Determination of *N*7-glycidamide guanine adducts in human blood DNA following exposure to dietary acrylamide using LC-MS/MS.

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Supplementary Information

Synthesis

(±)-Glycidamide

To a solution of acrylonitrile (13.2 mL, 10.6 g, 0.2 moles) in H_2O (100 mL) was added hydrogen peroxide ((27.5% aq.) 22.3 mL, 0.2 moles H_2O_2) and this was stirred at room temperature for 5 mins. To this solution was added sodium hydroxide ((6 M aq.) 4 mL) at a rate of 1 mL h^{-1} using a syringe and infusion pump. After which time palladium ((10% on activated carbon) 0.2 g) was added and the resulting suspension was stirred at room temperature overnight. The palladium was removed by filtration (Celite) and cold acetone (100 mL, 0 °C) was added to the filtrate and the solution re-filtered. The filtrate was concentrated *in vacuo* to afford a light orange viscous liquid that crystallised on cooling to 4 °C to give a waxy, orange solid (7.35 g, 42%). δ_H(300 MHz; D_2O) 2.86 (1H, dd, *J* 5.5 and 2.6, C(3)$H_a\underline{H_b}$), 3.01 (1H, apparent t, *J* 5.1, C(3)$\underline{H_a}H_b$), 3.48 (1H, dd, *J* 4.6 and 2.6, C(3)H); δc(75 MHz; D_2O) 47.12 (C(3)), 48.70 (C(2)), 174.05 (C(1)).

***N*7-GA-Gua**

To a stirred solution of 2'-deoxyguanosine monohydrate (1.0 g, 3.52 mmoles) in H_2O (180 ml) at 37 ºC was added glycidamide (3.1 g, 35.2 mmoles) in H_2O (20 ml). The resulting solution was stirred at 37 ºC for 7 days. During which time a white precipitate formed. The suspension was filtered and washed with H_2O (3 x 10 ml). The white product was air dried and then dried in a vacuum desiccator (silica/ 3Å molecular sieves) to yield *N*7-GA-Gua (300 mg, 36%). Mp 340ºC dec.; δ_H(300 MHz; D-6 DMSO) 4.09 (1H, dd, *J* 13.3 and 9.0, N(7)C$\underline{H_a}H_b$), 4.18 (1H, ddd, *J* 9.0, 6.1 and 3.1, N(7)$CH_2C\underline{H}$(OH)), 4.49 (1H, dd, *J* 13.3 and 3.1, N(7)C$H_a\underline{H_b}$), 5.85 (1H, d, *J* 6.1, N(7)$CH_2CH(O\underline{H})$), 6.05 (2H, s, $N^2\underline{H_2}$), 7.22 (1H, s, CON$\underline{H_a}H_b$), 7.24 (1H, CON$H_a\underline{H_b}$), 7.72 (1H, s, C(8)$\underline{H}$), 10.76 (1H, br s, N(1)$\underline{H}$); δ_C(100 MHz; D-6 DMSO) 50.1 (N(7)-$\underline{C}H_2$-CH(OH)-), 70.8 (N(7)-CH_2-$\underline{C}$H(OH)), 108.7 ($\underline{C}$(5)), 144.5 ($\underline{C}$(8)H), 153.1 ($\underline{C}$(6)), 155.3 ($\underline{C}$(2)), 160.4 ($\underline{C}$(4)), 174.0 (N(7)-CH_2-CH(OH)-$\underline{C}ONH_2$).

