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Carry-over of dietary organochlorine pesticides, PCDD/Fs, PCBs, and brominated flame retardants to Atlantic salmon (*Salmo salar* L.) fillets

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ABSTRACT

Information on carry-over of contaminants from feed to animal food products is essential for appropriate human risk assessment of feed contaminants. The carry-over of potentially hazardous persistent organic pollutants (POPs) from feed to fillet was assessed in consumption sized Atlantic salmon (*Salmo salar*). Relative carry-over (defined as the fraction of a certain dietary POP retained in the fillet) was assessed in a controlled feeding trial, which provided fillet retention of dietary organochlorine pesticides (OCPs), dioxins (PCDD/Fs), polychlorinated biphenyls (PCBs), and brominated flame retardants (BFRs). Highest retention was found for OCPs, BFRs and PCBs (31–58%), and the lowest retentions were observed for PCDD/Fs congeners (10–34%). National monitoring data on commercial fish feed and farmed Atlantic salmon on the Norwegian market were used to provide commercially relevant feed-to-fillet transfer factors (calculated as fillet POP level divided by feed POP level), which ranged from 0.4 to 0.5, which is a factor 5–10 times higher than reported for terrestrial meat products. For the OCP with one of the highest relative carry-over, toxaphene, uptake and elimination kinetics were established. Model simulations that are based on the uptake and elimination kinetics gave predicted levels that were in agreement with the measured values. Application of the model to the current EU upper limit for toxaphene in feed (50 μg kg^{-1}) gave maximum fillet levels of 22 μg kg^{-1}, which exceeds the estimated permissible level (21 μg kg^{-1}) for toxaphene in fish food samples in Norway.

1. Introduction

Farmed Atlantic salmon (*Salmo salar*) is known to contain relatively high levels of persistent environmental contaminants which are potentially hazardous to the consumers. These environmental contaminants include polychlorinated biphenyls (PCBs), dioxins [polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), (PCDD/Fs)], polybrominated diphenyl ethers (PBDEs), hexabromocyclodecane (HBCD), and organochlorine pesticides (OCPs) (Hites et al., 2004; Maule et al., 2007; Shaw et al., 2008; van Leeuwen et al., 2009). Farmed oily fish, such as Atlantic salmon, has higher concentrations of these persistent organic pollutants (POPs) than lean farmed fish species such as tilapia (*Oreochromis mossambicus, Oreochromis niloticus*), pangasius (*Pangasius hypophthalmus*) and gilt head seabream (*Sparus aurata*) (Nacher-Mestre et al., 2009; van Leeuwen et al., 2009). 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, 2010b). Estimated global production of farmed salmon (including *S. salar*, *Oncorhynchus kisutch*, *O. tshawytscha*) is ~1800 thousand metric tonnes with an expected production of ~2900 thousand tonnes in 2020 (Tacon and Metian, 2008), and the consumption of farmed fish is expected to exceed that of feral fish. Seafood, and oily fish in particular, is a dominant contributor to POP exposure such as PCDD/Fs and dioxin-like PCBs (DL-PCBs) in the human diet (Bergkvist et al., 2008; De Mul et al., 2008; Fattore et al., 2008; Voorspoels et al., 2008; Kvalem et al., 2009; van Leeuwen et al., 2009). The European Union (EU) has established upper limits for several POPs 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; EC, 2005, 2006; Ribo et al., 2009). Information on carry-over 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). In order to provide a pro-active approach to protecting consumer safety, feed legislation ought to assure compliance with food safety legislation. Whereas for terrestrial farmed animals (such as cows, pigs, and broilers) carry-over data is available (Kan and Meijer, 2007) and carry-over data bases have been established (Kan and Meijer, 2007; Leeman et al., 2007), relatively little

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is known about the carry–over of POPs in feed to farmed oily fish such as Atlantic salmon, despite the importance of this food product for human exposure to these potentially hazardous contaminants. Most knowledge on the dietary carry-over of POPs in fish comes from ecotoxicological studies on trophic transfer of POPs in the aquatic food chain (e.g. (Fisk et al., 1998; Maruya et al., 2005).

Several kinetic processes determine the extent of carry-over of contaminants from feed to the edible part of the fish. In addition to the magnitude of feed contamination, feed consumption and growth rates are important factors in determining the carry-over of feed contaminants, with increased contaminant levels in fish associated with decreased growth efficiency (growth per feed consumed) (Berntssen et al., 2005, 2007; Trudel and Rasmussen, 2006). Fish species, salinity, and temperature are also factors affecting dietary carry-over kinetics (Opperhuizen and Sijm, 1990; Seubert and Kennedy, 2000). Assessment of carry-over in large consumption sized fish is essential to provide appropriate risk assessments of feed contaminants for humans. The carry-over of several individual feed contaminants in farmed fish have been studied in separate studies, such as chlordane and toxaphene in freshwater reared rainbow trout (*Oncorhynchus mykiss*) (Karl et al., 2002), PCDD/Fs and PBDEs in seawater reared rainbow trout and Atlantic salmon (Isosaari et al., 2002, 2004, 2005; Berntssen et al., 2007), and methylmercury in freshwater rainbow trout (Ciardullo et al., 2008). Differences in experimental conditions (e.g. study duration, magnitude of feed contamination, fish species, temperature, fresh or seawater) among these studies make it difficult to compare the relative carry-over of the multitude of environmental feed contaminants present in feed and farmed fish.

The present study aims to conduct a comparative assessment of the relative carry-over of several potentially hazardous environmental contaminants by assessing dietary accumulation in a long-term feeding trial of market-size Atlantic salmon. Controlled experimental conditions were used with known and stable POP levels in the formulated feed, controlled feed intake, and measured fillet POP levels. For selected contaminants with the highest carry-over potential, uptake and elimination kinetic rates were established in a cross-over design and used in a one-compartmental carry-over model that includes aquacultural parameters such as relative feeding rate and growth. While relative carry-over assessment was based on experimental feed and fillet data, commercially realistic transfer factors were based on data from the National Monitoring programmes on contaminants in farmed Atlantic salmon and commercial salmon feeds. The transfer factors were assessed similar to those used in comprehensive databases for POP feed-to-fillet carry-over in terrestrial farmed animals (Leeman et al., 2007). Detailed information on congeners specific contamination levels in feeds and fish fillets from the present trial are published elsewhere (Berntssen et al., 2010a).

