Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2012, 24(2) 221–227
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Polybrominated diphenyl ether levels in wild and farmed Chilean salmon and preliminary flow data for commercial transport

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Received 02 March 2011; revised 30 May 2011; accepted 31 August 2011

Abstract

This pilot study documented the occurrence and levels of brominated flame retardants in the tissues of farmed and wild salmon in southern Chile. Samples of Coho salmon and rainbow trout were obtained from fish farms, rivers and lakes in the Patagonia in Aysen Region, Chile. The samples were analyzed by Gas Chromatography Negative Chemical Ionization Mass Spectrometry for the different polybrominated diphenyl ether (PBDE) congeners. Contaminants were observed in all the samples, and the congeners BDE 17, 28, 47 and 66 were observed in all both farmed and wild samples. The concentrations were higher in the farmed Coho salmon, presenting significant differences with wild salmon. The levels reached 182 pg/g wet weight (ww) vs. 120 ww. In the case of the rainbow trout, the concentrations were lower, although the congener profile was quite similar. The levels reached an average of 100 pg/g ww in the farmed fish versus 110 pg/g ww in wild fish, and no significant difference was observed between the species. In both species, the congener with the highest concentration was BDE 47. Based on this information, the BDE flow was estimated for commerce, which is a form of pollutant transport not usually considered in POP pollution studies. A preliminary estimation indicated that the quantity of PBDEs mobilized by commerce was in the order of kg, and in the case of Chile might reach almost 1 kg.

Key words: Polybrominated diphenyl ethers; farmed Salmon; Chile; POPs; Patagonia
DOI: 10.1016/S1001-0742(11)60761-4

Introduction

World aquaculture has significantly increased in the last 50 years, increasing from an annual production below a million of tons in the 1950s up to more than 59 million tons in 2004 (FAO, 2008). The increased supply per capita of aquacultural products increased from 0.7 kg in 1970 to 7.8 kg in 2006, representing an annual growth rate of 7% (FAO, 2008). Similarly, the percentage of farmed salmonids in world commerce has notably increased in the last decades, reaching 11% of total aquaculture production (FAO, 2008). The largest world producers of salmonids are Norway and Chile with 33% and 31% of world production, respectively. The remaining 19% is provided by other European producers (FAO, 2008).

Salmon farming as an industry was introduced in Chile at the beginning of the 1980s and was developed in relatively pristine aquatic environments with low productions costs and excellent farming conditions (Bjørndal, 2001). In 2008, production in Chile was 440,000 tons, where 51.6% corresponded to Atlantic salmon (*Salmo salar*), 28% to rainbow trout (*Oncorhynchus mykiss*) and 20% to Coho salmon (*Oncorhynchus kisutch*) (Salmonchile, 2008, www.salmonchile.cl). Even though salmonids are exotic species in the South American Pacific, they have notably adapted to local conditions and have even escaped and reproduced in wild conditions (Soto et al., 2001, 2006).

The Aysen Region has experienced the greatest growth in fish farming in Chile: the concession of new farming centers increased in more than 70% in 2009 (Sernapesca, 2008, www.sernapesca.cl). This new development is particularly interesting considering that this region is located in the Chilean Patagonia, which represents one of the last pristine locations on the planet (Brooks et al., 2006) and constitutes a Biosphere reserve (UNESCO, 1995, http://www.unesco.org).

According to the FAO, aquaculture is an increasingly important food source in the world (FAO, 2004). At the same time, it also represents a source of contamination as shown in studies of persistent toxic compounds, including the polybrominated diphenyl ethers (PBDEs), in farmed fish (Hites et al., 2004a; Schecter et al., 2006; Montory and Barra, 2006). PBDEs are ubiquitous pollutants, being classified as POPs, whose characteristics are toxicity, persistence, bioaccumulation and capacity for long distance

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transport (de Wit, 2002). These pollutants have been associated to endocrine disruption and alterations of neurological development in rodents (Birnbaum and Staskal, 2003), and there is growing concern for the potential risks to human health due to the evidence of its occurrence in maternal milk (Meironyté et al., 1999; Schecter et al., 2003; Toms et al., 2009).

