OSPAR COMMISSION

Protecting and conserving the North-East Atlantic and its resources

Mercury assessment in the marine environment

Assessment criteria comparison (EAC/EQS) for mercury

Hazardous Substances & Eutrophication Series

2016

OSPAR Convention

The Convention for the Protection of the Marine Environment of the North-East Atlantic (the "OSPAR Convention") was opened for signature at the Ministerial Meeting of the former Oslo and Paris Commissions in Paris on 22 September 1992. The Convention entered into force on 25 March 1998. The Contracting Parties are Belgium, Denmark, the European Union, Finland, France, Germany, Iceland, Ireland, Luxembourg, the Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Convention OSPAR

La Convention pour la protection du milieu marin de l'Atlantique du Nord-Est, dite Convention OSPAR, a été ouverte à la signature à la réunion ministérielle des anciennes Commissions d'Oslo et de Paris, à Paris le 22 septembre 1992. La Convention est entrée en vigueur le 25 mars 1998. Les Parties contractantes sont l'Allemagne, la Belgique, le Danemark, l'Espagne, la Finlande, la France, l'Irlande, l'Islande, le Luxembourg, la Norvège, les Pays-Bas, le Portugal, le Royaume-Uni de Grande Bretagne et d'Irlande du Nord, la Suède, la Suisse et l'Union européenne.

Acknowledgement

This report was prepared by Brendan McHugh with the help of Rob Berbee, Eivind Farmer, Rob Fryer, Norman Green, Martin Mørk Larsen, Lynda Webster, Peter Lepom, Evin McGovern, Thomas Maes and Eric Verbruggen and the ICES Marine Chemistry Working Group of ICES

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Introduction

Mercury is known for its worldwide environmental impact. Due to its characteristics, mercury is capable of traveling long distances by both atmosphere and ocean current transport means and is thus truly a global pollutant. Mercury is also addressed by several existing international agreements addressing atmospheric emissions (CLRTAP), the marine environment (OSPAR, HELCOM, Barcelona, Bucharest), waste (Basel), and export of chemicals (Rotterdam).

Mercury can be brought into the biosphere by humans by two different overall mechanisms: by 1) intentional extraction and technical use of mercury, and 2) as a natural constituent in other materials which are processed in a way that releases mercury to the biosphere (environment).

Mercury is extremely toxic to both man and biota and can be transformed within the aquatic environment into more toxic organic compounds (e.g. methyl mercury). A main pathway of mercury to the sea is atmospheric and it can be carried long distances from its source. The main sources of mercury to the environment are natural atmospheric emissions from volcanoes and anthropogenic emissions from coal-fired power stations and metal production and cement production. Mercury also enters into the environment through the disposal products containing mercury including: car parts, batteries, fluorescent bulbs, medical products, thermometers, and thermostats. Emissions from crematoria are a small but widespread source. Many of the releases of industrial mercury during the 1900s came from the mercury cell chlor-alkali process used to produce chlorine but with the introduction of new technologies, this source has largely been phased out over the last twenty years. The critical exposure routes of all mercury compounds are via their decomposition and natural formation of methylmercury in the aquatic environment. The primary risk to the general population is thus exposure to methylmercury via ingestion of aquatic foods.

OSPAR measures and subsequent EU measures regulate the main industrial sources for mercury releases to the environment. A suite of OSPAR measures control mercury emissions and discharges from the chlor-alkali industry, including the complete phase-out of mercury cell chlor-alkali plants by 2010[1]. Other OSPAR measures address a variety of important sources for mercury including dentistry, thermometers, batteries and dental filters, crematoria and other diffuse sources. OSPAR has promoted actions in other international forums, especially the EU, e.g. call for actions to prevent pollution from the disposal of large amounts of pure and waste mercury arising from the closure or conversion of mercury cell chlor-alkali plants and for control measures on the use and marketing of mercury in various products. Other measures in the EU address a series of other uses including in biocides, plant protection products and batteries, toys and ceramics. The initiative in the UNEP framework to develop a legally binding global instrument to reduce mercury releases worldwide will support the OSPAR's cessation target for mercury. Mercury is listed as a priority hazardous substance under the European Union's Water Framework Directive[2] (WFD), which sets a European-wide surface water standard, and more recently a European biota standard Directive 2013/39/EU (EC, 2013) has been set that limits the concentration of mercury in fish. This biota standard was set

[1] PARCOM Decision 90/3 on Reducing Atmospheric Emissions from Existing Chlor-Alkali Plants recommended that "existing mercury cell chlor-alkali plants be phased out as soon as practicable. The objective is that they should be phased out completely by 2010" although some plants continue to operate in the OSPAR Maritime Area.
[2] Directive 2000/60/EC

to protect predatory birds and mammals from adverse effects of mercury via food intake. European member states have to prove that mercury levels in fish are not exceeded.

Elemental mercury is a constituent of a large number of substances, broadly categorised in two groups, inorganic mercury compounds and organic mercury compounds, which each have some distinct group characteristics. The form of the mercury compound influences such characteristics as uptake in biological cells, bonding to organic and inorganic matter (bioavailability), atmospheric transport distances after emission, and retention efficiency of flue gas filters, among others. Being an element, no matter which form mercury is in, it may however ultimately be decomposed to elemental mercury in nature, which is in itself toxic to humans and in the environment.

A Danish evaluation of mercury mass flow in society shows that energy industries (including burning of coal and waste incineration) are responsible for 60% of the Danish emission followed by manufacturing and construction as second largest and by non-industrial combustion and waste, transportation and finally industrial processes.

Dental fillings and light and use of metal in laboratories (e.g. porosimetry) are likely to be significant sources of “intentional” use, with other small sources of mercury being switches, some thermometers and other equipment. Chlor-alkali production can be a large source while compounds of mercury are used in batteries, chemicals, other chemical applications and medical application. Coal based emissions in addition to cement, agricultural uses and foodstuff can all contribute to mercury impacts. Elemental mercury plus 202 mercury compounds were pre-registered by industry under the REACH regulation.

According to data reported to the Environmental Monitoring, Evaluation, and Protection agency (EMEP) there has been an overall reduction in total air emissions of around 20% in the period 1998 – 2006. The picture of reductions achieved across OSPAR countries is very varied. Total emissions from industrial processes, including manufacturing industries, remained fairly stable over this period with there being an increase in emissions from the metal production sector. The most consistent development since 1998 has been for mercury emissions from the chlor-alkali industry, which halved, as have the total losses of mercury from this industry through product, wastewater and air.

Recent estimates suggest that despite significant emission reduction in Europe and North America, global mercury emissions have not changed significantly over the past 15 years due to emissions growth in other parts of the world (e.g. Asia). Data on discharges of mercury to water reported to the European Pollutant Emission Register (EPER) give indication that discharges from heavily regulated point sources continue, but do not allow conclusions on trends. Direct and riverine inputs of mercury are the major input in Regions II (Greater North Sea), III (Celtic Seas) and IV (Bay of Biscay/Iberian Coast). Riverine inputs of mercury decreased significantly by 75% in Region II. Direct discharges were much smaller and showed a similar scale of decrease. Major reductions in riverine inputs (~85%) and direct discharges of mercury were also observed for the Celtic Seas. Data are not sufficient to allow conclusions on changes in either riverine or total waterborne mercury inputs in Region I (Arctic Waters) or IV. In Region I atmospheric deposition accounts for 99% of inputs.

In an overall OSPAR context almost all sediment temporal trends for mercury exhibit a downward direction. Measured concentrations in sediments indicate a risk of pollution effects in the southern North Sea, at many of the other locations monitored on coast of the UK, the west coast of Norway and some locations near urban industrialised areas in northern and southern Spain. Concentrations

around the Dogger Bank are also high, but elsewhere in offshore areas of the North Sea are lower, and at background in some locations. Background concentrations also occur in parts of northern Scotland and in northern Norway.

OSPAR has determined the presence of a number of upward trends of mercury in biota in southern Norway, but in general mercury concentrations in fish and shellfish are at background at a large proportion of stations on the Channel coast of France, and the French and Spanish coasts of the Bay of Biscay. Background concentrations are also found at some stations in Ireland, Scotland, and western Norway. Concentrations above EU dietary limits occur mainly around Denmark and in certain industrialised estuaries in Norway and the UK. Elevated concentrations close to Iceland may be a consequence of geological conditions.

The critical exposure routes of all mercury compounds are via their decomposition and natural formation of methylmercury in the aquatic environment. The primary risk to the general population is thus exposure to methylmercury via ingestion of aquatic foods.

There are three key assessment criteria thresholds against which mercury concentrations in biota can be assessed (and be utilised by OSPAR), namely:

- Environmental Assessment Criteria (EAC) values, which represent the contaminant concentration in the environment below which no chronic effects are expected to occur in marine species, including the most sensitive species, and which where appropriate information is available account for secondary poisoning effects.
- Environmental Quality Standards (EQS) which are set to represent the contaminant concentration in the aquatic environment below which no chronic effects are expected to occur, (including secondary poisoning and human health) and which serve as a benchmark to decide whether or not specific measures are required.
- EC 1881/2006 maximum concentrations in foodstuffs to protect public health.

The EQSs for priority (hazardous) substances are set on a European community level. For other compounds that are relevant to individual member states, standards are set on a national level. In respect of EACs, concentrations below the EAC are considered to present no significant risk to the environment, and to that extent may be considered as being related to the EQSs applied to concentrations of contaminants in water, for example under the Water Framework Directive (WFD). EAC and EQS threshold values have their derivation in differing origins, which are further described below.

i) Derivation of OSPAR assessment criteria approaches.

OSPAR Background Concentrations (BCs) and Background Assessment Concentrations (BACs).

In addition to assessment criteria corresponding to statutory limits, or to policy objectives aimed at avoiding unacceptable biological effects arising from contaminants in the environment, the OSPAR Hazardous Substances Strategy has “the ultimate aim of achieving concentrations in the marine environment near background values for naturally occurring substances and close to zero for manmade synthetic substances”. It is therefore appropriate, where possible, that assessment of contaminants data in an OSPAR context should take account of this additional policy aim.

In order to assess progress towards near background or zero concentrations, OSPAR has developed Background Concentrations (BCs), the definition for which is "the concentration of a contaminant at a 'pristine' or 'remote' site based on contemporary or historical data" (OSPAR Agreement 2005-6).

For naturally occurring substances, such as trace metals, BCs are the typical concentrations found in uncontaminated locations in the OSPAR maritime area (North-East Atlantic). In order to facilitate precautionary assessments of data collected under the OSPAR CEMP against BCs, OSPAR has developed Background Assessment Concentrations (BACs). Observed concentrations are said to be 'near background' if the mean concentration is statistically significantly below the corresponding BAC. BCs and BACs were developed using criteria as outlined above and they have been recommended for use throughout the OSPAR maritime area.

Concentrations below the EACs are considered to present no significant risk to the environment, and to that extent may be considered as being related to the EQSs applied to concentrations of contaminants in water, for example under the WFD. Concentrations below the EAC are unlikely to give rise to unacceptable biological effects. EACs have been developed for a range of matrices and contaminants through a combination of work by OSPAR and ICES groups. Some EACs have not been used in OSPAR assessments, mainly because the proposed EACs are less than the OSPAR Background Assessment Concentrations (BACs). In the case of trace metals, EACs for cadmium and lead in sediment, mercury in mussels and mercury and cadmium in fish are also below the corresponding BACs. It has been concluded that EACs for metals in biota cannot be used to describe the green/red transition.

