Occurrence of HBCDDs, bromophenols, tetrabromobisphenol A and tetrabromobisphenol S in milk, eggs, fish, offal and animal fat produced in Ireland in 2014

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Introduction

The Food Safety Authority of Ireland (FSAI) has conducted a number of collaborative studies over the last few years on the occurrence of persistent organic pollutants in food. These have included investigations on dioxins, PCBs, PCNs, brominated dioxins, mixed halogenated dioxins and a number of brominated flame retardants (BFRs) such as polybrominated diphenylethers (PBDEs), polybrominated biphenyls (PBBs), hexabromocyclododecanes (HBCDDs), tetrabromobisphenol A (TBBPA), hexabromobenzene (HBB), bis(246-tribromophenoxy)ethane (BTBPE) and decabromodiphenylethane (DBDPE) [1-7].

In 2014, the European Commission [8] recommended the monitoring of several BFRs, including PBDEs, HBCDDs, TBBPA, bromophenols and tetrabromobisphenol derivatives in food. Concerning the latter three compound classes, very little is known regarding occurrence in food and feed, production volumes, persistence in the environment.

This survey was carried out in collaboration with the Department of Agriculture, Food and the Marine, the Environment Protection Agency and the Marine Institute with a view to establish occurrence and background levels of 4-bromophenol, 2,4-dibromophenol, 2,6-dibromophenol, 2,4,6-tribromophenol, tetrabromobisphenol S (TBBPS), HBCDD (α-, β- and γ- isomers) and TBBPA in food produced in Ireland and to aid on-going global efforts in assessing potential risks to human health from these compounds.

Materials and methods

A total of fifty three composite samples were prepared after collection of individual sub-samples at the production or processing stage. These included 12 milk samples, 12 egg samples, 10 fish samples, 12 samples of carcass fat taken from beef cattle, pigs, lambs, chickens and turkeys, and 7 samples of liver (bovine, porcine, ovine, equine and avian). In the case of liver, the composites were prepared from between 10 and 40 subsamples, for carcass fat, 10 sub-samples were used, for the eggs, 24 sub-samples were used and the milk was taken from bulk tanks, reflecting regional production. All samples were supplied by officers of the Department of Agriculture, Food and the Marine at production level (fat and liver: slaughterhouse; eggs: packing station; fish: aquaculture and landings), except for milk samples, which were collected by the Environmental Protection Agency.

Samples were pooled, homogenised and freeze dried, as applicable, by the State Laboratory, Celbridge, Ireland and analysis of the samples was undertaken by Fera Science Ltd, York, UK, during 2015/2016 under contract to FSAI.

Methods for extraction, purification and analysis, using liquid chromatography tandem mass spectrometry (LC-MS/MS), were developed and validated.

Quality assurance procedures included duplicate analyses and use of appropriate reference materials.

Extraction - TBBPA and HBCDDs

Internal standards were added to the fat and freeze-dried samples (eggs, fish tissue, milk and liver) and blended using an Ultra Turrax with dichloromethane:hexane (4:6) and acid modified silica. The mixture was filtered through silanised glass wool and further washed with extraction solvent mixture. This was evaporated to just dryness under nitrogen and reconstituted with methanol containing β-HBCDD- d18 and water.

Extraction - TBBPS and bromophenols

Samples were extracted with hexane:acetone (1:1) by shaking for 21 hours. The mixture was filtered through silanised glass wool, and evaporated to approx. 1 mL and further washed with extraction solvent mixture. Hexane was added and the compounds were back extracted into acetonitrile containing 0.35% ammonia. This step was repeated and the acetonitrile aliquots combined. Extracts were evaporated to just dryness under nitrogen, reconstituted with methanol and water and micro-centrifuged before analysis.

Analysis

All extracts were analysed by LC-MS/MS in negative electrospray mode. Separation was achieved using an Agilent Rapid Resolution HD Zorbax Eclipse Plus C18 column (2.1 x 150 mm, 1.8 µm) held at 50°C. TBBPA and HBCDDs were separated isocratically over 8 minutes using water and methanol in a ratio (1:4) at 0.5 mL/minute. TBBPS and the bromophenol compounds were separated using a binary gradient at 0.4 mL/minute with water containing 10 mM ammonium acetate buffer (pH of 4.5, A) and methanol (B) starting at 30% A held for 2.2 minutes, 0% A by 8.1 minutes (held 2 minutes) and back to 30% A by 10.2 minutes (held for 1.8 minutes).

Results

Whilst several surveys covering HBCDDs and TBBPA have been carried out in Ireland, this is the first time milk, eggs, fish, fat and liver samples have been tested for bromophenols and tetrabromobisphenol derivatives.

HBCDD isomers were detected in two egg (yolk) samples, five fat samples, six fish samples and one liver sample ranging from 0.01 to 0.54 µg/kg. TBBPS, 2,4,6-tribromophenol and 2,6-dibromophenol were not detected in any of the food samples investigated.

Residues of 4-bromophenol were detected in all egg samples with concentrations ranging from 0.28 to 0.63 µg/kg of whole weight. Residues of 4-bromophenol and 2,4-dibromophenol were detected in two fish samples at concentrations ranging from 0.47 to 0.98 µg/kg, and TBBPA was detected in one fish sample at 0.01 µg/kg.

One porcine fat sample showed elevated levels of HBCDD (results not shown) and was subject to a follow up investigation.

Tables 1and 2 provide an overview of results for each BFR (µg/kg of whole weight).

