





national research centre for environmental toxicology

FINAL REPORT

# **INVESTIGATION OF CONTAMINANT LEVELS IN GREEN TURTLES FROM GLADSTONE**

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# **EXECUTIVE SUMMARY**

The objective of the present study was to measure the concentration of contaminants in blood of live green turtles captured in the Boyne River estuary near Gladstone, and to evaluate whether the contaminant levels are elevated and may pose a risk to the health of the turtle population.

During early 2011, Port Curtis experienced approximately 5 times higher mortality rates of sea turtles compared to previous years, as well as increased mortality rates of other wildlife species. In July 2011, an evaluation of the health status of live and diseased local green turtles was conducted. In parallel with this investigation, blood was collected from 40 live green turtles to assess exposure to a range of organic and inorganic contaminants that may be associated with agricultural, urban and industrial activities and that are known to accumulate in marine wildlife and may present a hazard to these species. Three of these 40 green turtles had to be euthanised due to poor diagnoses for survival, providing liver and kidney samples in addition to blood. Additional liver and fat samples were also obtained from stranded specimens.

The measured levels of contaminants in the Boyne River estuary turtle samples were compared to the levels reported in the peer-reviewed scientific literature for other green turtles, sea turtles and, where limited information was available, other vertebrates from both polluted and relatively low impacted areas. These levels were further evaluated against reported contaminant concentrations in a range vertebrate species where either chronic health effects (after long term exposure to contaminants) or acute health effects (after short term exposure) have been observed. Based on these assessments, the contaminants that were found in the turtle samples were classified into three categories:

1. Contaminants were considered of "relatively low concern" if they were detected in the Boyne River turtles at relatively low concentrations that were comparable to those reported for most other sea turtles and vertebrates, including those considered healthy and originating from relatively low impacted areas. At these levels, no associated adverse health effects have been reported in the scientific literature for turtles or other vertebrate species.

Contaminants assigned to this category were: bioaccumulative pesticides, organotins, flame retardants (polybrominated diphenyl ethers (PBDEs)), perfluorinated compounds (perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA)), aluminium (Al), iron (Fe), manganese (Mn), and zinc (Zn).

2. Contaminants were considered "possibly of concern" if they were present at concentrations that were comparable to the upper ranges of those reported for other sea turtles and vertebrates. Where relevant information was available, the contaminant levels in a proportion of the Boyne River green turtles were found to be above the concentrations where chronic effects occur in other vertebrates; i.e. long term exposure at these levels may result in adverse health effects. In contrast, the levels in the turtles were lower than the concentrations expected to result in adverse health effects after short term, acute exposure to these compounds.

Contaminants that fell within this category were: polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), dioxin-like polychlorinated biphenyls (PCBs), silver (Ag), copper (Cu), chromium (Cr), molybdenum (Mo), and lead (Pb).

3. Contaminants were considered "of concern" if their concentrations were clearly higher compared to most other green turtles and sea turtles, or within the upper levels reported from animals that were moribund and/or originated from areas considered relatively polluted. These contaminants were also present at higher levels compared to normal concentrations known for other vertebrates from low impacted or unpolluted areas. In particular, the measured concentrations in Boyne River turtles were found to be above or near the concentrations where acute adverse health effects have been observed across different vertebrate taxa. Although the sensitivity of sea turtles to these contaminants is mostly unknown, this suggests that adverse health effects are possible in the Boyne River estuary turtle population at the detected concentrations.

Contaminants that fell within this category were: arsenic (As), cadmium (Cd), cobalt (Co), mercury (Hg), nickel (Ni), selenium (Se), and vanadium (V).

It should be noted that information on the sensitivity of green turtles to contaminants are limited. For this study, as for other studies reported in the scientific literature, comparisons to other vertebrates were required in most instances. There is, therefore, an uncertainty involved when evaluating the effects that a particular concentration of contaminants may have on green turtles. Considering these results, it is recommended to monitor the health and contaminant levels in adult and juvenile green turtles from Gladstone as well as other, suitable control populations. Investigation of contaminants with strong tendencies to biomagnify in marine biota should be carried out across species of different trophic levels, and detailed speciation of metal/metalloids should be considered to provide a better understanding on the risks associated with the compounds of concern based on total metal/metalloid concentrations.

# **TABLE OF CONTENTS**





### **LIST OF FIGURES**



- [10 most abundant peaks \(numbered\) were identified based on the mass spectra](#page-25-1)  [of each peak..................................................................................................................26](#page-25-1)
- Figure 3 Box and whisker plots for metal and metalloid concentrations (ppb ww) in blood from individual (n=40) green turtles (*Chelonia mydas*[\) from Boyne River estuary](#page-32-0)  [near Gladstone, Queensland. Box plots show the mean \(red cross\) and median](#page-32-0)  (red line), the  $25<sup>th</sup>$  (bottom of box) and  $75<sup>th</sup>$  (top of box) percentiles, and 1.5 times the inter quartile range (whiskers). [....................................................................33](#page-32-0)
- **Figure 4** [Probabilistic distributions of body burden \(ng kg](#page-73-0)<sup>-1</sup> bw (x-axis)) in juvenile green [turtles from Gladstone; A\) derived using mammalian TEFs and B\) derived using](#page-73-0)  [avian TEFs. The blue portion of the graph depicts the fraction of the juvenile](#page-73-0)  population at or above the LOAEL of A) 3 ng  $kg^{-1}$  bw for biochemical effects in mammals (29%) and B) 9 ng  $kg^{-1}$  bw for developmental toxicity in chickens [\(5.0%\)............................................................................................................................74](#page-73-0)