2. Material and methods

2.1. Fish and diets

Details of the experimental conditions and production performance such as growth and feed conversions are presented in detail by Berntssen et al. (2010a) and Torstensen et al. (2008). Briefly, the feeding trial was carried out at Matre Aquaculture Research Station (Matredal, Norway; 60°52′N. 05°35′E). Atlantic salmon smolt with an initial weight of ~0.3 kg were fed a diet containing a high inclusion level of marine fish oil and fish meal, in triplicate tanks, over a period of twelve months until the fish reached a weight of ~ four kg. The detailed description of the feed formulation formulated diet is described by Torstensen et al. (2008). The composition was 28% fish oil, 56% fish meal, and 16% wheat. This feed was formulated to represent the upper range of POPs normally found in commercial feeds. The experimental feeds were produced by Skretting ARC, Stavanger, Norway. Seawater adapted Atlantic salmon smolt were kept in fibreglass tanks (500 fish per tank), with a continuous flow-through of seawater (salinity 34.9 ‰) from a deepwater inlet (Matrefjord). Temperature and oxygen were continuously recorded automatically, and kept at 8.9 ± 0.1 °C and 80% saturation, respectively. Fish were fed in excess twice a day with automatic feeders for half an hour. Excess feed was collected in a flow-over system and weighed in order to assess the daily feed intake.

2.2. Cross-over design

After seven months, a cross-over design was used to assess the accumulation and elimination kinetic rates of selected POPs. Atlantic salmon that were fed on the diet with a high inclusion of fish oil and fish meal ("high contaminant" feed) were transferred to a "low contaminant" feed where fish oil and fish meal were replaced with feed ingredients of plant origin that have low levels of POPs (for details see Berntssen et al., 2010a). Conversely, fish previously fed on "low POP" feed was transferred to the "high POP" marine feed. Half of the fish were randomly fin clipped, and transferred to tanks that received the opposite diet, while the non-fin clipped fish were maintained on their original diet. The cross-over of half of the fish per tank was performed in all triplicate tanks per experimental diets. Carry-over was assessed on the other half of the fish which were maintained on their original diet with triplicate tanks per diet. The cross-over feeding lasted for five months and ten fish per tank were sacrificed after 0, 24, 35, 65, 85, and 120 d of cross-over. The carry-over was assessed at the end of the twelve month trial (336 d).

2.3. Comparative carry-over assessment

In the present study, relative carry-over of dietary POPs was defined as fillet retention rates. The carry-over was assessed in Atlantic salmon fed on the experimental diets for twelve months. Retention was calculated as the percentage of contaminants in the edible part of the fish in relation to the total dose consumed according to Formula one.

$$\text{retention}(\%) = \frac{(\textit{Conc.}\ \text{fillet}_{t=end}(\mu\text{g kg}^{-1}) * \text{mass}_{t=end}(\text{kg})) - (\textit{Conc.}\ \text{fillet}_{t=0}(\mu\text{g kg}^{-1}) * \text{mass}_{t=0}(kg))}{\textit{Conc.}\ \text{feed}(\mu g\ \text{kg}^{-1}) * \text{amount feed consumed}(\text{kg})} \quad (1)$$

Transfer factors were calculated as described by Leeman et al. (2007) for the animal product carry-over data base and is expressed as the concentration of a contaminant in animal products (mg kg^{-1} wet weight) divided by the concentration of the contaminant in animal feed (mg kg^{-1} wet weight). To provide realistic feed-to-fillet transfer factors for salmon food products on the Norwegian market, National monitoring data on commercially-reared Atlantic salmon and commercially-produced salmon feeds were used (NIFES, 2007, http://www.nifes.no).

2.4. Kinetic toxaphene model

For the POP with one of the highest observed comparative carry-over, toxaphene, a feed-to fillet carry-over model was established based on the uptake and elimination kinetics obtained from the cross-over part of the trial. Fillet levels of toxaphene at different time points were corrected for growth and control levels. Growth rates were calculated by fitting fish weight to the equation; ln fish weight = $a + b^{*}t$, where a is a constant, b the growth rate (g d^{-1}), and t the time. All fillet toxaphene concentrations used to establish the toxaphene kinetics (C_{fillet}) were multiplied by the factor ($1 + b^{*}t$) to correct for growth dilution. The elimination constant (k_{el}), which includes non-metabolic and metabolic elimination, was determined by fitting concentration data to a first-order decay curve; ln $C_{fillet} = a + k_{el}{}^{*}t$. Elimination half-lives ($t_{1/2}$) was calculated as $\ln 2/k_{el}$. The uptake rates were calculated by fitting the concentration data (Statistica, Statsoft Inc., Tulsa USA, 1993) to the integrated form of the kinetic rate Eq. (2) for constant dietary exposure (Sijm et al., 1993; Berntssen et al., 2007).

$$\alpha = \frac{C_{fillet}(t) \cdot k_{el}}{F \cdot C_{feed}[1 - \exp(-k_{el} \cdot t)]} \quad (2)$$

where C_{feed} is the total toxaphene concentration (μg g^{-1} wet weight) in feed; α is the uptake rate constant; and F is the feeding rate (g feed g^{-1} fish d^{-1}). Fillet concentrations were modeled by using formula (two) re-written as Eq. (3), which is a simple model-based one compartment first-order rate kinetics (Sijm et al., 1993).

$$C_{fillet}(t) = \frac{\alpha F t}{K_{el} + b} C_{feed}(1 - e^{-(k_{el}+b)t}) + C_{fillet0} e^{-(k_{el}+b)t} \quad (3)$$

where b is the growth rate and $C_{fillet0}$ is the concentration in the fillet at the start of the exposure.

2.5. Sample treatment

Samples of the feeds (pooled samples from six different 25 kg bags per diet type) were collected in aluminium foil for POP determination and stored at −30 °C until analyses. The experimental feeds were homogenised in a blender and analyzed independently in triplicate ($n = 3$ for analytical replicates). Fish were sampled at five time points during the cross-over and at the end of the experiment, and fillet samples (skin-off but including subcutaneous fat) were taken from 10 fish per tank, with three separate treatment tanks per diet. The fillets from each tank were pooled, homogenised, frozen and stored at -30 °C until analysed (N = 3 for experimental tank replicates per diet).