In cases where the EACs have not been recommended, alternative approaches to appropriate criteria for the assessment of data on contaminant concentrations (in sediment) and biota need to be considered. In order to maintain consistency, wherever possible, when filling these gaps in the suite of assessment criteria, it is deemed helpful to employ as few alternatives as possible to the EACs. Where required, the use of alternatives needs to be consistent across groups of contaminants so that the output from the assessment process is readily understandable and features in the assessment can be interpreted (OSPAR 2009).

Derivation of EACs for metals in fish and shellfish:

There are no recommended EACs for metals in biota and at the time of derivation no equivalents were deemed available for fish and shellfish. Therefore an alternative approach to assessment criteria was required, which needed to be coherent across the range of species addressed in the CEMP programme. Two possible approaches were considered.

The first approach considered was the use of an added risk approach requiring the use of the sum of the BCs and the EACs to derive a maximum concentration within the organisms.

- The advantages of this approach include that the derived MPC involves the use of the OSPAR BCs and EACs, and that the process is described in Moffat *et al.* (2004) and has been discussed in WFD contexts.
- The disadvantages include that the EACs were not recommended for use in this way, and that the EACs are in some case only a small proportion of the BC/BACs so that the derived MPCs would not differ greatly from the BACs. The absence of proposed EACs for oysters prevents the derivation of MPCs for this species.

The second approach considered was an assessment of the contaminant concentrations in fish and shellfish with respect to their human health risk. The Commission Regulation (EC) No 1881/2006 (and subsequent additions and amendments) sets maximum concentrations for contaminants in foodstuffs to protect public health, i.e. to ensure that contaminant concentrations are toxicologically acceptable. This regulation includes maximum levels for mercury in bivalve molluscs and fish muscle on a wet weight basis.

- Advantages of this approach are that the dietary standards are firmly established within EC statute, and that they can be used to fill the gaps for metals in both fish and shellfish species.
- Disadvantages include that standards are not directly available for all the matrix/contaminant combinations required for the assessment. Standards for shellfish exist, and for application in assessments of concentrations in mussels and oysters, the standards were converted to a dry weight basis.

Overall it was considered that the advantages of having assessment criteria that covered (all three) metals in both fish and shellfish greatly outweighed the consequences of not having any criteria for the green/red transition for metals in biota. Without criteria, all assessments would default to red, and this would result in very significant loss of information.

As an interim position, until a more appropriate approach to assessment criteria for metals in biota becomes available, the EC dietary limits, as described above, were used for the purposes of the QSR 2010 assessment as a coherent suite of assessment criteria for trace metals in biota at an amber (replacing the green)/red transition. The use of amber rather than green takes account of concerns over the relevance of the EC dietary limits as criteria for environmental effects.

Thus a traffic light colour scheme is used to classify these criteria: red, amber/green, and blue, which would represent large, uncertain risk and small risk, respectively as per Moffat *et al* (2004). In the case of mercury exceedance of the food standard results in red classification, while concentrations below the BAC results in blue. Concentrations in between, i.e. result in amber, indicating the uncertainty in the classification due to lack of information.

It is recognised that natural processes such as geological variability or upwelling of oceanic waters near the coast may lead to significant variations in background concentrations of contaminants, for example trace metals. The natural variability of background concentrations should be taken into account in the interpretation of CEMP data, and local conditions should be taken into account when assessing the significance of any exceedance. This needs to be explained where it is a relevant factor in data interpretation.

The combination BCs, BACs and EACs are key assessment thresholds used in OSPAR based assessment of contaminant concentrations in (sediment and) biota. Final assessment outputs result in the generation of a metric corresponding to the achievement, or failure to achieve, statutory targets or policy objectives for contaminants in these matrices. Outcomes of these assessments generally being described by the transition in a traffic light scheme between green and red representing the contaminant concentration in the environment below which no chronic effects are expected to occur in marine species, including the most sensitive species with green indicating that the target/objective has been achieved; red that it has not.

ii) Derivation of EQS_{biota} for Mercury

For a good chemical status the WFD requires that EQSs are met. These EQSs serve as a benchmark to decide whether or not specific measures are required. The EQSs for priority (hazardous) substances are set on a European community level. For other compounds that are relevant to individual member states, standards are set on a national level.

The EQS for chronic exposure is aimed at the protection of ecosystems and human health. In the priority substances Directive 2013/39/EU (EC, 2013) there are 11 substances (or substance-groups) where EQS_{biota} have been defined. EQS dossiers, show that seven of these have an EQS based on risk to human health (brominated diphenylethers (PBDEs), fluoranthene, hexachlorbenzene (HCB), benzo[a]pyrene, perfluorooctane sulfonic acid and its derivatives (PFOS), dioxins and dioxin-like compounds, and heptachlor and heptachlor epoxide), whereas four are not (hexachlorobutadiene (HCBD), mercury and its compounds, dicofol, and hexabromocyclododecane (HBCDD)). Since the derivation of an EQS is based on the strictest threshold either for the ecosystem or human health, this would indicate that the seven are more protective of the ecosystem than necessary. Background dossiers further detail derivation of the EQS itself and threshold values for other protection goals.

The derivation of EQSs considers direct ecotoxicity to aquatic organisms, exposure of humans through consumption of fish and fishery products, and exposure of predators through secondary poisoning. The most critical of these routes determines the final standard. For compounds that have a strong potential to bioaccumulate in fish, human fish consumption and secondary poisoning routes are often most critical. Due to the characteristics of these compounds, concentrations increase along the food chain. Consumption of fish therefore leads to critical levels in humans or predators while at similar concentrations in water, aquatic organisms are not affected. For these compounds, concentrations in fish have been derived that will not cause adverse effects in humans or predatory birds and mammals upon lifetime consumption. The most critical of these routes determines the final standard. For the priority hazardous substance mercury the secondary poisoning route is considered to be the most critical, because of its high bioconcentration potential

According to Directive 2008/105/EC (EC, 2008), EU community level EQSs based on surface water concentrations are sufficient for the majority of substances. An EQS based on surface water concentrations of 0.07µg/L was set for mercury and its compounds. However, for the protection of fish eating birds and mammals it was considered appropriate to establish EQSs for biota at the EU community level, because for this substance *"it is not possible to ensure protection against indirect effects and secondary poisoning at Community level by EQS for surface water alone".*

A maximum concentration in biota for mercury of 20µg kg wet wt., expressed as total mercury , was set in Directive 2013/39/EU (amending Directives 2000/60/EC and 2008/105/EC), (EC, 2013), based on a substance data sheet compiled in 2005 (EC, 2005). This value represents a concentration in fish at which birds and mammals are protected against effects of mercury via secondary poisoning

The biota standard is based on the toxicity of mercury to birds and mammals. For human exposure via fish, the biota standard was set to 500µg/kg wet wt. based on the European legal food limit for fish as laid down in Commission Regulation (EC) 1881/2006 (and its predecessor Commission Regulation 466/2001). The rationale for setting standards based on concentrations in biota rather than concentrations in the water column was primarily the uncertainty surrounding both bioconcentration factor (BCF) and biomagnification factor (BMF).

The Quality Standard for biota based on the risk due to secondary poisoning ($QS_{biota,\ secpois}$) was derived in 2005 as 20 µg/kg wet weight. This is for total mercury based on chronic toxicity data for birds and mammals. The biota standard is maintained in the new priority substances Directive 2013/39/EU (EU, 2013). The motivation for setting a biota standard is phrased differently and focuses on the analytical challenges when setting water-based standards for biota: "*Some very hydrophobic substances accumulate in biota and are hardly detectable in water even using the most advanced analytical techniques. For such substances, EQS should be set for biota*."

The biota standards as defined in the priority substances directive apply to large fish that are consumed by humans or freshwater predators, such as cormorants or otters. This $QS_{biota,\ secpois}$ aims to protect these predators by setting a limit for their food, which is 1 trophic level below this predator. For freshwater ecosystems, assuming the trophic level (TL) for algae, zooplankton, small fish and large fish are TL1, TL2, TL3, and TL4, respectively, the $QS_{biota,\ secpois}$ is set on TL4 to protect birds and mammals at TL5.

Pollutant magnification through the marine web

Concentrations in TL4-fish (as discussed above) depend on the accumulation of substances from the aqueous phase by lower aquatic organisms (bioconcentration) and accumulation in the food chain from TL1-3 to TL4 (biomagnification). These processes are represented by a BCF and BMF. The combination of these processes is represented by the bioaccumulation factor (BAF).

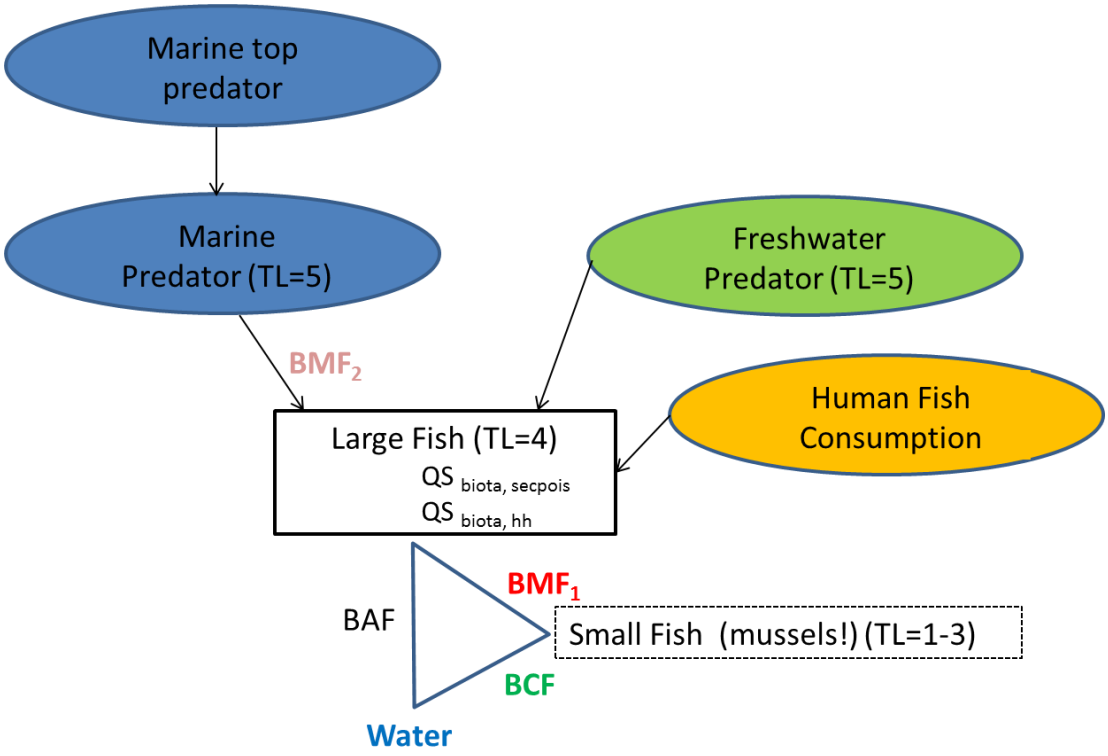

Figure 1: Accumulation mechanisms in the aquatic environment. Adapted from *Moermond et al. (2013)*

BMF1 describes the overall biomagnification from aquatic organisms to larger fish (TL4) in the aquatic environment that in turn are eaten by predators (including humans). For the marine

environment, BMF2 is included to account for accumulation in bird and mammals at TL5 (e.g. seals, dolphins, seabirds) that serve as food for top predators such as polar bears and killer whales.

