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A baseline study on levels of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, non-ortho and mono-ortho PCBs, non-dioxin-like PCBs and polybrominated diphenyl ethers in Northeast Arctic cod (*Gadus morhua*) from different parts of the Barents Sea

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ABSTRACT

This study is one of several baseline studies on commercially important Norwegian wild fish species that will provide information concerning metals and persistent organic pollutants (POPs) and food safety. The cod liver is a traditional food product in Norway and a potential source for POPs in the diet. The concentrations of dioxins and furans (PCDD/Fs), dioxin-like PCBs (DL-PCBs), non-dioxin-like PCBs (NDL-PCBs, PCB_6) and polybrominated flame retardants (PBDEs) were determined in the liver of 784 individual Northeast Arctic cod caught at 32 positions in the Barents Sea in the period from 2009–2010. In addition, muscle samples from 30 individual cod were analysed for the same substances. The mean concentration of the sum of PCDD/Fs and DL-PCBs for all samples was 14.2 ng $TEQ_{who\text{-}2005}$/kg ww with a variation between 1.0 and 151 ng TEQ/kg ww. The concentrations of POPs in the fillet samples were very low.

Atlantic cod (*Gadus morhua*) is distributed in the North Atlantic Ocean, and the Northeast Arctic cod stock is the population of Atlantic cod that dominates the Barents Sea ecosystem. This is currently, and possibly historically, the largest cod stock in the world (Yaragina et al., 2011) and the largest biomass (of fish aged three years and older) ever recorded was 4.2 million tonnes in 1946 (Øiestad, 1994). Migration over long distances is a characteristic of this species. Adult fish can migrate up to 2000 km annually, from feeding grounds in the Barents Sea and in the waters around Svalbard, to the spawning areas along the Norwegian coast and back (Yaragina et al., 2011). Cod are well adapted to the environment in the Barents Sea and, in 2011, cod was the commercially most valuable species of wild-caught fish in Norway (data from the Directorate of Fisheries in January 2012). The catch of Northeast Arctic cod has varied from 212,000 to 1,343,000 tonnes, with an average of 656,000 tonnes caught between 1946 and 2007 (Hylen et al., 2008; ICES, 2008).

In Norway, it is common practice to utilise not only the fillet but also the liver from codfishes (family Gadidae), particularly Atlantic cod (*Gadus morhua*), saithe (*Pollachius virens*) and haddock (*Melanogrammus aeglefinus*). These fish species have a lean fillet,

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with a fat content of less than 1 g/100 g, and energy storage primarily in the liver. The liver is very oily, with a lipid content that can vary between 31 and 75 g/100 g (www.nifes.no/seafooddata), and with ω-3 fatty acids accounting for up to 30% of the total fat. Fish liver is also rich in vitamins A and D (VKM, 2007). Extensive volumes of fish liver from the three codfish species are produced as a side-product during fishing with about 13,000 tonnes from cod, 23,000 tonnes from saithe and 5000 tonnes from haddock. Part of this volume is today used for production of cod liver oil, following extensive clean-up to minimise the content of undesirable substances, while the major part is discarded. A minor part is also used directly for human consumption (VKM, 2007).

Since the cod musculature is very lean, only very small amounts of lipophilic compounds, including POPs, can be accumulated in the fillet. However, the oily liver has the potential to accumulate relatively large amounts of lipophilic organohalogen compounds, such as polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) (collectively referred to as dioxins or PCDD/Fs), polychlorinated biphenyls (PCBs) and the brominated flame-retardants such as polybrominated diphenyl ethers (PBDEs). Of the PCBs, there are both dioxin-like PCBs (DL-PCBs), which exhibit dioxin-like toxicity (i.e. non-ortho and mono-ortho PCBs), and the non-dioxin-like PCBs (i.e. NDL-PCBs, PCB_6), which have a different toxicological action (EC, 2001). These organohalogen compounds are all defined as persistent organic pollutants (POPs) that are ubiquitous in the environment, and also present in low concentrations in food. To ensure that seafood products containing particularly high levels of these substances are not sold in the European market and hence to protect consumer safety, the EU has set a maximum limit for the concentrations of the sum of PCDD/Fs in fish muscle of 3.5 ng TEQ kg^{-1} wet weight (ww), and for the the sum of PCDD/Fs and DL-PCBs in muscle and liver of fish of 6.5 and 20 ng TEQ kg^{-1} ww, respectively, using re-evaluated Toxic Equivalency Factors (TEF-2005) (EC, 2011; Van den Berg et al., 2006). Recently, upper limits for the sum of six NDL-PCBs (PCB_6) in fish muscle and liver have also been established at 75 and 200 μg kg^{-1} ww, respectively (EC, 2011). To date, no upper limit has been set for the PBDEs, even though risk assessments have been performed (JECFA, 2006).

