	*6*	*4,3*
+	+	*160*	-	*5*	*4,3*
+	+	*170*	-	*30*	*4,4*
-	-	150	-	87	2,1
-	-	160	-	233	2,9
-	-	170	-	760	2,9
+	+	-	*150*	*ND*	*3,3*
+	+	-	*160*	*ND*	*3,5*
+	+	-	*170*	*4*	*3,5*
-	-	-	150	80	4,6
-	-	-	160	218	5,3
-	-	-	170	267	5,1

*Score 1-7, with 7 as the best   ND = Not Detected

Blanching and change of pH by immersion of sliced raw potatoes in citric acid solution (0.25%), had a strong influence for reducing the acrylamide content in crisps fried at atmospheric pressure and in vacuum. Acrylamide content of potatoes crisps with pre-treatments fried in vacuum were very low between non detected levels up to 4 ppb. At atmospheric pressure with pre-treatments, the acrylamide content was between 6 up to 30 ppb. Without pretreatments, the acrylamide content of the crisps varied between 80 – 267 ppb for vacuum frying and at between 87 – 760 ppb for frying at atmospheric pressure.

The sensory evaluation (trained and consumer panel) obtained as acceptability score (1 – 7), was better for the crisps with pretreatments fried at atmospheric pressure than in vacuum, 4.3 compared with 3.5. A commercial crisp sample sensory evaluated at the same time scored 4.8 with an acrylamide content of 536 ppb. The sensory panel concluded that the best crisps were obtained in the samples corresponding to control assays, submitted to vacuum frying processing at 160ºC and 170 C. Considering the assays with pre-treatments, the vacuum fried crisps got a better evaluation than the crisps fried at atmospheric pressure.

Conclusion: Vacuum frying is a processing method not commonly used in the industry, however, it can be a very good alternative in the near future (acrylamide free crisps)

Photos: Lilia Masson

2.2.1.4. Raw materials selection

Potato field in the south of Sweden. Photo: K. Olsson, Svalöf Weibull

Well characterised Swedish-grown potatoes, including two breeding clones that are resistant to low-temperature sweetening were studied and batches were obtained from three consecutive years. The content of free amino acids and sugars in potato were studied in potatoes from three growing seasons. In some potatoes, the levels of reducing sugars were practically the same the first two years, but almost doubled the third year, while in another variety; the level of reducing sugars decreased to half the second year and increased the third year. The concentrations of asparagine were generally not so much affected by the growing season. These observations make it difficult to draw conclusions on which variety processing conditions that are best for crisp production because this may vary with year.

Frying conditions were discussed with crisp producers and producers of frying equipment to establish laboratory conditions that would resemble industrial conditions. For industrial crisp production, potato slices are usually passed on a belt through a continuous fryer with a starting temperature around 180-190 C and an end temperature of around 150 – 175°C. Quality parameters such as crispiness and colour have to be taken in account. A water content below 2% is considered to give a good crispiness and shelf-life. Using genotypes with a wide range of precursor concentrations, we found that the asparagine levels affect the formation of acrylamide to a higher degree than earlier studies have shown. Crisps made from potatoes with the lowest asparagine content, generally had the lowest concentration of acrylamide regardless of storage temperature. In agreement with work by others, we found that storage at 4°C enhanced the concentration of sugars in he tubers and thus the acrylamide content compared with storage at 8°C.

Blanching before frying was shown to efficiently reduce the acrylamide content in crisps. For potatoes stored at 4 C, the acrylamide content was generally reduced by at least 50%. The mass transport of precursors during heating may be important for acrylamide formation, but this requires further investigation.

2.2.1.5. Home cooking

Much work has been devoted to the mitigation of acrylamide in potato crisps and French fries, but literature data on acrylamide in home-cooked potato dishes is scare.

Several home-cooking experiments were performed. Roasted potato wedges and pan fried boiled potato dices (a common Swedish dish) had an appetising appearance; the wedges were soft with a golden yellow surface. Acrylamide was found in all cooked samples. Blanching before oven roasting made it possible to reduce the heating time in the oven and yet obtain potato wedges with similar eating quality. Blanching combined with a shorter roasting time is an easy and efficient way of reducing the acrylamide content in oven roasted potato wedges.

Domestic fryer.
(Photo: University of Bologna)

The effect of frying in a commercial available domestic fryer was studied in relation to acrylamide formation in French fries. It was found that the frying temperature drops drastically and slowly reached its original temperature. The drop in temperature depends of the product/oil ratio and makes it difficult to define an initial oil temperature as a measure to reduce acrylamide.

The results showed that higher initial oil temperature causes a faster recover of oil temperature during frying, as well as a greater acrylamide formation rate. The choice of a lower initial frying temperature lowered the acrylamide content of the product. At the optimal cooking time in terms of culinary quality, the lower the potato quantity the lower acrylamide content was found.

Our results underline that the kind of frying equipment (heating power), frying time, oil temperature, amount of potato immersed in the hot oil (product/oil ratio) have to be taken into account for optimisation of product quality (colour, texture, water and oil content) and acrylamide content in French fries.

Since par-fried French fries do not contain acrylamide when leaving the factory, it is important to instruct users (both consumers and the out-of-home channel) to cook the products in a proper way. Time and temperature instructions should be clearly stated on the package, preferably at somewhat lower temperatures. Instructions should also consider the volume of product to be cooked. For frying the ratio of oil-to-product should be clear. For oven cooking, the electrical power-to-product ratio should be indicated. Secondly, we found that power of household equipment (ovens and deep fryers) varies and should be taken into account as well. Producers of consumer appliances could improve their product by using better time and temperature controls.

Most important perhaps is that many consumers overcook French fries, thereby increasing acrylamide levels substantially, simply because of specific liking (darker and dryer end product) or routine behaviour or sometimes negligence.

2.2.2. Bread

Studies have shown that asparagine is the limiting factor for formation of acrylamide in bread, and that asparagine is mainly concentrated in cereal bran. This means that high fibre flour will result in higher acrylamide content than sifted flour. New fermentation techniques could help in break down of asparagine and thus lowering acrylamide.

The effects of asparagine and fructose, added to the dough, were studied in an experiment with a full factorial design. More than 99% of the acrylamide was found in the crust. Added asparagine dramatically increased the content of acrylamide in crusts dry matter (from about 80 µg/Kg to between 600 and 6000 µg/Kg) while added fructose did not influence this content. Both time and temperature increased the acrylamide content in crust dry matter, and a significant interaction was found between these two factors. When baked at different conditions with the same ingredients, a highly significant relationship between colour and acrylamide content was found.

Added asparagine increased acrylamide content in yeast-leavened breads while added glycine decreased the content. The more asparagine in the dough, the stronger was the reducing effect of glycine. When glycine was applied on the surface of the fermented dough, also a significant reduction of acrylamide content in the bread was found. Addition of glycine but not of asparagine caused an increased browning reaction during baking.

Fermentation of doughs made with different milling fractions showed that most of the asparagine was used up after 2 hr of fermentation with bakers yeast. Sourdough, on the other hand, did not reduce the content of free asparagine as efficiently but had a strong negative impact on asparagine utilization by the yeast. This indicates that this type of fermentation may result in bread with higher acrylamide content than breads fermented with yeast only. Short fermentation time compared to longer fermentation reduced acrylamide content in bread made with whole grain wheat by 87%. For bread made with rye bran, the corresponding reduction was 77%.

Baking time and temperature increased acrylamide content in rye crisp bread. A significant interaction between time and temperature of baking was found. Added asparagine had a significant effect on the formation of acrylamide but fructose had not. There was a correlation between acrylamide content and colour of the milled bread in the time-temperature experiment but this correlation was not observed in the experiment with added precursors. Added oat-bran concentrate with high content of mixed-linkage β-glucan did not influence the acrylamide content in the breads.

2.2.2.1. New baking techniques

New baking techniques such as air jet impingement and infrared radiation baking has focussed on wheat based bread. Various baking technologies have been studied: Traditional baking; Steam baking; Jet air impingement baking, and Infrared radiation (IR) baking.

Baking by infrared radiation.
(Photo: Swedish Institute for Food and Biotechnology)

First the influence of baking parameters on acrylamide formation and bread characteristics at conventional baking was evaluated. We could show that the highest acrylamide levels normally were found in the outer part of the crust and that the levels increased with increased baking temperature (200-260 C) and prolonged baking time (10-25 min). However, at the highest temperature-time combination (260°C; 20 min) the acrylamide level decreased and was lower in the outer crust than in the inner crust. From this knowledge base, we worked along two lines: the application of steam to traditional baking and alternative heating techniques, such as infrared radiation and air jet impingement.

Steam baking
We studied the effects of using steam during the final part of baking. The reference baking parameters were 200 C baking temperature for 20 minutes, and this was compared to baking when steam was introduced after 5, 10, or 15 minutes of baking. The steam also lowered the temperature of the bread, so for comparison baking experiments were performed where similar temperature profiles were obtained by temperature adjustments in the oven. Both the steam baking and the "falling temperature" baking substantially lowered the acrylamide levels in the final bread crust. However the crust colour was more influenced in the falling temperature breads than in the steam baked breads. With steam baking it was possible to find conditions that gave bread of the same colour level as that achieved by traditional baking, but with considerably lower acrylamide levels. With falling temperature baking, however, though acrylamide levels were lowered, the bread colour was lighter. Using sensory analysis, we could show that it was possible to bake bread (using steam the final 5 minutes) that was not significantly different from the control regarding odour, appearance, texture, and flavour, but having 40% lower acrylamide level.

Alternative baking techniques
The effect of new baking techniques, such as air jet impingement and infrared radiation baking, on bread crust formation and characteristics was initially studied. From the knowledge of such experiments baking conditions were defined to reach similar crust colour with the various baking techniques. It was obvious that the acrylamide content in the bread crust could be reduced by using alternative baking technologies. Reduced levels were obtained with infrared radiation baking as well as with air jet impingement baking.

Preliminary experiments were also performed to study the flavour formation in bread baked by these new technologies. It turned out to be a close relationship between a large number of volatiles and the formation of acrylamide for the baking methods studied, especially those volatile and odorous compounds that are known to be formed via browning reactions in the bread crust, e.g. Strecker aldehydes and alkyl pyrazines. Obviously, a baking method that resulted in low acrylamide content in these experiments also tended to result in low contents of important flavour substances.

We then further focussed on IR baking to evaluate how the IR baking can be optimized with regard to acrylamide minimisation and sensory product quality. Two different IR wavelength regions (one in the shorter range, IRS; and one in the medium range, IRM) and two effect levels (100% and 50%) were compared, using traditional baking as a reference. Flat bread cakes were used for the study. We followed the development of crust colour, acrylamide, flavour compounds and sensory characteristics with baking time at the various baking conditions. One important finding from these experiments was that with IR baking it is possible to obtain a sensory profile almost identical to that of the conventionally baked bread, but with considerably lower acrylamide content. A reduction of the acrylamide content by 60% could be shown.

The main conclusion is that it is possible to reduce the acrylamide content while retaining the sensory quality both by introducing steam in traditional baking and by using new alternative baking techniques. In this work it was possible to reduce the acrylamide content by 40% in white bread, by applying steam during the final five minutes of baking, with no significant changes of the sensory quality. Using infrared radiation heating it was possible to reduce the acrylamide level in flat bread cakes by 60% with retained sensory properties.

2. Analysis

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Swedish Food Administration (Sweden), Universitat de Barcelona (Spain),
Institute of Chemical Technology Prague (Czech Republic), Universitat de Barcelona (Spain), National Food Institute (Denmark),
Norwegian Institute of Public Health (Norway), Stockholm University (Sweden), Lund University (Sweden)

3.1. Method development

Several experiments have been performed with the aim to optimize and validate analytical methods for heat-induced toxicants in different types of foods, of high accuracy and precision and suitable for use by producers and enforcement laboratories.

Methodology for acrylamide analysis is now mature. The available LC-MS and GC-MS methods are capable of testing all the food types of interest down to the concentrations of interest. Accuracy is acceptable although precision could be improved. Method development and validation has been assisted by an understanding of the potential interferences in particular food types, and how to avoid them.

To provide analytical data for intake estimates, the detection limit of the bromination-GC-MS method was improved to give a low (single-figure) µg/kg limit of detection. The improved method was applied to the analysis of UK total diet samples. The estimated dietary exposure to acrylamide was within, and at the low end of, estimates made in other countries.

This Br-GC-MS method was found to be more variable than wanted in between-lab trials and an LC-MS/MS method was developed and validated. The development work included systematic studies on extraction conditions, optimised extract clean-up by SPE, and chromatographic column separation. The method was subjected to inter-laboratory validation under the auspice of JRC-irmm. Repeatability (r) figures were 4-9% with potato crisp samples and 3-8% with bakery products. The reproducibility (R) values were 9-13% and 5-12%, respectively. For evaluation of trueness, the study included a spiked potato powder (500 µg/kg) and a candidate reference material from crisp bread (980±90 µg/kg). The mean results for these were 485 µg/kg (RSDR=9%) and 950 µg/kg (RSDR=5%), respectively, indicating that the method bias is low or nonexistent.

A variation of the method was developed for testing low acrylamide levels in baby food. The multimode step was omitted and proteins were precipitated by adding $KAl(SO4)2$ prior to SPE on ENV+. An LOQ of 0.5 µg/L was obtained.

A new GC/HRTOF-MS analytical procedure enabling direct determination of acrylamide (no derivatisation) in various food matrices was validated. Also, a new LC-MS/MS method employing a novel sample preparation strategy was implemented. For quantification, either D3-acrylamide (GC/HRTOF-MS) or 13C3-acrylamide (LC-MS/MS) are employed. The accuracy of these methods is checked regularly via proficiency test scheme FAPAS®. Satisfactory z-scores were obtained: -0.3 (Baby Rusk Test Material, series 30 Round 9); 0.2 (Potato Crisps; 30:11), 0.4 (Breakfast Cereals; 30:14) and 1.5 (Crisp bread; 30:15). Performance characteristics are as follows:

| GC/HRTOF-MS	LC-MS/MS
LoD = 10-30 µg/kg, depending on matrix repeatability (RSD) = 2% (chips, ca 500 µg/kg)	LoD = 10-25 µg/kg depending on matrix repeatability (RSD) = 3% (chips, ca. 700 µg/kg)

GC/HRTOF-MS
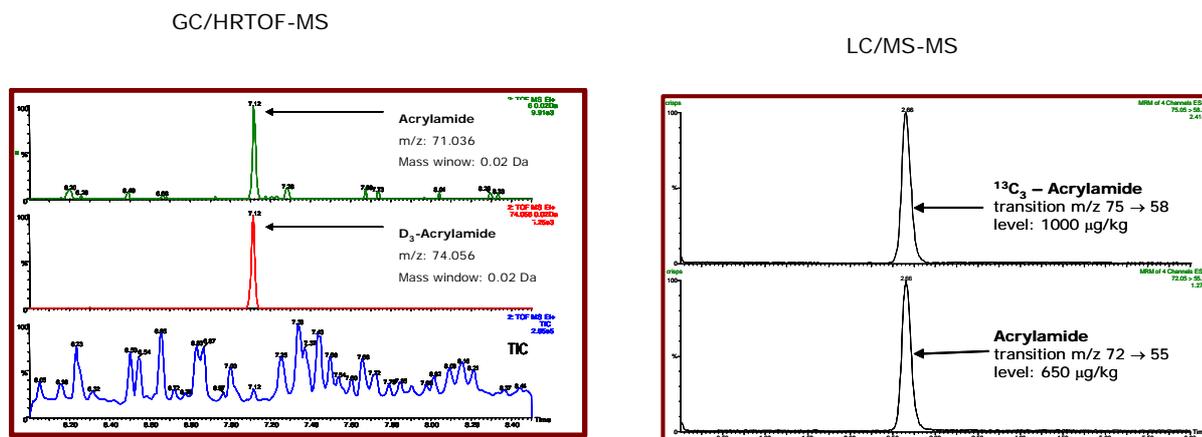
LC/MS-MS

Figure 5: *SPME-GC-ITD/MS and SPME-GCxGC-TOF/MS methods for determination of heat-generated volatile compounds.*

A method for the analysis of acrylamide by LC-MS/MS using an ion trap system with atmospheric pressure chemical ionization (APCI) was developed.

A micro-emulsion electro-kinetic chromatography (MEEKC) method has been established suitable for acrylamide analysis at high levels in certain food types.

Besides the direct analysis of acrylamide using LC-MS, a derivatisation method using 2-mercaptobenzoic acid was adopted that results in a substance with a higher molecular weight, which is more selective than the low molecular weight of acrylamide itself at 72. This method was used for the analysis of acrylamide in coffee and in model systems. The same derivatisation with mercaptobenzoic acid was used prior to a CZE analysis using FASI (field-amplified electrokinetic sample injection) as a pre-concentration on-line technique to increase sensitivity.

Additionally a method for an intermediate compound was developed namely 3-amino-propionamide. This substance could be identified in a model system as an intermediate but it was not found during the roasting of coffee.

A method for the determination of HMF (hydroxymethylfurfural) by GC-ITMS using N,O-bis(trimethylsylil) trifluoroacetamide as derivatising reagent has been developed and also an LC-MS method using an ion trap system with ESI as ionisation source.

A method for the analysis of a metabolite HMFA (5-hydroxymethyl-furanoic acid) was developed. This metabolite is excreted via the kidney to the urine. The method was applied to the analysis of urine to estimate the exposure. The results suggest that the exposure is higher than calculated from the exposure data (food analysis). Therefore, it is necessary to analyse other foods and identify any additional source(s) of exposure.

As part of method development for various heat-induced toxicants, different extraction techniques including solid phase extraction (SPE), solid phase micro-extraction (SPME) and pressurized solvent extraction have been explored to good effect.

In response to the report on "masked" acrylamide, the pilot study of its formation in model system was initiated and possible precursors of "masked" acrylamide were identified.

3.2. Analytical results on heat-induced toxicants levels in foods

The acrylamide levels in breast milk and Swedish baby food products were tested. The average levels for gruel, porridge and canned baby food, all ready to eat, were 1.4, 26, and 7.8 μg kg^{-1} respectively. For all breast milk samples except one, acrylamide was below the limit of quantification (0.5 μg kg^{-1}). The mean intake between seven and twelve months of age was estimated to be about 0.5 μg/kg body weight/day.

The acrylamide level in toasted bread was investigated in order to aid the estimation of intake from home-prepared foods. The amounts of acrylamide produced in the different breads by toasting were strongly related to the concentrations before toasting. There was hardly any acrylamide increase in the light toasted breads. The levels for medium toasting were about 2 to 5-times higher than the levels before toasting.

Table 3. Acrylamide concentrations in toasted bread (corrected for weight loss).

Toasting time	wheat		wheat		wheat + rye		wheat + rye	
	Weight loss (%)	Acrylamide (µg/kg)	Weight loss (%)	Acrylamide (µg/kg)	Weight loss (%)	Acrylamide (µg/kg)	Weight loss (%)	Acrylamide (µg/kg)
no toasting	0	3,0	0	8,4	0	28,8	0	41,6
1	11	3,9	10	8,7	11	29,7	11	43,9
2	14	4,2	12	9,6	12	28,3	13	60,5
3	17	15,9	15	21,6	14	57,7	15	84,6
4	20	31,0	19	57,8	18	113,5	17	118,0

Analysis of acrylamide in foodstuffs (n=98) from Spanish markets and homemade processed foods has been performed. Similarly, a total of 227 data were collected for acrylamide contents in foods from the Turkish market, and 40 data for furan content. The survey for acrylamide included traditional Turkish foods and home-cooked foods. Moreover, French fries collected from fast food restaurants and French fries that were prepared by using frozen potato were also analysed. The French fries from fast food restaurants had the higher acrylamide content. Compared to other food groups, the traditional Turkish foods and bakery products contained lower levels of acrylamide. The survey was performed over two years. The same brands of products were monitored during this period. Although there is not enough data for proving it statistically, there were indications that acrylamide levels in some specific products, including baby biscuits, toasted bread and potato chips, decreased during this period. Typical results are given in Table 4.

Table 4. Data for acrylamide content in some food products sold in Turkey.