2.6. Analysis

All fish samples were freeze-dried and well homogenised prior to analysis. The samples were analysed for the PCDDs, PCDFs, and DL-PCBs congeners which have been assigned a WHO-TEF_{1998} and are included in the current EU maximum limit (Van den Berg et al., 1998, 2006; Van den Berg, 2006), the six indicator PCBs for none dioxin-like PCBs (NDL-PCBs) and seven indicator congeners of PBDE which the European Food safety Authority have advised to monitor (EFSA, 2005, 2006), in addition to those OCPs with a current EU upper limit in feed (EC, 2002). For toxaphene the analytical method include the congeners recommended by EFSA (40, 41, 42a, 44, 32) (EFSA, 2005) as well as the congeners included in EU legislation (26, 50, 62)(EC, 2005). For PCDD/Fs and DL-PCB analyses, sample material was scaled to give approximately 25 pg sum TEQ, and maximum 3 g of fat. For PCB and OCPs, approximately 2–3 g wet weight material was solvent extracted while for PBDE and toxaphene 10 g approximately 10 g wet weight material was used. PCBs and DDTs. Surrogate internal standard PCB-53 (Cambridge Isotope Laboratories, Andover, MA, USA) was added to the samples. Sample material was pressure solvent extracted with hexane on a Dionex ASE 300 accelerated solvent extractor (Dionex Sunyvale, CA, USA). Acid-impregnated silica was added to the extraction cell for on-line clean-up, and co-extracted fat was removed in an external clean-up procedure by adding concentrated sulphuric acid to the extract. Determination of DDTs and NDL-PCBs was performed by gas chromatography/mass spectrometry (Thermo Quest Trace GC/MD800, Thermo Finnigan, Bremen, Germany) in electron impact, SIM mode. The GC was equipped with a fused silica capillary column (30 m × 25 mm i.d. 25 μm film thickness HP-5MS Column, Agilent J&W, Sanata Clara, CA, USA). Quantification was performed according to the internal standard (IS) method using congener-specific RRFs from a linear congener specific external standard curve relative to the internal surrogate standard. Recoveries were between 85% and 110% and limit of quantifications (LOQs) were between 0.06 μg kg^{-1} and 0.24 μg kg^{-1} ww for DDTs and its metabolites and the NDL-PCB congeners.

2.7. Toxaphene and brominated flame retardants (BFRs)

Surrogate internal standards [toxaphene-414 (LGC standards, Teddington, UK,) for toxaphene congeners and PBDE 139 EO-5100 for BFRs (Cambridge Isotope Laboratories, Andover, MA, USA)] were added prior to extraction. Sample material was pressure solvent extracted with 80:20 dichloromethane:hexane (v/v) on ASE 300. A clean-up procedure similar to the PCB/DDT analysis was performed. Determination of toxaphene and BFRs was performed by GC/MS (TRACE GC Ultra™/DSQ ™ Single Quadrupole GC/MS, Thermo Finnigan, Bremen, Germany) in negative chemical ionization, SIM mode. The instrument was equipped with a RTX-5MS fused silica capillary column (30 m × 0.25 mm i.d. 25 μm film thickness, Restek, Bellefonte, PA, USA). Quantification was performed according to the IS method from a five-point linear congener specific external standard curve relative to the internal surrogate standard. Recoveries were between 102% and 113% for toxaphene and between 81 and 118% for BFRs. LOQs were between 0.1 μg kg^{-1} and 2.5 μg kg^{-1} ww for toxaphene and 0.03 μg kg^{-1}–0.5 μg kg^{-1} ww for BFRs.

2.8. OCPs other than DDT and Toxaphene

Prior to extraction, surrogate internal standards were added to the samples [^{13}C-labelled HCB, trans-chlordane and heptachlor (Cambridge Isotope Laboratories, Andover, MA, USA)]. The samples were extracted with hexane on ASE 300 and further purified on three sequenced solid phase extraction (SPE) columns [Chem Elut™, BondElut® C18, and BondElut® Florisil columns, respectively, Varian Inc., USA, for solvent conditions see Berntssen et al. (2010a)] in an automated column system (ASPEC™ XL4, Gilson, USA). Determination of OCPs was performed by GC/MS (TRACE GC Ultra™/DSQ ™ Single Quadrupole GC/MS, equipped with an HP-5MS 30 m × 25 mm i.d. 25 μm film thickness capillary column). The instrument was run in negative chemical ionization, SIM mode. Quantification was performed according to the IS method from a four-point linear congener specific matrix matched standard curve. Recoveries for OCPs was between 75% and 136%, and the LOQs were between 0.07 μg kg^{-1} and 2.5 μg kg^{-1} ww.

2.9. PCDD/F and DL-PCBs

Sample material was pressure solvent extracted with hexane on ASE 300. Surrogate internal standards were added prior to extraction [^{13}C labelled EDF-4147, 6999, 7999, 8999, 9999, 4097, 9999-3-

4, 5999 for PCDD/F and EC-4935, 4937, 4976, 4976-3, 4979 for DL-PCBs (Cambridge Isotope Laboratories, Andover, MA, USA)]. The sample extracts were purified using a sequence of columns [H_2SO_4 on silica, multilayered silica, basic alumina and carbon column, respectively, on an automated PowerPrep system, FMS, Waltham, MA, USA, for solvent conditions see (Berntssen et al., 2010a)]. After elution, the samples were concentrated using Turbovap II™ (Zymark, USA). Prior to PCDD/F and DL-PCBs determination, a mixture of ^{13}C labelled performance standards [EDF 5999 for PCDD/F and EC-4979 for DL-PCBs, Cambridge Isotope Laboratories, Andover, MA, USA] was added. Separation and quantification was performed by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS, MAT 95XL Thermo Finnigan, Bremen, Germany), equipped with a fused silica capillary column (30 m × 0.25 mm i.d. and 0.25 μm film thickness, RTX-5SILMS, Restek, Bellefonte, USA). Recoveries were calculated as described in the USEPA methods (USEPA, 1994) and ranged from78% to 110%. Analyte concentrations of the natural compounds were quantified according to the internal standard isotope dilution method using congener-specific relative response factors (RRFs) determined from three point calibration standard runs (CS1–CS3, Cambridge Isotope Laboratories, Andover, MA, USA) according to the USEPA 1613 method (USEPA, 1994). Final quantified PCDD/F and DL-PCB values are expressed as pg upperbound WHO-TEQ g^{-1}wet weight (ww) in WHO-TEQ using the WHO-TEFs from 1998 according to EU legislation (EC, 2006). The limits of quantification for the test samples were in the range of 0.02 pg g^{-1}–0.83 pg g^{-1} ww for PCDD/Fs, 0.07 pg g^{-1}–1.5 pg g^{-1} ww for non-ortho PCBs and 0.6 pg g^{-1}–166 pg g^{-1} ww for mono-ortho PCBs.

