Chapter 50
Using Total Diet Studies to Assess Acrylamide Exposure

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Introduction

Acrylamide has been used as an industrial chemical since the mid-1950s, but its presence in food was only discovered in May 2002. Since then there has been extensive international effort to investigate how acrylamide forms in food and how formation could be reduced. In addition, efforts have been made to develop and refine risk assessment for dietary exposure to acrylamide. Acrylamide is known to be neurotoxic in humans as a result of occupational and accidental exposure. Studies in animals have shown that acrylamide can have reproductive effects, cause cancer and also damage DNA (i.e. it is genotoxic). It is not known whether dietary exposure to acrylamide could cause cancer in humans, but based upon the evidence from the animal studies, it is considered probable [1].

Following the Swedish announcement of the presence of acrylamide in foods in April 2002, an expert consultation was quickly convened by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) in June 2002 to review and evaluate existing data on acrylamide, and to provide interim advice to governments, industry and consumers. The European Scientific Committee on Food also assessed the implications for food safety posed by acrylamide. The Committee considered research conducted across Europe and endorsed the recommendations of the FAO/WHO expert consultation, including the recommendation that amounts of acrylamide in food should be reduced to as low as reasonably achievable (ALARA).

In 2005, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) carried out a risk assessment showing that current estimated exposure levels may

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indicate a human health concern [2]. The International Agency for Research on Cancer had previously classified acrylamide as “probably carcinogenic in humans” [3]. The European Food Safety Authority’s (EFSA) Scientific Panel on Contaminants in the Food Chain concluded in May 2008 that more data on the carcinogenicity of acrylamide in humans are required before it could fully re-evaluate its toxicity [4]. EFSA has plans to conduct a risk assessment by 2015.

Occurrence in Food

Acrylamide has been detected in a wide range of heat-treated foods especially dietary staples such as potatoes, cereals and cereal products. It is found in both foods processed by manufacturers and foods that are cooked in the home. Generally, acrylamide is formed when foods containing the natural amino acid asparagine and sugars, either present naturally or added, are heated at temperatures greater than 120 °C. It has been found in a wide range of home-cooked and processed foods, including potato chips (in the UK, potato crisps), French fries (in the UK, potato chips), bread, crackers (in Europe, crisp breads) and coffee. However, research has indicated that acrylamide does not occur in such foods subjected to lower temperatures and relatively short process times [5]. It is interesting to note that studies have shown acrylamide not to be formed in foods that have been boiled or microwaved [6]. Table 50.1 lists some of the foods that have been found to contain acrylamide.

Ingestion of excessive amounts of contaminants such as acrylamide through the food supply may potentially lead to detrimental effects on the health of consumers. Thus, it is essential to analyze the foods we eat for contaminants and other chemicals through regular monitoring and surveillance programs to assure that chemical levels found in foods remain safely within acceptable national and international reference values. As part of its mandate to ensure that chemicals are not present in foods at levels that would pose an unacceptable risk to health, the UK Food Standards Agency (FSA) carries out total diet studies (TDSs) to provide estimates of exposure for the UK population to chemicals through the food supply. TDSs involve the analyses of groups of foods that reflect the average food consumption patterns of a given population [7]. Results of the analyses can then be used in conjunction with national food consumption data to estimate the average exposure of the general population and certain subgroups to chemicals in foods. The data can also be used to identify changes or trends in exposure and to make assessments on the quality and safety of the food supply. The World Health Organization (WHO) supports TDSs as one of the most cost-effective means for assuring that people are not exposed to unsafe levels of toxic chemicals through food, while also recognizing the importance of TDSs to the development of Codex Alimentarius Commission standards and international trade [8].

The main purpose of a TDS is to protect consumers from chemical contaminants by monitoring exposure levels of the general population over time [9]. TDS programs can be set with varying levels of complexity and sophistication but usually have some degree of sampling to take account of geographic regions and seasonality.