One of the principal uptake pathways of PBDEs in humans is by food intake (Vonderheide et al., 2008), especially in fatty foods, due to the fact that salmon tissues present higher PBDE concentrations in comparison with other farm-raised animals such as chicken, beef, and pork (Schecter et al., 2004). Additionally, in general terms, the levels of persistent organic contaminants are significantly higher in farmed in comparison with wild salmon (Hites et al., 2004a, 2004b). This result is worrisome because consumption of farmed salmon has significantly increased in countries like the United States and Japan, where consumption is even twice weekly (Bjørndal, 2001), and these two countries are consistently the principal destinations of Chilean farmed salmon.

Given the size of the global aquaculture market, it is interesting to analyze the PBDE flow that is potentially being transported by exportation and importation of salmon. Different authors have cited the existence of atmospheric, aquatic, and biological (biotransport) transport for these types of contaminants (de Wit et al., 2010; Blais et al., 2007), although they have not generally considered the fact that large quantities of biomass (such as salmon products) are loaded in ships and airplanes from fish farms in order to reach the final consumers. Consequently, the objective of this work was to analyze the presence of PBDEs in wild and farmed salmon in southern Chile, comparing different species and studying as well the congeneric patterns in order to perform a preliminary study of contaminant flows transported by commerce of these salmonoids from Chile to the major consumer centres. The results and subsequent analysis should reaffirm the importance of good environmental practices in the aquaculture plants together with the availability of water, food, and "healthy" raw materials for the process. The data presented could be used as a reference for future spatial and temporal comparisons in order to improve the actual commercial standards of salmon products.

1 Materials and methods

1.1 Study area

The study area (Fig. 1) comprises the Aysen Region between 43°45′00″S latitude and 49°15′00″W longitude. This area is characterized by a unique morphology, climate, soil, hydrology vegetation and fauna, and because it is the larger reserve of freshwater resources in the southern part of the continent. Rivers in this region are relatively short (compared to those draining into the Atlantic) and are characterized by the steep slope and high flow rates, with little variation between seasons. This region presents the lowest population density and a low level of industrial activities in Chile, although fish farming has increased in the last few years.

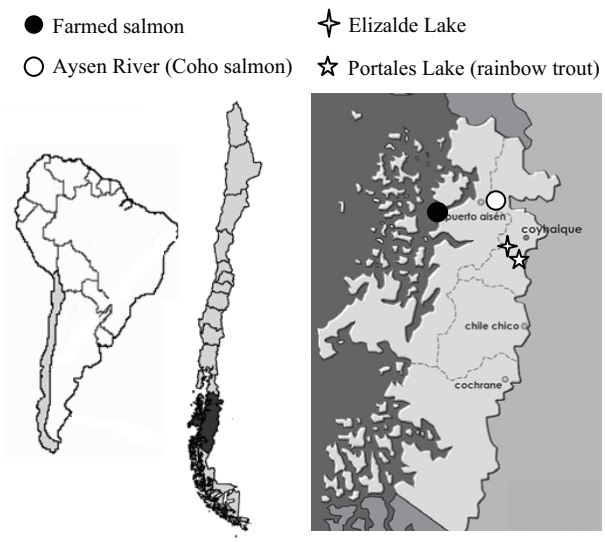

Fig. 1 Map of the Aysen Region.

1.2 Biological samples

The "wild" coho salmon (species that had escaped and reproduced in the wild) samples were obtained in the Aysen river (8 specimens); the wild rainbow trout were obtained from two lakes for a total of 10 specimens. The farmed salmon were obtained from companies in the area (Puerto Chacabuco), where 12 samples corresponded to Coho salmon and 6 to rainbow trout.

Body weight, fork length and sex were recorded for all samples. Dorsal muscle samples (4 cm^2) were taken in the field for subsequent residue analysis. Samples were stored in pre-cleaned aluminum foil and transported on ice until reaching the laboratory where samples were frozen at –20°C until analysis.

1.3 Chemicals

For residual analysis, *n*-hexane, dichloromethane, isooctane, 95%–97% concentrated sulfuric acid; acetone and anhydrous sodium sulfate were from Merck (Darmstadt, Germany). Cellulose extraction cartridges of 20 mm i.d. and 80 mm long were from Whatman (England). Sodium sulfate and cartridges were pre cleaned by Soxhlet extraction with *n*-hexane:dichloromethane (4:1, *V*/*V*)) for 24 hr before use. Anhydrous sodium sulfate was activated overnight at 400°C. PBDE standards (No. 28, 47, 99, 100, 153 and 154) were from Cambridge Isotope Laboratories, Inc. USA.

