CLIMATE AND POLLUTION AGENCY

Contaminants in fish from Etnefjord, Norway

TA
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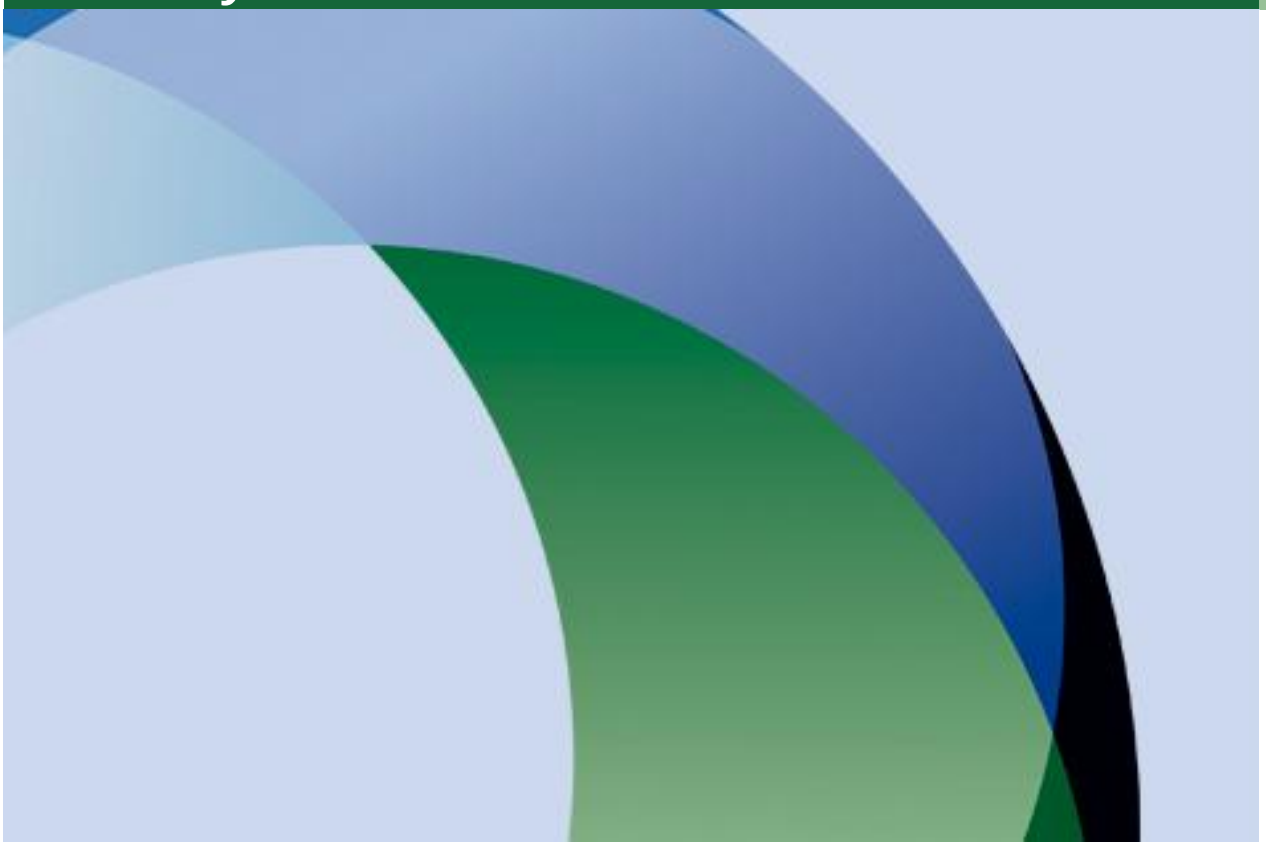

Prepared by/in collaboration with NILU – Norwegian Institute for Air Research

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TA-2821/2011
ISBN 978-82-425-2431-7 (trykt)
ISBN 978-82-425-2432-4 (elektronisk)

Client: Climate and Pollution Agency
Contractor: NILU – Norwegian Institute for Air Research

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Contaminants in fish from Etnefjord, Norway

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Authors: Espen Mariussen, Martin Schlabach

NILU project number.: O-111053
NILU report number : OR 50/2011

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Preface

NILU was engaged by The Norwegian Climate and Pollution Agency (Klif) to monitor the levels of hexabromocyclododecane (HBCDD), polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), hexachlorocyclohexane (HCH), dichlorodiphenyltrichloroethane (DDT), mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As) in cod and flounder from Etnefjorden. Etnefjorden is a branch of the Hardangerfjord at the southwest coast of Norway. Recently, high levels of HBCDD in fish from Etnefjorden were reported, which raised some concerns about the state of pollution in this fjord.

Cod and flounder were collected from Etnefjord in December 2010 by the local fisherman Lars Moe. Responsible for the sampling of fish was Sigurd Øxnevad from Norwegian Institute for Water Research (NIVA).

Responsible for the preparation and clean-up of the fish samples were Ellen Katrin Enge, NILU. Anders Borgen, Hans Gundersen and Mebrat Ghebremeskel were responsible for the instrumental analyses, Espen Mariussen was the main author of the report, and Martin Schlabach was project leader. Responsible at Klif was Bård Nordbø.

Kjeller, September 2011

Martin Schlabach
Senior scientist

Summary

1,2,5,6,9,10-hexabromocyclododecane (HBCDD) is one of the most frequently used brominated flame retardants (BFR) and is considered as an emerging environmental pollutant. Recently, high levels of HBCDD were reported in five different fish species from Etnefjord, which is a branch of the Hardangerfjord at the southwest coast of Norway. This report raised some serious concerns and the Norwegian Climate and Pollution Agency (Klif) therefore initiated a survey of HBCDD in cod and flounder from Etnefjorden in order to confirm the findings. In addition to HBCDD, the levels of other environmental pollutants were analyzed in the fish, namely polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), hexachlorocyclohexane (HCH), dichlorodiphenyltrichloroethane (DDT), mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As). The fish muscle filet and livers were prepared and analyzed using recognized and previously published methods. The levels of HBCDD in the fish were low, representing expected background concentrations. HBCDD was only detected in the cod livers with concentrations in the range 0.3-5.7 ng/g wet weight, corresponding to 1.3-8.2 ng/g on a lipid weight basis. Previously, it was reported a level of 30 000 ng/g lipid weight in cod filet, which is, as far as we know, the highest HBCDD concentration ever reported in cod. Our study could, therefore, not confirm the recent findings that showed high HBCDD concentrations in fish from the Etnefjord area. Also, the levels of the other pollutants were low. For example, the concentrations of DDT, PCB, HCH and Hg can, according to Klifs system for classification of environmental state, be regarded as background levels (class 1, slightly/negligible polluted). In conclusion, there are no reasons to believe that cod and flounder in Etnefjorden are exposed to considerable amount of environmental pollutants, neither of HBCDD nor other contaminants. Only further analysis of sediments from the inner site of the fjord can, however, positively reveal a possible HBCDD source.