# **LIST OF TABLES**





# **ABBREVIATIONS AND ACRONYMS**







# <span id="page-11-0"></span>**1.0 BACKGROUND**

Since early 2011, Port Curtis has been experiencing higher than usual mortality rates of sea turtles, with 260 reported strandings between 1 January 2011 and 28 February 2012 in the Gladstone region from Rodds Bay Peninsula to Sandy Point north of Yeppoon, compared to 50-51 reported strandings per year during 2008-2010 [\(DERM, 2012\)](#page-78-0). There has also been an increase in the number of other wildlife strandings, as well as outbreaks of diseases in fish in this region [\(DEEDI, 2011\)](#page-78-1).

The mass turtle stranding event was attributed partly to the significant loss of seagrass beds, which form important foraging habitats for resident populations of green turtles [\(DERM, 2012\)](#page-78-0). The summer of 2010-2011 witnessed unprecedented extensive flooding in the Gladstone Harbour region as well as across much of Queensland, resulting in increased freshwater and sediment outflow and subsequent reduced seagrass cover [\(Sankey](#page-84-0) *et al.*, 2011). These events are compounded by the large-scale industrial development in the Gladstone Harbour region. As a major port city along the Queensland coast, Gladstone hosts a variety of industries, including mining and processing of minerals, and liquefied natural gas, a large fishing industry, as well as agricultural activities within the catchment. Since 20 May 2011, the city has been undergoing substantial development of its port resources, including dredging and land reclamation [\(Gladstone Ports Corporation, 2011\)](#page-79-0).

In response to the wildlife strandings, a Scientific Advisory Committee was formed at the request of the Queensland Minister for the Environment, and recommended the investigation of the health status of green turtles within the Gladstone Harbour. This investigation commenced with an on-site survey and sample collection during 8-11 July 2011 by a team from Queensland Department of Environment and Resource Management (DERM) and the School of Veterinary Sciences, The University of Queensland. Clinical examination of 56 green turtles revealed that the juvenile turtle population from this region were generally in poor health, due most likely to chronic malnutrition (Eden *et al.*[, 2011\)](#page-78-2). Diseases of the digestive, respiratory, and circulatory systems were found and, in most cases, may have developed secondary to chronic debilitation. Spirorchiid fluke infection was the most commonly identified infectious agent on complete necropsy of 10 green turtles, with other infectious diseases diagnosed as fibropapillomatosis and bacterial gastroenteritis.

In parallel with the health assessment, a comprehensive contaminant exposure assessment was conducted for blood of live captured green turtles. Of interest were a range of inorganic and organic contaminants that may have been brought downstream from the catchment with flood waters or have arisen from industrial activities.

#### <span id="page-11-1"></span>**1.1 BLOOD AS EXPOSURE SURROGATE**

Blood has been demonstrated to be an appropriate matrix for assessing exposure to a broad range of chemical groups, including both organic and inorganic compounds [\(Hermanussen](#page-80-0) *et al.*, 2008[; van de](#page-85-0)  [Merwe](#page-85-0) *et al.*, 2010). Blood provides a logistically feasible, ethical and nonlethal option for exposure assessment of free ranging wildlife. Despite this, blood and tissue concentrations of contaminants are dependent on a number of factors that need to be considered when interpreting analytical results for exposure and risk assessment.

The contaminant's physico-chemical properties and its speciated ion (molecular form) affect the toxicokinetics (uptake, distribution, metabolism and excretion) in organisms. Persistent lipophilic contaminants are accumulated in body lipids, and their concentrations in blood, when normalised to a lipid basis, are typically comparable to those in other tissues [\(Hermanussen, 2009;](#page-80-1) [van de Merwe](#page-85-0) *et al.*[, 2010\)](#page-85-0). Thus, blood concentrations of persistent lipophilic contaminants can inform on tissue or body burdens, and long-term exposure regimens. Many metals and metalloids exist as different reduced and oxidised species, ranging from water-soluble ions to relatively lipophilic metalorganic compounds. While the more water soluble ionic species are mostly circulated through the body via the blood stream after absorption, they are often stored predominantly in the liver and kidney and can be rapidly eliminated through faeces or urine. Therefore, blood analysis often provides a snapshot of the most recent exposure to most metals and metalloids (in the order of days to months, depending on the element and speciation), while storage tissues can inform on longer term exposure regimens. Understanding of toxicokinetics of individual metals is thus particularly important for interpreting blood concentrations of metals [\(Grillitsch and Schiesari, 2010\)](#page-80-2). At constant exposure, the concentrations of such contaminants in blood and organs are often correlated, with blood containing considerably lower levels, except during initial phases of high-level exposure [\(Grillitsch and Schiesari,](#page-80-2)  [2010\)](#page-80-2). However, changes in exposure will be reflected rapidly in blood, with the levels depending on time of exposure relative to time of sampling (Day *et al.*[, 2010\)](#page-78-3).

