Abstract

The fate and transportation of mercury in the marine environment are driven by combinations of anthropogenic atmospheric and aquatic sources, as well as natural geological inputs. Mercury bioaccumulates and biomagnifies up the food chain and can result in the accumulation of toxic concentrations in organisms even when the concentrations in the marine environment remain below the threshold level for direct toxicity. As a result, mercury exposure has been recognised as a health concern for both humans and top marine predators, including cetaceans. There appears to be no overall trend in the global measured concentrations reported in cetaceans between 1975 - 2010, although differences between areas show that the highest concentrations in recent decades have been measured in the tissues of Mediterranean odontocetes. There is increasing concern for the impacts of mercury in the Arctic marine ecosystem with changes in water temperatures, ocean currents and prey availability all predicted to impact the exposure of Arctic species to mercury. The accumulation of mercury in various tissues has been linked to kidney and liver damage as well as other neurotoxic, genotoxic and immunotoxic effects. These effects have been documented through studies on stranded and by-caught individuals as well as in vitro cell culture experiments. Demethylation of methylmercury and protection by selenium have been suggested as possible mercury detoxification mechanisms in cetaceans that can help to explain the very high concentrations measured in tissues of some species with no apparent acute toxicity. Thus, the ratio of selenium to mercury is of importance when aiming to determine the potential toxicity of the contaminant load at an individual level. The long-term population level effects of mercury exposure are unknown, and continued monitoring of odontocete populations in particular is advised in order to predict the uptake and impact of mercury on marine food chains in the future.
Key Words
detoxification, health, marine mammals, methylmercury, toxicity

1. Introduction

Mercury (Hg) in the marine environment originates from both natural and anthropogenic inputs (Gworek, et al. 2016). Natural inputs include volcanic activity, and the weathering of rocks and soils enriched with mercuric minerals (Gworek, et al. 2016), while anthropogenic inputs historically included a range of industrial processes including the manufacture of paints, pesticides and catalysts (Horowitz, et al. 2014). Currently, approximately a quarter of the environmental mercury inputs are through mercury vapour as a by-product of coal-fired power stations (Chen and Driscoll 2018; Obrist, et al. 2018), and small-scale gold-mining operations which still use mercury to separate the pure metal from silt (Chen and Driscoll 2018; Obrist, et al. 2018). Since the 1950s, mercury has been recognised as a health concern for exposed humans (Ha, et al. 2017) and marine biota (Dietz, et al. 2013), and a recent review concluded that radical emission reductions need to be put in place on a global scale in order to significantly reduce exposure in both humans and wildlife (Sonke, et al. 2013). In August 2017, the “Minamata Convention on Mercury” was ratified by 91 countries with the aim of reducing global emissions and thus protecting human health and the environment. Continued monitoring of mercury in the marine environment is therefore required to determine whether new measures agreed by this treaty do, in fact, reduce the uptake and impact of mercury on marine food chains in the future.

In this review, the transport and fate of mercury in the marine environment is discussed, with emphasis on its bio-magnification, the process by which mercury is transferred and accumulated up the food chain at higher concentrations, in cetaceans as marine top predators. Cetaceans have a limited ability to eliminate mercury, and therefore sequester it in their tissues
As a result, mercury concentrations in cetaceans may be between 10 and 100 times higher than those measured in other predators at the same trophic level that have a similar average life span and dietary intake, like tuna species for example (Nigro and Leonzio 1993). Cetaceans are therefore considered as sensitive and reliable tracers of environmental mercury contamination (Capelli et al. 2000). Here, the current understanding of the exposure to, and impacts of high mercury concentrations on cetacean health in terms of the effects on kidney and liver function, immunocompetence, and the nervous system in particular is reported. Key priorities for further research are identified, particularly within the framework of understanding how mercury exposure may play a role in contributing to the other cumulative anthropogenic stressors that can have population level impacts for cetaceans.

2. The Mercury Cycle: Transportation and Fate

Mercury exists in three forms: elemental (metallic), inorganic (e.g. mercury salts like mercuric sulphate (HgSO₄) and mercuric chloride (HgCl₂)), and organic (e.g. methylmercury (MeHg / CH₃Hg⁺)) (Bjørklund, et al. 2017). Most of the mercury that enters the ocean directly from either land-based sources or the atmosphere (Fig. 1), is mercury in its elemental form. Elemental mercury can then be adsorbed onto sediment particles where, both as a result of chemical reactions and biological factors (such as the activity of sediment-bound, sulphate-reducing bacteria), the organic forms of mercury, namely methylmercury and dimethylmercury are produced (King, et al. 2001; Mazrui, et al. 2016; Chouvelon, et al. 2018) (Fig. 1). Organic mercury is also produced from inorganic mercury within the water column itself (Cossa, et al. 2009; Sunderland, et al. 2009) (Fig. 1). The methylation of mercury resulting from these abiotic and biotic processes is affected by a range of factors including pH, temperature, solar radiation,
organic matter remineralisation, and the availability of sulphates and organic carbon (Lee and Fisher 2017). Overall, through a combination of these processes, methylmercury is the most common form of organic mercury in the marine environment and bio-magnifies most readily up the food chain (see below). The transportation and fate of marine mercury is therefore complex because of the size and open nature of the ecosystem, the multiple import and export pathways, the transformations between its elemental, inorganic and organic forms, and the diversity of marine habitats (Braune, et al. 2015).