Examples of Foods	Acrylamide (µg/kg)	Examples of Foods	Acrylamide (µg/kg)
Roasted almond	207-313	Fried vegetables (eggplant, pepper, squash)	<10
Pop corn	170	Pickled olives	<10-139
Baby biscuits	36-613	Corn bread	<10
Corn chips	198-835	Turkish coffee	227-300
Potato chips	59-2336	Instant coffee*	336
Bread (wheat)	<10-79	Fried potato – home made	60-66
Toasted bread	58-205	Fried potato – frozen potato	72-76
Pilav (ready to eat rice)	<10	Fried potato – fast food	375-428

The carcinogenic substance furan 'emerged' as a heat-induced toxicant in foods during the planning and conduct of the HEATOX project. The issue originated from work by the US-FDA. Our studies made an important contribution in helping to understand aspects of the analysis of foods for furan – crucially the need to avoid strong heating during headspace analysis to avoid misleadingly high results. This provided acceptance criteria and so gave confidence in the analytical data on furan occurrence and concentrations that is being complied by EFSA. The headspace GC-MS method of testing for furan is straightforward and accurate. It can be used both by producers and official laboratories. Studies were then conducted to understand the chemical precursors, the mechanisms of formation, the nature of the

foods affected by formation of furan, and the effect of normal consumer practice of heating and cooking before consumption. This work confirmed that there are different precursors and mechanisms of formation.

On other furan compounds, HMF (hydroxymethylfurfural) was found at high levels in foods containing high amounts of carbohydrates that are stored for a long time or heated strongly. These included dried fruits, coffee, Madeira wine, caramel, balsamic vinegar, non alcoholic and alcoholic beverages.

Comprehensive databases on (i) the reaction products from Maillard browning, and (ii) lipid oxidation products, were set-up and populated with occurrence data and with hazard indications derived from QSAR (quantitative structure-activity relationship) considerations.

Similarly, a database for specified heat-induced toxicants (acrylamide, ethylcarbamate, furan, heterocyclic amines, hydroxymethylfurfural, polycyclic aromatic hydrocarbons and nitrosoamines) has been designed. Concentration levels of heat-induced toxicants in cooked food samples have been introduced in the database. Sample food and cooking data together with analytical information (sample treatment, identification and determination details) have been included in the database. As an example, in Table 5, there is the information about acrylamide in baby food products

Table 5.
Baby foods

	Nº Samples	Nº ND Samples	MIN	MEDIAN	MAX	Units	Determination Technique	Extraction and clea-up procedures	Ref.
Breast milk	4	0	0,25	0	0,65	µg/kg	LC-MS/MS	Precipitation of macromolecules (KAl $(SO_4)_2$)	49
Cereals	12	4	1	10	24	µg/kg	LC-MS/MS	S-L (H_2O), L-L (AcOEt), SPE	49,80,125
Infant formulas	67	64	-	10	-	µg/kg	LC-MS/MS	S-L (H_2O:MeOH 95:5), SPE	123,125
Purées (Fruits, potatoes, vegetables, meat)	22	3	1	22	121	µg/kg	LC-MS/MS	S-L (H_2O), SPE	49,125
Gruels and porridges	10	0	0,25	2	34	µg/kg	LC-MS/MS	S-L (H_2O)	49
Others (Biscuits, cakes, crackers, toasts)	10	0	19,9	118	1568,9	µg/kg	LC-MS, LC-MS/MS	S-L (H_2O), L-L (AcOEt), Carrez, SPE	27,70,80,125

3.3. Analytical method for biomarkers
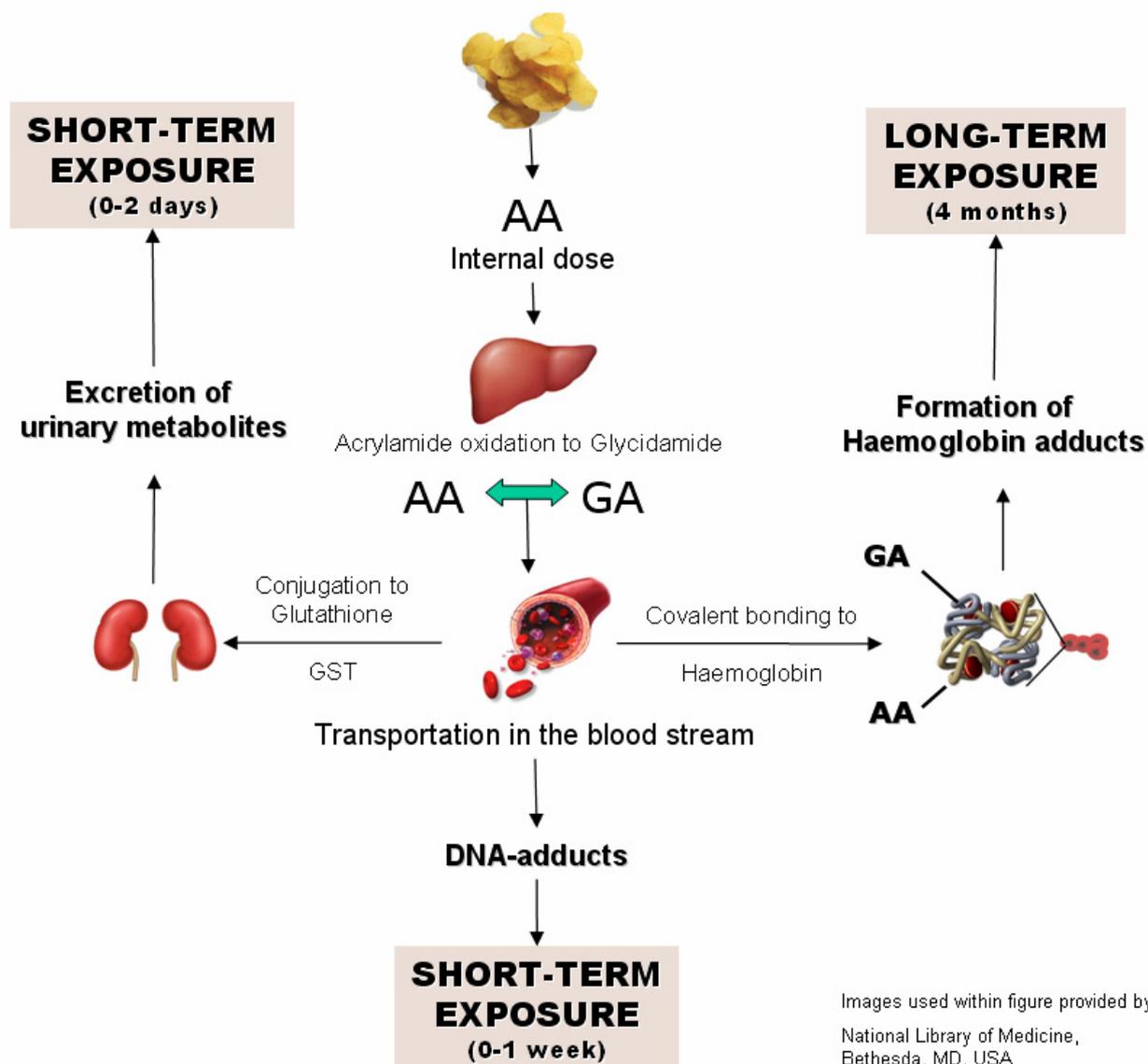
Figure 6. *Biomarkers of AA for determination of short-term and long-term exposure (Thomas Bjellaas - Doctoral Thesis)…*

3.3.1. Identification of DNA-adducts in vivo
An analytical method including the use of LC/MS has been developed for the detection of genotoxic compounds in a rodent bioassay. 2-methylfuran was detected as a possible genotoxic Maillard reaction product in this bioassay. More unknown genotoxins may be identified using this procedure.

Mixtures of single amino acids and carbohydrates were suspended in phosphate buffer and heated to various temperatures for the production of Maillard reaction products as visualized by the colour formation. The Maillard reaction has been upscaled in order to produce sufficient material for rat feeding studies and a method for a fairly homogeneous incorporation of the Maillard reaction product into rat feed pellets has been developed. A study of the palatability in rats showed that two grams of Maillard product in 15 grams of rat feed was well tolerated after a short adaptation period.

Rats were given feed with reaction products from different amino acids and after 5 days DNA was isolated from different organs. The DNA was hydrolysed and analysed by HPLC/MS.

Chromatographic data of DNA from dosed animals were compared with data from control animals and differences, suggesting the presence of DNA-adducts, were detected by use of "metabolite detect" computer software.
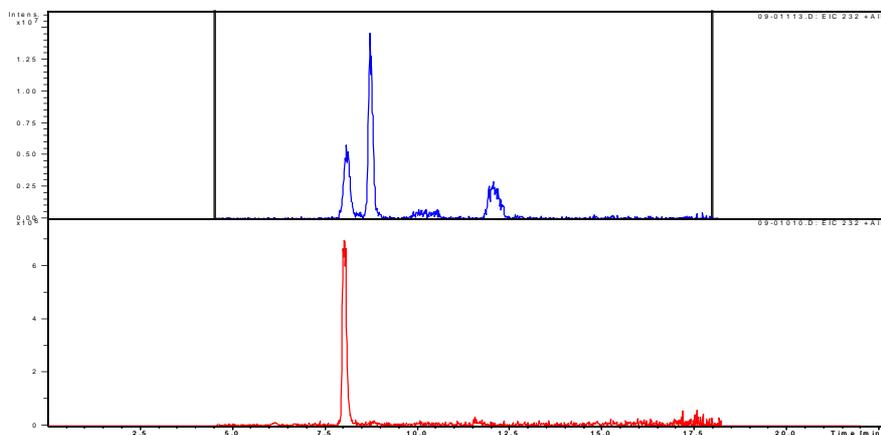
Figure 7. Extracted ion chromatograms m/z=232 of hydrolyzed intestinal DNA (upper) and a synthesised standard (lower).

At least two possible DNA adducts with mass 232 were detected by HPLC/MS analysis of hydrolysed DNA extracted from rodents following feeding with Maillard reaction products (Figure 7, upper panel). Analysis of the adducts by high resolution TOF-MS gave exact masses of 232.08345 for both adducts which is in accordance with the molecular formula $C_{10}H_{10}N_5O_2$.

The molecular formula suggested a guanine adduct to which $C_5H_6O_2$ had been added followed by elimination of water.

A possible candidate to one of the adducts 4-oxo-2-pentenal was synthesised by oxidation of the known Maillard reaction product, 2-methylfuran with m-chloro-perbenzoic acid.

Reaction of 4-oxo-2-pentenal with deoxyguanosine gave a product which after acid hydrolysis had identical retention time and mass as the first eluting adduct found in rodent DNA (Figure 7, lower panel).

3.3.2. Acrylamide metabolites as biomarkers of exposure
Exposure to acrylamide from food has been estimated using records of food consumption combined with knowledge on acrylamide content of various foods. However, these intake estimates needs to be validated against biological markers of exposure.

The concentration of haemoglobin adducts of acrylamide reflects the internal dose of acrylamide during the last 3-4 months, while we had no markers for short term intake of acrylamide. The bioavailability of acrylamide from food is an important issue which was addressed in experiments on mice.

In one study, 53 persons answered a food frequency questionnaire about the daily food intake and also had answered a 24 h food recall. Blood and 24h urine samples were collected.

In another study, mice were kept in metabolic cages and were exposed to crisp bread with different concentrations of acrylamide, subcutaneous injections of corresponding doses of acrylamide, including ^{14}C-labeled acrylamide.

Acrylamide and its oxidation product glycidamide are conjugated to glutathione and these conjugates are stepwise converted into N-mercapturic acids which are excreted into urine fairly rapidly. The mercapturic acid metabolites including [2]H-labelled standards as well as an external standard were synthesised and characterised (Figure 8).
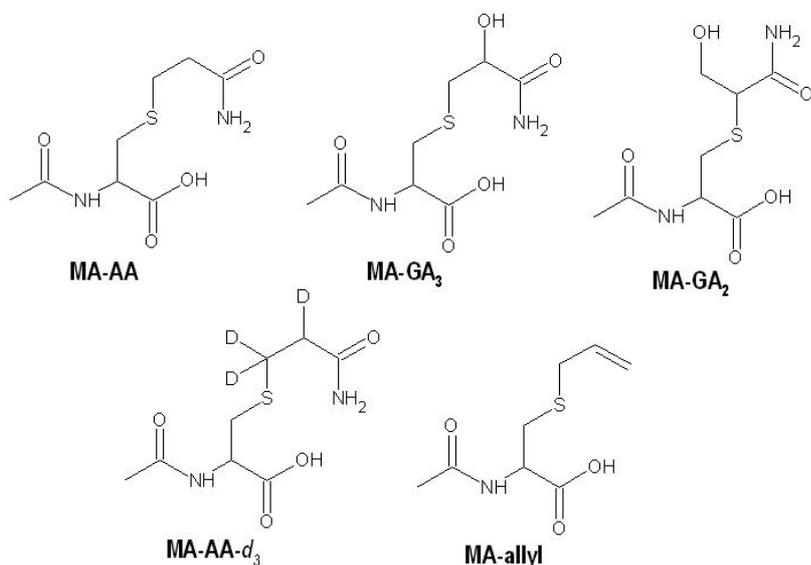
Figure 8. *Structures of standards used in the study.*

An analytical method of determination was developed based on solid-phase extraction of urine followed by LC-MS/MS separation and quantification (Figure 9). The method of analysis was applied in a pilot study of six persons. Fasting caused a 50 percent reduction in excretion of mercapturic acid metabolites clearly showing that food is an important source. Smoking was associated with a high excretion of mercapturic acids.
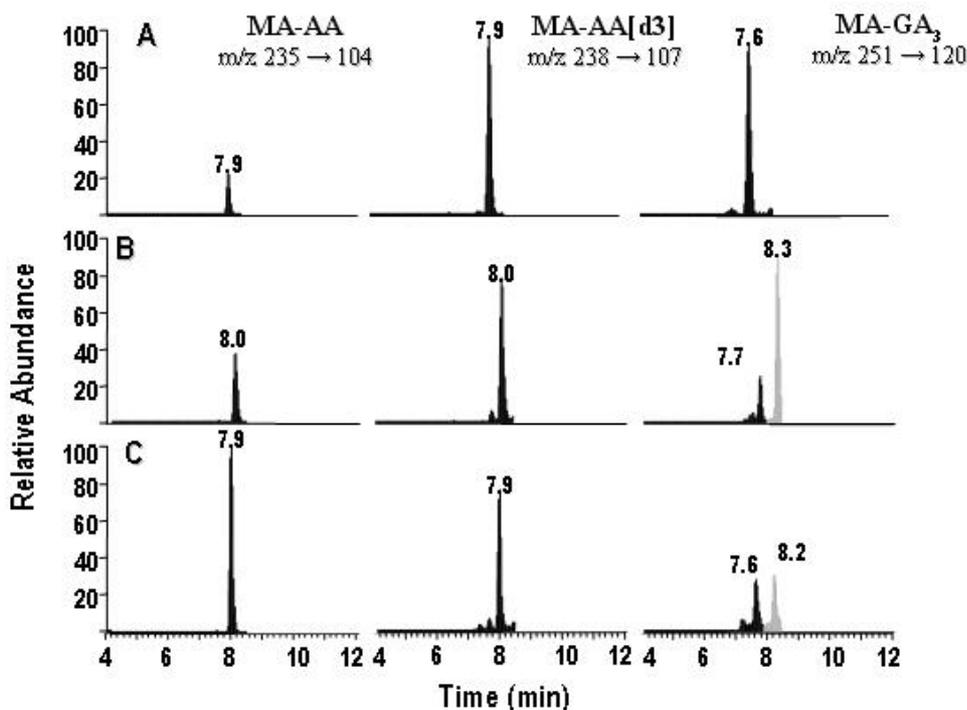
Figure 9. *HPLC-MS/MS chromatogram of (A) a spiked urine sample at 40 µg/L for MA-AA and MA-GA3, (B) urinary metabolites from a non-smoker and (C) urinary metabolites from a smoker. Peak intensities are set relative to the spiked urinary sample. The grey shaded peak at 8.2 min might possibly be the sulfoxide analogue of MA-AA.*

It was shown that excretion of urinary mercapturic acid metabolites was related to food intake, the biomarker responded quickly to food with a high content of acrylamide, e.g. potato crisps (Figure 10).
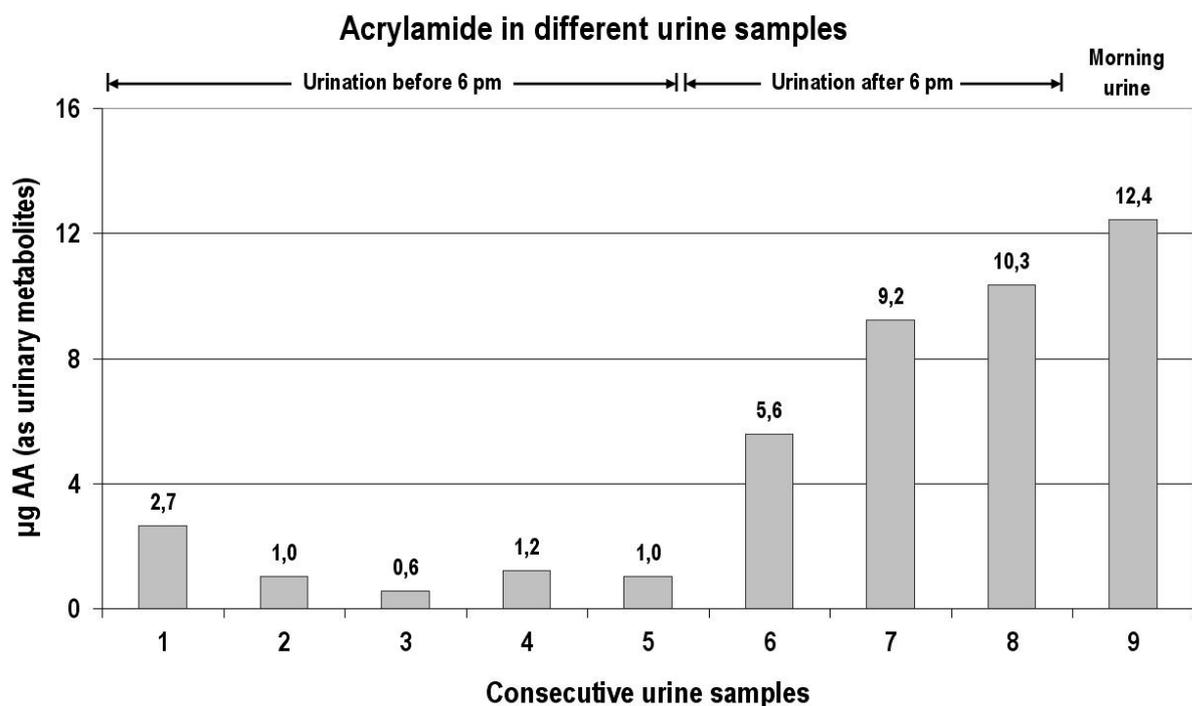
Figure 10. *The total amount of AA found as urinary metabolites in consecutive urine samples in a participant who reported intake of potato crisps during the evening.*

On the assumption that 55% of the acrylamide dose is excreted as mercapturic acids, the average dose in the 53 persons was 30 µg/day. Excretion correlated with estimated recent intake, but not with the total estimated amount during the last 24 hrs. Multiple regression analysis showed that the intake of aspartic acid (including also asparagine), starch and coffee were significant predictors of AA metabolite excretion. The method of urinary excretion could also be used for the identification of particular foods contributing to acrylamide exposure.

Acrylamide haemoglobin (Hb) adducts were determined in the same study subjects. The level decreased with age, possibly explained by a lower intake of foods with high acrylamide content, such as potato chips and snacks, in middle aged and older persons. A 3.5-fold higher adduct concentration was found in smokers in comparison with non-smokers.

No correlation was found between Hb adduct levels and total dietary acrylamide intake estimated from reported food consumptions and levels of acrylamide in foods. After adjusting for age significant predictors for Hb adduct formation were intake of chips, snacks and crisp bread.
Haemoglobin adduct concentrations did not correlate with urinary excretion of mercapturic acid derivatives in the same subjects.