The levels of undesirable substances such as PCB_7 in fish and other seafood in Norway have been regularly monitored by spot checks since 1994 (Julshamn et al., 2004; www.nifes.no/seafooddata). To establish more reliable surveillance data on undesirable substances, comprehensive baseline studies, including a large number of stations over a broad geographical area throughout the seasons, are needed for commercially important species such as Arctic cod. Such baseline studies can also provide recommendation to food safety authorities with recommendations on how future monitoring should be performed (e.g. frequency of sampling and number of sampling sites). Several fish stocks from Norwegian waters (Greenland halibut (*Reinhardtius hippoglossoides*), Norwegian spring spawning (NSS) and North Sea herring (*Clupea harengus*), mackerel (*Scomber scombrus*), saithe and different stocks of Atlantic cod) have been subjected to baseline studies where the levels of undesirable substances, i.e. heavy metals and POPs, have been determined for a large number of fish. Results from these studies have earlier been reported for the baseline study of POPs in NSS herring (Frantzen et al., 2011) and for metals in Northeast Arctic cod (Julshamn et al., 2013). This study reports on the baseline study of POPs in livers from 784 Northeast Arctic cod and a few muscle samples (30) of Northeast Arctic cod (the same samples as reported for metals by Julshamn et al., 2013). Muscle and liver samples were analysed for PCDDs, PCDFs, DL-PCBs, NDL-PCBs (PCB_6) and PBDEs ($PBDE_7$). Biological parameters such as age, weight, length, gender, condition factor and liver index were recorded in order to help explain the observed patterns.

The sampling of Northeast Arctic cod was carried out in 2009 and 2010, covering an area in the Barents Sea from approximately 70° to 75°N and from 16° to 41°E (Fig. 1). This area mainly covered the Norwegian part of the Barents Sea, although some samples were also taken in the Russian zone. Six commercial fishing vessels belonging to the reference fleet of the Institute of Marine Research (IMR) were involved in collecting the fish samples, and trawl was the major fishing gear in addition to nets, longlines and handlines. In total, samples were retrieved from 32 positions from February 2009 to May 2010. At most positions, 25 cod were sampled according to a protocol given to the fishermen and, in total, 807 fish were collected.

Whole fish were individually frozen before being shipped to the laboratory. The fish were defrosted and the length, weight and gender for each individual fish were determined and the otoliths were removed for subsequent age determination. To obtain as much information as possible, all analyses were performed on samples from individual fish. The liver was removed from each fish and weighed, and then homogenised using a food processor. The homogenate was stored in closed glass bottles at −20 °C until analysis. Approximately 200 g muscle tissue without skin from 30 individual fish (i.e. 20 fish from the southwest area and 10 fish from the east area with high dioxin contents in the liver) was removed for analysis. The wet subsamples were homogenised and freeze-dried, and the dry matter content was calculated. The dried muscle samples were ground to a fine powder, which was again homogenised and kept dry prior to analysis. A sample of the remaining wet homogenates was prepared for the determination of the fat content. Fish age was determined by otolith reading. The otoliths were broken, and the annual growth zones visible on the broken sections were counted using a binocular microscope with light transmitted from the side and the broken surface shadowed.

The fat was extracted from the wet sample homogenate by shaking with 30% isopropanol in ethyl acetate. The solution was then filtered before the solvent was evaporated and the fat residue weighed. Measurement uncertainties ranged between 5% and 10% and the limit of quantification (LOQ) was 0.1 g/100 g. The method was tested by performing three proficiency tests (PT) in 2010, analysing three different seafood samples having fat contents between 2 and 20 g/100 g. The z-scores obtained were between −0.3 and +2.0. This method is accredited in accordance with ISO-EN 17025 and is registered as a Norwegian Standard, NS 9402.

The same method of sample clean-up and extraction was used for dioxins and furans (PCDD/Fs), DL-PCBs, NDL-PCBs and PBDEs. The applied method was slightly modified from the method described in detail by Berntssen et al. (2010), based on method numbers 1613 and 1668 of the United States Environmental Protection Agency (USA EPA, 1994,1999). Briefly, wet and homogenised liver sample or dried homogenised muscle sample was mixed with hydromatrix (Agilent Technologies) and ^{13}C-labelled internal standards were added (27 standards for dioxins, furans and dioxin-like PCBs, CIL, and one standard for PBDEs, Sigma–Aldrich). The mixture was transferred to an Accelerated Solvent Extractor 300 (ASE, Dionex Corp.) or a Pressurised Liquid Extraction System (PLE, FMS Inc.), with a layer of acidic silica gel (Merck), and extracted with hexane under elevated pressure and temperature (100 °C, 1500 psi). The extract was further purified chromatographically using PowerPrep (FMS Inc.) over three columns packed with multilayer silica, basic alumina and carbon, respectively, and was eluted with different solvents. Two fractions were collected: fraction 1 contained PBDEs, PCB_6 and mono-ortho PCBs and fraction 2 contained dioxins, furans and non-ortho PCBs. Determinations of PCDD/Fs and non-ortho-PCBs were performed by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) using a DFS-MS, HRGC-HRMS, Ther-