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Levels of omega 3 fatty acids, vitamin D, dioxins and dioxin-like PCBs in oily fish; a new perspective on the reporting of nutrient and contaminant data for risk–benefit assessments of oily seafood

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A B S T R A C T

Oily seafood is an important food source which contains several key nutrients beneficial for human health. On the other hand, oily seafood also contains persistent organic pollutants (POPs), including the dioxin-like compounds (DLCs) polychlorinated dibenzo-p-dioxins/polychlorinated dibenzofurans (PCDD/Fs) and dioxin-like-polychlorinated biphenyls (dl-PCBs), potentially detrimental to human health. For a comprehensive comparison of the beneficial and potentially adverse health effects of seafood consumption, risk–benefit analyses are necessary. Risk-benefit analyses require reliable quantitative data and sound knowledge of uncertainties and potential biases.

Our dataset comprised more than 4000 analyses of DLCs and more than 1000 analyses each of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and vitamin D in the three most important Norwegian commercial oily seafood species: Atlantic herring (Clupea harengus), Atlantic mackerel (Scomber scombrus) and farmed Atlantic salmon (Salmo salar). The levels of several DLC congeners were below the limit of quantification (LOQ), making estimation of true levels challenging. We demonstrate that the use of upper bound substitution of censored data will overestimate, while lower bound substitution will underestimate the actual levels of DLCs. Therefore, we implement an alternative robust statistical method by combining Maximum Likelihood Estimation, Regression on Order Statistics and Kaplan-Meier analyses, which is better suited for providing estimations of levels of these contaminants in seafood. Moreover, we illustrate the impact of the toxic equivalency factor (TEF) system on estimation of the sums of DLCs by comparing the TEF system to an alternative system of relative effect potency (REP) factors (Consensus Toxicity Factors).

The levels of nutrients and contaminants were related to adequate intake (AI) and tolerable weekly intake (TWI), respectively. We used AI and the TWI values established by the European Food Safety Authority (EFSA). The benefit and the risk were further viewed in the context of the Norwegian average intake of oily fish, and the Norwegian governmental official dietary recommendations of oily fish. Our results showed that both benefit and risk are met at the levels found of nutrients and DLCs in oily seafood. The comprehensive quantitative data presented here will be a key for future risk–benefit assessment of oily fish consumption. Together, our results underline that a refined formalized integrative risk–benefit assessment of oily fish in the diet is warranted, and that the data and methodology presented in this study are highly relevant for future integrated and multidisciplinary assessment of both risks and benefits of seafood consumption for human health.

1. Introduction

Seafood provides several health-promoting nutrients including: high quality protein, minerals, vitamins and polyunsaturated long chain omega 3 fatty acids (n-3PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Consumption of oily fish has been associated with reduced blood pressure (Bernstein et al., 2019), lower cholesterol (Lim et al., 2012), and improved cardiovascular function and
reduced risk of death from coronary heart disease (FAO/WHO, 2011; Mozaffarian and Rimm, 2006). Fish consumption has been shown to reduce all-cause mortality, with consumption of 60 g of fish per day associated with a 12% reduction in risk (Zhao et al., 2016). Consuming fish has also been associated with improved cognitive development and higher IQ (Hibbels et al., 2006; Hibbeln et al., 2019), and to prevent certain allergies, as well as positive behavioral and mental health outcomes (Bernstein et al., 2019). However, seafood also contains hazardous environmental contaminants such as methylmercury (MeHg) and dioxin-like compounds (DLCs), polychlorinated dibenzo-p-dioxins/ polychlorinated dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs). Of these, the levels of lipid soluble DLCs are particularly relevant in oily fish. Exposure to DLC has been associated with chloracne and other dermal effects, cancer, hepatic disorders thyroid disease, type 2 diabetes and obesity as well as detrimental effects on the cardiovascular, nervous and immune systems, digestion, reproduction, teeth and bones as reviewed by EFSA (2018). Chloracne caused by dioxins is the most reliable and specific indicator of toxicity in humans, but it occurs following high levels of exposure for example in accidental, deliberate or occupational incidents. An association between dioxin exposure during early childhood and impaired semen quality is the most sensitive effect that has been observed in epidemiological studies (Minguex-Alarcon et al., 2017; Mocarelli et al., 2011; Mocarelli et al., 2008).

The combination of nutrients and contaminants in the same food matrix has led to several risk-benefit analyses of fish consumption. Based on tolerable weekly intakes (TWIs) available at the time, previous evaluations performed both by risk assessment bodies as well as independent researchers, have generally concluded that the benefits of the nutrients in fish outweigh the risks of the contaminants (FAO/WHO, 2011; Hellberg et al., 2012; VKM, 2014). However, the European Food Safety Authority (EFSA) recently performed a re-evaluation of DLCs and reduced the TWI for these contaminants from 14 to 2 pg WHO TEQ/kg bw (EFSA, 2018). This reduction of the TWI for these contaminants will potentially impact the human intake of oily fish, since these are among the highest dietary exposure sources to DLCs (EFSA, 2018). However, oily seafood is also a particularly good source for marine omega 3 and vitamin D. Atlantic mackerel, (Scomber scombrus), Atlantic herring (Clupea harengus) and farmed Atlantic salmon, (Salmo salar), are among the most important commercial oily fish species available for human consumption (FAO, 2018). Since these species store fat in the fillet, they also provide high levels of the marine n-3PUFAs, and different governmental health authorities have specific dietary guidelines suggesting that some of the weekly intake of seafood should be in form of oily fish species (Becker et al., 2007; VKM, 2014). Still, since oily fish are among the main sources of DLCs for humans, a re-evaluation of the dietary recommendations following the reduction in the TWI is warranted.