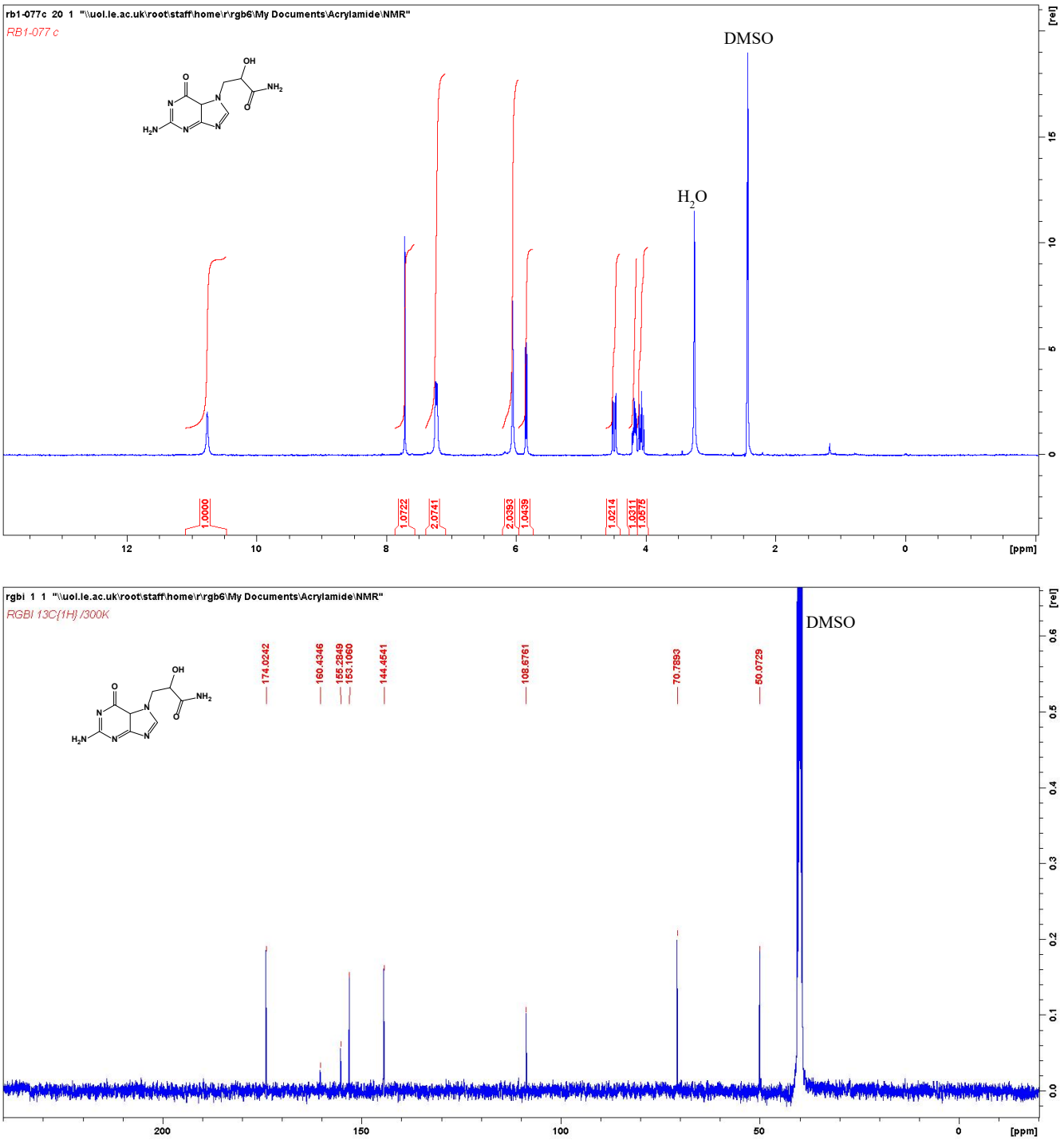
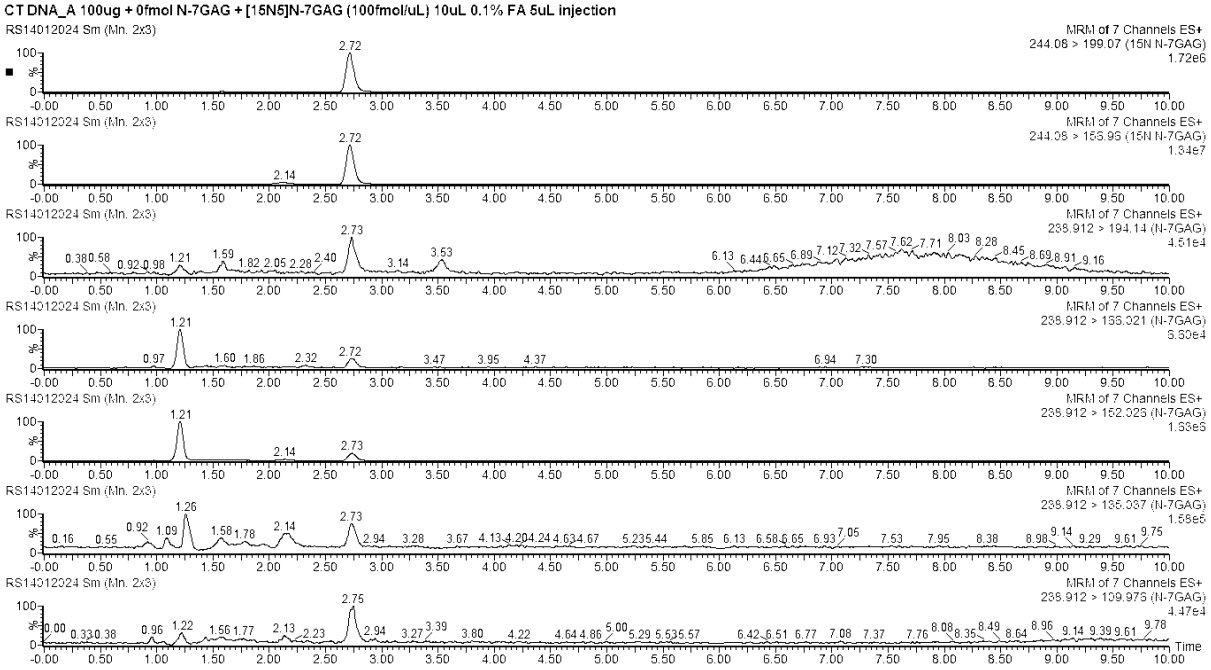
