Health impacts of organochlorines and trace elements in humpback and snubfin dolphins in the Port of Gladstone

Report to Gladstone Ports Corporation

March 2019

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Email: j.vandemerwe@griffith.edu.au</td><td>Document: A desktop study to advise on the research to estimate the health impacts of organochlorines and trace elements in dolphins in the Port of Gladstone</td></tr>
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<tr><td colspan="2">Synopsis: This report provides a review of organochlorine and trace element concentrations in humpback and snubfin dolphins in the Port of Gladstone. Adverse effects of contaminants in dolphin species are also discussed and recommendations for further toxicological assessments in the dolphin populations in the Port of Gladstone are provided.</td></tr>
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Executive summary

Gladstone Ports Corporation (GPC) Limited completed the study *Increase understanding of the status of the Australian snubfin and Australian humpback dolphins within Port Curtis and Port Alma* under the Ecosystem Research and Monitoring Program (ERMP) from 2014 to 2016. The study provided population estimates of the Australian humpback (*Sousa sahulensis*) and the Australian snubfin (*Orcaella heinsohni*) dolphins in the ERMP survey area and measured the presence of contaminants in dolphin tissue samples. Organic and inorganic contaminants were found in many of the tissue samples collected from these dolphins. However, due to the small sample sizes and limited knowledge of adverse effects in these species, the potential health impacts of contaminants on dolphin populations in this region could not be assessed. The aim of this desktop study was to review the accumulation and effects of organic contaminants and trace elements in dolphins, and provide recommendations on the research required to assess the potential health impacts of these contaminants in humpback and snubfin dolphins in the Port of Gladstone.

Dolphins are exposed to contaminants primarily through their food. Contaminants can bioaccumulate and biomagnify in dolphins to concentrations well above what is in their surrounding environment. A literature review revealed that humpback and snubfin dolphins in the Port of Gladstone have elevated levels of organochlorine contaminants compared to other species and populations around Australia. The humpback dolphins from Port Curtis had organochlorine concentrations comparable with humpback dolphins in other Indo-Pacific regions, while concentrations from humpback dolphins in Port Alma were elevated above concentrations found elsewhere. Trace element concentrations were also elevated in both species of dolphins compared to other species in Australia and other locations. Many of these concentrations were above those known to cause adverse effects on the immune system and reproduction in other species of marine mammals. There is, however, a considerable lack of knowledge on the effects of contaminants in dolphins, and in particular species-specific effects in humpback and snubfin dolphins.

Recommendations for further toxicological analysis to better understand the impacts these contaminants are having on dolphin health in the Port of Gladstone include:

1. Continued monitoring of contaminants in dolphin skin biopsies and carcasses, including consideration of major flooding or dredging activities - to identify changes in chemical exposure over time.
2. The application of ecological risk assessments - to provide important information on the likelihood of adverse effects occurring at current and future contaminant exposures.
3. Development and application of *in vitro* (cell-based) toxicity bioassays - to provide species-specific data on the effects of contaminants in dolphins and improve risk assessments.
4. Toxicokinetic-toxicodynamic modelling - to develop better understanding of exposure and effects at the organism and population levels.
5. Further development of biomarkers of chemical exposure and effect - to provide tools for early detection of chemical exposure.

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Glossary of abbreviations and acronyms

Abbreviation	Meaning
<DL	Below the detection limit
<LOQ	Below the limit of quantification
Ag	Silver
AhR	Aryl hydrocarbon receptor
Al	Aluminium
As	Arsenic
BPMO	Benzo(a)pyrene monooxygenase
Cd	Cadmium
Cr	Chromium
Cu	Copper
CYP1A1	Cytochrome P450 1A1
CYP2B	Cytochrome P450 2B
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
ER	Estrogen receptor
ERMP	Ecosystem Research and Monitoring Program
Fe	Iron
GPC	Gladstone Ports Corporation
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
Hg	Mercury
HSP70	Heat shock protein 70
lw	Lipid weight
MeHg	Methylmercury
Mn	Manganese
Mo	Molybdenum
NA	Not analysed
Ni	Nickel
OC	Organochlorine
PAH	Polycyclic aromatic hydrocarbon
PBDE	Polybrominated diphenyl ether
PCB	Polychlorinated biphenyl
PCDD/F	Polychlorobenzodioxin/furan
PFAS	Per and polyfluorinated alkyl substance
PFC	Perfluorinated compound
Se	Selenium
TBT	Tributyltin
ww	Wet weight
Zn	Zinc

Glossary of species names

Common name	**Species name**
Australian humpback dolphin	*Sousa sahulensis*
Australian snubfin dolphin	*Orcaella heinsohni*
Common bottlenose dolphin	*Tursiops truncatus*
Indo-Pacific bottlenose dolphin	*Tursiops aduncus*
Irrawaddy dolphin	*Orcaella brevirostris*
Indo-Pacific humpback dolphin	*Sousa chinensis*
Short-beaked common dolphin	*Delphinus delphis*
Pantropical spotted dolphin	*Stenella attenuata*
Harbour porpoise	*Phocoena phocoena*
Striped dolphin	*Stenella coeruleoalba*
Beluga	*Delphinapterus leucas*
Atlantic spotted dolphin	*Stenella frontalis* (formally: *Stenella plagiodon*)
Long-finned pilot whale	*Globicephala melas*
Killer whale	*Orcinus orca*

1 Introduction

The Port of Gladstone receives input from the three major catchments of the Fitzroy, Calliope and Boyne Rivers, which contain a diverse range of land uses. Grazing and irrigated cropland have expanded in this area, resulting in modification of land and river systems (GBRMPA 2013). There is also a long standing history of mining in the area, including legacy gold and copper mines, and ongoing substantial coal mining. Mining is supported by large scale refineries and smelting in the area and the Port of Gladstone functions as a major industrial port of Australia. Additionally, the town of Gladstone supports industry workers and has a steadily increasing population. These activities can all lead to increased quantities and types of contaminants entering the waterways of the port, via runoff and direct discharges.

Concerns regarding contaminant loads within the Port of Gladstone have instigated a small number of studies investigating water and sediment quality. Studies conducted prior to 2011 found few exceedances of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality trigger values for trace elements in Port Curtis (Jones et al. 2005, Angel et al. 2010), with the exception of tributyltin (TBT), which was identified as a potential concern (Jones et al. 2005). However, sediment concentrations of As, Cr, Ni and TBT were found to have several exceedances of sediment quality guideline trigger values (Jones et al. 2005). Further assessments began in September 2011 in response to weather-induced flooding and an increase in marine wildlife disease and mortality (Meager & Limpus 2012, Meager et al. 2012, Wesche et al. 2013, Flint et al. 2015, Dennis et al. 2016), which led to concerns about the quality of the waterways and potential influx of contaminants into the port (EHP 2013). Additionally, concerns were raised about dredging activities that had commenced in May 2011 as a potential source of contaminants to the aquatic biota in this area (EHP 2013). Subsequent assessment of sediment and water quality in the port found organic contaminants at low concentrations in the sediment, with no exceedances of Interim Sediment Quality Guidelines. Similarly, sediment trace element concentrations were almost all below guideline trigger values with the exception of As at a number of locations. However, a decrease in water quality was observed, with Australian and New Zealand Guidelines for Fresh and Marine Water Quality trigger values exceeded for dissolved Al, As, Cu, Mo, and Zn on one or more occasions (EHP 2013). Similar exceedances were identified by Arango et al. (2013) during February 2013 sampling.