An organism's trophic level, age, and breeding status can considerably affect the distribution and levels of many contaminants in tissues and blood. Concentrations of chemicals that are only poorly metabolised typically (at constant exposure) increase with age (bioaccumulation) until a steady state is reached where their rate of uptake is equal to the rate of metabolism or transformation. Such chemicals may accumulate over time to levels that may be harmful, even at relatively low exposure regimens (van den Berg *et al*., 2006). Some of these compounds also have strong tendencies to biomagnify through the food chain, whereby the highest trophic levels contain the highest concentrations. However, low trophic benthic feeders, such as green turtles, may take in substantial amounts of such contaminants sorbed to seagrasses or sediments [\(Hermanussen, 2009\)](#page-80-1).

Health and nutritional states are additional factors that may affect the toxicokinetics of contaminants in organisms [\(Eisler, 2007\)](#page-79-1). Nutrient deficient states and declining health of organisms can disturb contaminant equilibria through mobilisation of lipid stores and associated chemicals, and may influence the metabolic capacity of liver and kidneys, thus affecting storage, detoxification and elimination pathways. This is particularly relevant for the present study, which focused on an area where a large proportion of green turtles were found to be near or at emaciated states.

# <span id="page-13-0"></span>**2.0 OBJECTIVES AND SCOPE**

The present study focused on assessing contaminants in live green turtles collected from the Boyne River estuary, Gladstone, using mainly blood as anexposure surrogate. The objectives were:

- To quantify a range of contaminant groups that are known to bioaccumulate in marine wildlife and may present a hazard to green turtles in Gladstone
- To evaluate whether detected contaminant concentrations in green turtles from Gladstone are elevated and may present a risk to the turtle population.

Analysis was carried out using a tiered approach [\(Figure 1\)](#page-13-1) whereby pooled samples were initially screened for the presence of relatively high levels of nonpolar organic chemicals, in order to identify contaminant groups that should be covered. In a second tier, pooled samples were analysed for a broad range of known and potentially hazardous bioaccumulative pollutants to direct further prioritisation. Based on information from these screenings, individual samples were analysed in the third tier for compounds that may be elevated and/or have relatively high toxic potency.



<span id="page-13-1"></span>

To evaluate whether contaminants detected in the green turtle samples are elevated, a literature review was carried out to compare concentrations with those reported for other green turtles, and where necessary due to a lack of data for green turtles, other sea turtles, marine biota or reptiles, birds and mammals in general.

To evaluate whether contaminants present at elevated levels may present a risk to green turtles, reptile specific toxicological studies were reviewed and, where insufficient information was available, contaminant concentrations in green turtles from Gladstone were compared to effect concentrations across a range of vertebrate taxa. Where possible, green turtle contaminant body burdens were estimated using probabilistic approaches and compared to body burdens that elicit physiological effects in a dose-dependent manner, to estimate the proportion of green turtles that may be at risk of adverse effects. Where such approaches were not feasible, contaminant levels in green turtles were compared with available tissue based effect concentrations to identify whether adverse effects may be possible at the determined exposure levels.

# <span id="page-15-0"></span>**3.0 METHODOLOGY**

#### <span id="page-15-1"></span>**3.1 SAMPLING**

Green turtles (*Chelonia mydas*) were collected from the Boyne River estuary near Gladstone (-23.9 °S, 151.3 °E) during 8-11 July 2011. This population is characterised by turtles in poor health and associated elevated incidence of mortality. The samples were collected using best practice Australian standard procedures developed by DERM, and were stored at The University of Queensland School of Veterinary Science and Entox.

For contaminant analysis, blood samples were collected from 40 live green turtles [\(Table 1\)](#page-16-0). The animals were collected while basking on land (n=31) or captured using a rodeo technique (n=9) described in Limpus [\(1978\)](#page-82-0). All specimens underwent assessment as described in Limpus et al. [\(1994\)](#page-82-1) including measurements of size (curved carapace length, CCL) and body weight, as well as determination of age class (new recruit, juvenile, sub-adult or adult), gender and body condition, the latter informing on body mass for a given CCL (according to Limpus and Chalaupka [\(1997\)](#page-82-2)).

For contaminant analysis, 13-24 mL blood, depending on the individual's size and body condition and up to a maximum of 4% body weight, was collected from each turtle. Blood samples were taken from the dorso-cervical sinus using an 18 gauge 38 mm needle and 10-25 mL syringe. Whole blood was transferred to solvent-washed Schott bottles, with Teflon lined caps, containing 1.5 mL of heparinised saline (50 international units (IU)), and stored at -20°C until analysis.