2.10. Quality assurance

All analytical methods used have been validated in-house according to the Nordic Committee on Food Analysis (NMKL) Procedure No. 4 Validation (2005). The limit of detection (LOD) was statistically estimated as the analyte concentration giving a peak signal of three times the background noise from an internal surrogate standard spiked procedural blank. The 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. The trueness of the methods has been 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 z-score $< +2.0$ and repeatability as RSD (%) of 10% and better). Further references to the results of interlaboratory proficiency test and quantification quality assurance procedures are given by Berntssen et al. (2010a). The analytical methods for determination of PCDD/F, DL-PCBs, NDL-PCBs, DDTs and BFRs are accredited in accordance with ISO-EN 17025 by the Norwegian accreditation authority.

3. Statistics

All statistics were performed using the programme STATISTICA™ (Statsoft Inc. USA, 1993). Differences among POPs carry-over were assessed using one-way analysis of variance (ANOVA) (Zar, 1984). The Kolmogrov–Smirnov test was used to assess normality of distribution of each treatment (Zar, 1984). Dependent variables were checked for homogeneity of variance by the Levene F-test and transformed when necessary (Zar, 1984). Where the null hypothesis (H_0: no difference between treatments or within treatment at different time intervals) was rejected the position of significant differences among treatments were tested using Tukey's HSD test ($P < 0.01$). Regression analysis was used to assess the relationship between biomagnifications and physical–chemical properties by least square regression analyses.

4. Results and discussion

4.1. Concentrations and legislation

Detailed information on congeners specific contamination level in feeds and fillet is given by Berntssen et al. (2010a). Concentrations of sum PCDDs, PCDFs, and DL-PCBs assigned WHO-TEF, non dioxin-like PCBs (PCB-6), brominated flame retardants [hexa bromocyclododecane (HBCD), and polybrominated diphenyl ether mixtures (PBDEs)], and organochlorine pesticides (HCB, toxaphene, and DDT) in fish feeds and Atlantic salmon fillets are given in Table 1. The level of persistent organic pollutants (POPs) in fillets of Atlantic salmon in the current study were in the upper range compared with commercially-reared Atlantic salmon fillets on the Norwegian market (Table 1). Replacement of fish oil with vegetable oils is known to reduce the level of POPs in fish feed and fish fillets (Bell et al., 2005; Berntssen et al., 2005; Drew et al., 2007). This replacement has lead to a reduced inclusion of fish oil in commercial salmon feeds. Over the last decade, fish oil has been partially replaced by vegetable oils in commercially-produced salmon grower feeds. Salmon feeds (for *S. salar, O. kisutch*, and *O. tshawytscha*) produced in 2006 contained 20–30% fish oil whereas it was predicted that in 2010 this would be reduced to 12–15% fish oil. By 2020 salmon grower feeds are expected to contain only approximately 8% fish oil (Tacon and Metian, 2008). An additional way to reduce POPs in farmed Atlantic salmon is the use of commercially available purification techniques, which remove the POPs from the fish oils and consequently the Atlantic salmon production chain (Usydus et al., 2009; Berntssen et al., 2010b; Oterhals et al., 2010; Sprague et al., 2010). In the present trial the fish oils were not purified and a relatively high (28%) inclusion level of fish oils was used. Hence, the contaminant levels in the salmon feed were relatively high with contaminant levels just under, or for PCDD/Fs and DL-PCBs just above, the EU upper limits in fish feed (Table 1). In contrast, the levels found in Atlantic salmon fillets reared on these high contaminant feeds were considerably (threefold) below the current EU upper limits for sum PCDD/Fs and DL-PCBs for fish as a food products. Feed legislation is often based on the precautionary principle where upper limits for contaminants in feed are related to background levels normally observed in commercial feeds applying the principle of as low as reasonably achievable (ALARA). Whereas maximum limits for POPs in food are often based on the internationally established tolerable weekly intake of a contaminant and the relative contribution of a food product to the total dietary exposure to the contaminants (van Raamsdonk et al., 2009) in addition to the ALARA principle. Empirical assessment of feed-to fillet carry-over would provide the basis for a harmonized feed and food legislation which links feed safety limits with food limits.

4.2. Carry-over of POPs from feed to fillet

The carry-over rate is the fraction of a certain contaminant which is retained in the edible tissue, in the case of fish this is generally the fillet. Expressing carry-over as retention partly compensates for differences in growth efficiency as concentrations in fish and feed are multiplied by mass growth (kg) per consumed feed (kg) (Formula one). Retention calculations can only be performed when feed consumption and growth is known, as in the present study. Earlier trials have reported on the feed to fillet transfer of individual POPs in farmed fish (Isosaari et al., 2002, 2004, 2005;

Table 1
Concentrations and EU upper limits (values in parentheses are newly suggested limit for dioxins and dioxin like-PCBs using the WHO-TEQ values of 2005) of dioxins (PCDD/Fs) and dioxin-like PCBs (DL-PCBs), non dioxin-like PCBs (NDL-PCBs), brominated flame retardants [hexa bromocyclododecane (HBCD), and polybrominated diphenyl ether mixtures (PBDEs)], organochlorine pesticides (i.e. HCB, toxaphene, and DDT), in feeds and Atlantic salmon fillets (*Salmo salar*) fed diet for 12 months (mean ± standard deviation in parentheses, $n = 3$ for analytical replicates for feed and experimental replicates for fish), NQ = not quantified. For comparison surveillance data is given for commercially Atlantic salmon fillets on the Norwegian market in 2007 (mean, min–max, and number of samples, n in parentheses under).