Table 50.1 Foods found to contain acrylamide [16]

Food group	Food subgroup	Number of samples	Mean	Acrylamide (μg/kg)	
				Minimum	Maximum
Bakery wares	White bread	7	84	60	117
	Wholemeal bread	6	17	15	33
	Rye bread	38	140	10	397
	Other bread	9	24	15	60
	Crackers excluding sweet crackers	43	301	10	830
	Other ordinary bakery products	32	137	10	1,987
	Crispbread and crisp rolls	212	411	10	2,380
	Bread type products	29	140	10	514
	Cakes, cookies and pies	135	202	10	1,080
	Other fine bakery products	5	557	169	1,491
Beverages (coffee and beer)	Coffee (as reported)	273	600	80	2,932
	Beer and lager	37	5	10	10
	Cider	1	5	10	10
	Malt drinks	3	107	90	130
Biscuits	Miscellaneous biscuits	189	303	10	1,950
	Gingerbread	680	569	10	7,834
	Ginger biscuits	139	585	15	2,220
	Almond based	79	310	10	1,234
	Shortbread	151	409	10	6,798
Cereals and cereal products	Whole, broken or flaked grain	4	15	10	30
	Flours and starch	3	42	13	112
	Muesli	51	64	10	258
	Maize-based cereals	110	98	10	545
	Rice-based cereals	11	251	20	1,649
	Wheat-based cereals	22	132	30	532
	Mixed grain cereals	15	137	50	260
	Oat-based cereals	6	95	10	274
	Bran based	15	304	20	640
	Rice cakes	8	137	15	250
Confectionery	Chocolate confectionary	47	138	10	826
	Sugar-based confectionary	23	151	10	548
Fruits, vegetables and nuts	Dried fruit	73	42	10	258
	Fruit in vinegar, oil or brine	32	169	3	1,548
	Fresh vegetables	5	13	15	20
	Dried vegetables	3	303	220	439
	Canned vegetables	27	10	10	68
	Nuts and seeds	1	200	200	200
Potatoes and potato products	Potato chips	349	626	10	4,215
	Reformed potato snack products	22	818	50	1,680
	French fries	723	299	10	3,428
	Fried potato products and roast potatoes	35	320	5	1,428
	Miscellaneous potatoes	1	66	66	66
Infant foods	Rusks	215	143	10	1,060
Snack products	Maize-based snacks	42	201	40	820
	Pretzels	10	165	60	273
Sugar syrup	Sugar-based syrups	3	156	15	438

The extent to which ethnic groups are taken into account may depend on the resources available and the diversity of the population and its diet. It is also important to include in a TDS provision to differentiate age and sex groups to be able to give a full picture of the exposure of different categories of consumers to the substances of concern.

TDS data differ from other chemical surveillance programs because they focus on chemicals in the diet and not on individual foods. Additionally, the foods are processed as for consumption in the home, thus they take into account the impact of cooking on the decomposition of less stable chemicals and the formation of new ones, e.g. acrylamide. As such, it is background levels of chemicals that are sought, not regulatory compliance.

This type of study is recommended by the WHO as an important activity for its member nations to undertake, as it provides reliable estimates of dietary intakes of contaminants.

Methods of Calculating Exposure to Acrylamide

The FAO/WHO expert consultation in June 2002 reviewed and evaluated available data on acrylamide, and provided interim advice to governments, industry and consumers [10]. Several preliminary exposure estimates were consolidated allowing the consultation to estimate that long-term acrylamide exposures would be in the range of 0.3–0.8 μg/kg bw/day [5]. The consultation stressed that the data available were sparse and that further work should be undertaken to produce more robust exposure estimates taking into account other dietary sources of acrylamide. JECFA, in their assessment in 2005 [11], estimated the average acrylamide exposure for the general population (1 μg/kg bw per day) and the exposure for consumers with high dietary exposure (4 μg/kg bw per day). JECFA, in their re-evaluation of acrylamide in 2010, concluded that new data on the levels of acrylamide in food did not significantly change the 2005 exposure estimates either for the general population or for consumers with high dietary exposure. The MOE values, too, were similar and thus the extensive new data from a variety of sources and studies supported their previous 2005 evaluation. Exposure assessments carried out in the UK estimated the mean adult UK consumer dietary exposure at 0.61 μg/kg bw/day and the high level adult consumer exposure at 1.29 μg/kg bw/day; both being well within the reported JECFA ranges [12].