In mice exposed systemically to ^{14}C-acrylamide, about 92% of the radioactivity was excreted into urine during the following 48 hrs. There were linear dose response curves both for acrylamide from crisp bread and systemically given (11). The bioavailability of AA from crisp bread was about 100%.
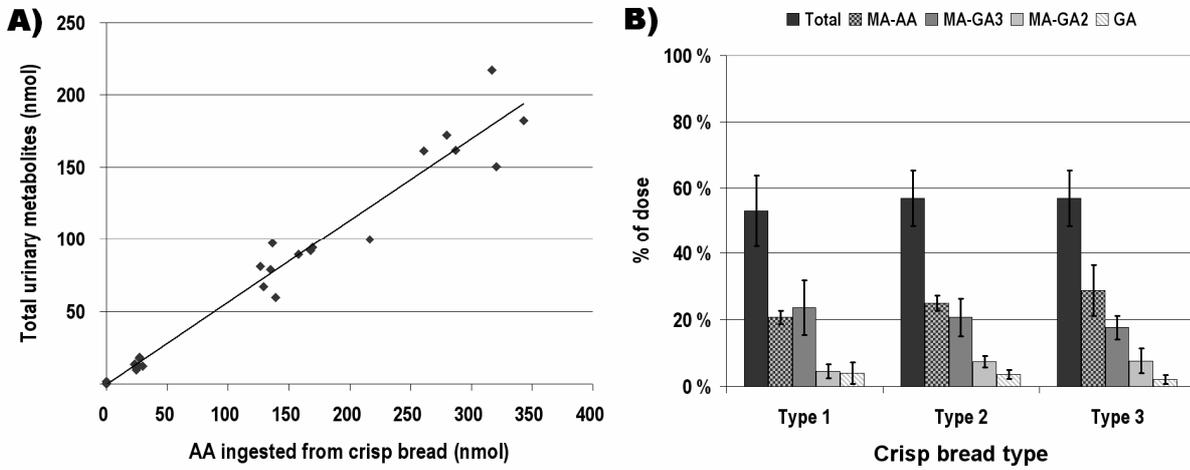
Figure 11. A) *The linear relationship between the amount of acrylamide (AA) ingested from crisp bread and the amount of AA recovered as urinary metabolites. B)* *The percentage of dose recovered in urine for total and the different urinary biomarkers MA-AA, MA-GA3, MA-GA2 and GA*

3. Hazard characterisation

Swedish Food Administration (Sweden), Lund University (Sweden), Stockholm University (Sweden),
German Institute for Human Nutrition (Germany), Norwegian Institute of Public Health (Norway),
*University of Zürich (Switzerland)*

4.1. In-vivo genotoxicity and carcinogenicity

In absence of data on the effects of a chemical on humans it is often necessary to extrapolate the effect from high doses given to animals to lower dose regions for exposed humans. Therefore when the effect of very low experimental doses can be determined the extrapolation is more reliable and makes the risk quantification more reliable. The work has been concentrated around the uptake and the effect in the low dose region of acrylamide and other chemicals produced when heating foodstuffs.

To clarify the shape of the dose response curve in the low dose region the biomarkers for DNA adducts, haemoglobin adducts for acrylamide and glycidamide, and the micronucleus assay (two-lazer-beam flow cytometer) were used. The mice (35) were given by gavages 14 different doses (control included) of acrylamide. The doses were ranged from 0 to 60.0 mg/kg body weight.

Data from the measurement of haemoglobin adducts, Figure 12, and from the micronucleus (MN) assay, Figure 13, are given below. It is clearly shown that there is a dose related increase of the frequency of MN (fMNPCE). The fMNPCE levels for the different mice are about the same as after intraperitoneal injection. The dose response is linear.

Also the analysis of haemoglobin adducts showed a linear dose related increase. However, the lowest level of the quantification of haemoglobin adducts was about 1 mg/kg.
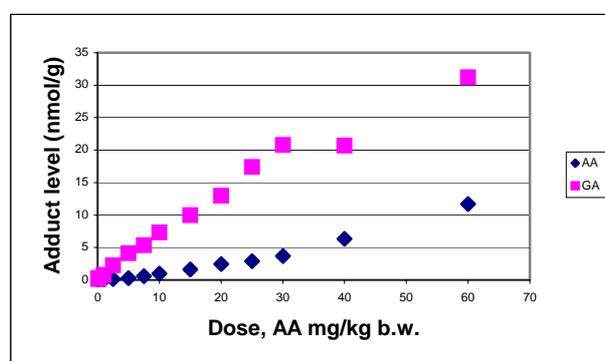
Figure 12. Measurement of hemoglobine adducts
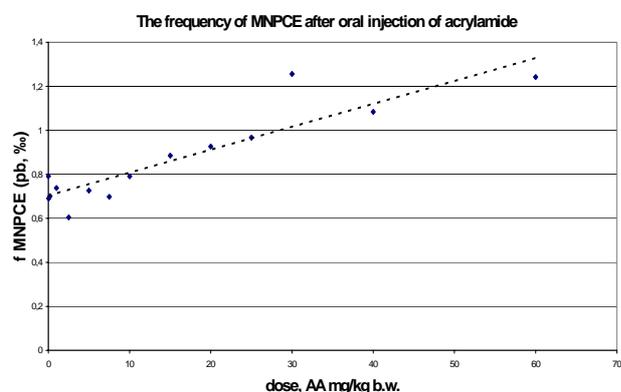
Figure 13. Micronucleus assay

Results from studies using accelerated mass spectrometry (AMS) show that glycidamide binds to DNA in mice at a dose of about 1.5 μg/kg body weight.

In a human study where twenty-four healthy persons eating either
a) boiled (max 100 ºC) and fresh food or
b) strongly heated (fried) and fresh food,
there was a significant difference between the groups regarding the frequency of micronucleated young erythrocytes. From each of the subjects blood samples were drawn twice, before and after an "eating period" of four days. Blood samples were analysed for both the frequency of micronucleus (MN) in young erythrocytes and the haemoglobin adduct levels of acrylamide and glycidamide.

The estimated intake of acrylamide in the two groups was about 25 μg and 3000 μg, respectively. This difference in calculated acrylamide intake was verified by a significant difference (p<0.001) in the level of haemoglobin adducts between the two groups. After the eating period the difference in the mean fMN for the persons belonging to the high-heated-food group (b) was +0.17‰. The difference for the persons belonging to the low-heated-food group (a) was –0.15‰. These two groups are significant separated from each other, p<0.005 (one tailed, paired student t-test), 14.
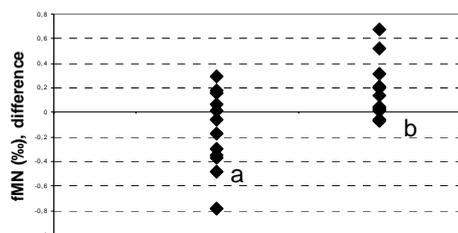
Figure 14. *frequency of micronucleated young erythrocytes in twenty-four healthy persons eating either a) boiled (max 100°C) and fresh food or b) strongly heated (fried) and fresh food …*

There are different possibilities to increase the sensitivity of the micronucleus assay in human erythrocytes; one way is to e.g. discriminate between clastogenic and aneugenic effects. This can be done by a fluorescent *in situ* hybridisation (FISH) of the DNA in the micronuclei. Another way (without FISH) is to optimize the fixation method, in order to better define the cell population (very young erythrocytes) when analysing the cells in the flow cytometer. This latter option was used as a new refined method when the human study was done.

Also other chemicals than acrylamide have been studied upon the potency to increase the frequency of micronuclei, for example furan, metylfuran, HMF, its metabolite SMF, phytosterol oxides, metylacrylamide and also extracts of potato chips. Among these chemicals only SMF showed a positive effect in the *in vivo* micronucleus assay in mice. A comparison of the genotoxicity of acrylamide and three heterocyclic amines (PhIP, IQ, and MeIQx) was made. In the case of PhIP, we found a significant dose-response relationship, while MeIQx and IQ did not give an increased fMPCE level. A comparison between the heterocyclic amines and acrylamide revealed that the slope of the dose-response curve is about ten times steeper for PhIP than acrylamide. In spite of this, acrylamide probably constitutes a higher human risk than heterocyclic amines since the intake of acrylamide is about a hundred to a thousand fold higher.

In conclusion, the results points to an increased risk already at the lowest doses. In the human study the acrylamide dose was about a thousand times lower than the lowest effective doses in earlier studies in mice. It is likely that the explanation of the increase of chromosome instability (micronuclei) is something else than acrylamide and it is possible that the high heating temperature of foodstuffs is responsible for the production of chemicals other than acrylamide inducing a higher frequency of micronuclei.

4.1.1. Data on the relationship between internal dose and estimated intake of acrylamide in blood samples from a biobank

Associations between estimated acrylamide-intake, based on self-reported data on usual food consumption combined with data on published acrylamide content in foods, and Hb adducts were examined with linear regression and correlation analysis. A sample from the Malmö Diet and Cancer cohort was selected to obtain a large variation in acrylamide-intake. The estimated acrylamide intakes ranged from 3.5 to 190 μg/day, with a median of 45 μg/day. In randomly selected individuals the estimated intake was 28 μg/day. The estimated intake thus varied with a factor of ca 50. In contrast to this, the internal doses as measured by acrylamide adduct levels in Hb varied with a factor of 5 in the non-smoking group (see 15). Hb adduct levels are expected to give a more exact estimate of the intake than the estimates from questionnaires. Positive partial correlations between dietary acrylamide-estimates and Hb adducts were seen in non-smoking men but not in non-smoking women. In conclusions, two results from this study cast doubt on the validity of dietary intake estimates used in cancer epidemiology: a) The lack of correlations between adduct levels and intake estimates in non-smoking women, b) The much lower variation in internal doses compared to estimated intakes from food frequency questionnaires.
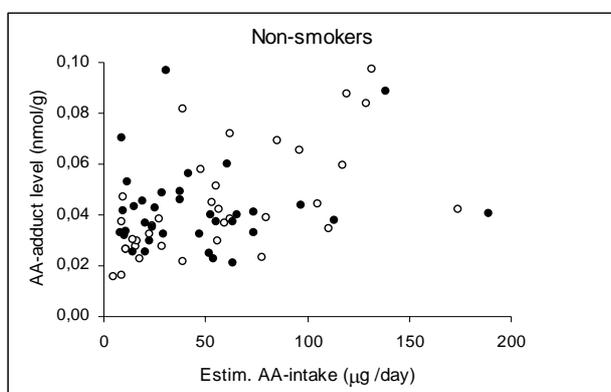
Figure 15. *Relation between estimated dietary AA-intakes from foods (μg/day) and Hb-adducts from acrylamide (nmol/g) in non-smoking men and women in a sub-sample from the Malmö Diet and Cancer cohort. Filled circles represent men and unfilled circles represent women.*

We studied some non-acrylamide compounds that form when carbohydrates are heated, in particular 5-hydroxymethylfurfural (HMF) and related compounds. These compounds are ingested at extremely high levels (1000 times higher than acrylamide) and it has been reported that rodent sulfotransferases (SULTs) can covert HMF *in vitro* to a mutagen, 5-sulphooxymethylfurfural (SMF). We wanted to find out whether human SULTs can mediate this reaction *in vitro* and *in vivo* and to study resulting adverse effects . The objectives included the development of biomarkers for this activation pathway in animals and humans. Our results may be summarised as follows:

A major human SULT form (SULT1A1) was very efficient in converting HMF to SMF. In rat and mouse, only minor SULT forms showed high sulfation activity towards HMF. Therefore, mice and rats may be poor models for humans when studying SULT-mediated toxicity of HMF and congeners.

SMF was identified as a urinary HMF metabolite in a mouse line engineered for expression of human SULT1A1, but not in wild-type mice, corroborating that human SULT1A1 may be a risk factor in exposures to HMF.

SMF, unlike HMF, formed DNA adducts in cell-free systems, was mutagenic in conventional bacterial and mammalian cell test systems, and was strongly nephrotoxic in mice (Figure 16).

HMF and other hydroxymethylfuran congeners present in foods were mutagenic in bacteria and mammalian cells engineered for expression of human SULT1A1. Whereas HMF was only mutagenic when SULT1A1 was expressed, some analogues were also mutagenic in control V79 cells (but not in control bacteria) suggesting the existence of additional bioactivation mechanisms in mammalian cells (*e.g.* mediated by unidentified kinases).

Externally added furfuryl sulphate (unlike SMF) was only cytotoxic, but not mutagenic, to bacterial and mammalian cells. However, when furfuryl sulphate was generated by SULT from its alcohol within target cells, it was strongly mutagenic. This difference in biological activity between internal and external furfuryl sulphate might be due to its charge and short half-life time preventing permeation of the cell membranes.

Adenine was identified as the major nucleobase forming DNA adducts with SMF in cell-free systems. This and other adducts were not detected in target cells and tissues of SMF despite increased mutant frequencies and massive histopathological alterations. Unfortunately we did not succeed developing sufficiently powerful enrichment methods for adducts, as required for their demonstration in cells and tissues. Moreover, the presence of the carbonyl group in 2-postion of HMF/SMF, expected to be still present in the adducts, complicated the analysis, as it might form cross-links with DNA and/or other cellular constituents.

We have demonstrated that major human SULTs, unlike major rat and mouse SULTs, efficiently convert HMF to SMF, and that SMF forms DNA adducts in cell-free systems, is mutagenic in bacterial and mammalian cells in vitro, and is strongly nephrotoxic in mice. The negative result of HMF in conventional in vitro systems is due to a inappropriate metabolic situation, as HMF is positive in the same tests after expression of human SULT1A1 at levels occurring in human tissues. Likewise, the low biological activity of HMF in laboratory animals should be regarded with caution, as these models do not truly reflect the human sulfation capacity. A similar situation was found with other hydroxymethylfuran congeners present at high levels in food, such as furfuryl alcohol.
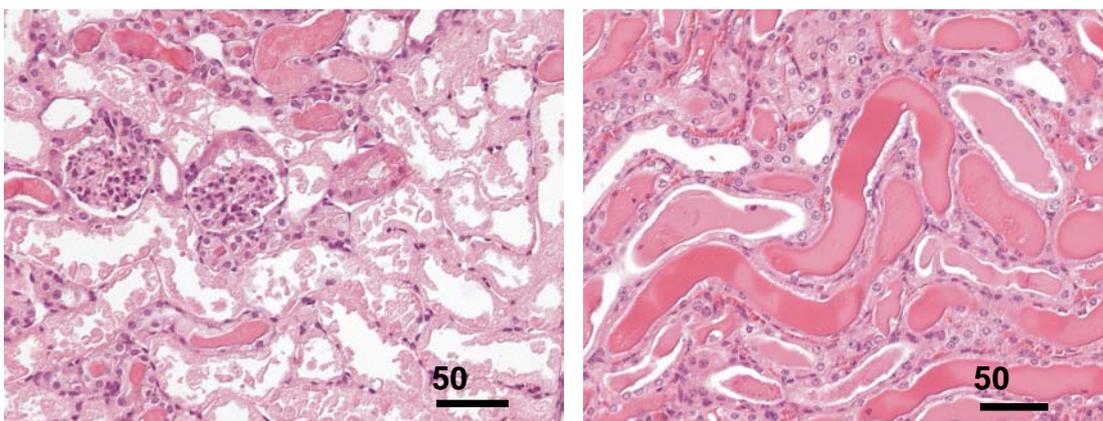

Figure 16. Kidney of an SMF-treated mouse. Note that the proximal tubules were completely destroyed, whereas the glomeruli were damaged much less. The right panel shows abundant proteinaceous casts.

4.2. Tumorigenicity

Two experiments on intestinal tumourigenesis of acrylamide and glycidamide in *Min*/+ mice and their wt litter mates have been completed.

In the first experiment the animals were exposed to acrylamide or glycidamide (10 or 50 mg/kg bw) week 1 and 2 after birth, and in the second experiment they were exposed to acrylamide or glycidamide one week before birth to the dam, alone or in combination with exposure of the pups at week 1 and 2 after birth (50 mg/kg bw). *Min*/+ mice and wild type mice have been treated postnatal with subcutaneous injections of 5-hydroxy-methyl-furfural (HMF) or its reactive metabolite 5-sulfoxy-methyl-furfural (SMF).

Eight weeks old female *Min*/+ and wt mice were exposed to glycidamide at the dose 50 mg/kg dissolved in 0.9% NaCl or to NaCl-solution alone for 6 and 24 hours. RNA was isolated from the epithelium and cDNA was made by *in vitro* transcription of total RNA, and Affymetrix GeneChipswere used for the expression analysis.

A weak tumourigenic activity in the intestine of *Min*/+ mice and their wt litter mates was observed after exposure to acrylamide or glycidamide. The weak tumourigenicity observed was probably related to the perinatal exposure since such effects never previously have been observed in rodents exposed later in life. SMF significantly increased the number of adenomas in the small intestine and the number ACF in the colon of *Min*/+ mice. HMF did also show a tendency to increase the number of lesions.

Gene expression profiles showed a high degree of variability and only one gene was significantly up-regulated by glycidamide in wt mice exposed for 6 h (metallothionein-2, 3.27-fold, adjusted p<0.05). No other genes were significantly regulated in each of the treatment. We investigated differences in gene expression between 6 and 24 hours, expecting different expression of genes involved in circadian exercises. We had from 75 to 1790 significantly regulated genes (adjusted p < 0.05) in each of the four data sets, and indeed, genes involved in circadian rhythms came out as a major functional cluster.

4.2.1. Reproductive toxicity

Isolated mouse testicular cells and lymphocytes (primary cells) from BigBlue[TM] C57BL/6 male mice, either mOgg1^{+/+} or mOgg1^{-/-} (a transgenic mouse strain deficient in oxidated lesion repair) were used. Peripherial blood lymphocytes from humans were also used.

The alkaline single cell gel electrophoresis (comet assay) has been used to measure levels of DNA lesions as single strand breaks and alkali labile sites (baseless sites) after *in vitro* exposure of acrylamide (AA) and glycidamide (GA) in mouse and human cells (see above). To further study and characterise the DNA lesions formed, a modified comet assay was used. The modified assay includes a treatment step in which cellular DNA is subjected to an endogenous DNA repair enzyme that recognises and cleaves certain DNA lesions thereby converting them to single strand breaks, so that they are measurable in the comet assay. The CYP2E1 AA metabolite glycidamide was shown to be genotoxic in mouse testicular cells and lymphocytes *in vitro*, at concentrations similar to those producing genotoxicity in human lymphocytes. The parent compound AA was as expected very inactive. The DNA lesions induced by GA exposure are recognised by repair enzyme which dramatically increases the lesion levels, implicating that human testicular cells might be particularly susceptible to the active metabolite of acrylamide.

4.2.2. Neurotoxicity

Neonatal mice were exposed to different low doses of acrylamide at an age of 10 days. This study indicates that neonatal exposure to acrylamide causes developmental neurotoxic effects that are persistent and also get worse with age. These disturbances are both dose-response and time-response related. The spontaneous motor behavior data showed a disruption of habituation in animals exposed to acrylamide. Habituation, here defined as a decrease in the locomotion, rearing, and total activity variables in response to the diminishing novelty of the test chamber over a 60 min. period, was displayed in the control animals, but the animals exposed to acrylamide were clearly hypoactive during the beginning of the 60 min. period, while towards the end of the test period they were hyperactive. Furthermore, the lowest dose not causing any developmental neurotoxic effects (NOEL) on spontaneous behaviour is 0.1 mg acrylamide/kg body weight.

Acrylamide is tumorigenic at high doses in rodents and has been classified as a probable human carcinogen. However, cancer risk projections in the general population remain problematic because the molecular pathogenesis of acrylamide at the low level of dietary uptake is not understood. In particular, the question of how non-genotoxic (epigenetic) responses such as changes of gene expression may modulate the known genotoxicity of acrylamide has never been examined. To close this gap of knowledge, we used high-density DNA microarrays and real-time PCR validations to determine genome-wide transcriptional profiles induced by acrylamide and glycidamide, the oxidative metabolite of acrylamide.

Human MCF7 cells (derived from the mammary epithelium) and human CaCo-2 cells (derived from the intestinal epithelium) were exposed to acrylamide or glycidamide. After 24-h exposures, the cells were collected, RNA was extracted and processed for genome-wide expression profiling on Affymetrix microarrays that display the sequences of 47'000 human transcripts.