Three of the animals were severely emaciated and moribund, with poor clinical diagnosis for survival; these were euthanized (by intravenous injection of sodium pentobarbitone (325 mg/mL)) by a registered veterinarian and necropsied at The University of Queensland's School of Veterinary Science. Necropsies included gross pathology and histopathology examinations, and the results are reported in Eden et al [\(2011\)](#page-78-2). Blood, liver, kidney and fat samples were collected from these three specimens [\(Table 1\)](#page-16-0).

Additional stranded animals were collected by Queensland Parks and Wildlife Service staff and underwent necropsy at the School of Veterinary Science, The University of Queensland, as described above. Liver and fat tissues were collected from six additional specimens and pooled, together with liver and fat from euthanized specimens described above, for analyses (n=9). Tissues were wrapped in aluminium foil and stored frozen at -20°C until analysis.

<span id="page-16-0"></span>**Table 1** Sample information for green turtles (*Chelonia mydas*) from Boyne River estuary near Gladstone, Queensland



ND No data

FP\* Fibropapilloma codes according to DERM classifications

### <span id="page-17-0"></span>**3.2 ANALYSES**

## <span id="page-17-1"></span>3.2.1 Description of tiered analysis approach

#### **TIER 1 - QUALITATIVE (NON-TARGET) SCREENING**

This analysis was undertaken as a non-target-screening for the purpose of identifying the presence of possible contaminants at high levels, to inform subsequent Tiers 2 and 3 [\(Figure 1\)](#page-13-1). Analysis was carried out on a high resolution gas chromatograph low resolution mass spectrometer at Eurofins GfA.

One gram of liver was pooled from stranded and euthanized specimens that underwent necropsy (n=9, including the three specimens EX2, 3, and 22 for which blood samples were also available; [Table 1\)](#page-16-0). Approximately 0.5 g homogenised sample was extracted with n-hexane using ultrasonication. The raw extract was then directly used for injection on an Agilent 6890/5973 GC-MS system using a non-polar DB5-type capillary column. An electron ionisation mode was used to scan a mass range of m/z 50-600. For evaluation, the 10 most abundant peaks were baseline subtracted and evaluated with the assistance of spectra libraries (Wiley 75K; NIST) by manual spectra interpretation and judgement for presence of artefacts or contaminants from the process.

#### **TIER 2 - QUANTITATIVE (TARGET) SCREENING**

Tier 2 screening comprised target chemical analysis using pooled samples of blood, liver and fat to provide initial information on the type and levels of contaminants to be expected, and thus estimation of minimum sample volume required for each contaminant group, as well as further prioritisation for Tier 3 analysis [\(Figure 1\)](#page-13-1).

For blood pools, 1 mL blood was sub-sampled and combined from each specimen (n=40). Liver and fat pools comprised of 1 g tissue, respectively, from each necropsied specimen (n=9). These pools underwent quantitative analyses for a set of contaminants listed in Table 2, except for metals and metalloids (which were analysed for each sample under Tier 3).

Pooled turtle fat was analysed for polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), dioxin-like polychlorinated biphenyls (WHO-PCBs) and a set of 7 indicator PCBs listed in [Table 2.](#page-23-0) Pooled turtle liver was analysed for organotins, polybrominated flame retardants (PBDEs), bioaccumulative pesticides and perfluorinated compounds. Pooled turtle blood was analysed for perfluorinated compounds. These analyses were carried out at an accredited laboratory according to standardised protocols which are described briefly below.

#### **TIER 3 - QUANTITATIVE TARGET ANALYSIS**

In the third tier, blood samples from individual turtles underwent quantitative target analyses for selected contaminant groups as prioritised based on the two screening Tiers (i.e. based on contaminant type and expected concentrations, taking into account toxic potency) [\(Figure 1\)](#page-13-1). In addition to blood, liver and kidney from euthanized specimens (n=3) were analysed for individual compound groups to evaluate tissue distributions and facilitate comparisons to literature data.

Individual blood samples were analysed for metals and metalloids (n=40), organotins (n=7), WHO-PCBs (n=22), PCDDs and PCDFs (n=22), and bioaccumulative pesticides (n=7). These analyses were carried out and evaluated on a batch-by-batch approach. Where Tier 1, 2 and 3 confirmed the presence of low levels, the limited volume for blood samples was prioritised for other analytes. Hence, a varying number of samples have been analysed for the different contaminant groups. Individual analytes for each of these groups are listed in [Table 2,](#page-23-0) and a brief description on the analytical methods, and associated quality assurance and quality control procedures are provided below.

Analysis for organic compounds were performed at Eurofins GfA in Hamburg, Germany, which is accredited for the determination of PCDD/F, PCB, chlorinated pesticides, PBDE and polyfluorinated compounds (PFC) in biological material in accordance with DIN EN ISO/IEC 17025:2005. Analysis for metals and metalloids was undertaken at the National Research Centre for Environmental Toxicology (Entox) according to standardised protocols.