Norsk sammendrag

1,2,5,6,9,10-hexabromocyclododecane (HBCDD) er en av de mest benyttede bromerte flammehemmerne og regnes for å være en potensiell miljøgift. Nylig ble det, av en tysk forskningsgruppe, rapportert svært høye HBCDD-konsentrasjoner i forskjellige typer fisk hentet fra Etnefjorden. Etnefjorden er en fjordarm tilknyttet Hardangerfjorden på sørvestlandet. Den tyske rapporten vakte bekymring og Klima og forurensningsdirektoratet (Klif) tok derfor initiativ til å måle innholdet av HBCDD i torsk og flatfisk hentet fra Etnefjord for å få bekreftet funnene. I tillegg til HBCDD ble det også analysert på andre miljøgifter som polybromerte difenyletere (PBDE), polyklorerte bifenyler (PCB), heksaklolorosykloheksan (HCH), diklorodifenyltrikloroetan (DDT), kvikksølv (Hg), bly (Pb), kadmium (Cd) og arsen (As). Miljøgiftene ble bestemt i fiskefilet og/eller i lever med anerkjente og tidligere publiserte metoder. Nivåene av HBCDD i fisken viste seg å være lave og representerer forventede bakgrunnskonsentrasjoner. HBCDD ble kun detektert i torskelever med konsentrasjoner fra 0,3-5,7 ng/g våtvekt som tilsvarer 1,3-8,2 ng/g på fettvektsbasis. Tidligere ble det rapportert en konsentrasjon på 30 000 ng/g på fettvektsbasis i torskefilet. Dette er vesentlig høyere enn det vi målte. Vi kunne derfor ikke bekrefte funn av høye HBCDD-konsentrasjoner i fisk fra Etnefjord. Nivåene av de andre miljøgiftene var også lave og representerer forventede bakgrunnskonsentrasjoner. Nivåene av for eksempel DDT, PCB, HCH and Hg kan klassifiseres, i henhold til Klifs klassifiseringssystem for miljøtilstand, som ubetydelig (Klasse 1). Konklusjonen er at det ikke er grunn til å tro at torsk og flatfisk fra Etnefjorden er eksponert for betydelige nivåer av miljøgifter, verken for HBCDD eller andre viktige miljøgifter. Det er vanskelig å spekulere rundt årsakene til at våre funn avviker fra det som tidligere ble funnet. En analyse av sediment hentet fra det indre fjordbassenget vil kunne bekrefte eller avkrefte et utslipp av HBCDD.

1. Introduction

1,2,5,6,9,10-hexabromocyclododecane (HBCDD) is one of the most frequently used brominated flame retardants (BFR) with an estimated annual demand of 16 700 metric tons in 2001 (de Wit, 2002; Law et al., 2006). The industrial application of HBCDD has increased during the last decade concomitantly with restrictions on the use of polybrominated diphenyl ethers (PBDEs) (Prevedouros et al. 2004, Morf et al. 2007). HBCDD is primarily used in extruded and expanded polystyrene for thermal insulation in buildings, but is also used in certain textiles (Remberger et al., 2004; UNEP, 2010; Posner and Säll, 2011). The European Union has recently implemented HBCDD into the authorization procedure under REACH as a substance of very high concern (Commission regulation (EU) No 143/2011).

Recent investigations have revealed that HBCDD must be considered as an ubiquitous environmental contaminant. The ubiquitous presence of HBCDD is attributed to long-range atmospheric spread, as shown by use of air sampling and the findings of HBCDD in polar bears from the arctic (de Wit et al., 2010; Morris et al., 2004; Remberger et al, 2004). HBCDD is further found in guillemot eggs and marine mammals at concentration up to 6.8 mg/kg lipid weight (Morris et al., 2004; Lindberg et al., 2004). This is even higher than what is found of polybrominated diphenly ethers (PBDEs) in trout in Lake Mjøsa and PCB in char from Lake Ellasjøen in Bear Island (Mariussen et al., 2008; deWit 2006). In Åsefjorden, at the west coast of Norway, which is a source area with extremely high levels of HBCDD in the sediments, it has been reported a level of 200 ng/g lipid weight in eggs from common eider (Haukås et al., 2009) and 114 ng/g lipid weight in cod liver (Berge et al., 2006). Elevated levels of HBCDD are also found in the blood of workers, which have come into contact with the substance (Thomsen et al, 2007).

In a recent work by Köppen et al., (2010) very high levels of HBCDD were reported in fish caught from Etnefjorden, southwest in Norway (Fig 1) indicating a source of HBCDD contamination in the area. In codfish filet it was reported a level of approximately 30 μg/g on a lipid weight basis. No lipid content was reported in their report. Cod is a lean fish, but with a lipid content of 0.5% this is equivalent to a wet weight concentration of approximately 0.15 μg/g, which is, as far as we know, the highest concentration ever reported in cod. In flounder, Köppen et al., (2010), further reported a concentration of approximately 0.7 μg/g lipid weight. No sources of HBCDD were identified in the surrounding area in their study. Both cod and flounder are lean fish species, and lipid based concentrations may give a false impression of the actual levels. Nevertheless, the work of Köppen et al. (2010) in Etnefjorden has raised some serious concerns. The Norwegian Climate and Pollution Agency (Klif) therefore initiated a monitoring survey of HBCDD in cod and flounder from Etnefjorden in order to confirm the recent findings by Köppen et al., (2010). Increased levels of one pollutant may be followed by elevated levels of other environmental toxicants. To identify the general level of pollutants in these fish species from the fjord, the fish were in addition analyzed for the level of polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), hexachlorocyclohexane (HCH), dichlorodiphenyltrichloroethane (DDT), mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As).

2. Materials and Methods

2.1 Sampling

Fish, 10 individuals of Atlantic Cod (*Gadus morhua*), 9 individuals of the flatfish Common dab (*Limanda limanda*), and 1 individual of the flatfish Torbay sole (*Glyptoceophalus cynoglossus*), were collected in Etnefjorden at the southwest coast of Norway in December 2010 by fish net. The cods were sampled at a depth of approximately 40 m at the Holmaseid area (approximate GPS position: UTM32 6616926N 321685E) and the flounders were sampled at depth of approximately 15 m at the Osvågen area (approximate GPS position: UTM32 6616073N 325572E) (Fig 1). Near Holmaseid, it has previously been found dumped car wrecks, which may be suspected to contain HBCDD (Hamre and Westrheim, 2009). Responsible for the sampling of fish was Norwegian Institute for Water Research (NIVA). Sex, size, weight and liver weight for each individual was determined (Table 1). Samples of filets and livers were taken, put into glass containers and kept frozen at –20°C until preparation for analysis. The samples were analyzed either as individual fish or as pooled samples of multiple organisms.