While contaminant concentrations in water and sediment in the Port of Gladstone have, to date, been typically low, there can be significant enrichment in biota, as indicated by concentrations in plants and animals well above what is found in water and sediment. For example, many trace element concentrations in Port Curtis seagrass meadows were elevated compared to reference locations (Prange & Dennison 2000, Jones et al. 2005). Similarly, some trace element concentrations in whelks, oysters and crabs from Port Curtis were elevated relative to reference locations (Andersen & Norton 2001, Jones et al. 2005). Contaminants have also been found in green sea turtles from the mouth of the Boyne river, with concentrations of some organics and trace elements raising health concerns (Gaus et al. 2012). Contaminants can be taken up by organisms at low trophic levels and can be transferred to higher trophic levels through prey ingestion (Hickie et al. 2013, Gui et al. 2014b). Dolphins are top predators and accumulate contaminants through ingestion of prey species (Monteiro et al. 2016). Elevated levels of contaminants have been found in skin biopsies of dolphins within the region (Cagnazzi et al. 2013, Cagnazzi 2017), indicating contaminants are a significant hazard to dolphins. These contaminants can have harmful effects in wildlife, causing disruptions to the immune system (Ross et al. 1996), genotoxicity (Boon et al. 1998), and endocrine disruption (Tanabe 2002). However, there is still very little known about the species-specific effects of contaminants in dolphins. There is therefore a need to better understand these effects and to characterise the risk posed to dolphin species in the Port of Gladstone.

2 Background

2.1 Port of Gladstone

The Port of Gladstone extends from Port Alma (in the north) to just past Wild Cattle Island (in the south). A previous study conducted on dolphins within the region under the Ecosystem Research and Monitoring Program extended the research area past the limits of Port Alma and the southern limits of Port Curtis to include Rodd's Bay (Figure 1).

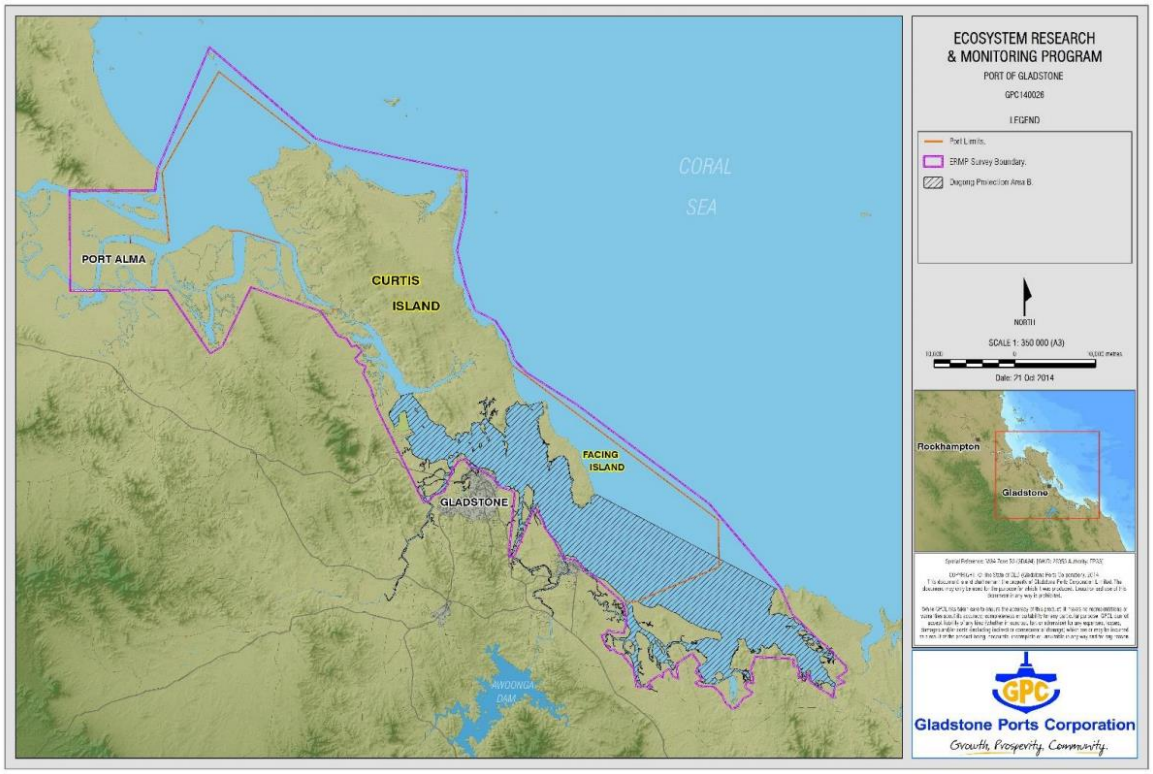

Figure 1. Map delineating the Port of Gladstone (yellow boundary line) and the Ecosystem Research and Monitoring Program area (pink boundary line).

2.2 Dolphins in the Port of Gladstone

The Port of Gladstone supports three species of dolphins, the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*), the Australian humpback dolphin (*Sousa sahulensis*), and the Australian snubfin dolphin (*Orcaella heinsohni*) (Cagnazzi 2017). This report will focus on the latter two species.

Australian humpback dolphins were described as a species in 2014, following a separation from the Indo-Pacific humpback dolphin, *Sousa chinensis* (Jefferson & Rosenbaum 2014), and distribution of the species is uncertain in some locations (Parra et al. 2017b). Humpback dolphins are distributed throughout the Port of Gladstone from Port Alma/Keppel Sands to Rodd's Bay (Cagnazzi 2017). Between 2014 and 2016, 181 individuals were identified, of which 128 were identified as female, 14 as male and 39 of unknown sex (Cagnazzi 2017). The majority of dolphins were sighted in Port Curtis (n=92), followed by Rodds Bay (n=53) and Port Alma/Keppel Sands (n=53) (Cagnazzi 2017). Sightings of individual humpback dolphins indicate significant movement between Port Curtis and Rodd's Bay and limited movement between Port Alma/Keppel Sands and Port Curtis/Rodds Bay (Cagnazzi 2017). The population was estimated to be 140-160 adult humpbacks plus ~36% of juveniles and calves (Cagnazzi 2017). These estimates are similar to what was found in Port Curtis between 2007 and 2010, and indicate a significant decline in the Port Alma/Keppel Sands population, compared to estimates between 2007 and 2010 (Cagnazzi 2017).

Australian snubfin dolphins were described as a new species in 2005, following a separation from the Irrawaddy dolphin, *Orcaella brevirostris* (Beasley et al. 2005). As a result, distribution and abundance of the species in some areas remains uncertain (Parra et al. 2017a). Within the Port of Gladstone, the known distribution of snubfin dolphins is limited to Port Alma (Cagnazzi 2017). Between 2014 and 2016, 127 individuals were identified, of which 41 were identified as females, 21 as males and 65 of unknown sex (Cagnazzi 2017). The population of snubfin dolphins was estimated to be 110-140 adults plus ~17% of juveniles and calves (Cagnazzi 2017). These estimates are similar to what was found in the Port Alma/Kessel Sands between 2007 and 2011 (Cagnazzi 2017).