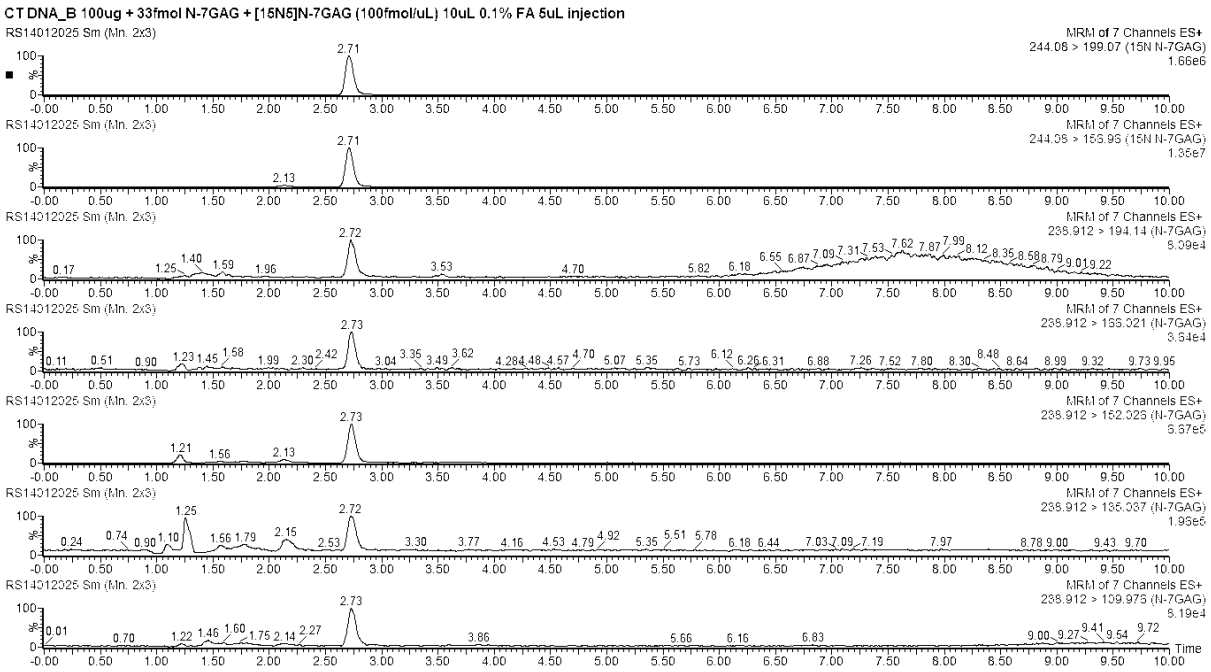