#### <span id="page-18-0"></span>3.2.2 Trace element analysis

Analysis for the trace elements Al, As, Cd, Cr, Co, Cu, Fe, Pb, Mn, Hg, Mo, Ni, Se, Ag, V, and Zn was undertaken using inductively coupled plasma mass spectrometry (ICP-MS).

Samples were prepared according to in-house standardised protocols. Briefly, a subsample of 0.5 mL of whole blood was diluted to 10 mL with high purity MilliQ water (Millipore, Australia), and then vortex mixed and centrifuged to remove precipitate. Liver and kidney samples were freeze-dried and homogenised using a mortar and pestle. As tissue samples were stored in aluminium foil, the outer tissue was removed; nevertheless, cross-contamination with aluminium cannot be excluded. Aliquots of approximately 0.10 g homogenised tissue was then transferred into Teflon vessels and mixed with 1 mL of concentrated nitric acid (HNO3; 70% AR grade, BioLab (Aust) Pty Ltd). Tissue samples were then digested in a water bath at 60-70 °C for 4 to 6 hours until the solution was clear. After cooling down to room temperature, digested solutions were diluted (x50) with MilliQ water and filtered through  $0.45 \mu m$  filters prior to analysis.

Blood and tissue solutions were then spiked with an internal standard solution containing the elements Ge, Rh, Sc, Y, In and Bi (Agilent) to a final concentration in the samples equivalent to 10 g/L. Analysis and quantification was performed using an Agilent 7500CS ICP-MS equipped with a quartz torch, and a quartz double-pass spray chamber fitted with a Micro Flow nebulizer. Quantification was performed using the relative response of each trace metal to internal standards against an external 5-point calibration curve.

For quality assurance and quality control, duplicates, reagent blanks, blank spikes, analytical spikes were run with each batch of samples. Certified reference materials were analysed with each batch of samples to ensure accuracy; these included DORM-3 fish protein standard reference material (National Research Council, Canada), an in-house certified reference material (human blood reference material provided by Queensland Health and Forensic Scientific Services) and Seronorm L-1 and L-2 whole blood trace elements (SERO, Norway). The limit of quantification (LOQ) for each element was defined as three times the standard deviation of blank replicates (n=10) expressed in g/L. The LOQs for each element in blood and tissue samples ranged from 0.11 (As) to 5.76 (Fe) and

0.020 (Cr) to 22 (Fe) µg/L, respectively. Recovery was calculated using a triplicate analysis of certified reference material DORM 3 (for tissue samples) and were generally between 70% and 130%, which is considered acceptable for this analysis.

#### <span id="page-19-0"></span>3.2.3 Analysis for organotins

The organotin compounds monobutyltin (MBT), dibutyltin (DBT), tributyltin (TBT), tetrabutyltin (TTBT), monooctyltin (MOT), dioctyltin (DOT), triphenyltin (TPhT), tricyclohexyltin (TCHT) were analysed using high resolution gas chromatography low resolution mass spectrometry (HRGC-LRMS).

Prior to extraction, all samples were spiked with internal standard substances (monoheptyltintrichloride, diheptyltindichloride, tripropyltinchloride, tetrapropyltin). The samples were homogenized, mixed and conditioned over night with methanol and trimethyl ammoniumhydroxid, then buffered with an acetic acid/acetate buffer and extracted and simultaneously derivatized with hexane and sodium tetraethylborate. The hexane phase was used for clean-up by column chromatography on alumina, deactivated with 10% water and eluted with hexane. The cleaned extract was evaporated and tetrapentyltin was added as injection standard for the determination of recovery rates.

Analytical measurement was performed on an Agilent 6890/5973 HRGC-LRMS system with a DB-XLB fused silica column. Quantification of the organotin compounds was carried out via the internal standard method and based on daily instrument calibration.

For quality control, method blanks were run with each sample batch to monitor for possible background contamination. Reference materials (pooled samples) are regularly monitored and the laboratory participates in respective interlaboratory comparisons (e.g. QUASIMEME).

#### <span id="page-19-1"></span>3.2.4 Analysis for bioaccumulative pesticides

Target analytes for pesticides were o,p'-DDT, p,p'-DDT, α-HCH, β-HCH, γ-HCH (lindane), δ-HCH; three main toxaphene compounds (Parlar #26, #50 and #62), α-chlordane, γ-chlordane, oxychlordane, heptachlor, *cis*-heptachlor epoxide, *trans*-heptachlor epoxide, aldrin, dieldrin, endrin, α-endosulfan, β-endosulfan, endosulfan sulfate, mirex, hexachlorobenzene (HCB) and pentachlorobenzene. The analysis was carried out by high resolution gas chromatography high resolution liquid mass spectrometry (HRGC-HRMS), and high resolution gas chromatography tandem mass spectrometry (HRGC-MS-MS).

Tissue samples were homogenized, mixed with sodium sulphate to create a free flowing mixture, after which ultrasonic extraction was carried out with a mixture of *n*-hexane/acetone. Blood samples were extracted by a specialised liquid-liquid extraction with *n*-hexane, followed by *n*-hexane/*i*propanol. All samples were spiked with quantification standards (internal standards) before extraction using the following  $^{13}$ C-labeled compounds: β-HCH, γ-HCH, p,p'-DDT, p,p'-DDE, pentachlorobenzene, hexachlorobenzene, endosulfan sulfate, β-endosulfan, dieldrin.