The Australian humpback and snubfin dolphins are both listed as 'vulnerable' in Queensland under the *Nature Conservation Act (1992)*, are protected under the *Environment Protection and Biodiversity Conservation Act 1999* as 'cetaceans' and 'migratory species', and are listed as 'vulnerable' internationally by the International Union for Conservation of Nature, with declining population trends (Parra et al. 2017a, Parra et al. 2017b). Both species are listed under the Department of Environment and Science 'Back on Track' species prioritisation framework as a critical priority. As a result, a number of recovery actions have been set for these species, including sustainable fisheries management, reduction of human interactions with dolphins (e.g. fishing, boating), protection of habitat, improvement of water quality, and continued research and monitoring of the species (DERM 2010).

2.3 Overview of contaminants in dolphins within the Port of Gladstone

Between 2014 and 2015, biopsy samples from 17 Australian humpback and 18 snubfin dolphins from the Port of Gladstone were analysed for organochlorine contaminants (polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes (DDTs) and hexachlorobenzene (HCB)) (Cagnazzi 2017). In Port Alma, humpback dolphins had higher levels of these contaminants than snubfin dolphins, and humpback dolphins from Port Alma had higher levels of these contaminants than humpback dolphins from Port Curtis (Cagnazzi 2017). This indicates both species and location differences in the chemical contamination of Port of Gladstone dolphins. Comparisons with similar data collected in 2010/2011 (Cagnazzi et al. 2013) found a significant increase in ΣPCBs, ΣDDTs and HCB for both species over this 4-5 year period (Cagnazzi 2017). While Cagnazzi et al. (2013) did not identify the sex of the dolphins they sampled in 2010/11 (and hence the influence of biological factors cannot be completely disregarded), these increases in contaminant concentrations over time most likely indicate increased duration of chemical exposure in these dolphin populations, and/or increased contaminant levels in the Port of Gladstone over this period.

While concentrations found in both Port of Gladstone species were comparable to concentrations found in dolphins worldwide, levels sometimes exceeded those known to cause adverse effects. Concentrations of ΣPCBs from seven snubfin and nine humpback dolphins exceeded blubber threshold concentrations for adverse health effects in marine mammals of 17,000 ng/g lipid weight, based on PCB-induced immunological and reproductive effects (Kannan et al. 2000). PCB concentrations in many of the dolphins from the Port of Gladstone were also above threshold concentrations for altered lymphocyte proliferation (an indication of immunotoxicity), and within the range of modulation of respiratory burst in cetacean species (Desforges et al. 2016). Similarly, ΣDDT concentrations in 33 samples were within the range associated with altered immune function in common bottlenose dolphins (Lahvis et al. 1995, Cagnazzi 2017). HCB concentrations were low; however, toxicity of this chemical in cetaceans is unknown.

Biopsy samples from 17 Australian humpback and 22 snubfin dolphins were also analysed for eight essential elements (Zn, Cu, Cr, Se, Ni, Al, Mn, Fe) and four non-essential elements (Hg, Cd, As, Ag) (Cagnazzi 2017). No significant differences in trace element concentrations were found between snubfin and humpback dolphins with the exception of Zn and Ni, which were higher in snubfins (Cagnazzi 2017). Similarly, no differences in trace element concentrations were found between

humpback dolphins from Port Alma and Port Curtis. Concentrations of trace elements in most samples exceeded upper baseline (or reference) values established from free ranging common bottlenose dolphins (Bryan et al. 2007, Stavros et al. 2007, Cagnazzi 2017). However, these baseline values were established for a different species of dolphin (common bottlenose) foraging in a different location (south-east coast of the United States) and may be influenced by biological and ecological factors. Regardless, concentrations of trace elements in dolphins from the Port of Gladstone are higher than those found in Australian humpback dolphins from Moreton Bay (Weijs et al. 2016). Concentrations of Hg, Cd and Al in dolphins from the Port of Gladstone were within the range of, or above, concentrations found to have immunological effects in common bottlenose dolphins (Pellissó et al. 2008, Desforges et al. 2016). However, it is important to note that there is very limited information on the effects of trace elements in dolphins, which prevents meaningful assessments of the risks that these chemicals pose to dolphins.

2.4 Purpose of this report

The overall purpose of this report was to provide a clear understanding of the current state of chemical contamination in the humpback and snubfin dolphins living in the Port of Gladstone, and how to best assess the impacts this is having on their health. Specifically, this report presents:

- a desktop review of available literature related to chemical accumulation and toxicity of organochlorines and trace elements in dolphins, and
- recommendations on how further toxicological analysis could be conducted in the Port of Gladstone to improve understanding of the impacts of chemical contaminants on humpback and snubfin dolphins living in this area.

3 Desktop review

3.1 Exposure pathways

Industrial, urban and agricultural chemicals can enter coastal habitats via runoff and direct discharge. Dolphins, particularly inshore species and populations, may be exposed to high concentrations of these contaminants as they inhabit areas closer to human development and activities. Land use can influence contaminant exposures in dolphins, with differences in contaminant profiles found in dolphins that inhabit areas adjacent to different types of human activity (Monaci et al. 1998, Hansen et al. 2004, Stavros et al. 2007, Kucklick et al. 2011). Even fine-scale variation in habitat preference within the same bay can result in differences in contaminant levels in dolphins in coastal areas (Litz et al. 2007). These fine scale variations may influence interpretation of contaminant levels from dolphins across larger areas considered as one population.

Patterns of contamination in dolphins often closely match prey species (Senthilkumar et al. 1999, Weisbrod et al. 2001, Yeung et al. 2009), indicating that dolphins are primarily exposed to chemicals through their intake of food. Contaminants can accumulate within different tissues at different concentrations (André et al. 1990, Aubail et al. 2013, Gui et al. 2014c). For example, lipophilic organic compounds accumulate at high concentrations in blubber (Gui et al. 2014c), which may influence bioavailability of these compounds (Yordy et al. 2010a). For trace elements, tissue accumulation patterns vary by element (Cardellicchio et al. 2000, Carvalho et al. 2002). These tissue-specific differences in accumulation patterns can have significant effects on toxicity that may not be accurately interpreted using skin, blubber or blood as an estimate of body burden. Furthermore, contaminant accumulation patterns may vary between dolphin species due to differences in prey, habitat use or internal toxicokinetic processes (Watanabe et al. 1989, Yordy et al. 2010a, Hansen et al. 2016).

Dolphins are long-lived and some contaminants can show increased accumulation with age (Gui et al. 2014c), particularly in males (Hansen et al. 2004, Hickie et al. 2013, Gui et al. 2014b). Females

offload organic contaminants during gestation and lactation (Tanabe et al. 1982, Houde et al. 2005, Hickie et al. 2013). Due to this maternal transfer, females often have lower contaminant loads than males and even juveniles (Hansen et al. 2004). Calves receive high doses of lipophilic contaminants through milk, which is then followed by a transition to a fish diet and a period of rapid growth during which a growth dilution of contaminants occurs (Hansen et al. 2004). Contaminant loads in both male and females show a decline during this time until growth slows down or stops. Both sexes may then show an increase in contaminant levels associated with continued consumption of contaminated prey. Once females reach sexual maturity, contaminant loads decrease through maternal offloading, though continue to increase over time in males (Hickie et al. 2013).

Contaminants, particularly those that are resistant to breakdown and/or transformation, can biomagnify in top predators like dolphins, in concentrations well above what is found in their prey. Concentrations of PCBs, DDTs and HCB in Indo-Pacific humpback dolphins have been found to be 99, 212 and 68 times the concentrations found in prey fish species (Gui et al. 2014b). Other organochlorine concentrations ranged from 3.4 – 184 times the concentrations in fish (Gui et al. 2014b). Biomagnification can also occur with a variety of other chlorinated and non-chlorinated organics, such as polybrominated diphenyl ethers (PBDEs), perfluorinated compounds (PFCs), hexachlorocyclohexane (HCHs), chlordanes and butyltin (Johnson-Restrepo et al. 2005, Houde et al. 2006, Yeung et al. 2009). Biomagnification has largely been investigated for organics, though it can also occur with trace elements. For example, methylmercury (MeHg) and inorganic Hg have both been found to biomagnify in dolphin species (Gui et al. 2014a, Seixas et al. 2014).

