HEXABROMOCYCLODODECANE (HBCD) IN EGG YOLKS FROM MARKET BOUND CANADIAN CHICKEN EGGS.

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Abstract
Eggs from three regions of Canada were collected at grading stations and analysed for a number of persistent organic pollutants (POPs), including the flame retardant, hexabromocyclododecane (HBCD). Four categories of eggs (conventional, omega-3 enriched, organic, free range) were collected from all regions of Canada, while free run eggs were collected from two regions based on availability. At least one isomer of HBCD was detected in the majority (85 %) of the 162 egg yolks analysed from all regions of Canada. In general, α–HBCD was detected the most frequently (83%) and was the dominant contributor to ΣHBCD levels. γ-HBCD was observed in 27% of the eggs tested and β-HBCD was detected in only 9% of the samples. ΣHBCD concentrations in the yolks ranged from below the limits of detection to 1110 ng/g lipid. Of the categories tested, the highest ΣHBCD concentrations (3.36 - 1110 ng/g lipid) were observed in organic egg yolks from the Maritime region, although organic eggs from other regions of Canada had similar concentrations to other egg types (<detection limits to 0.234 ng/g lipid). Region of production did not significantly impact the HBCD residue levels, although variability was associated with differences in egg yolk type.

Introduction
Hexabromocyclododecanes (HBCDs) are additive flame retardants used in extruded and polyurethane foams in upholstery textiles and as thermal insulation in buildings [1, 2]. They are not, however, generally added to electrical equipment, which is in contrast to the polybrominated diphenyl ethers[1, 2]. HBCD has been studied in abiotic samples (e.g., air, sediments and soils) in addition to fish and wildlife tissues including eggs from predatory birds[3]. Limited residue data in humans are found in the published literature, with the exception of some human milk data[3, 4]. Similarly, literature reports of residue levels of HBCDs in food are scarce, although isomeric determination of less than 1 ng/g levels of HBCDs has been reported from total diet pooled samples from the UK in foods of plant and animal origin[5].

HBCD was positively identified in 2 of 75 free range chicken eggs from Germany where the highest total HBCD and metabolite concentration were in the high ng/g lipid range[6]. Concentrations were given as total HBCD owing to the analyses being performed by GC/MS rather than LC-MS/MS. Similarly, ΣHBCD was observed in 11 of 20 home-produced chicken eggs in Belgium[7] and the median concentration in eggs collected during the spring of 2007 was 2.85 ng/g lipid although the median level was below the limit of quantification in eggs collected during autumn of 2006. Detection frequency was higher in eggs obtained during the spring collection suggesting seasonality associated with HBCD levels.

Chicken eggs are produced throughout Canada, with greatest production occurring in the central part of the country, in the provinces of Ontario and Quebec. Egg production is mostly by conventional means (caged and feed controlled) with average Canadian consumption about 3-4 eggs per week. As part of our on-going studies of the occurrence of POPs in a number of foods including domestic eggs produced under a variety of conditions[8], we analyzed egg samples for the flame retardant, HBCD. Eggs were collected for the present study from British Columbia (BC), Quebec and the Maritimes to represent each of the major regions (i.e., western, central and eastern Canada) as a means of capturing spatial differences in residue levels. The collection included eggs produced by chickens raised under conventional, free range and free run conditions, in addition to those identified as being organic and omega-3 enriched. Analyses have been carried out using isomeric measurement techniques.

Materials and Methods

Canadian Food Inspection Agency (CFIA) Inspectors collected eggs (n = 162) from grading stations in BC, Quebec and the Maritimes in 2005/06. The samples included at least 10 large eggs belonging to each category (conventional, omega-3 enriched, organic and free-range) from each region of collection. Eggs belonging to the free run category were sampled in the regions where they were available (Quebec, Maritimes). Upon receipt in the laboratory, eggs were frozen and retained at -20°C until extraction and analysis was initiated. Given that the yolk, about one-third of the weight of the edible part of the egg, contributes 100% of the lipid content in whole eggs and POPs are generally associated with lipids, it was decided that yolks would be the focus of investigation for this work. When ready for analysis, samples were thawed, separated into yolk and whites. $^{13}C_{12}$ analogues of α-, β-, and γ-HBCD were added prior to yolks being extracted using acetone: hexane (2:1) following the method for milk described previously[4, 9]. Due to the high cholesterol levels in egg yolks, sample preparation was adapted to include an initial clean up using non-acidified silica gel (2 g), eluted with 4 mL hexane prior to digestion with sulphuric acid[10].