Contaminants	Feed	Consumer sized (~4 kg) salmon	Commercially-reared Atlantic salmon 2007
Dioxins (WHO-TEQ pg g^{-1} ww^{-1})			
Sum PCDDs	0.61 ± 0.1	0.14 ± 0.02	
Sum PCDFs	1.4 ± 0.2	0.25 ± 0.01	
Sum PCDD/Fs	2.05 ± 0.2	0.39 ± 0.02	0.33 (0.18–0.64)
EU limit PCDD/Fs	2.25	4 (2.25)	($n = 39$)
Sum DLPCBs	6.08 ± 0.3	2.1 ± 0.1	
Sum PCDDF + DLPCBs	8.13 ± 0.4	2.4 ± 0.1	1.3 (0.7–2.2)
EU limit PCDD/F + DLPCBs	7	8 (4)	($n = 39$)
Non dioxin-like PCBs (ng g^{-1} ww)			
sum PCB-6	38 ± 3	13 ± 1	13 (4.1–20) ($n = 31$)
Brominated flame retardants (ng g^{-1} ww)			
Sum – 7 PBDEs	7.3 ± 0.9	2.6 ± 0.7	1.3 (0.4–2.0)
HBCD	3.1 ± 0.8	NQ	($n = 24$)
Organochlorin pesticides (ng g^{-1} ww)			
HCB	9.8 ± 0.	2.9 ± 0.4	1.4 (0.8–3.5)
EU limit	10		($n = 27$)
Sum toxaphene	25 ± 3	12 ± 0.4	(<5.7–16.9)
EU limit	50		($n = 27$)
Sum DDT	49 ± 2	21 ± 0.6	19 (13–28)
EU limit	50		($n = 12$)

Table 2
Fillet retention (% of consumed, mean ± SD) of brominated flame retardants [hexa bromocyclododecane (HBCD), and polybrominated diphenyl ethers (PBDEs)], organochlorine pesticides (HCB, toxaphene, and DDT), dioxins (PCDD/Fs) and dioxin-like PCBs (DL-PCBs), non dioxin-like PCBs (NDL-PCBs) in consumer sized filet of Atlantic salmon (mean ± standard deviation, n = 3 for experimental replicates).

BFRs	Retention (%)	OCPs	Retention (%)	PCDD/Fs	Retention (%)	PCBs	Retention (%)
HBCD	35 ± 1	HCB	34 ± 1	2378-TCDD	34 ± 3	PCB 101	50 ± 2
PBDE-100	45 ± 3	Toxaphene-26	46 ± 5	12378-PeCDD	29 ± 5	PCB 138	42 ± 3
PBDE-153	41 ± 2	Toxaphene-50	45 ± 2	123678-HxCDD	19 ± 1	PCB 153	47 ± 3
PBDE-154	34 ± 2	Toxaphene-62	42 ± 2	123789-HxCDD	22 ± 3	PCB 180	42 ± 3
PBDE-28	42 ± 3	Toxaphene-40 + 41	32 ± 4	1234678-HpCDD	16 ± 1	PCB 28	46 ± 1
PBDE-47	49 ± 2	Toxaphene-44	31 ± 5	OCDD	–	PCB 52	47 ± 3
PBDE-66	39 ± 4	Sum tox. (26 + 50 + 62)	48 ± 2	Sum PCDD	24 ± 5	Sum NDL-PCBs 6	46 ± 3
PBDE-99	41 ± 3	op – DDD	41 ± 4	2378-TCDF	33 ± 3	PCB 105	49 ± 2
Sum $PBDE_7$	42 ± 2	op – DDE	50 ± 9	23478-PeCDF	34 ± 3	PCB 114	48 ± 2
		op – DDT	48 ± 4	12378-PeCDF	27 ± 2	PCB 118	46 ± 2
		pp – DDD	51 ± 4	123478-HxCDF	22 ± 1	PCB 123	47 ± 3
		pp – DDE	58 ± 5	123678-HxCDF	19 ± 1	PCB 126	45 ± 2
		pp – DDT	46 ± 4	123789-HxCDF	21 ± 3	PCB 156	46 ± 2
		Sum DDT	49 ± 9	234678-HxCDF	19 ± 1	PCB 157	47 ± 2
				1234678-HpCDF	10 ± 2	PCB 167	45 ± 3
				1234789-HpCDF	10 ± 2	PCB 169	45 ± 2
				OCDF	–	PCB 189	46 ± 3
				Sum PCDF	24 ± 4	PCB 77	–
						PCB 81	–
						Sum DL-PCBs	46 ± 4

Karl et al., 2002, 2003; Ciardullo et al., 2008), but a comparative carry-over rate assessment of a wide range of POPs (including OCPs as well as PBDEs, PCDD/Fs and PCBs) has not previously been conducted. The fillet retention calculations for the different POP congeners are given in Table 2. The fillet retention of PCDDs (16–34%) and PCDFs (10–34%) was lower than the other POPs congeners such as DL-PCBs (45–50%) and PBDEs (34–45%). Earlier trials in Atlantic salmon also showed a higher accumulation efficiency for PBDE and DL-PCB compared to PCDD/Fs (Isosaari et al., 2004, 2005). Among the PCDD/Fs, the higher chlorinated (hexa and hepta) congeners had a relatively lower retention (10–22%) compare to the lower chlorinated (tetra and penta) PCDD/F congeners (27–34%). This is in line with earlier findings, where a higher accumulation for low chlorinated PCDD/Fs was observed in Atlantic salmon compared to higher chlorinated PCDD/Fs (Isosaari et al., 2004; Berntssen and Lundebye, 2008). The bioaccumulation of POPs is curvelinear related to their lipophilicity, expressed as octanol–water coefficient (log K_{ow}), with increase bioaccumulation for hydrophobic organochlorines compounds with increasing log K_{ow} values (from five to seven log K_{ow}) (Fisk et al., 1998). Compounds with a low hydrophobicity (log K_{ow} < five) have a low bioaccumulation due to higher respiratory elimination of these more water-soluble compounds, whereas highly hydrophobic compounds (log K_{ow} > seven) have a low bioaccumulation due to a high elimination by feces (Gobas et al., 1993) and/or or limiting membrane diffusion over the gut (Opperhuizen and Sijm, 1990). Hexa- and hepta-chlorinated PCDD/Fs have a log K_{ow} exceeding seven, which explains the low retentions of these highly chlorinated PCDD/Fs