The negative health effects of DLCs are derived from dose-additive toxicities using toxic equivalence factors (TEFs) (Van den Berg et al., 2006), a concept based on these compounds ability to interact with and activate the Aryl hydrocarbon Receptor (AhR) (Haws et al., 2006; Van den Berg et al., 2006). TEF values are derived using: expert judgement, point estimates and relative effect potency (REP) to TCDD based on in vivo or in vitro studies (Van den Berg et al., 2006). As the putative toxicity of any food item containing DLCs will be based on the sum of several TEF values, accurate TEF values are essential to evaluate potential acute health effects. The latest evaluation of TEFs was performed by the WHO in 2005 (Van den Berg et al., 2006). However, EFSA called for a re-evaluation of the TEF system in their recent risk assessment (EFSA, 2018), to take into account studies of congener-specific toxicity performed after 2005.

In this study, we present the levels of EPA, DHA and vitamin D, as well as the levels of DLCs in herring, mackerel and farmed salmon, the most commercially important oily fish species from Norway. Since these three species are minor sources of methylmercury (Azad et al., 2019; Nostbakken et al., 2015), another important marine contaminant, we have focused on DLCs in this study. We relate the intake of nutrients and contaminants from these species to recommended intake data, and we further visualize the impact that the current TEF system has for each congener’s impact on toxicity, and consequently on the risk assessment. The levels of several congeners of DLC are close to, or below, the limits of quantification (LOQ). Hence, we show how choice of data treatment of left censored data can affect estimation of toxicity, by using: lower bound (LB), upper bound (UB) and a robust statistical protocol for the estimation of DLCs. The comprehensive quantitative data presented here will be key for future risk-benefit assessment of oily fish consumption.

2. Material and methods

2.1. Sampling

The data presented in the current study comprise measurements of DHA, EPA, vitamin D, and DLCs in fish fillet from Norwegian surveillance and monitoring: farmed salmon sampled between 2012 and 2017, wild caught Northeast mackerel sampled between 2008 and 2016, wild caught North Sea (NS) herring sampled between 2009 and 2014 and wild caught Norwegian spring spawning (NSS) herring sampled between 2006 and 2017. The two stocks of herring are considered together as one group for this study. In total, we show results from 1096 analyses of EPA and DHA, 1105 analyses of vitamin D and 4056 analyses of DLCs.

Samples of farmed salmon were obtained from all regions along the Norwegian coast with aquaculture activity, by the Norwegian Food Safety Authority, and analysed for DLCs (n = 884), EPA, DHA (n = 455) and vitamin D (n = 455) at the Institute of Marine Research (IMR). Farmed salmon were sampled by three different sampling techniques: Norwegian Quality Cuts (NQCs) were sampled from 2012 to 2017 (n = 358), these were pooled in samples of five fish prior to homogenization and analyses, as described in Nostbakken et al. (2015). Single sampled NQC homogenates (n = 228) were prepared in 2012, 2013 and 2017. Homogenates from whole skin-free fillets of individual farmed salmon (n = 298) were sampled from 2014 to 2017. For verification that sampling technique did not alter the mean concentration of DLCs, whole fillet homogenates (n = 100) and individual NQC homogenates (N = 52), from the overlapping year of sampling (2015) were compared (Fig. 1). Since these samples are not from the same fish, it does not rule out potential effects of the sampling technique, but it gives a good
Samples of NSS herring were collected throughout the Norwegian Sea by research vessels or commercial fishing vessels contracted by the IMR. However, the amount sampled in this area would not affect overall median levels. Sampled fish were filleted and shipped to the laboratory. Individual fish were filleted and skin-fillets homogenized using a food processor. Homogenized samples were analysed for DLCs (n = 2018) at the IMR. EPA, DHA (n = 196) and Vitamin D (n = 196) were only analysed in NSS herring. Since both NS- and NSS herring are commercially marketed as “Atlantic herring”, the results from these samples were combined and statistically treated as one group.

Mackerel was sampled in the North Sea, Skagerrak, the Norwegian Sea and in the Northeast Atlantic west of Scotland during the baseline survey from 2008 to 2009 (N = 798) and a subsequent monitoring study between 2012 and 2016 (N = 366). Individual fish were filleted and skin-fillets homogenized using a food processor. Homogenized samples were analysed for DLCs (n = 1154), EPA, DHA (n = 345) and Vitamin D (n = 345).

2.2. Analyses

Analyses for DLCs were conducted as described by Lundebye et al. (2017). In brief, the analyses were extracted with hexane using an accelerated solvent extractor and purified by an automated PowerPrep system. PCDDs, PCDFs and non-ortho-PCBs were analysed by high-resolution gas chromatography/high resolution- mass spectrometry (HRGC-HRMS). Mono-ortho-PCBs were analysed by gas chromatography/tandem mass spectrometry (GC-MS/MS).