3.2 Contaminant levels in Australian dolphins

3.2.1 Organochlorine (and other organic) contaminants

Organochlorine contaminants have been found in a number of dolphin species within Australia (Table 1). Cagnazzi et al. (2013) found high levels of PCBs, DDTs and HCB in humpback and snubfin dolphins from the Fitzroy River catchment, and a more recent study suggests concentrations have since increased (Cagnazzi 2017). Humpback and snubfin dolphins from the Fitzroy catchment have elevated PCBs compared to dolphins from other regions of Australia, though may be more comparable to PCB concentrations in humpback dolphins elsewhere (Table 1). Within the Port of Gladstone more specifically, humpback dolphins from Port Alma show particularly elevated levels of PCBs compared to humpback dolphins from Australia and other Indo-Pacific regions (Weijs et al. 2016). However, inter-study comparisons of PCBs should be considered carefully, as the number and types of congeners analysed may differ between studies. PCB profiles in Australian humpback dolphins in South-East Queensland indicate the sum of PCBs is likely dominated by PCB 118, 138, 153 and 180 congeners (Weijs et al. 2016). DDTs are also elevated in humpback dolphins from Port Alma and Port Curtis, and snubfin dolphins from Port Alma. As is typical of other marine mammals, *p,p'*-DDE is the major contributing congener to the sum of DDTs in dolphins (Weijs et al. 2016, Cagnazzi 2017). Though concentrations of HCB were typically low, the mean HCB concentration in Port Alma humpback dolphins was an order of magnitude above means from other locations around Australia.

Other organochlorines such as HCHs, heptachlor, lindane, mirex, pentachlorobenzene, 2-endo,3-exo,5-endo,6-exo,8,8,9,10,10-nonachlorobornane (B9-1679), endrin, aldrin, as well as some non-chlorinated compounds such as brominated diphenyl ethers (BDEs), butyltins and a number of naturally occurring organohalogen compounds have been measured in dolphin species from Australia, though have all been found in low concentrations (Kemper et al. 1994, Vetter et al. 2001, Vetter et al. 2002, Vetter et al. 2007, Weijs et al. 2016). In addition, Cagnazzi et al. (2013) found high levels of polycyclic aromatic hydrocarbons (PAHs) in dolphins from the Fitzroy region (mean ΣPAHs of 50879.9 ng/g lw and 40782.4 ng/g lw for humpback and snubfin dolphins, respectively), though these compounds were not investigated in the subsequent study (Cagnazzi 2017). Per and

polyfluorinated alkyl substances (PFAS) have also been found in high concentrations in Indo-Pacific bottlenose dolphins from southern Australia, particularly from the Swan River (mean PFOS of 6975 ng/g) (Gaylard 2017).

3.2.2 Trace elements

Trace elements have been found in a number of dolphin species within Australia (Table 2). A number of trace elements (Cd, Zn, Se, As, Cr, Cu, Ni, Al, Mn, Fe, Ag) in Port Alma and Port Curtis dolphins were elevated above humpback dolphins in other locations (Cagnazzi 2017), and other species in Australia (Table 2). Mercury may also be elevated in the Port of Gladstone humpback dolphins, although comparisons between different tissues become difficult as different tissues can accumulate trace elements to varying degrees. To illustrate this, concentrations from Weijs et al. (2016) for three different tissues (skin, blubber and liver) were included in Table 2. Some tissues accumulate different elements, for example cadmium concentrations were higher in the liver than skin or blubber; however chromium concentrations were higher in the blubber and skin compared to the liver. Therefore, meaningful comparisons of metal concentrations can only be made within the same tissue type. Furthermore, many elements are not measured in every study, and therefore cannot be compared between populations. Some elements, Ba, Ca, Co, K, Mg, Mo, Na, Sn, Sb, Th, Ti, Tl, U, and V, were only measured in one study (Weijs et al. 2016), and were detected in low concentrations or below detection limits in all tissues.

3.2.3 Consideration of small sample sizes

Many studies on chemical contamination in dolphins within Australia are based on stranded animals, and have small sample sizes for individual species, age classes, sex or locations. Comprehensive data for detailed comparisons is therefore lacking for contaminants in Australian dolphins. Consequently, comparisons between small sample sizes, particularly of one or two stranded individuals, should be considered carefully. Small sample sizes are particularly important to consider in wildlife toxicology investigations, as contaminant concentrations may vary significantly between individuals depending on biological factors such as age, sex, reproductive status, body weight, diet, body condition and disease (André et al. 1990, Aguilar et al. 1999, Yordy et al. 2010b). Despite the limitations of small sample sizes, analysis of contaminants in the internal tissues of stranded animals can provide important information on the distribution of contaminants in dolphins; for example, cadmium is generally higher in kidney compared to other organs (Cardellicchio et al. 2002). This is important information for supporting toxicokinetic modelling (see Section 4.4) and for ecological risk assessments (see Section 4.2). This knowledge can also help prioritise investigations into species-specific cell-based assessments of contaminants in dolphins (see Section 4.3).

Table 1. Mean concentrations and/or range of organochlorine contaminants in blubber or skin biopsies from dolphin species in Australia. Only the range is given when the mean was not provided, sample size was 2 or the location of the dolphins was unknown. Values are reported as ng/g lipid weight (lw), unless otherwise stated. The sum of PCBs, DDTs, CHLs and PCDD/Fs may include different numbers of congeners, depending on what was analysed in the study. Where the sample size of the analysis differs from the study sample size, it is given in square parentheses.

