Toxicokinetics and carry-over model of α-hexabromocyclododecane (HBCD) from feed to consumption-sized Atlantic salmon (Salmo salar)

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A two-compartmental model for the kinetics of carry-over of the brominated flame retardant α-hexabromocyclododecane (HBCD) from feed to the fillet of farmed harvest-sized Atlantic salmon (Salmo salar L.) was developed. The model is based on a fat compartment for storage of the lipophilic α-HBCD and a central compartment comprising all other tissues. Specific for this model is that the salmon has a continuous growth and that fillet contaminant levels are explained by both the fat and the central compartments. The uptake and elimination kinetics are obtained from experimental data where consumer sized (start weight approximately 1 kg) Atlantic salmon was fed α-HBCD spiked feed (280 ± 11 μg kg⁻¹) for 2 months followed by a depuration period of 3 months. The model was used to simulate the HBCD feed-to-fillet transfer in Atlantic salmon under realistic farming conditions such as the seasonal fluctuations in feed intake, growth and fillet fat deposition. The model predictions gave fillet concentrations of 0.2–1.8 μg kg⁻¹ depending on the level of fish oil inclusion in the salmon diets when using fish oil with high POP background levels. Model simulations show that currently farmed Atlantic salmon can contribute to a maximum of 6% of the estimated provisional food reference dose for HBCD.

Keywords: LC/MS; exposure; risk assessment; toxicology; pharmokinetics; bromate; feeding; animal feed; fish and fish products

Introduction

Farmed Atlantic salmon (Salmo salar) may contain relatively high levels of persistent organic pollutants (POPs) such as dioxins, PCBs and organochlorine pesticides, which are potentially hazardous to consumers. These POPs are fat-soluble and persistent compounds that are ubiquitous to the environment and readily bioaccumulate along the aquatic food chain. Fish oils, obtained from pelagic fish species and used in the high-energy salmonid feeds, are the main source of POPs in farmed Atlantic salmon fillets (Easton et al. 2002; Jacobs et al. 2002; Berntssen et al. 2005; Berntssen, Olsvik, et al. 2010). Hexabromocyclododecane (HBCD) is an emerging environmental risk POP after its extensive use in the fire prevention of textiles, building materials and electrical equipment for the last two decades (Covaci et al. 2006). Elevated HBCD levels are found worldwide in oily pelagic fish (Morris et al. 2004; Remberger et al. 2004; Law, Allchin, et al. 2006; Jenssen et al. 2007; Fernandes et al. 2008; Tomy et al. 2008; Shaw et al. 2009; Roosens, Geeraerts, et al. 2010) as well as farmed oily fish such as Atlantic salmon and rainbow trout (Oncorhynchus mykiss) (Knutsen et al. 2008; van Leeuwen and de Boer 2008; van Leeuwen et al. 2009). Lean farmed fish species such as tilapia (Oreochromis mossambicus, Oreochromis niloticus) and pangasius (Pangasius hypophthalmus) contain far lower HBCD levels (van Leeuwen et al. 2009). The technical HBCD mixture consists mainly of three diastereoisomers (α-, β- and γ-HBCD) of which the γ-isomer is the dominant isomer (approximately 75–90%) (Heeb et al. 2005). Whereas the γ-isomer is the dominant form found in sediment near point pollution site, the α-isomer is the dominant form found worldwide in aquatic organisms (Morris et al. 2004; Tomy et al. 2008; van Leeuwen and de Boer 2008; Haukas, Hylland, et al. 2009; Koppen et al. 2010). In-house determinations of HBCD isoforms in more than 30 samples of crude oil from different fish species showed that only α-HBCD was present in levels above the limit of quantification (1 μg kg⁻¹) (unpublished...
As for most POPs, the consumption of oily fish, such as farmed Atlantic salmon and rainbow trout, can be an important source for human α-HBCD exposure for European consumers (Knutsen et al. 2008; Roosens, Cornelis, et al. 2010; Thomsen et al. 2010), while exposure from the indoor atmosphere is an additional source (Roosens et al. 2009; Roosens, Cornelis, et al. 2010; Thomsen et al. 2010).