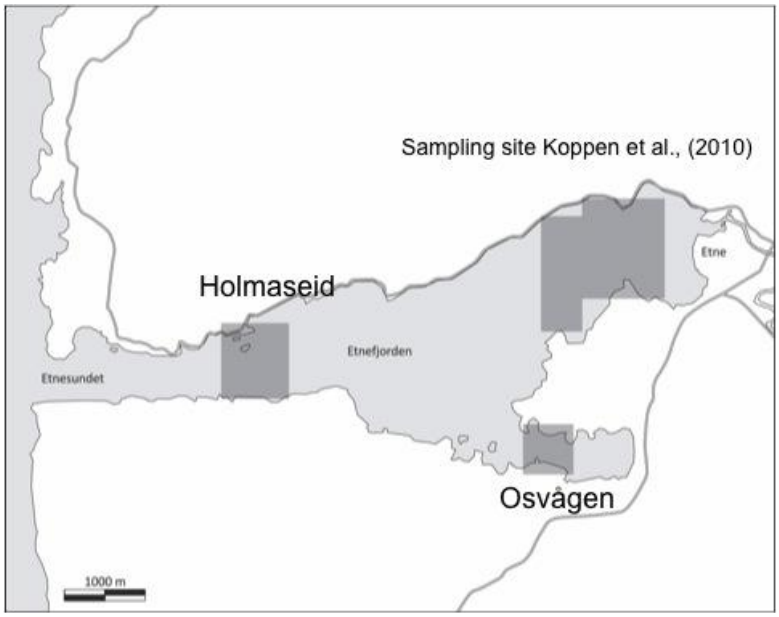

Figure1. *The sampling sites of fish in the area of Holmaseid and Osvågen respectively, and the sampling site of fish as reported in Köppen et al., (2010).*

Table 1. *Sampling data for the fish species investigated in the present work (m = male; f = female).*

Species	m/f	Weight (g)	Lenght (cm)	Liver weight (g)
Atlantic cod	5/5	2000 ± 150 (2000)	57 ± 2.3 (58)	60 ± 22 (65)
Common dab	3/6	290 ± 200 (163)	29 ± 4.7 (26)	6.5 ± 8.1 (1.8)
Torbay sole	f	355	38	2

2.2 Sample preparation

2.2.1 Sample preparation for HBCDD, PCB, DDT, HCH and PBDE analysis

Individual livers from flounder and cod were prepared for HBCDD-analysis. Pooled samples of the ten filets from cod and flounders were prepared for HBCDD, PBDE, PCB, HCH and DDT analysis. Pooled samples of the ten livers from cod and flounders were prepared for PBDE, PCB, HCH and DDT analysis. The torbay sole was pooled together with the 9 individuals of the common dab. Both livers and filets were prepared similarly. Aliquots of fish filet (5g) and liver (0.5-1g) were homogenized and mixed with sodium sulphate (pretreated 12 h at 600 C) to remove water. The samples were spiked with internal standards: d_{18}-labeled α-, β-, and γ-HBCDD; ^{13}C-labelled PBDE-28, -47, -99, -153, -183 and –209; ^{13}C-labeled α-, β-, and γ-HCH; ^{13}C -labeled p,p'- DDE and p,p'-DDT; ^{13}C -labeled PCB-28, -52, -101, -105, -114, -118, -123, -138, -153, -156, -157, -167, -180, -189, and -209. The sodium sulphate homogenates were then subjected to cold-column extraction with cyclohexane and ethyl acetate (1:1). To remove lipids, 5 ml of the crude extracts were subjected to gel permeation chromatography with cyclohexane and ethyl acetate (1:1) as mobile phase. The extracts were evaporated to dryness and dissolved in approximately 1ml *n*-hexane and subjected to further clean-up on a silica column with 4 g activated silica eluting with 60 ml of ether/hexane (1:9, by volume). For the extracts that were subjected to HBCDD-analysis, ^{13}C-labeled α- β and γ-HBCDD was added as recovery standard before solvent change to methanol and attributed to analysis on LC-MS (Haukås et al., 2009). The purified extracts subjected to PBDE, PCB, HCH and DDT analysis were added 1, 2, 3, 4-tetrachloronaphtalene (1, 2, 3, 4-TCN) as recovery standard before solvent change to iso-octane and subjected to GC/HRMS-EI analysis (Mariussen et al., 2008; Halse et al., 2011).

2.2.2 Sample preparation for Hg, Pb, Cd and As analysis

Aliquots of the pooled samples of cod and flounder filet were prepared for Hg analysis. Aliquots of the pooled samples of cod and flounder liver were prepared for Pb, Cd and As analysis. Approximately 0.5g of the samples were subjected to microwave assisted digestion with final volume of 5 ml of concentrated nitric acid and 3 ml hydrogen peroxide. After digestion the samples were diluted to approximately 1% final concentration of nitric acid and subjected to metal analysis.

2.2.3 Lipid extraction

The lipid content of the samples was determined gravimetrically. Aliquots of the fish and samples were homogenized in Na_2SO_4 to remove water and extracted with 100ml dichloromethane. The solvent was evaporated into dryness, and the residue weighed. The residue was considered as the lipid fraction of the samples.

2.3 Analysis

2.3.1 Analysis of HBCDD

The methanol extracts were analyzed for α-, β-, and γ-HBCDD using a Waters 2690 HPLC coupled to a single quadrupole Micromass z-spray mass detector (ZMD) in electro spray negative mode (ESI-) as described in detail in Haukås et al., (2009). The HBCDD diastereomers were separated on a reversed phase C_{18}-column from Atlantis (150 mm, 2.1 mm id, 3.0 μm particle size) employing a trinary gradient of methanol (A), acetonitril (B) and water (C) as eluent. The initial mobile phase (time zero) composition of 30 % A, 10 % B and 60 % C was changed to 83 % A, 15 % B and 2 % C after 12 minutes. The HBCDD

diastereomers were monitored at mass-to-charge ratio (*m/z*) of the molecular ions $[M\text{-}H]^-$. *m/z* of the selected primary/secondary ions were 640.64/638.64, 652.64/650.64 and 657.74/655.74 for the ^{12}C-diastereomers, ^{13}C - and d_{18}- labelled standards, respectively. Sum of the primary and secondary ions were used in the quantification with the ratio between the two ions as verification.

