Monitoring of Chlorate in Fruit and Vegetables

Report to Food Standards Agency

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Executive Summary

1. Chlorate is a naturally occurring and manmade contaminant that can affect the thyroid gland and haematological system functions. An initial risk assessment by EFSA recommended that further monitoring and more information on the impact of food processing on chlorate residues should be collected.
2. Although chlorate was used as a herbicide, it has not been authorised for use in Europe since 2008, and was banned in all EU countries in 2010. It is formed as a by-product after disinfection of drinking water, irrigation and food processing and may be present on surfaces coming into contact with food. Fertilisers, some man-made industrial products and natural sources (rainwater and certain mineral deposits) are also considered potential sources of chlorate. The EU MRL of 0.01 mg/kg is the default maximum level for residues of unauthorised plant protection products in food.
3. This work was commissioned by the Food Standards Agency (FSA) to measure chlorate levels in fruit and vegetables. Samples of liquid milk were also analysed.
4. This investigation was carried out over a nine month period.
5. Chlorate was detected at or above the EU MRL of 0.01 mg/kg in 29 of the 89 food samples tested. (A further 3 samples had detectable levels of chlorate <0.01 mg/kg).
6. The incidence of chlorate was highest in liquid milk. It was also frequently found in leaf, legume and root vegetables, and herbs.
7. It was not possible to establish correlation between the chlorate found and the source of the contamination.

Key messages

Chlorate was found in the UK food supply. 29 of the 89 samples tested were in excess of the default MRL currently set at 0.01 mg/kg. These findings were similar to those reported by the EURL.

1. Introduction

Chlorate is formed as a by-product when using products containing chlorine, chlorine dioxide or hypochlorite for disinfection. These products are used for the disinfection of drinking water, water used during food production and surfaces used during food processing. Chlorination of animal-derived food is not allowed in the EU, while washing of plant derived food with chlorine disinfected water is permitted under national regulations. The World Health Organisation (WHO) has established a guideline level for chlorate in drinking water of 0.7 mg/L, while no such level has been set in the European Union (EU). In many fruit and vegetable commodities, chlorate levels exceeding the default MRL of 0.01 mg/kg have been reported. Based on the available information, it is believed that chlorate contamination in food results mainly from the use of chlorinated water for food processing (e.g. washing) and from the disinfection of surfaces and food processing equipment coming into contact with food [1].

In 2012-13, chlorate was found in >20% of plant-based food samples tested in Germany [2]. Further surveillance in Germany in 2014 found that approximately 10% of plant-based foods contained chlorate [3].

The work described in this report was undertaken to investigate chlorate levels in UK food commodities (fruit, vegetables and milk), as requested by EFSA. A range of samples (89) were collected and supplied to Fera by Hallmark Ltd in accordance with a sampling plan provided by the FSA.

The analysis involved extraction of chlorate using a method developed by the EU reference laboratory [4] and LC-MS/MS determination using an in-house ion chromatography technique.

2. Methodology

2.1 The analytical procedure was adapted from a method published by the European Union Reference Laboratory [4]. This method involved extraction of highly polar compounds from samples using acidified methanol / water. Any chlorate present in the extracts was determined by an in-house ion chromatography LC-MS/MS method developed at Fera.

2.2 The determination step involved the use of matrix matched, multi-level, bracketed calibration standard solutions, using stable isotope labelled chlorate as an internal standard.

2.3 Chlorate analysis was carried out on a Metrohm Professional IC coupled to an Agilent 6460 mass spectrometer. An AG19 (2 x 50mm) guard column and AS19 (2 x 250mm) analytical column was used for the separation, with an injection volume of 20 µL (Metrohm-Agilent) full loop fill injections. The column flow rate was 0.3 mL/min with a potassium hydroxide (aqueous) gradient as detailed below.

0 mins	20 mM KOH (aq)
7 mins	20 mM KOH (aq)
8 mins	60 mM KOH (aq)
20 mins	60 mM KOH (aq)
20.1 mins	60 mM KOH (aq)
25 mins	20 mM KOH (aq)

Transitions collected

Chlorate internal standard ($^{18}O_3$): 89>71

Chlorate: 85>69 (quantification), 83>67 (confirmation), 83>51 (confirmation)

3. Results

3.1 Method Validation

The method was validated in a single laboratory to meet the requirements of DG SANCO guidelines [5]. This involved analysis of replicate samples, spiked with known levels of chlorate. This was done at the start of the study to establish within-lab method reproducibility. In addition, replicate samples, spiked at known chlorate levels, were analysed in each analytical run (batch) to establish method repeatability. The method was successfully validated for food samples (see Tables 1 & 2). Table 3 shows Fera proficiency results for an EURL PT for the analysis of chlorate (and perchlorate) in milk.