The European Union has established upper limits for several POPs, but not brominated flame retardants, in feed ingredients and fish feed, aiming to ensure that food is safe for consumers and to control the level of these substances in the food production chain (McEvoy 2002; European Commission 2005; Covaci et al. 2006; Ribo et al. 2009). European Union risk assessments on HBCDs are currently in progress (Covaci et al. 2006) and a request has been made for data on HBCD in feed and foodstuffs (European Food Safety Authority (EFSA) 2006). Carry-over assessment of contaminants from feed to animal food products is essential for appropriate human risk assessment of feed contaminants (Leeman et al. 2007) as well as for harmonization of legislation of contaminants throughout the food production chain (van Raamsdonk et al. 2009). Toxicokinetic models provide a means of interpreting the experimental results in terms of processes as the substances absorption across the gut wall, its distribution over body tissues and its elimination from the body. Results of experiments to assess the carry-over of dioxin-like compounds from feed to farmed animals (growing pigs and egg laying hens) have been modelled and applied successfully (van Eijkeren et al. 2006; Hoogenboom et al. 2010). Assessment on uptake and elimination is needed in modelling feed-to-fillet carry-over (Hoogenboom et al. 2010; van Eijkeren et al. 2006). The four dietary exposure studies on HBCD toxicokinetics in fish report on the biotransformation of γ- and β-HBCD into α-HBCD in juvenile rainbow trout (Law, Palace, et al. 2006; Haukas, Mariussen, et al. 2009), while no such biotransformation of γ- into α-HBCD could be observed in mirror carp (Cyprinus carpio morpha noblis) (Esslinger et al. 2010). All HBCD isoforms are readily distributed to liver, brain and muscle (Haukas, Mariussen, et al. 2009). Highest muscle uptake was observed for α-HBCD (Law, Palace, et al. 2006) but no muscle α-HBCD elimination rates constants could be established in any of these studies (Law, Palace, et al. 2006; Haukas, Mariussen, et al. 2009). Other dietary HBCD exposure studies report on metabolism and thyroid disturbance in rainbow trout (Palace et al. 2008, 2010).

The present work aimed to develop a toxicokinetic two-compartment model to describe the feed-to-fillet carry-over of α-HBCD in harvest-sized and lipid-rich Atlantic salmon. The uptake and elimination kinetics were determined in a controlled feeding trial (daily feed intake registration), where adult Atlantic salmon was fed α-HBCD spiked diets followed by a prolonged depuration period in which all fish were fed no-spiked control feed. Earlier dietary fish trials include exposures with technical HBCD mixture or γ-HBCD (Haukas, Mariussen, et al. 2009; Esslinger et al. 2010). The only dietary exposure study on α-HBCD includes uptake assessment in the juvenile fish stage of rainbow trout and no elimination rates constants could be gleaned for the α-isomer (Law, Palace, et al. 2006). The present trial focused on the uptake and elimination rate kinetics of the dominant HBCD isomer present in the salmon food chain (α-HBCD) and adult consumption-sized Atlantic salmon, which has the highest muscle lipid content of all life stages of the farmed Atlantic salmon. The toxicokinetic model is partly based on physiological data such as tissue volumes and cardiac output. The calibrated and validated model was applied to estimate fillet α-HBCD residue levels during realistic farming conditions.

**Material and methods**

**Experimental conditions and sampling**

The dietary trial was carried out at Lerang Research Station, Skretting ARC, Stavanger, Norway (58°56’N, 06°02’E). The trial started on 15 March 2010 and ended on 8 September 2010. A total of 200 adult seawater-adapted Atlantic salmon with a mean (±1 × standard deviation) weight and length of 0.73 ± 0.14 kg and 0.39 ± 0.03 m, respectively, were stocked in two 7-m³ land-based seawater flow-through tanks. One group of fish received a control (not fortified) diet and the other group an α-HBCD fortified diet. Prior to the start of the trial, all fish were fed the control diet during a 1-month period to acclimate to the holding facilities. An uptake period of 56 days, where fish were fed fortified or control diets, was followed by an 84-day depuration period, in which both groups received the control diet. The fish were reared under a 12:12 light-to-dark regime and the fish were fed twice a day in slight access of appetite corresponding to a level of approximating 0.7% of body weight (bw) day⁻¹. Feeding rate was adjusted after each sampling based on the mean weight of the fish sampled. Uneaten pellets were collected in a flow-over system and registered daily thus providing exact daily feed intake assessment. Each tank received aerated and temperature regulated seawater at a constant flow-through of 851 min⁻¹. The dissolved oxygen levels was kept at 10 mg l⁻¹, salinity was 33%, and temperature was regulated at 10 ± 0.1°C. Three fish per tank were sacrificed on days 0, 8, 14, 26, 41 and 54 of the uptake period and on days 0, 1, 3, 5, 7, 14, 28, 42, 55 and 83 of the depuration period. The fish were
killed by an overdose of anaesthesia (MS-222 at 0.5 g l\(^{-1}\)) and then whole left skin-free fillet \((n=3)\) was dissected, homogenized and stored at \(-30^\circ\text{C}\) until analysis.