Glycidamide induced gene expression changes of much higher amplitude than acrylamide itself. We identified > 100 human transcripts, coding for proteins with known biological functions that are significantly up- or down-regulated by exposure to glycidamide. The expression profiles resulting from glycidamide treatment are characterized by the dose-dependent up-regulation of cytoprotective factors including the glutathione system, enzymes that reduce toxic carbonyl compounds as well as diverse antioxidants. Low-dose experiments indicate that the transcript coding for epoxide hydrolase-1 represents the most sensitive biomarker for glycidamide exposure. At higher concentrations, glycidamide induces typical markers of tumour progression such as steroid hormone activators, positive regulators of nuclear factor-kB, growth stimulators and apoptosis inhibitors. Concomitantly, transcripts coding for growth suppressors and cell adhesion molecules are down-regulated. Many of these transcriptional changes have been validated by quantitative real-time reverse-transcriptase PCR assays in both MCF7 and CaCo-2 cells.

In the case of epoxide hydrolase-1, a significant transcriptional induction was observed at a glycidamide concentration of as low as 0.1 µM. This is a glycidamide level that may be reached transiently in the blood or tissue of humans after dietary uptake of acrylamide. The micromolar doses of glycidamide that induce many other transcriptional responses might seem too high to be relevant for human exposure. However, it cannot be excluded that the sensitivity of target cells depends on the capacity of their detoxification systems and, considering that the uptake of acrylamide is normally accompanied by other toxic food constituents that may saturate these cytoprotective systems, adverse effects may occur at lower concentrations than those expected from threshold experiments performed with a single compound.

This functional genomic study revealed that acrylamide and, in particular, glycidamide exert a plethora of transcriptional effects that should be included in risk assessment studies. We propose to implement a threshold level that prevents the induction of potentially hazardous transcriptional responses mediated by acrylamide or glycidamide in target tissues such as the mammary gland or the intestinal epithelium.

The results of the *in vitro* study suggest that low-dose responses, primarily the induction of epoxide hydrolase-1, exert beneficial effects by promoting inactivation of the toxicant. Other detoxification processes observed at the low-dose glycidamide level of 0.1 μM include its inactivation through conjugation with glutathione (see Figure 17). Adverse effects, such as for example the down-regulation of cell-adhesion molecules (CDH12, ANXA1, ITGB6), the up-regulation of factors that lead to dysregulation of steroid hormone synthesis (AKR1C2, AKR1C3), or factors that promote cell survival and cell cycle progression (NF-kB, RRM2, Cyclin-1, MAPK12) are detected at concentrations above the range that appears to be relevant for human populations. An induction of the DNA repair enzyme EME1, indicative of the formation of DNA damage, is also observed in the upper level of the tested dose range. Thus, the main implication of these findings for risk assessment is that transcriptional signatures associated with DNA damage or tumour cell progression may be expected only at doses that exceed the range of ordinary dietary exposure.
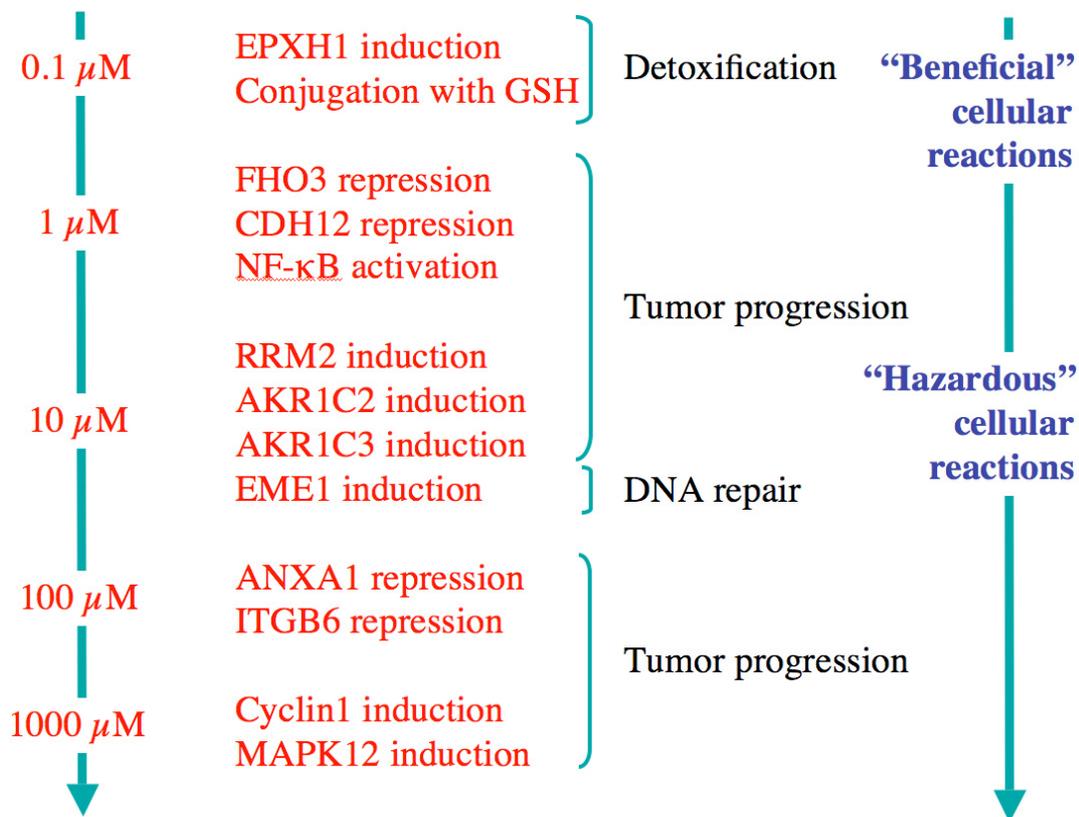
Figure 17. *Summary of the main findings from the transcriptomic profiling of glycidamide effects in human cells. Beneficial responses are observed at the low-dose level, potentially hazardous effects are detected at high glycidamide concentrations. Abbreviations: EPXH1, epoxide hydrolase-1; GSH, glutathione; FHO3, formin homology-3; CDH12, cadherin-12; NF-kB, nuclear factor-kB; RRM2, ribonucleotide reductase polypeptide-M2; AKR1C2, aldo-keto reductase-C2; AKR1C3, aldo-keto reductase-C3; EME1, essential meiotic endonuclease-1; ANXA1, annexin-A1; ITGB6, integrin beta-6; MAPK12, mitogen-activated protein kinase-12.*

Consequences for risk assessment: these findings indicate that the standard procedure of extrapolating from high-dose effects in rodents or other experimental systems to the very low-dose level of human exposure would lead to an overestimation of the toxicological risk, thus exaggerating the health hazards resulting from the presence of acrylamide in food and its metabolic conversion to glycidamide in the human body.

4. Exposure Assessment

RIKILT Institute of Food Safety (The Netherlands), Swedish Food Administration (Sweden),
Stockholm University (Sweden), National Food Institute (Denmark)

5.1. Monte Carlo Risk Assessment

For the work on the exposure assessment the Monte Carlo Risk Assessment (MCRA) software has been used. MCRA was developed in other projects, but adapted slightly for HEATOX purposes e.g. an additional option has been developed to study the effect of brand loyalty on exposure.

5.1.1. Dietary exposure assessment

The long-term exposure of the Dutch population has been calculated, using the EU database on acrylamide concentrations. Exposure of the total population ranged from 0.4-1.8 μg/kg bw/day (P50-P99), exposure of children aged 1-6 years ranged from 1.0-2.4 μg.kg bw/day.

Also a preliminary calculation of the exposure to furan has been made. With the data available from US FDA in 2004, the exposure to furan of the Dutch population was estimated at 0.25-0.87 μg/kg bw/day (P50-P97.5).

With the purpose to validate the MCRA software duplicate diets of infants have been collected and analysed. Since only one of the diets contained a quantifiable amount of acrylamide it was not possible to use this study for validation.
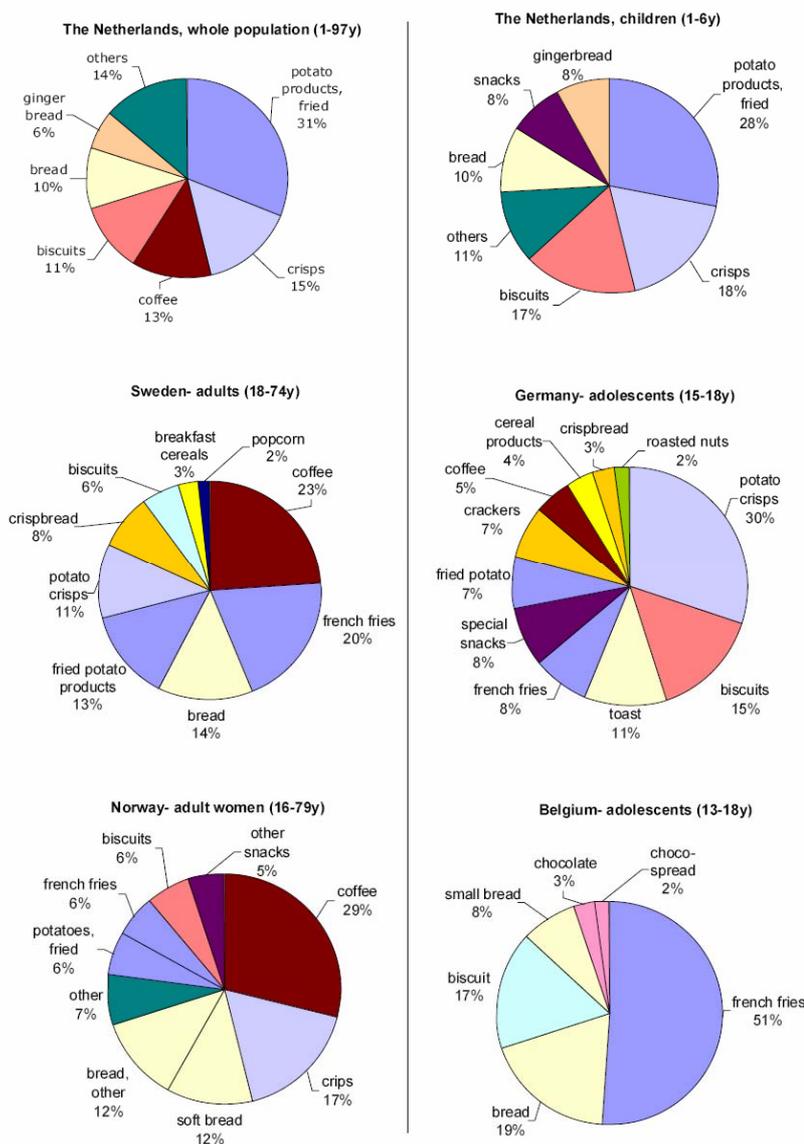
Figure 18. Contribution of food groups to acrylamide exposure in different countries for different age groups.

5.1.2. Scenario-analysis of effect of new processing techniques

Within the HEATOX-project research is performed to develop new or adapted processing techniques to reduce acrylamide (AA) levels in the following foods; bread, crispbread, coffee, crisps and French fries. These reduced acrylamide levels were applied in scenario analyses with the aim to give insight in the theoretical reduction in AA exposure. The scenario-analysis showed that the reduction of the AA levels in single food groups had small effects on the exposure. When reductions of all food groups are applied in one scenario 'total' this resulted in a reduction of the exposure of the total Dutch population by approximately 40%, to 0.3 μg/kg bw/ day (P_{50}) and to 1.2 μg/kg bw/ day ($P_{99.9}$).For children the exposure decreased to 0.6 μg/kg bw/ day. Figure 19 shows the exposure expressed as Margin of Exposure per scenario for the upper 50% of the population. It should be noted that the new processing techniques which were applied in the scenarios were developed on lab scale and are not yet applied on industrial scale; thus the shown reduction is only potential.

5.1.3. Brand loyalty

With the developed brand loyalty model the issue of brand loyalty in the scenario-analysis of acrylamide exposure assessment. The model was applied on the case of potato crisps, for which data on levels of different brands was used from a German data source (www.foodwatch.de), and market shares and brand loyalty were simulated. These calculations showed that brand loyalty has influence on the higher exposure levels and that brands with high acrylamide levels can increase exposure levels depending on the market share.
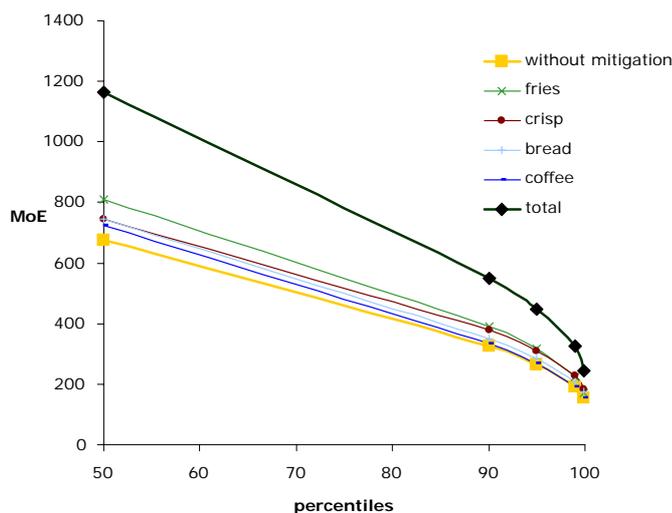
Figure 19. *Distribution of Margin of Exposure (MoE) of the acrylamide reduction scenarios over the percentiles.*

5.1.4. Home cooking

Only limited data was available on the levels in home-cooked foods and no information was available on the amount of home-cooked foods in the diet. The levels reported for home-cooked foods are mostly within the range of levels in the EU-database, thus the intake from home-cooked foods is at least partly covered by intake calculated with the EU-database.

However, from the first estimates we may conclude that home-cooking can have (a probably small) influence on the acrylamide exposure, both in increasing and decreasing exposure, therefore it is important that consumers follow the advice not to overcook their food and follow the available cooking guidelines. From the experiments known today it appears that by following the cooking guidelines acrylamide intake form home-cooked foods can be kept as low as possible.

5.1.5. Intake in British and Dutch infants

A comparison has been made between the acrylamide exposure of Dutch and UK infants using the same exposure assessments methodology. It appears that UK infants have a two times higher intake based on the difference in their diet of acrylamide containing food items. A standardized international methodology for exposure assessment is recommendable.

5.2. Training on probabilistic exposure assessment

A training on probabilistic modelling of exposure to acrylamide was organised for European National Food Safety Authorities, including EFSA. The training was aimed at those working at the authorities and who are involved in risk assessment of acrylamide or other heat-toxicant research.

In the training participants learned about the probabilistic approach and learned to work with the Monte Carlo Risk Assessment software in relation to acrylamide. Specific acrylamide issues were discussed and studied through exercises, like the grouping of foods, variability in the acrylamide levels, effect of mitigation measures and the possibility to study and how to quantify these aspects with MCRA.

The training was very well received, and all participants were able to use the Monte Carlo Risk Assessment (MCRA) software for probabilistic exposure calculations.

The calculation performed on acrylamide exposure gave a good estimate of the exposure of the Dutch population. Work on brand loyalty and home-processing showed that these issues may influence exposure, especially the exposure of the high-end consumers may be increased.

The effectiveness of different mitigation possibilities to reduce acrylamide exposure levels has been studied using probabilistic models.

Some of the mitigation measures showed a significant reduction on a particular food item, but overall the effect on exposure levels was limited. A significant reduction in exposure can only be received when mitigation measures are applied in a variety of products.

Comparing acrylamide levels of different countries has been done, and the methodology of how to perform probabilistic intake calculations and scenario studies regarding the effectiveness of different mitigation options has been trained to different European National Food Authorities. This was received very well, and has resulted in new proposals for future cooperation.

5.3. Estimation of breast cancer risk from exposure to heat-induced toxicants

An LC/MS/MS method for the analysis of haemoglobin adducts of acrylamide (AA) and glycidamide (GA) was established. The procedure involves isolation of reed blood cells, lysis and isolation of haemoglobin. Normal and adducted N-terminal valine amino acids are liberated by a modified Edman degradation followed by column purification. Normal and adducted valine residues are separated by gradient HPLC with MS/MS detection.

The LC/MS/MS method has been optimised and the use of deutorated internal standards (Figure 20) has been included in the analytical procedure. Standard curves for AA-Val-PTH are linear from 0.3 pmol/ml to 28 pmol/ml and for GA-Val-PTH (two diasteriomers) standard curves are linear from 0.8 pmol/ml to 48 pmol/ml.

Different sample purification methods were evaluated, including solid phase extraction (SPE) with different columns, columns with diatomaceous earth and liquid/liquid extractions. A useful and reproducible method using SPE was established.
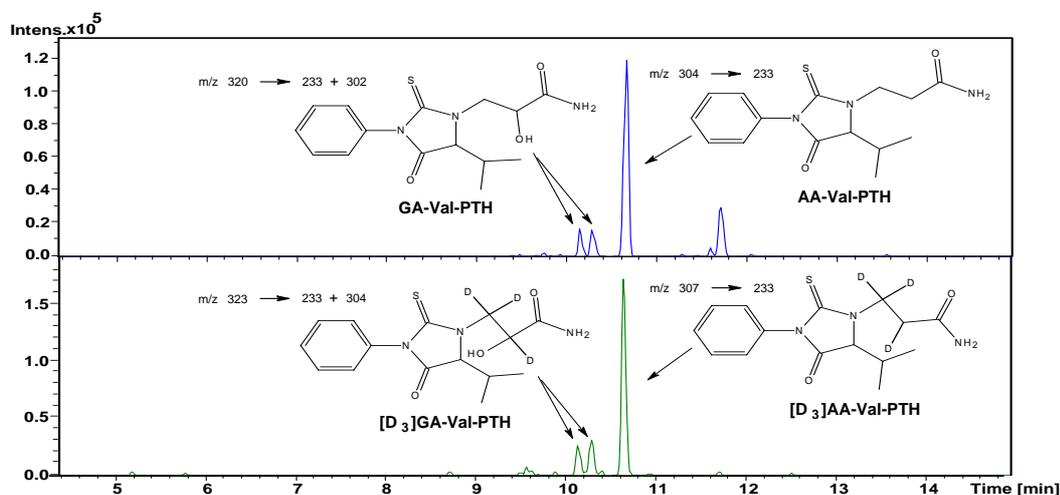
Figure 20. Chromatograms showing GA-Val-PTH and AA-Val-PTH (upper) as well as the deutorated internal standards (lower)

From 1993 to 1997 blood samples were collected from approx 30.000 women in Denmark. Since then about 430 postmenopausal women had been diagnosed with breast cancer.

Globin was purified from the 430 blood samples from the cancer patients as well from the same number of controls. The globin samples were analysed for content of AA-ValPTH and GA-Val-PTH by the LC/MS/MS method.

Results of the unadjusted statistical analysis showed that neither acrylamide [IRR (95% CI) 1.01 (0.65-1.57)] nor glycidamide [IRR (95% CI) 0.79 (0.45-1.38)] concentrations were found to be associated with breast cancer incidence. Inclusion of potential confounding factors in the model did not have clear effects on the association between glycidamide and breast cancer, whereas the association between acrylamide and breast cancer was strengthened after inclusion of information on smoking at blood sampling [IRR (95% CI) 1.43 (0.75-2.71)], further inclusion of former smoking, duration of smoking and amount of tobacco currently smoked [IRR (95% CI) 1.70 (0.85-3.38)], as well as additional inclusion of baseline risk factors for breast cancer [IRR (95% CI) 2.04 (1.00-4.18)].

When the concentrations of glycidamide and acrylamide were evaluated with estrogen receptor (ER) specific breast cancer as the outcome, all associations was found to be strongest with regard to ER+ breast cancer. In the fully adjusted model, women with the highest concentrations of acrylamide adducts were found to be at almost 3-times increased risk of ER+ breast cancer when compared to women with the lowest concentrations (p=0.01).

5.4. Measurement of internal doses of acrylamide and glycidamide – a tool for exposure assessment and risk assessment

5.4.1. Haemoglobin adducts as a measure of internal dose

Doses in blood of reactive compounds, like acrylamide and its metabolite glycidamide, could be inferred from levels of their respective adducts to haemoglobin (Hb). Hb adduct levels from acrylamide and glycidamide were quantified as a measure of internal doses in samples from humans and animals in different studies. Preparation of samples and analysis of adducts were performed according to an established method (the N-alkyl Edman procedure).