2.3.2 Analyses of PCB, DDT, HCH and PBDE

Analyses of the PCB and PBDE congeners, α-HCH, β-HCH, γ-HCH, p,p'-DDE, p,p'-DDD, o,p'-DDT and p,p'-DDT, HCB, were performed with GC/HRMS on an Agilent 6890N gas chromatograph coupled to a Waters AutoSpec mass spectrometer in electron impact (EI) mode. The compounds were detected by monitoring at m/z of the molecular ions. The GC was operating in splitless mode with helium as a carrier gas. The PCB congeners were separated using a HT-8 (50 m×0.22 mm inner diameter (SGE)) fused silica capillary column. Separation of α-HCH, β-HCH, γ-HCH, p,p'- DDE, p,p'-DDD, o,p'-DDT and p,p'-DDT, compounds were done by use of a HP-1 (25 m×0.2 mm inner diameter (J&W Scientific)) fused silica column. The PBDEs were separated with a fused silica capillary column from Zebron (ZB-1, 15m, 0.25mm id, 0.1 µm film thickness). The congeners were quantified with the labelled compounds added as internal standards. Procedure blanks were regularly prepared to control background contamination. Detailed descriptions of the analyses are given in Mariussen et al., 2008 for the PBDEs and in Halse et al. (2011) for the chlorinated compounds.

2.3.3 Analyses of Hg, Pb, Cd and As

The fish extracts were analyzed for Pb, Cd and As on an ICP-MS (Element 2, Thermo Scientific). Each sample was added internal standard and quantified with the use of an external standard curve. Mercury in the extracts was determined as Hg-total. All mercury species in the sample were oxidized to Hg^{2+} with BrCl. Determination of Hg-total was then performed using the fully automated Tekran 2600 system (CV-ASS, cold vapor atomic fluorescence spectrometer. To ensure correct quantification of the metals, a reference solution of a known metal concentration was analyzed. A deviation of 10% from the given concentration in the reference solution was accepted. Blanks were regularly analyzed to control for background contamination.

2.3.4 Measurement uncertainty

With the exception of HBCDD and PBDE NILU is accredited according to EN/NS-17025 for the analysis of all measured compounds in the fish samples. For all compounds NILU is regularly participating in international laboratory intercalibrations (e.g. Liane and Becher, 2010). NILUs performance in these intercalibration studies is satisfactory. Based on this the total expanded measurement uncertainty is estimated to be in the range of 20 to 30 %.

3. Results and Discussion

3.1 HBCDD concentrations in fish

The observed levels of HBCDD in cod livers ranged from 0.3-5.7 ng/g wet weight, which corresponds to 1.3-8.2 ng/g on a lipid weight basis Table 2. Only α-HBCDD was detected in the cod livers, whereas the concentrations of β- and γ-HBCDD were below detection limits of approximately 50 pg/g wet weight. Neither of the HBCDD congeners was detected in the flounder livers or in the pooled filet samples. Detection limits in flounder livers were 80 pg/g (± 40 SD), 80 pg/g (± 40 SD) and 50 pg/g (± 30 SD) for α- β- and γ-HBCDD respectively on a wet weight basis, which corresponds to detection limits of 0.6 ng/g (± 0.4 SD), 0.6 ng/g (± 40 SD) and 0.4 ng/g (± 0.2 SD) on a lipid weight basis. The mean lipid content in the flounder livers was 22% (± 12 SD), ranging from 6.2-41%. Detection limits of the cod and flounder filets were 5 pg/g for all three congeners, which corresponds to detection limits of 1.7 and 0.9 ng/g respectively on a lipid weight basis. The lipid content in cod and flounder was low and the lipid contents in the pooled samples were 0.29% and 0.55% in the cod and flounder samples respectively.

Table 2. *The levels of HBCDD and the lipid content in the cod livers (nd = not detected).*

		Cod liver (ng/g wet weight)			**Cod liver (ng/g lipid weight)**		
	n	Mean ± SD	Range	Median	Mean ± SD	Range	Median
α-HBCD	10/10	2.5 ± 1.8	0.3 - 5.7	2.4	4.5 ± 2,7	1.3 - 8.2	4.5
β-HBCD	nd	<0.06 ± 0.04			<0.1 ± 0.04		
γ-HBCD	nd	<0.04 ± 0.02			<0.07 ± 0.02		
Lipid (% ± SD)		54 ± 20	18 - 74	56			

Köppen et al (2010) reported high HBCDD levels in fish filets, especially in cod with a reported lipid based concentration of approximately 30 000 ng/g. The results were, however, only reported on a lipid weight basis and lipid content were not presented. Cod is a lean fish with low lipid content in the muscle tissue; most lipids are stored in the liver. In our study, the mean lipid content in the cod filet was 0.44% (± 0.25 SD) and 0.60 % (± 0.33 SD) in the flounder filet. The conversion of contaminant level from wet weight to lipid weight, may give the impression of a disproportionately high level of the pollutant in the animal. The concentration level should, therefore, be reported both on a wet weight and lipid weight basis. Taking into account an approximate lipid content of 0.5% in cod and flounder, and 7.5% in mackerel (Grégoire et al., 1994), Köppen et al (2010) found an approximate level of ΣHBCDD of 150 ng/g wet weight in cod filets (30 000 ng/g lipid weight), ΣHBCDD of 3.5 ng/g wet weight in flounder filets (680 ng/g lipid weight) and 16 ng/g wet weight in mackerel (220 ng/g lipid weight), which are substantially higher levels than found in our investigation.

Our findings indicated that the fish from Etnefjorden, contained HBCDD at concentrations close to environmental background levels (de Wit et al., (2006) and Law et al., (2006)). Other recent studies have reported similar levels. Shaw et al., 2009 reported a mean HBCDD concentrations in mackerel from the northwest Atlantic marine food web of 14 ng/g lipid weight, corresponding to a wet weight concentration of 1.4 ng/g. Jenssen et al., (2007) reported a HBCDD concentration of 22 ng/g lipid weight in Atlantic cod and 12 ng/g lipid weight in polar cod. In the study by Jenssen et al (2007), HBCDD levels were analyzed in

whole fish and no lipid content was reported, but with an approximate lipid content of 2%, the concentrations corresponds to approximately 0.5 ng/g wet weight. Montie et al., (2010) reported a level of 0.8-15 ng/g lipid weight in whole winter flounder from the Northwest Atlantic Ocean. The reported levels in cod by Köppen et al., (2010) were even higher than the levels reported in cod sampled from the Norwegian fjord, Åsefjorden, which is highly contaminated by HBCDD from a polystyrene factory (Berge et al., 2006; Haukås et al., 2009). Here, HBCDD was detected in both cod filet and liver. A concentration of 17 ng/g lipid weight (0.05 ng/g wet weight) was reported in filet, and 114 ng/g lipid weight (44 ng/g wet weight) was reported in liver (Berge et al., 2006). Köppen et al (2010) measured HBCDD in fish filet, but did not analyze fish livers. The levels of lipid soluble contaminants, such as HBCDD, in different organs usually differs, but are nevertheless expected to correlate. Also, from the limited data available, HBCDD levels in cod are reported higher in liver than in muscle (Berge et al. 2006). Moreover, in a separate study, Sellstrøm et al., (1998) reported a level of 8000 ng/g lipid (40 ng/g wet weight) in pike filet sampled downstream of a possible HBCDD point source, which is a factor of four less than what was reported by Köppen et al., (2010) in cod..