For biomagnifying substances, only the first trophic level of primary consumers is in equilibrium with the water phase with magnification primarily dictated by BCF. The next trophic levels deviate from equilibrium if biomagnification occurs. The overall BMF up to the fourth trophic level in the aquatic environment thus actually comprises three biomagnification steps. To apply a BMF in combination with a BCF value, the BMF should include all steps from the organisms that are in thermodynamic equilibrium with the water phase up to the trophic level that corresponds to the biota standard (TL4). Usually, only algae (TL1) are in equilibrium with the water concentration, if biomagnification occurs (e.g. Burkhard *et al.*, 2013). Few data on biomagnification factors over the entire pelagic food chain are reported. If biomagnification is expressed as the trophic magnification factor (TMF, which is the average increase in concentrations per trophic level) then the overall biomagnification step to TL 4 is equal to TMF^3 (Burkhard *et al.*, 2013; Verbruggen, 2014).

Deriving different biota standards for freshwater and marine waters has (apparently) not been considered in the EQS-dossier on mercury, since one value is presented for all waters, including marine. Therefore, in this report also a single value is derived, based on the EQS_{biota} for mercury fish.

Mercury specific trophic biomagnification

As previously discussed the TMF is the average factor change in contaminant concentration between two trophic levels (Hallanger *et al.* (2011), Kidd *et al. (*2012), Jardine *et al., (*2012)). TMFs are generally calculated as the antilogarithm of m; ($TMF = 10^m$), where m is the slope of the regression of log_{10}-transformed-concentration data vs. the trophic level of the sample analysed. A TMF above 1 indicates an increase in contaminant concentration with increasing trophic position (i.e. food web biomagnification) whereas, a TMF < 1 indicates trophic dilution (Hallanger *et al.,* (2012), Arnot & Gobas, (2006). Lavoie *et al* (2013) note differences in TMF when either freshwater and/or marine species were included, or whether the potential influence of the latitude where species reside and/or coastal versus open sea influences were evaluated. It is clear that the choice of TMF has huge impact subsequent calculations. The influence of such factors is further discussed below and the need for ongoing research in this area is additionally noted. A generic TMF of 4.0 as reported within Verbruggen *et al* (2015) was utilised for the purposes of this assessment, derivation is further detailed below.

Uncertainty in derivation of standards

Uncertainty about published BAFs is one of the reasons for not setting a water-based EQS for secondary poisoning of mercury. The EQS datasheet reports BAFs for methylmercury that span four orders of magnitude (EC, 2005). Contributory sources of some of this variation include the complex chemistry of mercury itself, complex dietary assimilation and in the numerical assignation of trophic level itself. In natural waters, mercury is predominantly present in its metallic and inorganic forms and about 1-10% is present as organic methylmercury. In fish, 80- 99% is present in the methylated form due to the biomagnification of methylmercury from food, but also due to internal and external methylation of inorganic mercury (Slooff *et al.*, 1995). Normally, for deriving a BAF, the concentrations measured in the organism and the corresponding water concentrations should be based on the same compound. For mercury, however, a BAF could be based on the summed concentration of all dissolved mercury forms in water, indicated as dissolved total mercury, because

all mercury forms in water will contribute to the internal methylmercury levels in fish. If BAFs are based solely on methylmercury concentrations in water, resulting values will be much higher, because methylmercury concentrations in water are only small compared to the dominant inorganic mercury species. Whether total mercury or methylmercury concentrations in fish are used is less relevant, because the fraction of methylmercury is high in fish. However, at lower trophic levels, fractions of methylmercury will be lower as well. This may partly explain a wide range and high values of observed BAF values based on methylmercury as described in the EQS dossier (EC, 2005).

Another major influence on the value of the BAF values is the trophic level of the species. In the EQS dossier no distinction is made between the trophic level for the reported BAF values. Mercury is known for its high biomagnification potential, with average increase in concentration per trophic level for aquatic ecosystems worldwide by a factor of 3.5 for total-mercury and 6.5 for methylmercury (Verbruggen et al (2014)). From these values, also the increase in the fraction methylmercury with trophic level becomes apparent. The influence of trophic level will be discussed further below.

Indeed, these observations are confirmed in a recent analysis of mercury bioaccumulation of mercury in fish to derive a water-based EQS on the biota standard for secondary poisoning (Verbruggen *et al.*, 2014). The BAF values based on methylmercury concentrations in water are much higher than those based on total-mercury concentrations in water, due to the lower water concentrations. BAF values significantly increase with increasing trophic level. BAF values still are rather variable, but the data do deviate from the relationship between BAF and trophic level by not more than about one order of magnitude in both directions, and this improves further if BAFs are based on aqueous methylmercury concentrations. This is a considerable reduction compared to the four orders of magnitude as mentioned in the EQS factsheet for mercury (EC, 2005).

The BAF based on aqueous methylmercury concentrations increased stronger with trophic level than the BAF based on aqueous total-mercury concentrations. However, as both datasets were based on total-mercury concentrations in fish, this effect could not be due to increase in fraction methylmercury with trophic level. Rather, it appeared to be influenced by the smaller subset of data, but also to the improved correlation between BAF and trophic level, if BAFs were based on aqueous methylmercury concentrations. The data underlying this study were solely based on fish for which mostly only total-mercury concentrations are available. However, for the vast majority of fish, for which both total-mercury and methylmercury concentrations were reported, the contribution of methylmercury to total-mercury exceeds 50%, even at trophic level 2 up to trophic level 5. The influence of the increase in fraction methylmercury with trophic level on trophic magnification should thus be rather limited if only fish are considered.

Methodologies used for the application of EQS_{biota}: Scientific methodologies

The collaborative **T**echnical **G**uidance **D**ocument on Biota Monitoring (i.e. Guidance Document No. 32 on biota monitoring and the implementation of EQS_{biota} under the WFD, (EC 2014) , and herein referred to as TGD, notes that due to the variation in chemical residues that will result from sampling and analysing biota of different species and trophic levels, steps may need to be taken to constrain as much of that variability as possible, and to make corrections to the measured chemical concentrations to account for the major influences on bioaccumulation (i.e. lipid content, dry weight content and trophic status).

The TGD recognises the importance of the availability of appropriate field based bioaccumulation studies for priority substances. These data are essential to enable translation from a standard in one type of biota (e.g. fish) to another (e.g. mussels) and from a biota standard into an equivalent concentration in water, or to adjust monitoring data from biota at different trophic levels for comparison with the established EQS_{biota}.

The TMFs that should be used for this purpose are those that refer solely to the pelagic food chain. This would exclude birds and mammals. Hence, only the relative accumulation in species in the pelagic food chain is relevant and an extra magnification step would be required to cover for accumulation in birds and mammals that serve as food for the marine top predators for the biota quality standard for the marine environment (EC 2014).

It should be noted that Directive 2008/105/EC as amended by Directive 2013/39/EU contains the provision: *" Member States may opt, in relation to one or more categories of surface water, to apply an EQS for a matrix other than that specified in paragraph 2, or, where relevant, for a biota taxon other than those specified in Part A of Annex I. Member States that make use of the option referred to in the first subparagraph shall apply the relevant EQS laid down in Part A of Annex I or, if none is included for the matrix or biota taxon, establish an EQS that offers at least the same level of protection as the EQS laid down in Part A of Annex I."*

In summary, establishing an equivalently protective EQS for another biota taxon needs to take into account the trophic level and consequent adjustment of the monitoring data. The EU TGD further describes a procedure for data preparation prior compliance assessment to ensure consistency in approaches throughout the union.

Framework for converting OSPAR monitoring data to TL4

A total of 455 meanLY and 354 cILY time series datasets were available for assessment (see **table 1**). The completed assessment uses OSPAR muscle and whole organism data, namely;

meanLY: = mean concentration of the last year of the time series,

cILY: = upper confidence limit for the last year of the time series,

Data as reported to OSPAR up to and including 2015 were utilised. No data filtering (for the potential effects of sample size/age or for data concerns) was employed. The majority of data (ca. 68%) comprised of mussel and/or oyster species (mainly *Mytilus edulis and Crassostrea gigas),. Limanda limanda* provided the greatest number of fish based time series ca. 11%).

Mercury concentrations in environmental samples tend to be low. As a consequence a large number of values can be reported that are greater than the limit of detection but that are lower than the limit of quantification of routine analytical methods. The process by which mercury data were treated, i.e. for individual time series, Log concentration was modelled as a function of time, with less-than measurements treated as left-censored data (methodological based supporting information is detailed in Appendix 1).

Table 1: The number of mercury concentration time series available for EQS_{biota} assessment.

Species	meanLY (wet weight)	clLY (wet weight)
Cerastoderma edule - Common cockle	1	
Clupea harengus– Atlantic herring	2	2
Crangon crangon - Shrimp	1	1
Crassostrea gigas – Pacific oyster	35	28
Gadus morhua- Cod	20	14
Lepidorhombus whiffiagonis- Megrim	2	2
Limanda limanda - Dab	51	40
Merlangius merlangus -Whiting	1	
Merluccius merluccius -European Hake	3	3
Mya arenaria - Soft Shell Clam	1	1
Mytilus edulis- Blue Mussels	272	222
Mytilus galloprovincialis - Mediterranean Mussels	11	7
Ostrea edulis- European Flat Oyster	4	2
Platichthys flesus- European Flounder	25	22
Pleuronectes platessa- European plaice	20	9
Scomber scombrus - Atlantic mackerel	3	
Zoarces viviparous - Eelpout	3	1
Total	455	354

The stepwise process of adjusting monitoring data to an appropriate trophic level (TL=4) requires that monitoring data (concentrations in muscle or whole organism on a wet weight basis) be adjusted to account for a number of factors. The process/rationale to derive EQS-adjusted concentration OSPAR data reported for mercury is divided into five steps, *i* to *v* as follows:

i) Establishment of an appropriate species and tissue type

Due to the different bioaccumulation potential of substances among species, the EU EQS_{biota} for mercury refers only to whole fish. The TDG notes that there should be a clear link between the EQS and the tissue that is analysed for comparison with the EQS.

The choice of appropriate tissue can be influenced by *inter alia*: the monitoring purpose (detection of spatial and/or temporal trends or assessment of compliance with suitable effect thresholds or guideline concentrations); the classes of investigated chemicals (lipophilic contaminants which differentially partition into fatty tissue, or contaminants with high affinity for protein-rich tissue/organ); and tissue availability (quantity of biological material compatible with minimum performance criteria for methods of chemical analysis laid down by Directive 2009/90/EC (EC, 2009)).

For smaller species, such as most invertebrates, the only practical option is to measure contaminants in the whole organism. For crustaceans, the edible parts of crustaceans (i.e. muscle from appendages and abdomen) are generally sampled if the main objective includes human health concerns. For fish, one of several tissue types is typically monitored: homogenised whole fish, muscle, liver and/or, occasionally, kidney. The choice between them depends on the goal of the monitoring programme and the type of EQS used for compliance assessment. Fish are thus

considered an appropriate organism for checking compliance against biota EQS. Invertebrates represent a good compromise in terms of feasibility and fulfilling the objectives of the WFD, since they also represent a food source for secondary predators and humans, and their smaller size facilitates handling and caging.

When assessing compliance using fish, contamination is usually evaluated either by analysing the fillet in regards to risk to human health or whole fish in regards to risk to wildlife. With respect to human health, fillet data are usually those most readily available. There are few whole-fish datasets that can be used to address questions regarding bioaccumulation, food-web transfer and to assess the risk toward piscivorous wildlife (birds and mammals).

In relation to the substances for which EQS_{biota} exist, the TGD notes that the use of whole-fish contaminant concentrations may overestimate the risk toward human health for PBDEs, HCB, PFOS, dioxins and dioxin-like compounds, and heptachlor/heptachlor epoxide.