The UK Total Diet Study

The UK Food Standards Agency, following the discovery of acrylamide in foods in 2002 and the health concerns raised by the toxicity of acrylamide, conducted a TDS in 2003 to estimate dietary exposure of the general UK population to acrylamide in food [13]. A TDS representing the average UK diet has been carried out on an annual basis since the 1960s to estimate dietary exposure and possible trends in exposure to a wide range of contaminants in food. The results of the UK TDS on acrylamide were considered as part of the wider international body of evidence that contributed to the 2005 JECFA safety evaluation of acrylamide in food.

Methodology

Details concerning the design and conduct of the UK TDS is described in an earlier chapter (see Chap. 40 – Total Diet Studies—United Kingdom's Experience) and therefore will not be repeated here. Foods relevant for the exposure assessment of acrylamide include the miscellaneous cereals group with products such as biscuits and breakfast cereals. The majority of carcase meats were baked. Meat products which were all prepared as for consumption included sausages and pies. Poultry was mostly baked or grilled. Sugars and preserves included chocolate and confectionery and potatoes contained a range of cooked fresh and processed potatoes.

Homogeneity

The entire portion of each 2003 TDS sample was homogenized and divided into four parts for analysis. To ensure sufficient homogeneity, samples were tested by measuring the content of water-soluble metals. The method of homogenization varied according to sample type: oils and fats were stirred; milk and beverages were shaken; cereals and sugars were ground using a coffee grinder; and the remaining groups were homogenized in a blender. Each homogenized sample was then tested for sodium, magnesium, potassium, calcium and manganese using acid digestion followed by inductively coupled plasma mass spectrometry as a marker for homogeneity.

Acrylamide Analysis

In order to minimize degradation of acrylamide, samples were kept at room temperature and in the light for the minimum amount of time during preparation and analysis. Prepared samples were returned to storage in the dark at −20 °C. The TDS samples were analyzed for acrylamide in duplicate using a United Kingdom Accreditation Service accredited gas chromatography–mass spectrometry (GC-MS) method, by the Central Science Laboratory (now the Food and Environment Research Agency). A third sub-sample was spiked with a known amount of acrylamide, to determine the recovery of acrylamide by the method of determination. The full method is given in the laboratory report [14].

A portion of the food sample was extracted with hot water and then brominated to form 2,3-dibromopropionamide. The brominated derivative was extracted using ethyl acetate and the organic layer concentrated before analysis by GC-MS [13]. C_{13}-acrylamide was used as the internal standard. For analysis of the TDS samples, two modifications were made from the standard operating procedure [14]. In addition, calibration standards were added to cover the range 1, 5, 10, 20, 30, 40 and 50 μg/kg.

Four of the food groups analyzed i.e. bread, eggs, sugars and preserves and beverages, required additional clean-up by solid phase extraction before bromination, in order to remove extraneous compounds which are known to reduce sensitivity in these matrix types. Details of the analytical methods can be obtained from the laboratory report [14].

Quality Control

The quality control criteria used were as follows: Results of duplicate analysis were accepted if they had a relative standard deviation of less than or equal to 20 %. Acrylamide data were accepted only if the recovery of spiked samples were in the range 60–140 % with at least 75 % of the spiked samples within the range 80–120 %. The laboratory participated in Food Analysis Proficiency Assessment Schemes (FAPAS), namely, Series 30 (acrylamide) Round 1 (crisp bread) and Round 3 (breakfast cereal), as part of its quality control check. The FAPAS acceptance criterion was that the results should be within the range that would give a z-score of ±2. The measurement of uncertainty was determined from the analysis of the FAPAS test materials on a batch-by-batch basis. The limit of quantification (LOQ) was established for each food group and varied from 1 to 5 μg/kg, depending on the food group. Samples containing levels of acrylamide that could not be quantified were reported as having less than the LOQ.

Results

All results were corrected for recovery. Levels ranged from <1 to 112 μg/kg with the lowest detectable levels occurring in poultry (6 μg/kg) and the highest levels occurring in the 2001 potato group (112 μg/kg). Acrylamide was not quantified in 13 of the 20 food groups, namely, offal, fish, oil and fats, eggs, green vegetables, other vegetables, canned vegetables, fresh fruit, fruit products, beverages, milk, dairy products and nuts. These results are generally consistent with occurrence and formation of acrylamide and are consistent with results of other international research such as those recorded in the European Commission's Joint Research Centre-Institute of Reference Materials and Measurements database [15].