HBCD was quantified using an Acquity HPLC coupled to a Quattro Premier MS/MS (Waters) with electrospray ionization in the negative ion detection mode using a 2.1mm x 100 mm, Hypersil Gold C18, 1.9 µm column (Thermo Scientific). Water (mobile phase A) and acetonitrile: methanol (2:1) (mobile phase B) were the mobile phases used in separation of the HBCD isomers and the gradient was as follows: 40% B for 1 min, 40 to 80% mobile phase B by 4 min, 80 to 90% B by 13 min, then returned to 40 % B over 0.5 min where it remained until 18 min. The flow rate was maintained at 0.175 mL/min and the column temperature was maintained at 15°C. The capillary and cone voltage were -3.5 KV and 20 V, respectively. The source temperature and desolvation temperature were 140°C and 400°C, respectively. Cone gas flow and desolvation gas flow were 47 L/hr and 947 L/hr, respectively. Argon was the collision gas and resolution was established at 90% valley at base for both quadrupole analyzers. Transitions monitored for native HBCD were 639 > 79, 641 > 79, 641 > 81 and 643 > 81. Dwell times were set to 0.05 s. Completely deuterated HBCD, $C_{12}{}^2H_{18}Br_6$, was used as a performance standard. For each group of 8-10 unknown samples, a quality control butter sample and a laboratory blank sample were processed through the method to check for precision and laboratory background contamination. The lipid content of each yolk was determined gravimetrically and ranged from 12.8% to 27.9% and the mean lipid content was 21.1%.

The reagent blanks tested for HBCD levels were consistently observed to have non-detect or negligible levels of both native and ^{13}C labelled HBCD analogues which we attributed to the lack of matrix in the purified extract. We are currently working to improve HBCD recoveries in reagent blank samples in addition to samples that seem to have lower matrix contribution, which effects the elution of HBCD from the initial silica column. Recoveries of α-, β-and γ-HBCD were 67%, 46% and 66%, respectively in the butter quality control samples tested. Average α-, β-and γ-HBCD recoveries from egg yolks were 34%, 13% and 14%, respectively. We also observed that extracts that had been prepared well in advance of analysis resulted in lower recoveries, which is consistent with the reduction in HBCD levels associated with time sitting between sample preparation and analysis reported in the literature[6]. In general, a reduction in ^{13}C β-HBCD recoveries was the first indicator that samples had been stored too long prior to analysis while α- and γ-HBCD recoveries would remain as usual. The limit of detection ranged from to 3.6 – 7.7 pg/injection, 3.0 – 6.4 pg/injection and 2.2 – 4.5 pg/injection for α-, β-, and γ-HBCD, based on 15 µL injection volumes, respectively. The average detection limit was determined to be 0.01 ng/g lipid for each of α-, β- and γ-HBCD.

For some time, the Norwegian Institute of Public Health has organized international interlaboratory studies to measure several classes of POPs in foods. Health Canada has participated in these exercises for which the last four years have also involved measurement of HBCD isomers. Our results in biotic samples for the α-isomer have been within 15-20 % of the median values of reporting laboratories.

Results and Discussion

Total HBCD (α-, β-, and γ-) was detected in 85% of the samples analysed with α-HBCD being present in the majority of egg yolks. Each of the diastereoisomers was detected with different frequency, with the α- isomer

being most prevalent (83%) in egg yolks. The β- and γ- isomers were observed in 9% and 27% of the yolks tested, respectively.
In the vast majority of samples where this brominated flame retardant was detected, α-HBCD was the dominant contributor (89%) to ΣHBCD levels (Figure 1), while γ–HBCD was the dominant contributor to ΣHBCD levels in only 11% of the samples analysed. The high relative contribution of α-HBCD to ΣHBCD levels in predatory birds and eggs has been observed by other researchers[11], and although the β- isomer is generally not observed in eggs, it was detected in some yolks analysed in the present study (Table 1).