3.2 Sampling

- 89 fruit, vegetable, herbs and milk samples were collected during 2014 - 15.
- 22 of these samples were collected from farms. For these samples, irrigation practices, fertilizers used and growing conditions were recorded.

3.3 Analytical Results

- 12 fruit samples were tested. One apple sample was found to contain chlorate at 0.018 mg/kg. Chlorate was also detected in a strawberry sample below 0.01 mg/kg (<LoQ) (Table 4a).
- 8 samples of fruiting vegetables were tested, of which one tomato sample contained chlorate at 0.050 mg/kg. Chlorate was also found in a paprika pepper sample below 0.01 mg/kg (<LoQ) (Table 4b).
- 9 samples of fresh herbs were tested, of which 5 were found to contain chlorate at levels of between 0.011 and 0.33 mg/kg (Table 4c).
- 11 leafy vegetable and salad samples were tested, of which four were found to contain chlorate between 0.017-0.18 mg/kg. In addition, chlorate was detected in one sample below 0.01 mg/kg (<LoQ) (Table 4d).
- 14 root vegetable samples were tested of which chlorate was found in five at levels of between 0.026 and 0.11 mg/kg (Table 4e).
- 27 samples of miscellaneous vegetables (brassica and legume) were tested, of which six were found to contain chlorate at levels of between 0.017 and 1.89 mg/kg. Five of these positive samples were legume vegetables, including a sample of beansprouts at 1.89 mg/kg (Table 4f).

- 8 liquid milk samples were tested, of which seven contained chlorate at levels of between 0.017 and 0.058 mg/kg (Table 4g).

In total, 89 food samples were tested, 29 of which were contaminated with chlorate. The highest incidences were associated with fresh milk (7/8), herbs (5/9) and legume vegetables (5/10). This is broadly similar to residues found during a study in Germany by CVUA [2, 3], although the number of samples tested in most of the commodity groups was relatively low.

The incidence of detectable chlorate by country of origin was as follows:

UK; 23 of the 59 samples collected.

Spain; 3 of the 13 samples collected.

Italy; 1 of 2 the samples collected.

USA; 1 of 2 the samples collected.

Unknown; 1 of 3 the samples collected.

No clear relationship was found between positive findings in the food samples and irrigation methods, fertilizer use, the growing environment or country of origin. There are a number of factors that make it difficult to determine relationship between residues and treatment/origin.

- Limited number of samples with measurable levels of chlorate
- Possible chlorate presence at levels below the LoD
- Likely variation in the degree of possible uptake of chlorate between different crop types
- Level of chlorate in process and wash water
- Contact with surfaces cleaned using chlorine-based disinfectants and sanitisers

4. Conclusions and Recommendations

A method was successfully adopted and validated to meet the requirements of DG SANCO guidelines.

89 food samples were tested of which 29 samples were found to be contaminated with chlorate. The highest frequency was in milk (7 out of 8), herbs (5 out of 9,) and legume vegetables (5 out of 10). 23 out of 59 samples (39%) from the UK contained chlorate. The high incidence of chlorate in milk samples may be due to the use of chlorine-based disinfectants to clean milking and processing equipment [1]. It may also arise from the use of sodium chlorite as a post-milking teat dip treatment for mastitis in dairy cattle [6], which degrades to sodium chlorate and other chlorinated compounds.

It was not possible to establish a clear relationship between positive findings in the food samples and irrigation methods, fertilizers used or the growing environment. This ambiguity is partly due to the limited number of samples with positive findings.

If establishing the possible sources of chlorate is a priority, a more targeted sampling approach is recommended.

a. More samples should be collected directly from farms and premises where food is washed and packaged. This will allow collection of more information about irrigation methods, fertilizers used, the growing environment and method used for disinfection.
b. More samples of herbs, leaf and salad vegetables should be collected. This is likely to increase the number of positive findings and thus provide a bigger data set of positive samples to help identify possible sources of chlorate.
c. More samples of produce originating from southern Europe should be tested. This should increase the percentage of positive findings, and thus provide a larger data set of positive samples to help identify possible sources of chlorate.