Concentrations of DLCs were converted into toxic equivalents (TEQ), ng TEQ kg\(^{-1}\) wet weight using the WHO2005 TEFs (Van den Berg et al. 2006). For illustrational purposes, we additionally implemented Consensus Toxicity Factors (CTF), ng CTF kg\(^{-1}\) wet weight (Larsson et al. 2015). CTFs are based on the same principle as TEFs, but are based on in Silico models and in Vitro screening of AhR mediated effects in human and rodent cells (Larsson et al., 2015). CTFs were developed as a novel alternative to the globally accepted TEF values, and although the term “consensus” might be misleading since these were suggested by a single study, the CTFs illustrate the uncertainty in the impact of relative effect potency (REP). Unless otherwise specified, the CTF system is used. If concentrations without the use of TEF or CTF are discussed, these are specified as “original concentration”.

Fatty acids were analysed as described by Lundebye et al. (2017). Briefly, fatty acids were analysed on a HP-7890A gas chromatograph using a flame ionization detector (GC-FID), and with nonadecanoic acid (19:0) as an internal standard. The fatty acids methyl esters (FAME) were extracted using hexane. The fatty acids were identified by retention time in combination with FAME standards and a fatty acid standard. Chromatographic peak areas were corrected by empirical response factors calculated from the areas of the GLC-463 mixture. The chromatograms were integrated using EZChrom Elite software from Agilent Technologies.

Vitamin D was analysed as described previously (Horvll and Lie, 1994). Briefly, the samples were saponified and the unsaponifiable material was extracted and then purified on a preparative high-performance liquid chromatography (HPLC) column. The fraction containing D2 (ergocalciferol) and D3 (cholecalciferol) was pooled (normal phase). This fraction was injected on an analytical HPLC column (reverse phase). Vitamin D2/D3 was determined by a UV detector. The content of vitamin D3 was calculated using vitamin D2 as internal standard (CEN, 2009).

2.3. Nutrient and DLC levels in a human health context

The levels of marine nutrients, and DLCs in the analysed species, were evaluated in the context of exposure from both reported and recommended seafood consumption in Norway. Data on intake of oily fish (meals and spread) was obtained from the Norwegian dietary survey: Norkost 3 (Totland et al., 2012). Recommended intake was obtained from the Norwegian Directorate of Health (2014). For simplicity, in the calculations each species was considered separately as the sole source contributing to the overall intake of oily fish as provided by the dietary survey.

To evaluate the levels of DLCs and nutrients in the Norwegian diet, we compared them with the TWI and the adequate intake (AI), respectively. The TWI for DLCs was 2 pg WHO TEQ-05 per kg per week (EFSA, 2018) and the AI for adults and children (aged 1–17 years of age) was 15 μg per day for vitamin D (EFSA, 2016) and 250 mg per day for EPA and DHA (EFSA, 2010b). For comparative reasons we also evaluated the levels using the JECFA provisional monthly intake (PTMI) (JECFA, 2002). Here the PTMI was converted to weekly intake by dividing by 4.34. A complete risk–benefit assessment was not feasible within the scope of this study.

2.4. Statistical treatment of data

To account for values below the limit of quantification (LOQ), and to visualize the impact of results below the LOQ, we computed summary statistics and sums of PCDDs, PCDFs, dl-PCBs or total DLCs using different methods. In upper bound (UB) calculations, levels below the LOQ were substituted with the LOQ; in lower bound (LB) calculations levels below the LOQ were substituted with zero. In addition, we implemented different statistical procedures described by Tekindal et al. (2017). In short, data was subjected to parametric (Maximum Likelihood Estimation; MLE), semi-parametric (Regression on Order Statistics; ROS), and non-parametric (Kaplan–Meier; KM) analyses in the statistical programming language R (version 3.6.1) (R Core Team, 2019) running in RStudio (version 1.2.5019; RStudio Team, 2019). The chosen statistical methods were implemented following and adapting R-scripts presented in Bolks et al. (2014). Summary statistics of all tests performed are presented in the supplementary material (see Suppl. 1), and a further description of our statistical approach is illustrated in supplement 2. Based on the estimated level of censoring for each congener, we chose to report ROS if censoring was below 50%, MLE if censoring was between 50% and 80%; if more than 80% of data were censored, it was deemed unfeasible to estimate summary statistics, as recommended by Helsel (2012). Medians were chosen as the main descriptors of the average of DLCs, since the data showed a tailed distribution for all species (Fig. 2). ROS/MLE was, however, not applied to the sum of DLCs since these data already include UB LOQ. Therefore, in order to estimate sum for DLCs, each congeners median was summed (supplement 2). Data on EPA, DHA and vitamin D were not censored; i.e. all measurements were above the LOQ. Therefore, the statistical approach did not require an additional robust approach. Data on nutrients did not show a Gaussian distribution, and did not pass the D’Agostino-Pearson normality test. Therefore, the median was chosen as the descriptor of the average and variation visualized using interquartile range and full range. Basic statistical analyses were performed using Graphpad Prism 8 (Graphpad software Inc., San Diego, CA, USA).
3. Results