Table 50.2 gives the mean concentrations of acrylamide quantified in each of the 20 food group samples of the 2003 UK TDS and also includes the 2001 TDS potato group

Table 50.2 Mean concentrations of acrylamide in the UK total diet study 2003 samples, including 2001 potato group sample [13]

Food group	Acrylamide[a] (μg/kg)	Acrylamide levels used in exposure assessment (μg/kg) and rationale	
1. Bread	12	12	
2. Miscellaneous cereals	57	57	
3. Carcass meat	10	10	
4. Offal	<3	3	Acrylamide quantified in poultry and carcass meat therefore potential to form in offal [1]
5. Meat products	13	13	
6. Poultry	6	6	
7. Fish	<5	5	Food group may contain breaded and battered products which may contain acrylamide [18]
8. Oils and fats	<3	0	
9. Eggs	<1	0	
10. Sugars and preserves	23	23	
11. Green vegetables	<2	0	
12. Potato 2003 Group	53[b]		
12. Potato 2001 Group	112[c]	112	
13. Other vegetables	<5	5	Food group may contain roasted vegetables, which may contain acrylamide
14. Canned vegetables	<5	0	
15. Fresh fruit	<1	0	
16. Fruit products	<1	1	Food group may contain cooked components, which may contain acrylamide [16, 18]
17. Beverages	<1	1	Food group may contain coffee which may contain acrylamide [16]
18. Milk	<1	0	
19. Dairy products	<1	0	
20. Nuts	<3	3	Food group may contain peanut butter and roasted nuts, which may contain acrylamide [16]

[a] Mean of duplicate analyses and corrected for the analytical recovery. Analytical recoveries lay in the range 60–140 %

[b] Measurement uncertainty = ±28 %

[c] Measurement uncertainty = ±32 %

sample. The measurement uncertainties for these analyses are ±28 % and ±32 %, respectively. The 2001 TDS potato sample was used to estimate dietary exposure to acrylamide in this product group; this gave a more conservative, i.e. worse case, estimate of exposure, given it was made up of products more likely to contain acrylamide.

Discussion

The level of acrylamide quantified in the 2003 TDS potato group sample was lower than expected and lower than in the 2001 TDS potato group sample. No crisps or fried potato products were included in the 2003 potato products category and only a third of the potato and potato products included were baked, grilled or microwaved. The rest of the sample was boiled, steamed or prepared from instant mash potato. Since 2002, various initiatives to reduce acrylamide levels in food have been explored by the food industry and other researchers. Any impact of such initiatives will not be reflected in the acrylamide levels found in the 2001 TDS potato group sample, which did include crisps as well as baked and grilled potatoes/potato products.

Levels of acrylamide reported in toasted bread are generally higher than in fresh bread [16]. However toast was not included in the 2003 TDS. High levels of acrylamide were quantified in the sugar and preserves group; with the likely source of this acrylamide being chocolate, which was shown to contain significant levels of acrylamide. The current understanding of the formation of acrylamide suggests that acrylamide is mainly formed in starch-rich foods; however, acrylamide was also found in the carcass meat and poultry groups albeit at very low levels. Of the food groups tested, none was found to contain significant levels of acrylamide.

The dietary exposure estimates were derived from the TDS and are given in Table 50.3. Data show that cereal-based products and potatoes are the main sources of acrylamide in the UK diet. UK consumers' estimated exposure to dietary acrylamide, based on the TDS results, was similar to exposure estimates in other countries.

However, it should be noted that these dietary exposures are not directly comparable because of the different methods used (for example: different age groups; whole populations or consumers of particular products; using limited food groups rather than the whole diet). There have been many other estimates of acrylamide intake, some of which are detailed in Table 50.4.

When comparing dietary exposure estimates it is important to acknowledge their limitations. One of the intriguing characteristics of acrylamide contamination of foods is the variability in acrylamide levels; it is not unusual to find a large variation in the levels of acrylamide found in samples of the same products, and even between samples originating from the same batch. Table 50.5 gives the ranges for a variety of products sampled in the UK during November 2010 and April 2011 [17], while Fig. 50.1 shows the variability in acrylamide levels in more detail for different samples of French fries [18]. This variability is important when considering dietary exposure to acrylamide.