3. Bio-magnification: Mercury in Marine Food Webs

Marine wildlife species are exposed to mercury largely through their diet because low concentrations in air and water lead to minimal transfer through dermal exposure and inhalation (Rodgers 1994; Hall, et al. 1997; Duffy, et al. 2001). Bacteria and phytoplankton are the main entry points for the uptake of mercury into marine food webs (Atwell, et al. 1998; Campbell, et al. 2005). Mercury is then bio-magnified up the higher trophic levels to marine top predators including marine mammals and seabirds (Fig. 2). The rate of bio-magnification of mercury through the food chain has been estimated to be about 6.0 ± 3.7 times for each trophic level in polar marine food webs (Lavoie, et al. 2013), and 5.4 for each trophic level in tropical marine food webs (Kehrig, et al. 2013). In top predators, mercury concentrations are often higher in older and larger individuals because they consume larger prey that are, themselves, at a higher trophic position (Kehrig, et al. 2017).

Methylmercury, and other organic mercury compounds, are highly lipophilic and are therefore transferred up the food chain into fish and other vertebrates more readily than other forms of mercury because they are more efficiently assimilated into tissues following absorption. Methylmercury is also relatively slowly eliminated from the body with a half-life of between 10 and 15 days from different organs (Evans, et al. 2016). Once methylmercury is absorbed, it
enters the blood stream and is distributed quickly to various tissues and organs as it binds to
cysteine in fluids mimicking methionine, which makes it easily transported across cell
membranes by amino acid transporters (Clarkson, 1993). First, it is distributed to the liver,
kidney and spleen, and is then later to muscle and the brain (Oliveira Ribeiro, et al. 1999). In
contrast, inorganic mercury (e.g. mercuric chloride) is poorly absorbed in the vertebrate
gastrointestinal tract and is mostly excreted fairly rapidly in urine and faeces following
ingestion (Clarkson 1997). In addition, inorganic mercury does not bind as efficiently with
cysteine and therefore does not travel around the body as efficiently as organic forms of
mercury (Clarkson, 1993). However, methylmercury is slowly metabolised to inorganic
mercury, and has also been shown to accumulate in various tissues and organs (Bridges and
Zalups, 2010).

4. Exposure in Cetaceans: Measured Concentrations and Temporal Trends

4.1 Target Organs and Tissues

The main target organ for mercury in cetaceans is the liver. Other tissues such as brain, kidney,
blubber, muscle, blood, skin, teeth and even cetacean earplugs also contain measurable
coeeruleoalba*) and bottlenose dolphins (*Tursiops truncatus*) that stranded along the Italian coast
were highest in the liver compared to the heart, kidney, muscle and lung of both species (8 -
1,752 µg/g dry weight for striped and 10 - 1,404 µg/g dry weight for bottlenose dolphins)
(Bellante, et al. 2012). A similar result was reported in the tissues of bottlenose, striped and
Risso’s (*Grampus griseus*) dolphins from the Croatian waters of the Adriatic Sea where mean
concentrations in the liver were 11 - 34 times higher than in the other tissues (Bilandžić, et al.
2012). Highest total mercury concentrations were also measured in the liver of a range of
cetacean species sampled from Japanese meat markets compared to raw blubber, muscle, intestine and tongue (Simmonds, et al. 2002).

Monitoring mercury exposure in these target organs obviously requires lethal sampling, so there are increasing efforts to determine how these concentrations are related to concentrations in tissues accessible for sampling from live animals; skin and blubber (Reif, et al. 2017). Mercury concentrations were measured in the skin, blubber, liver and kidneys of four species of stranded and/or bycaught small cetaceans (common dolphin (*Delphinus delphis*), harbour porpoise (*Phocoena phocoena*), bottlenose dolphin and striped dolphin), and significant correlations were shown between all tissue types (Aubail, et al. 2013). In 2014, Monk and colleagues reported concentrations of mercury in the blubber of both live and dead-stranded individuals of a newly identified species of bottlenose dolphin (*Tursiops australis*), and the relatively high levels were attributed to chronic low dose exposure. These studies therefore demonstrate the potential use of blubber and skin from biopsy samples to make inferences about mercury exposure in live cetaceans.

### 4.2 Highest Measured Concentrations

A number of studies have reported the concentrations of total mercury (organic and inorganic) in the tissues of a wide variety of stranded cetacean species since the 1990s (for review see Marsili, et al. 2017). In terms of regional exposure, the highest concentrations in recent decades have been reported in the liver of striped dolphins from the Mediterranean Sea with a maximum of 5,374 µg/g dry weight in one individual (mean 514 µg/g d.w., n = 50, (Wafo, et al. 2014)). Individuals that stranded on the French coasts showed significantly higher levels compared to those from the other Mediterranean areas, and overall, individuals from the eastern Mediterranean basin showed the lowest concentrations. It has been hypothesised that the high mercury levels measured in Mediterranean dolphins originate from natural sources because of
the weathering of cinnabar ores throughout the Mediterranean Basin (André, et al. 1991), but other studies have suggested that the high concentration are as a result of industrial pollution (Bellante, et al. 2012). Liver and kidney concentrations of mercury were higher in 1990 - 1993 than in 2007 - 2009 in Mediterranean striped dolphins, which suggests that measures to reduce emissions specifically in western European countries have been somewhat effective in reducing mercury pollution in open waters (Borrell, et al. 2014).

Two short-finned pilot whales (Globicephala macrorhynchus) stranded on the coast of New Caledonia in the South Pacific also showed extremely high concentrations of total mercury up to 1,452 µg/g dry weight in the liver (Bustamante, et al. 2003). Concentrations were up to 1,980 µg/g wet weight in the livers of small odontocetes sold for human consumption in Japan (Endo, et al. 2002), and high concentrations (max. 1,571 µg/g wet weight) were also found in the liver of an adult female false killer whale (Pseudorca crassidens) from the Hawaiian Islands region (Hansen, et al. 2016). High concentrations were measured in the livers of a small number of bottlenose dolphins stranded in the Canary Islands between 1997 and 2013 (max. 700 µg/g dry weight), and unlike in the western Mediterranean, displayed an increasing temporal trend over the sampling period (Garcia-Alvarez, et al. 2015).