In the work by Köppen et al (2010) it was also reported high levels of the β- and γ-HBCDD diastereomers, indicating that the fish was sampled near a source area with considerable release of HBCDD. Technical mixtures of HBCDD contain 70-90% γ-HBCDD, 10-20% β-HBCDD and 1-10% α-HBCDD. In the flounder the level of γ-HBCDD was nearly similar to the level of α-HBCDD. This is previously only reported in invertebrates living close to sediments (e.g. Haukås et al., 2009). If detected, only trace amounts of the β- and γ-HBCDD are usually found in vertebrates (Covaci et al., 2006; Law et al., 2006, Haukås et al., 2009). Near a point source area, however, it has been reported up to 20% γ-HBCDD and 3% β-HBCDD in eggs from common eider (Haukås et al., 2009). In our work only α-HBCDD was detected, which is in accordance with previous reports in fish sampled from background areas or from locations with no clear sources. Köppen et al (2010) reported somewhat lower detection limits of the HBCDD diastereomers than in our study. The measured concentrations of the two diastereomers in their study were, however, substantially higher than the detection limits reported in our study. A similar level should, therefore, be discovered. Etnefjorden is a small branch of the Hardangerfjord in the municipality Etne. The municipality centre, Etne, has approximately 1000 inhabitants at the head of the fjord. With the exception of findings of dumped car wrecks near Holmaseid (Fig 1), there are, as far as we know, no obvious sources suspected for HBCDD emissions in this area.

It is difficult to speculate about the reasons for the discrepancy between our findings and the findings by Köppen et al., (2010). According to Köppen et al (2010) the fish were caught in the inner area of Etnefjorden (Fig 1). This is approximately 4 km further into the fjord than the cod sampled in our study. Although coastal cod is relatively resident with limited migration there is no reasons to believe that the cod analyzed by Köppen et al (2010) belongs to a different stock, explaining the differences in HBCDD concentrations. In a study by Jorde et al., (2007) with use of genetic markers it was showed that the population structure of coastal cod is limited in geographic area of approximately 30 km, which is on the scale of local fjords. Köppen et al (2010) also found elevated levels in mackerel and pollack, which probably migrates larger areas than the coastal cod. The elevated concentrations in the codfish and flounder, as reported in their study, should, therefore, have been discovered even at the site of catchment in our study. Åsefjorden, at the west coast of Norway, is a HBCDD point source area, which covers a similar area as Etnefjorden. Haukås et al., (2009) reported a spatial distribution of the HBCDD levels in eggs from common eider and three different

invertebrates in this fjord. Another difference between our study and the study by Köppen et al. (2010) is the sample preparation. They dried the fish samples beforehand and extracted aliquots of the dried materials, whereas we extracted aliquots of fresh materials. An error in the conversion of dry material from fresh weight may lead to an error in the results. NILU is regularly participating in international laboratory intercalibrations (e.g. Liane and Becher, 2010). The performance in these studies is satisfactory indicating that our method for analysis and preparation of HBCDD is adequate. Only, analysis of sediments from the inner site of the fjord, in addition to sediment living organisms, can positively reveal possible sources of HBCDD pollution in this area.

3.2 Concentrations of other halogenated pollutants in fish

Increased levels of one pollutant may be followed by elevated levels of other environmental toxicants. The levels of PBDEs, PCBs, HCH and DDT were, therefore, analyzed in pooled filets and livers from the cod and flounder samples from Etnefjorden. Below is a summary of the levels found in the fish samples (Table 3). Detailed information about the levels of single congeners is found in the Appendix.

Table 3. *The levels of halogenated pollutants in the in the pooled fish samples. Detailed information about the levels of single congeners is found in the Appendix. (dl = detection limit, mg/kg lipid weight in brackets).*

	Cod liver	**Cod filet**	**Flounder liver**	**Flounder filet**
	(mg/kg wet weight (mg/kg lipid weight))			
∑HCH[a]	1.4 (2.4)	< dl	0.8 (2.6)	< dl
∑DDT[b]	168 (290)	0.49 (171)	65 (208)	0.94 (171)
∑PBDE[c]**$_{28\text{-}183}$**	14 (24)	0.02 (7.2)[d]	5.8 (19)	0.05 (9.3)[d]
∑PBDE[e]**$_{196\text{-}209}$**	< dl	< dl	< dl	< dl
∑PCB[f]**$_{7}$**	238 (410)	0.55 (189)	110 (353)	1.1 (208)
∑PCB[g]**$_{total}$**	344 (593)	0.85 (294)	161 (515)	1.7 (302)

[a] Σ α-HCH, β-HCH,γ-HCH.
[b] Σ o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT.
[c] Σ PBDE-28, -47, -66, -49 + -71, -77, -85, -99, -100, -119, -138, -153, -154, -183.
[d] In cod filet and flounder filet only BDE-47 was detected.
[e] Σ PBDE-196, -206, -209.
[f] Σ PCB-28, -52, -101, -118, -138, -153, -180.
[g] Σ PCB-18, -28, -31, -33, -37, -47, -52, -66, -74, -99, -101, -105, -114, -118, -122, -123, -128, -138, -141, -149, -153, -156, -157, -167, -170, -180, -183, -187, -189, -194, -206, -209.

3.2.1 Polybrominated diphenyl ethers (PBDEs)

In cod filet, only trace of the single PBDE-congener 47 was found (0.02 ng/g wet weight corresponding to 7.2 ng/g lipid weight). In the pooled cod liver there was found somewhat higher levels and 10 out of 16 PBDE-congeners were identified (see Appendix). ΣPBDE in cod liver was 14 ng/g wet weight corresponding to 24 ng/g lipid weight. Also in the pooled flounder filet only trace of BDE-47 was found (0.05 ng/g wet weight corresponding to 9.3 ng/g lipid weight). In the pooled flounder liver 9 out of 16 PBDE-congeners were identified (see Appendix), and ΣPBDE was 5.8 ng/g wet weight corresponding to 19 ng/g lipid weight.

No traces of the decabrominated PBDE (BDE-209) was found. These figures are low and show that the fish have been exposed only to background concentrations of PBDEs.