Common name	Australian humpback dolphin			Indo-Pacific humpback dolphin			Australian snubfin dolphin			Common bottlenose dolphin				Indo-Pacific bottlenose dolphin		Short-beaked common dolphin			Pantropical spotted Dolphin
Species	*Sousa sahulensis*			*Sousa chinensis*			*Orcaella heinsohni*			*Tursiops truncatus*				*Tursiops aduncus*		*Delphinus delphis*			*Stenella attenuata*
Reference	Cagnazzi (2017)		Weijs et al. (2016)	Cagnazzi et al. (2013)		Gaus et al. (2005)	Cagnazzi (2017)	Cagnazzi et al. (2013)		Law et al. (2003a)	Gaus et al. (2005)	Kemper et al. (1994)	Vetter et al. (2001)	Law et al. (2003b)		Kemper et al. (1994)	Vetter et al. (2001)	Law et al. (2003b)	Kemper et al. (1994)
Location	Port Alma	Port Curtis	South-east Queensland	Fitzroy river catchment	Mackay-Whitsunday catchment	Darwin	Port Alma	Fitzroy river catchment	Mackay-Whitsunday catchment	Queensland	Port Adelaide	Australia	East coast Australia	Gold Coast	Gippsland Lake	Victoria	East coast Australia	Gold Coast	Australia
Tissue	Skin	Skin	Blubber	Skin	Skin	Blubber	Skin	Skin	Skin biopsy	Blubber	Blubber	Blubber	Blubber	Blubber	Blubber	Blubber	Blubber	Blubber	Blubber
Sample Size	6	11	6	13	5	1	18	8	9	2	1	6	4	1	1	1	1	1	1
∑PCBs	51750 (9254 – 222511)	16209 (516 – 32527)	1600 – 370000	13143.9 (776.75 – 93522.5)	3753.9 (1382.4 – 7431.9)	900	16216 (7465 – 33886)	6674.3 (1382.4 – 19135.4)	3894.3 (168.6 – 21424.6)	NA	280	60ª	792 – 25524	1020ª	500ª	180ª	627	870ª	820ª
∑DDTs	37490 (26402 – 53344)	32930 (1552 – 74195)	1800 – 17000	2616.6 (491.99 – 6163.6)	969.2 (308.4 – 2452.2)	NA	22167 (6371 – 54648)	5285.3 (178.4 – 16073.4)	930.6 (231.1 – 2121.9)	NA	NA	180 – 2410ª [2]	682 - 52549	900ª	269ª	10000ª*	575	548ª	1190ª
∑CHLs	NA	NA	270 – 1000	NA	NA	NA	NA	NA	NA	NA	NA	NA	323 - 7686	NA	NA	NA	284	NA	2ª‡
∑PCDD/F	NA	NA	0.0097 – 3.02	NA	NA	0.14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
HCB	166 (32 – 307)	145 (5 – 402)	9.4 - 17	6.5 (0 – 45.5)	5.6 (0 – 28.3)	NA	72 (22 – 293)	25.55 (0 – 47.7)	6.79 (0 – 27.2)	NA	NA	NA	5.9 - 23	1ª	33ª	NA	40	38ª	9ª
Dieldrin	NA	NA	87 – 700	NA	NA	NA	NA	NA	NA	NA	NA	2ª	47 - 425	767ª	45ª	NA	74	140ª	NA

Abbreviations: PCB: polychlorinated biphenyl; DDT: dichlorodiphenyltrichloroethane; CHL: chlordane; PCDD/F: polychlorobenzodioxins/furans; HCB: hexachlorobenzene; NA= not analysed; <LOQ=below limit of quantification

ª ng/g ww; *DDT only; ‡oxychlordane only; °muscle

Table 2. Mean concentrations and/or range of inorganic contaminants in blubber, skin biopsies and liver from dolphin species in Australia. Only the range is given when the sample size was 2 or the location of the dolphins was unknown. Values are reported as ng/g wet weight (ww). Where the sample size of the analysis differs from the study sample size, it is given in square parentheses.

Common name	Australian humpback dolphin				Australian Snubfin dolphin	Common bottlenose dolphin		Indo-Pacific bottlenose dolphin			Short-beaked common dolphin			Pantropical spotted dolphin
Species	*Sousa sahulensis*				*Orcaella heinsohni*	*Tursiops truncatus*		*Tursiops aduncus*			*Delphinus delphis*			*Stenella attenuata*
Reference	Cagnazzi (2017)	Weijs et al. (2016)			Cagnazzi (2017)	Kemper et al. (1994)	Lavery et al. (2008)	Lavery et al. (2008)	Law et al. (2003b)		Kemper et al. (1994)	Lavery et al. (2008)	Law et al. (2003b)	Kemper et al. (1994)
Location	Port of Gladstone*	South-east Queensland	South-east Queensland	South-east Queensland	Port of Gladstone*	Australia	South Australia	South Australia	Gold Coast	Gippsland Lake	Australia	South Australia	Gold Coast	Australia
Tissue	Skin	Skin	Blubber	Liver	Skin	Blubber	Liver	Liver	Liver	Liver	Blubber	Liver	Liver	Blubber
Sample Size	39	6	6	1	39	10	11	63	1	1	2	69	1	2
Ag	<DL	<DL	<DL – 45	276	<DL	NA	NA	NA	540	<10	NA	NA	50	NA
Al	<DL - 921160	<DL – 51836	241 – 462	<DL	<DL - 921160	NA	NA	NA	NA	NA	NA	NA	NA	NA
As	<500 – 3700	202 - 700	416 – 2794	68	<500 – 3700	NA	NA	NA	760	200	NA	NA	320	NA
Cd	10 – 13230	<DL - 7	<DL	389	10 – 13230	50	4100 (<DL – 20000)	6450 (<DL – 99950)	3700	70	60 – 110	1120 (<DL – 10920)	20	50
Cr	650 – 15290	15 – 274	11 – 24	16	650 – 15290	NA	NA	NA	1200	970	NA	NA	NA	NA
Cu	1860 - 10660	1127 – 1477	80 – 288	4813	1860 - 10660	NA	21280 (4940 – 85040)	19670 (620 – 73710) [50]	8500	180	NA	11350 (2960 – 71180) [48]	11000	NA
Fe	14850 – 328330	5808 – 229177	1553 – 5340	247393	14850 – 328330	NA	NA	NA	NA	NA	NA	NA	NA	NA
Hg	1670 - 15720 [17]	NA	NA	NA	<DL – 7220 [22]	10 – 3810	213940 (2500 – 771900) [10]	475780 (280 – 2110680) [59]	32000	720	150 – 980	31210 (150 – 165280) [68]	850	420 – 460
Mn	250 - 13030	47 – 2127	10 – 30	1192	250 - 13030	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ni	410 - 9570	<DL – 151	<DL	<DL	410 - 9570	NA	NA	NA	560	NA	NA	NA	NA	NA
Pb	NA	<DL - 218	<DL - 2	40	NA	50 - 3410	74 (10 – 110) [9]	455 (4 – 13570) [54]	170	40	50 – 100	63 (<DL – 130) [47]	<40	50 – 60
Se	8710 - 72110	2048-9576	555-1609	67808	8710 - 72110	NA	70190 (<DL – 253380)	178850 (<DL – 1188400)	12000	1500	NA	14140 (<DL – 63430)	1600	NA
Zn	245000 - 1373000	26574-258245	3134 - 26279	31591	245000 - 1373000	NA	40200 (26160 – 68000)	93880 (24020 – 452880)	41000	144000	NA	62220 (26000 – 175220)	77000	NA

*The element concentrations were grouped for the Port Alma and Port Curtis humpback and snubfin dolphins, with the exception of mercury which was provided for each species.
DL= Detection limit

3.3 Effects of contaminants on dolphins

Environmental contaminants can have a wide range of detrimental effects on humans and wildlife (Pal et al. 2010). There are thousands of contaminants present in the environment, with many different modes of action and effects. While the focus of this report is on organochlorines and trace elements, effects data are so scarce for dolphins that studies related to effects of non-chlorinated organic contaminants have also been included in this section. In marine mammals, contaminants have been linked to endocrine disruption (Tanabe 2002), effects on reproduction (Reijnders 2003), effects on the immune system (Ross 2002), genotoxicity (Taddei et al. 2001) and differences in gene and protein expression that may lead to disruptions to normal physiological processes (Fossi et al. 2014, Lunardi et al. 2016). However, there is a considerable lack of information concerning the effects of contaminants in Australian dolphin species.

To date, research into understanding the effects of contaminants on dolphins can be separated into five main categories: 1) correlations between contaminant concentration and effect, 2) threshold values and ecological risk assessments, 3) *in vitro* (cell-based) studies, 4) toxicokinetic-toxicodynamic modelling, and 5) biomarkers of chemical exposure and effect.