A review of published concentration data from the literature was conducted by searching ScienceDirect, Google Scholar, and additional references from relevant articles. The mean total mercury concentrations measured in cetacean livers reported in 101 technical reports and peer-reviewed articles published between 1972 and 2016 were collated (for the full list of references see the Supplementary Material Reference List). This produced a total of 284 liver total mercury measurements in 43 different cetacean species. Values are reported here as µg/g dry weight either as reported in the original study, or converted to dry weight using the correction factor (w.w. / d.w.) of 0.25 (Becker, et al. 1995). Across these studies, the means were calculated based on varying sample sizes ranging from multiple samples from just
one individual, up 129 individuals sampled in a single study. A number of studies separated samples based on age and sex class, while others also separated samples into discrete time periods. The separation of samples, and thus the reporting of mean total mercury concentrations was not consistent over the 101 studies. For this reason, these data were grouped here broadly by region and species group, and the mid-point of the data collection period used as a “time-stamp”.

Where there were more than 10 mean concentration measurements reported for a particular region (eg. of the 284 reported concentrations use here, there were 35 from the North Sea, while there were only 7 from the Baltic Sea), these were plotted by species group and over time (Fig. 3). Overall, between 1975 and 2010, the highest concentrations have been measured in the Mediterranean, and the lowest in the Arctic (Fig. 3). The delphinids dominated the datasets from the majority of these eight regions, and there is no apparent change in reported concentrations over time across these regions (Fig. 3). Overall, the highest mercury concentrations have been reported particularly in odontocetes, and these may result in adverse health effects (see below).

4.3 Arctic Species

There is increasing concern for the impact of mercury in the Arctic marine ecosystem (Braune, et al. 2015) and its top predators, including cetaceans (Dietz, et al. 2013). Specifically, mercury concentrations have been measured in the livers of belugas (Delphinapterus leucas) (5 - 53 μg/g wet weight) (Lockhart, et al. 2005), narwhals (Monodon monoceros) (7 to 17 μg/g wet weight) (Braune, et al. 2015), walruses (Odobenus rosmarus) (<3 μg/g wet weight) (Braune, et al. 2015)) and polar bears (Ursus maritimus) (~ 5 – 60 μg/g wet weight) (Routti, et al. 2011)). Liver concentrations have also been reported in Arctic phocids: ringed (Phoca hispida), bearded (Erignathus barbatus) and harbour (Phoca vitulina) seals (mean wet weight
concentrations of 0.2 μg/g, 0.1 μg/g and 2.2 μg/g for each species respectively) (Young, et al. 2010). A significantly higher mean concentration of total mercury in western, compared to eastern Arctic marine mammals was first reported in 1995 by Wagemann and colleagues, and was attributed, partly, to geological differences in the sediments between the two regions (Westgate and Johnson 1995). Of particular concern is that even though direct anthropogenic inputs into the ecosystem are thought to be minimal, longitudinal studies monitoring mercury in the Arctic have shown that there has been an increase in some marine biota (Braune, et al. 2015).

The most extensively studied Arctic cetacean is the beluga. Teeth were collected from various regions of the Canadian Arctic to investigate temporal trends from the pre-industrial period in the 15th and 17th century up until 1993, and showed that much of the anthropogenic increase of mercury in Beaufort Sea belugas had already taken place by 1960 (Outridge, et al. 2009). In the central Canadian Arctic, between the late 1800s and the 1990s specifically, there was a 1.2 to 5.5 fold increase in total mercury measured in teeth, but teeth from the 1920s - 40s contained similar mercury concentrations to those from the 1890s, suggesting that modern increases occurred after the early decades of the 20th Century (Outridge, et al. 2005). Later, in the 1990s, liver mercury levels in Beaufort Sea belugas tripled in comparison with levels measured in the 1980s, and were the highest relative to other Canadian Arctic beluga populations (Lockhart, et al. 2005). By the early 2000s, although concentrations were still higher than in the 1980s, mercury levels dropped and were comparable to other Arctic populations (Lockhart, et al. 2005). Most recently, no changes in liver mercury concentrations were observed between 2002 and 2012 for young belugas in the Beaufort Sea, but a significant decrease was seen in adults (Loseto, et al. 2015). It was concluded that these most recent declines do not follow trends in mercury emissions, and are not easily explained by diet markers, thus highlighting the
complexity of the relationships between foraging, food web dynamics and mercury uptake in this species (Loseto, et al. 2015).

A number of studies have also investigated mercury concentrations in narwhals (Wagemann, et al. 1998; Wagemann and Kozlowska 2005; Braune, et al. 2015). The average concentration of methylmercury in narwhal skin is nearly identical to that measured in the skin of eastern Arctic belugas (~0.5 µg/g wet weight) (Wagemann, et al. 1998), and between 1978 and 2004, narwhal liver mercury concentrations appear to have remained stable off Baffin Island (Braune, et al. 2015). There are few data regarding the mercury concentrations in Arctic baleen whales, but muscle samples from minke whales (*Balaenoptera acutorostrata*) taken as part of whaling operations in the Barents Sea in 2011, showed that total mercury concentrations varied from 0.05 to 0.5 µg/g wet weight, all of which was methylmercury. Interestingly, mean concentrations were slightly lower than measured in animals sampled from the same area nine years earlier, in 2002 (Kleivane and Børsum 2003).