The results of this preliminary investigation confirm the occurrence of a wider range of BFRs in food, in addition to the PBDEs which have been widely measured over the last decade or so. Although the sample numbers are small, they may provide an initial indication of dietary exposure to these chemicals.

Table 1. Summary data for HBCDDs and TBPPA

Food	Food	*n*	Unit	α-HBCDD	β-HBCDD	ɣ-HBCDD	TBBPA
Eggs (Yolk only)	caged	4	% detected	n.d.	n.d.	n.d.	n.d.
			Min-Max	<0.01	<0.01	<0.01	<0.01
	free range	4	% detected	n.d.	n.d.	n.d.	n.d.
			Min-Max	<0.01	<0.01	<0.01	<0.01
	organic	4	% detected	50%	n.d.	n.d.	n.d.
			Min-Max	<0.01 - 0.02	<0.01	<0.01	<0.01
Milk	Milk	12	% detected	n.d.	n.d.	n.d.	n.d.
			Min-Max	<0.01	<0.01	<0.01	<0.01 - <0.09
Carcass Fat	Avian	3	% detected	33%	n.d.	n.d.	n.d.
			Min-Max	<0.02 - 0.055	<0.01 - <0.02	<0.01 -< 0.02	<0.01 - <0.04
	Bovine	3	% detected	33%	n.d.	n.d.	n.d.
			Min-Max	<0.04 - 0.085	<0.01 - <0.02	<0.01 - <0.02	<0.01
	Ovine	3	% detected	n.d.	n.d.	n.d.	n.d.
			Min-Max	<0.04	<0.01	<0.01	<0.01
	Porcine	2	% detected	100%	50%	50%	n.d.
			Min-Max	0.19 – 0.54	<0.02 - 0.02	<0.02 - 0.03	<0.04 - <0.06
Offal	Liver	7	% detected	14%	n.d.	n.d.	n.d.
			Min-Max	<0.01 - 0.05	<0.01	<0.01 - <0.04	<0.01 - <0.04
Fish	Oily	6	% detected	100%	50%	83%	17%
			Min-Max	0.065 - 0.39	<0.01 - 0.02	<0.01 - 0.02	<0.01 - 0.01
	White	4	% detected	n.d.	n.d.	n.d.	n.d.
			Min-Max	<0.01	<0.01	<0.01	<0.01 - <0.07

n = Number of (pooled) samples (see *Materials and Methods* for number of samples per pool); n.d. = not detected

Table 2. Summary data for bromophenols and TBBPS

Food	Food	*n*	Unit	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	TBBPS
Eggs (Yolk only)	caged	4	% detected	100%	n.d.	n.d.	n.d.	
			Min-Max	0.28 - 0.63	<0.45 - <0.98	<3 -< 4	<0.45 - <1.5	
	free range	4	% detected	100%	n.d.	n.d.	n.d.	
			Min-Max	0.49 - 0.6	<0.26 - <0.64	<1.5 - <3.9	<0.47 - <0.94	
	organic	4	% detected	100%	n.d.	n.d.	n.d.	
			Min-Max	0.34 - 0.57	<0.24 - <0.58	<0.83 - <2.6	<0.46 - <2.6	
Milk	Milk	12	% detected	n.d.	n.d.	n.d.	n.d.	n.d. (*n*=7)
			Min-Max	<0.02	<0.01 - <0.04	<0.1 - <0.29	<0.01 - <0.03	<0.03 - <0.24

Food	Food	*n*	Unit	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	TBBPS
Carcass Fat	Avian	3	% detected	n.d.	n.d.	n.d.	n.d.	n.d.
			Min-Max	<0.19 - <0.29	<0.81 - <1.5	<4.9 - <6.9	<0.12 - <0.21	<0.09 - <0.14
	Bovine	3	% detected	n.d.	n.d.	n.d.	n.d.	n.d.
			Min-Max	<0.06 - <0.08	<0.23 - <0.36	<1.2 - <3.4	<0.05 - <0.07	<0.14 - <0.71
	Ovine	3	% detected	n.d.	n.d.	n.d.	n.d.	n.d.
			Min-Max	<0.08 - <0.11	<0.22 - <0.63	<1.6 - <3.5	<0.05 -<0.12	<0.12 - <0.24
	Porcine	3	% detected	n.d.	n.d.		n.d. (*n*=2)	n.d. (*n*=2)
			Min-Max	<0.13 - <0.44	<0.95 - <2.4		<0.08 - <0.09	<0.33 - <0.36
Offal	Liver	7	% detected	n.d.	n.d.	n.d. (*n*=6)	n.d.	
			Min-Max	<0.03 - <0.21	<0.08 - <0.27	<0.39 - <1.3	<0.05 - <0.21	
Fish	Oily	6	% detected	n.d.	n.d.	n.d. (*n*=2)	n.d.	
			Min-Max	<0.34 - <1.05	<0.88 - <1.7	<3.1 - <3.5	<0.09 - <0.44	
	White	4	% detected	50%	50%	n.d.	n.d.	n.d. (*n*=1)
			Min-Max	<0.1 - 0.91	<0.09 - 0.98	<0.8 - <1.9	<0.03 - <0.06	<0.42

Acknowledgements: This work was funded by the Food Safety Authority of Ireland. Food samples were taken by officers of the Department of Agriculture, Food and the Marine, the Marine Institute and the Environmental Protection Agency. Sample preparation was carried out by the State Laboratory prior to dispatch to Fera.

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