3.3.1 Correlations between contaminant concentration and effect

Persistent organic pollutants have been correlated with high rates of infertility in common dolphins and inhibited ovulation in harbour porpoises (Alonso Farré et al. 2010). Similarly, concentrations of PCBs and PBDEs were negatively correlated to pregnancy rate in common dolphins (Pierce et al. 2008). In common bottlenose dolphins, females whose calves were stillborn, or died within 12 days after birth, had significantly higher concentrations of DDTs and HCB than females whose calves survived over 6 months (Reddy et al. 2001). Of the calves that died in this study, 67% were first born calves (Reddy et al. 2001), which may indicate a significant effect maternal offloading of organic contaminants, particularly during a dolphin's first pregnancy. This is further supported by risk assessments performed by Schwacke et al. (2002) that found the risk of reproductive failure was higher in females that had not previously reproduced and this risk was reduced with each subsequent birth.

A number of organic compounds have also been linked to effects on the immune system in dolphin species. Activity of lymphocytes isolated from wild common bottlenose dolphins exhibited a strong negative correlation with PCBs and DDTs, indicating immunosuppressive effects of these contaminants (Lahvis et al. 1995, Schwacke et al. 2011). Innate immune response, neutrophil and monocyte phagocytosis, were also negatively correlated with PCB concentrations (Schwacke et al. 2011). Conversely, PFCs show a positive correlation with some immune parameters (Fair et al. 2013), indicating a possible immune-stimulatory response. Contaminant-induced dysfunction of the immune system can have detrimental effects, such as increased susceptibility to pathogens, and has been suspected as a factor in disease outbreaks and mortality in dolphins and other marine mammals (Ross 2002).

Despite some strong links established between contaminant exposure and adverse outcomes in dolphins, it is important to note that correlation does not always equal cause. There are a number of other biological and environmental factors other than, or in combination with, chemical contamination that may be responsible for the effects in dolphins described above. As such these correlative studies should be treated with some caution.

3.3.2 Threshold values and ecological risk assessments

Indisputable cause-effect relationships between contaminants and adverse effects have not been established for dolphins. In many cases, direct exposure experiments are not practical, logistical or

ethical, particularly with large, long lived, often threatened species, such as dolphins. Thus, studies assessing the risk of contaminants in dolphins and other marine mammals often involve an extrapolation of effects from laboratory or captive studies on other animals that can be more easily handled and manipulated (Kannan et al. 2000, Ross 2002). However, differences in sensitivity between species are often apparent, associated with differences in metabolism and other physiological processes. Even between cetacean species there are differences in cytochrome P450 enzymes, which are involved in metabolism (Watanabe et al. 1989). Effect concentrations or guideline values established for other species in laboratory or captive studies therefore may not be applicable to dolphins. Nonetheless, laboratory and captive studies on other animals have provided valuable information for assessing effects of chemicals in marine mammals, such as the ability to establish threshold values or perform risk assessments.

Threshold values have been established for suppression of lymphocyte proliferation in cetaceans by PCBs (5.42 ± 2.15 ppm), Cd (0.047 ± 0.059 ppm), Hg (0.016 ± 0.0049 ppm) and MeHg (0.21 ± 0.45 ppm), and for suppression of phagocytosis by PCBs (1.1 ± 0.7 ppm) and Hg (1.88 ± 36.16 ppm) (Desforges et al. 2016). These values were calculated from the concentration giving a 1% response in beluga and common bottlenose dolphin lymphocytes and neutrophils *in vitro* (Desforges et al. 2016). However, variability in lymphocyte proliferation between the two species suggests that threshold values should be established on a species-by-species basis. Threshold values for the effects of PCBs (17 ug/g lw in blubber) on reproduction and the immune system have also been established for marine mammals, based on effect values in seals, mink, otters, polar bears and some cetacean species using a combination of semi-field, *in vivo* and *in vitro* exposure studies (Kannan et al. 2000).

Risk assessments allow for an estimation of the likelihood of an adverse effect associated with contaminant exposure, and are a valuable tool for identifying significant effects of contaminants in wildlife. Risk quotients (RQs) can be calculated from the contaminant concentration in an animal (either directly measured or estimated via measurement of concentration in prey) divided by an effect concentration. An individual, or population, is considered to be at risk from a particular chemical if the calculated RQ >1. In the absence of species-specific toxicity data, risk assessments have been performed for dolphins using effect-concentration data from a number of other mammalian species, in some cases including the application of a safety or uncertainty factor to account for inter-species differences (Schwacke et al. 2002, Hung et al. 2004, Hung et al. 2006a, Hung et al. 2006b, Hung et al. 2007, Wirth et al. 2015). It should be noted that ecological risk assessments calculated from concentrations in prey species often indicated no risk (Hung et al. 2004, Hung et al. 2006a, Wirth et al. 2015). However, bioaccumulation and biomagnification of contaminants in top end predators suggest a preference for calculating RQs from concentrations found within the dolphins themselves. Although, this is not always possible, and in the absence of such data, prey-based risk assessments can provide valuable information on the potential risks of chemical exposure in dolphins.

3.3.3 *In vitro* (cell-based) studies

Species-specific data may provide more useful information for risk assessments. For dolphins, this can be obtained from *in vitro* studies using species-specific cell cultures. *In vitro* techniques can help to elucidate direct cause-effect relationships between contaminants and specific effects. *In vitro* techniques have been used to demonstrate the immunotoxic effects of PCBs, DDTs, PBDEs and trace elements on phagocytosis, lymphocyte proliferation and natural killer cell activity, often in the range of concentrations found in free-ranging dolphins (De Guise et al. 1998, Levin et al. 2004, Pellissó et al. 2008, Wirth et al. 2015). Levin et al. (2004) further demonstrated effects of co-planar PCBs on the immune system, independent of the aryl-hydrocarbon receptor (AhR), which may indicate the

presence of an unidentified receptor for co-planar PCBs specific to common bottlenose dolphins. Contaminants can have other effects in immune-related cells beyond altering their function. For example, organochlorines, MeHg, Hg and Cd can affect cell viability at environmentally relevant concentrations in common bottlenose dolphins (Betti & Nigro 1996, Levin et al. 2004, Pellissó et al. 2008). Genotoxic effects have also been found in common bottlenose dolphin lymphocytes following exposure to MeHg (Betti & Nigro 1996, Taddei et al. 2001). *In vitro* assays are therefore valuable tools for investigating species-specific mechanisms of toxicity. However, this area of science is still in its infancy, and there are many more toxicological endpoints that warrant further investigation.

In vitro techniques can also be used for investigating expression of biomarkers of toxicological stress, some of which could be used as biomarkers of exposure and effect in wild populations. For example, enzymes involved in metabolism and detoxification (e.g. CYP1A1 and CYP2B), AhR and HSP70 can be upregulated or downregulated following exposure to persistent organic pollutants in striped, common bottlenose and Indo-Pacific humpback dolphin fibroblasts (Fossi et al. 2006, Marsili et al. 2008, Jia et al. 2015). Furthermore, metabolic and detoxification enzyme expression showed differences between species that may relate to differences in susceptibility (Fossi et al. 2006, Marsili et al. 2008). These studies have helped to validate the use of these proteins as biomarkers in wild populations by demonstrating direct effects of contaminants on expression, and supporting correlations found in field studies.