Clean-up was performed by column chromatography applying a combination of columns with basic alumina and Florisil. Hexane was used for elution of the main fraction and toluene for a second fraction for endosulfan compounds which underwent an additional clean-up step using acetonitrile: hexane partitioning. The fractions were evaporated and  $^{13}$ C-PCB #105 was added as

injection standard for the analytes of the first fraction and <sup>13</sup>C-PCB #28 for the analytes of the second fraction. Analyses for compounds of the first fraction was performed by HRGC/HRMS on a Thermo DFS at mass resolution R  $\geq$  8,000 on a DB5-type fused silica column (60m x 0.25 mm i.d. x 0.25 µm dF). Endosulfan compounds were determined on an Agilent 7000 triple quadropole HRGC-MS-MS. Quantification was carried out by isotope dilution and internal standard methods against daily calibration points, together with a multipoint calibration.

For quality control, method blanks were run with each sample batch to monitor for possible background contamination. Reference materials (pooled samples) are regularly monitored and the laboratory participates in respective interlaboratory comparisons (e.g. AMAP).

# <span id="page-20-0"></span>3.2.5 Analysis for polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs)

Target analytes were the 17 2,3,7,8-substituted PCDD/Fs and the 12 dioxin-like PCBs (WHO-PCBs; PCB #77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189). Analyses were carried out using a high resolution gas chromatograph high resolution mass spectrometer (HRGC-HRMS).

Tissue samples were homogenised, mixed with sodium sulphate to create a free flowing mixture after which ultrasonic extraction was carried out with a mixture of n-hexane/acetone. Blood samples were extracted by a specialised liquid-liquid extraction with n-hexane followed by n-hexane/ipropanol. All samples were spiked with quantification standards (internal standards) prior to extraction using all PCDD/F and PCB analytes as  $^{13}$ C-labeled compounds (exception: 1,2,3,7,8,9-HexaCDD). The obtained raw extract was gently evaporated for fat determination and the yielded lipids were used for clean-up.

The clean-up consisted of a sulfuric acid treatment and a fractionation on active carbon for separation of PCDD/Fs and PCBs. This was followed by column chromatography with a combination of columns using silica modified with sulfuric acid, basic alumina (activity super I) and florisil. Elution was carried out with hexane, toluene and dichloromethane. The fractions were evaporated and a set of four  $^{13}$ C-PCDD/Fs and four  $^{13}$ C-PCBs were added as injection standards. Analytical measurement was performed by HRGC/HRMS on a Waters Autospec HRMS at mass resolution R ≥ 10,000 equipped with a DB5ms-type fused silica column (60m x 0.32mm i.d. x 0.25µm dF). Quantification was carried out by isotope dilution against daily calibration points together with a multipoint calibration.

For quality control, method blanks were run with each sample batch to monitor for possible background contamination. Reference materials (pooled samples) are regularly monitored and the laboratory participates in respective interlaboratory comparisons (e.g. Norway/ Norwegian Institute of Public Health).

Analytes were accepted for quantification if their retention times were within 2 seconds of the retention times of the relevant labelled internal standards and the ratios for the area of the two most abundant isotopes were within 20% of their calculated values. The limit of quantification for PCDD/F and PCB congeners was defined as a signal–to-noise ratio greater than 3 times the average baseline variation. Analytes were marked with '<' when the sample concentration did not exceed 3 times the concentration found in the batch blank. Toxic equivalencies (TEQs) were calculated using mammalian toxic equivalency factors (TEFs) adopted by the World Health Organisation [\(van den Berg](#page-85-1) *et al.*, [2006\)](#page-85-1), unless otherwise stated, and are reported using middle bound concentrations (i.e. half the concentration of the limit of quantification (LOQ) or values marked with a "<"), unless otherwise stated.

### <span id="page-21-0"></span>3.2.6 Analysis for polybrominated diphenyl ethers (PBDEs)

Target analytes for PBDEs were the congeners #17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, 196, 197, 206, 207, 209. The analyses were carried out using high resolution gas chromatography tandem mass spectrometry (HRGC-MS-MS).

Tissue samples were homogenised, mixed with sodium sulphate to create a free flowing mixture after which ultrasonic extraction was carried with a mixture of n-hexane/acetone. Blood samples were extracted by a specialised liquid-liquid extraction with n-hexane followed by n-hexane/ipropanol. All samples were spiked with isotope-labelled quantification standards prior to extraction using the six  $^{13}C_{12}$ -PBDEs #28, 47, 99, 153, 154, 183 and 209. The obtained raw extract was gently evaporated and the yielded lipids were used for clean-up. The clean-up consisted of a sulfuric acid treatment followed by column chromatography and fractionation on alumina, preconditioned with hexane and toluene, and eluted with dichloromethane. The eluate was evaporated and <sup>13</sup>C-HexaBDE #138 was added as injection standard. Analytical measurement was performed by HRGC/MS-MS on an Agilent 7000 with a Restek RTX1614 column (15m x 0.25 mm i.d. x 0.1  $\mu$ m dF). Quantification was carried out by isotope dilution against daily calibration points together with a multipoint calibration.