3.1. Levels of EPA, DHA, vitamin D, and DLCs in Norwegian oily fish

The measured concentrations of EPA, DHA and vitamin D in herring, mackerel and farmed salmon are shown in Fig. 3. Median levels of DHA + EPA were 1.2 g 100 g$^{-1}$ in salmon, 2.2 g 100 g$^{-1}$ in herring, and 5.2 g 100 g$^{-1}$ in mackerel. The median levels of vitamin D were 4 µg 100 g$^{-1}$ in mackerel, 7 µg 100 g$^{-1}$ in salmon and 23 µg 100 g$^{-1}$ in herring. The levels of the sums of PCDD/F, dl-PCB and sum DLCs in mackerel, herring and salmon are shown in Fig. 4. Briefly, the median UB levels of sum DLCs were 0.51 ng kg$^{-1}$ WHO TEQ-05 for salmon, 0.77 ng kg$^{-1}$ WHO TEQ-05 for mackerel and 0.82 ng kg$^{-1}$ WHO TEQ-05 for herring. The levels of DLCs, particularly in the wild species, showed a wide intra-species variation. In mackerel, the measured values ranged from 0.12 to 9.7 ng kg$^{-1}$ WHO TEQ-05, in herring from 0.21 to 10 ng kg$^{-1}$ WHO TEQ-05 or with a maximum value of 5.4 disregarding the contaminated local spring spawning stock from the coast of Telemark, whereas the values in salmon ranged from 0.18 to 1.6 ng kg$^{-1}$ WHO TEQ-05.

3.2. Estimation of DLCs; congener distribution, censored data and the TEF system

Congeners of DLC which have been assigned TEFs include seven dibenzo-p-PCDDs (PCDDs), ten dibenzofurans (PCDFs), four non-ortho PCBs and eight mono-ortho PCBs. All of these 29 congeners were measured, and the results revealed that the congener distribution in the muscle was comparable in the three species (Fig. 5). Using ROS/MLE, we calculated the median ng kg$^{-1}$ WHO-05 TEQ and original concentrations (measured concentrations not converted to TEQ) of each congener in each fish species (Fig. 5 B and C). In all species, the dl-PCBs were present at much higher original concentrations than the PCDDs and PCDFs. PCB-105, PCB-118 and PCB-156 comprised approximately 90% of the total sum of DLC in original concentrations. The congener present in highest concentrations was the mono-ortho PCB-118, representing approximately 60% of the sum of DLCs (Fig. 5 C). When TEQs were applied, 2378-TCDD, 12378-PeCDD, 2378-TCDF, 23478-PeCDF, PCB-126 and PCB-169 comprised approximately 90% of the total TEQ (Fig. 5 B). The non-ortho PCB-126 alone comprised approximately 50% of the total TEQ in all fish species. Despite low concentrations in all species, PCDD/Fs made a somewhat higher contribution to the total sum of DLCs in herring than in mackerel and salmon (Fig. 5).

The censoring, meaning results below LOQ, of DLCs occurs at congener level. Hence, the estimation of medians using a robust statistical approach was calculated for each individual congener. When ROS/MLE were used to estimate medians of each congener, the degree of
censoring in the dataset became apparent (Fig. 5 A). Only 3 of 17 congeners of PCDDs and PCDFs showed less than 80% censoring in mackerel and salmon, whereas for herring 10 of 17 congeners showed less than 80% censoring. All dl-PCB congeners, except PCB-114 and PCB-189 in salmon, showed less than 80% censoring. Therefore, for further assessment of sum DLCs using ROS/MLE, two approaches were used to deal with the high degree of censored data: Either the censored data was set as LOQ (UB), or it was set as zero (LB).

To exemplify the impact of the choice of estimation method and treatment of left censored data, we compared the traditional UB TEQ-05 median for sums of PCDD/Fs, dl-PCBs and sum DLCs to other calculation methods, such as: sums of pre-calculated UB-medians of each single congener (sum of congener medians), sums of each congener using ROS/MLE both UB and LB, as well as the traditional LB TEQ-05 median of sums (Table 1) (Suppl.2). For clearer visualisation, the concentrations obtained by traditional calculation was set to 100% and the results from the other methods were calculated relative to this (Table 1). Comparing sum of congener medians to the traditional median of sum DLCs reduced all the sums, except for dl-PCB in mackerel. However, by including UB-ROS/MLE calculations the median levels were slightly further reduced for sum PCDD/F for all species. However, the reduction was only between 1 and 9% compared with UB median of sums, and the reduction was not observed for dl-PCB in any of the species. By excluding congeners where more than 80% were censored (LB ROS/MLE), the decrease in sum PCDD/F amounted to almost 70% for Atlantic mackerel and Atlantic salmon, and 22% for Atlantic herring. No decrease was observed for dl-PCBs, but sum DLCs combined decreased 31% for Atlantic mackerel, 27% for Atlantic salmon and 13% for Atlantic herring. Regular LB calculation showed a similar tendency (Table 1).