In vitro studies have also been used to assess the relative sensitivity of dolphins to contaminant exposure. While little is known about the sensitivity of dolphins to environmental contaminants, there is some suggestion that marine mammals may be less sensitive than other mammals to the toxic effects of some metals due to detoxification mechanisms. For example, the Atlantic spotted dolphin may be less sensitive to mercury exposure, due to detoxification mechanisms involving selenium (Wang et al. 2001). Efficient detoxification and sequestration of Cd by bottlenose dolphins have also been suggested (Hansen et al. 2016). To support this, laboratory based studies, using lymphocyte cells harvested from a range of mammals, illustrated common bottlenose dolphin lymphocytes were less sensitive to the genotoxic and cytotoxic effects of MeHg compared to rat and humans lymphocytes (Betti & Nigro 1996), and showed greater resilience to genotoxic effects through more efficient DNA repair (Taddei et al. 2001). Detoxification and sequestration may be adaptive mechanisms to high accumulation of chemical contaminants in marine mammals.

3.3.4 Toxicokinetic-toxicodynamic modelling

To fully understand the effects of contaminants in any species, there is a need to understand the uptake of contaminants, how they are distributed through the body, how these contaminants are metabolised and eliminated from each tissue and what physiological effects take place in the process. This can be accomplished with the use of physiologically-based toxicokinetic-toxicodynamic modelling. Toxicokinetic models require in-depth physiological knowledge of the target species including routes and duration of exposure, blood flow, organ volumes, growth characteristics, rates of metabolism and elimination, while toxicodynamic models require information on the effects of contaminants including mechanisms of toxic injury and repair (Andersen 1995), which can often be obtained *in vitro*.

Toxicokinetic modelling has accurately explained the accumulation of PCBs (Weijs et al. 2010, Weijs et al. 2011), PBDEs (Weijs et al. 2012) and DDTs (Weijs et al. 2013) in tissues, by age and gender, of harbour porpoises. Models developed in long-finned pilot whales were limited by a lack of information about PCBs in tissues other than blubber, however have the ability to be updated (Weijs et al. 2014b). Similar models have been developed for PCBs in common bottlenose dolphins and killer whales that included an assessment of risk by age and gender based on adverse effect

threshold concentrations (Hickie et al. 2013). Toxicokinetic models can also predict changes over time, for example with decreasing exposure to banned contaminants (Hickie et al. 2007, Weijs et al. 2012). These models can be useful in understanding internal tissue concentrations in relationship to those measured in non-destructive samples, such as skin biopsies and blood, and can be valuable in risk assessments by providing information on accumulation during current and future exposures (Weijs et al. 2012, Weijs et al. 2014a). Finally, toxicokinetic modelling can be combined with toxicodynamic and population modelling to predict changes at the population level (Desforges et al. 2018). Such models, based on calf survival and immune suppression associated with PCB accumulation, have predicted declines in many of the world's killer whale populations, with total collapse of the most exposed populations expected in the next 100 years (Desforges et al. 2018).

3.3.5 Biomarkers of chemical exposure and effect

Biomarkers of chemical exposure and effect can be useful in assessing if wild populations are experiencing any adverse effects from exposure to contaminants (Chaousis et al. 2018). A large number of studies have investigated a wide variety of biomarkers in marine mammals, including cetaceans (Fossi & Marsili 1997). Protein or gene expressions are commonly used biomarker techniques. For example, cytochrome P450 1A1 (CYP1A1) is a commonly used protein biomarker of chemical exposure due to its involvement in metabolism of halogenated and polycyclic aromatic hydrocarbons. Altered metabolism can affect regular endogenous processes and some contaminants are metabolised to more toxic intermediates (Tanabe et al. 1994, Letcher et al. 2000). High benzo(a)pyrene monooxygenase (BPMO) activity, indicative of CYP1A1 induction, has also been linked to higher levels of DDTs and PCBs (Fossi et al. 1992, Fossi et al. 2000, Fossi et al. 2003, Fossi et al. 2004). A suite of gene expression biomarkers involved in stress, metabolism, and reproduction (CYP1A, aryl hydrocarbon receptor, heat shock protein 70, E2F-1 transcription factor, catalase and the estrogen receptor) have also been used to compare toxicological stress between dolphins from locations of different chemical exposures (Panti et al. 2011, Fossi et al. 2013).

A small number of studies have also used *ex vivo* techniques, based on exposure of tissue biopsies to contaminants, to investigate biomarkers of chemical exposure and effect in dolphins. For example, gene expression of typical biomarkers (CYP1A, CYP2B, HSP70, and ER) and protein expression of CYP1A and CYP2B have been investigated following exposure of striped dolphin skin biopsies and liver slices to organochlorines (OCs), PBDEs and PAHs (Fossi et al. 2014). Upregulation was detected following many of the exposures, with induction of CYP1A and CYP2B showing upregulation at lower concentrations and downregulation at higher concentrations following exposure to OCs and PBDEs (Fossi et al. 2014). In addition, transcriptomics found a large number of differentially expressed genes in common bottlenose dolphins skin biopsies exposed to bisphenol A and perfluorooctanoic acid, including genes involved in immune response, response to stress, developmental processes, lipid homeostasis and metabolic and cellular processes (Lunardi et al. 2016). These studies provide important information for application of the biomarkers in wild populations.

Despite the potential use of biomarkers, particularly as an early warning sign of chemical exposure, there are some limitations to using biomarkers for assessing effects of contaminants. In particular, it may be difficult to predict the effect of chemical mixtures, and the effects of contaminants on biomarker expression may vary between species or individuals (Chaousis et al. 2018). Consequently, it is advantageous to use a suite of biomarkers to better assess the effects of chemical exposure and effect. It may also be necessary to compare results with relatively unexposed populations to better elucidate baseline biomarker levels. Finally, biological significance must be considered when selecting a biomarker, as changes in biomarker expression may not necessarily indicate a significant biological effect within the organism.

4 Recommendations for further toxicological analysis in Port of Gladstone dolphins to assess the impacts of contaminants on dolphin health

Information regarding chemical exposure to Australian dolphins is limited, and information regarding toxicity in Australian species is entirely lacking. Decreases in dolphin populations and increases in contaminant loads in the Port of Gladstone therefore warrant a more detailed investigation into the effects of contaminants on the health of dolphins in this area. It is recommended that further research to understand the health impacts of contaminants in dolphins within the port include:

- Continued monitoring of contaminants in dolphin skin biopsies and carcasses, including consideration of major flooding or dredging activities - to identify changes in chemical exposure over time.
- The application of ecological risk assessments - to provide important information on the likelihood of adverse effects occurring at current and future contaminant exposures.
- Development and application of *in vitro* (cell-based) toxicity bioassays - to provide species-specific data on the effects of contaminants in dolphins and improve risk assessments.
- Toxicokinetic-toxicodynamic modelling - to develop better understanding of exposure and effects at the organism and population levels.
- Further development of biomarkers of chemical exposure and effect - to provide tools for early detection of chemical exposure.

4.1 Continued chemical monitoring

It is recommended that continued analysis of chemical contamination in skin/blubber biopsies of humpback and snubfin dolphins within the Port of Gladstone be carried out at a minimum frequency of every 3-5 years. This frequency is based on the significant differences found in PCBs, DDTs and HBC in Port of Gladstone dolphins between 2010/2011 (Cagnazzi et al. 2013) and 2014/2015 (Cagnazzi 2017), and is supported by increases in PCB 153 and DDTs (in particular DDE) in harbour porpoises over a similar period (Weijs et al. 2010, Weijs et al. 2013). A higher frequency of sampling will allow for earlier detection of increases in contaminants before a significant effect can occur. It is also recommended that additional sampling and analysis occur immediately prior to, and following, major flooding or dredging events, which have the capacity to increase exposure of dolphins to chemicals.