For quality control, method blanks were run with each sample batch to monitor for possible background contamination. Reference materials (pooled samples) are regularly monitored and the laboratory participates in respective interlaboratory comparisons (e.g. Norwegian Institute of Public Health and QUASIMEME).

#### <span id="page-21-1"></span>3.2.7 Analysis for perfluorinated compounds (PFCs)

Target analytes for PFCs were perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). The analyses were carried out using high performance liquid chromatography tandem mass spectrometry (HPLC-MS-MS).

The whole blood sample was homogenized and extracted using acetonitrile with ultrasonic extraction. The tissue sample was homogenized, mixed with sodium sulphate and extracted with acetonitrile. All samples were spiked with quantification standards (internal standards) before extraction using <sup>13</sup>C-labeled C8-PFOA and C4-PFOS.

Clean-up was performed by acetonitrile-hexane distribution and interference adsorption on activated carbon (Envicarb). After removal from the carbon the extract was evaporated, dissolved in methanol and  $^{13}C_4$ -PFOA was added as injection standard for monitoring of the quantification standard recoveries. Analytical measurement was performed on an Agilent Triple Quad 6460 LC-MS-MS system equipped with a 100 x 2mm Phenomenex Synergi 4u Fusion RP80A column. Mobile phase was methanol (0.05% acetic acid) and reagent water (2 mmol ammonium acetate), run with a gradient programme. Quantification was carried out by isotope dilution against multiple daily calibration points together with a multipoint calibration.

For quality control, method blanks were run with each sample batch to monitor for possible background contamination. Reference materials (pooled samples) are regularly monitored and the laboratory participates in respective interlaboratory comparisons (e.g. University of Erlangen).

#### <span id="page-22-0"></span>3.2.8 Statistical analyses

All statistical analyses were performed using XLSTAT Version 2012.2.01. Descriptive statistics (mean, maximum, minimum, standard error, percentiles and median) were determined for analytes and analyte groups, and are provided as box and whisker plots for metals and metalloids. A box plot combines multiple information that can be obtained from a group of data points. Box plots used in this report show the mean (red cross) and median (red line). The box represents the  $25<sup>th</sup>$  (bottom) and  $75<sup>th</sup>$  (top) percentiles and whiskers represent 1.5 times the inter quartile range (i.e. the difference between the 75<sup>th</sup> and 25<sup>th</sup> percentiles). All individual data points are also provided on these plots.

Nonparametric one-way analysis of variance was performed using the Kruskal Wallis test to evaluate statistically significant (p<0.05) differences of contaminant concentrations between turtles with very poor, poor and normal body conditions.

Correlations between % lipid, turtle weight, turtle size (CCL) and contaminant concentrations were tested using Pearson correlation coefficient with significance determined at p<0.05.

<span id="page-23-0"></span>**Table 2** List of target contaminant groups and individual analytes quantified under Tier 2 and Tier 3 analysis; note: organotin concentrations are reported on the basis of their organic forms as well as normalised to tin (Sn).



# <span id="page-24-0"></span>**4.0 RESULTS**

#### <span id="page-24-1"></span>**4.1 TURTLE BIOMETRICS AND HEALTH STATES**

Among the forty green turtles sampled for this study, 39 were in their juvenile, neretic life stage (average CCL 46; range 39-62 cm); the remaining specimen was an adult of unknown gender (CCL 100 cm) (Table 1). A large proportion of the animals (55%; n=22) were evaluated to have poor (35%; n=14) or very poor (20%; n=8) body conditions, with the latter showing signs of emaciation; three of these specimens were considered to have no chance of survival and were euthanized by a registered veterinarian (Table 1). The remaining 18 animals (45%) appeared to have normal body conditions. Accordingly, body weight was significantly (p<0.05) lower in green turtles with very poor (average 8.1; range 5.4-9.3 kg) and poor (average 9.8; range 8.0-22 kg), compared to normal (average 11; range 7.8-25 kg) body conditions; curved carapace length (CCL) did not differ significantly between these three groups. Despite this, no significant differences were observed for blood lipid content between animals with normal (0.14 ±0.038; range 0.087-0.20%; n=9), poor (0.13 ±0.047; range 0.070- 0.20%; n=5) or very poor (0.16  $\pm$ 0.064; range 0.062-0.23%; n=8) body conditions. This suggests that the blood lipids consisted mainly of fats not used for storage (e.g. lipoproteins, cholesterol).