Figure S1. ^{1}HNMR and ^{13}CNMR of *N*7-GA-Gua

Analyte recovery from ctDNA

Briefly, calf thymus (ct) DNA (Sigma-Aldrich, UK. D4764) was spiked with *N*7-GA-Gua and then hydrolysed and isolated as described in the methods section. The sample was then spiked with [$^{15}N_5$]*N*7-GA-Gua immediately prior to LC-MS/MS analysis as described in the methods section.





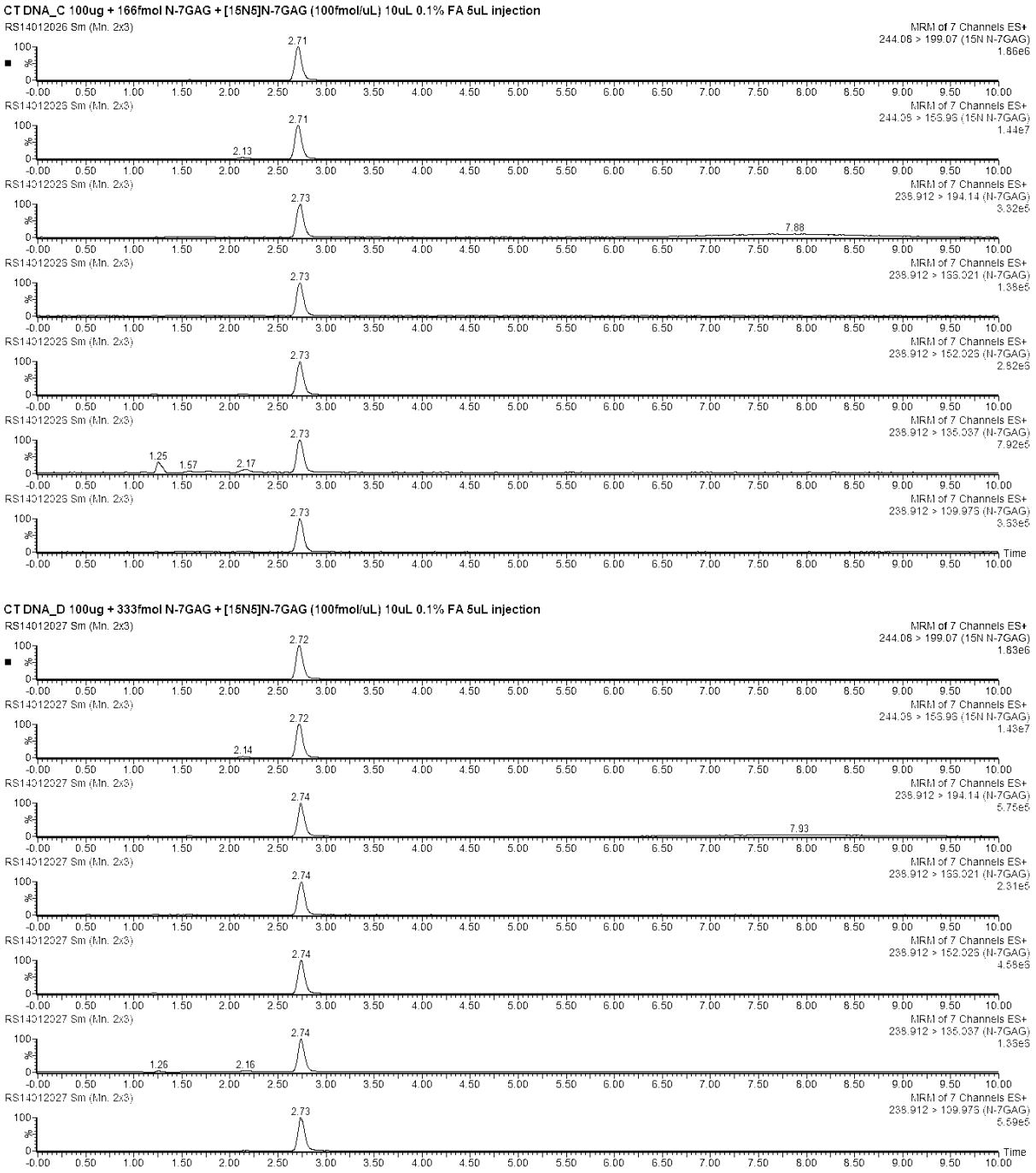


Figure S2. LC-MS/MS chromatograms of ctDNA spiked with *N*7-GA-Gua prior to thermal hydrolysis and spiked with [$^{15}N_5$]*N*7-GA-Gua immediately prior to analysis.
NB Top 2 chromatograms show transitions for [$^{15}N_5$]*N*7-GA-Gua; the bottom 5 show transitions for *N*7-GA-Gua.