Table 50.3 Exposure[a] to acrylamide from food groups of the UK total diet study (all food groups combined) [13]

Dietary survey	Age range	Exposure estimate[b] (μg/kg bw/day)[c]	
		Average consumer[d]	High level consumer[d]
Adults		0.3	0.6
Female	19–64	0.3	0.6
Male		0.4	0.6
Young people	15–18	0.5	0.9
	11–14	0.6	1.1
	7–10	0.8	1.4
	4–6	1.0	1.6
Toddlers		1.0	1.8
Elderly (free living)		0.3	0.6
Elderly (institutional)		0.4	0.7
Vegetarians		0.3	0.7

[a]To calculate dietary exposure to acrylamide, the occurrence data from the analysis of the TDS samples was used together with consumption data from the following dietary surveys; the National Diet and Nutrition Survey of British adults, young people aged 4–18 years, toddlers aged 1.5–4.5 years and people aged 65 years and over and the British Marketing Research Bureau's dietary survey of vegetarians

[b]To estimate these dietary exposures the following acrylamide levels were used: For food groups where acrylamide was quantified, the quantified level was used. For food groups where acrylamide was not quantified but where it is known to be present in components of that group or has the potential to form in that group, an acrylamide level of the LOQ was presumed. Food groups where acrylamide was not quantified and where the group does not have the potential for acrylamide formation; it has been assumed that acrylamide is present at 0 μg/kg

[c]Body weight consumption is calculated using each dietary survey participant's body weight

[d]Consumer estimates are based only on those people who ate the food in question. The term "average consumer" refers to UK consumers who eat an average amount of food (for the UK). "High level consumers", also referred to as 97.5th percentile consumers, are UK consumers who eat in excess of the average amount of food

Conclusions

The estimated dietary exposure to acrylamide was 0.34 g/kg bw/day for the average adult consumer and 0.62 g/kg bw/day for high level (97.5th percentile) adult consumers. The highest estimate of exposure on a body weight basis was 1.84 μg/kg bw/day at the 97.5th percentile for toddlers aged 1.5–4.5 years. These are within the range reported in the JECFA evaluation of 2005 and confirmed in 2010 by the same committee as still valid.

The FSA has issued advice to UK consumers that people should eat a healthy balanced diet, including plenty of fruits and vegetables, bread, rice, potatoes, pasta and other starchy foods, some meat, fish, eggs, beans, milk and dairy foods, and just a small amount of foods and drinks high in salt, fat and/or sugar (including chips and crisps). The advice remains unchanged as a result of this TDS investigation for acrylamide and following subsequent surveillance results, up to and including the results from the 2012 published data [17].

Table 50.4 Summary of some exposure estimates for acrylamide

		Estimated dietary intake (μg/kg bw/day)	
Organization, country	Population/sex (age)	Mean	95th percentile; [a]90th percentile; [b]97.5th percentile
BfR, Germany	All, 15–18	1.1	3.2
SNT, Norway	Males	0.49	1.04[a]
	Females	0.46	0.86[a]
	Males (13)	0.52	1.35[a]
	Females (13)	0.49	1.2[a]
AFSSA, France	All	0.5	1.1
	All	1.4	2.9
SNFA, Sweden	All (18–74)	0.45	1.03
NFCS, Netherlands	All (1–97)	0.48	0.60
	All (1–6)	1.04	1.1
	All (7–18)	0.71	0.9
FSA, UK	Males (19–64)	0.4	0.6[b]
	Females (19–64)	0.3	0.6[b]
	All (1.5–4.5)	1.0	1.8[b]
FDA, USA	All (2+)	0.44	0.95[a]
	All (2–5)	1.06	2.33[a]

The UK estimate is for people who consumed particular foods; other estimates are mostly for the entire population

BfR Federal Institute for Risk Assessment, *SNT* Norwegian Food Control Authority, *AFSSA* French Food Safety Agency, *SNFA* Swedish National Food Authority, *NFCS* Netherlands Food and Consumer Products Safety Authority, *FSA* Food Standards Agency, *FDA* Food and Drug Administration