References

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Scientific Opinion on Risks for public health related to the presence of chlorate in food, EFSA Panel on Contaminants in the Food Chain (CONTAM)

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[4] *http://www.eurl-pesticides.eu/library/docs/srm/meth_QuPPe.pdf*
Quick Method for the Analysis of Residues of numerous Highly Polar Pesticides in Foods of Plant Origin involving Simultaneous Extraction with Methanol and LC-MS/MS Determination (QuPPe-Method) - Version 7 (updated Jan 2013); M. Anastassiades; D. I. Kolberg; D. Mack; Chr. Wildgrube; I. Sigalova; D. Roux; D. Fügel

[5] *http://www.eurl-pesticides.eu/library/docs/allcrl/AqcGuidance_Sanco_2013_12571.pdf*
Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. SANCO/12571/2013

[6] *http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015951.pdf*
Summary Report on Maximum Residue Level Assessment for Sodium Chlorite, The European Agency for the Evaluation of Medicinal products, Committee for Veterinary Medicinal Products; 1 January 1996

Tables:

Table 1: Repeatability validation data for chlorate

Job: 46907	worksheet: 21358r	Apple
spike level	mean recovery	CV
(mg/kg)	n=5	
0.01	104%	2%
0.02	101%	3%
0.05	99%	5%

Job: 44483	worksheet: 21355	lettuce
spike level	mean recovery	CV
(mg/kg)	n=5	
0.01	107%	8%
0.02	103%	16%
0.05	92%	9%

Job: 47147	worksheet: 21362	MILK
spike level	mean recovery	CV
(mg/kg)	n=5	
0.01	99%	12%
0.02	97%	7%

Table 2: Routine QC data (% Recovery) from individual batches

			Spike level	
Batch	**Job**	**Matrix**	**0.01 mg/kg**	**0.02 mg/kg**
PRA14-05080	46841	lettuce	82	94
PRA14-05080	44652	lettuce	85	93
PRA14-05089	47079	lettuce	120	99
PRA14-05090	44651	lettuce	107	97
PRA14-05093	46837	lettuce	122	113
PRA14-05344	46906	apple	94	95
PRA14-05993	47149	potato	129	130
PRA14-05995	47148	milk	88	89
PRA14-06351	47288	lettuce	104	99
average			103	101
%CV			16.8	12.6

Table 3: Fera Proficiency data for chlorate and perchlorate

compound	PT round	Lab number	Sample	Result reported (mg/kg)	z-score
chlorate	EUPT SRM 09	53	Milk	0.152	-0.7
perchlorate	EUPT SRM 09	53	Milk	0.156	-0.6

Table 4	Measured concentrations in fruit, vegetables and milk				
~ indicates the measured value is between LOD (0.005 mg/kg) and LOQ (0.01 mg/kg)					
Table 4a	**Fruit samples**				
Fera LIMS Code	**Sample ID**	**Description**	**Chlorate (mg/kg)**	**Commodity Group**	**Country of Origin**
S14-052491	1418267	Grapefruit	<0.005	Citrus fruit	South Africa
S15-006755	1613211	Lemons	<0.005	Citrus fruit	Italy
S15-006756	1613210	Melons	<0.005	Citrus fruit	Honduras
S15-050626	1613197	Lemons	<0.005	Citrus fruit	Spain
S14-052478	1614538	Apple	**0.018**	Fruit	United Kingdom
S15-023700	1614971	Pineapples	<0.005	Fruit	Unknown
S15-050627	1613195	Pineapples	<0.005	Fruit	Costa Rica
S15-050629	1613196	Pineapples	<0.005	Fruit	Unknown
S14-052475	1614540	Blueberries	<0.005	Soft Fruit	Argentina
S15-006795	1613205	Blueberries	<0.005	Soft Fruit	Chile
S15-050612	1613194	Strawberries	<0.005	Soft Fruit	Spain
S15-051732	1613929	Strawberries	**~0.006**	Soft Fruit	United Kingdom