### Diet preparation

All feeds were produced by Skretting ARC, Stavanger, Norway. The control diet was composed of fish meal (South American, Peru, 180 g kg\(^{-1}\) diet), wheat meal (Skretting, Norway, 70 g kg\(^{-1}\)), wheat gluten (Cargill Nordic, Denmark, 162 g kg\(^{-1}\) diet), soybean meal (Felleskjøpet, Norway, 97 g kg\(^{-1}\)), soya concentrate (Imcopa, Brazil, 150 g kg\(^{-1}\) diet), fish oil (Nordic, decontaminated FF Skagen, Denmark, 310 g kg\(^{-1}\) diet), and premixes including vitamins and minerals according to NRC (1993) recommendations (32 g kg\(^{-1}\) diet). To reduce the natural background levels of POPs in the control diet the fish oil was decontaminated with a commercially available decontamination technique which is based on a two-step process with active carbon followed by steam deodorisation (Berntssen, Olsvik, et al. 2010). The \(\alpha\)-HBCD fortified diets had the same composition as the control diets, and \(\alpha\)-HBCD was supplemented by top coating (1%) the control diet with \(\alpha\)-HBCD enriched oil. The control diets were top-coated (1%) with the same fish oil, which was not fortified. The level of \(\alpha\)-HBCD supplementation was chosen so as to (1) cause no acute toxic effects based on earlier studies (Haukas, Hylland, et al. 2009) and (2) to give detectable levels of \(\alpha\)-HBCD in fish muscle in order to establish uptake and elimination rate constants. The feeds were analysed before and during the experimental trial. Measured \(\alpha\), \(\beta\) and \(\gamma\)-HBCD levels in control feed were under the limit of quantification (LOQ; 1 \(\mu\)g kg\(^{-1}\)), and measured levels in the \(\alpha\)-HBCD spiked feeds were 280 ± 11 \(\mu\)g kg\(^{-1}\) for \(\alpha\)-HBCD and under the limit of quantification (< LOQ; 1 \(\mu\)g kg\(^{-1}\)) for \(\beta\)- and \(\gamma\)-HBCD. Feed was stored in closed bags at 4\(^\circ\text{C}\) to prevent degradation.

### Alpha HBCD production

The \(\alpha\)-HBCD was produced from \(\gamma\)-HBCD by thermal rearrangement as described by Smith et al. (2005). Briefly, approximately 10 g of 1,2,5,6,7,10-hexabromo-cyclododecane (Sigma-Aldrich, Germany; cas 3194-55-6, > 98% purity) was heated in an oven at 185°C for 45 min in a dry 10 ml round-bottom flask. After cooling to room temperature the heat-treated HBCD was triturated with 50 ml of ethanol:acetonitrile (60:40 v/v) and filtered to give a yield of 85% thermal-rearranged HBCD. The HBCD was dissolved in 50 ml acetonitrile and stirred with 400 mg activated carbon (Supereclean ENVI-Carb 120/400, Supelco Ltd, Bellefonte, PA, USA) for 120 min then filtered and concentrated to give a white crystal HBCD (yield of 56%). The thermal rearranged HBCD was dissolved in toluene at a concentration of 100 ng ml\(^{-1}\) and quantitatively analysed for the \(\alpha\), \(\beta\) and \(\gamma\)-HBCD isoforms. Quantitative LC-MS/MS analyses (see the section on HBCD isoform analyses) showed that the original HBCD mixture contained 71% \(\gamma\)-HBCD and 29% \(\alpha\)-HBCD, while no \(\beta\)-HBCD was detected (Figure 1a). After thermal rearrangement, all (100%) of the HBCD was in the \(\alpha\)-isoform (Figure 1b), the chromatogram of \(\alpha\), \(\beta\) and \(\gamma\)-HBCD standards is given in Figure 1(c) (Cambridge Isotope Laboratories, Andover, MA, USA).

### HBCD isoform analyses

Suprasolv-grade acetone, cyclohexane and hexane were used. HPLC-grade methanol was used. Concentrated sulphuric acid was obtained from Merck. Ammonium acetate (99.999% trace metal basis) was obtained from Sigma-Aldrich. Native and \(^{13}\)C-labelled HBCD standards were obtained from Cambridge Isotope Laboratories. All samples of feed and fish were weighed, and spiked with internal standards \((^{13}\text{C}_1\alpha-,^{13}\text{C}_1\beta-\text{ and }^{13}\text{C}_1\gamma\text{-HBCD})\) before ultrasound extraction (10 min) with 10:15:2 (v/v ml) acetonitrile:cyclohexane:3% aqueous sodium chloride. The centrifuged (10 min at 3000 rpm) extract was evaporated to 1 ml (TurboVap LV, Caliper, Hopkinton, MA, USA) and re-dissolved in hexane. Co-extracted fat was removed in an external clean-up procedure by adding concentrated sulphuric acid. The cleaned extract was evaporated and re-dissolved in 1 ml of 20 mM ammonium acetate in methanol. Samples were analysed by LC-MS/MS (Waters ACQUITY UPLC coupled to Waters Quattro Premier XE, Waters, Milford, MA, USA) in negative electrospray mode. The HBCD diastereomers were separated on a reversed-phase C18 column (150 mm × 2.1 mm i.d., 1.7 \(\mu\)m particle size, BEH C18, Waters) with isocratic elution (0.5 ml min\(^{-1}\), 60°C) using 2 mM ammonium acetate in methanol (79%) and 2 mM aqueous ammonium acetate (21%) as the mobile phase. Capillary voltage was set at 2.5 kV, source temperature was set at 120°C and the desolvation temperature was set at 400°C. The native HBCD diastereomers were monitored using the following MRM transitions: 640.4 > 78.9 and 640.4 > 80.9. The internal standards were monitored using the 652.4 > 80.9 transition. Cone voltage and collision voltage were set at 15 V for all compounds monitored. Quantification was performed according to the internal standard (IS) method using congener-specific relative
response factors (RRFs) from a linear congener-specific external standard curve relative to the internal surrogate standard. The method limit of detection (LOD) was statistically estimated during validation as the analyte concentration giving a peak signal of three times the background noise from an internal surrogate standard spiked procedural blank. The method limit of quantification (LOQ) was determined for each congener using three times the LOD (nine times the signal-to-noise level). Recovery was validated for each congener by spiking of sample matrix with standards for all congeners at three concentrations. Recovery was between 80 and 120% and the validated linear range was between 1 and 25 μg kg⁻¹ w/w. Spiked (native and mass labelled α-, β- and γ-HBCD) control samples were analysed in each run to monitor the quality of the method. Method blanks were run in each series of samples analysed. The trueness of the method was further established by participating in proficiency tests of calibration material and spiked sample material (i.e. satisfactory trueness was set on a z-score of > -2.0 and < +2.0). The quality of the method was controlled regularly by participation in the interlaboratory proficiency test organized by the Norwegian Institute of Public Health (see http://www.fhi.no/; Interlaboratory comparison on dioxins in food).