Furthermore, the TGD notes that the use of fillet contaminant concentrations may underestimate the risk toward top predators for priority substances for which $QS_{biota,secpois}$ is the "critical" QS, with the notable exception of mercury. It is generally recognised that mercury, binds to muscle proteins thus fillet tissue is considered as an appropriate matrix. Normalisation to lipids is not applicable. The TGD notes that fillet contaminant concentrations may underestimate the risk toward top predators for: "*Priority substances for which $QS_{biota,secpois}$ is the "critical" QS, with the notable exception of mercury*". In accordance with the JAMP guidelines mercury data are generally reported to OSPAR based on a muscle tissue and/or on a whole organism basis in the case of crustaceans and molluscs. With these rationale OSPAR fillet and/or whole body crustaceans and molluscs (wet weight) data were deemed to be a suitable tissue for the purposes of assessment relative to the EQS_{biota}.

ii) Normalisation for tissue dry weight

The TGD notes that where a substance does not accumulate by hydrophobic partitioning into lipids (e.g. mercury), but via another mechanism of accumulation, normalisation against another parameter, such as dry weight, may be appropriate. If only fish are considered, differences in moisture content are considered to be limited (EFSA, 2009; Smit, 2005) and wet weight concentrations could be used for mercury in fish. In line with TGD methodologies, the appropriate metric to use for normalisation will usually follow from the normalisation used in the bioaccumulation studies used to derive the standard and thus in the case of mercury normalisation relative to default dry weight contents of 26% in fish and 8.3% in bivalves. The TGD recommends that the actual dry weight content of the sampled biota be determined alongside the contaminant concentrations, or that a generic value for the particular biota species are used, such as those available in FishBase[3]. For this assessment (see tables 1 to 4) dry weights as reported to OSPAR for individual samples were utilised for normalisation (see table 1) in accordance with equation 1 below. It should be noted that default values as suggested within the TGD for the purposes of normalisation (26% for fish and 8.3% for mussels) are generally different from those actually measured in marine samples reported in this paper. Mussels having a median dry weight of 17.3% and fish from 18.5% (whiting) to 26.5% for herring (see table 1). Dry weight differences of this magnitude can have dramatic effects on the normalisation factor to be used to generate the final $Conc_{TL\text{-}adj}$. Where

[3] Example of TL = 4.1 for *Gadus Morhua* (Cod) http://www.fishbase.org/summary/SpeciesSummary.php?ID=69&AT=cod

individual sample dry weights were unavailable, median dry weights based on available OSPAR data were used.

Equation 1: $\mathbf{Conc_{norm,dry\ wt.} = Conc_{meas} * 0.26\ (fish)\ or\ 0.083\ (mussels) / species\ specific\ dry\ wt.}$

$Conc_{meas}$ = Concentration of mercury (CILY or MeanLY) in individual samples.

$Conc_{norm,\ dry\ wt.}$ = Conc after normalisation to dry weight (0.26 for fish or 0.083 for mussels).

Species specific dry wt =Median species specific values derived from OSPAR database

iii) Trophic level adjustment

The biota standard should be applied to the most 'important' link in the food chain. In this context, 'important' means the trophic level where concentrations peak, such that the predator of species of that level is exposed to the highest food concentrations. In general, for substances subject to biomagnification, the critical concentrations are attained at TL= 4 in freshwater food webs, and TL=5 for marine food webs.

In the case of mercury the biota standards refer to fish. As mentioned above an alternative biota taxon, or another matrix, may be monitored instead as long as the EQS applied provides an equivalent level of protection. This implies that if, for example, a monitoring program with mussels (TL = 2) is implemented, the monitoring data should be compared with biota standards that have been adjusted for this trophic level. However it is well documented that trophic positions are not fixed values for each species, but may vary from one ecosystem to another and even from one individual to another. Therefore, instead of this approach (which may be adequate for certain biota, e.g. certain fish species) the TGD notes the requirement to adjust the monitoring data to correspond to a more appropriate trophic level before comparing them with EU's EQS_{biota}. To determine trophic level for the monitored organism the TGD recommends the measurement of stable isotopes in the biota samples. Using the nitrogen isotope ratio ($\delta^{15}N$) this should be done together with the characterisation of a baseline of the food-web from which the monitored organism originates. The baseline is determined through measurements of primary consumers (e.g. mussels) with all components then combined as per equation 2 (see Post (2002) and section A.8 of TGD).

Equation 2: $\mathbf{Trophic\ level = (\delta^{15}N_{(fish)} - \delta^{15}N_{(mussels))} / 3.4 + 2}$

$\delta^{15}N_{(fish)}$ = measured isotope ratio in sampled species.

$\delta^{15}N$(mussels)= measured isotope ratio in baseline species (e.g. mussels).

3.4‰ = mean enrichment in $\delta^{15}N$ per trophic level.

2 = Trophic level of baseline primary consumer species (e.g. mussels).

The value of 2 represents the trophic level of primary consumers. On occasion it may be more appropriate to utilise other primary producers (aquatic vegetation, algae i.e. TL=1), in such cases equation 2 should be adapted accordingly. It should be noted that regardless of the method, the determination of trophic level introduces considerable (biologically associated) variability into EQS_{biota} assessments.

Many factors impact the determination of trophic level, such as individual animal size, gender, condition factor, spatial considerations, not to mention the differences between the methods. These factors were not incorporated into this assessment. Because stable isotope data is not currently

reported with contaminant monitoring data, a default trophic level for each species was used based on the data extracted from Fishbase (see table 6).

iv) Accounting for contaminant trophic magnification.

Tropic magnification factors to cover for accumulation in birds and mammals that serve as food for marine top predators, should be incorporated in the biota quality standard for the marine environment. Different trophic magnification factors (TMF) are required to be used for this process to account for the extra magnification step in the marine environment, compared to the TMF to account for trophic magnification in the pelagic environment.

Establishing an equivalently protective EQS for another biota taxon in the pelagic food chain necessarily involves taking account of the combined effects of sample trophic level and contaminant TMFs. As discussed above, a wide range of TMF values are reported in the literature for mercury.

For the purposes of this assessment a generic TMF of 4.0 for total-mercury was used for all species sampled in order to derive trophic level adjusted mercury concentrations ($Conc_{TL\text{-}adj}$ as per equation 3), using OSPAR reported data. This TMF value was derived using the slope of log BAF vs. TL (=0.605). With TMF = $10^{\wedge(slope\ =0.605)}$ =TMF = 4.0 and is based on in excess of 2000 fish originating from 59 ecosystems (Verbruggen et al 2015).

Equation 3: $Conc_{TL\text{-}adj} = Conc_{biota} * TMF^{(4\text{-}TL(x))} *$(default dry wt. / actual dry wt.)

Where:

TMF for mercury = 4.0 (Verbruggen et al (2013),

TL(x) = Species TL value from Fishbase,

default dry wt. (from TGD =26.3% for fish and 8.3% for crustaceans/molluscs),

actual dry weight = generic species dry weights from OSPAR data (table 6).

The QS_{biota} of 20 µg/kg total-mercury is based on wet weight concentrations in Directive 2013/39/EU, which according to the TGD are considered to represent the 4th trophic level (EC (2014). Mussels are filter feeders and as such are deemed to occupy the 2nd trophic level. Trophic magnification slopes based on wet weight concentrations are on average 0.16 based on a worldwide analysis of mercury biomagnification, including all kinds of fresh and marine water types (Lavoie et al., 2013), where such slopes represent the increase in log [total-mercury] with $\delta^{15}N$. The most widely used value (originally proposed by Post (2002)) for the enrichment of $\delta^{15}N$ per trophic level is that of 3.4‰.

Because the presented slopes by Lavoie et al (2013) are already based on wet weight, a correction between the moisture content of mussels and fish should not be needed. It is assumed that these differences are already captured within the slope. By way of example utilising a range of TMFs (e.g. 3.5 and 4.7) it can be demonstrated that a substantial differences in calculation of the biota standard in mussels would result, see worked examples below;

TMF =3.5 =>20 µg/kg total-mercury/(3.5^2)=1.6 µg/kg

TMF =4.7 => 20 µg/kg total-mercury/(4.7^2)= 0.9 µg/kg.

Further context on the complexities of BAF based approaches is detailed in a study by Meng et al (2015) who report mercury and associated BAF values for a number of marine molluscs including the Asian hard clam (*Meretrix meretrix*), where a dry weight BAF of 20000 is reported for total-mercury. Corrected to a default dry weight content of 8.3% (as per TGD) the BAF on wet weight is reduced to 1660, compared to 29900 (and 2481 @ 8.3%) for *Mytilus* species.

Another important aspect of BAF studies is the derivation of the trophic level of the test species, and in that context it should be noted that not all molluscs occupy exactly trophic level 2. The lowest trophic level reported in the Meng et al (2015) study for *Meretrix meretrix*, at TL = 2.61 followed by *Mytilus edulis* and *Mya arenaria* at 2.72 and 2.74. However, these mollusc species can be considered to occupy trophic level 2, and therefore the higher recorded TL for these species may be a function of a low recorded zooplankton baseline. Further to this the authors note differentiation between $d^{15}N$ for the different mollusc species, with the carnivorous *Rapana venosa* having a trophic level that is on average 0.7 higher than that of *Meretrix meretrix*. For *Rapana venosa* the BAF is much higher 46400 for total-mercury (compared to methylmercury and methylmercury). With a default wet weight content of 8.3%, this value can be recalculated to 3850 for total-mercury.

In the case of fish species the BAF of total-mercury on wet weight basis for fish species as a function of trophic level was measured as 298000 for trophic level 4, 73900 for trophic level 3, and 18400 for trophic level 2. With a default dry weight content of 26.3% these values for total-mercury are 1,130,000, 281,000 and 69,800 for trophic level 4, 3, and 2 respectively. It should thus be noted that these values are much higher than the values for the mollusc, regardless whether wet weight or dry weight is considered. It appears that the wet weight BAFs for trophic level 2 molluscs are one order of magnitude lower than the equivalent BAFs for trophic level 2 fish, and a factor 3.5 based on dry weight, both for total-mercury and methylmercury, with differences in the routes of dietary assimilation of mercury (e.g. filter feeding of mollusc versus predatory magnification in fish) likely to be a key contributor to these differences. Using this information this would equate to an EQS in molluscs that would be only 0.11 $\mu g/kg_{wet\ weight}$ or 1.3 $\mu g/kg_{dry\ weight}$.

Considering this information it can be concluded that, the translation from an EQS in fish to an equivalent value in molluscs has high uncertainty, the rationale being that the earlier derived value of 1.6 $\mu g/kg_{wet\ weight}$ was based on a general generic relationship not specific for certain species. Where a study specifically deals with molluscs, it might indicate a rather big difference between molluscs and fish. The low accumulation of mercury in molluscs is also in accordance with the low percentage of methylmercury (21%). With a number of exceptions it is generally considered that the higher accumulation equates to greater methylmercury tissue content. In fish at trophic level 2, the contribution of methylmercury to total-mercury is still around 80%, i.e. much higher than in molluscs.

Derivation of a standalone EQS_{biota} for mussels ignores the fact that many birds and mammals forage at a higher trophic level than the trophic level 2 of mussels; for example, sharks, killer whale and sperm whale feed at high trophic levels and do not eat mussels. Besides that, the caloric content of mussels is much lower than that of fish. In the derivation of the EQS_{biota} a factor that is reasonable for the caloric content of fish has been applied. For mussels, this is insufficient (RIVM letter report 2014-0097) and a lower value than 20 (e.g. ≅6) could be derived for those animals consuming

mussels (e.g. some duck species), leaving the rest of the ecosystem unprotected. Further context on possible food chain effects on real top predators is reported in Jepson et al (2016).

v) Assessment of monitoring data relative to the EQS_{biota}

Assessing compliance with biota standards is subject to the same statistical considerations as any other standard (ISO 2008). Decisions about compliance with the standard may be taken on the basis of a 'face value' assessment (i.e., comparing the mean of a number of samples with the EQS), or statistical approaches that take account of uncertainty in measured values. These are required if the assessment of compliance is to be supported by an estimate of the confidence in the decision (i.e. whether a site has passed or failed the EQS). Thus, a conservative approach would be to use the upper confidence limit. This would give the benefit of doubt to the environment but false positives are more likely. Alternatively, a pass/fail decision could be made on the basis of the lower confidence limit, where false negatives are more likely to occur.