1.4 PBDEs analysis

Muscle tissues were analyzed as described elsewhere (Berdié and Grimalt, 1998; Vives et al., 2004). Briefly, muscle samples were ground with activated sodium sulfate until a fine powder was obtained. A Soxhlet extraction was done with *n*-hexane:dichloromethane (4:1, *V*/*V*) for 18 hr. Lipid content was determined gravimetrically using 20% of the extract. The rest of the extract was cleaned up with sulfuric acid (5 times), concentrated by vacuum

rotatory evaporation (20°C, 20 Torr) and concentrated to near dryness under a gentle flow of nitrogen. Finally, the extract was redissolved in 50 μL of isooctane. Before chromatographic analysis, an internal standard of PCB142 was added to correct for instrument variability.

PBDEs were analyzed using an Agilent 6890N GC coupled to a 5975 mass spectrometer (Agilent Technologies, USA) operating in negative chemical ionization mode (NICI). The instrument was equipped with a low bleed SGE-BPX5 MS fused silica capillary column (15 m in length, 0.25 mm internal diameter and 0.10 μm film thickness). The oven temperature program was from 110°C (held for 1 min) to 180°C at 8°C/min (held for 1 min), then to 240°C at 2°C/min (held for 5 min) and finally to 310°C at 2°C/min (held for 15 min). Helium was used as a carrier gas (10 psi) and ammonia as ionization gas (1.6×10^{-4} Pa). Transfer line and quadrupole temperatures were 280 and 150°C, respectively. Quantification was performed at a m/z value of 79 $[Br]^-$ which is the base peak of all PBDEs monitored. Confirmation was done at m/z values of 81 $[Br]^-$, 161 $[HBr_2]^-$, 327, 405, 483, 563 and 643, corresponding to $[M]^-$ or $[M\text{-}HBr_2]^-$. Retention time shifts could not be higher than 1 sec.

1.5 Quality assurance

Procedural blanks were analyzed for every set of six samples, which correspond to 3-day periods of sample handling. Average values ranged 13–38 pg/g wet weight (ww) for PBDEs. These levels accounted for 4%–11% of the individual PBDE concentrations. Identification and quantification of all studied compounds were performed by injection of external standards at different concentrations PBDE Analytical Standard Solution EO-5099 (Cambridge Isotope Laboratories, Inc., USA).

Limits of detection (LOD) were calculated from real samples as the mean of noise signal plus 3 times the standard deviation (n = 5). They were in the range of 13–16 pg/g ww for PBDEs. More information of the quality control procedure to quantity PCBs and PBDEs can be found in (Berdié and Grimalt, 1998; Vizcaino et al., 2009; Montory et al., 2010).

1.6 Statistical analysis

The test of multiple comparisons of Tukey was used for the a posteriori comparisons. This study considered a significance level of 0.05.

2 Results

2.1 PBDE concentrations in salmonids

The PBDE congeners found differed between the farmed trout and Coho salmon, therefore this study only included congeners found in both species, corresponding to BDE 17, 28, 47 and 66, similar situation occurs with the samples obtained in the wild.

The concentration of ∑PBDEs (BDE 17, 28, 47 and 66) for Coho salmon was almost twice as (182.3 pg/g ww) that of rainbow trout's concentration (96.2 pg/g ww) as shown in Fig. 2.

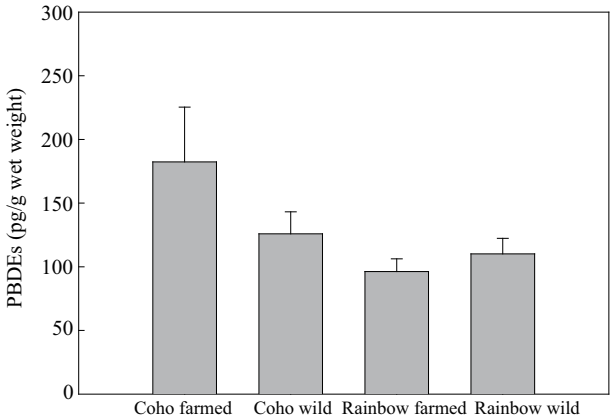

Fig. 2 Concentrations of PBDEs in Coho salmon and rainbow trout by the type of "farmed" and "wild". The bars correspond to standard error.

The congeners presented a similar distribution (Fig. 3), except for the congener BDE 66, which presented a higher concentration in trout although this difference is not significant. The highest concentration was observed for BDE 47, which corresponds to 53% and 51% of all the congeners for Coho salmon and rainbow trout respectively (both farmed).