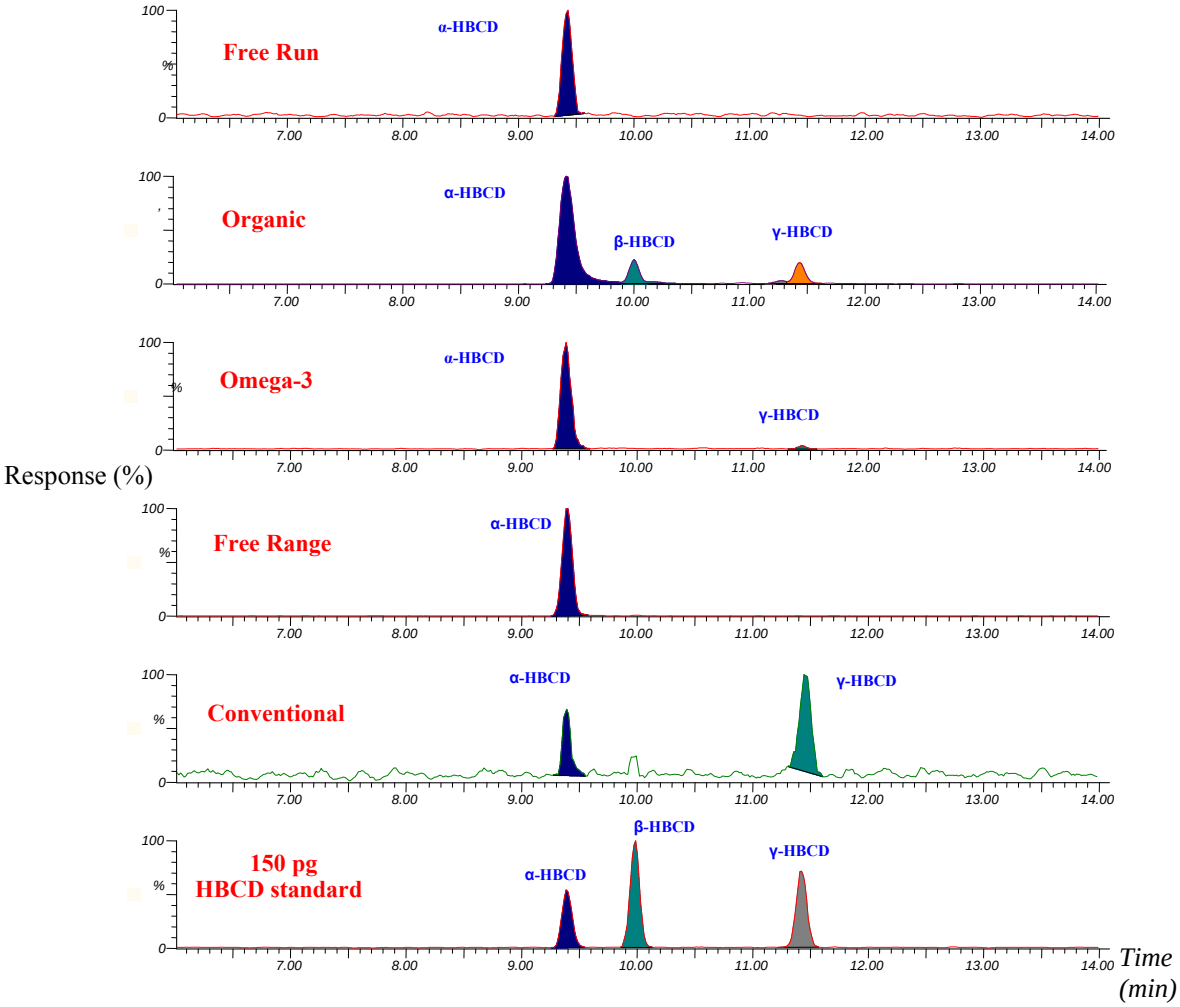

Figure 1: Chromatographic separation of HBCD diastereoisomers in a standard mixture and chicken egg yolks from the Maritimes (eastern Canada).

Recovery corrected ΣHBCD concentrations in the yolks ranged from below the level of detection to 1100 ng/g lipid (Table 1). The highest concentrations (131 – 1110 ng/g lipid) were, however, observed in four samples from the Maritimes with low surrogate recoveries of α-HBCD (<10%) which resulted in increased uncertainty in the results for these samples. The majority of egg yolks from across Canada had ΣHBCD levels below 1 ng/g lipid, regardless of which type of egg was sampled (Figure 2) and concentrations in Canadian egg yolks are generally within the range reported for eggs of predatory birds[11, 12]. Similarly, Canadian egg yolks had levels

corresponding to home produced eggs from Belgium[7]. The region of Canada from which eggs were collected did not significantly impact the ΣHBCD levels (p = 0.766).