Modelling approach
The toxicokinetic model for the carry-over of dioxin-like compounds of van Eijkeren et al. (2006) was adopted and extended to a model for the growing salmon. In this modelling approach the body is considered to consist of a fat compartment for the storage of highly lipophilic compounds and a central compartment comprising all other tissues. Specific for the salmon model is that the salmon has a continuous growth and that fillet contaminant levels are explained by both the fat and central compartment levels. First the body growth and body composition in light of the two-compartment modelling approach will be presented followed by an introduction of the model equations. Then the alternative body composition corresponding to the observed tissue is discussed and finally matters concerning the identifiability of unknown model parameter values are considered.

Body composition
The salmon model was based on continuous changing of growth and both fat and central muscle compartment as occurs during the farming of an Atlantic salmon. Body weight data from a large-scale farming
trial with Atlantic salmon were modelled by a relation between data and time involving the hyperbolic tangent (Berntssen, Olsvik, et al. 2010; Lock et al. 2011) (see Supplementary Data Figure 1). In addition, both the muscle and fat deposition data during a large-scale farming production cycle was modelled likewise (see Supplementary Data Figure 1 and describing relations). Relative liver weight growth was modelled from data of the experiment and was modelled as a hyperbolic tangent relation between weight and body weight from start of the experiment (see Supplementary Data). Liver weight is of importance as elimination of HBCD from the body is assumed to be proportional to it (Equation 2). Cardiac output was found to be linear with body weight from the literature (Olson 1998). Cardiac output is of importance as the rates of HBCD transfer between the central and the peripheral compartment are proportional to this. Figure 2(a) describes a two-compartmental model which is based on the partitioning of the lipophilic α-HBCD in the fat part of the fish (fat compartment) and the non-fat part (central compartment), the transfer of α-HBCD between these compartments and clearance from the central compartment. The edible part of farmed Atlantic salmon, the fillet, contains both fractions of the fat and central compartment. Figure 2(b) gives the body composition of the different compartments as a percentage of total volumes of a whole fish; as an example relative volumes are given of an approximately 1 kg Atlantic salmon. The whole fish can be divided into a central (87%) and fat compartment (13%). The central compartment can be divided into the muscle fibre fraction of the fillet (‘fillet muscle’, 58%) and other non-fat organs such as liver, kidney, gills etc. (‘others’, 29%). The fat compartment in which α-HBCD will accumulate can be divided into a fillet part (fillet fat, 8%) and fat in other organs such abdominal fat and bone marrow (‘rest fat’, 5%).

Figure 2. (a) Two-compartment PBPK model for the disposition of lipophilic compounds in muscle of adult consumption-sized Atlantic salmon (Salmo salar). A denotes the amount of contaminant, V the compartments’ volumes, and P the partition with respect to blood. The fat compartment serves as storage for the highly lipophilic contaminants. Fraction F of the dose is absorbed ($F_{\text{abs}}$, D) over the gut wall into the central compartment. K represents the elimination through liver clearance. Q represents the compartment transfer parameters from central to fat compartment and vice versa. (b) Body composition as a percentage of total volumes of a whole fish; as an example relative volumes are given of an approximately 1 kg Atlantic salmon. The whole fish can be divided into a central (87%) and fat compartment (13%).
Model equations

Figure 2(a) can be formulated mathematically as a set of two mass balances representing the changes in absolute amounts of \( \alpha \)-HBCD in respectively the central and fat compartment of the fillet.

\[
\begin{align*}
\frac{dA_c}{dt} &= F_{abs} \cdot D - (q_c(t) + k_c(t)) \cdot A_c + q_f(t) \cdot A_f \\
\frac{dA_f}{dt} &= q_c(t) \cdot A_c - q_f(t) \cdot A_f
\end{align*}
\]