The wild Coho salmon and rainbow trout presented a much smaller difference in the ∑PBDEs concentration in comparison with the farmed salmonids (Fig. 2). The dominant trend was that the Coho salmon presented higher concentrations than rainbow trout, the concentration found in the wild fish was lower than found in the farmed fish. This difference is significant for the farmed versus wild Coho salmon because the trout only marked a tendency (the difference between farmed and wild trout is not significant).

For the wild salmonids, the congener with the highest concentration was BDE 47, which contributed 59% of the total for the Coho salmon and rainbow trout. The congeneric distribution is similar to that found in the farmed salmon, and the difference lies in that the congener BDE 66 was not found in the wild fish although BDE 71 was present and presents a higher concentration in trout, contrasting with the other congeners 17, 28, 47 (Fig. 3).

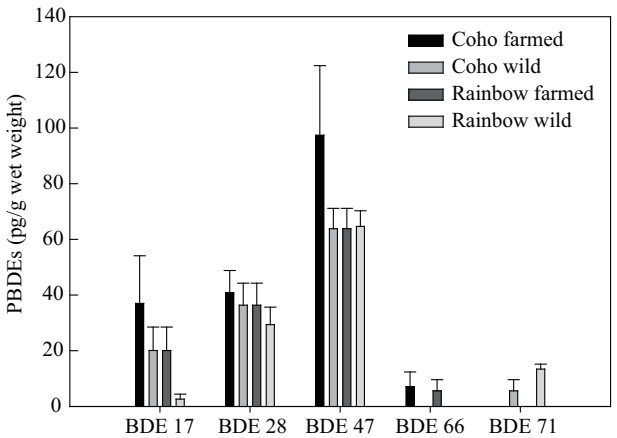

Fig. 3 PBDE congeneric distribution in Coho salmon and rainbow trout tissue by the type of "farmed" and "wild". The bars correspond to standard error.

2.2 Production and exportations of Chilean salmonids

Considering the important Chilean salmon production (657,390 tons, Salmonchile, 2009, www.salmonchile.cl) and PBDE occurrence, Table 1 shows that together with the exports of salmon to different destinations, there is a flow of PBDEs toward the destination markets (where the receptor countries are those that import this product). The species *Salmo salar* contributes the greatest mass of PBDEs, followed by rainbow trout, and finally by Coho salmon. This difference is due to the differences found in contaminant concentrations for each species (Table 1).

Table 2 presents global data on potential commerce transport of PBDEs produced by the principal salmon producers that supply almost 100% of world demand, where Norway and Chile are the largest producers (839 $\times$ 10^6 and 657 $\times$ 10^6 tons, respectively). Consequently, the PBDE flow is almost twice for Norway, while Chile presents a flow comparable to United Kingdom, Canada and the Faroe Islands. This flow is related to the quantity of exported product, and the PBDE concentrations observed in each country. Observed values account for the same order of magnitude even when Chile presents almost half the PBDE concentrations for United Kingdom, Canada and the Faroe Islands. Consequently, the lower the concentrations observed in the farmed salmon tissues, the lower the human transport due to commercial flows.

Table 2 Levels of PBDEs in the largest salmon exporters of Atlantic salmon (*Salmo salar*)

	Average concentration (ng/g)	Production ($\times$ 10^6 tons)	Estimated PBDE mass (g)
Norway	2.5[a]	839	2097.5
Chile	1.5[b]	657	985.5
Scotland	3.8[c]	137	520.6
Canada	3.5[c]	126	441
Faroe Island	3.0[c]	40	120

[a] Bethune et al., 2005; [b] Montory and Barra, 2006; [c] Hites et al., 2004a.

3 Discussion

3.1 PBDE concentrations in salmonids

The obtained results agree with the descriptions made by our group for farmed Atlantic salmon (*Salmo salar*) in southern Chile, which presented more elevated PBDE concentrations (Montory and Barra, 2006) than found in this study. This difference could be due to the different species (distinct species present differences in concentrations) or the distinct stages of life cycles where the fish have been sampled, among other possibilities. On the other hand, the PBDE concentrations found for farmed trout in Chile is much lower than those found in Swiss fish farms (740–1300 pg/g ww) (Zennegg et al., 2003). Similarly, the value is lower than the trout in remote lakes of the United States where the concentration reaches 1.4 μg/kg ww, although this concentration reaches 1250 μg/kg ww in urbanized rivers (Johnson and Olson, 2001).