The relatively high contribution of the dl-PCBs, and particularly PCB-126, on total TEQ highlights the importance of the TEF-values in estimating the toxicity of sum DLCs. To further visualize the impact of the TEF, we also calculated sum PCDD/Fs, sum dl-PCBs and sum DLCs using Consensus Toxicity Factors (CTF). Applying CTFs instead of TEF resulted in the estimation of UB sums of DLCs being increased by 9% for herring, but decreased 34% for mackerel and 28% for salmon (Table 1). When CTF were applied the level of PCDDs and PCDFs increased for all species, while the dl-PCBs were reduced for all species, compared to the TEF system.
To assess the levels of nutrients and DLCs in herring, mackerel, and farmed salmon, we demonstrate that the congener distribution, the impact of left censoring on such data as well as the use of different estimation methods: - UB median of sums in TEQ-05, UB ROS estimated median in TEQ-05, LB ROS estimated median in TEQ-05 and UB median of sums in CTF (Fig. 7). Use of UB-ROS led to a slightly reduced risk, whereas the estimated risk using LB-ROS was reduced to a level allowing safe consumption of the recommended 200 g for all species. Using CTF led to an estimated reduced risk for mackerel and salmon, but augmented the risk of herring.

To visualize the effect of estimation method and the TEF system, we also assessed the recommended intake in a TWI context using four different estimation methods: UB median of sums in TEQ-05, UB ROS estimated median in TEQ-05, LB ROS estimated median in TEQ-05 and UB median of sums in CTF (Fig. 7). Use of UB-ROS led to a slightly reduced risk, whereas the estimated risk using LB-ROS was reduced to a level allowing safe consumption of the recommended 200 g for all species. Using CTF led to an estimated reduced risk for mackerel and salmon, but augmented the risk of herring.

### 3.3. Benefit of nutrients, or risk of DLCs?

To assess the levels of nutrients measured in mackerel, herring, and salmon in a dietary benefit context, we assessed the contribution of each of these species to the recommended adequate intakes (AI) of marine omega 3 (DHA and EPA) and vitamin D (EFSA, 2010b; EFSA, 2016). Weekly intake was estimated by multiplying median level of each nutrient with the average consumption of fatty fish in the adult Norwegian population, which for men is 105 g week⁻¹ and for women is 98 g week⁻¹ (Totland et al., 2012). The median total contents of DHA and EPA in mackerel, herring and salmon were 2.2, 5.2 and 1.2 g/100 g, respectively (Fig. 3A). The reported intake of fatty fish would be more sufficient to meet the weekly AI of 1.75 g for DHA and EPA given the fatty fish consumed was herring or mackerel (Fig. 6B). The same would provide 71% of the AI for DHA and EPA for males and 66% for females (Fig. 6B). The Norwegian health authorities recommend a weekly intake of 200 g fatty fish (The Norwegian Directorate of Health, 2014). An intake of 200 g of each of the three species would provide more than the weekly AI for EPA and DHA. For vitamin D, an average intake of fatty fish would contribute to 4% of the AI for both sexes (15 μg d⁻¹) if mackerel was consumed, 7% for men and 6% for women of the AI if salmon was consumed, and 23% for men and 22% for women of the AI if herring was the fatty fish source (Fig. 6C). A consumption of 200 g of either herring, mackerel or salmon would provide 44%, 7% or 13% of the AI, respectively.

To assess the levels of DLCs from herring, mackerel and salmon in a risk context, the new TWI of 2 pg WHO TEQ-05/kg bw set by EFSA was introduced (EFSA, 2018). For comparison, we also evaluated the levels using the JECFA PTMI (supplement 3). The intake of DLCs was derived from their respective median levels in each fish species and the average consumption of fatty fish obtained from the Norkost 3 study (Totland et al., 2012). For assessment of risk in the Norwegian diet, traditional UB TEF calculations were used. When excluding other sources of exposure, an average Norwegian intake of oily fish would lead to a DLCs intake of less than 50% of the TWI if either one of the three species were consumed (Fig. 6A). However, given the variation in the oily fish intake, the TWI will be exceeded in fractions of the population. The recommended intake of 200 g oily fish would lead to a slight exceedance of the TWI for DLCs in the case of herring (110% of the TWI) and mackerel (104% of the TWI), whereas consumption of 200 g salmon would contribute to 73% of the TWI. When assessing our data using the JECFA PTMI, no exceedance of the TMI was found (supplement 3). All calculations are for simplicity and illustrative purposes based on the assumption that each individual fish species comprises the entire weekly oily fish intake.

We here report the levels of EPA, DHA, vitamin D, and DLCs in the most important commercial Norwegian oily fish species: herring, mackerel and farmed salmon. We demonstrate that the congener distribution, the impact of left censoring on such data as well as the use of the TEF system are of importance when assessing the risk of fish consumption. We have also shown that Norwegian oily fish can be an important source of beneficial nutrients, but at the same time, a highly relevant source of exposure for DLCs. The latter was shown using representative dietary intake data for oily fish consumption in Norway, a country with a relatively high seafood intake (FAO, 2017).