Variable HBCD levels were observed among egg yolks from Quebec and the Maritimes belonging to different categories (e.g., conventional, free range, etc.), however, those collected from BC had similar concentrations regardless of category. Egg yolks identified as omega-3 enriched from Quebec had somewhat elevated levels of α-HBCD (median = 0.246 ng/g lipid) relative to those belonging to other categories (median = 0.022 – 0.035 ng/g lipid). Greater variability was observed among the egg yolks from the Maritimes, where organic yolks were determined to contain the highest ΣHBCD levels (median = 70.7 ng/g lipid), followed by free range yolks (median = 0.341 ng/g lipid). Levels of HBCD in the other categories of yolks were lower and within the same order of magnitude (median = 0.024 – 0.080 ng/g lipid).

Table 1: HBCD concentrations [median} (ng/g lipid) in egg yolks by region from Canada.

Location	α-HBCD	β-HBCD	γ-HBCD	ΣHBCD
BC (Western)	<DL – 1.52 [0.032]	<DL – 0.112 [<DL]	< DL – 1.23 [<DL]	<DL – 1.59 [0.046]
Quebec (Central)	<DL – 1.60 [0.043]	<DL [<DL]	<DL – 28.1 [<DL]	<DL – 28.4 [0.049]
Maritimes (Eastern)	<DL – 1060 [0.050]	<DL – 34.8 [<DL]	<DL – 15.8 [<DL]	<DL – 1110 [0.087]

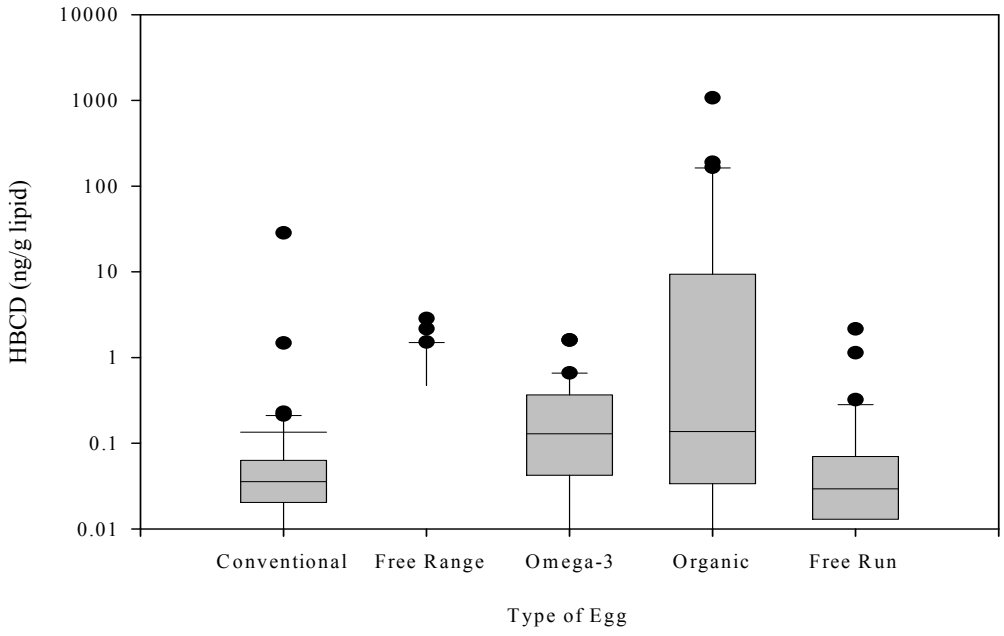

Figure 2: ΣHBCD recovery corrected concentrations (ng/g lipid) in egg yolks from Canada. Box indicates 25th, 50th and 75th percentiles. Points indicate data outside of 10th (⊥) or 90th (⊤) percentiles.

The results of the present study are consistent with levels observed in eggs from Europe. The levels of HBCD in Canadian egg yolks varied by three orders of magnitude, with the majority of eggs remaining at the very low ng/g levels. This wide variation in HBCD concentrations is similar to reported observations in Germany where a single chicken egg tested was found to contain 2000 ng/g lipid HBCD, as determined using GC/ECD. Only one other egg from the 77 tested during the study had detectable levels[6]. Home produced eggs tested in Belgium also showed a wide range. Although food obtained by predatory birds differs considerably from chickens that are provided an established feed, a wide variability is also observed in eggs from other avian species.

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