While, overall, mercury exposure in Arctic cetaceans is lower than in other areas, how exposure will be affected by climate change is uncertain. Unprecedented changes have taken place in the Arctic over the last few decades in terms ocean warming and the resulting loss of sea ice. These environmental changes modify the planktonic ecosystem which has knock-on effects from the lowest to highest trophic levels. Large-scale environmental change could therefore trigger ecological responses including shifts in the availability, abundance and types of prey species, which in turn, can influence mercury exposure in Arctic cetaceans. The combination of environmental changes and shifts in diet could therefore make Arctic species especially susceptible to the cumulative effects of mercury exposure together with the other increasing anthropogenic pressures in these particularly vulnerable environments including increased shipping traffic, increased industrial fishing activities and anthropogenic noise for example.
5. Toxicity in Cetaceans

5.1 Mercury Detoxification: Methylmercury Demethylation and Selenium Binding

Marine mammals are capable of detoxifying methylmercury through the demethylation of methylmercury in the liver (Caurant, et al. 1996; Wagemann, et al. 1998) and its subsequent binding to selenium to form insoluble and toxicologically inert mercuric selenide (HgSe) crystals. The toxicologically inert HgSe crystals then accumulate in the tissue. These crystals were first detected using a combination of electron microscopy and histology in cetacean liver samples (Martoja and Viale 1977; Martoja and Berry 1980; Nigro and Leonzio 1996). Later, Nakazawa and colleagues (2011) investigated the formation of HgSe in various other cetacean tissues and organs (kidney, lung, spleen, pancreas, muscle and brain) using micro-X-ray fluorescence imaging and micro-X-ray diffraction. The authors confirmed the presence of HgSe in all the tissues examined suggesting that selenium could be involved in the detoxification process of mercury in tissues other than just the liver. It is hypothesised that this capacity to demethylate and sequester mercury with selenium in a non-toxic form may give cetaceans a greater tolerance to dietary mercury exposure than terrestrial animals, and therefore reduces some of the direct toxic effects of mercury in different organs and on various physiological processes described in detail below (Fig. 4).

As individuals reach their adult size, they demethylate methylmercury from their diet more efficiently, and in the case of high mercury exposure, a close to 1:1 molar ratio of Hg:Se is maintained in adulthood (Sakamoto, et al. 2015; Hansen, et al. 2016). In fact, many studies have reported a significant correlation between selenium and mercury concentrations in both cetacean liver and kidney samples, with molar ratios of close to 1 (Bustamante, et al. 2003; Yang, et al. 2007; Capelli, et al. 2008; Cáceres-Saez, et al. 2013; Hansen, et al. 2016) or below 1 (Krone, et al. 1999). It is thought that an animal with a liver molar excess of selenium (Hg:Se
1) is likely to be at lower risk of direct mercury toxicity, whereas an animal with a molar excess of mercury (Hg:Se > 1) is at greater risk (Hansen, et al. 2016). The toxicological significance for individuals and populations from studies reporting levels of mercury without associated selenium levels are therefore hard to interpret. Future monitoring efforts should always report mercury and selenium ratios to better understand which populations, or specific groups within populations are potentially most at risk of direct mercury toxicity.

However, while selenium binding appears to act as a defensive mechanism against the direct toxic effects of mercury exposure, this binding process itself may cause other indirect physiological problems. As methylmercury has such a strong binding affinity for selenium, Spiller (2018) suggests that the previously suggested “protective effect” of selenium against mercury toxicity may in fact be backwards in that the effect of mercury is to produce a selenium deficient state. For example, as methylmercury sequesters selenium, it directly affects both the synthesis and activity of important selenium-dependent enzymes (selenoenzymes) (Ralston, et al. 2012). As a result, methylmercury is now recognised as a highly specific, irreversible inhibitor of selenoenzymes (Ralston, et al. 2012). Oxidative damage, particularly in the brain and neuroendocrine tissues, are prevented due to the activity of these selenoenzymes which inhibit many inflammatory mechanisms (Forceville 2006). Inhibition of their synthesis and their protective activities when selenium levels are depleted therefore appears to contribute to the neuro-toxic effects of methylmercury (Ralston and Raymond 2010).

A recent study investigating the formation of HgSe clusters in the brain and the liver of long-finned pilot whales supports this theory as it provided evidence of the depletion of bioavailable selenium (Gajdosechova, et al. 2016). So, while cetaceans, and perhaps other top marine predators, have the capacity to demethylate mercury and then form toxicologically inert HgSe crystals, this protective effect is only maintained if equally high levels of selenium can also be maintained from the diet. It is therefore critical that an adequate selenium status can be
maintained in mammals exposed to high levels of mercury in order to mitigate its toxicity. This is a problem for cetaceans as top predators, as it has been shown that mercury bio-accumulates up the food chain at a higher rate than selenium (2.4 times for selenium and 5.4 times for mercury) (Kehrig, et al. 2013). A key research priority moving forward is thus a better understanding of these indirect effects of mercury toxicity caused by the generation of a potentially selenium-deficient state, and how they interact with the direct effects of mercury exposure itself. A better understanding of these two toxicity pathways is imperative for future risk assessments of mercury exposure.

5.2 Health Effects

An understanding of the links between contaminant concentrations, including mercury, and health effects largely comes from studies on laboratory animals where the underlying cellular mechanisms that cause harm can be assessed in experimental set-ups in which mercury exposure to individuals or cell lines can be controlled. The current understanding of these processes from various laboratory studies on model species and in humans is summarised below for context. To date, the only experimental studies on marine mammals, with regards to the effects of ingesting trace metal contaminated food items, were conducted on harp seals (Phoca groenlandica) in the 1970s (Freeman, et al. 1975; Ronald, et al. 1977). In these studies, seals were given a dietary intake of mercury of between 0.25 and 25.0 mg/kg body weight per day for 60 and 90 days. They showed a reduction in appetite and mass loss (Ronald, et al. 1977), auditory damage and altered steroid metabolism (Ramprashad and Ronald 1977).