(1)

where \( A \)’s denote HBCD amounts (ng) in tissue and subscripts \( c \) and \( f \) the central and peripheral fat compartment, respectively; \( D \) is the daily dose of HBCD; and \( F_{abs} \) is the fraction of ingested HBCD that is absorbed across the gut wall. In this equation, the time dependent rates

\[
q_c(t) = \frac{f \cdot Q(t)}{P_c \cdot W_c(t)}, \quad q_f(t) = \frac{f \cdot Q(t)}{P_f \cdot W_f(t)}, \quad k_c = \frac{CL \cdot W_f(t)}{P_c \cdot W_A(t)}
\]

denote HBCD transfer from the central to the peripheral compartment \( q_c \) and vice versa \( q_f \) and HBCD elimination from the body \( k_c \). These rates vary in time because of the salmon’s body growth. The parameter \( f \) denotes the fraction of cardiac output \( Q \) flowing to and from the fat compartment, \( P_c, P_f \) are partition coefficients and \( CL \) denotes clearance per unit of liver weight.

Observed compartment

Most often in toxicokinetic modelling the compartment of observation is (proportional to) a state variable. However, in this case the observed tissue is fillet which is composed of muscle, which is only part of the model’s central compartment, and part of the fat compartment (Figure 2b). Therefore, the amount of HBCD in fillet is:

\[
A_{\text{fillet}} = W_{\text{fillet}} \cdot C_{\text{fillet}} = A_{\text{muscle}} + \varphi \cdot A_f
\]

(3)

where only \( A_f \) is a state variable and \( \varphi \) is the fraction of total body fat in fillet. Muscle is part of the central compartment:

\[
A_{\text{muscle}} = \frac{P_{\text{muscle}} V_{\text{muscle}}}{P_{\text{muscle}} V_{\text{muscle}} + P_{\text{others}} V_{\text{others}}} \cdot A_c
\]

\[
= \frac{1}{1 + \frac{P_{\text{others}}}{P_{\text{muscle}}} \cdot \frac{V_{\text{others}}}{V_{\text{muscle}}} A_c} = \gamma \cdot A_c
\]

(4)

and so, the fillet concentration is modelled to be:

\[
C_{\text{fillet}}(t) = \frac{\gamma \cdot A_c + \varphi \cdot A_f}{W_{\text{fillet}}(t)}
\]

(5)

Parameter identification

The model contains seven unknown parameters: the fraction absorbed, the fraction of cardiac output to the fat compartment, the central and fat compartment partition coefficients, and the partition coefficients for the ‘muscle’ and ‘others’ central compartment subcompartments. This number can be reduced to six by noting that the last two partition coefficients are related to the central compartment partition coefficient by:

\[
P_{\text{others}} = \frac{V_{\text{other}}}{V_{\text{muscle}}} P_{\text{muscle}} \cdot V_{\text{muscle}} = P_c \cdot V_c.
\]

The model formulation leads to a concentration–time curve that contains only four parameters. Two of the unknown parameters concern the rate of change of concentration values, with an initial phase of relatively fast increase (decrease during depuration) followed by a terminal phase of relatively slow increase (decrease during depuration) (van Eijkeren et al. 2006; Hoogenboom et al. 2010). The other two parameters concern the corresponding concentration levels. The constant rate parameters case is described in detail by van Eijkeren et al. (2006). The current model is over parameterized by two parameters. We can further reduce the number of model parameters by considering the parameter value ratios \( f/P_c, f/P_f, CL/P_c \) and \( p = P_{\text{others}}/P_{\text{muscle}} \). This leaves still one unknown parameter too much. It is assumed that of the central compartment the ‘others’ tissue generally will contain more lipids than muscle so that the ratio of partition coefficients \( p \) will lie in the vicinity of two, will not be smaller than one and is not likely to exceed the value of four.

The model was implemented in ACSL (currently http://www.acs-lim.com) and model parameter optimization was performed with ACSL-Optimise by fitting the fraction absorbed \( F_{abs} \) and the parameter value ratios \( f/P_c, f/P_f, CL/P_c \) to the experimental data.