3.2.2 Polychlorinated biphenyls (PCBs)

According to Klifs system for classification of environmental state (Molvær et al., 1997), the levels of PCBs found in cod can be regarded as background levels (class 1, slightly/negligible polluted). The concentration of ΣPCB_7 found in cod filet was 0.55 ng/g wet weight, corresponding to 189 ng/g lipid weight. The concentration in cod liver was 238 ng/g wet weight corresponding to 410 ng/g lipid weight. ΣTE_{PCB} was calculated to 0.01 pg/g in cod filet and 1.48 in cod liver. The levels of ΣPCB_7 in flounder filet were similar as in cod with a wet weight concentration of 1.1 ng/g corresponding to 208 ng/g lipid weight. The concentration in flounder liver was 110 ng/g wet weight corresponding to 353 ng/g lipid weight. ΣTE_{PCB} was calculated to 0.01 pg/g in flounder filet and 0.57 pg/g in liver. Detailed information about the levels of single PCB congeners is found in the Appendix.

3.2.3 Dichlorodiphenyltrichloroethane (DDT) and DDT metabolites

The levels of (o,p'; p,p')-DDT and its metabolites, (o,p'; p,p')-DDE and (o,p'; p,p')-DDD, in the fish were also low and can be put into class 1 in Klifs classification system of environmental state. ΣDDT in cod filet and liver was 0.5 ng/g wet weight (171 ng/g lipid weight) and 168 ng/g wet weight (290ng/g lipid weight) respectively. ΣDDT in the flounder filet and liver was 0.94 ng/g wet weight (171 ng/g lipid weight) and 65 ng/g wet weight (208 ng/g lipid weight) respectively.

3.2.4 Hexachlorocyclohexane (HCH)

The three HCH diastereomers, α-, β- and γ-HCH, were only detected in the livers of cod and flounder. ΣHCH in cod and founder liver was 1.4 ng/g (2.4ng/g lipid weight) and 0.8 ng/g wet weight (2.6ng/g lipid weight), respectively. According to Klifs system for classification of environmental state, these levels can be regarded as background levels (class 1, slightly/negligible polluted).

3.3 Concentrations of Hg, Pb, Cd and As

In addition to the halogenated compounds, some metals of interest were analyzed in the pooled filet and liver samples. The elements analyzed were As, Cd, and Pb in the pooled liver samples of cods and flounder, and Hg was analyzed in the pooled filet samples. The analyzed levels are summarized in Table 3. In general, the level of Hg in fish filet is of particular concern to Norwegian authorities as fish from several areas in Norway are subject to consumption advices by the Norwegian food authorities due to high Hg levels. However, the concentrations of Hg in the cod and flounder filets from the Etnefjord as detected in this study were relatively low and can be regarded as background levels (class 1, slightly/negligible polluted) according to Klifs system for classification of environmental state. The National Institute of Nutrition and Seafood Research (NIFES) has collected data on Hg-levels in seafood and mean level of Hg in wild caught Atlantic cod is approximately 0.04 mg/kg wet weight with a range of approximately 0.01-0.08 mg/kg wet weight (http://www.nifes.no/sjomatdata/). In brown trout (*Salmo trutta*) from the Norwegian Lake Mjøsa, the mean Hg level in filet was 0.59 mg/kg wet weight (Fjeld et al., 2009).

Arsenic, Cd and Pb in fish are still not subjected to any classification system of environmental state. Arsenic is a priority pollutant and was, in Norway, previously used in pressure

impregnated wood (Bakke et al., 2007). Little is known about the levels of As in seafood, but available literature indicates that the level varies substantially. In Bakke et al., (2007) As was analyzed in cod liver with concentrations ranging from 2.5-19. The highest levels were found in cod caught in the Fjord of Oslo. According to data collected by NIFES, however, even higher concentrations of As is measured in cod liver, but mean concentrations range from approximately 1 mg/kg to 16 mg/kg (http://www.nifes.no/sjomatdata/). The concentration of 24 mg/kg As in the flounder sample analyzed in this study is probably within expected variation.

The levels of Cd and Pb in cod liver were also low, representing background levels. According to data collected by NIFES, the mean level of Cd in wild caught Atlantic cod liver is 0.2 mg/kg wet weight, with a range of approximately0.03-0.4 mg/kg wet weight (http://www.nifes.no/sjomatdata/). In cod filet the levels are even lower. The levels of Pb in the cod liver analyzed in this study resemble what is found in livers from farmed fish (http://www.nifes.no/sjomatdata/). Maximum allowable levels of Cd and Pb in fish filet for human consumption in EU is 0.05 mg/kg and 0.2 mg/kg wet weight respectively (Commission regulation 78/2005). In a recent study on perch (*Perca fluviatilis*) which were sampled from a water nearby a small arms shooting range (Mariussen et al., 2010) reported concentrations of 0.46 mg/kg wet weight in liver and 0.19 mg/kg wet weight in filet. In freshwater fish a Pb-concentration of 0.1-0.2 mg/kg wet weight in filet and liver respectively is considered normal (Grande, 1987; 1997).

Table 3. *The levels of the elements As, Cd, Hg and Pb in the pooled fish samples.*

	Cod liver	**Cod filet**	**Flounder liver**	**Flounder filet**
	(mg/kg wet weight)			
As	5.9		24	
Cd	0.03		0.4	
Hg		0.07		0.09
Pb	0.01		0.3	

4. Conclusions

In this survey the levels of selected environmental contaminants were analyzed in cod and flounder from Etnefjord in Sunnhordland, Norway. The levels of HBCDD detected in this study were low and comparable to environmental background levels and levels previously reported in fish elsewhere (Covaci et al., 2006; Law et al., 2006, de Wit, 2006, 2010). The results, however, contrasts with previous findings by Köppen et al. (2010), which showed very high levels of HBCDD in cod, flounder, mackerel, pollack, and thorny skate from the same area.

A reason for this discrepancy in results could be that the fish analyzed in this study were not collected at the exact same site as in Köppen et al., (2010), but approximately 4 km further out of the fjord. However, given the very high levels of HBCDD that were found in fish in the Köppen study, elevated levels of HBCDD should also have been discovered at the site of catchment in this study. In addition, based on studies on population structure of coastal cod (Jorde et al., 2010), there are no reasons to believe that the cod analyzed by Köppen et al. (2010) belongs to a different stock, explaining the difference in HBCDD concentration.

Another reason for the difference in the results may be the sample preparation of the fish. Köppen et al. (2010) extracted aliquots of dried fish materials, whereas we extracted aliquots of fresh materials. NILU regularly participates in interlaboratory studies on POP analysis in food (e.g. Liane and Becher, 2010). The performance in these studies is satisfactory, indicating that our method for analysis and sample preparation of HBCDD is adequate.

Only analysis of sediments and sediment living organisms from the inner site of the Etnefjord can reveal possible sources of HBCDD pollution. Additional analysis of HBCDD in cod collected at the same sampling site as Köppen et al., (2010) would, in addition, give supplementary information of whether the high HBCDD levels reported in fish from the Etnefjord by Köppen et al., (2010) were a result of a real environmental exposure to HBCDD.