OSPAR assessment approaches incorporate uncertainty elements by comparing the upper confidence limit associated in the last year of the time series (cILY) relative to the assessment threshold.

Results of the assessment

This working document reports an assessment completed using a method that is consistent with the approach taken under the WFD and that is consistent with the approach taken by OSPAR and its Hazardous Substances Strategy.

Following conversion in line with the process above a number of assessment products were developed, namely;

1) A summary status assessment of mercury concentrations in biota utilising OSPAR criteria in addition to EQS_{adj_conc} for both OSPAR meanLY and cILY assessment concentrations.
2) A summary of the frequency of OSPAR cILY and $cILY_{TL\text{-}Adj}$ concentration values as referenced against EAC/EC and EQS_{biota} assessment criteria.
3) Regionalised status assessment (data up to 2015) for mercury in biota utilising OSPAR criteria in addition to EQS_{adj_conc} for both OSPAR meanLY and cILY assessment concentrations.

Table 2: Summary status assessment (Post MIME 2015) for mercury in biota utilising OSPAR criteria in addition to EQS_{adj_conc} for both OSPAR meanLY and cILY assessment concentrations

		Vs EQS_{adj} meanLY		Vs EQS_{adj} cILY		Current OSPAR Status (2015)			
	Species	FAIL	PASS	FAIL	PASS	Red	Green	Blue	Black
BE	*Crangon crangon*	1		1					1
	Mytilus edulis	3		3			3		
	Platichthys flesus	1		1			1		
DK	*Mya arenaria*	1		1					1
	Mytilus edulis	12		10			9	3	
	Platichthys flesus	1		1				1	
	Zoarces viviparus	2						2	
FR	*Crassostrea gigas*	19		19			7	12	
	Mytilus edulis	25		24			21	4	
DE	*Limanda limanda*	4		3			4		
	Mytilus edulis	2					2		
	Platichthys flesus	1					1		
IC	*Mytilus edulis*	1						1	
IE	*Cerastoderma edule*	1							1
	Crassostrea gigas	13		7				13	
	Mytilus edulis	30		20			23	7	
	Ostrea edulis	4		2				4	
NO	*Gadus morhua*	18		13		2	15	1	
	Lepidorhombus whiffiagonis	2		2			2		
	Limanda limanda	4		3			4		
	Mytilus edulis	51		36			31	20	
	Platichthys flesus	3		3			3		
	Pleuronectes platessa	2		2				2	
PO	*Mytilus galloprovincialis*	11		7			9	2	
ES	*Merluccius merluccius*	3		3			2	1	
	Mytilus edulis	40		39			27	13	
SE	*Clupea harengus*	2		2				2	
	Gadus morhua	1		1			1		
	Mytilus edulis	2		2				2	
	Zoarces viviparus	1		1				1	
NL	*Crassostrea gigas*	2		1				2	
	Mytilus edulis	2		2			2		
	Platichthys flesus	4		4			4		
	Pleuronectes platessa	3					2	1	
UK	*Crassostrea gigas*	1		1				1	
	Gadus morhua	1					1		
	Limanda limanda	43		34			43		
	Merlangius merlangus	1					1		
	Mytilus edulis	104		86			93	11	
	Platichthys flesus	14	1	13			10	5	
	Pleuronectes platessa	15		7				15	
	Scomber scombrus	3						1	2
	Total	**454**	**1**	**354**	**0**	**2**		**337**	**113** **3**

Table 3: The frequency of OSPAR meanLY and meanLY$_{TL\text{-}Adj}$ concentration values as referenced against EAC/EC and EQS$_{biota}$ assessment criteria. The frequency is reported as a percentage in parenthesis.

Number (percentage) of time series relative to EQS$_{biota}$ and EAC/EC criteria	% of Assessment criteria	meanLY relative to EQS$_{biota}$ (%)	meanLY relative to EAC/EC (%)
	0	0 (0)	0 (0)
	10	0 (0)	341 (75.4)
	25	0 (0)	82 (18.1)
	50	0 (0)	22 (4.9)
	75	1 (0.2)	5 (1.1)
	100	0 (0)	0 (0)
	200	4 (0.9)	1 (0.2)
	300	14 (3.1)	1 (0.2)
	400	31 (6.8)	0 (0)
	500	43 (9.5)	0 (0)
	750	113 (24.8)	0 (0)
	1000	68 (14.9)	0 (0)
	2500	125 (27.5)	0 (0)
	5000	44 (9.7)	0 (0)
	>5000	12 (2.6)	0 (0)
	Total	455	452

Table 4: Regionalised status assessment for mercury in biota utilising OSPAR criteria in addition to EQS_{adj_conc} for both OSPAR (EC/EAC) using meanLY and cILY assessment concentrations

			EQS $_{adj}$ meanLY (cILY)		Current OSPAR Status vs EC/EAC (500)			
Country	**EcoRegion**	**Species**	FAIL	PASS	**Red**	**Green**	**Blue**	**Black**
NO	BSea	*Gadus morhua*	5 - (4)		1	3	1	
NO	BSea	*Mytilus edulis*	3 - (3)				3	
NO	BSea	*Pleuronectes platessa*	2 - (2)				2	
FR	CH	*Crassostrea gigas*	6 - (6)			1	5	
UK	CH	*Limanda limanda*	4 - (4)			4		
FR	CH	*Mytilus edulis*	21 - (20)			17	4	
UK	CH	*Mytilus edulis*	6 - (2)			5	1	
UK	CH	*Platichthys flesus*	2 - (2)			1	1	
IE	CS	*Crassostrea gigas*	1 - (1)				1	
UK	CS	*Limanda limanda*	3 - (2)			3		
IE	CS	*Mytilus edulis*	7 - (3)			6	1	
UK	CS	*Mytilus edulis*	5 - (2)			3	2	
UK	DB	*Limanda limanda*	3 - (3)			3		
ES	GCad	*Merluccius merluccius*	1 - (1)			1		
PO	GCad	*Mytilus galloprovincialis*	3 - (3)			2	1	
IC	GSR	*Mytilus edulis*	1				1	
FR	IBS	*Crassostrea gigas*	1 - (1)				1	
ES	IBS	*Merluccius merluccius*	2 - (2)			1	1	
ES	IBS	*Mytilus edulis*	40 - (39)			27	13	
PO	IBS	*Mytilus galloprovincialis*	8 - (4)			7	1	
IE	IRSea	*Cerastoderma edule*	1					1
IE	IRSea	*Crassostrea gigas*	6 - (2)				6	
UK	IRSea	*Crassostrea gigas*	1 - (1)				1	
UK	IRSea	*Limanda limanda*	14 - (12)			14		
UK	IRSea	*Merlangius merlangus*	1			1		
IE	IRSea	*Mytilus edulis*	9 - (8)			8	1	
UK	IRSea	*Mytilus edulis*	26 - (23)			24	2	
UK	IRSea	*Platichthys flesus*	4 - (3)			4		
UK	IRSea	*Pleuronectes platessa*	7 - (3)			7		
UK	IRSea	*Scomber scombrus*	3			1	2	
IE	ISC	*Crassostrea gigas*	6 - (4)				6	
UK	ISC	*Limanda limanda*	2 - (1)			2		
IE	ISC	*Mytilus edulis*	14 - (9)			9	5	
UK	ISC	*Mytilus edulis*	17 - (17)			17		
IE	ISC	*Ostrea edulis*	4 - (2)				4	
UK	ISC	*Platichthys flesus*	2 - (2)				2	
UK	ISC	*Pleuronectes platessa*	2 - (2)			2		
FR	NBB	*Crassostrea gigas*	12 - (12)			6	6	
FR	NBB	*Mytilus edulis*	4 - (4)			4		

Table 4 (cont): Regionalised status assessment for mercury in biota utilising OSPAR criteria in addition to EQS_{adj_conc} for both OSPAR (EC/EAC) using meanLY and cILY assessment concentrations

			EQS $_{adj}$ meanLY (cILY)		Current OSPAR Status vs EC/EAC (500)			
Country	**EcoRegion**	**Species**	FAIL	PASS	**Red**	**Green**	**Blue**	**Black**
UK	NNS	*Gadus morhua*	1			1		
UK	NNS	*Limanda limanda*	11 - (6)			11		
UK	NNS	*Mytilus edulis*	40 - (36)			36	4	
UK	NNS	*Platichthys flesus*	3 - (3)	1		3	1	
UK	NNS	*Pleuronectes platessa*	4 - (2)			4		
NO	NT	*Gadus morhua*	5 - (4)			5		
NO	NT	*Lepidorhombus whiffiagonis*	2 - (2)			2		
NO	NT	*Limanda limanda*	2 - (2)			2		
NO	NT	*Mytilus edulis*	16 - (15)			12	4	
NO	NT	*Platichthys flesus*	2 - (2)			2		
NO	NWSea	*Gadus morhua*	3 - (2)		1	2		
NO	NWSea	*Mytilus edulis*	2			1	1	
NN	SK	*Clupea harengus*	2 - (2)				2	
NO	SK	*Gadus morhua*	5 - (3)			5		
NNS	SK	*Gadus morhua*	1 - (1)			1		
NO	SK	*Limanda limanda*	2 - (1)			2		
DK	SK	*Mytilus edulis*	5 - (4)			3	2	
NO	SK	*Mytilus edulis*	30 - (18)			18	12	
NNS	SK	*Mytilus edulis*	2 - (2)				2	
NO	SK	*Platichthys flesus*	1 - (1)			1		
DK	SK	*Zoarces viviparus*	2				2	
NNS	SK	*Zoarces viviparus*	1 - (1)				1	
BE	SNS	*Crangon crangon*	1 - (1)					1
NL	SNS	*Crassostrea gigas*	2 - (1)				2	
DE	SNS	*Limanda limanda*	4 - (3)			4		
UK	SNS	*Limanda limanda*	6 - (6)			6		
DK	SNS	*Mya arenaria*	1 - (1)					1
BE	SNS	*Mytilus edulis*	3 - (3)			3		
DK	SNS	*Mytilus edulis*	7 - (6)			6	1	
DE	SNS	*Mytilus edulis*	2			2		
NL	SNS	*Mytilus edulis*	2 - (2)			2		
UK	SNS	*Mytilus edulis*	10 - (6)			8	2	
BE	SNS	*Platichthys flesus*	1 - (1)			1		
DK	SNS	*Platichthys flesus*	1 - (1)				1	
DE	SNS	*Platichthys flesus*	1			1		
NL	SNS	*Platichthys flesus*	4 - (4)			4		
UK	SNS	*Platichthys flesus*	3 - (3)			2	1	
NL	SNS	*Pleuronectes platessa*	3			2	1	
UK	SNS	*Pleuronectes platessa*				2		

Table 5: Variability (RSD %) measured between species after normalisation using the TGD#32 approach and as observed with (un-normalised) OSPAR meanLY data

Species	Conc EQS_{adj}			meanLY			#Series
	Average	Stdev	RSD (%)	Average	STDEV	RSD (%)	
Cerastoderma edule	108	-	-	10.0	-	-	1
Clupea harengus	28.6	1.11	**3.90**	22.0	0.10	**0.43**	2
Crangon crangon	313	-	**-**	84.9	-	**-**	1
Crassostrea gigas	168	56.7	**33.7**	26.4	9.42	**35.6**	35
Gadus morhua	289	397	**138**	165	233	**141**	20
L. whiffiagonis	290	33.4	**11.5**	114	15.0	**13.2**	2
Limanda limanda	770	446	**58.0**	113	65.7	**58.4**	51
Merlangius merlangus	154	-	**-**	110	-	**-**	1
Merluccius merluccius	73.8	29.9	**40.4**	43.1	21.4	**49.6**	3
Mya arenaria	131	-	**-**	16.7	-	**-**	1
Mytilus edulis	186	135	**72.5**	26.1	16.7	**63.9**	272
Mytilus galloprovincialis	137	33.1	**24.1**	22.4	4.93	**22.0**	11
Ostrea edulis	114	17.9	**15.7**	22.3	5.18	**23.2**	4
Platichthys flesus	204	142	**69.5**	83.1	57.4	**69.1**	25
Pleuronectes platessa	401	223	**55.6**	67.3	38.6	**57.4**	20
Scomber scombrus	58.4	13.0	**22.2**	38.2	10.5	**27.4**	3
Zoarces viviparus	72.5	22.7	**31.2**	24.2	9.87	**40.8**	3

One of the key aims of the normalisation approach proposed within the TGD is to even out the influences of biological factors (e.g. trophic level, dietary differences etc.) between species to enable better comparison in different species fish and between locations. Within this concept (and where individual isotope data are available) normalisation should reduce data variability, provided no age or other effects interfere with the normalisation process.