Table 4	Measured concentrations in fruit, vegetables and milk				
~ indicates the measured value is between LOD (0.005 mg/kg) and LOQ (0.01 mg/kg)					
Table 4b	**Fruiting vegetables**				
Fera LIMS Code	**Sample ID**	**Description**	**Chlorate (mg/kg)**	**Commodity Group**	**Country of Origin**
S14-052476	1614537	Tomatoes	<0.005	Fruiting vegetables	United Kingdom
S14-052485	1418271	Cucumbers	<0.005	Fruiting vegetables	Spain
S15-000400	1614645	Tomatoes	**0.05**	Fruiting vegetables	United Kingdom
S15-006759	1613208	Tomatoes	<0.005	Fruiting vegetables	Morocco
S15-050622	1613189	Tomatoes	<0.005	Fruiting vegetables	Spain
S15-051736	1613927	Tomatoes	<0.005	Fruiting vegetables	United Kingdom
S15-051781	1613930	Peppers, paprika	**~0.006**	Fruiting vegetables	United Kingdom
S15-051785	1613919	Tomatoes	<0.005	Fruiting vegetables	United Kingdom

Table 4	Measured concentrations in fruit, vegetables and milk				
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Table 4c	**Herbs**				
Fera LIMS Code	**Sample ID**	**Description**	**Chlorate (mg/kg)**	**Commodity Group**	**Country of Origin**
S14-052477	1614541	Dill, herb	<0.005	Herbs	Morocco
S14-052499	1438	Basil, herb	**0.33**	Herbs	United Kingdom
S14-052538	1614656	Tarragon, herb	<0.005	Herbs	United Kingdom
S15-000425	1784	Parsley, herb	**0.033**	Herbs	United Kingdom
S15-006718	1779	Basil, herb	**0.050**	Herbs	United Kingdom
S15-006760	1613214	Parsley, herb	**0.011**	Herbs	Spain
S15-050615	1613192	Coriander, herb	<0.005	Herbs	Spain
S15-050620	1914	Parsley, herb	<0.005	Herbs	United Kingdom
S15-051618	1916	Basil, herb	**0.068**	Herbs	United Kingdom

Table 4	Measured concentrations in fruit, vegetables and milk				
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Table 4d	**Leaf vegetables and salad**				
Fera LIMS Code	**Sample ID**	**Description**	**Chlorate (mg/kg)**	**Commodity Group**	**Country of Origin**
S14-052469	1613116	Lettuce	<0.005	Leaf vegetables	United Kingdom
S14-052493	1613119	Lettuce	<0.005	Leaf vegetables	United Kingdom
S14-052520	1614662	Lettuce	**0.017**	Leaf vegetables	United Kingdom
S15-000383	1614638	Lettuce	<0.005	Leaf vegetables	United Kingdom
S15-006688	1982	Spinach (fresh)	**0.12**	Leaf vegetables	Italy
S15-006794	1613213	Iceberg lettuce	<0.005	Leaf vegetables	Spain
S15-050613	1921	Spinach (fresh)	<0.005	Leaf vegetables	Spain
S15-050621	1905	Iceberg lettuce	**0.18**	Leaf vegetables	Spain
S15-050890	1613924	Iceberg lettuce	<0.005	Leaf vegetables	United Kingdom
S15-051616	1918	Lettuce	**~0.008**	Leaf vegetables	United Kingdom
S14-052533	1418262	Florette Classic Crispy Salad	**0.12**	Salad	United Kingdom

Table 4	Measured concentrations in fruit, vegetables and milk				
~ indicates the measured value is between LOD (0.005 mg/kg) and LOQ (0.01 mg/kg)					
Table 4e	**Root Crops**				
Fera LIMS Code	**Sample ID**	**Description**	**Chlorate (mg/kg)**	**Commodity Group**	**Country of Origin**
S14-052480	1614539	Sweet potatoes	**0.026**	Root crops	United States
S14-052486	1418269	Carrots	**0.081**	Root crops	Unknown
S14-052492	1418264	Carrots	<0.005	Root crops	United Kingdom
S14-052530	1614653	Potatoes and potatoes products	**0.034**	Root crops	United Kingdom
S14-052535	1614668	Potatoes and potatoes products	<0.005	Root crops	United Kingdom
S15-000403	1614644	Swedes	<0.005	Root crops	United Kingdom
S15-000428	1614648	Carrots	<0.005	Root crops	United Kingdom
S15-000431	1614650	Parsnips	<0.005	Root crops	United Kingdom
S15-009526	1613206	Sweet potatoes	<0.005	Root crops	United States
S15-023699	1614970	Beetroot	<0.005	Root crops	United Kingdom
S15-050614	1613193	Carrots	**0.11**	Root crops	United Kingdom
S15-050624	1613180	Beetroot	**0.033**	Root crops	Spain
S15-050887	1613922	Parsnips	<0.005	Root crops	United Kingdom
S15-051786	1613921	Celeriac	<0.005	Root crops	United Kingdom