In cetaceans, most work to date has focused on reporting measured concentrations of mercury in different tissues, rather than cause and effect relationships associated with different health effects. However, there is evidence from stranded and harvested animals that link tissue and organ mercury concentrations to specific pathologies (Fig. 4). The direct effects of mercury
toxicity on key organs and processes are discussed below, and while few data are available to assess toxicity thresholds for environmentally-exposed wildlife, published effect thresholds for a small number of cetacean studies are summarized in Table 1. Future risk assessments for the effects of mercury exposure on cetaceans need to consider both these direct effect thresholds and the indirect effects associated with the generation of a selenium deficient state discussed above.

5.2.1 Central Nervous System

In mammals generally, methylmercury toxicity is manifested primarily as central nervous system damage (Das, et al. 2003). Transport of methylmercury around the body is facilitated by complexes formed with cysteine groups which are able to cross the blood–brain barrier and may accumulate in brain tissue (Roos, et al. 2010). Thus, mercury in the brain is often predominantly (Basu, et al. 2009), but not exclusively methylmercury (Squadrone, et al. 2015). Typically, damage results in sensory and motor deficits and behavioural impairment as animals become anorexic and lethargic (Das, et al. 2003; Oken, et al. 2005). These deficits are caused as methylmercury has the potential to block neurotransmitter release, interfere with the transport of amino acids and ions, bind to sulphydryl groups and inhibit protein synthesis. Together, these effects result in the neuropathological damage including focal necrosis of neurons in regions of the cerebral cortex, which, overall, results in cerebral oedema (Nagashima 1997; Castoldi, et al. 2001). In fact, methylmercury exposure has been shown to result in the widespread loss of neurons and gliosis, with the hypertrophy of a number of different glial cells including astrocytes, microglia, and oligodendrocytes in the human and rodent cerebellum and midbrain, as well as the cerebral cortex (Mottet, et al. 1997). Of particular concern is that methylmercury is transferred across the placenta (Wagemann, et al.
and concentrates in the fetal brain (Wolfe, et al. 1998) resulting in developmental alterations in the fetus and/or fetal death.

New evidence suggests that despite previous assumptions regarding its poor ability to cross biological barriers, inorganic mercury, as well as methylmercury, can cross the blood-brain barrier (Evans, et al. 2016) and result in neurotoxic effects in mammals. In rats, it was observed that chronic, low-dose exposure to inorganic mercury resulted in a reduction in both balance and fine motor coordination (Teixeira, et al. 2018). In the same study, at the cellular level, it also resulted in the formation of mercury deposits and oxidative stress through a decrease in the total antioxidant capacity. It was concluded that exposure to continued, low-doses of inorganic mercury caused cell death through a combination of cytotoxicity and induction of apoptosis which resulted in a decreased number of neurons and astrocytes in the motor cortex (Teixeira, et al. 2018). This has potential implications for other mammals too, although the extent to which inorganic mercury can cause brain damage in other species requires further investigation.

In cetaceans, odontocetes appear to be one of the most vulnerable groups, with high concentrations of mercury recorded in brain tissue with associated signs of neurochemical effects (Dietz, et al. 2013). In fact, belugas exhibit brain concentrations of total mercury that are an order of magnitude higher than those measured in polar bears and Arctic seals (Lemes et al. 2011). Threshold concentrations for total mercury for neurotoxic endpoints detected in laboratory animals and field observations established from the literature were collated by Krey and colleagues (2015) (Table 1), and were compared to measured concentrations in the brains of belugas. It was seen that they exceeded all four of these neurotoxicity thresholds (Krey, et al. 2015). Another study on belugas explored the relationships between mercury and selenium concentrations and neurochemical biomarkers in different brain regions (Ostertag, et al. 2014). It was found that methylmercury exposure is associated with neurochemical variation in the
cerebellum of belugas and that selenium may partially protect it from methylmercury associated neurotoxicity (Ostertag, et al. 2014). Interestingly, while high concentrations of total mercury were measured in both the liver and the lymph nodes of 50 Atlantic bottlenose dolphins, no significant neuropathology was documented in these cases (Turnbull, et al. 1998). The authors therefore hypothesised that the dolphins have unique mechanisms for tolerating persistently high mercury concentrations that are neurotoxic in other mammals (Turnbull, et al. 1998). This, together with other evidence suggests that the high Se:Hg molar ratio in the brain of these species could, at least to some extent, protect the animals from mercury-associated neurotoxicity (Krey, et al. 2015).

5.2.2 Liver

Unlike terrestrial animals, in marine mammals and seabirds, the main organ where mercury accumulates at the highest concentrations, as well as being demethylated, is the liver. Studies on humans have shown that at the cellular level, methylmercury-related toxic effects are thought to be caused by binding of methylmercury to the cysteiny1 groups of proteins, which can have severe implications for the synthesis of cellular glutathione, and lead to oxidative damage (Choi, et al. 2017). Oxidative stress has thus been identified as an important reason for hepatotoxicity. The mechanisms of its toxicity have been suggested to also involve degeneration, and changes in the energy metabolism of renal cells, but these mechanisms are not fully understood (Choi, et al. 2017). Overall, hepatotoxicity occurs through cell death, mitochondrial dysfunction, endocrine disruption, and metabolic disorders though combinations of the deregulation of oxidative stress, intrinsic apoptotic pathways, and nuclear receptor and kinase activity (Choi, et al. 2017).

few studies have determined threshold concentrations for health effects in marine mammals, although it was first reported that concentrations around 60 μg/g total mercury (wet weight) in
the liver of marine mammals were damaging to hepatic processes (Law, et al. 1991). In another study using HgSe concentration data collected from the livers and respiratory systems of 25 stranded bottlenose dolphins, it was calculated that the minimum body burden to produce mild lesions, specifically mild fatty liver, was 600mg for a 300kg dolphin (Rawson, et al. 1995). This is approximately 7 times the threshold required to cause mild lesions in humans. Chronic mercury accumulation has been associated with liver abnormalities in bottlenose dolphins (Rawson, et al. 1993; Rawson, et al. 1995). For example, a fourfold increase in active liver disease in the dolphins suggested a significant health effect associated with liver mercury concentrations above 61µg/g wet weight of tissue (Rawson, et al. 1993). In this study, deposits of a brown pigment, identified as lipofuscin, were observed in the livers of nine animals with high hepatic mercury levels (>60 µg/g wet weight).