The muscle assimilation efficiencies (\( \alpha \)) were calculated according Tomy et al. (2004) by the following equation:

\[
\alpha = \frac{(\text{Control corrected concentration in muscle})}{(\text{Control corrected concentration in feed})} \ast \frac{(\text{mass of muscle})}{(\text{mass feed consumed})}
\]

Results and discussion

Uptake and elimination kinetics

Due to its lipophilic character (log \( K_{ow} \) 5.5; Hayward et al. 2006), \( \alpha \)-HBCD would be expected to partition to lipid-rich tissues. The fillet is one of the main organs for lipid storage in Atlantic salmon which accounts for up to approximately 60% of whole body fat (Torstensen et al. 2008). This accounts only for adult Atlantic salmon as muscle fat deposition rapidly
increases when the fish has reached a weight of approximately 1 kg (Berntssen et al. 2005). Since the kinetics of the lipophilic α-HBCD are likely to depend on muscle lipid content, the present trial used adult salmon with a start weight of approximately 0.7 kg and a final weight of approximately 2.5 kg. The fillet final lipid content was 13.8% of wet weight, which is representative for the fillet fat content of Norwegian-farmed Atlantic salmon (range = 12.6–18%; National Institute of Nutrition and Seafood Research (NIFES) 2009). Concurrent with the difference in lipid storage, farmed Atlantic salmon has higher fillet α-HBCD levels than lean farmed fish species (van Leeuwen et al. 2009). The dietary α-HBCD accumulated significantly between all sampling points, and no steady-state in α-HBCD accumulation was observed in fillet HBCD concentrations during the exposure period (Figure 3). The muscle assimilation, which is the product of both muscle uptake and elimination, was 31%, which is in the same range as found for juvenile rainbow trout (Law, Palace, et al. 2006). The spiked diets in the present study will likely contain a racemic mixture of the (−) and (+) α-HBCD enantiomers. A selective enrichment of the (−) α-HBCD enantiomer compared with the (+) α-HBCD enantiomer (Janak et al. 2005; Tomy et al. 2008; Harrad et al. 2009) indicates a difference in bioaccumulation and/or metabolism of the α-HBCD enantiomers. In the present trial, however, enantiomeric differentiation in accumulation was not assessed. The measured fillet α-HBCD total amount followed first-order elimination kinetics in adult Atlantic salmon (Figure 3b), with a continuously exponential decline in muscle α-HBCD during the depuration period. Earlier α-HBCD spiked dietary trials with juvenile rainbow trout did not show such first-order elimination kinetics; instead a clear two-phase elimination was observed with an initial rapid elimination (about 50% reduction of the accumulated α-HBCD within 7–14 days) followed by constant levels over prolonged periods (about 100 days) (Law, Palace, et al. 2006). The monoexponential elimination observed in the present study is typically for elimination from fat storage which behaves as a large capacitor with slowly reacting kinetics (van Eijkeren et al. 2006). A biphasic elimination is more typical for low-fat tissue which has a small capacitance for HBCD storage and often fast initial kinetics that reflect blood levels (van Eijkeren et al. 2006). The difference in elimination kinetics between the present trial and the trial with juvenile rainbow trout (Law, Palace, et al. 2006) is most likely due to differences in muscle lipid content. No muscle fat content is given for the juvenile rainbow trout used, but for other salmonids such as Atlantic salmon the flesh lipid content of juvenile fish (50–500 g) is around 3–8%, whereas adult Atlantic salmon (>1000 g) contains more than 14% fat (Berntssen et al. 2005).

**Model performance and parameter identification**

The two-compartment model as described in Figure 2 was applied to the data and the unknown scaling parameter values for intercompartment flow (f/Pc and f/Pf for transfer to the fat compartment and back to the central compartment, respectively), elimination (CL/Pc) as well as the fraction absorbed (Fabs) were fitted. Figure 3(a) shows the residue concentration data and the model computed concentration–time curves for α-HBCD in the fillet of consumption-sized Atlantic salmon based on the fitted parameter values. Figure 3(b) shows the model simulation of the total amount of α-HBCD in the fillet compartment and of...
the fat and non-fat central department. The model is made for growing salmon including a growth dilution over time giving a more curved fillet $\alpha$-HBCD concentration over time fit (Figure 3a) compared with total fillet $\alpha$-HBCD amount over time fit (Figure 3b). Comparing the decline of the amount of HBCD in fillet during depuration with the decline in HBCD concentration, it can be seen that a substantial part of the decline in concentration is caused by salmon growth. Indeed, the final HBCD amount is 59% of maximum at 56 days, while the final HBCD concentration is only 29%. Figure 4 gives residue concentration–time curves for $\alpha$-HBCD in salmon fillet at different partition coefficients ratios for the model parameter $p = P_{\text{other}} / P_{\text{muscle}}$ in Equation (4). The use of a partition coefficient of 2 gave the best fit on experimental data compared with higher (4) or lower (1) partition coefficients.

The kinetics in muscle tissue of the fillet (the central compartment of the fillet) are clearly biphasic and convex during contamination and concave during depuration (Figure 3b). The kinetics in the fat part of the fillet are almost monophasic and show the opposite convex/concave pattern (Figure 3b). In the present study, the contribution of the concentration in fat to the concentration in fillet masks the biphasic nature of the muscle and the fillet shows a more monophasic elimination (Figure 3b). This suggests the reduction of the two-compartment model to a one-compartment model where all kinetics are explained by the fat compartment. However, such an approach is invalid as it would only poorly account for the growth differentiation in body composition as is a dominant factor in the farming of Atlantic salmon. Moreover, the two-compartment approach can be applied on the monophasic experimental dataset by fixing a reasonable range for the parameters $f / P_c$ and $f / P_f$ (0.006–0.06 and 0.05–0.2, respectively) for fitting the values for $CL / P_c$ and $F_{\text{abs}}$. Applying the range of different initial values of $f / P_c$ and $f / P_f$ gave stable fitted values with little variation (for example, CV was 4% for fitted values $F_{\text{abs}}$). The low variation shows the robustness of the two-compartment model for the experimental fillet $\alpha$-HBCD elimination data, which are dominated by the fat fraction of the fillet.