The farmed Coho salmons have a much lower concentration than that found by Hayward et al. (2007) who found 300 pg/g wet weight; however, the same author did find concentrations of 40 pg/g ww for free Coho salmons which are similar to values found by Hites et al. (2004a) in Alaska (87–112 pg/g ww), and which are still lower than the concentrations found for free Coho salmon. This increased concentration in free Patagonian Coho salmon could be because free salmon feed near the fish farms and thus could also feed on the wastes of the farmed fish as described by Soto (1997), who reported that feed pellet remains were found in the stomachs of free living fish.

On the other hand, the difference found between farmed and wild fish agrees with the literature where the farmed salmonids present higher concentrations than the wild ones (Hites et al., 2004a, 2004b). This difference could be due, as indicated in the literature, to the feed consumed by farmed species (Hites et al., 2004b; Montory and Barra, 2006).

Generally, BDE 47 is the congener with the highest concentration found in environmental samples (Johnson and Olson, 2001; Zennegg et al., 2003), and this result is confirmed in our study. This result also confirms the conclusions that BDE 47 is rapidly assimilated in comparison with other congeners (Boon et al., 2002). On the other hand, the difference between species for the BDE 47 concentration would be given for the ability of salmonids to metabolize PBDEs (Stapleton et al., 2004). Rainbow trout, as other fish species can also debrominate BDE, although there are differences in the patterns of the less brominated congeners that are formed during the metabolism (Stapleton et al., 2004).

3.2 Production and exportation of Chilean salmonids

Exports of salmon with presence of PBDEs is one type of anthropogenic transport, but there are others that vary in their routes and the quantity of contaminants transported. One example is Long Range Atmospheric Transport

Table 1 Chilean exports of salmon and trout by destination and flow the PBDEs by salmon and trout*

	Exports				PBDE		
	2008 (ton)	Salar (ton)	Coho (ton)	Trout (ton)	Salar (g)	Coho (g)	Trout (g)
Japan	162812	84662.24	32562.40	45587.36	123.61	21.34	24.10
United States	108093	56208.36	21618.60	30266.04	82.06	14.17	16.00
Europe	43171	22448.92	8634.20	12087.88	32.78	5.66	6.39
Asia (excluding Japan)	43857	22805.64	8771.40	12279.96	33.30	5.75	6.49
Latin America	52747	27428.44	10549.40	14769.16	40.05	6.91	7.81
Others	34404	17890.08	6880.80	9633.12	26.12	4.51	5.09
Total	445084	231443.68	89016.80	124623.52	337.91	58.34	65.89

* Salmonchile, 2008.

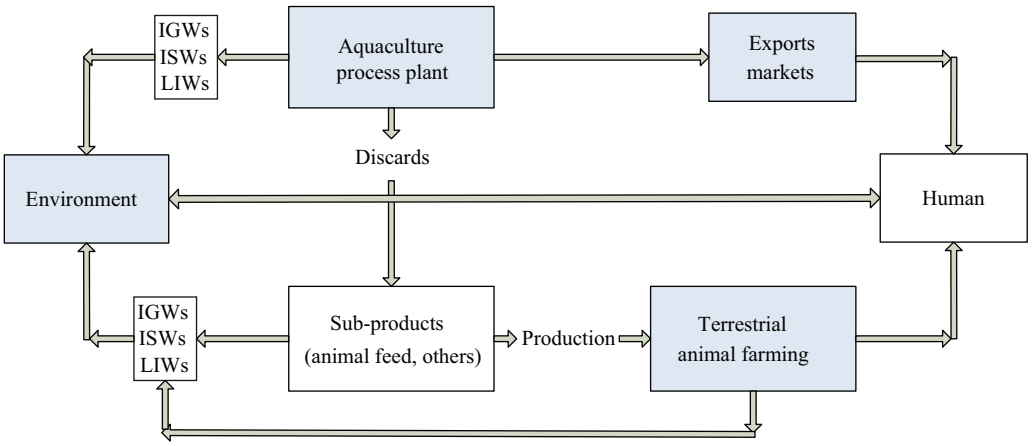

Fig. 4 Conceptual diagram for the flow of PBDEs from aquaculture process to humans. GIWs: industrial gas waste; ISWs: industrial solid waste; LIWs: liquid industrial waste.