Lipofuscin is derived from damaged subcellular membranes, and these deposits were strongly correlated with mercury concentrations. As mercury inhibits the activity of lysosomal digestive enzymes, this reduces the degradation of proteins, which in turn, leads to excessive accumulation of lipofuscin within cells and results in cell death (Rawson, et al. 1995). Interestingly, while liver and kidney damage have been documented in bottlenose dolphins, lesions characteristic of acute or chronic mercury exposure were not found in harbour porpoises from the North and Baltic Seas with high mercury concentrations in the liver and kidney (upper range 449 µg/g and 160 µg/g wet weight, respectively) (Siebert, et al. 1999). The threshold for, and effects of hepatotoxicity may therefore be somewhat species specific, and/or a function of mercury-selenium interactions which have not been reported in these studies.

5.2.3 Kidneys
In humans and other terrestrial mammals, the kidneys are the primary organs where mercuric ions accumulate after exposure to elemental, organic and inorganic forms of mercury (for
review see Zalups, 2000). While all forms of mercury are nephrotoxic, the inorganic forms of mercury are most acutely nephrotoxic (Zalups 2000). Specifically, mercuric chloride leads to acute tubular necrosis where the tubular epithelial cells that form the renal tubules of the kidneys die (Zawada, et al. 1998). As a result of the high bonding affinity between mercury and sulphur, interactions between mercuric ions and the thiol group(s) of proteins, peptides and amino acids including albumin, metallothionein, glutathione, and cysteine have been implicated in the mechanisms involved in the proximal tubular uptake, accumulation, transport, and toxicity of mercuric ions in the kidneys of mammals (Zalups 2000).

In cetaceans, an increase in blood urea nitrogen was observed in bottlenose dolphins with increased mercury concentrations in both the skin and the blood suggesting a decrease in kidney function in these animals (Schaefer, et al. 2011). Varying doses of mercuric chloride were shown to induce apoptosis in vitro in cultured Atlantic Spotted Dolphin (Stenella plagiodon) renal cells (Wang, et al. 2001) (Table 1). In the same study, the protective effects of sodium selenite against the toxic effects of mercuric chloride were documented, and it was concluded that inhibition of mercury-induced apoptosis in renal cells, provided by selenium, may contribute to the in vivo protection in this organ.

5.2.4 Immune System Function

A large body of literature regarding in vitro experimental investigations has clearly shown that mercury compounds can have immunomodulatory effects (Moszczyński 1997). Specifically, both mercuric chloride and methylmercury have been shown to inhibit most lymphocyte functions including proliferation, expression of cell activation markers on the cell surface and cytokine production (Moszczyński 1997). In vivo studies on rats injected with mercuric chloride exhibit immunosuppression, and showed increased susceptibility to challenge with infectious agents or tumour cells (Moszczyński 1997). In marine mammals specifically,
methylmercury was shown to alter the in vitro synthesis of steroid hormones which play an important role in modulating both inflammatory and immune responses (Freeman and Sangalang 1977). These kinds of in vivo and in vitro studies have not been carried out to the same extent in cetaceans, but potentially similar immunosuppressive effects have been documented in various species using strandings data in case-controlled approaches to investigate the prevalence of infectious diseases in mercury-contaminated animals.

Siebert and colleagues (1999) examined the possible relationship between mercury tissue concentrations and disease in harbour porpoises from the North and Baltic seas. Higher mercury concentrations were found in porpoises from the North Sea compared to the Baltic Sea and were associated with an increased prevalence of parasitic infection and pneumonia.

Bennett and colleagues (2001) also used this indirect approach to investigate the hypothesis that increased exposure to toxic metals results in a lower resistance to infectious disease in harbour porpoises from the coasts of England and Wales. Mean liver concentrations of mercury, selenium, zinc and the Hg:Se ratio were significantly higher in the porpoises that died of infectious diseases compared to porpoises that died from physical trauma. As previously discussed, the authors concluded that the Hg:Se balance is a complex phenomenon that might be more important for the general health status of porpoises than absolute concentrations of mercury alone. Similarly, Mahfouz and colleagues (2014) also found that harbour porpoises stranded along the French coast between 2006 and 2013 that died from infectious disease had significantly higher hepatic concentrations of cadmium, mercury, selenium and zinc compared to healthy porpoises that died from physical trauma.

In order to better understand the mechanisms of immunosuppression associated with high mercury concentrations, Pellissó and colleagues (2008) studied the effects of varying mercury exposure on bottlenose dolphin lymphocyte and phagocyte function in vitro. A significant reduction in the lymphoproliferative response was found following exposure to just 1 mg/L of...
mercury and decreased phagocytosis was observed at 5 mg/L (Table 1). The authors concluded that their results support the hypothesis that exposure to mercury could lead to a reduction in host disease resistance. Desforges and colleagues (2016) used a combination of field and laboratory data to determine effect threshold levels for suppression of lymphocyte proliferation. These were between 0.002 - 1.3 ppm for mercury and 0.009 - 0.06 ppm for methylmercury in polar bears and several pinniped and cetacean species combined. Finally, in another study on bottlenose dolphins, after controlling for age, a significant inverse relationship was observed between mercury concentrations measured in the blood, and several markers of endocrine function and hematologic parameters (Schaefer, et al. 2011). Specifically, an inverse relationship was observed between blood and skin mercury concentrations and thyroid hormone concentrations (total thyroxine and triiodothyronine), as well as absolute numbers of lymphocytes, eosinophils, and platelets (Schaefer, et al. 2011). Mercury is not specifically recognized as an endocrine-disrupting chemical, but it has been suggested that continuous exposure of the brain to mercury could affect the hypothalamic–pituitary axis which regulates thyroid activity, and thus the circulating concentrations of total thyroxine and triiodothyronine (Sin, et al. 1990). Further investigation of the roles of both mercury and selenium in the mechanisms that lead to reduced thyroid hormone production in marine mammals is necessary to confirm these results, and better interpret the implications for the reduced immunocompetence of individuals.