It should be noted in the absence of individual isotope data for these OSPAR time series data , this pilot study utilises “generic” trophic level for each species.

Table 5 documents variability (RSD %) as measured in species after normalisation using the TGD#32 approach and as observed with non-normalised OSPAR meanLY data. The generic trophic level value (e.g. derived from FISHBASE) applied to individual species across the convention area masks the potential influence of local TL on test species. The absence of “real” isotope data or trophic level information for the OSPAR time series evaluated hinders the normalisation process. As such with this pilot assessment, and with the application of a generic TL value, it is not currently possible to further evaluate whether local/ecosystem influences in the trophic level would bring about a reduction in variability.

Discussion and conclusions

Mercury is known for its worldwide environmental impact. Due to its chemical characteristics it is deemed to be highly toxic and with high biomagnification potential with a relatively wide range of concentration increases through all trophic level compartments of aquatic ecosystems reported in the literature.

A number of key thresholds namely; the combination of OSPAR, BCs, BACs and EACs (currently the EC food safety level for mercury as the EAC equivalent (500 µg/kg ww)) and EU derived Environmental Quality Standard (EQS_{biota}) are available to OSPAR for the purposes of assessing mercury concentrations in aquatic biota. This latter threshold (EQS_{biota}) having been set to protect predatory birds and mammals from adverse effects (via secondary poisoning) of mercury via food intake.

In order to establish an equivalently protective EQS for all biota, taxon adjustment of monitoring data to account for trophic magnification is required. To ensure consistency in approaches between individual countries the EU has recently described a procedure (via Technical Guidance Document #32) for data preparation prior to compliance assessment. This stepwise process of adjusting monitoring data to an appropriate trophic level (TL=4) requires that monitoring data (concentrations in muscle or whole organism on a wet weight basis) be adjusted to account for a number of factors.

It is widely recognised that data correction to incorporate trophic level biomagnification exhibits a number of biological based (e.g. age/sex, trophic level assignment and derivation of appropriate trophic magnification factors) and spatially related (e.g. migration of fish) variables. The correction for trophic level has known insecurities, but these are smaller than the uncertainty in other parameters/units. It is expected that not correcting for trophic level would equate to gives much greater uncertainty in the outcomes. Greater variability is associated with the biological based parameters involved in the conversion process itself as against that associated with the generation of the analytical data measurement and sampling components. Such concerns have been documented by a number of authors including Post et al (2002) and have additionally been elaborated upon by the MSFD Expert Network on Contaminants (MSFD-ENC) when evaluating the applicability under the MSFD of this TGD (see Appendix 2).

The current EQS_{biota} of 20 $\mu g/kg_{ww}$ is based on a data set of chronic toxicity values of methylmercury for 4 mammal and 7 bird species. This data set originates from RIVM report 601501009 from 2000, but all data in this report in its turn originate from an older RIVM report 601014008 from 1995. The resulting toxicity data are from 1987 or older. The most sensitive species were the rhesus monkey and the mallard duck, with NOECs of 0.22 and 0.25 mg/kg_{food}. It should be noted that similar studies with the rhesus monkey of half a year instead of a year were considered as sub-chronic. The assessment factor applied to the lowest NOEC was 10. This assessment factor also includes the default factor of 3 to account for the differences in energy content between laboratory food and fish, and in principle this assessment factor is lower than the lowest assessment factor to be applied to the lowest NOEC.

The RIVM report suggests that this EQS_{biota} could be refined in 3 ways.

1) Firstly, the factor 3 to account for the differences in energy content and laboratory food and the conversion factor from dose to diet concentration could be refined. However, as the difference in energy content between laboratory food and fish is in general close to a factor

of 3, this refinement will probably not lead to a large change in the EQS_{biota}. The conversion from dose to diet appears sometimes rather conservative, but this factor is not needed for each study (Verbruggen, 2014(2)).

2) Instead of applying a deterministic approach, all toxicity data for birds and mammals could be used for a species sensitivity distribution (SSD). With the current data in the EQS factsheet for mercury, the HC5 will be slightly below the lowest value for all species. As the applied assessment factor, remaining after taking account of the factor 3, is 3.33 and considering that to the HC5 also an assessment factor varying from 1 to 5 has to be applied, the application of the SSD method will also not lead to a much different EQS_{biota} (with only eleven species the assessment factor would be more towards 5).
3) As the data set is now relatively old, new data could be added, including data for new species. A recent review was undertaken by WCA in order of the European Commission (report not published). The conclusion from this review was that new data were available, of which several species were noting the data set in the current EQS data sheet for mercury. Their analysis revealed that some of these species (white ibis, common loon) were more sensitive to mercury than the ones included in the EQS derivation. Most likely, taking into account more recent data would lead to a reduction in the EQS_{biota} for mercury.

Data with typical marine species are not available. This is of course not surprising, because marine species cannot be held in laboratory condition and they are not suitable for toxicological experiments for practical and ethical reasons. The only study that is known is a historic study with a few harbour seals that were fed methylmercury. The derivation of EQS_{biota} based on marine species will therefore be impossible. The assumption of in the EQS derivation is that the diversity in the species used is diverse enough to cover also marine birds and mammals (Verbruggen 2014(2)).

MCWG 2016 noted the value of completing this pilot study to identify and quantify some of the key issues that need to be considered in order to normalise data relative to trophic level and tropic magnification. EQS_{biota} are intended to protect fish eating birds and mammals and assumes that animals feed at trophic level 4. MCWG 2016 recommends that extension of the EQS_{biota} concept to protect top marine predators (e.g. seals and cetaceans) is therefore not applicable. MCWG strongly recommends that a more integrated assessment that accounts for these animals is completed by appropriate expert groups. Additionally future assessments should consider the relationship between whole body mercury burdens and concentrations of mercury in muscle tissue. MCWG referred to the food safety value for mercury as a proxy for EAC but MCWG does not consider this as a suitable threshold.

Use of “generic” TMF (e.g. 4.0 for mercury) and assigning “generic” species specific trophic levels based on information available (e.g. from FISHBASE) was discussed. MCWG 2016 referred to the fact that in the literature TMFs can often have a high uncertainty and thus upper and lower-bound assessments would have large error factors limiting the potential power of normalisation process.

The determination of Trophic level is ecosystem specific and depends on a number of variables such as age and/or feeding habits and even within species. This can vary by up to one trophic level between locations. Where this assessment approach is applied, the MCWG recommends that the trophic level should be determined experimentally on a site specific basis using stable isotope measurements in e.g. mussels as a baseline and covering the entire associated ecosystem/food-web. It was also noted that the proposed dry weight normalisation value for mussels (8.3%) is unrealistic

and the MCWG recommends that this default value should be reviewed and consider that dry weight normalisation is not necessary for the purposes of assessment.

The EQS approach itself was not challenged but it is noted that EQS (which were developed primarily for freshwater) are not readily extendable to the marine environment. The use of generic values (TMF and TL) as "biological" variables adds additional unquantifiable uncertainty to assessments and does not improve the results of assessment. It was concluded that use of generic values in the absence of measured information is not advisable. Ecosystem specific TMF and TL data are required. Literature values where they are suitable for the study area may be a good proxy. Thus MCWG recommends that in the case of mercury that OSPAR should concentrate its assessment outputs without normalisation of concentrations to trophic level 4.

As part of this science-led, process-based "framework" approach (MIME 15/04/04a1 see Appendix 5), MIME 2015 evaluated the applicability/suitability of OSPAR monitoring data for the purposes of compliance assessment relative to EQS_{biota}. Application of the MIME framework took into consideration that as mercury primarily binds to muscle proteins that OSPAR fish fillet/muscle tissue and whole organism (mollusc and crustacean) data were deemed suitable as an appropriate matrices in respect of completing an EQS_{biota} assessment.

Further to the framework and while taking cognisance of the associated uncertainties/variabilities, available OSPAR mercury contaminant data in biota was compiled and tropic level adjustment was completed using a number of "adjustment factors" (i.e. sample dry weight, TMF and generic species specific trophic levels) which were derived from existing OSPAR data and/or from literature. These factors were then used for the purpose of assessing OSPAR data in line with the methodology reported in the TGD.

OSPAR meanLY and upper cILY concentration data were assessed relative to the current OSPAR assessment threshold and were then "adjusted" to derive values for comparison to the EQS_{biota}. Regionalised and ecoregion based status assessments for mercury were then completed.

It can be concluded that when utilising the current OSPAR EC food safety level for mercury that greater than 95% of biota time series would exhibit a better than red status. When referenced against the EQS_{biota} of 20 µg/kg wet weight less than 1% of biota data would be deemed to be compliant.

It is the opinion of MIME 2015 that:

1) Even in the absence of tropic adjustment elements (i.e. direct comparison of data to the EQS_{biota} that a significant number of OSPAR time series data would fail the EQS_{biota} threshold.

2) While the EQS_{biota} reference value is close to or below current OSPAR BC (18 for mussels) it will be generally be impossible to reach good status when the TGD approach is applied, even at "pristine" locations.

3) Derivation of a standalone EQS_{biota} for mussels is not recommended as it ignores the fact that many birds and mammals forage at a much higher trophic level than the trophic level 2 of mussels.

4) The EQS approach is not readily extendable to the marine environment.

5) Hg concentrations over the whole food chain are influenced by intrinsic physical and chemical characteristics, but it is unclear what their role is on the biomagnification process (and thus

on trophic magnification). The use of generic values for TMF and TL (as completed in this pilot assessment) does not improve the results of assessment and thus the use of generic values in the absence of measured information is not advisable.

6) Ecosystem specific TMF and TL data are required to strengthen future assessment outputs.

7) Without global based intervention measures there is unlikely to be a means (and especially in the short to medium time frame) to dramatically reduce mercury inputs to the marine environment and to reach good status as measured under this TGD based approach.

8) The use of generic values in the absence of measured information is not advisable. Ecosystem specific TMF and TL data are required. Literature values where they are suitable for the study area may be a good proxy.