Table S1. Recovery of *N7*-GA-Gua spiked into ctDNA as the matrix

Sample	*N7*-GA-Gua spike (fmol)	% Recovery
CTDNA_A	0	-
CTDNA_B	33	113.1
CTDNA_C	166	106.4
CTDNA_D	333	89.1

Table S2. *N7*-GA-Gua adduct levels detected in control (non-spiked) and spiked ctDNA

Sample	*N7*-GA-Gua spike (fmol)	Adducts per 10^8 nucleotides
CTDNA_A	0	6.9
CTDNA_B	33	15.0
CTDNA_C	166	58.0
CTDNA_D	333	95.0

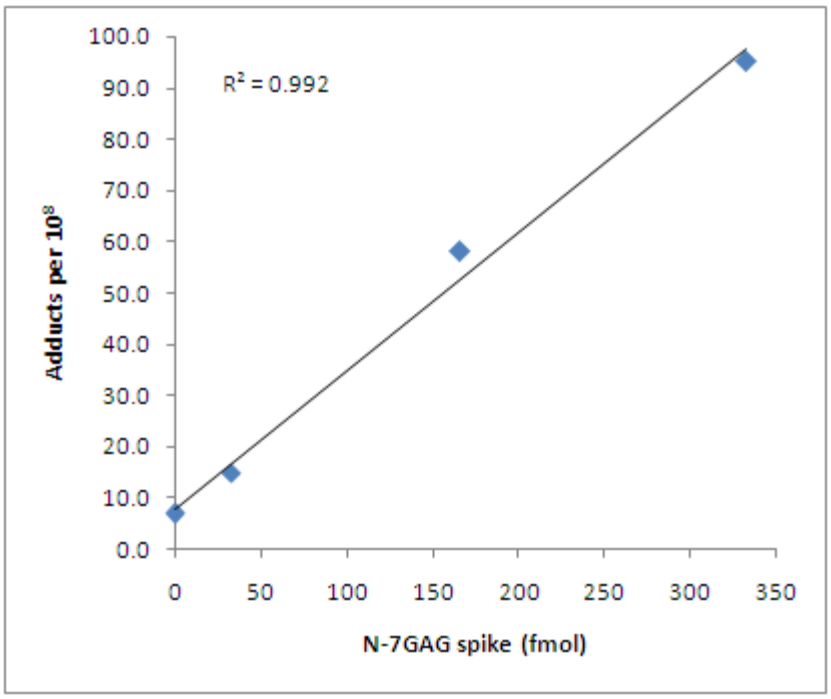


Figure S3. Calibration for *N*7-GA-Gua using ctDNA as the matrix

Table S3. FSA food categories and AA levels reproduced from Hamlet *et al* 2019
https://www.food.gov.uk/sites/default/files/media/document/acrylamide-and-furans-survey-summary-report-2014-18_0.pdf

Food category / sub category[a]		Description	Sampling date[b]	n	Acrylamide (µg/kg)					n>IV
					mean	min	max	SE	IV[c]	
1		**French fries sold as ready to eat**	**2014**	**40**	**157**	**12**	**587**	**21**	**-**	**-**
	1.1	French fries from fresh potatoes	Mar-14[b]	20	186	29	587	37	600	0
			Nov-14[b]	20	129	12	326	19	600	0
2		**Potato crisps and potato-based crackers**	**2014**	**27**	**779**	**88**	**2542**	**122**	**-**	**-**
	2.1	Potato crisp from fresh potatoes	Mar-14[b]	10	861	329	1873	157	1000	4
			Nov-14[b]	10	551	126	1010	83	1000	1
			Dec-14[b]	1	673	-	-	-	1000	0
	2.4	Potato-based crackers	2014	6	1039	88	2542	467	1000	2
3		**Pre-cooked French fries, potato products for home cooking**	**2014**	**29**	**256**	**6**	**1156**	**54**	**-**	**-**
	3.1	Fries baked in the oven (oven fries)	Mar-14[b]	6	405	103	1156	157	-	-
			Nov-14[b]	6	87	13	175	26	-	-
	3.2	Deep fried fries	Mar-14[b]	2	62	19	104	-	-	-
			Nov-14[b]	2	11	6	15	-	-	-
	3.3	Unspecified pre-cooked French fries, potato products for home cooking	Mar-14[b]	6	477	92	1116	143	-	-
			Nov-14[b]	7	211	23	522	62	-	-
4		**Soft bread**	**2014**	**20**	**14**	**4**	**37**	**2**	**-**	**-**
	4.1	Wheat based bread	2014	20	14	4	37	2	80	0
	4.2	Soft bread other than wheat based bread	2014	0	-	-	-	-	150	0
5		**Breakfast cereals (excluding porridge)**	**2014**	**22**	**174**	**41**	**541**	**26**	**-**	**-**
	5.1	Maize, oat, spelt, barley and rice based products	2014	4	77	58	94	7	200	0
	5.3	Bran products and whole grain cereals, gun puffed grain	2014	18	196	41	541	29	400	2