### 5.2.5 Genetic Effects

Mercury exposure has been recognised to have both mutagenic and teratogenic effects (Aggarwal, et al. 2014). Once inside the cell, damage is thought to be caused by methylmercury compounds that bind to sulphydryl groups of glutathione, leading to the formation of free radicals that cause DNA damage. When these compounds bind to sulphydryl groups in the
microtubules responsible for providing structure and shape to the cytoplasm, it leads to impairment of spindle formation which then causes chromosomal aberrations (structural abnormality in one or more chromosomes) and aneuploidy (an abnormal number of chromosomes) (for review see Aggarwal et al. 2014). In humans, the genotoxic effects of methylmercury compounds have been assessed by quantifying chromosome aberrations and polyploidy cells in cultures of whole blood exposed to varying mercury concentrations. The number of polyploidy cells increased with the increasing mercury concentration while the mitotic index, and thus the cells’ ability to divide normally, decreased at just 100 µg/L (Silva-Pereira, et al. 2005). Mercury therefore has strong genotoxic and cytotoxic effects at low concentrations in humans.

There is currently very little data on the extent to which mercury or methylmercury is genotoxic in cetaceans. Betti and Nigro (1996) evaluated the genetic effects of methylmercury in bottlenose dolphin lymphocytes in vitro using single cell microgel electrophoresis (the Comet assay). Lymphocytes were isolated from the blood of a single dolphin, and were exposed to methylmercury concentrations naturally occurring in the blood of wild dolphins in the Mediterranean (Betti and Nigro 1996). This induced DNA single-strand breaks and cytotoxicity in a dose-dependent manner. However, dolphin lymphocytes were more resistant to the genotoxic and cytotoxic effects of methylmercury than either human or rat cells (Betti and Nigro 1996). This resistance was interpreted as an adaption to limit the damage caused by methylmercury exposure. Further in vitro testing is therefore required to fully assess the potential genotoxicity of methylmercury in cetaceans.
6. Conclusions and Future Directions

Both natural and anthropogenic sources of mercury contribute to its accumulation in the tissues and organs of cetacean species around the world. This accumulation, together with the potential for toxic effects highlight how further monitoring of mercury in the environment, and in these top marine predators, is required to better understand its potential health effects and how these could ultimately lead to population-level impacts. Recent evidence demonstrating the potential use of blubber and skin samples from biopsies of live animals to quantify tissue mercury concentrations has important implications for large-scale population monitoring.

Given the reported ‘protective’ effects of selenium binding, of particular importance in future monitoring efforts is the need to measure both selenium and mercury concentrations in tandem in order to obtain a more accurate indicator of what measured concentrations mean in terms of compromising cetacean health. There is still limited data regarding the mechanisms of toxicity specific to cetaceans, and while comparing mercury concentrations in cetaceans with concentrations in appropriate laboratory studies can be used as a tool for risk characterization, using published thresholds and established cellular mechanisms of mercury toxicity in other animals adds uncertainty regarding the assessment of risk to cetaceans. Future investigations should prioritise a better understanding of both the direct effects of mercury toxicity and the indirect effects associated with the development of selenium deficiency. An improved understanding is imperative in order to better evaluate risk to individual and population-level health. In addition, efforts have been focused on understanding mercury toxicity in isolation, whereas there are likely important health effects associated with exposure to several, possibly interacting contaminants (Filipak Neto, et al. 2008). More research is therefore needed before the effects of both mercury alone, and its cumulative health effects in combination with other
heavy metals and persistent organic pollutants for example, can be addressed adequately in top
marine predators.

A recent, highly comprehensive study investigated how the effects of climate change and other
potential anthropogenic stressors are likely to modulate the bioaccumulation and bio-
magnification of mercury in marine ecosystems in the future (Eagles-Smith, et al. 2018). In
terms of potentially the most vulnerable environments, further research is especially required
to identify the changing underlying processes linking the biogeochemical cycle responsible for
methylation rates in Arctic seawater and bioaccumulation through the Arctic food web which
will ultimately affect top marine predators (Braune, et al. 2015).

Marine mammal species, including cetaceans are thought to be good sentinels for human health
for two main reasons; firstly, because they consume many of the same species of fish caught
by commercial fisheries for human consumption, and secondly, they share similar life history
traits including a high trophic level, low reproductive output and a long life-span. Together,
these can make them particularly susceptible to the negative impacts of anthropogenic activities
and environmental pollution and contamination. With the signing of the Minimata Convention
on Mercury in 2017 there is a clearly an appetite for reducing the use and therefore the impact
of mercury contamination at a global scale. But without continued, long-term monitoring of
concentrations in species of concern, or those that are important ecosystem indicators (such as
the odontocete cetaceans), it will be impossible to determine if any mercury exposure
mitigation measures have been successful, and predict how mercury will affect the marine
environment into the future.

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Declarations of Interest

None.

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Table 1 – Reported thresholds for toxic effects of mercury exposure. N.B. Thresholds for neurotoxic endpoints have not been published specifically for cetaceans.