5.4.2. Bioavailability of acrylamide in food

The bioavailability of acrylamide from two different kinds of diets, one containing potato and one containing fibre-rich bread, has been studied in mice. No difference in the bioavailability of acrylamide between the different types of diets was observed. Furthermore, it was shown that the "extra acrylamide", obtained during alkaline extraction of the fibre-rich diet, does not correspond to bioavailable acrylamide.

5.4.3. Endogenous formation of acrylamide

Internal dose of acrylamide in blood were measured in free-living animals with assumed no dietary intake of acrylamide; common hare, red deer, roe deer, red fox and wild boar. In all these species the adduct levels were shown to be very low (below or close to the limit of quantification). The conclusion is that there is no or very low endogenous production of acrylamide in mammals, and that possible endogenous formation gives insignificant contribution in humans compared to intake through diet.

5.4.4. Inter- and intra-variation of internal doses in humans

Inter-individual variation in acrylamide- and glycidamide doses was studied in blood samples from 142 smoking and non-smoking individuals in the general population. The acrylamide adduct levels varied between 0.02 to 0.1 nmol/g globin (a factor of 5) in non-smokers. In smokers the range of the acrylamide-adduct level was 0.03 - 0.43 nmol/g globin. The variation in glycidamide doses was slightly higher. The results indicate a non-linearity in the internal doses of glycidamide at low exposure doses of acrylamide. This observation has to be more carefully investigated.
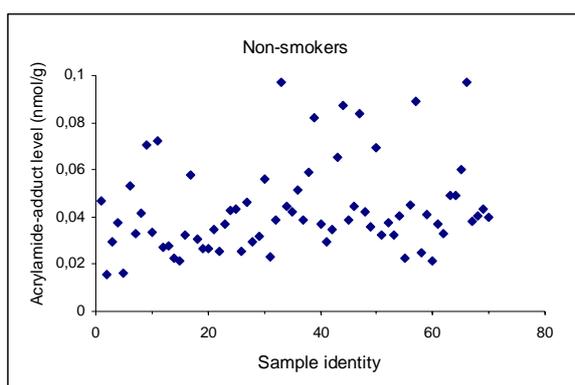
Figure 21. Variation in acrylamide-adduct levels in Hb between non-smoking individuals (n=70).

The intra-individual variation of adduct levels from acrylamide was studied in blood samples collected from 15 non-smokers every second month during 1.5 years. There was an intra-individual variation in the acrylamide adduct levels in Hb over time, assumed to reflect a variation in background exposure. This variation was, however, lower than the inter-individual variation.

5.4.5. Relationship between internal dose and estimated acrylamide intake from food

The average acrylamide-intake, estimated from food frequency questionnaires (FFQ), varied nearly with a factor of 50 between the donors of the blood bank samples mentioned above. This means nearly 10 times larger individual variation compared to the measured internal doses (vary with a factor of 5). This illustrates the difficulties with estimation of intakes from FFQ.

In two human studies volunteers were given controlled intake of food with known acrylamide content. Levels of Hb-adducts from acrylamide were compared with estimated intakes. In each study, about 20 individuals, smokers and non-smokers were having a 28- respectively 5-days period of controlled intake of acrylamide-rich diet (in average 3 and 11 μg acrylamide/kg and day, respectively). In blood samples, collected before and after the exposure periods, Hb-adducts from acrylamide was measured. Both studies clearly showed increased Hb-adduct levels corresponding to the acrylamide intake in both non-smokers and smokers.

6 Risk assessment and risk communication

Swedish Food Administration (Sweden), Norwegian Institute of Public Health (Norway), Stockholm University (Sweden), National Food Institute (Denmark), RIKILT Institute of Food Safety (The Netherlands), BEUC - European Consumers' Organisation (Belgium), National Veterinary Institute (Norway), Lund University (Sweden)

6.1. Risk assessment

6.1.1. Models for extrapolation of cancer risk

According to a relative cancer risk model (applied/developed by the partner) the species extrapolation from cancer tests in rodents should be based on internal doses of the ultimate genotoxic agent in the test species and in humans. Internal doses are obtained from Hb-adduct levels.
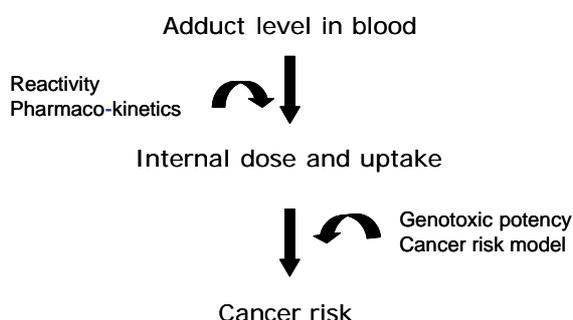
Figure 22. *Important corner-stones in the approach for cancer risk estimation.*

Rats were exposed to acrylamide in drinking water in an experiment simulating the conditions used in published cancer tests, and after one week blood samples were collected. In other studies blood samples from humans with background exposure to acrylamide (samples from blood banks), or from humans with increased exposure to acrylamide (experiments with dietary exposure), were studied. Hb-adduct levels from acrylamide and glycidamide were measured. Complementary experiments have been done to allow calculations of internal doses from the measured adduct levels in rodents and humans.

Results with non-linearity of the internal doses of glycidamide at low exposure doses of acrylamide were strongly indicated in the human experimental studies. Taken together, the results indicate a relatively lower dose of glycidamide at low acrylamide doses. However, as these low exposure doses are associated with a larger measurement error, the indicated results of non-linearity has to be further strengthened. The result, if true, considerably complicates the cancer risk estimation. Therefore, further verifying measurements are required.

6.1.2. Final Risk Characterisation

The work performed in HEATOX together with results obtained outside the project are summarised and commented below. For non-carcinogenic effects of acrylamide, the most sensitive endpoint is neurotoxicity with a NOAEL (No Observable Adverse Effect Level) in experimental animals of 0.2 mg/kg bw/day. The margins of safety relative the NOAEL for the average and high exposure were 200 and 50, respectively. The safety margins for other effects were larger. Comparing estimated NOAEL for neurotoxicity in humans of 10-40 μg/kg bw/day with exposure from food resulted in a safety margin in the range of 2.5 to 40. Given these margins any risks of neurotoxic and other non-carcinogenic effects following dietary acrylamide exposure are likely to be very small.

There are obvious problems with epidemiological studies, particularly those addressing low level exposure through food in the general population using Food Frequency Questionnaires (FFQ). First of all none of the studies published so far were designed for investigating the carcinogenic risk of acrylamide. There is no validation of the exposure assessments, and in some of them all dietary sources of acrylamide had not been taken into account. A comparison of intake estimates based on FFQ with that of acrylamide haemoglobin adducts showed large overlaps; a major problem is the correct classification according to exposure. Misclassifications would strongly weaken a hypothetical association between acrylamide exposure and cancer. The next limitation is the ability of the dietary studies, and even the occupational cohort study, to detect the small increases in risk expected from the lowest to the highest exposure groups. Indeed, neither the epidemiologic study nor the studies on dietary exposure to acrylamide did have the power to detect small increases in cancer risk and should be regarded as non-positive and not negative studies.

Another issue is the mechanism of cancer induction by acrylamide. There are examples of chemicals that can induce heritable epigenetic modifications to DNA and thereby change gene expression without altering DNA sequence. So far there are no data to prove that epigenetic changes are important in acrylamide carcinogenesis although, for example, tumour location in animals might indicate a hormonal component in the aetiology. All data reported support a genotoxic mechanism, mediated by glycidamide, for acrylamide carcinogenesis. However, this needs further investigations.

JECFA compared exposure estimates of 1 and 4 µg/kg bw/day with the BMDL10 of 30 mg/kg bw/day (BMDL10=Bench Mark Dose Lower confidence limit for 10% increased incidence) and obtained margins of exposure (MOEs) of 300 and 75. JECFA considered these exposure margins to be low for a compound being both carcinogenic and genotoxic and that they indicate a human health concern (WHO, 2005). The Scientific Committee of EFSA (EFSA, 2005) also endorsing a MOE approach for compounds that are both carcinogenic and genotoxic is of the view that a MOE of 10,000 or larger would be of low concern from a public health point of view and might be considered as a low priority for risk management actions. The MOEs for acrylamide are clearly below 10,000, but the Scientific Committee of EFSA gives no guidance for risk managers how to interpret a MOE less than 10,000. However, if a MOE lower than 10,000 had no meaning, the entire exercise would be worthless. A reasonable conclusion is that the MOE for acrylamide gives rise for concern and merits risk management action.

Although open for criticism, using the life time risk estimates based on extrapolation and inclusion of the scaling factor the average and high exposure estimates is associated with life time risks of 1.4 to $6.4 \cdot 10^{-3}$, a risk that would be considered a public health concern.

The theoretical risk estimates given above are compatible with the non-positive outcome for cancer risk of acrylamide in the epidemiological studies based on FFQ.

The cancer risk associated with food borne acrylamide exposure is probably low, and can at most only explain a small fraction of cancers associated with diet. However, for other chemicals a risk level to consumers comparable with that of acrylamide would not be ignored. Actions to reduce the risk by reducing acrylamide in foods and thereby food borne exposure are therefore warranted and generally agreed upon. Lastly, as it is known that a great number of proven or suspected carcinogens, e.g. other Maillard reaction products, such as heterocyclic amines, furfural and furan derivatives together with lipid peroxidation products can be formed during heat treatment of food, it is important to further study the health risk associated with heat-generated food toxicants foods. Acrylamide is not the sole problem.

Acrylamide is not the sole problem.
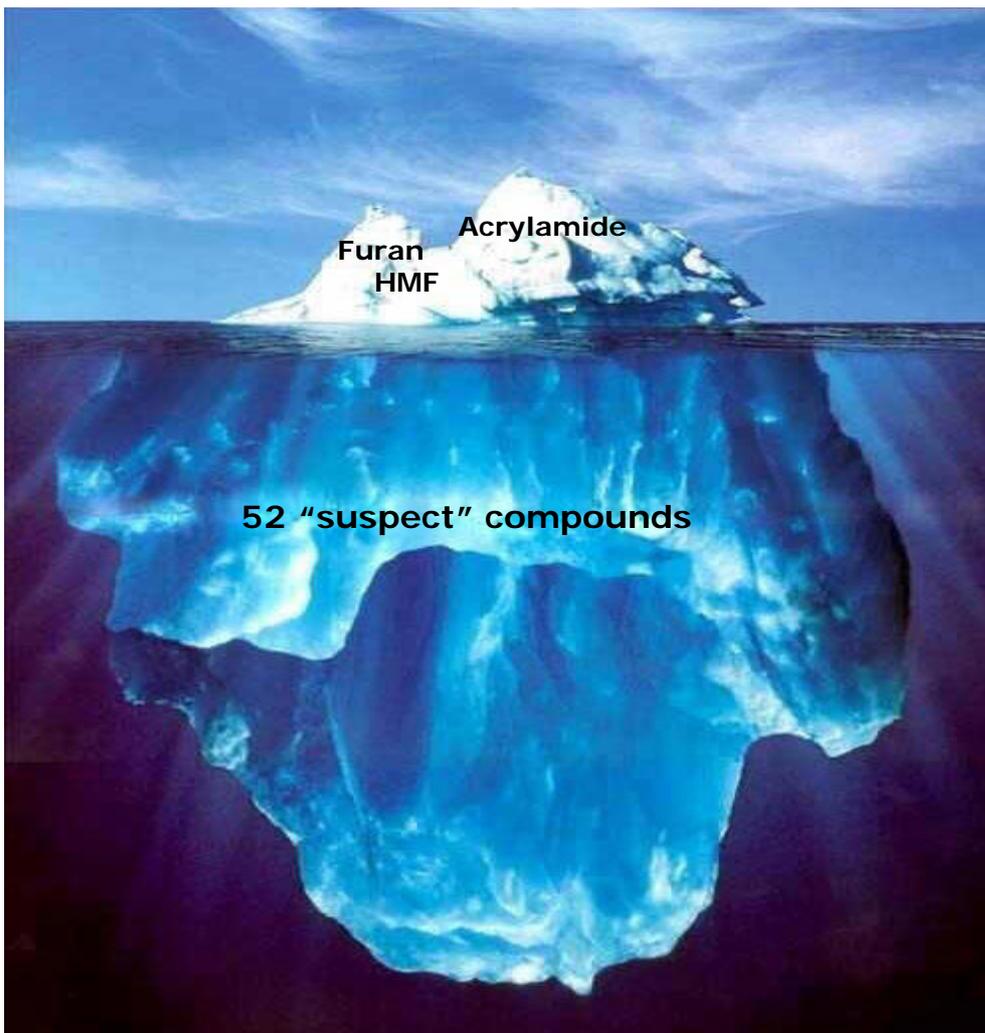
To summarize, the main conclusions are:

- Acrylamide has been classified by WHO, World Health organisation. This conclusion is strengthened by the project. Following exposure to acrylamide via food the primary concern is the possible risk of cancer.
- Compared with many regulated food carcinogens, the exposure of acrylamide poses a higher estimated risk to European consumers.
- Risk assessments and recommendations to minimize exposure to acrylamide made by WHO are still valid.
- Other compounds formed during cooking of food, for example HMF, Furan, and a variety of Maillard reactants and lipid oxidation products may also constitute an increased cancer risk for consumers. Approximately 50 substances that would require risk assessment have been theoretically identified within the project.
- Current knowledge does not allow for a risk-benefit assessment of cooking with respect to acrylamide or other heat-induced toxicants.

6.2. Risk communication

Risk communication strategy and actions have been integrated with research activities in the project. Different kinds of strategic communication tools and channels have been applied.
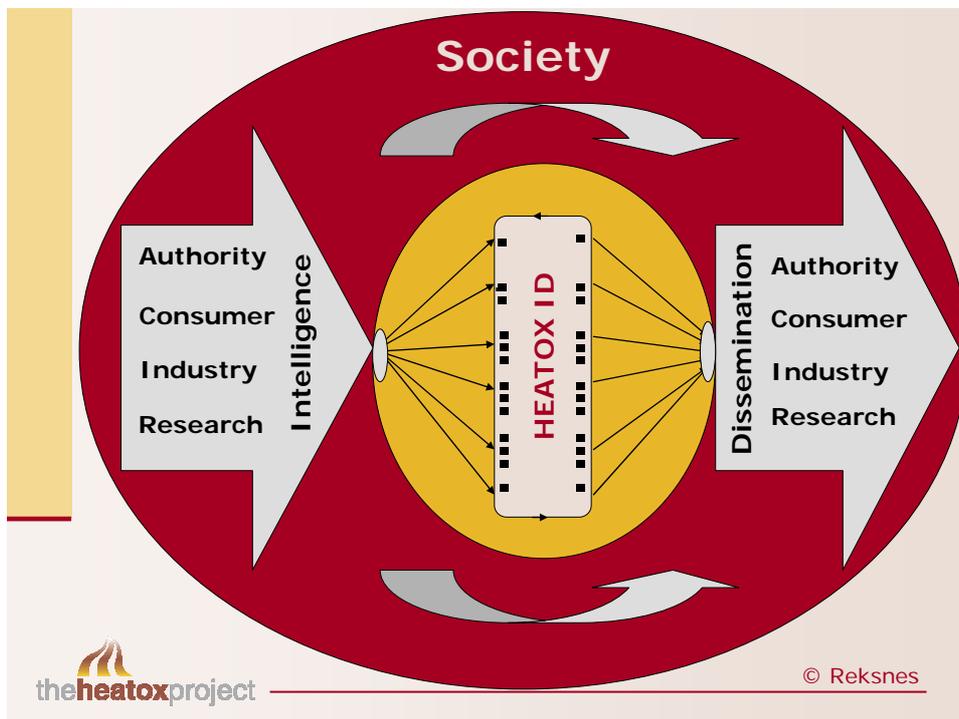
Figure 23. *The HEATOX ID was created early in the project to illustrate the main strategic grips and to help researchers focus on the communication tasks.*

6.2.1. Risk Communication Strategy

To support and strengthen the project profile a Risk Communication Strategy was developed.

The strategy was based on the overall HEATOX objectives and an initial analysis of communication challenges based on interviews with key persons within the project and representatives from interested parties. The strategy framework was set up in 2004 and offered as a guideline for risk communication within the project and between the project and the outside world.

Main channels and a visual profile for the project was outlined and developed early in the project. Based on own observations and comments in Midterm Review Report, efforts were taken to strengthen risk communication and dissemination activities within HEATOX. During summer 2005, a dialogue was initiated with HEATOX External Panel. The aim being to ensure that end deliverables were as useful and focused as possible and that dissemination from HEATOX to the outside world was as effective as possible.

Special risk communication training sessions have been organised targeting the PhDs and young researchers in the project. Considerable attention was given to the designing and organising of the main risk communication event of HEATOX, the Workshop in June 2006. The conclusions from the HEATOX workshop have given clear indications as to how some of the end deliverables should be constructed to meet end user's needs and expectations.

Did risk communication play the intended role in the HEATOX project? What lessons can be learned? Can HEATOX experiences contribute to making Guidelines for good risk communication practice related to heat-induced toxicants?

At the end of the project, evaluations have been organised to try to answer these questions and give further insight into the nature of practical risk communication. To assess the risk communication efforts within HEATOX, two limited web-based surveys were conducted during January 2007. To assess the risk communication efforts between HEATOX and the outside world, short personal interviews with members of the External Panel were conducted in February 2007. The interviews focused on possible beneficiaries from HEATOX work, to what extent HEATOX has succeeded in networking with the outside world. The Panel members were also asked to give their personal reflections related to being members of the Panel.

6.2.1.1. HEATOX Advice to Industry and Catering

Industry and catering represent important food chain levels where effective risk minimisation initiatives could contribute to reducing the intake of acrylamide. HEATOX research covers many aspects related to the three main food sectors;

- Potato products; from growing conditions to crisp or French fry processing
- Cereal products; mainly focused on bread and baking
- Coffee; roasting process and influence of raw material parameters.

There has been active and close contact between CIAA (the Confederation of the Food and Drink Industries of the EU) and HEATOX during the entire project period. The first version of the CIAA Acrylamide Toolbox was evaluated internally within HEATOX by relevant research groups prior to the decision to build further on this basis rather than developing an alternative HEATOX system. Following advice from the HEATOX External Panel and the outcome of the HEATOX Workshop in 2006, HEATOX results relevant to industry and catering sector have been fed into the CIAA Toolbox.

6.2.1.2. HEATOX Advice to Authorities and Consumer Organisations on Home Cooking and Consumption

Acrylamide formation and minimisation strategies have been quite extensively studied in the laboratory and in industrial environments where the processing/heating conditions are better controlled than in home cooking. HEATOX researchers have conducted a number of experiments related to deep-fried French fries, crisps, oven-roasted potato wedges and toasted bread.

The general intake of acrylamide for adults is quite similar across Europe. Due to the fact that a large number of foods contributing to acrylamide intake are industrially produced the contribution from home cooking is probably quite small in the general population.

The HEATOX Workshop in Graz 2006 identified significant differences in home cooking methods and availability of ingredients within countries and certainly across the different European regions and member states. The national differences in dietary habits and cooking practices, as well as the different availability of ingredients, need to be taken into account by national authorities and consumer organisations when developing material like brochures, web pages, and presentations for consumers.

The HEATOX advice is aimed as a tool for National Authorities and Consumer organisations for handling the acrylamide issue in relation to home-cooking. This might include giving cooking advice directly to consumers as well as influencing providers of raw materials, pre-fried products and frying equipment for domestic use.

Home cooking
National authorities should highlight the following:

Potatoes low in sugar
- Low sugar potato varieties
- Maintenance of suitable storage temperature during the supply chain
- Low sugar levels in prefabricated potato products for domestic frying

Best frying temperature
- Frying temperature in the range 145 to 170°C for deep frying potatoes
- Clear and accurate cooking instruction on the package of pre-fried products
- Clear and accurate instruction for fryers for domestic use

Golden, not brown!
- French fries and roast potatoes cooked to a golden-yellow rather than golden-brown colour
- Bread toasted to the lightest colour acceptable

Consumption
Balance the diet as proposed in national dietary recommendations and integrate acrylamide considerations into the "normal" dietary recommendations.

6.2.1.3. Guidelines for Good Risk Communication Practice related to heat-induced toxicants

Objectives related to integrating risk communication activities into HEATOX form the basis for one deliverable: Guidelines to Good Risk Communication Practice related to heat-induced toxicants.