Table 4	Measured concentrations in fruit, vegetables and milk				
~ indicates the measured value is between LOD (0.005 mg/kg) and LOQ (0.01 mg/kg)					
Table 4f	**Miscellaneous Vegetables**				
Fera LIMS Code	**Sample ID**	**Description**	**Chlorate (mg/kg)**	**Commodity Group**	**Country of Origin**
S14-052470	1613118	Cauliflower	<0.005	Vegetable	United Kingdom
S14-052479	1614542	Broccoli	<0.005	Vegetable	Spain
S14-052484	1418273	Legumes, beans, green, with pods	<0.005	Vegetable	Morocco
S14-052488	1613115	Legumes, beans, green, with pods	**0.017**	Vegetable	United Kingdom
S14-052496	1614549	Legumes, beans, green, with pods	<0.005	Vegetable	United Kingdom
S14-052528	1614661	Head cabbage	<0.005	Vegetable	United Kingdom
S14-052531	1614654	Cauliflower	<0.005	Vegetable	United Kingdom
S14-052534	1614667	Head cabbage	<0.005	Vegetable	United Kingdom
S14-052536	1614671	Head cabbage	<0.005	Vegetable	United Kingdom
S15-000379	1614633	Brussels sprouts	<0.005	Vegetable	United Kingdom
S15-000396	1614640	Cauliflower	<0.005	Vegetable	United Kingdom
S15-000397	1614646	Chinese cabbage	**0.071**	Vegetable	United Kingdom
S15-000402	1614543	Head cabbage	<0.005	Vegetable	United Kingdom
S15-000404	1614639	Kale	<0.005	Vegetable	United Kingdom
S15-006689	1444	Broccoli	<0.005	Vegetable	Spain
S15-006758	1613212	Trimmed fine beans	<0.005	Vegetable	Kenya
S15-009524	1613207	Peas, green, without pods	**0.083**	Vegetable	United Kingdom
S15-009525	1613200	Beans, green, without pods	**0.093**	Vegetable	United Kingdom
S15-050623	1613188	Head cabbage	<0.005	Vegetable	Spain
S15-050625	1613190	Peas, green, without pods	<0.005	Vegetable	United Kingdom
S15-050628	1613198	Beans	<0.005	Vegetable	Egypt
S15-050630	1613186	Beansprouts	**1.89**	Vegetable	United Kingdom
S15-050783	1613120	Broccoli	<0.005	Vegetable	United Kingdom
S15-051617	1613178	Broad bean	**0.027**	Vegetable	United Kingdom
S15-051735	1613925	Head cabbage	<0.005	Vegetable	United Kingdom
S15-051794	1613911	Broccoli	<0.005	Vegetable	United Kingdom
S15-051796	1613912	Head cabbage	<0.005	Vegetable	United Kingdom

Table 4	Measured concentrations in fruit, vegetables and milk				
~ indicates the measured value is between LOD (0.005 mg/kg) and LOQ (0.01 mg/kg)					
Table 4g	**Milk**				
Fera LIMS Code	**Sample ID**	**Description**	**Chlorate (mg/kg)**	**Commodity Group**	**Country of Origin**
S14-052498	1364353	Liquid milk	**0.020**	Milk	United Kingdom
S14-052521	1614663	Liquid milk	**0.037**	Milk	United Kingdom
S14-052532	1614655	Liquid milk	**0.039**	Milk	United Kingdom
S14-052541	1614658	Liquid milk	<0.005	Milk	United Kingdom
S15-000380	1614634	Liquid milk	**0.019**	Milk	United Kingdom
S15-050784	1613122	Liquid milk	**0.029**	Milk	United Kingdom
S15-050889	1613923	Liquid milk	**0.058**	Milk	United Kingdom
S15-051797	1613177	Liquid milk	**0.017**	Milk	United Kingdom

Glossary:

Aq	Aqueous
EFSA	European Food Standard Agency
EU	European Union
EURL	European Union Reference Laboratory
Fera	Fera Science Ltd
FSA	Food Standard Agency
KOH	Potassium Hydroxide
LC-MS/MS	Liquid Chromatography – Tandem Mass Spectroscopy
<LoQ	Less than Limit of Quantification
mg	Milligram
kg	Kilogram
mins	Minutes
mL	Millilitre
mM	Millimolar
PT	Proficiency Testing
UK	United Kingdom
µL	Microlitre