<table>
<thead>
<tr>
<th>Directly Toxic Effects</th>
<th>Species</th>
<th>Study System</th>
<th>End Point</th>
<th>Reported Exposure Threshold for Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurotoxicity: Central Nervous System</td>
<td>Mouse, rat, mink, river otter, cat, dog, horse, pig, macaque, squirrel monkey, harp seal, polar bear</td>
<td><em>in vivo</em></td>
<td>clinical changes neuropathological changes neurochemical changes neurobehavioural changes</td>
<td>Brain Concentration THg*: &gt; 6.75 mg/kg w.w. &gt; 4 mg/kg w.w. &gt; 0.4 mg/kg w.w. &gt; 0.1 mg/kg w.w.</td>
<td>Krey et al. 2015*</td>
</tr>
<tr>
<td>Hepatotoxicity: Liver</td>
<td>Bottlenose dolphin</td>
<td><em>in vivo</em></td>
<td>liver disease</td>
<td>Liver Concentration THg: 61μg/g wet weight</td>
<td>Rawson et al. 1993</td>
</tr>
<tr>
<td></td>
<td>Bottlenose dolphin</td>
<td><em>in vivo</em></td>
<td>liver disease</td>
<td>Whole Body Burden THg: 2mg/kg</td>
<td>Rawson et al. 1995</td>
</tr>
<tr>
<td>Nephrotoxicity: Kidneys</td>
<td>Atlantic spotted dolphin</td>
<td><em>in vitro</em></td>
<td>apoptosis of renal cells</td>
<td>20 μM HgCl₂</td>
<td>Wang et al. 2001</td>
</tr>
<tr>
<td>Immune System Function</td>
<td>Bottlenose dolphin</td>
<td><em>in vitro</em></td>
<td>suppression of lymphocyte proliferation suppression of phagocytosis</td>
<td>1mg/L Hg 5 mg/L Hg</td>
<td>Pellissó et al. 2008</td>
</tr>
<tr>
<td></td>
<td>Beluga</td>
<td><em>in vitro</em></td>
<td>suppression of lymphocyte proliferation</td>
<td>0.067 ± 0.094 ppm Hg 0.016 ± 0.0049 ppm MeHg</td>
<td>Desforges et al. 2016</td>
</tr>
<tr>
<td>Genetic Effects</td>
<td>Bottlenose dolphin</td>
<td><em>in vitro</em></td>
<td>DNA single-strand breaks and cytotoxicity</td>
<td>0-1 μg/ml MeHg</td>
<td>Betti and Nigro, 1996</td>
</tr>
</tbody>
</table>

* Authors conducted a literature review of threshold concentrations for toxic endpoints detected in laboratory animals and field observations in order to establish potential thresholds in marine mammals.
Fig. 1. Mercury in the marine environment is cycled through biogeochemical processes with both anthropogenic and geological (natural) inputs from land-based sources, and deposition from the atmosphere. Mercury enters sediments through the actions of sediment-fixing bacteria. Methylmercury is produced through a combination of methylation of elemental mercury and inorganic mercury in sediments and in the water column itself through both abiotic and biotic processes. Methylmercury then enters, and bioaccumulates up the food chain from zooplankton up to top marine predators, including cetaceans.

Fig. 2. Total mercury concentrations in example marine species in the Mediterranean show how bio-magnification occurs up the food chain. Total mercury values in µg/g dry weight are indicated based on published concentrations (Cresson, et al. 2014; Wafo, et al. 2014; Živkovic, et al. 2017). The total mercury concentrations here include both inorganic and organic mercury, and are shown as examples of the most widely available published data for comparison, rather than methylmercury alone.

Fig. 3. Mean total mercury concentrations measured in the livers of cetaceans worldwide between 1975 and 2010 collated from 101 reports and published peer-reviewed articles. N.B. The total mercury concentrations on the y-axes are on different scales as minimum and maximum reported values vary between regions.

Fig. 4. Mercury exposure in cetaceans has the potential to cause neurotoxicity, nephrotoxicity, hepatotoxicity, immunotoxicity and genotoxicity. The main toxicology findings from in vivo and in vitro investigations in cetaceans are summarised together with the proximate mechanisms described in model species where cetacean data are lacking.
Figure 1.

Figure 2.
Figure 3.

Central Nervous System 

Memory loss is manifested primarily as central nervous system damage. 

Mild depression of memory and 

loss of memory occurs with irreversible 

neuronal damage in the hippocampus. 

Serious and more severe with 

behavioral impairment. 

Some level of cognitive 

greathen through higher Hg 

serum ratios in the brain. 

Hypoxic Effects 

There is very little data on the 

overall effect of Hg on brain 

function in humans. 

Some evidence suggests 

Mg may cause DNA-altered 

development. 

Further testing is required.

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Figure 4.

Kidney: Nephrotoxicity 

While all forms of mercury are nephrotoxic, the inorganic forms of mercury are most toxic to the kidneys. 

High levels of Hg can lead to some level of protection. 

Nephron and system dysfunction (renal failure) 

Mg and Ca may inhibit 

transmembrane 

function, including expression of cell activation markers, and cytokine production. Mg is also involved in protection. 

High levels of exposure can lead to a reduction in most disease resistance. 

Mg is a key element in the 

management of diabetes and decrease inflammation. 

Inflammation and other processes require further investigation.

User: Hypersensitivity 

High levels of exposure are rare and in the few cases which do occur, susceptibility of Hg may be species-specific. 

Disease course, induction of hyperalgesia, sensitivity to antihypertensive membranes and 

destruction of connective tissue. 

Excessive accumulation of fat, tissue and muscle 

reaction to a laser treatment. 

Effects of exposure may be species specific.