Risk communication within HEATOX has had positive effects on the outcome of the project. On a short term basis, the results from the project will be more clearly disseminated towards the targeted audiences. On a long term basis, the HEATOX experience has probably motivated most of the younger researchers and many of the senior researchers to engage in risk communication activities and thus bridging the gap between science and the end users of scientific results.

The multidisciplinary and holistic approach has been recognised as important and this will probably influence the final project results. Both senior scientists in capacity of theme and workpackage leaders and the PhDs have taken actively part in risk communication within HEATOX.

The planned actions taken towards different stakeholders throughout the project at specific occasions have probably influenced the outcome in a positive way. Many HEATOX scientists have taken actively part in numerous risk communication activities directed towards other stakeholders.

The HEATOX network, with the External Panel and Scientific Committee as core has probably had some, but limited, effects on the outcome of the project. Several of the members have been engaged in the scientific dialogue at the HEATOX meetings. The Panel played an important role in recruiting stakeholders to the HEATOX workshop and several of them also played important roles during the actual workshop. The External Panel has contributed to bringing outside knowledge and experiences to the HEATOX table and to some extent also disseminated results on behalf of HEATOX. Some have definitely functioned as HEATOX ambassadors while others have played a less significant role in the HEATOX network.

The Guidelines to Good Risk Communication Practice related to heat-induced toxicants have been constructed on the basis of experiences from earlier and ongoing risk communication projects and practices, updated risk communication research and experiences from the HEATOX project. If communicated properly, it is anticipated that these guidelines could have crossover effect on other similar project, enhancing the integration of risk communication into risk-related research even further.

Acknowledgement

The HEATOX project has been carried out as a wide international collaboration with integration of different and complementary research fields, creating a big, interesting and useful network. The success of the HEATOX project has not only been the completion of the tasks and deliverables, but the wide dissemination of results for the benefit of industry, authority and consumers. Dissemination has been achieved (and will continue) as scientific papers for refereed journals; totally around 60 papers so far, or by attendance at national and international meetings and workshops with posters and paper presentations; papers in other journals and presentations in radio and TV. Totally around 20 PhD students or young researchers have been involved in the project, and contributed with enthusiasm, curiosity and thus helped in creating a warm and stimulating atmosphere. Some have already got their Doctoral degree and several Thesis will be presented during the coming years, and I am very proud of the thought that the HEATOX project has been a part of this research.

I wish to express my sincere gratitude to all HEATOX partners and members of the External panel for all work and discussions, and to our scientific officers, Achim Boenke who was in charge at the start of the HEATOX project, and Jürgen Lucas who has been our guide throughout most of the project time. It has been a pleasure to work with you and I am looking forward to seeing you again.

Lund, April 2007

Life's truest happiness lies in the friendship we make on the way…

Publishable results of the Final plan for using and disseminating the knowledge

Exploitable Knowledge (description)	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use	Patents or other IPR protection	Owner & Other Partner(s) involved	Comments
	Publications	Scientific community	2007 and forward		See Partner list, page 6.	Most partners will present the results in scientific journals and at international conferences
Knowledge on the role of precursors and cooking conditions for the formation of acrylamide in potato products		Potato industry Scientific community			P1	The knowledge will be actively disseminated and used as a basis in future projects and collaborations.
Measurement of ^{14}C-labelled acrylamide (AA) in biological samples.		Toxicological studies			P1	The knowledge will be actively disseminated and used as a basis in future projects and collaborations.
Formation of HMF during coffee roasting	Reduction of HMF formation	1. Industry			P2	
Database containing potential toxicants found in heated foods		Toxicological studies Chemical composition			P3 and P8	The database lists 800 volatile products of the Maillard reaction and lipid oxidation together with predicted toxicological data. In the future, the database will be converted into a suitable form for viewing on the CSL web site where it will be maintained and updated, as appropriate
Assessment of optimal set-up of frying end-point	Electronic device and appropriate algorithm	Equipments for home cooking and catering frying	2008	Yes	Partner 5	

Exploitable Knowledge (description)	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use	Patents or other IPR protection	Owner & Other Partner(s) involved	Comments
Indications or suggestions to be addressed to the equipment user	Guidelines to be printed on equipment manuals	Equipments for home cooking and catering frying	2007-2008	No	Partner 5	
New knowledge on the application of steam during baking.		Baking industry	May be introduced by companies quite soon		P6	The knowledge will be actively disseminated. Then each company has to test, evaluate and optimise the technique for each specific application (taking acrylamide reduction and other quality aspects into consideration). SIK is prepared to support on a consultant basis.
New knowledge on the application of IR technology in baking		Baking industry	Investments in new types of baking ovens needed before industrial use		P6	As above.
Mitigation strategies that are helpful to reduce AA intake related to home preparation of French fries	Leaflet	Consumers	2007	none	P11	Leaflet will be produced in the course of 2007 and made publicly available in many languages.

Exploitable Knowledge (description)	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use	Patents or other IPR protection	Owner & Other Partner(s) involved	Comments
Computational model to calculate acrylamide levels under various processing conditions	Fry Simulator model	Dutch Potato Industry	Negotiations start Q4/2006	None	P11 & P7	
Tabulated information on known cooking/ processing contaminants	Publication	Food Industries Food Agencies	2007 - 2008		P12	Tabulated information.
Knowledge of metabolism and internal doses of acrylamide (and its metabolite glycidamide)	Improved cancer risk assessment for acrylamide	1. Authorities (exposure limits, e.g. ADI) 2. Cancer research (epidemiology)			P14	A main component in an improved cancer risk assessment of acrylamide is comparison of internal doses of glycidamide - the genotoxic metabolite of acrylamide - in humans and animals that have been used in cancer tests. Other parameters that are necessary (intermediate goals) for estimation of the range of risk: inter- and intra-individual variation in internal doses, bioavailability of acrylamide in different foodstuffs, possible endogenous formation of acrylamide.
Probabilistic exposure calculation	Monte Carlo Risk Assessment programme (MCRA) for scenario analysis of the effect on exposure of acrylamide reduction.	1. Policy 2. Industry	2007 2008	- -	P17	MCRA has been developed in other projects and is in the consortium agreement defined as pre-existing knowledge. The use of MCRA in acrylamide and other heat-treated toxicants has been exploited and will be further exploited for research questions of Food Industry (in cooperation with ILSI) regarding the effectiveness of several mitigation steps as proposed in the TOOLBOX of the CIAA. Further application of the MCRA software also in relation to quantitative risk-benefit evaluations are foreseen. There is also an interest of national Food Standard Agencies to use the MCRA software in cooperation with the RIKILT in the nearby future.

Exploitable Knowledge (description)	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use	Patents or other IPR protection	Owner & Other Partner(s) involved	Comments
Identification of SULT1A1 as the principal human enzyme involved in the toxification of HMF	Mouse lines humanized for SULT1A1	1. Pharmaceutical Industry 2. Toxicological contract rsearch	2008 or 2009	Application patent envisaged for 2008	P18	
Toxicokinetics related to acrylamide or glycidamide					P22	Expression profiling of human MCF7 cells exposed to different concentrations of acrylamide or glycidamide Real-time reverse-transcriptase PCR validation of 25 transcripts in MCF7 cells and human CaCo-2 cells Measurement of intracellular glutathione levels after treatment with acrylamide or glycidamide Determination of nuclear factor-•B activity using a reporter gene assay Standard toxicity and cell viability assays.
Knowledge on vacuum frying to reduce acrylamide level in potatoes crisps		Industry			P23	Vacuum frying with and without pre-treatments has shown to produce low level acrylamide crisps. It is possible to improve their quality characteristics. The pre-treatments represent a practical industrial alternative to reduce acrylamide level in potatoes crisps

Dissemination of Knowledge

SCIENTIFIC JOURNAL

Author	Journal	Title	Partner (See list, page 6.)
		Year 2004	
Murkovic, M.	J. Biochem. Biophys. Meth. 61, 161-167, 2004	Acrylamide in Austrian Foods	P2
Surdyk, N., Rosen, J., Andersson, R. and Åman, P	J. Agric. Food Chem.	Effects of asparagines, fructose, and baking conditions on acrylamide content in yeast-leavened wheat bread	P4
Fredriksson, H., Tallving, J, Rosen, J. and Åman, P.	Cereal Chem.	Fermentation reduces free asparagines in dough and acrylamide content in bread	P4
Husøy, T., Abramsson-Zetterberg, L., Ølstørn, H., Paulsen J E., and Alexander J.	*Mutation Research*, 580, 103-110.	Adenomatous polyposis coli influences the effect of the heat induced mutagens, PhIP and acrylamide, on the production of micronuclei in mice erythrocytes.	P9
Durling, L. and Lilianne Abramsson-Zetterberg, L.*	*Mutation Research, 580*, 103-110.	A comparison of genotoxicity between three common heterocyclic amines and acrylamide.	P9
Dunovská L., Hajšlová J., ajka T., Holadová K. and Hájková K.	Czech Journal of Food Science, Vol. 22, 283-286	Changes of Acrylamide Levels in Food Products during Technological Processing	P10
E. Bermudo, V. Ruiz-Calero, L. Puignou, M.T. Galceran	Electrophoresis	Microemulsion electrokinetic chromatography for the analysis of acrylamide in food.	P12
		Year 2005	
Hellenäs K.-E., Abramsson-Zetterberg L., and Skog K.	Journal of AOAC International, January/February Vol. 88, No. 1, 242-245.	The HEATOX project,	P1 P9
Bagdonaite, K., Murkovic, M.	Czech J. Food Sci. 22, 22-24, 2005	Factors affecting the formation of acrylamide in coffee	P2
Mustafa, A., Adersson, R., Rosen, J., Kamal-Eldin A. and Åman P.	J. Agric. Food Chem. 2005, 53, 5985-5989	Factors influencing acrylamide content and color in rye crisp bread	P4
Olsson, E.E.M., Trägårdh, A.C. & Ahrné, L.M.	J. Food Sci. 70(8), E484-491 (2005)	Effect of Near-infrared Radiation and Jet Impingement Heat Transfer on Crust Formation of Bread	P6

Authors	Title	Journal	Ref
Knol,J.J; Van Loon,W.A.M. van; Linssen,J.P.H.; Ruck,A.L.; Van Boekel,M.A.J.S.; Voragen,A.GJ.	Toward a Kinetic Model for Acrylamide Formation in a Glucose-Asparagine Reaction System.	J. of Agric. Food Chem. 2005, 53, 6133-6139.	P7
L. Castle and S. Eriksson	Analytical methods used to measure acrylamide concentrations in foods.	Journal of AOAC International, 2005, 88, 274-284.	P8
L. M. Owen, L. Castle, J. Kelly, L. Wilson and A. S. Lloyd	Acrylamide analysis: Assessment of results from six rounds of Food Analysis Performance Assessment Scheme (FAPAS) proficiency testing.	Journal of AOAC International, 2005, 88, 285-291.	P8
P. Fohgelberg, J. Rosén, K.-E. Hellenäs, L. Abramsson-Zetterberg*	The acrylamide intake via some common baby food for children in Sweden during their first year of life - (an improved method for analysis of acrylamide).	Food and Chemical Toxicology, 43: 951-959.	P9
Petersson E.V., Rosén J., Turner C., Danielsson R., Hellenäs KE.	Critical factors and pitfalls affecting the extraction of acrylamide from foods: An optimisation study	Analytica Chimica Acta	P9
Dunovská L., ajka T. and Hajšlová J.	Acrylamide Levels in Foodstuffs from Czech market	Chemické listy Journal, 99 279-280, 2005, Special Issue 14	P10
E. Bermudo, O. Núñez, L. Puignou, M.T. Galceran	Analysis of acrylamide in food samples by capillary zone electrophoresis	Journal of Chromatography A	P12
E. Barceló-Barrachina, F.J. Santos, L. Puignou, M.T. Galceran	Determination of heterocyclic amines in a meat extract as dimethylformamide dialkylacetal derivatives by gas chromatography mass spectrometry.	Analytica Chimica Acta	P12
Hagmar, L., Wirfält E., Paulsson, B., Törnqvist, M.	Differences in hemoglobin adduct levels of acrylamide in the general population with respect to dietary intake, smoking habits and gender	Mutation Research 580, 157.165	P14
Thomas Bjellaas, Karel Janak, Elsa Lundanes, Georg Becher	Determination and quantification of urinary metabolites after dietary exposure to acrylamide	Xenobiotica	P16
Boon PE, Mul A de, Voet H van der, Donkersgoed G van, Brette M, Klaveren JD van	Calculations of dietary exposure to acrylamide	Mutation Research 580, 143-155; 2005	P17
H. R. Glatt, H. Schneider, Y.-G. Liu	V79-hCYP2E1-hSULT1A1, a cell line for the sensitive detection of genotoxic effects induced by carbohydrate pyrolysis products and other food-borne chemicals	Mutation Research 580 (2005) 41-52	P18
H. R. Glatt, W. Meinl	Sulfotransferases and acetyltransferases in mutagenicity testing; technical aspects	Methods in Enzymology 400 (2005): 230-249	P18

Authors	Journal	*Year 2006*	Code
Bagdonaite, K., Viklund, G., Skog, K., Murkovic, M.	J. Biochem. Biophys. Methods, **69**, 215-221, 2006	Analysis of 3-aminopropionamide: A potential precursor of acrylamide	P2, P1
Murkovic, M., Pichler, N.	Molecular Nutrition & Food Research, **50**, 842-846 (2006)	Analysis of 5-hydroxymethylfurfural in coffee, dried fruits and urine.	P2
Murkovic, M., Derler, K.	J. Biochem. Biophys. Methods, **69**, 25-32, 2006	Analysis of amino acids and carbohydrates in green coffee.	P2
Low, M. Y.; Koutsidis, G.; Parker, J. K.; Elmore, J. S.; Dodson, A. T.; Mottram, D. S.	Journal of Agricultural and Food Chemistry	Effect of citric acid and glycine addition on acrylamide and flavor in a potato model system. *NB Work partly supported by Heatox*	P3
Martin Fink, Roger Andersson, Johan Rosén, and Per Åman	Cereal Chemistry	Effect of Added Asparagine and Glycine on Acrylamide Content in Yeast-Leavened Bread	P4
S. Romani*, A. Gasparri*, M. Bacchiocca*, E. Cocci*, P. Rocculi*, M. Dalla Rosa*	Industrie alimentari (chiriotti ed., pinerolo – to – italy)	Contenuto in acrilammide e caratteristiche qualitative di patate fritte in differenti apparati tecnologici – Acrylamide content and characteristics of potato fried in different fryers	P5
S. Romani, M. Bacchiocca, P. Rocculi, M. Dalla Rosa	Accepted to be published on European Food Research And Technology	Effect of frying time on acrylamide content and quality aspects of French fries	P5
Wenzl, T., Karasek, L, Rosen, J., Hellenaes, K-E., Crews, C., Castle, L. and Anklam, E.	Journal of Chromatography A. 2006 1132, 211-21.	Collaborative trial validation study of two methods, one based on high performance liquid chromatography–tandem mass spectrometry and on gas chromatography–mass spectrometry for the determination of acrylamide in bakery and potato products.	P8
Hasnip, S., Crews, C. and Castle, L.	Food Additives and Contaminants, 2006, 23, 219-227.	Some factors affecting the formation of furan in heated foods.	P8
Golmann, T.,Perisset, A., Bertholet, M.-C., Stadler, R. H., Petersson, E. V., Hellenäs, K.-E.	Food Addit. Contam., 23(5), 437-445	Impact of extraction conditions on the content of acrylamide in model systems and food.	P9
Wenzl, T., Karasek, L, Rosén, J., Hellenaes, K.-E., Crews, C., Castle, L., and Anklam, E.	J. Chrom. A., doi:10.1016/j.chroma.2006.07.007	Collaborative trial validation study of two methods, one based on high performance liquid chromatography–tandem mass spectrometry and on gas chromatography–mass spectrometry for the determination of acrylamide in bakery and potato products.	P9
Dunovská L., ajka T., Hajšlová J., Holadová K.	Analytica Chimica Acta, Volume 578, Issue 2, 25 September 2006, 234-240	Direct determination of acrylamide in food by gas chromatography-high-resolution time-of-flight mass spectrometry	P10

Author	Title	Reference	Code
E. Bermudo, O. Núñez, L. Puignou, M.T. Galceran	Analysis of acrylamide in food samples by capillary zone electrophoresis	J. Chromatogr. A, 1120 (2006) 199-204	P12
E. Bermudo, O. Núñez, L. Puignou, M.T. Galceran	Analysis of acrylamide in food products by in-line preconcentration capillary zone electrophoresis	J. Chromatogr. A, 1129 (2006) 129-134	P12
E. Teixidó, F.J. Santos, L. Puignou, M.T. Galceran	Analysis of 5-hydroxymethylfurfural in foods by gas chromatography-mass spectrometry	J. Chromatogr. A, 1135 (2006) 85-90	P12
T Bjellaas, LH Stølen, M Haugen, JE Paulsen, J Alexander, E Lundanes and G Becher	Urinary acrylamide metabolites as biomarkers for short-term dietary exposure to acrylamide.	Food and Chemical Toxicology 2006 Dec 19; [Epub ahead of print]	P16
Year 2007			
G. Viklund, F. Mendoza, I. Sjöholm and K. Skog	An experimental set-up for studying acrylamide formation in potato crisps	LWT - Food Science and Technology, 40, issue 6, 1066-1071	P1
G. Viklund,. Olsson, K., Sjöholm I. and K. Skog	Effect of potato variety, year and storage conditions for the formation of acrylamide in crisps	submitted	P1
G. Viklund,. Olsson, K., Sjöholm I. and K. Skog	Blanching reduces precursor content and acrylamide formation in crisps	submitted	P1
Mustafa, A., Åman, P., Andersson, R. Kamal-Eldin A.	Analysis of free amino acids in cereal products	Food Chemistry, submitted	P4
Ahrne, L., Andersson, C.-G., Floberg, P., Rosén, J. & Lingnert, H.	Effect of crust temperature and water content on acrylamide formation during baking of white bread: steam and falling temperature baking	LWT – Food Science and Technology, In press	P6
J.J. Knol, G. Viklund, J.P.H. Linssen, I. Sjöholm, K. Skog, M.A.J.S. van Boekel	Kinetic Modeling: A Tool to Understand the Formation of Acrylamide in Food *In preparation*	Journal of Molecular Nutrition and Food Research	P7, P1
C. Crews and L. Castle	Review of the occurrence, formation and analysis of furan in heat-processed foods	Trends in Food Science and Technology, in press	P8
C. Crews, S Hasnip, D. P. T. Roberts and L. Castle	Factors affecting the analysis of furan in heated foods.	Food Additives & Contaminants, in press.	P8
C. Crews and L. Castle.	The determination of furan in foods- challenges and solutions.	LC-GC Europe, in press	P8
D. P. T. Roberts, C. Crews and H. Grundy	Effect of consumer cooking on furan in convenience foods	*Manuscript in preparation.*	P8

Authors	Journal	Title	Code
Vollebregt, H.M.; Zondervan, C.	J. Heat Transfer	A kinetic and thermodynamic model to predict levels of acrylamide in heated food products (tentative)	P11
M.S. Altaki, F.J. Santos, M.T. Galceran	J. Chromatogr. A	Analysis of furan in foods by headspace solid-phase microextraction-gas chromatography-ion trap mass spectrometry	P12
E. Bermudo, O. Núñez, E. Moyano, L. Puignou, M.T. Galceran	J. Chromatogr. A	Field Amplified Simple Injection-Capillary Electrophoresis-Tandem Mass Spectrometry for the análisis of acrylamide in foodstuffs	P12
A Vikström, S Eriksson, B Paulsson, P Karlsson and M Törnqvist	submitted	Comparison of bioavailability of acrylamide in different foods-a study in mice	P14
E Wirfält, B Paulsson, M Törnqvist, A Axmon and L Hagmar	European Journal of Clinical Nutrition (in press, doi 10.1038/sj.ejcn.1602704)	Associations between estimated acrylamide (AA) intakes, and Hb AA-adducts in a sample from the Malmö Diet and Cancer cohort	P14
T Bjellaas[*†], P Thonning Olesen[‡], H Frandsen[‡], M Haugen[*], LL-H Stølen[*], J E Paulsen[*], J Alexander[*], E Lundanes[†] and G Becher[*†]	Toxicological Sciences, submitted	Comparison of estimated dietary intake of acrylamide with haemoglobin adducts of acrylamide and glycidamide	P15
R.V. Hedegaard, H. Frandsen, K. Granby, A. Apostolopoula and L. Skibsted	Journal of food and Agricultural Chemistry, submitted	Influence of water activity on acrylamide formation from glucose and asparagines in aqueous glycerol	P15
Clement F, Dip R, Naegeli H	Cancer Epidemiology Biomarkers and Prevention (Submitted)	Expression Signature of Glycidamide, the Reactive Metabolite of the Widespread Food Carcinogen Acrylamide	P22