The levels of the halogenated organic compounds PCB, PBDE, DDT and HCH and the elements As, Cd, Hg and Pb were also analyzed in the fish from the Etnefjord. The levels of these compounds were low, representing background levels. Thus, on the basis of this study there are no reasons to believe that cod and flounder in Etnefjorden are exposed to considerable amount of environmental pollutants.

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Appendix

Raw Data

Composite samples of:		Cod filet	Cod liver	Flatfish filet	Flatfish liver
Sample amount in g		5	1	5	1
Compound	IUPAC-no.	ng/g fresh weight			
PeCB		<0,01	0,85	<0,01	0,40
HCB		0,06	11,2	0,06	3,60
2,2',5-TriCB	18	<0,01	0,37	<0,01	0,08
2,4,4'-TriCB	**28**	**<0,01**	**2,44**	**0,01**	**0,88**
2,4',5-TriCB	31	<0,01	0,78	<0,01	0,50
2',3,4-TriCB	33	<0,01	0,16	<0,01	0,03
3,4,4'-TriCB	37	<0,01	0,01	<0,01	0,02
Sum-TriCB		***0,02***	***5,02***	***0,02***	***1,91***
2,2',4,4'-TetCB	47	0,01	3,08	0,01	0,95
2,2',5,5'-TetCB	**52**	**0,02**	**6,02**	**0,03**	**2,51**
2,3',4,4'-TetCB	66	0,03	7,09	0,02	2,09
2,4,4',5-TetCB	74	0,01	4,33	0,01	1,38
Sum-TetCB		***0,09***	***31,1***	***0,12***	***11,9***
2,2',4,4',5-PenCB	99	0,05	16,9	0,08	7,05
2,2',4,5,5'-PenCB	**101**	**0,05**	**19,2**	**0,11**	**10,4**
2,3,3',4,4'-PenCB	105	0,03	9,97	0,04	3,49
2,3,4,4',5-PenCB	114	<0,01	0,93	<0,01	0,42
2,3',4,4',5-PenCB	**118**	**0,08**	**28,3**	**0,12**	**11,1**
2'3,3',4,5-PenCB	122	<0,01	<0,02	<0,01	<0,01
2',3,4,4',5-PenCB	123	<0,01	0,47	<0,01	0,18
Sum-PenCB		***0,22***	***75,8***	***0,36***	***32,6***
2,2',3,3',4,4'-HexCB	128	0,02	8,91	0,04	3,42
2,2',3,4,4',5'-HexCB	**138**	**0,14**	**59,9**	**0,29**	**28,5**
2,2',3,4,5,5'-HexCB	141	<0,01	3,13	0,02	1,49
2,2',3,4',5',6-HexCB	149	0,03	8,83	0,05	5,30
2,2',4,4',5,5'-HexCB	**153**	**0,21**	**93,9**	**0,46**	**44,8**
2,3,3',4,4',5-HexCB	156	0,01	4,67	0,02	1,71
2,3,3',4,4',5'-HexCB	157	<0,01	1,07	<0,01	0,42
2,3',4,4',5,5'-HexCB	167	<0,01	3,30	0,01	1,40
Sum-HexCB		***0,41***	***184***	***0,89***	***87,1***
2,2',3,3',4,4',5-HepCB	170	0,02	8,85	0,03	3,60
2,2',3,4,4',5,5'-HepCB	**180**	**0,06**	**28,6**	**0,11**	**12,3**
2,2',3,4,4',5',6-HepCB	183	0,01	5,55	0,02	2,79
2,2',3,4',5,5',6-HepCB	187	0,02	10,9	0,07	9,42
2,3,3',4,4',5,5'-HepCB	189	<0,01	0,52	<0,01	0,21
Sum-HepCB		***0,11***	***54,4***	***0,24***	***28,3***
2,2',3,3',4,4',5,5'-OctCB	194	<0,01	2,93	0,01	1,70
2,2',3,3',4,4',5,5',6-NonCB	206	<0,01	1,63	0,01	1,54
DecaCB	209	<0,01	1,92	0,02	1,73
Sum 7 PCB		**0,55**	**238**	**1,15**	**110**
Sum PCB		***0,87***	***356***	***1,68***	***167***

Sum 7 PCB: Sum of PCB-28, 52, 101, 118, 138, 153, and 180. Sum PCB: Sum of all detected PCB congeners (not including mono- and dichloro congeners).

Composite samples of:	Cod filet	Cod liver	Flatfish filet	Flatfish liver
Sample amount in g	5	1	5	1
Compound	ng/g fresh weight			
α-HCH	< 0.01	0,66	< 0.01	0,34
β-HCH	< 0.01	0,42	< 0.01	0,27
γ-HCH	< 0.01	0,32	< 0.01	0,19
o,p'-DDE	< 0.01	0,31	< 0.01	0,30
p,p'-DDE	0,40	131	0,81	55,4
o,p'-DDD	< 0.01	1,04	< 0.01	0,43
p,p'-DDD	0,05	21,1	0,07	5,23
o,p'-DDT	< 0.01	1,19	0,01	0,51
p,p'-DDT	0,03	13,7	0,04	3,09
Sum DDT	0,48	168	0,93	65,0

Sum DDT: Sum of o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, and p,p'-DDT,

Composite samples of:		Cod filet	Cod liver	Flatfish filet	Flatfish liver
Sample amount in g		5	1	5	1
Compound	IUPAC-no.	ng/g fresh weight			
TBA		<0,01	1,21	0,01	0,57
2,4,4'-TriBDE	28	<0,01	0,47	<0,01	0,09
2,2',4,4'-TetBDE	47	0,02	8,71	0,05	3,94
2,3',4,4'-TetBDE	66	<0,01	0,10	<0,01	0,03
2,2',4,5' + 2,3',4',6-TetBDE	49 + 71	<0,01	1,73	<0,01	0,49
3,3',4,4'-TetBDE	77	<0,01	<0,01	<0,01	<0,01
2,2',3,4,4'-PenBDE	85	<0,01	<0,01	<0,01	<0,11
2,2',4,4',5-PenBDE	99	<0,01	0,20	<0,01	0,10
2,2',4,4',6-PenBDE	100	<0,01	1,55	<0,01	0,48
2,3',4,4',6-PenBDE	119	<0,01	0,06	<0,01	<0,07
2,2',3,4,4',5'-HexBDE	138	<0,01	<0,02	<0,01	<0,02
2,2',4,4',5,5'-HexBDE	153	<0,01	0,05	<0,01	0,15
2,2',4,4',5,6'-HexBDE	154	<0,01	0,87	<0,01	0,39
2,2',3,4,4',5',6-HepBDE	183	<0,01	0,03	<0,01	0,03
2,2',3,3',4,4',5,6'-OctBDE	196	<0,01	<0,07	<0,01	<0,04
2,2',3,3',4,4',5,5',6-NonBDE	206	<0,02	<0,11	<0,01	<0,06
DecaBDE	209	<0,04	<0,23	<0,02	<0,13