### 4. Discussion

The levels of nutrients and DLCs described for herring, mackerel and farmed salmon are comparable to previously reported data (Fernandes et al., 2018; Nøstbakken et al., 2015; Saini and Keum, 2018). The levels of DLCs in farmed salmon declined from the beginning of the 2000s to...
around 2010 as a result of reduced inclusion of marine feed ingredients \cite{nibelakensmoller2015}. Since then, the levels have remained stable at around 0.5 – 0.6 ng TEQ-05 kg^{-1} w.w. The levels of DLCs in wild mackerel and herring depend on the levels in the marine environment and particularly, on their prey, which mainly comprises zooplankton \cite{bachiller2016, norgestbakken2015}. Variations in levels of nutrients, and DLCs in the fish due to geography, temporality, size or age will likely affect the distribution in our data. However, due to the large sample-size in this study we assume that the median level will describe the level of nutrients, and DLCs the general consumer of commercially caught herring or mackerel is exposed to. Beyond an initial assessment of temporal and spatial trends of DLCs these factors have not been the focus of this study and will not be further addressed.

4.2. Estimation of DLCs; congener distribution, censored data and the TEF system

The EU, Regulation (EC) No 1881/2006, specifies the use of upper-bound (UB) calculation of DLCs for reporting levels in general surveillance. By reporting UB levels, one will implement a precautionary approach ensuring consumer safety, which will very likely overestimate the levels of contaminants actually present in a food source. Another

![Fig. 6. Norwegian relative exposure to dioxin and dl-PCBs, vitamin D, or marine omega 3 from oily seafood in relation to Tolerable Weekly Intake (TWI) or Adequate Intake (AI). Consumption data is based on intake of fatty fish for men and women from the Norkost 3 survey \cite{totland2012}. Recommendation is the recommended intake of fatty seafood as specified by Norwegian authorities https://helsedirektoratet.no/folkehelse/kosthold-og-ernering/kostrad-fra-helsedirektoratet#5.-spis-fisk-til-middag-to-til-tre-ganger-i-uken-bruk-ogat-gjerne-fisk-som-pilleg. A) The intake of PCDD/PCDF and dl-PCB compared to TWI, assuming that all intake of fatty fish is of each fish species exclusively and other sources are excluded, mean fish intake is shown by columns and the whiskers are p95 of Norwegian intake of fatty fish. B) The intake of DHA + EPA compared to AI, assuming that all intake of fatty fish is of each fish species exclusively and other sources are excluded, mean fish intake is shown by columns and the whiskers are p95 of Norwegian intake of fatty fish C) The intake of Vitamin D compared to AI, assuming that all intake of fatty fish is of each fish species exclusively and other sources are excluded, mean fish intake is shown by columns and the whiskers are p95 of Norwegian intake of fatty fish.](image-url)
AHR responsiveness can vary when the TEFs were established, suggested that the REP may be lower. However, human studies with different endpoints, different models, different dosing regiments and different mechanisms (Van den Berg et al., 2006). REPs are determined through in vitro or in vivo studies with different endpoints, different models, different dosing regimens and different mechanisms (Van den Berg et al., 2006). Naturally, a large variation in REPs will be observed even for single congeners, however, for PCB-126 in particular, the variation of the REP is relatively low in rats (Haws et al., 2006). The REP, and consequently the TEF system on risk assessment and underlines the importance of well-founded TEFs. TEFs are derived using expert judgement, point estimates and relative effect potency (REP) (Van den Berg et al., 2006). A database of REPs has been constructed based on several studies which have investigated each congener’s relationship, indirectly or directly, to TCDD (Haws et al., 2006). REPs are determined through in vitro or in vivo studies with different endpoints, different models, different dosing regimens and different mechanisms (Van den Berg et al., 2006). Naturally, a large variation in REPs will be observed even for single congeners, however, for PCB-126 in particular, the variation of the REP is relatively low in rats (Haws et al., 2006). The REP, and consequently the TEF, is set at 0.1 for PCB-126 (Haws et al., 2006; Van den Berg et al., 2006). However, human in vitro studies published already at the time when the TEFs were established, suggested that the REP may be lower than 0.1 (Van den Berg et al., 2006). AHR responsiveness can vary significantly among species, hampering the transfer of effects from animal studies to the determination of human TEFs (Flaveny et al., 2009; Flaveny and Perdew, 2009). A recent in vitro study, with REPs based on human bioassays, showed that their estimated REPs, or Consensus Toxicity Factors (CTFs), demonstrate that sensitivity to PCB 126 in particular, may be significantly lower in humans than in rats (Larsson et al., 2015). These authors also showed that the corresponding CTF for some dibenzo-furans in fact show more sensitivity in humans than previously assumed in their TEFs. Studies investigating human “in vivo” response from peripheral blood mononuclear cells and thyroid endpoints, performed in a Slovak population living in a known exposure site for DLCs, assessed the correlation between serum levels of DLCs and induction of CYP1A1, CYP1B1, thyroid volume and free serum thyroxin (FT₄). They observed that the human response in CYP1B1 to PCB 126 was below the established TEFs. However, they also observed a higher response in CYP1B1 for the other PCBs in humans, except for PCB 118, compared to the TEF (Trnovec et al., 2013; Wimmerova et al., 2016). Conversely, a study using gestational diabetes mellitus and fasting blood glucose as endpoints for determining REPs in a population of pregnant women found a REP for PCB 126 similar to the WHO TEF (Liu et al., 2019).