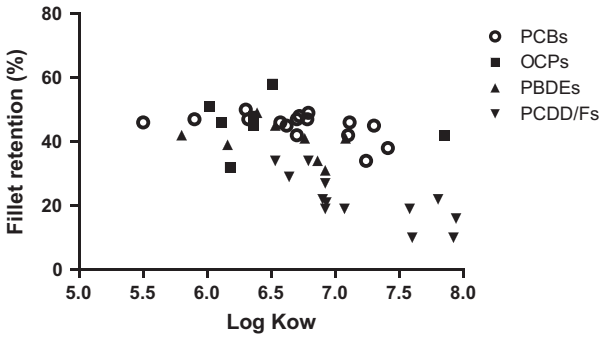

Fig. 1. Retention rates of consumed contaminant (% of consumed) of PCBS, OCPs, PBDEs, and PCDD/Fs congeners in the fillet of consumer sized (~4 kg) farmed Atlantic salmon versus the congener specific octanol–water partition coefficient (K_{ow}). Log K_{ow} values after (Mackay et al., 2006).

compared to the lower chlorinated PCDD/Fs. In the present trial, none of the POPs analysed had a log K_{ow} lower than five, which are the more water-soluble compounds with a low bioaccumulation potential (Fisk et al., 1998). Fig. 1 gives the correlation between fillet retention of dietary POPs and their corresponding K_{ows}. The POPs are classified into PCBs, PCDD/Fs, PBDEs and OCPs compound groups. The organochlorine pesticides include DDT, HCB, and toxaphene of which individual fillet retentions are given in Table 2. Other than a tendency towards reduced retention of POPs with a K_{ow} above seven, no clear relationship was observed between carry-over rate and log K_{ow} (Fig. 1). Similarly, Kelly et al. (2008) did not find a relationship between bioaccumulation and congeners specific K_{ow}s for PCBs or PCDD/Fs in hatchery reared Pacific salmon smolts. Differences in physical properties such as K_{ow} may not be sensitive enough to discern differences in retention of PCB isomers in aquatic organisms (Niimi, 1996). The elimination of PCB isomers from rainbow trout was best described by combined quantum chemical descriptors such as positive charge of a hydrogen atom, standard heat of formation, core-core repulsion, electronic energy, molecular polarisability, and molecular surface area (Wang et al., 2009). The dietary uptake efficiencies of highly chlorinated PCDD/Fs can also be explained by molecular configuration, such as effective molecular cross section, rather than hydrophobicity (Opperhuizen and Sijm, 1990). The present trial includes a wide range of POPs with different ground structures in addition to isomers of these ground structures. A more detailed quantitative structure activity relationship (QSAR) modeling of all relevant quantum chemical descriptors would be needed to make a sensitive model, which describes the retention of these POPs in Atlantic salmon.

The retention of dietary POPs in the present study is in the same range as reported for commercially farmed salmonids. For example, 42–59% of the sum PBDE consumed by Atlantic salmon accumulated in the skinned fillet (Isosaari et al., 2005), compared to 42% in the present study. Approximately 29% of the PCDD/Fs and 52% of DL-PCBs consumed has been reported to be retained in the edible part of marketable-sized cultured rainbow trout (Isosaari et al., 2002; Karl et al., 2003), compared to 24% and 46% for respectively PCDD/F and DL-PCBs in the present study. Whole body retention of PCDD/Fs and DL-PCBs was higher (80–90%), because non-fillet parts (viscera) of the fish has higher concentrations of PCDD/Fs and PCBs than the skinned fillet, which contained approximately 30% of the total PCDD/F and PCB content of whole Atlantic salmon (Isosaari et al., 2004). In addition to retention, carry-over assessment for terrestrial farmed animals is also often expressed as the concentration (mg kg^{-1} wet weight) of the compound in animal products divided by the concentration of the compound in animal feed (mg kg^{-1} wet weight) (Kan and Meijer, 2007; Leeman et al., 2007).To provide similar transfer factors for commercially-reared Atlantic salmon, data are used from the Norwegian national monitoring programmes in 2007 for commercially available salmon grower feeds and commercially-reared Atlantic salmon fillet were used (NIFES, 2007). Table 3 gives the transfer factors for POPs in farmed salmon, which ranged from 0.52 for PCDD/Fs to 0.8 for DL-PCBs and PBDEs. As for the present trial the transfer rates for PCDD/Fs were lowest among the POPs. The transfer factors for POPs in commercially-reared Atlantic salmon were several orders of magnitudes higher than for meat from terrestrial farmed animal (Table 3).

4.3. Kinetic model for toxaphene

Toxaphene is one of the most heavily applied organochlorine insecticides (Vetter and Scherer, 1999). Toxaphene is a POP with a high carry-over from feed to fish (EFSA, 2005) and fish is a dominant source of toxaphene in dietary human exposure (EC, 2005). In the present study, the toxaphene congeners that are currently included in EU feed legislation (Parlar toxaphene congeners 26, 50 and 62) had among the highest fillet retentions of the POPs examined (Table 2). These toxaphene congeners represent the group of octa- and nona-chloroboranes with a low heats of formation (ΔH^0), which explains the high persistency of these congeners and hence their ability to bioaccumulate (Vetter and Scherer, 1999). Models for simulating carry-over is an approach to describe the transfer of contaminants from animal feed to food products (van Raamsdonk et al., 2009). Kinetic models which include input data on expected daily feeding rate, growth rate, and production duration have been shown to be able to accurately predict fillet levels based on known contaminant concentrations in fish feed (Berntssen et al., 2007).The congener uptake and elimination kinetic rates have to be empirically established in order to model the feed-to-fillet carry-over (Sijm et al., 1992; Berntssen et al., 2007). In the present trial, the uptake and elimination kinetics were established in a cross-over designed feeding study with Atlantic salmon. Toxaphene kinetics were assessed for the congeners currently included in feed legislation (parlar toxaphene congeners 26, 50 and 62), as well as the additional congeners that the European Food Safety Authority (EFSA) recommend to include in food monitoring programmes and in future legislation (Parlar number congeners 32, 40, 42a, 41, 44) (EC, 2005). The congeners 32 and 42a were not detected in feeds and fish, and are hence not included in the present assessment. The accumulation and elimination of toxaphene congeners 26, 50, 62, 40, 41, and 44 in Atlantic salmon is given in Fig. 2. Highest accumulation was found for toxaphene 50 followed by 26 and 62, respectively. Table 4 gives the quantified uptake and elimination rates for the different congeners. No significant differences were observed in the half-lifes of