Book Chapters

Author	Book	Title	Partner
		Year 2006	
Mottram, D. S; Low, M. Y.; Elmore, J. S	*In:* Acrylamide and other Hazardous Compounds in Heat-Treated Foods. K Skog; J Alexander (Eds), Woodhead Publishing	The Maillard reaction and its role in the formation of acrylamide and other potentially hazardous compounds in foods	P3
Q. Chaudhry, J. Cotterill, R. Watkins and N. Price	In '*Acrylamide and other health hazardous compounds in heat-treated foods*'. pp. 132–159. K. Skog and J. Alexander (Eds), Woodhead Publishing, 2006. ISBN 978-1-84569-011-3.	A molecular modelling approach to predict the toxicity of compounds generated during heat treatment of food.	P8
L. Castle	In '*Acrylamide and other health hazardous compounds in heat-treated foods*'. pp. 117-131. K. Skog and J. Alexander (Eds), Woodhead Publishing, 2006. ISBN-13: 978-1-84569-011-3.	Analysis for acrylamide in foods.	P8

Other Publications

Author	Book	Title	Partner
		Year 2004	
Hülya Erol Gezgin	Dünya GIDA-Mayıs 2004	Isıl i lem Gören Gıdalarda Kanser Riski (Cancer Risk in Heat Treated Foods)	P13
K Granby, H Frandsen, K Kack, BBB Jensen, JA Nielsen	Alimenta	Acrylamid i fødevarer	P15
		Year 2005	
Per Åman	Livsmedel I Fokus, 2005, 7 pp 57	Akrylamid i bröd	P4
J.J. Knol, J.P.H. Linssen and M.A.J.S. van Boekel	Bi-/triennial periodical on current research of the graduate school VLAG	Kinetic Modeling of Acrylamide Formation in Model Systems	P7

Author	Publication	Title	Code
L Castle	FSA Food Survey Information Sheet 71/05. January 2005. http://www.food.gov.uk/multimedia/pdfs/fsis712005.pdf#page=5	Analysis of Total Diet Study samples for acrylamide.	P8
Hülya Ölmez-Songün Demirel	Dünya GIDA-Ocak 2005	Tahıl Bazlı Gıdaklarda Akrilamid (Acrylamide in cereal based products)	P13
Jacob D. van Klaveren, Polly E. Boon and Anika de Mul	Woodhead publishing. (submitted)	Modelling of dietary exposure to acrylamide, Acrylamide and other health hazardous compounds in heat-treated foods.	P17
H. R. Glatt	In: *Human Sulphotransferases* (G. M. Pacifici, M. W. H. Coughtrie, eds.) Taylor & Francis, London, p. 281-306	Activation and inactivation of carcinogens by human sulfotransferases	P18
H. R. Glatt, W. Teubner, W. Meinl	In: *15th International Symposium on Microsomes and Drug Oxidations*, held in Mainz (Germany) (F. Oesch, ed.), Medimond/Monduzzi, Bologna, p. 9-15	Sulfotransferases expressed in the human gastro-intestinal tract and their ability to activate promutagens/carcinogens	P18
		Year 2006	
Skog K. and Alexander J. (Eds)	Acrylamide and other hazardous compounds in heat-treated foods, Woodhead Publishing Ltd	BOOK with contributions of many HEATOX partners	P1, P16
Skog K, Hellenäs K-E. and Lingnert H.	Livsmedel i fokus	CIAA-workshop kring akrylamid	P1, P6, P9
Skog K.	Food Science and Technology, in press	The HEATOX project: Heat-generated food toxicants: Identification, Characterisation and Risk Minimisation,	P1
Murkovic M	Acrylamide and other hazardous compounds in heat treated foods, Skog K, Alexander J (Eds.) Woodhead Publishing Ltd, Cambridge	Mechanism for the formation of PhIP	P2
Murkovic M	Schadstoffe in Lebensmitteln und Futtermitteln, Cichna-Markl M, Sontag G, Stidl R (Eds.) Gesellschaft Österreichischer Chemiker, pp. 2-10, Wien, 2006	Bildung von heterozyklischen aromatischen Aminen beim Erhitzen von Fleisch	P2

Author	Reference	Title	Code
Bagdonaite K, Murkovic M		Formation of acrylamide in coffee	P2
Low, M. Y.; Elmore, J. S.; Mottram, D. S.	*In*: Flavour Science – Recent Advances and Trends. W. Bredie; M. Petersen (Eds), Elsevier.	Relationship between acrylamide formation and the generation of flavour in heated foods *NB Work partly supported by Heatox*	P3
Pehrsson, S	NyTeknik, 48(2006), 5	Så bakar du akrylamidfritt	P6
M Törnqvist, B Paulsson and S Osterman-Golkar	Acrylamide and other hazardous compounds in heat-treated foods, Kerstin Skog and Jan Alexander	Bio-monitoring of acrylamide	P14
SH Hansen, EJ Søderlund, D Anderson, A Baumgartner, R Gopalan and G Brunborg	14th European Testis Workshop, 2006. Programme and miniposters	Abstract: DNA damaging effects of the food contaminant acrylamide in male germ cells	P16
Jacob D. van Klaveren, Polly E. Boon and Anika de Mul	Book: Acrylamide and other health hazardous compounds in heat-treated foods (Woodhead publishing)	Modelling of dietary exposure to acrylamide, Acrylamide and other health hazardous compounds in heat-treated foods	P17
H. R. Glatt, Y. Sommer	In: *Acrylamide and Other Health Hazardous Compounds in Heat-treated Foods* (K. Skog, J. Alexander, eds.), Woodhead Publishing, Cambridge, p. 328-357	Health risks by 5-hydroxymethylfurfural (HMF) and related compounds	P18
Flurina Clement, Susanne Haller-Brem	UNIMAGAZIN, University of Zürich	Carcinogenic breakfast cereals?	P22
		Year 2007	
Skog K	Food Science and Technology, *21*,25-27	The HEATOX project: Heat-generated food toxicants: Identification, Characterisation and Risk Minimisation	P1
Murkovic, M.,	Consumer Protection through Food Process Improvement a& Innovation in the Real World, Ed. E. Lazos; Vol 3, 2007, pp. 502-510	Toxic substances in heated foods	P2

Author	Publication	Title	Code
J.S.Elmore & Q.Chaudhry	Database in Microsoft Access	The Heatox database of potential toxicants resulting from lipid oxidation	P3
J.S.Elmore & Q.Chaudhry	Database in Microsoft Access	The Heatox database of potential toxicants resulting from the Maillard reaction	P3
J.J. Knol, J.P.H. Linssen and M.A.J.S. van Boekel	Bi-/triennial periodical on current research of the graduate school VLAG	Kinetic Modeling of Acrylamide Formation in Model Systems	P7
J.J. Knol	PhD Thesis, Wageningen University, The Netherlands	Kinetic Modeling of Acrylamide Formation *(In preparation)*	P7
C. Zondervan	Voedingsmiddelen Technologie (2007, in preparation)	Acrylamide in Nederlandse voedselproducten	P11
Zondervan, C.; De Mul, A.	Voedingsmiddelentechnologie	*Acrylamide in verhitte voedselproducten: inname en voorkomen van inname* (final title to be confirmed by publisher). [*Acrylamide in heated food products: intake and reduction of intake*]	P11
T Bjellaas	Thesis, Disertation for the degree of Philosophiae Doctor, University of Oslo, 2007	Biomarkers of dietary exposure to acrylamide	P16
Wageningen University, The Netherlands	Publication: Bi-/triennial periodical on current research of the graduate school VLAG	Higher education Industry Research	P7
Wageningen University, The Netherlands	Publication in preparation: Journal of Molecular Nutrition and Food Research	Higher education Research	P7/P1
Wageningen University, The Netherlands	Submitted Abstract: 9th International Symposium on the Maillard Reaction	Higher education Industry Research	P7

POSTERS / ORAL	PRESENTATIONS		
Author	**Event**	**Title**	**Partner**
		Year 2003	
Kerstin Skog	Experts on Contaminants In Food", Workshop - Ways to Reduce Levels of Acrylamide in Food, Brussels, 20-21 October 2003	"The HEATOX project"	P1
Kerstin Skog, Ingegerd Sjöholm	Workshop on Acrylamide Formation in Food, (EFSA), 17 November 2003, Brussels.	"The HEATOX project"	P1
Lilia Masson	Conferencia Sociedad Chilena de Tecnología de Alimentos Santiago, Chile	"Acrilamida En Alimentos, ¿Un Cancerigeno Potencial" y "The HEATOX project"	P23
		Year 2004	
Kerstin Skog	Meeting before the start of COST 927 action, 28 February 2004, Vienna.	"The HEATOX project"	P1
Kerstin Skog	Network meeting "Akrylamid i maten", LI (Swedish Food Industries)	"The HEATOX project"	P1
Kerstin Skog	"Livsmedelsforskardagarna", Gothenburg, 17 November 2004	"The HEATOX project",	P1
Gunilla Viklund	Livsmedelsforskardagarna, Göteborg, Sweden	Acrylamide in potato crisps	P1
Bagdonaite	Österreichische Lebensmittelchemiker Tage, 11.5. – 12.5.2004	Formation of acrylamide in roasted coffee	P2
Ahrné, Andersson, Floberg, Rosén, Lingnert	Swedish Food research Days, Gothenburg, Sweden	Effect of temperature and water content on acrylamide formation during baking of white bread (Poster)	P6
Lingnert, Hans	JIFSAN Acrylamide Update, Chicago, USA	Acrylamide in food – Mitigation options	P6
Lingnert, Hans	Leatherhead Key Issue Day – Acrylamide Up-date, Leatherhead, UK	Acrylamide in food – Mitigation options	P6
Lingnert, Hans	Nordic Starch Network Meeting, Gothenburg, Sweden	Acrylamide and starch – State of the art	P6

Lingnert, Hans	CIAA acrylamide Technical Expert Workshop, Brussels	HEATOX Update	P6
Lingnert, Hans	Swedish Industry Acrylamide Network, May 2004, Gothenburg	HEATOX Update	P6
Lingnert, Hans	Swedish Industry Acrylamide Network, October 2004, Gothenburg	HEATOX Update	P6
Lingnert, Hans	NORDACRYL – Nordic Network Project Meeting, Oslo, Norway	HEATOX Update	P6
Hellenäs, K.-E.	JRC-irmm work-shop, Geel,17 December.	The HEATOX project	P9
Dunovská L., Hajšlová J., ajka T., Holadová K. and Hájková K.	Chemical Reactions in Foods V. Prague, Czech Republic, 29.thSept - 1.stOct., 2004	Changes of Acrylamide Levels in Food Products during Technological Processing	P10
J. van Gijssel	Potato convention, June 2004 Amsterdam	EU HEATOX and Potato Processing	P11
E. Teixidó, F.J. Santos, M.T. Galceran	SECyTA (Madrid 2004)	Analysis of hydroxymethylfurfural in honey by gas chromatography mass spectrometry	P12
Elisabet Bermudo, Encarna Moyano, Luis Puignou, Maria Teresa Galceran	Reunión Nacional de Espectrometría de Masas	LC-APCI-MS/MS (LTQ) for the determination of acrylamide in foodstuffs.	P12
Elena Barceló-Barrachina, Encarna Moyano, MTGalceran	Reunión Nacional de Espectrometría de Masas	Accurate mass measurement using a Q-TOF for characterisation and determination of heterocyclic amines in food.	P12
H Frandsen, P Olesen	Meeting with Danish Food Industry	Acrylamide, risk assessment	P15
Siri Helland Hansen and Gunnar Brunborg	Reproductive Toxicology in the Adult, ReproSafe Summer School, Sweden.	Short presentation of the PhD project - "Acrylamide in food - effects on reproduction"	P16
H. R. Glatt, W. Meinl, G. Dobbernack, R. Kollock, U. Pabel, Y. Sommer, et al	*15th International Symposium on Microsomes and Drug Oxidations*, Mainz, July 2004	Sulphotransferases. (invited oral presentation)	P18
Y. Sommer, H. Schneider, H. R. Glatt	*First European Nutrigenomics Conference*, Wageningen, September 2004	Hazard identification of the mutagen 5-sulfoxymethyl-2-furfural (SMF) formed from the common Maillard product 5-hydroxymethyl-2-furfural (HMF) by individual human sulfotransferases. (poster)	P18
H. R. Glatt	Seminars of Institute of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Giessen, Germany, September 2004	Sulfotransferasen als toxifizierende Enzyme [Sulfotransferases as toxifying enzymes] (seminar talk)	P18

H. R. Glatt	Course on Drug Metabolism, Organized by the German Society of Toxicology, in Dortmund, Germany, September 2004	Sulfotransferasen und Hydrolasen (lecture)	P18
H. R. Glatt	19th European Workshop on Drug Metabolism, Antalya, Turkey, October 2004	Activation of genotoxicants by cDNA-expressed cytochromes P450, alcohol dehydrogenases, acetyltransferases and sulfotransferases: differences between rodent and human enzyme forms (lecture)	P18
H. R. Glatt	Postgraduate study course on Toxicology, University of Leipzig, Germany, December 2004	Sulfotransferasen (lecture)	P18
H. R. Glatt	Seminars of Institute for Food Chemistry and Environmental Toxicology, Technical University of Kaiserslautern, Germany, December 2004	Humanisierte Modellsysteme zum Erfassen gentoxischer Wirkungen von Pyrolyseprodukten und Naturstoffen in Lebensmitteln [Humanized model systems for the detection of genotoxic effects of heat-induced and natural food constituents] (seminar talk)	P18
Lilia Masson	Congreso Nacional de Tecnología de Alimentos, Mar del Plata, Argentina	Curso de Fritura en Profundidad, Modulo 4: Formación de Acrilamida y "The HEATOX project"	P23
Lilia Masson	XIII Seminario Latinoamericano Y Del Caribe Ciencia Y Tecnología De Alimentos, Montevideo, Uruguay	"Acrilamida En Alimentos, ¿Un Cancerigeno Potencial" y "The HEATOX project"	P23
		Year 2005	
K Skog	ILSI Europe, workshop	Acrylamide task force meeting brainstorming session on risk positioning	P1
K Skog	Nordic Ministry Council, Mölle (Sweden), 8 September, 2005	"The HEATOX project"	P1
G Viklund, K Olsson, I Sjöholm, K Skog	16th Triennial Conference of the EAPR	Effect of storage on acrylamide in potato crisps from five Swedish grown potato varieties	P1
Skog K	Fruit, vegetable and potato processing, Brugge,	Formation of acrylamide in crisps	P1
Murkovic	Miniworkshop at University of Helsinki	Formation of HMF	P2

Author	Conference	Title	Code
M. Murkovic	1st International Symposium of the Human Nutrition & Metabolism Research and Trainings Center, Graz Austria	Effects of heating on food	P2
Pichler, Murkovic	In vino analytica sciencia 2005, 7.7. – 9.7.2005, Montepellier, France	Determination of HMF in wines and balsamic vinegars by RP-HPLC	P2
Derler K., Murkovic M.	XVIIth International Botanical Congress, 17.7. – 23.7.2005, Wien, Austria	Determination of mono and disaccharides in green coffee and their nutritional significance	P2
Bagdonaite, Murkovic	8th Symposium on Instrumental Analysis, 259. – 28.9.2005	3-APA a possible intermediate in the pathway leading to acrylamide	P2
Derler, Murkovic	8th Symposium on Instrumental Analysis, 259. – 28.9.2005	Analysis of amino acids and carbohydrates in green coffee	P2
Mei Yin Low, Donald S. Mottram and J. Stephen Elmore	11th Weurman Flavour Research Symposium, Roskilde, Denmark	Relationship between Acrylamide Formation and the Generation of Flavour in Heated Foods	P3
Åman P.	NI meeting in Iceland	Acrylamide in Bread	P4
Mustafa A.	Food Science Day in Uppsala	Strategies to Reduce Acrylamide in Bread	P4
M. Dalla Rosa	Workshop on bread and bakery products – Verona (Italy) – Italian Association in Food Technology (AITA)	UE research activity on acrylamide and toxicants in heat treated foods	P5
S. Romani	7th Italian Congress in Food Science and Technology (CISETA) – Cernobbio (Italy)	Comparison among different fryers on some quality characteristics and acrylamide content of fried potatoes	P5
Lingnert	Swedish Industry Acrylamide Network, March 2005, Uppsala	HEATOX Update	P6
Lingnert	Safe Food for the Future; Öresund Food Network, Lund, Sweden	Acrylamide	P6
Lingnert	NORDACRYL – Nordic Network Project Meeting, Reykjavik, Iceland	HEATOX Update	P6
Lingnert	Swedish Industry Acrylamide Network, October 2005, Gothenburg	HEATOX Update	P6

Author	Venue	Title	Partner
Lingnert	KSLA Seminar, Stockholm, Sweden	Events in foods during heating – Maillard reaction products, including acrylamide	P6
Wageningen University	Bi-/triennial periodical on current research of the graduate school VLAG	One-page summary of project of partner 7	P7
J.J. Knol, J.P.H. Linssen and M.A.J.S. van Boekel	Socrates IP 'Food & Health', International Advanced Course	Toward a Kinetic Model for Acrylamide Formation in Model Systems (Poster presentation)	P7
J.J. Knol, J.P.H. Linssen and M.A.J.S. van Boekel	Meeting with members of Dutch Potato Processors' Association (DPPA/VAVI)	Toward a Kinetic Model for Acrylamide Formation in Model Systems (Poster presentation)	P7
Petersson E.V., Rosén J., Turner C, Danielsson R, Hellenäs K.-E.	1st International Food and Nutrition Congress, Istanbul, Turkey, 15-18 June, 2005	Critical factors and pitfalls affecting the extraction of acrylamide from foods (an optimisation study)	P9
Wageningen University	Industry	Poster: Meeting with members of Dutch Potato Processors' Association (DPPA/VAVI)	P7
Petersson E.V., Rosén J., Turner C, Danielsson R, Hellenäs K.E.	2nd International Symposium on Recent Advances in Food Analysis, Prague, Czech Republic, 2-4 November, 2005	Identification of critical factors for extraction of acrylamide from foods (an optimisation study using analysis by LC-MS/MS)	P9
Hellenäs, K.-E.	SANCO Expert committee on environmental and industrial contaminants, Brussels, 14 January.	The HEATOX project update.	P9
Hellenäs, K.-E.	SANCO Expert committee on environmental and industrial contaminants, Brussels, 9 June.	Update on recent HEATOX activities on acrylamide and Furan.	P9
Hellenäs, K.-E.	BEUC meeting, Brussels, 13 November.	The HEATOX project.	P9
Hellenäs, K.-E.	Food safety in a European perspective, Seminar and work-shop, Oslo, 7-8 December	Acrylamide – Still a matter of concern?	P9
Gibala P., Novotný O., Petersson E.V., Dunovská L., Václavík L. Hajslová J., Hellenäs K.-E., Rosén J.	2nd International Symposium on Recent Advances in Food Analysis, Prague, Czech Republic, 2-4 November, 2005	Can alkaline extraction release masked acrylamide?	P9 P10