The daily absorbed dose in the central compartment ($F_{\text{abs}}$), i.e. uptake over the gastrointestinal tract into the systemic blood circulation, is estimated to be approximately 70% of the dietary intake. The corresponding terminal half-life time of the total amount of $\alpha$-HBCD in fillet is not constant due to changing body composition. The terminal total amount of $\alpha$-HBCD in fillet half-life was calculated to range from 310 days for juvenile Atlantic salmon to 165 days for adults (>5 kg salmon). The terminal half-life time of the concentration of $\alpha$-HBCD in fillet ranges from 53 days for juvenile Atlantic salmon to 160 days in adult Atlantic salmon. During the duration of the experiment, the HBCD concentration decreases more than twice as fast as the total amount contained in fillet. The decrease in HBCD concentration is the result of both elimination and growth, while the decrease in total amount HBCD represent only elimination. During the end of a long-term (>300 days) farming of harvest size Atlantic salmon, the contribution of the relative fillet weight growth, which accounts for the shorter half-life time of concentration of $\alpha$-HBCD in fillet, becomes negligible with respect to the terminal elimination rate of the total amount of $\alpha$-HBCD in fillet and both half-lives (of HBCD concentration and total amount) converge to the same value.

**Salmon feeds and model simulation of HBCD carry-over from feed to fillet**

Fish oils is the dominant source for POPs in high energy salmon grower feeds and hence farmed salmon (Easton et al. 2002; Jacobs et al. 2002; Berntssen et al. 2005; Berntssen, Olsvik, et al. 2010) and both the source of fish oil and inclusion level in fish feed determine the degree of feed contamination. Large variations exist in background fish oil POP levels, depending on season, fish species and geographical origin of the pelagic fish used to produce fish oil (Berntssen and Lundebye 2009). An HBCD level of

![Figure 4. Measured concentrations in fillet (data points from three fish per time point) and model-simulated concentration–time curve based on fitted parameters for Atlantic salmon (Salmo salar) fillet. Atlantic salmon was fed $\alpha$-HBCD spiked feed for 54 days, followed by a depuration period of 84 days. The model-simulated curves are based on a standard and best-fit partition coefficient of two (straight line), as well as one low (one; dashed line) or high (four; dotted line) partition coefficients.](image-url)
11 µg kg⁻¹ has been reported for a Northern Atlantic fish oil, which has relatively high background levels of POPs but still under the European Union permissible levels for dioxins, dioxin-like PCBs and organochlorin pesticides in fish oils (Berntssen, Julshamn, et al. 2010). Traditional high-energy feeds for adult (>2 kg) Atlantic salmon can contain up to 36% fish oils (Tacon and Metian 2008). However, the rapid growth of aquaculture and increasing pressure on feral fish stocks, and hence limited access to fish meal and oil, has lead to the development of salmon feeds that rely less on marine ingredients (Espe et al. 2006; Drew, Borgeson, et al. 2007; Tacon and Metian 2008; Torstensen et al. 2008). Estimated fish oil inclusion in salmon feeds in 2010 was 12–15%, while in 2020 a predicted 6% fish oil will be used (Tacon and Metian 2008). Based on these fish oil inclusion levels, the use of high background POP (11 µg kg⁻¹ HBCD) fish oils in salmon feeds would give HBCD levels of approximately 4 µg kg⁻¹ for maximum fish oil feeds, while current salmon feed will give 1.3 µg kg⁻¹, and future replacement feeds would have levels of approximately 0.7 µg kg⁻¹. Earlier studies on the replacement of fish oil with vegetable oils showed a reduction in the levels of notorious POPs in farmed fish (Bell et al. 2005; Berntssen et al. 2005; Drew, Ogunkoya, et al. 2007; Friesen et al. 2008; Nacher-Mestre et al. 2009; Berntssen, Julshamn, et al. 2010).