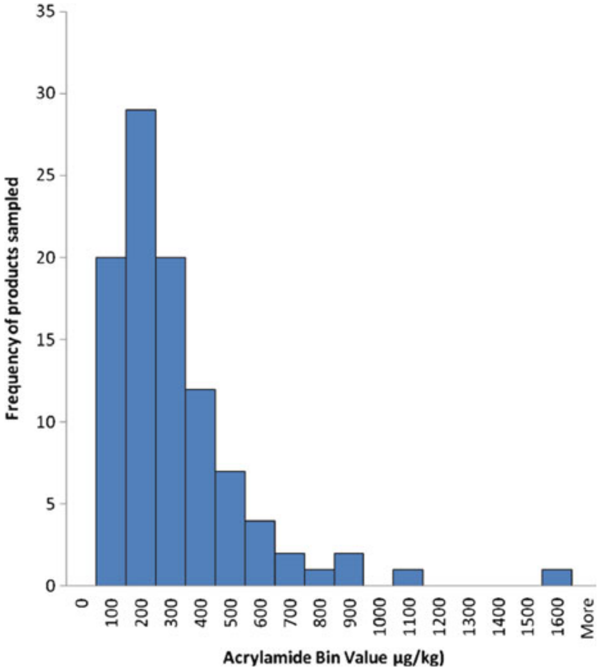

Fig. 50.1 Histogram of acrylamide levels found in French fries sampled as part of a 2009 Food Standards Agency Survey (Data based on 99 samples of French Fries collected from outlets as ready-to-eat between March 2008 and March 2009 [18])

Table 50.5 Summary of acrylamide concentrations in the UK (mean and range) [17]

Product category	n	Mean (µg/kg)	Min (µg/kg)	Max (µg/kg)
Group 1 – French fries sold as ready to eat	**42**	**239**	**41**	**1,285**
Nov-'10 delivery	21	255	41	660
Mar-'11 delivery	21	223	53	1,285
Group 2 – Potato crisps	**20**	**835**	**220**	**2,061**
Nov-'10 delivery	10	738	220	1,859
Mar-'11 delivery	10	933	444	2,061
Group 3 – Pre-cooked French fries for home-cooking	**16**	**194**	**21**	**1,155**
Nov-'10 delivery	8	206	21	1,155
Mar-'11 delivery	8	183	39	491
Group 4 – Soft bread	**20**	**16**	**3**	**51**
White	8	15	7	37
Wholemeal	4	24	15	33
Others (e.g. *in-store bakery, rye, linseed, rolls, etc.)*	8	14	3	51
Group 5 – Breakfast cereals	**20**	**149**	**35**	**325**
Group 6 – Biscuits & crackers	**20**	**380**	**27**	**1,573**
Crackers	9	275	48	473
Crispbread	3	197	120	326
Wafers	1	154	154	154
Other (sweet)	7	625	27	1,573
Group 7 – Coffee	**20**	**501**	**49**	**1,009**
Roast coffee	8	212	172	243
Instant coffee	6	865	724	997
Coffee substitutes	6	521	49	1,009
Group 8 – Baby food other than processed cereal based	**20**	**13**	**3**	**27**
Group 9 – Processed cereal baby food	**20**	**65**	**3**	**598**
Biscuits and rusks for infants and young children	10	110	3	598
Other processed cereal-based foods for infants and young children	10	18	6	68
Group 10 – Others	**50**	**311**	**5**	**3,972**
Vegetable crisps	2	2,651	1,330	3,972
Canned black olives	1	884	884	884
Other potato products for home-cooking	6	579	44	1,604
Cocoa	2	442	176	707
Prefabricated crisps	2	364	285	443
Popcorn	2	328	205	451
Microwave French fries	2	327	327	328
Canned prunes	2	305	247	362
Novelty gingerbread	2	247	51	443
Cereal bars & granola	4	135	82	259
Tortilla/corn chips	2	103	79	127
Prefabricated potato products for home-cooking	4	68	18	108
Ethnic foods	6	64	25	120
Dried fruit	2	59	49	68
Cakes	5	33	12	86
Pastries	5	29	5	57
Chocolate	1	24	24	24

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