9) In the case of mercury ongoing work should concentrate on assessment outputs without normalisation of concentrations to trophic level 4.

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Glossary

BAC – Background assessment criteria
BAF- bioaccumulation factor
BC – background concentration
BCF - bioconcentration factor
BMF - biomagnification factor
CEMP - Coordinated Environmental Monitoring Programme
cILY – upper confidence limit for the last year of the time series
CLRTAP - Convention on Long Range Transboundary Air Pollution
EAC – Environmental Assessment Criteria
EMEP - Environmental Monitoring, Evaluation, and Protection
EPER -The European Pollutant Emission Register
EQS – Enironmental Quality Standards
EQS_{biota} EQS for biota
$EQS_{biota,secpois}$ EQS for biota taking account of secondary poisoning
$EQS_{adj,conc}$ EQS for biota adjusted for basis, TL and TMF
EU – European Union
HBCDD - hexabromocyclododecane
HCB - hexachlorbenzene
HCBD - hexachlorobutadiene
HELCOM - Helsinki Commission for the Protection of the Marine Environment of the Baltic Sea
meanLY – mean concentration of the last year of the time series
MeHg - methylmercury
MPC - maximum permissible concentration
OSPAR – OSPAR Commission
PBDE - polybrominated diphenylethers
PFOS - perfluorooctane sulfonic acid and its derivatives
REACH - Registration, Evaluation and Authorisation system for Chemicals
TGD – technical guidance document
THg – total mercury
TL – trophic level
TOR – Terms of reference
WFD – Water Framework Directive

Appendix 1: Data methodologies utilised for this assessment

For each time series, a mixed model was used to describe the change in log mercury concentration over time.

The fixed part of the model can be written log concentration ~ f(year), where the form f() depended on the number of years with data (ny) and the number of years with measurements above the detection limit (npos).

When $2 \leq npos \leq 4$ and $ny > 3$, log mercury concentrations were assumed to be stable over time: $f(year) = \alpha$ = constant.

When $5 \leq npos \leq 6$, log mercury concentrations were assumed to change linearly over time: $f(year) = \alpha + \beta$ year.

When $npos \geq 7$, log mercury concentrations were assumed to change smoothly over time, with the amount of smoothing estimated from the data: f(year) = smooth function of year.

The random part of the model had three variance components: a between-year component, a between-sample component (only estimated when there was more than one sample in any one year), and an analytical component (estimated from the reported uncertainty of each measurement). The model was fitted by maximum likelihood, with any less-than measurements treated as left-censored data. In the fitting process, the analytical variance component was assumed known. Status was assessed by comparing the upper one-sided 95% confidence limit on the fitted value in the last monitoring year to the assessment concentration.

Appendix 2: Considerations and recommendations of the MSFD Expert Network on Contaminants (MSFD-ENC) regarding the applicability under the MSFD of the WFD Guidance document #32 on Biota Monitoring. Application of WFD guidance in respect of the MSFD

The MSFD Expert Network on Contaminants (MSFD-ENC) regarding the applicability under the MSFD of the WFD Guidance document #32 on Biota Monitoring (The Implementation of EQS_{BIOTA}) making the considerations as below, these were additionally discussed at MIME 2015 with further supporting information post MIME 2015 detailed in **bold** below. ;

- As part of the MSFD CIS work programme, a common understanding on issues related to descriptors 8 and 9 should be developed together with the WFD issues and that WFD implementation guidance should be used for the MSFD to the largest extent possible.
- Although the general philosophy of WFD and MSFD, the environmental protection, is common in both directives, there are significant differences in the environmental thresholds for the environmental assessment. In fact, EQS in WFD were defined to protect freshwater and marine ecosystems, as well as protecting human health from adverse effects via drinking water or the intake of food originating from aquatic environments. Consequently different protection goals were considered in the derivation of EQS. , including human health for seven priority substances **with the EQS for mercury based on secondary poisoning of birds and mammals, not on human health**.
- However, in the MSFD there are two different descriptors dealing with contaminants, and they have different objectives: the protection of the environment (D8) versus protection of human health (D9). In this context, although the EQS proposed using human health criteria could be applied for Descriptor 9, they would not be useful for Descriptor 8. In fact, current EQS are sometimes lower than calculated Background Concentrations (BCs) in the marine environment. The MCWG noted that this is probably as a consequence of not having used environmental toxicology criteria for the EQS derivation and/or the application of assessment factors when few data were available. **It should be noted that all EQS are based on toxicological information. The AF used for mercury is only a factor of 10 (including the factor of 3 to account for caloric differences). Recent literature shows that the data set probably did not even cover the most sensitive species.**
- In order to adequately assess good environmental status (GES), the environmental criteria for Descriptor 8 in the MSFD should be based on toxicological criteria, preferentially marine ones, **although data are scarce**.
- Trophic Level Correction: The environmental criteria for the assessment of the marine environment should be species- and tissue-specific, considering the most common suitable species, as it is applied by Regional Conventions – high relevance. It is noted that this would greatly increase the uncertainty of the data to be compared with the EQS that are, for most of the substances, derived for a fish in trophic Level 4. While MCWG note that this could make sense for freshwater but in the marine environment it would be very helpful to, at least, have EQS derived for mussels that are the most widely used species in marine pollution monitoring. This would allow a simple assessment means for compliance **MIME 2015 further note that this approach may not deliver on the aim at protecting the ecosystem nor would it adequately work for freshwater or the marine environment.**

Uncertainty is rather limited (i.e. TMF should be close to 4. After all, correction for trophic level only should be within a factor of 25 (maximum value over a maximum of two trophic levels with a high TMF of 5. If a TMF value of 3 would be taken, the maximum factor would be 9, leaving a window of a factor of 2.5.

- If generic Trophic Level values are assumed for each species (e.g. based on the values in www.FishBase.org), then this process is **likely to be a significant source of variability and noise in the site average TL-corrected concentrations**. This is partly because the derivation of these TL values has not necessarily been done to the same level of rigour as the chemical analyses, but mainly because the values are generic and it is unrealistic to expect all individuals of a species, regardless of size, age, location, etc. to have the same TL. Indeed, the TL of a fish species varies both within and between sites (http://www.sciencedirect.com/science/article/pii/S0045653514015100) leading to variability in bioaccumulation factors. Stable nitrogen isotope analyses of the biota sample and of the site-specific base of food chain food item are required to accurately determine TL, but few monitoring authorities have access to stable isotope mass spectrometry. TL correction is therefore either a significant additional cost, or a significant additional source of error in compliance assessments**. MIME 2015 note that costs associated with isotope analysis are low relative to contaminant analysis and provides valuable supporting information at relatively low cost.**
- Tissue-whole organism conversion factors: Species-specific conversion factors are required for each contaminant to allow conversion of concentration measurements between tissues and whole organism.
- Greater sampling effort is problematic (ethics and population depletion). If we routinely analyse whole organisms and then convert those contaminants that need to be expressed on flesh, then we need appropriate conversion factors (and to know their uncertainty) for each species.
- For OSPAR analysis of flesh (mercury) or liver (organics and metals other than mercury) is employed; these tissues have the highest concentrations, meaning that trends can be detected with greater power and sensitivity. Factors to convert between liver-flesh or liver-WO will also be required for countries reporting to OSPAR. The development of conversion factors should be co-ordinated to ensure a common experimental approach is used. .
- Uncertainty: The QA/QC Directive (2009/90/EC) requires a measure of uncertainty of <50% at the EQS. The biota EQS's are expressed for organisms of TL4. It would appear therefore that the expanded uncertainty should include the uncertainty associated with conversion to TL4 (whether by generic conversion factors or by stable isotope determination), and should also include the uncertainty on tissue conversion factors. This is likely to be impossible for the likes of the PBDEs. Indeed the cumulative effects of these conversions will be more noise and greater uncertainty, making it more likely that EQSs are failed and the level of uncertainty may lead to challenge if there are significant costs imposed by way of measures. **MIME 2015 noted that uncertainty should be considered in both directions, i.e. an equal chance of failing EQS as to passing.**
- Specific comments: TGD notes that the most reliable summary statistic (for comparison with an EQS_{biota}) is therefore the antilog of the mean of log-transformed concentrations, after normalisation as described in Section 6.1 if appropriate, in individual samples". In the marine environment, there is often significant variability inter-individuals and, consequently, mean

concentration in biota could limit the information about the desired GES. The knowledge of the proportion of population that gets over the environmental criteria is also relevant for the proposal of measures designed to achieve or maintain GES. Consequently, attending to maximum protection level, all available data, and not only the mean of the log-transformed value, should be compared individually with the environmental criteria, as a way to establish which proportion of the ecosystem/population is affected. Another option could be to compare mean values but considering also 90% confidence interval as it is applied by OSPAR.

- Target species: No specific recommendation in the TGD regarding which species should be sampled, because flexibility in target species is required. However, EQS are specific for each species/tissue and should be derived to 4th trophic level for each case. Consequently it should be helpful to have a recommendation from the European Commission to use some widely distributed and representative target species (3-4 species) at regional or subregional level and to derive the specific EQS for these species, as is the case in the Regional Conventions (mussels, oyster, red mullet, cod, etc.). OSPAR has proposed EACs for some marine species that could be adopted by MSFD.
- In the marine environment the EQS for marine mussels (whole body), demersal fish (whole body or selected tissue) and sediments (whole fraction) should be proposed for the assessment as a minimum. If those environmental criteria are not available, the ones proposed by Regional Conventions could be useful for the marine environment (EACs for biota and ERLs for sediments).
- Specific tissues/organs should be recommended for the most widely distributed species in order to get comparable results among countries and regions. The conversion factors for fillet-to-whole fish contaminant levels could introduce high errors and uncertainty due to their high variability depending on the area and environmental conditions. It would be therefore preferable to have a recommendation for specific tissues in specific species at EU level, improving the inter-comparability.
- In general conversion factors (fillet-to-whole fish, trophic level, liver-to-whole fish) can modify significantly concentrations to be checked/assess with EQS and they should be only applied if factors were derived from similar species and environmental conditions.
- The TGD notes that “Using the exact lipid or dry weight content of the biota samples is always preferred over generic values for the species (such as those available from FishBase)”.
- If using FishBase as a reference, clear instructions are required as for which species data are verified in FishBase. The establishment of equivalently protective EQS for another biota taxa involves taking into account the trophic level, using the trophic magnification factor (TMF) is strongly dependent on the TMF value. The available data about TMF are limited and show a high variability depending on the ecosystems studied. Two species at the same trophic level can show very different concentration of contaminants. If the goal is environmental protection, the pollutant concentration should be compared directly with the criteria proposed for this species, not being necessary to apply conversion factors attending to trophic level. For this reason the (MSFD-ENC) report that it is more adequate that the European Commission establishes/proposes environmental criteria for the most common species using all available marine TMF data for these species and/or environmental toxicological data. **MIME 2015 additionally adds that, the largest variability can more likely be assigned to intra-species variability, rather than interspecies variability. TMFs are**

generated for ecosystem protection and not individual species protection. Toxicological data specific for marine mammals is extremely rare.

Appendix 3: Minimising natural variability for EQS_{biota} compliance

Minimising Variability

Regardless of species selection, natural variability within and between samples should be minimised as far as possible. Contaminant levels are known to be influenced by a range of biological factors including; feeding strategy/trophic level, lipid levels, age/size, gender, migration behaviour, and season (Pulkrabova *et al.* 2007; Gewurtz *et al.* 2011; Brázová *et al.* 2012), (see Annexes A.7 and A.9 of TGD).