Studies comparing activation of human AHR in transgenic mice with activation of the endogenous mouse AHR, reveal up to a 10 fold higher affinity of the mouse AHR for typical AHR ligands (Flaveny et al., 2009). Studies investigating the AHR sensitivity in hepatocytes from human donors, observed a lower sensitivity in human cells than in cells from animals, such as rats to TCDD, PCB-126 and a commercial mixture of PCB (Aroclor 1254) by factors ranging from 10 to 10 000 (Silkworth et al., 2005). The authors suggested that the species difference in AHR sensitivity can explain the lack of conclusive evidence that PCBs have affected human health (Silkworth et al., 2005). However, even though there are uncertainties associated with individual TEFs, the TEF system has proven to be predictive of the additive effects of DLCs (Van den Berg et al., 2006). But as stated by Haws et al (2006), the TEF system is an interim system requiring updating as new studies emerge.

The TFI established by EFSA in 2018 is based on both animal and human studies, where the study showing the most sensitive effects is a study of total sperm count and its association with PCDD and PCDF levels in blood from boys from Chapaevsk, Russia (Minguez-Alarcon et al., 2017). This study showed that a reduction in sperm count occurred already at a median PCDD level of 7 pg TEQ g⁻¹ lipid in serum. However, the authors also showed no correlation between sperm count and total TEQ of sum DLCs up to serum levels of 70.5 pg TEQ g⁻¹ lipid. The other human studies providing the basis for the revised/new TEF established by the EFSA originated from the Seveso accident, where the population was exposed to TCDD only (Mocarelli et al., 2011; Mocarelli et al., 2008). The lack of clear evidence on the effects of dl-PCBs on human sperm count has therefore prompted detailed debate about the applicability of this endpoint for all DLC congeners (BR, 2018). The lack of effects of dl-PCB in the “Russian boys study” may either suggest that the TEF system does not sufficiently describe the toxic effects of all congeners, or that the observed effects in this particular study are TEF independent. Therefore, in the recent risk assessment performed on DLCs by EFSA (2018), it was suggested that new studies should focus on re-evaluating the TEF value for PCB-126. However, since furans may be more toxic than previously assumed (Larsson et al., 2015), also these should be further investigated. As we have shown in this study, the impact of the TEF system compared to for example CTF, can affect final risk assessment outcomes.
4.3. Risk and benefit of Norwegian oily seafood

Seafood is considered an important food group for humans, providing several health promoting nutrients, which has been shown by both national and international food safety organizations, such as Norwegian Scientific Committee for Food and Environment (VKM), United States Environmental Protection Agency (US-EPA)/U.S. Food and Drug Administration (FDA), EFSA and Joint FAO/WHO Expert Committee on Food Additives (JECFA) (EFSA, 2014; FDA/US-EPA, 2017; JECFA, 2010; VKM, 2014). Seafood consumption has, for example, been associated with: improved cardiac health (Rimm et al., 2018), increased fertility (Gaskins and Chavarro, 2018) and improved neurodevelopment (Starr et al., 2015). By comparing estimated nutrient intake from mackerel, herring and salmon with AIs set by the EFSA, we show that these oily fish are good dietary sources of EPA, DHA and vitamin D. However, oily seafood consumption is also a major route of exposure to lipophilic environmental contaminants such as DLCs that have potential detrimental effects on the same endpoints as mentioned above (EFSA, 2012; Minguez-Alarcon et al., 2017). We show that the levels of DLCs in mackerel, herring and salmon comprise a substantial part of the TWI at current intake levels, hence these oily fish species represent a significant source of exposure. Our results further showed that individuals consuming 200 g mackerel or herring per week, the current recommended weekly oily fish consumption in Norway, will lead to an intake of DLCs exceeding the TWI. Hence, oily fish is associated with both benefits and risks even at the current intake in the Norwegian population. Therefore, it is necessary to expand an initial assessment of risk–benefit to a refined assessment as described by EFSA (EFSA, 2010a), since both risk and benefit are present in the same matrix. A recent qualitative benefit-risk assessment on nutrients and pollutants (DLCs and MeHg) in Baltic herring and salmon concluded that consumption of Baltic fish are safe and healthy for most population subgroups in the Nordic countries (Tuomisto et al., 2020), despite 20–75% of respondents exceeding the EFSA TWI for DLCs. In comparison with that study, the levels of DLCs found in our study were considerably lower, suggesting that benefits outweigh risk also for consumption of Norwegian oily seafood even though the TWI is exceeded. Our study provides an extensive dataset on nutrients and DLCs in oily seafood that can be used in future risk–benefit analyses. However, a proper risk–benefit analysis would require data on other dietary sources and more extensive data on human consumption and is therefore not feasible within the scope of this study.

Noteworthy, we found no risk when evaluating the levels of DLCs found in this study using the PTMI established by JECFA. The pivotal study forming the basis for the JECFA PTMI is a rat study showing reduced sperm in Wistar rats by Faqi et al. (1998), which is also the most sensitive animal study used in the EFSA evaluation. However, the JECFA assessment was performed in 2002 and could not include the human studies evaluated by EFSA which were performed later.