(LRAT), which uses modeling of regional movements of air mass to predict the presence of PBDE concentrations in remote sites (Vonderheide et al., 2008). Atmospheric mediated transport generally presents low concentrations, although it is the most important transport in remote zones, where there are no other forms that these compounds are deposited. Biota can also redistribute PBDEs in the environment once they are absorbed and accumulated. This redistribution is produced at a regional and global level due to long distance migration and dispersion of animals, especially fish and birds (Blais et al., 2007). Even when this biotransport is spatially concentrated (the animals transport these contaminants to a specific point), it can even be more important than atmospheric transport. For example, Evenset et al. (2007) demonstrate that marine bird excrement is responsible for up to 80% of the PCBs present in an Arctic lake, presenting transport efficiency 30 times greater than atmospheric transport.

It is complicated to compare the different transport types (anthropogenic, atmospheric, biotransport) with respect to contaminant flow because it depends on a variety of factors. To illustrate the importance, we will indicate the average order of magnitude according to literature reviews, which indicates an average atmospheric transport in the order of magnitude (pg/m^3) (Gouin et al., 2002; Cheng et al., 2007). The salmonids – presents an order of magnitude (ng/g); and in the particular case of this anthropogenic transport, it also presents an order of magnitude (ng/g) and depends directly of the quantity of individuals.

Another important point is that atmospheric transport and biotransport act differently on the environment and human health: atmospheric transport directly affects the environment and indirectly human health, while biotransport is directly related to human intake.

Even when there are other contaminant entry points into humans, the primary sources of exposure include food intake, dust inhalation, exterior air that varies from 2×10^{-9} to 77×10^{-9} g/m^3 in the United States (ATSDR, 2004) and occupational exposure. The most important factors vary geographically and between different individuals in a population. Dietary exposure is an important pathway for PBDE entry into humans and is principally attributed to the consumption of fatty fish (Vonderheide et al., 2008). When comparing the potential flows of contaminants, intake of food with high protein content and animal origin should be considered because the contaminants can act as substitutes for protein provided by the salmon. It is important to consider diverse factors, such as daily intake (quantity), contaminant concentrations, food price, and its accessibility. FAO (2008) reports the importance of fish as an inexpensive protein source that can be used to address food scarcity in the world.

Another important fact is that in addition to "PBDE exportation" mediated by salmonids, there is also a contaminant flow associated to effluent and waste of materials in the processing plants (Fig. 4). In 2008, this value corresponded to 212,312 tons (Salmonchile, 2008), a value that did not include the large amount of salmonids not used for human consumption, for example when they are discarded because of diseases or other reasons. Normally, these discarded products and subproducts (Fig. 4) are used to prepare feed for other farmed animals, such as cows and chickens. This is especially important when considering that chemical residues are one of the principal contamination sources of food for cattle (Brambilla et al., 2004). Consequently, feed of animal origin plays an important role in the determination of human exposure to biological and chemical contaminants (Lievaart et al., 2005; Brambilla et al., 2008) (Fig. 4).

Even when the number of residues and contaminants evaluated in animal feed (to comply with standards) has dramatically increased, PBDEs are not presently evaluated.

The regulation in different countries establishes maximum allowed limits and tolerable intake levels for many but not all the contaminants contained in food. Additionally, there is no definitive international consensus on the risks associated with the different contaminants.

4 Conclusions

Considering the results presented, we can conclude that there is appreciable anthropogenic transport for the salmonids (order of magnitude of ng/g with respect to the type and quantity of exported fish) and that human beings

export an endless quantity of food elements (whose PBDE and other contaminant flow has not been analyzed) that can also be contributed by the destination country. Clearly, this type of transport is increasingly important when considering the reach of global markets and commercial agreements.

For example, Chile has 20 commercial agreements with 56 countries, and thus has access to a market of more than 4 billion habitants (www.gobiernodechile.cl). In the scientific literature to date, there is no information on PBDE concentrations in products like fabric, wood, or oils, and this is motive of an important international debate.

The data provided by this study are the first of its type for the Southern hemisphere and are necessary to develop better control of PBDEs in the salmon farming process. They also contribute to a better understanding of PBDE flow in global markets.

Acknowledgments

This work was supported by Fondecyt No. 1080294 and Fondecyt 1110719 (R Barra). This work was made possible with the cooperation of Paulina Bahamondes and Ignacio Rudolph. The authors acknowledge the participation and help of Victor Hugo Coñuecar during the sampling.

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