Contaminant levels have been shown to be linked to the age, and therefore size, of the fish sampled (Burger *et al.* 2001; Dušek *et al.* 2005; Boscher *et al.* 2010; Gewurtz *et al.* 2011) and, alongside trophic level, this is the most important biological variable (McIntyre and Beauchamp. 2007). The TGD notes that the length of the individuals of each species collected should be constant from year to year at each sampling location, or should at least fall within a consistent range. A pragmatic choice of fish age is between 3-5 years, but practical considerations in the field and laboratory (e.g. tissue volume requirements) may override this (see TGD Annex A.5). For the purposes of this report **all biota** as reported to OSPAR in accordance with JAMP (Joint Assessment and Monitoring Programme) guidelines (JAMP Rev 2012) were included for assessment.

Migration behaviour

Many species undertake seasonal migration during their life cycle (e.g. for reproduction, foraging or overwintering), with some species covering tens to hundreds of kilometres. Hence, to be able to report on the local pollution pressure it is essential to choose a relatively sedentary, non-migratory species. In most species, migration behaviour is relatively well studied, and may be deduced from scientific literature. In sedentary species, individuals taken at one site should show similar levels/profiles of contamination (e.g. Belpaire *et al.* 2008). Sampling should therefore be directed at sedentary species most likely to be representative of the sampling location. However, for the purposes of the MSFD, less sedentary species can be relevant since the areas to assess under the MSFD are generally larger than water bodies under the WFD.

Sample/species condition factor

The condition factor of fish has often been associated with the contaminant levels in some studies (e.g. Farkas *et al.* 2003) but has shown weaker/no correlation in others (e.g. Noel *et al.* 2013). The relationship between contaminant load and condition factor may be substance specific. For example, Noel *et al.* (2013) observed no correlation between condition factor and the trace elements arsenic, cadmium and lead, but a positive correlation with mercury levels. As variation in condition factor may be closely associated with the seasonality of sampling (Farkas *et al.* 2003), the K value is unlikely to be a large contributor to variation except where fish are in extremely poor condition, providing that appropriate control measures are employed. Fish measurement data (length and weight) collected during field sampling should allow condition factor to be determined and taken into account if necessary (e.g. widely varying measurements).

Species Gender

Contaminant loads may differ between the different sexes of fish (Sharma *et al.* 2009) especially in the case of the potential elimination of lipophilic pollutants by females in roe at spawning (Sharma *et al.* 2009), differences in habitat utilisation leading to sex differences in substance concentrations

of prey, or sex differences in gross growth efficiency (Madenjian *et al.* 2011). Different mechanisms may operate in different species for influencing the degree of variation between sexes (Madenjian *et al.* 2011). The TGD notes that directing sampling to a particular sex would obviously control for any potential gender differences, and some biota monitoring guidance (e.g. JAMP guidance for the marine environment) suggests sampling all female fish. However, this could potentially result in an underestimation of contaminants should contaminant levels be reduced by spawning. Conversely, sampling all males may overestimate contamination if higher metabolic demands of males lead to increased food consumption (Madenjian *et al.* 2011). Considering that sex cannot be differentiated in most species prior to sampling, no recommendation is made on standardising for gender. Best practice would be to determine the sex of individuals analysed and use the data gathered to inform future guidance.

Seasonality

Chemical residues accumulated by biota can be affected by season, particularly when fish are approaching the breeding season. In cases where females are used, contaminant levels may have dropped during reproduction through maternal transfer into the eggs. Significantly lower levels of PBDE and PCBs have been measured in roach and perch in July after spawning compared with earlier in the year (Noel *et al.* 2013). Considerable seasonal variations in contaminants have also been reported in bream (Farkas *et al.* 2003). JAMP guidelines incorporate seasonality elements.

Appendix 4: HELCOM's CORESET process

The EQS for mercury has been adopted as GES for mercury at HELCOM HOD 48-2015 Refer to outcome of HOD 48. At HELCOM HOD 48 the GES boundary was agreed for metals as follows:

GES-boundary

Cadmium: EQS water (AA) 0.2 µg/l
Mercury: EQS biota secondary poisoning 20 µg/kg wet weight
Lead: EQS water (AA) 1.3 µg/l

Further it was agreed to have secondary boundaries, as data is available in some areas only for other matrices as follows:

Cd: QS sediment 2.3 mg/kg dry weight OR biota BAC blue mussel 960 µg/kg dm
Pb: QS sediment 120 mg/kg dry weight OR biota BAC mussel 1300 µg/kg dry weight, BAC fish 26 µg/kg wet weight (liver)

The core indicator report for the metal indicators has been agreed to be published on the HELCOM website but it is not yet available there. Extract from the report on GES:

Good Environmental Status: HELCOM

The concentrations of metals are used to evaluate whether an area reflects a good environmental status (GES) compared to the specified concentration levels. The GES-boundaries are Environmental Quality Standards (EQS) for water and biota (Table A3.1).EQS are derived at EU level as a substance included on the priority list under the Water Framework Directive (2000/60/EC, amended by directive (2013/39/EU).

The GES-boundary is applicable if the concentrations are measured in the appropriate matrix. For historical reasons, the Contracting Parties around the Baltic Sea have differing monitoring strategies. As a pragmatic approach, a GES-boundary is defined in this indicator however if suitable monitoring data is not available in a region the secondary GES-boundary can be used for the evaluation for alternative matrices (Table A3.1). Under the WFD Member States may establish other values than EQS for alternative matrixes if specific criteria are met (see Art 3.3. in 2008/105EG revised though 2013/39/EU).

Table A4.1. HELCOM GES-boundary for metals

Metal	GES-boundary			secondary GES-boundary
	ref	matrix	concentration	
Cadmium and its compounds	**EQS** water	**water**	**AA 0.2 µg/l**	$QS_{sediment}$[4], 2.3 mg/kg dw BAC blue mussel 960 µg/kg dw
Mercury and its compounds	**EQS_{biota}** secondary poisoning	**fish**	**20 µg/kg ww** *(CPs' national legislation differ regarding the consideration of background concentrations)*	
Lead and its compounds	**EQS** water	**water**	**AA 1.3 µg/l**	**$QS_{sediment}$ 120 mg/kg dw** BAC blue mussel 1300 µg/kg dw, BAC fish 26 µg/kg ww (liver)

The EU food safety limits are meant for fish meat (i.e. muscle samples). The liver concentrations are generally higher than muscle (except for mercury), so the higher values of food safety limits for bivalves are used instead for Pb and Cd. This follows the OSPAR (2010) approach (see Law et al. 2010 for discussion). If the indicator is used to evaluate the protection goal of human health, then the boundary values presented in Table A4.2 can be applied.

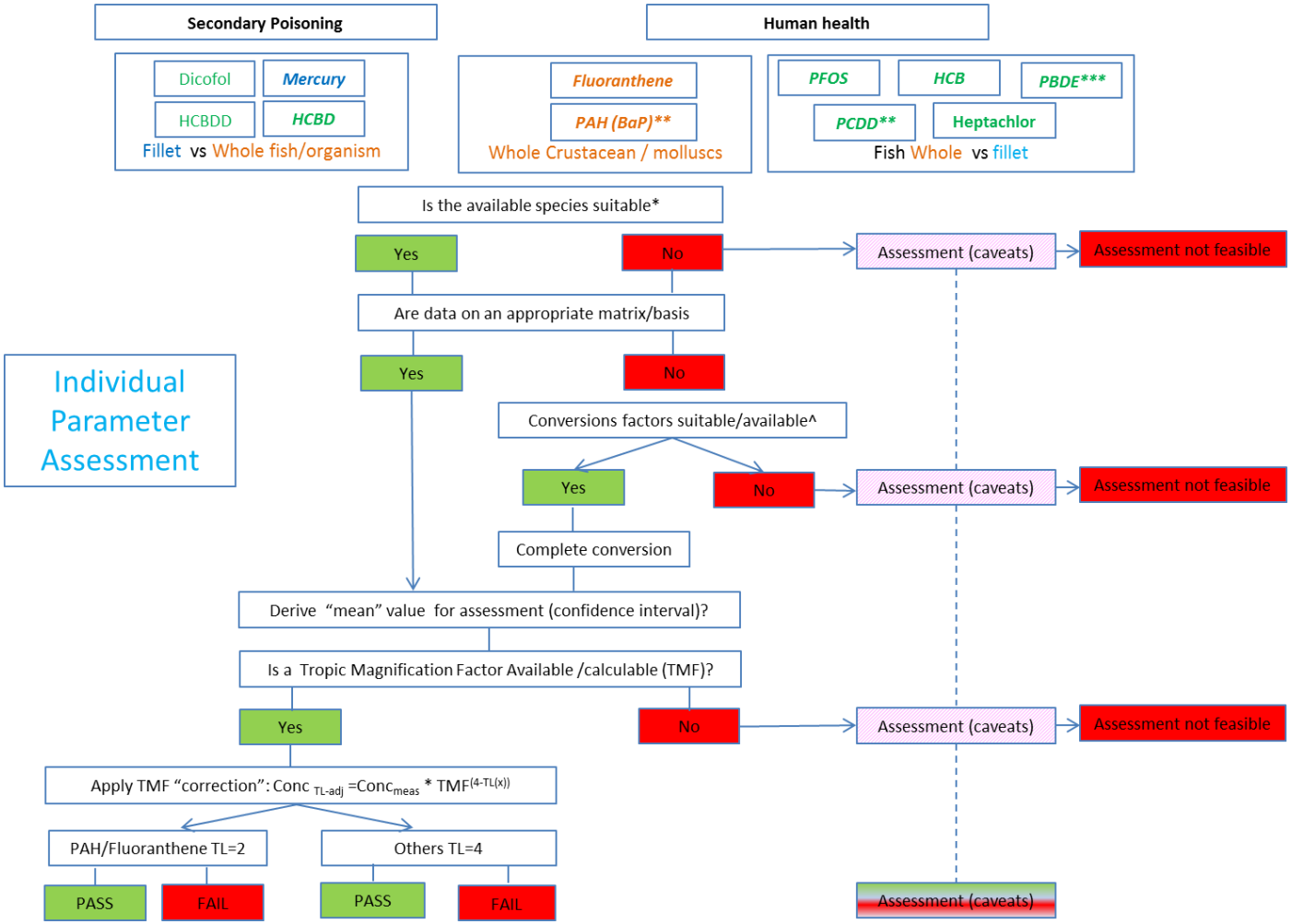
Table A4.2. Boundary value concentrations that can be applied to evaluate human health

Cadmium and its compounds	EU foodstuff Dir. (EU/1881/2006) mussel 1000 µg/kg dw, fish muscle 50 µg/kg ww (*fish liver 1000 µg/kg ww bivalve value, see Law et al. 2010 for discussion*)
Lead and its compounds	EU foodstuff Dir. (EU/1881/2006) mussel 1500 µg/kg dw, fish muscle 300 µg/kg ww, fish liver 1500 µg/kg ww
Mercury and its compounds	EU foodstuff Dir. (EU/1881/2006) fish muscle 500 µg/kg ww *(mussel 2500 µg/kg dw)*

The EU directive on environmental quality standards (2008/105/EC), Article 3, states that also long-term temporal trends should be assessed for substances that accumulate in sediment and/or biota.

[4] Applies to freshwater sediment (standard for marine sediment is currently not available). Sweden however considers this standard to be applicable also for assessment of the marine environment

Appendix 5: Framework for assessing suitability of OSPAR datasets for the purpose of compliance testing against EQS_{biota}. (MIME 2015 0404a1)



^Conversion dry or lipid weight and e.g. liver to whole fish, *Migratory or otherwise unsuitable? ** Sum PAHs ***Sum PBDEs PFOS and HCB underestimated by fillet

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ISBN: 978-1-911458-09-8
Publication Number: 679/2016