We show that estimation method and the TEF system have a clear impact on risk assessment of DLCs. Using UB-ROS reduced the risk, while using LB ROS reduced the risk further. The use of CTF instead of TEF also reduced the risk for mackerel and salmon, but augmented the risk for herring. The CTFs, as demonstrated by Larsson et al. (2015), showed a higher REP for the PCDDs and PCDFs than for the dl-PCBs using human cell bioassays. In herring, the PCDDs and PCDFs constituted a larger part of the total TEQ than dl-PCBs, than in mackerel and salmon. This explains why the risk was augmented rather than reduced for herring when the CTF system was applied. It is also noteworthy, that in the Russian boys study used by EFSA for establishing the new TWI, toxic effects were observed for TCDD and PCDD, but not for dl-PCBs (EFSA 2018; Minguez-Alarcon et al., 2017). This may suggest that PCDDs and PCDFs play a more important role in the toxic effects of the sum of DLCs in humans.

4.4. Study limitations and statistical considerations

Although this study first and foremost describes the levels of selected nutrients and DLCs in oily fish, and the issues concerning estimation of actual levels, we also set these data into a risk–benefit context. However, this study should not be viewed as a risk–benefit study since we only present data for Norwegian oily fish, and do not include any other dietary sources of nutrients or DLCs. Further, we do not attempt to reflect the actual composition of seafood in the Norwegian diet, but analyse each species separately in TWI and AI calculations to exemplify effects of calculations of true levels in the seafood. Furthermore, seafood also contains other contaminants than DLCs such as methylmercury, brominated flame retardants, pesticides and more, which have not been taken into account in this study. For a full risk benefit assessment of seafood consumption other contaminants must be considered, but this is beyond the scope of this study. Still, the results presented are highly relevant for future risk–benefit assessments, and underscores the importance of also considering nutrient contents when assessing risks of foods.

In our study, the levels of DLCs have been related to seafood consumption data from dietary surveys in Norway. It could be of interest to access data gathered by EFSA in their comprehensive database. However, as is stated by EFSA: “due to methodological differences in the studies used in their database including 32 different surveys, it is proposed to perform exposure assessment at a national level” (EFSA, 2011). Since the population in Norway has a relatively high seafood intake (Guillen et al., 2019), it was assumed that the levels observed in a Norwegian diet could be regarded as relatively high background exposure.

Here, we have implemented a robust statistics protocol in an attempt to better determine true levels of DLCs in oily fish. However, this is not without challenges. The main challenge using ROS/MLE is that it only calculates a distribution for each congener and does not impute numbers for each sample, and it is therefore not possible to estimate sum congeners for each sample (supplement 2). So, in order to assess the sums of DLCs as a whole, medians for each congener in each species were summed. This is challenging since it does not provide a spread for the sums. Further, when summing pre-calculated medians of each congener this does not correspond to the median of pre-summed samples. A possible explanation for this is related to the variable LOQ, meaning that the LOQ is determined for every run of the analytical instrument and is dependent on background noise. If the levels of DLCs show higher consistency between actual levels in the samples, than the LOQ does for each analysis, the range for the LOQ will be larger than the actual range for the levels in the sample. Consequently, when each sample is summed the combined worst cases of LOQs in such a dataset will be included in the sum for every sample, but not for the median of each congener. Therefore, the worst cases will be taken more into account in median of sums (e.g. sum DLCs) than in sum of medians of each congener, and hence the median of sums tend to be higher than sum of medians. In this study, we have addressed this issue by also including sum of medians for each congener for traditional UB calculations.

4.5. Conclusion

In this study, we have shown that mackerel, herring and farmed salmon are good dietary sources for EPA, DHA and vitamin D, but they also contain levels of DLCs that can lead to the EFSA TWI being exceeded if these fish species are consumed in accordance to the recommendation. The data provided in this paper is highly relevant for future risk–benefit assessments of seafood consumption. We have also demonstrated challenges in estimating the presence and toxic effects of DLCs accurately, by utilizing robust statistics and alternative toxic equivalent factors. The nutrients EPA, DHA and vitamin D, as well as the contaminants DLCs were found to be present in fatty fish at levels causing both risk and benefit, underlining the importance of accurate determination of actual
levels. A refined assessment of nutrients and contaminants from a diet containing seafood, particularly oily seafood, is warranted.

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Author contributions

O.J.N. has assembled data from previously performed surveillance, performed statistical analyses, and been in charge of writing the manuscript.

J.D.R. performed statistical analyses and contributed to writing and discussion of the manuscript.

R.H. led the surveillance of contaminants in salmon, and contributed to the writing of the manuscript.

M.S. contributed with critical feedback to the final version of the manuscript.

L.F. contributed with critical feedback to the final version of the manuscript.

A.D. has led the study of contaminants in North Sea herring, was involved in the sampling of herring and mackerel for nutrition analyses and has contributed to writing of the manuscript.

S.F. led the studies of contaminants in Norwegian Spring Spawning herring and Northeast Atlantic mackerel and reviewed and edited the manuscript.

A.K.L. participated in discussions, review and editing of the manuscript.

A.D. has led the study of contaminants in North Sea herring, was involved in the sampling of herring and mackerel for nutrition analyses and has contributed to writing of the manuscript.

S.F. led the studies of contaminants in Norwegian Spring Spawning herring and Northeast Atlantic mackerel and reviewed and edited the manuscript.

L.D. participated in data collection of nutrients from the oily fish, review and editing of the manuscript.

A.K.L. participated in discussions, review and editing of the manuscript.

L.M. participated in discussions, review and editing of the manuscript.

All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.106322.

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