Table 3
Feed-to-edible part transfer factors for dioxins (PCDD/Fs), dioxin-like PCBs (DL-PCBs), polybrominated diphenyl ethers (PBDEs), organochlorine pesticides (DDT, HCB and heptachlor) in commercially-reared Atlantic salmon fillet and for comparison meat from terrestrial animals as reported by Leeman et al. (2007).[a]

	Fish fillet	Terrestrial meat[a]	
	Median	Median	Max
Sum total PCDD/Fs	0.52	0.08	0.33
Sum DL-PCBs	0.79	0.12	0.36
SumPCDD/F + DL-PCBs	0.68		
Sum PBDE-7	0.81		
Old OCP pesticides		0.029	0.07
Sum DDT	0.56		
HCB	0.80		
Sum hepatchlor	0.43		

[a] Leeman et al. (2007).

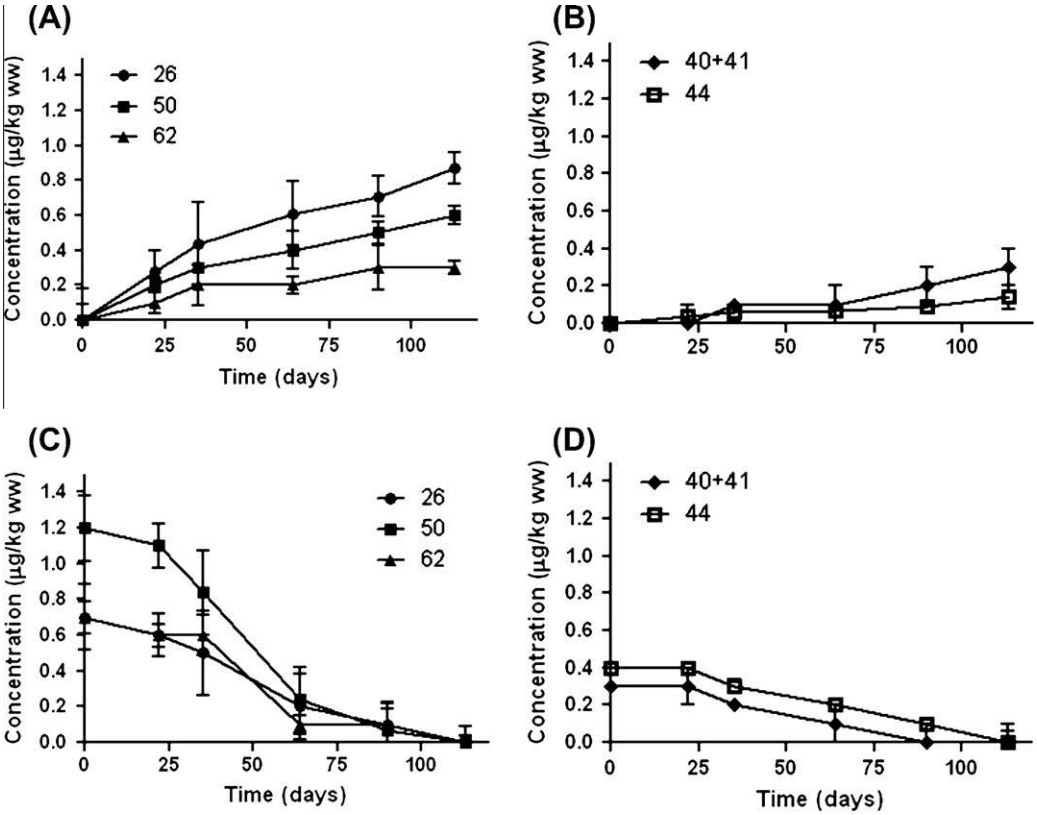

Fig. 2. A–D. Control corrected accumulation and elimination of toxaphene congeners 26, 50, and 62 (A and C, respectively) and toxaphene congeners 40 + 41 and 44 (B and D, respectively) in consumer sized Atlantic salmon. Figures A and B represent a shift from a low contaminant diet to a high contaminant diet and D and C the simultaneous shift from a high contaminant diet to a low contaminant diet. Time point at 0 d indicate the start of the shift of diets in a cross-over design.

Table 4
Estimated elimination rate constants (Half-life; Kel), and uptake rates (α) for toxaphene congeners (mean ± SD). Values with the different superscripts are significantly ($P < 0.005$) different from each other (ANOVA, Tukey's HSD-test).

Toxaphene (CHB) congeners	CHB 40 + 41	CHB 44	CHB 26	CHB 50	CHB 62
Elimination					
Half-life (T1/2, days)	198 ± 122	177 ± 151	113 ± 22	113 ± 54	112 ± 37
Uptake					
Uptake rate (α, d^{-1})	0.130 ± 0.018^{b}	0.180 ± 0.051^{b}	0.39 ± 0.09^{a}	0.34 ± 0.11^{a}	0.28 ± 0.10^{a}