Authors	Venue	Title	Code
Gibala P., Petersson E., Dunovská L., Václavík L., Novotný O., Hajšlová J., Hellenäs K. E., Rosén J.	2nd International symposium on recent advances in food analysis, Prague, Czech Republic, 2-4 November, 2005	Can alkaline extraction release masked acrylamide?	P10
Dunovská L., ajka T. and Hajšlová J.	3rd Meeting on Chemistry & Life Brno, Czech Republic, 20-22 September, 2005	Acrylamide Levels in Foodstuffs from Czech market	P10
Dunovská L., Hajšlová J., Holadová K., Gibala P., Sch rek J.	2nd International symposium on recent advances in food analysis, Prague, Czech Republic, 2-4 November, 2005	Analytical strategies for examination of roasted coffee: volatile markers of acrylamide formation	P10
J. van Gijssel	Acrylamide stakeholders meeting, 14 January 2005, Brussel	HEATOX WP 2. Formation and Processing, Progress in the first year.	P11
E. Bermudo, O. Núñez, L. Puignou, M.T. Galceran	XI Jornadas de Análisis Instrumental (Barcelona 2005)	Development of new capillary electrophoresis methodologies for the analysis of acrylamide in foodstuffs	P12
E. Teixidó, F.J. Santos, M.T. Galceran	XI Jornadas de Análisis Instrumental (Barcelona 2005)	Gas chromatography coupled to mass spectrometry for the analysis of hydroxymethylfurfural in foods	P12
M. S. Altaki, F.J. Santos, M.T. Galceran	XI Jornadas de Análisis Instrumental (Barcelona 2005)	Analysis of furan in food by headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry.	P12
Elisabet Bermudo, Oscar Núñez, Luis Puignou, Maria Teresa Galceran	HPLC 2005	Capillary Zone Electrophoresis for the determination of acrylamide in foodstuffs	P12
Elena Barceló-Barrachina, Encarnación Moyano, Lluís Puignou and Maria Teresa Galceran	HPLC 2005	Development of methacrylate monoliths for the analysis of heterocyclic amines by capillary electrochromatography	P12
Maria Teresa Galceran	1st International Workshop of MS/MS spectrometry	Tandem Mass Spectrometry and Accurate Mass Measurement for the analysis of genotoxic heat-induced compounds in food.	P12
Hülya Ölmez	TÜB TAK MRC 1st International Food and Nutrition Congress-15-18 June 2005, Istanbul / Oral presentation	Gıdalarda Akrilamid (Acrylamide in foods)	P13
Hülya Ölmez	TV Programme - ATV Channel / speaker	Acrylamide risk in foods	P13

Author	Event	Title / Description	Code
Hülya Ölmez	TV Programme – CNBC-e Channel / speaker	Acrylamide risk in foods	HP13
P Olesen	Oral presentation internally in DFVF	Akrylamid – analyse af globinaddukter I humane prøver (project HEATOX)	P15
Thomas Bjellaas	Norwegian Society for Mass Spectrometry, Hafjell January 2005	Poster – "Determination of acrylamide metabolites in human urine using solid-phase extraction and LC-MS/MS detection"	P16
Thomas Bjellaas, Hege B. Ølstørn, Jan Erik Paulsen	Nordic meeting for NORDACRYL – june, Iceland	Presentation of bioavailability of acrylamide from foods study in mice using metabolic cages	P16
Thomas Bjellaas	European meeting for HEATOX – (Netherlands), May 2005	Poster – "Determination of acrylamide metabolites in human urine using solid-phase extraction and LC-MS/MS detection"	P16
Thomas Bjellaas	Norwegian Institute of Public Health	Presentation – A pilot study on urinary excretion acrylamide metabolites in humans	P16
Hege B. A. Ølstørn, Jan Erik Paulsen, Jan Alexander	Norwegian Institute of Public Health	Presentation of preliminary results – Acrylamide and intestinal cancer	HP16
Siri Helland Hansen, Erik Søderlund, Adolf Baumgartner, D Anderson and G Brunborg	3rd Copenhagen Workshop on Environment, Reproductive Health and Fertility, 15-18 January 2005	Poster and abstract – "The paternal reproductive toxicity of the food contaminant acrylamide	P16
Siri Helland Hansen and Gunnar Brunborg	Norwegian Institute of Public Health, Division of Environmental Medicine	Presentation (in Norwegian) – "Kan akrylamid påvirke den mannlige reproduksjonen?"	P16
Jan Alexander	9th International Conference on Environmental Mutagens, San Francisco, Sept. 2005	Risk Assessment of Acrylamide in Food	P16
Jacob D. van Klaveren	Heatox 18th month meeting, Symposium with Dutch potato processors Association	Exposure assessment of HEATOX compounds;	P17
Jacob D. van Klaveren, Anika de Mul	Dutch Food Authority (VWA) work-meeting	Exposure assessment of heat-treated toxicants	P17
Anika de Mul	Food Standard Agency (UK), work-meeting	Probabilistic acrylamide intake calculations with MCRA and Comparison of acrylamide intake by British and Dutch toddlers	P17
H. R. Glatt	Symposium of Seminars of Bundesinstitut für Risikobewertung ([German] Federal Institute of Risk Assessment), February 2005	Gentoxizitäts-Untersuchungen in vitro mit verschiedenen Kohlenhydrat-Pyrolyseprodukten [Genotoxicity studies in vitro on various carbohydrate pyrolysis products] (talk)	P18

Name	Title	Venue	Code
H. R. Glatt	Heatox: Genotoxizitäts-Untersuchungen mit verschiedenen Kohlenhydrat-Pyrolyseprodukten [Heatox: Genotoxicity studies on various carbohydrate pyrolysis products] (talk)	Annual Meeting of the "Arbeitskreis Gesundheit und Lebensmittelsicherheit" [work group Health and Food Safety] of the "Bundesverbandes der Deutschen Süßwarenindustrie e.V." [Federal Association of the German Industry on Sweet Products], Bergholz-Rehbrücke, Germany, June 2005	P18
H. R. Glatt	Gentoxische und kanzerogene Wirkungen von Lebensmittel-Inhaltsstoffen in humanisierten Modellsystemen und deren Prävention/Potenzierung durch weitere Ernährungsfaktoren [Genotoxic and carcinogenic effects of food-borne substances in humanized model systems, and their prevention/potentiation by other food factors] (seminar talk)	Seminars of the Institute for Cancer Research, University of Vienna, Austria, June 2005	P18
H. R. Glatt	Genetisch veränderte Zellen und Tiere zur Untersuchung des Fremdstoff-Metabolismus [Genetically engineered cells and animals for investigations on xenobiotic metabolism] (lecture)	Postgraduate study course on Toxicology, University of Vienna, Austria, June 2005	P18
H. R. Glatt	Genotoxische Pyrolyseprodukte und Naturstoffe in Lebensmitteln [Genotoxc pyrolysis products and natural compounds in foods] (lecture)	Continuous Education in Toxicology, Symposium „Rückstandstoxikologie, neue Aspekte und Entwicklungen 2005" University of Zurich, Switzerland, November 2005	P18
Barbara Gallani	Acrylamide: regulatory approach and communication to consumers	BEUC Food officers' meeting 29-30 November 2005	P20
Helga Odden Reksnes	The role of risk communication policy in Europe in assuring food safety to consumers (manuscript and ppt-presentation)	Food practices at home; what are the risks? Workshops Ireland and Northern Ireland 17 and 18 May 2005	P21
Helga Odden Reksnes	HEATOX – Deliverables – Rsik Communication and dissemination (ppt-presentation)	DG Sanco, CCAB meeting	P21
Hanspeter Naegeli	The application of "-omic" technologies in food toxicology	Course in Toxicology Swiss Federal Institute of Technology	P22
Flurina Clement	Gene expression profiles induced by glycidamide in human cells	International Symposium of Pharmacology and Toxicology. Vienna	P22

Flurina Clement	Annual Meeting of the Swiss Toxicology Network (XERR)	Gene expression profiles induced by glycidamide in human cells	P22
Flurina Clement	Annual Meeting of the Xenobiotic and Environmental Risk Research Center Zürich, Switzerland	Transcriptomics of Acrylamide and Glycidamide	P22
Lilia Masson	VI Seminario Latinoamericano de Ciencia de Alimentos, Campinas, Brasil	"Acrilamida En Alimentos, ¿Un Cancerigeno Potencial" y "The HEATOX project"	P23
Lilia Masson	Congreso Federación Farmacéutica Sudamericana, Santiago, Chile	"Acrilamida En Alimentos, ¿Un Cancerigeno Potencial" y "The HEATOX project"	P23
		Year 2006	
Skog K	Environmental and Industrial Contaminants workshop- acrylamide, (CIAA) Brussels	The HEATOX project - Heat – generated Food Toxicants. Identification, Characterization and Risk Minimisation	
Skog K	HEATOX workshop, Graz	Home-cooking and acrylamide	P1
Skog K	Workshop at Nestlé, Lausanne	The HEATOX project	P1
Skog K	Formas visit to LU	Bildning av toxiska ämnen vid livsmedelsprocessning Bildningsmekanismer och hälsoeffekter	P1
G Viklund, K Olsson, I Sjöholm, K Skog	Livsmedelsforskardagarna, November 21- 22, 2006, Uppsala, Sweden	Acrylamide in crisps from Swedish grown Saturna potatoes	P1
G Viklund, K Olsson, I Sjöholm, K Skog	Meeting NORDACRYL	Effect of storage on acrylamide in potato crisps from five Swedish grown potato varieties	P1
Bagdonaite K, Murkovic M	Österreichische Lebensmittelchemiker Tage, 12.9. – 14.9.2006, Wien	Acrylamide formation in coffee	P2
Bornik MA, Jöbstl D, Derler K, Murkovic M	Österreichische Lebensmittelchemiker Tage, 12.9. – 14.9.2006, Wien	Kinetik der HMF- und HMFS-Bildung im Laufe der Kaffeeröstung, in Modellsystemen	P2
Per Åman	Arranged a meeting in collaboration with Nordic Innovation Centre (NICe Meeting NORDACRYL)	Acrylamide	P4

Author	Title	Event	Code
Arwa Mustafa	Analysis of Free Amino Acids	NICe Meeting NORDACRYL	P4
Arwa Mustafa	Acrylamide in Food Products	Lecture at Sudan University, Khartoum, Sudan	P4
Arwa Mustafa	Acrylamide in Food Products	Lecture at Ahfad University, Omdurman, Sudan	P4
M. Dalla Rosa	Heat – generated food toxicants. Identification, characterization and risk minimisation. Home cooking	CIAA Brusselles Meeting	P5
S. Romani, M. Bacchiocca, P. Rocculi, A. Angioloni, M. Dalla Rosa	Effect of frying time on some quality aspects and acrylamide content of French fries	CEFood Congress: 3th Central European Congress on Food. (Sofia - Bulgaria)	P5
S. Romani, M. Bacchiocca, P. Rocculi, M. Dalla Rosa	Effect of some frying conditions on acrylamide content in french fries	IUFOST, 13th World Congress of Food Science and Technology (Nantes – France)	P5
Lingnert, Hans	HEATOX Update	Swedish Industry Acrylamide Network, March 2006, Uppsala	P6
Lingnert, Hans	Minimisation Options - Industry	HEATOX Workshop, June 2006, Graz, Austria	P6
Lingnert, Hans	HEATOX deliverables in relation to the CIAA Toolbox	CIAA Acrylamide Group, 5 July 2006, Brussels, Belgum	P6
Lingnert, Hans	HEATOX Update	Swedish Industry Acrylamide Network, October 2006, Gothenburg	P6
Hellenäs, K.-E.	Acrylamide extraction at high pH.	SANCO/CIAA Workshop, Brussels, 16 March.	P9
Lingnert, Hans	Akrylamid – på gott och ont	Swedish Food Research Days, Uppsala, 21-22 November 2006	
Václavík L., Bartá ková V., Holadová K., Hajšlová J.	Acrylamide – new food carcinogen	JAKOST OBILOVIN - 2006 (Quality of cereals-2006), Krom íž, Czech Republic, 9.11.2006	P10
Hajšlová J., Novotný O., Václavík L, Dunovská L.	Study of "masked" acrylamide origin	COST IMARS Joint Workshop, Napoli, Italy, 24. - 27. 5. 2006	P10
Václavík L, ajka T., Dunovská L, Hajšlová J.	LC-MS/MS and GC/HRTOF-MS methods for analysis of acrylamide in foodstuffs	COST IMARS Joint Workshop, Napoli, Italy, 24. - 27. 5. 2006	P10
Václavík L., Bartá ková V., Holadová K., Hajšlová J.	Acrylamide – new food carcinogen	JAKOST OBILOVIN - 2006 (Quality of cereals-2006),	P10

Author	Event	Title	Code
Zondervan, C.	Workshop with DPPA Nov 28, 2006	Instructions on using the french fry modelling tool: Fry Simulator v.2	P11
E. Teixidó, E. Moyano, F.J. Santos, M.T. Galceran	III Reunión de la Sociedad Española de Espectrometría de Masas (Oviedo 2006)	Analysis of hydroxymethylfurfural in foodstuffs by liquid chromatography coupled to tandem mass spectrometry	P12
L. Puignou	Jornada de Genotòxics en Aliments (Reus 2006)	Tóxicos alimentarios endogénicos en alimentos procesados	P12
E. Bermudo	Jornada de Genotòxics en Aliments (Reus 2006)	Análisis de acrilamida en alimentos	P12
E. Teixidó	Jornada de Genotòxics en Aliments (Reus 2006)	Análisis de hidroximetilfurfural mediante técnicas cromatográficas y espectrometría de masas	P12
M. S. Altaki	Jornada de Genotòxics en Aliments (Reus 2006)	Furan: Formation and analysis	P12
E. Bermudo, O. Núñez, E. Moyano, L. Puignou, M.T. Galceran	SECyTA (Vigo 2006)	Application of electrophoretic techniques for the determination of acrylamide in foods	P12
M. S. Altaki, F.J. Santos, M.T. Galceran	SECyTA (Vigo 2006)	Analysis of furan in food by on-line headspace solid-phase microextraction and gas chromatography ion trap mass spectrometry.	P12
A Vikström, S Eriksson, B Paulsson, P Karlsson and M Törnqvist	Presentation at Åbo Academy	Study of relative bioavailability of acrylamide in different foodstuffs	P14
A Vikström	Seminar at Stockholm University	Background exposure to acrylamide and its genotoxic metabolite glycidamide	P14
A Vikström, B Paulsson, & M Törnqvist	Course at Karolinska Institutet, Solna,Sweden (ECNIS), poster presentation	Possible influence of polymorphism on internal dose of acrylamide and glycidamide	P14
M Törnqvist	HEATOX workshop 13-14 June	The acrylamide story	P14
Anika de Mul	ILSI, Expert group meeting 'Risk Benefit Analysis of Mitigation Measures'	Acrylamide; Exposure and Reduction scenarios	P17
Jacob D. van Klaveren	Norwegain Food Authority (VKM)	Probabilistic modeling in perspective of future risk assessment	P17
Jacob D. van Klaveren	Naam van het symposium. The HEATOX Workshop	Exposure and Reduction Scenarios	P17

Author(s)	Venue	Description	Code
Jacob D. van Klaveren	Television programme for consumers (Kassa)	Acrylamide levels in food and its risks	P17
H. R. Glatt	Continuous Education for Medical Doctors, Course "Ernährungs-medizin", Potsdam-Rehbrücke, Germany, March 2006	Krebserzeugende Stoffe in Lebensmitteln [Food-borne carcinogens] (lecture)	P18
H. R. Glatt	Course on Drug Metabolism, Organized by the German Society of Toxicology, in Dortmund, Germany, September 2006	Sulfotransferasen und Hydrolasen (lecture)	P18
H. R. Glatt, Y. Sommer, G. Dobbernack, W. Meinl	43rd Congress of the European Societies of Toxicology and 6th Congress of Toxicology in Developing Countries, Cavtat/Dubrovnik, Croatia, September 2006	Heterologous and transgenic models for studying genotoxic effects of contaminants produced by heat-treatment of food (invited talk), Abstract published in: Toxicol. Lett. 164 S (2006) 62-63.	P18
Jacob D. van Klaveren	Training for Norwegian Food Authority (VKM)	Probabilistic dietary intake calculations for acrylamide	P17
H. R. Glatt, F. Taugner, S. Florian, Y. Sommer	COST 926/927 Conference: International Conference on Molecular and Physiological Effects of Bioactive Food Components, Vienna, Austria, October 2006	Health risks by 5-hydroxymethylfurfural (HMF) and related compounds (invited talk), Some data accessible at COST926 web page: http://www.uochb.cas.cz/Zpravy/COST_926/	P18
H. R. Glatt, G. Dobbernack, W. Meinl	COST 926/927 Conference: International Conference on Molecular and Physiological Effects of Bioactive Food Components, Vienna, Austria, October 2006	Expression of human phase-II enzymes in model systems for detecting genotoxicants and protective agents (invited talk), Some data accessible at COST926 web page: http://www.uochb.cas.cz/Zpravy/COST_926/	P18
Barbara Gallani	CIAA/EC Workshop 16-17 March 2006	Acrylamide: information and communication to consumers	P20
Helga Odden Reksnes	Acrylamide Workshop EU/CIAA	Minutes from working group on home cooking	P21
Helga Odden Reksnes	CCFAC Workshop on Risk communication as a management tool for contaminants	Consumption Advice on Contaminants - a brief communication case study on Acrylamide	P21
Working Group, HEATOX Workshop	The HEATOX Workshop	Report	P21

Code	Title	Meeting	Author
P21	The HEATOX Aquarium	The HEATOX Workshop	Helga Odden Reksnes
P21	Summary Workshop Graz	The HEATOX 30 M meeting Graz	Helga Odden Reksnes
P22	Transcriptomics of Glycidamide, the Genotoxic Metabolite of Acrylamide	Meeting of the DNA Repair-Recombination Network Zürich, Switzerland	Flurina Clement
	Year 2007		
P1	Acrylamide in crisps from Swedish grown Saturna potatoes	4th EAPR/FNK/UEITP Potato Processing Conference and EAPR Engineering & Utilisation Section Meeting, January 17, 2007, Grantham, UK	G Viklund, K Olsson, I Sjöholm, K Skog
P1	Acrylamide in potato crisps from Swedish grown potatoes	First European Workshop on Food Engineering and Technology, EFFoST EFChE Food Working Party Congress, May 2007, Berlin, Germany	G Viklund, K Olsson, I Sjöholm, K Skog
P1	Acrylamide in roasted potato wedges	COST 927 Action Workshop, Sofia, Bulgaria	G Viklund, K Olsson, I Sjöholm, K Skog
P1	Heat derived toxicants in food – some findings of a collaborative European research project	Symposium on Chemistry and Toxicology of Acrylamide, Boston, US, August, 2007.	Skog K
P2	Formation of potentially toxic substances during heating of foods	5th International Congress on Food Technology, 9.3.-11.3.2007 Thessaloniki/Greece	Murkovic, M.
P6	Att undvika akrylamid när man bakar (To avoid acrylamide when baking)	Färsk Forskning, SIK, Göteborg, 7 February 2007	Andersson, C-G & Lingnert, H
P7	Kinetic Modeling: A Tool to Understand the Formation of Acrylamide (Oral presentations)	PhD study trip USA, presentations at different universities in the USA	J.J. Knol, J.P.H. Linssen and M.A.J.S. van Boekel
P7	Kinetic Modeling: A Tool to Understand the Formation of Acrylamide in Food (Oral presentation)	COST 927 Action Workshop, Sofia, Bulgaria	J.J. Knol, G. Viklund, J.P.H. Linssen, I. Sjöholm, K. Skog, M.A.J.S. van Boekel
P10	Fast and simple method for acrylamide analysis and content of acrylamide in Czech breads	Heatox Project Meeting Prague, Czech Republic, 21.-23.1.2007	Václavík L., Bartá ková V., Holadová K., Hajšlová J.

Siri Helland Hansen and Gunnar Brunborg	Internal seminar at the Division of Environmental Medicine, Norwegian Institute of Public Health	Intake of crisp bread and coffee while making children – mutually exclusive? (in Norwegian)	P16
Siri Helland Hansen, Minh Hoang, Erik Søderlund and Gunnar Brunborg	HEATOX 36 month meeting, Prague, The Czech Republic, 21st-23rd of Jan. 2007	Genotoxic effects of glycidamide on mouse testicular cells	P16
J.J. Knol, J.P.H. Linssen and M.A.J.S. van Boekel	9th International Symposium on the Maillard Reaction	Fate of acrylamide, melanoidins, sugars, asparagine, organic acids and pH in an aqueous fructose-asparagine reaction system at pH 5.5: a kinetic analysis (Submitted for Oral presentation)	P17
Other ways of dissemination			
Anika de Mul, Polly E. Boon, Jacob D. van Klaveren	Training for EFSA and National Food Authorities	Training program on probabilistic exposure assessment for international and national food safety authorities	P17