The HBCD carry-over model was employed to estimate fillet HBCD levels in Atlantic salmon under realistic farming conditions with the use of a fish oil with permissible POP levels in full fish oil-based feeds (4 µg kg⁻¹ feed), current fish oil inclusion level feeds (1.3 µg kg⁻¹ feed), or high fish oil replacement feeds (0.7 µg kg⁻¹ feed). Farming conditions from a commercial scaled feeding trial were used as input data in the HBCD carry-over model (Berntssen, Olsvik, et al. 2010; Lock et al. 2011). The input data to the model included seasonal variations in daily HBCD intake, growth rate and fat deposition in Atlantic salmon farmed from about 80 g to about 5 kg during an 18-month period (Lock et al. 2011). The daily dietary HBCD intake per salmon, weight gain and relative fillet fat deposition during a seawater production cycle are given in Figure 5. The gradually increased HBCD intake over time is due to an

![Figure 5](image-url)

**Figure 5.** Time-course farming condition data of a sea water production cycle of farmed Atlantic salmon (Berntssen, Olsvik, et al. 2010; Lock et al. 2011) which is used as input data to model simulation, including daily HBCD intake (a), weight gain (b), and relative fillet fat deposition as a percentage of whole body fat present in the fillet (c). Sea water temperature data are given to identify cold and warm water periods (d).
increased inclusion level of fish oil in feeds for larger salmon (Berntssen, Olsvik, et al. 2010; Lock et al. 2011). The temporary reduction in HBCD intake during the winter periods (January–March) is due to a reduced feed intake during these cold water (about 4°C) periods (Figure 5a and d). The weight increase, and hence growth dilution, is maintained during these cold water periods (Figure 5b) due to more efficient feed to muscle conversion (Lock et al. 2011) (Figure 5b). The lipid content in both whole fish and fillet is increasing rapidly in adult Atlantic salmon (approximately >1 kg) (Berntssen et al. 2005), with fillet accounting for a more increasing portion (approximately 55%) of the total fat deposition in adult fish compared with juvenile fish (Figure 5c). The model predictions of total HBCD amount in fillet (even line) and HBCD concentration in fillet (broken line) in farmed Atlantic salmon are given in Figure 6 (a, b and c for high, current and low fish oil inclusion in salmon feeds, respectively). The fillet HBCD concentration increases gradually over time and follows an oscillating pattern with decreased accumulation during the low temperature winter periods with low feed intake. The rapid increase of total HBCD during the last (>300 days) part of the farming coincides with the increased relative fat deposition in the fillet of adult (>2 kg) Atlantic salmon.

**Farmed salmon and human exposure to HBCD**

Employment of the HBCD model simulates fillet levels of about 1.8, about 0.5 and about 0.2 µg kg⁻¹ in harvest sized (about 5 kg) salmon farmed on full fish oil feeds, current fish oil inclusion feeds and replacement feeds (Figure 6a, b and c, respectively). Average values found on the Norwegian market for salmon in 2007 were 0.5–0.4 µg kg⁻¹ w/w (Knutsen et al. 2008; Schecter et al. 2010) with maximum reported levels of 1 µg kg⁻¹ w/w for Norwegian farmed Atlantic salmon.

![Figure 6](image-url)

Figure 6. Model-simulated levels total amount (µg; straight line, left y-axis) and concentration (µg kg⁻¹; broken line, right y-axis) of α-HBCD over time in the fillet of Atlantic salmon farmed under realistic conditions during a full seawater production cycle with either a (a) high, (b) moderate or (c) low background levels of α-HBCD in the feeds.
in 2008 (NIFES 2008). The model-predicted HBCD fillet concentrations of salmon reared on current (12%) fish oil inclusion feeds are similar to average surveillance data. No upper limits for HBCD have been set in feed or food products. However, the United States Environmental Protection Agency (USEPA) has set a reference dose (RfD) for $\Sigma$HBCD of 200 ng kg$^{-1}$ body weight day$^{-1}$ (Roosens, Cornelis, et al. 2010). Model simulation fillet levels will contribute to about 3%, about 0.8% and about 0.3% when one portion of 200 g salmon is consumed by a 60-kg adult. Average HBCD levels (0.5 $\mu$g kg$^{-1}$ w/w) and maximum levels found on the Norwegian market (1 $\mu$g kg$^{-1}$ w/w) would contribute to <1% of RfD. Both food and atmospheric exposure (dust and air) are the important sources for human exposure, and estimates of contribution of atmosphere or food to HBCD exposure varies largely. In a duplicate diet study in Belgium food intake was the most important contributor to total HBCD intake (mean = 67%, range = 23–93%) at an average dust intake, while at high dust intake food and dust had an equal contribution to the total $\Sigma$HBCD intake (Roosens et al. 2009). In another Belgium study the daily intake of HBCD in adults from food was estimated to be nearly 12-fold higher than the intake from dust (Roosens, Cornelis, et al. 2010). Of food products, highest levels of HBCD was reported for oily fish, which was predominantly as $\alpha$-HBCD (Knutsen et al. 2008). The Norwegian population has a relatively high intake of seafood, consequently consumption of oily fish such as Atlantic salmon and trout are important dietary source for $\alpha$-HBCD exposure (Knutsen et al. 2008). When assuming an equal exposure by atmosphere and food at high dust intake (Roosens et al. 2009), the RfD for food intake would be 100 ng kg$^{-1}$ bw day$^{-1}$. The highest model predicted fillet concentrations will contribute to 6% of the food RfD when fish oils with a relatively high POP level are used in feed to farmed Atlantic salmon.

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