the different toxaphene congeners in the present trial (Table 4). The half-life of these congeners varied from 113 to 198 d, which was shorter than previously reported for the sum of toxaphene 26 and 50 in single intraperitoneal injected lake trout (*Salvelinus namaycush*) where a half-life of 232–322 d was found (Delorme et al., 1999). Faster elimination rates in Atlantic salmon in the present study compared to injected lake trout may be due to species difference, physiological status, and/or route of exposure. Maruya et al. (2005) reported a considerably faster elimination (half-life of 7–27 d) of the low chlorinated (penta and hexa) toxaphene congeners compared to higher chlorinated (octa and nona) congeners. In the present trial, only the highly chlorinated toxaphene congeners (octa-chlorinated parlar congeners 26, 40, and 41, and nona-chlorinated parlar congeners 50 and 62) were investigated, which all are classified as toxaphene congeners with a slow elimination process (Maruya et al., 2005). The uptake rates were significantly higher for toxaphene congeners 26, 50 and 62 compared to toxaphene congeners 40 + 41 and 44. The differences in retention among the toxaphene congeners, with lower retentions for the 40 + 41 and 44 toxaphene congeners compared to the 26, 50 and 62 toxaphene congeners (Table 2), can be explained by differences in uptake rates rather than elimination and/or biotransformation. Fig. 3 gives the model simulated fillet toxaphene accumulation (for ∑#26/#50/#62 and∑#40/#41/#44, respectively) based on feed levels in this trial as well as the current upper limits for commercially produced fish feed. Model predictions for twelve months of feeding with trial dietary levels of 25 µg kg^{-1} gave fillet levels of 11.8 µg kg^{-1} for ∑#26/#50/#62 and 15.1 µg kg^{-1} for ∑#26/#50/#62/#40 + 41/#44. This agrees well with observed fillet levels of 12 ± 0.36 µg kg^{-1} and 15.3 ± 0.4 µg kg^{-1}, respectively in the present trial (Fig. 3). Using the legal EU feed upper limit of 50 µg kg^{-1} for ∑#26/#50/#62 and commercially relevant growth and feeding rates (0.81% d^{-1} and 0.93% body $weight^{-1}$ d^{-1}, respectively, data Centre of Aquacultural Compentence, CAC, Hjelmeland, Norway), the model simulated fillet levels after 12 or 22 months of rearing were 19 µg kg^{-1} and 22 µg kg^{-1} ww, respectively (Fig. 3). Highest observed ∑#26/#50/#62 levels in commercially-reared Atlantic salmon fillet on the Norwegian market was 16.9 µg kg^{-1} ww (NIFES, 2007). Although an EU upper limit for toxaphene in animal feeds

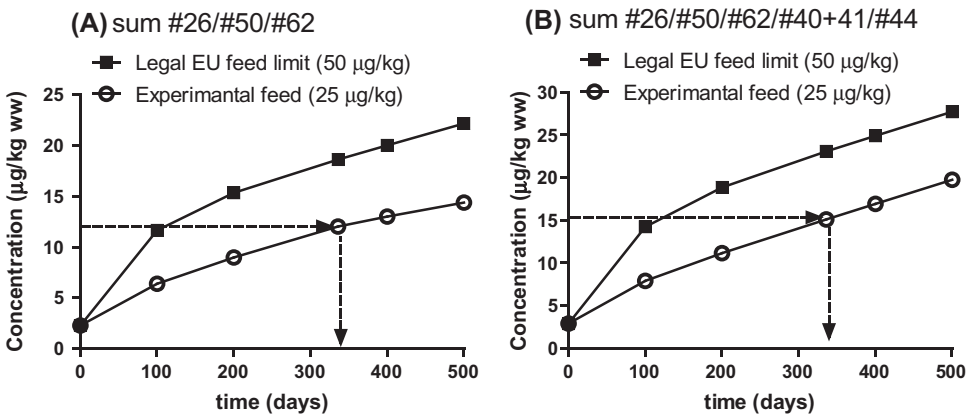

Fig. 3. A and B, model simulated fillet toxaphene accumulation of dietary toxaphene at present experimental feed levels as well a current EU feed limit for the current toxaphene congeners included in present EU feed legislation (∑#26/#50/#62, (EC, 2005), Fig. 3A) as well as the recommended toxaphane congeners for future legislation and monitoring (∑#26/#50/#62/#40 + 41/#44, (EFSA, 2005), Fig. 3B). Dotted arrows indicate observed experimental values after 12 months feeding.

exists, no EU upper limit for toxaphene (∑#26/#50/#62) in food products have yet been established. However, based on a toxaphene induced tumour promotion reference dose (RfD) for the three major toxaphene congeners (∑#26/#50/#62), average fish consumption, and relative occurrence of the three toxaphene congeners in fish, it was calculated that a permissible level of 90 µg kg^{-1} in seafood is recommendable in fish on the German market (Ekid et al., 2008). This estimate is made for the general German population, which has an estimated daily fish consumption of 21.4 g d^{-1} per capita (Ekid et al., 2008). In Norway, the estimated annual fish consumption is considerably higher and is estimated to be 65 g d^{-1} per capita, or 0.92 g kg^{-1} body weight d^{-1} for a 70 kg person (NORKOST, 1997-1999). Using the established reference dose of 2E-05 mg kg^{-1} d^{-1} for ∑#26/#50/#62 (Simon and Manning, 2006), an estimated daily fish intake of 0.92 g kg body $weight^{-1}$ d^{-1}, and assuming that the concentration of toxaphene is represented by ∑#26/#50/#62, the acceptable toxaphene levels in fish food products on the Norwegian market can be calculated by equation four as described by Simon and Manning (2006) and Ekid et al. (2008).

$$\text{Acceptable Conc}_{\text{fish}} = \frac{RfD(2E-05\ \text{mg/kg}-\text{day})}{0.92\ \text{g/kg}-\text{day}\cdot 0.001\ \text{kg/g}\cdot 100\%} = 0.021\ \text{mg/kg} \quad (4)$$

The acceptable toxaphene levels in fish food products on the Norwegian market can be set at 21 µg/kg ww. Highest observed ∑#26/#50/#62 levels in commercially-reared Atlantic salmon fillet on the Norwegian market was 16.9 µg kg^{-1} ww (NIFES, 2007). Model simulation shows that current upper limit for ∑#26/#50/#62 in feed (50 µg kg^{-1}) will give fillet levels of 22 µg kg^{-1} (Fig. 3A), which exceeds the estimated permissible level of 21 µg kg^{-1} for fish food samples in Norway. However, this model simulated excess is less than 5% of the estimated permissible toxaphene concentration in fish.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2011.01.017.

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