Sample type and number		Sample amount	Compound		
			α-HBCDD	**β-HBCDD**	**γ-HBCDD**
		g	ng/g fresh weight		
Cod liver	Sample 1	1	5,74	-0,11	-0,05
Cod liver	Sample 2	1	1,12	-0,08	-0,05
Cod liver	Sample 3	1	5,47	-0,10	-0,05
Cod liver	Sample 4	1	2,99	-0,08	-0,05
Cod liver	Sample 5	1	2,29	-0,05	-0,03
Cod liver	Sample 6	1	2,42	-0,13	-0,06
Cod liver	Sample 7	1	2,64	-0,03	-0,02
Cod liver	Sample 8	1	0,60	-0,04	-0,02
Cod liver	Sample 9	1	1,88	-0,02	-0,02
Cod liver	Sample 10	1	0,27	-0,02	-0,02
Flatfish liver, Common dab	Sample 1	0,5	-0,08	-0,08	-0,05
Flatfish liver, Common dab	Sample 2	1	-0,04	-0,04	-0,03
Flatfish liver, Common dab	Sample 3	1	-0,04	-0,05	-0,03
Flatfish liver, Common dab	Sample 4	1	-0,04	-0,04	-0,03
Flatfish liver, Common dab	Sample 5	0,5	-0,09	-0,10	-0,06
Flatfish liver, Common dab	Sample 6	0,5	-0,08	-0,08	-0,05
Flatfish liver, Common dab	Sample 7	0,5	-0,07	-0,08	-0,05
Flatfish liver, Common dab	Sample 8	0,4	-0,12	-0,13	-0,08
Flatfish liver, Common dab	Sample 9	0,4	-0,18	-0,15	-0,09
Flatfish liver, Torbay sole	Sample 10	1	-0,06	-0,08	-0,05
Cod filèt	Composite sample of 10 samples	5	-0,01	-0,01	-0,01
Flatfish filèt	Composite sample of 10 samples	5	-0,01	-0,01	-0,01

Prosjektnr:	O-111053		
Prøve ID	**Journal-nr**	**Kons. Hg**	**Enhet**
Fisk			
Torskefilet	11-137-1	0.067	mg/kg
Flatfiskfilet	11-137-3	0.090	mg/kg

Norsk Institutt for Luftforskning
Avdeling for Uorganisk Analyse
2007 KJELLER

N I L U I C P M S R A P P O R T

Dato: 11/05/06
Side: 1

Prøveidentifikasjon	Prøve dato	Nilu ID	Provetyp	Fort. faktor	Vekt	Ut.vol	Enhet	As	Sn	Be	Mg	Al	Ca	Rb	Sr	Cs	Ba	B	Na	
Torskelever		O-111053 11-137 2		1.	0.321		ng/g	5920.25												22
Flatfisk lever		O-111053 11-137 4		1.	0.318		ng/g	24194.65												22

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N I L U I C P M S R A P P O R T

Dato: 11/05/06
Side: 1

Prøveidentifikasjon	Prøve Dato	Nilu ID	Provetyp	Fort. faktor	Vekt	Ut.vol	Enhet	Pb	Cd	V	Cr	Mn	Co	Ni	Cu	Zn	Fe	
Torskelever		O-111053 11-137 2		1.	0.321	50.	ng/g	14.174	32.165									11
Flatfisk lever		O-111053 11-137 4		1.	0.318	50.	ng/g	270.912	412.657									11

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Utførende institusjon	ISBN-nummer
NILU – Norsk institutt for luftforskning	978-82-425-2431-7 (T) 978-82-425-2432-4 (E)

Oppdragstakers prosjektansvarlig	Kontaktperson i Klima- og forurensningsdirektoratet	TA-nummer
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	2011	35	5011106

Utgiver	Prosjektet er finansiert av
NILU – Norsk institutt for luftforskning	Klima- og forurensningsdirektoratet

Forfatter(e)
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Tittel - norsk og engelsk
Contaminants in fish from Etnefjorden, Norway
Miljøgifter i fisk fra Etnefjorden, Norge

Sammendrag – summary
1,2,5,6,9,10-hexabromocyclododecane (HBCDD) is one of the most frequently used brominated flame retardants (BFR) and is considered as an emerging environmental pollutant. Recently, high levels of HBCDD were reported in five different fish species from Etnefjord, which is a branch of the Hardangerfjord at the southwest coast of Norway. This report raised some serious concerns and the Norwegian Climate and Pollution Agency (Klif) therefore initiated a survey of HBCDD in cod and flounder from Etnefjorden in order to confirm the findings. In addition to HBCDD, the levels of other environmental pollutants were analyzed in the fish, namely polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), hexachlorocyclohexane (HCH), dichlorodiphenyltrichloroethane (DDT), mercury (Mg), lead (Pb), cadmium (Cd) and arsenic (As). The fish muscle filet and livers were prepared and analyzed using recognized and previously published methods. The levels of HBCDD in the fish were low, representing expected background concentrations. HBCDD was only detected in the cod livers with concentrations ranging 0.3-5.7 ng/g wet weight, corresponding to 1.3-8.2 ng/g on a lipid weight basis. Previously, it was reported a level of 30 000 ng/g lipid weight in cod filet, which is, as far as we know, the highest HBCDD concentration ever reported in cod. Our study could, therefore, not confirm the recent findings that showed high HBCDD concentrations in fish from the Etnefjord area. Also, the levels of the other pollutants were low. For example, the concentrations of DDT, PCB, HCH and Hg can, according to Klifs system for classification of environmental state, be regarded as background levels (class 1, slightly/negligible polluted). In conclusion, there are no recons to believe that cod and flounder in Etnefjorden is exposed to considerable amount of environmental pollutants, neither of HBCDD nor other contaminants. Only further analysis of sediments from the inner site of the fjord can, however, positively reveal a possible HBCDD source.

4 emneord	4 subject words
Etnefjord, Fisk, Miljøgifter	Etnefjord, Fish, Contaminants,

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Climate and Pollution Agency

The Climate and Pollution Agency reports to the Ministry of the Environment and has 325 employees, based mainly in Oslo. We implement government policy on pollution. We act as advisors, guardians and stewards for the environment. Our most important fields of work include climate change, chemicals, marine and freshwater environment, waste management, air quality and noise. Our vision is a future without pollution.
We are working to

- reduce greenhouse gas emissions
- reduce the spread of hazardous substances harmful to health and the environment
- achieve integrated and ecosystem-based management of the marine and freshwater environment
- increase waste recovery and reduce emissions from waste
- reduce the harmful effects of air pollution and noise

TA-2821/2011
ISBN 978-82-425-2431-7 (Trykt)
ISBN 978-82-425-2432-4 (Elektronisk)