Biopsy samples collected during routine and event-based sampling should continue to be analysed for organochlorines and trace elements. Inclusion of other organic contaminants such as PFAS, PFCs and PAHs in future monitoring may also be beneficial, as these compounds can accumulate in dolphins (see Section 3.2.1), and potentially exert adverse effects (Fair et al. 2013, Fossi et al. 2014). It may also be prudent to include a larger number of trace elements in the chemical analysis. This may be done by increasing the number of trace elements included in quantitative analytical methods. Alternatively, new methods established for sea turtles that can semi-quantitatively screen up to 70 different trace elements (Villa et al. 2015) may facilitate detection of metals that are not typically included in these analyses. It is also important to include size and gender of the dolphins sampled, where possible, due to differences in chemical accumulation and effect at different life cycle stages. Continued sampling and chemical analysis in this manner will allow detection of changes in bioaccumulation and biomagnification of organic and inorganic contaminants in Port of Gladstone dolphins over time. It will also provide information on the impacts of weather and dredging events on the exposure of dolphins to contaminants in this area.

It is also recommended that opportunistic sampling of dolphin carcasses continue in the Port of Gladstone, as information on contaminant loads in internal tissues of both humpback and snubfin

dolphins is scarce. Stranded individuals may not be representative of the population as chemical concentrations may be abnormally high due to mobilisation of fat stores in sick or unhealthy individuals. However, concentrations from internal tissue can provide important information for species-specific toxicokinetic modelling (see Section 4.4), which can be used to better understand the relationship between internal tissue concentrations and concentrations measured in biopsy samples. Contaminant data from internal tissues can also be used to better prioritise *in vitro* toxicity studies (see Section 4.3).

4.2 Ecological risk assessments

Ecological risk assessments evaluate the likelihood of adverse effects occurring as a result of chemical exposure. Typically, exposure is estimated through the intake of contaminated food (Hung et al. 2004, Hung et al. 2006a); however, due to biomagnification of contaminants in top end predators such as dolphins, internal tissue contaminant concentrations may be more meaningful in risk assessment calculations. Risk quotients (RQs) can be calculated by dividing exposure concentrations by an effect concentration, where values > 1 represent potential risk. Risk assessments for cetaceans have often been performed using effects data from other mammals, such as mice (Wirth et al. 2015) and mink (Schwacke et al. 2002), due to the lack of toxicity data for cetaceans. While there is some merit in estimating risk using effect-values from other species, estimates may not accurately depict risk within a different species, and often a safety factor is applied to account for these species-specific differences (Hung et al. 2006b, Hung et al. 2007). Species-specific effects data is more desirable and can be obtained via *in vitro* experiments when *in vivo* data are not available. This method has proved successful in identifying risk in other species such as marine turtles (Finlayson et al. 2019b, Finlayson et al. 2019c). For humpback and snubfin dolphins in the Port of Gladstone, species-specific risk assessments using *in vitro* toxicity bioassays would be a valuable tool for estimating risk associated with differences in exposure and effect between the species.

4.3 Species-specific *in vitro* toxicity assessments of dolphins

In vitro (or cell-based) techniques provide an ethical, cost effective and high throughput alternative for assessing the effects of contaminants in wildlife, especially when *in vivo* exposure studies are not practical, logistical or ethical. Data from *in vitro* studies can be used to assess the toxicity of contaminants (and mixtures of contaminants) of concern, as well as comparisons between populations, and can provide important information for toxicokinetic modelling and risk assessments.

Dolphin cells have been used in a number of *in vitro* studies (see Section 3.3.3) and future research should focus on developing that knowledge-base with additional toxicological endpoints. Species-specific cell cultures can be established from skin biopsies and used in toxicity bioassays that have already been adapted for primary cells of other species (e.g. marine turtles) to assess cell viability, oxidative stress and DNA damage (Finlayson et al. 2019a, Finlayson et al. 2019b, Finlayson et al. 2019c). Additionally, samples of internal tissues from fresh carcasses can be used to establish cell cultures from internal organs (e.g. liver, kidney, gonads), providing a wider range of tissue types for which toxicity can be assessed. *In vitro* chemical exposures to date have largely focused on common bottlenose dolphins and to a lesser extent, striped dolphins. Applying these techniques to Australian humpback and snubfin dolphins would represent the first cell cultures and species-specific toxicity data for these species.

The majority of *in vitro* chemical exposures are done with a single contaminant. These exposures are important to understand relative toxicity of individual compounds and compare species sensitivities, and could be used to assess the effects of chemicals known to accumulate in the Port of Gladstone

dolphins. However, contaminant exposure in biota usually occurs in a complex mixture, and the interactions between contaminants, whether additive, synergistic or antagonistic, should be considered. Mixture effects between accumulated contaminants can be significant and testing single contaminants may underestimate the effects of real world exposures (Desforges et al. 2017). To address this, contaminants can be extracted from blood (e.g. Dogruer et al. 2018) or blubber from wild ranging dolphins (Desforges et al. 2017) and the toxicity of the extracts tested *in vitro*. Given the significant effects of chemical mixtures and the benefit of testing environmentally relevant mixtures, it is highly pertinent to obtain blood and/or blubber samples for this kind of analysis where possible. Within the Port of Gladstone, this approach could be used to assess the level of toxic stress that dolphins are currently experiencing, and how this changes over time in response weather events and human activity.

New bioassays can also be developed with clearer links to chronic endpoints, such as development, growth and reproduction. These could involve the investigation of gene or protein expression involved in development and reproduction. Additionally, this could include the development of species-specific reporter gene assays that can measure endpoints such as endocrine disruption. In this regard, a dolphin-specific CALUX (or CAFLUX) assay would be of high interest. This assay focuses on the AhR pathway and is developed to respond to insults from AhR-inducing stressors or pollutants, such as dioxins and PCBs. In lieu of species-specific reporter gene assays, established human and rodent cell-based reporter gene assays (e.g. the GeneBLAzer battery) can also be used for assessing the impacts on endpoints, such as endocrine disruption, that are generally highly conserved between vertebrate species.

4.4 Toxicokinetic-toxicodynamic modelling

Toxicokinetic-toxicodynamic modelling has obvious merit in understanding the effects of contaminants at the organism level, by providing a clearer link between external and internal exposure. Models for humpback and snubfin dolphins would therefore be valuable tools in identifying species-specific effects of contaminants on dolphins within the Port of Gladstone. Concentrations of contaminants measured during opportunistic sampling of carcasses would provide important information for these models. Importantly, these toxicokinetic models would aid in understanding accumulation under future exposure regimes, for example following flooding or dredging events, and provide important information for risk assessments. Incorporating a toxicodynamic (effects-based) element to the models (for example, using *in vitro* toxicity bioassays) would provide a link between the effects observed *in vitro* and adverse effects *in vivo*. These models are critical in linking *in vitro* effects to *in vivo* effects, and can help to establish species-specific threshold values for adverse effects that would aid in future risk assessments.

4.5 Investigations into biomarkers of chemical exposure and effect

As discussed in Section 3.3.5, biomarkers of chemical exposure and effect can provide a minimally invasive technique for assessing whether animals, including dolphins, have been exposed to, and are been affected by, environmental contaminants. While there are some promising candidate biomarkers for dolphins, particularly proteins in blood and blubber (e.g. cytochromes P450), there is still some research to be done to develop further and more meaningful biomarkers of chemical exposure and effect in dolphins. In particular, development and validation of a whole suite of biomarkers would present a minimally invasive technique for comprehensively assessing chemical exposure in dolphins and providing early warning signs of effect.

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