6		**Biscuits, crackers, crisp bread and similar (excluding pastry and cake)**	**2014**	**24**	**347**	**27**	**1324**	**67**	**-**	**-**
	6.1	Crackers with the exception of potato based crackers	2014	6	233	90	544	73	500	1
	6.2	Crisp bread	2014	3	210	107	327	64	450	0
	6.3	Biscuits and wafers	2014	8	301	30	1056	114	500	1
	6.4	Gingerbread	2014	4	803	360	1324	199	1000	1
	6.5	Products similar to the other products in this category	2014	3	227	27	407	110	500	0
7		**Coffee and coffee substitutes**	**2014**	**20**	**455**	**7**	**940**	**70**	**-**	**-**
	7.1	Roasted coffee (dry)	2014	8	222	157	271	14	450	0
	7.2	Instant coffee (dry)	2014	6	820	629	940	47	900	2
	7.3	Substitute coffee (dry) mainly based on cereals	2014	4	560	310	896	136	2000	0
	7.4	Other coffee substitutes (dry)	2014	2	81	7	155	-	4000	0
8		**Baby foods , other than processed cereal based foods**	**2014**	**20**	**12**	**3**	**27**	**1**	**-**	**-**
	8.1	Baby foods not containing prunes	2014	20	12	3	27	1	50	0
	8.2	Baby foods, containing prunes	2014	0	-	-	-	-	-	-
9		**Processed cereal-based foods for infants and young children**	**2014**	**20**	**60**	**4**	**577**	**29**	**-**	**-**
	9.1	Biscuits and rusks for infants and young children	2014	8	65	4	165	20	200	0
	9.2	Other processed cereal-based foods for infants and young children	2014	12	56	4	577	47	50	1
10		**Other products, based on cereals, potatoes, cocoa and coffee**	**2014**	**17**	**135**	**6**	**425**	**29**	**-**	**-**
	10.2	Cake and pastry	2014	6	114	6	332	48	-	-
	10.3	Savoury snacks	2014	2	72	18	126		-	-
	10.4	Other products, based on cereals	2014	5	153	7	233	41	-	-

Food category / sub category[a]	Description	Sampling date[b]	n	Acrylamide (µg/kg)					
				mean	min	max	SE	IV[c]	n>IV
10.5	Other products, based on potatoes	2014	0	-	-	-	-	-	-
10.6	Other products, based on cocoa	2014	4	175	22	425	88	-	-
11	**Other products, not based on cereals, potatoes, cocoa and coffee**	**2014**	**12**	**436**	**12**	**1324**	**125**	**-**	**-**
	Vegetable crisps	2014	4	803	360	1324	199	**-**	**-**
	Black olives, canned	2014	2	650	269	1031	-	-	-
	Prunes, canned	2014	2	189	82	295	-	**-**	**-**
	Liquorice candies	2014	2	157	139	175	-	**-**	**-**
	Dates / prunes	2014	2	14	12	16	-	**-**	**-**

[a] according to EFSA[14,15]; [b] products prepared from seasonal potatoes; [c] EC Indicative Values[16]

[14] European Food Safety Authority, 2014; Specific requirements for chemical contaminants' data submission. EFSA supporting publication 2014:EN-604. 25 pp.

[15] European Food Safety Authority, 2015; Specific requirements for chemical contaminants' data submission. EFSA supporting publication 2015: 2015:EN-833.

[16] Commission Recommendation of 8 November 2013 on investigations into the levels of acrylamide in food (2013/647/EU), oj L 301, 15-17, 12.11.13