### <span id="page-24-2"></span>**4.2 SCREENING**

#### <span id="page-24-3"></span>4.2.1 Tier 1 - Qualitative (non-target) screening

[Figure 2](#page-25-1) shows all signals obtained in the total ion count for the liver pool. No signals were identified that could be traced to halogenated compounds or other common environmental pollutants. Using the mass spectra, the most abundant peak was identified as the barbiturate pentobarbital (1), which originates from the use of pentobarbitone to euthanize specimens included in the liver pool. The phthalate, diisobutyl phthalate (2), was identified but possibly originates from the use of materials to collect the samples (e.g. syringe) or materials in contact with the sample (e.g. heparinised saline). The remaining peaks are associated with lipids and sterols naturally occurring in biological samples: several fatty acid derivatives (3-6), a derivative of the hydrocarbon squalene (7), and derivatives of the steroid cholestadien (8-10). Peaks 11a-e could not be identified but are likely to represent sterols or similar compounds.



<span id="page-25-1"></span>**Figure 2** Total Ion Count (TIC) GC-MS chromatogram of a green turtle liver pool (n=9). The 10 most abundant peaks (numbered) were identified based on the mass spectra of each peak.

## <span id="page-25-0"></span>4.2.2 Tier 2 – Quantitative (target) screening

Detailed results from Tier 2 analysis of pooled blood (n=40 individuals), liver (n=9 individuals) and fat (n=9 individuals) are presented in [Table 3,](#page-26-0) [Table 4](#page-26-1) and [Table 5.](#page-27-0) The lipid content in pooled carapace fat was determined to be 1.7% and the water content 89%; the water content of the liver pool was not determined, but averaged 78% (range 76-80%) in liver of three of the specimens included in this pool.

The concentrations for the majority of contaminant groups analysed in these pooled samples were below the limit of quantification (LOQ).

In pooled blood, middle bound total PFOS and PFOA levels were 100 ppb ww; upper bound concentrations were 200 ppb ww [\(Table 3\)](#page-26-0).

Middle bound concentrations for toxic equivalency (TEQ) of PCDD/Fs and PCBs in pooled fat were 6.1 ppt lw and 4.9 ppt lw, respectively [\(Table 4\)](#page-26-1). Respective upper bound estimates were 12 and 22 ppt lw. The middle and upper bound concentrations for sum indicator PCBs in pooled fat were 13,000 and 25,000 ppt lw, respectively [\(Table 4\)](#page-26-1).

The middle to upper bound concentration ranges for sum tri- to deca-brominated flame retardants (PBDEs) and sum organotins in pooled liver were 2.4 to 3.0 ppb dw (approx. 0.53 to 0.66 ppb ww) and 29 to 58 ppb dw (approx. 6.4 to 13 ppb ww), respectively, while perfluorinated compounds were present at 0.65 to 0.70 ppb dw (approx. 0.14 to 0.15 ppb ww) [\(Table 5\)](#page-27-0).

<span id="page-26-0"></span>**Table 3** Concentrations of perfluorinated compounds (PFOS/PFOA; ppb ww) in pooled green turtle (*Chelonia mydas*) blood (n=40) from Boyne River estuary near Gladstone, Queensland. Water content approximately 89%.



<span id="page-26-1"></span>**Table 4** Concentrations of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F; ppt lw), WHO-PCBs (ppt lw) and indicator PCBs (ppt lw) in pooled green turtle (*Chelonia mydas*) fat (n=9) from Boyne River estuary near Gladstone, Queensland. Lipid content 1.7%.



< Below the limit of quantification (LOQ)

< Below the limit of quantification (LOQ)

PCB 138 <4400 PCB 153 <4700 PCB 180 <3400

SIndicator PCBs (Lower) <LOQ SIndicator PCBs (Middle) 13000 SIndicator PCBs (Upper) 25000 <span id="page-27-0"></span>**Table 5** Concentrations of brominated flame retardants (PBDEs; ppb dw), organotins (ppb dw), perfluorinated compounds (PFOS/PFOA; ppb dw) and bioaccumulative pesticides (ppb dw) in pooled green turtle (*Chelonia mydas*) liver (n=9) from Boyne River estuary near Gladstone, Queensland. Water content approximately 78%.





< Below the limit of quantification (LOQ)



< Below the limit of quantification (LOQ)



< Below the limit of quantification (LOQ)

#### <span id="page-28-0"></span>4.2.3 Tier 3 – Quantitative target analysis

[Table 6,](#page-29-0) [Table 7](#page-30-0) and [Table 8](#page-31-0) provide a summary of the mean, minimum, maximum and median concentrations, as well as other descriptive statistics, for PCDD/Fs, PCBs, bioaccumulative pesticides, organotins and metals and metalloids analysed in blood of individual green turtles from Boyne River estuary. [Table 9](#page-31-1) provides mean, minimum and maximum concentrations for metal and metalloid concentrations obtained in liver and kidney samples.

The mean lipid content in blood was determined to be 0.15% (n=22, range 0.062-0.23%). In liver, the mean water content was 78% (range 76-80%) and in kidney 88% (range 85-92%).

Combined blood TEQ levels for PCDD/Fs and PCBs (TEQ $_{df+pcb}$ ) in Gladstone green turtles ranged from 7.1-130 ppt lw on a mammalian TEF basis (middle bound). Using avian TEFs, the levels were 13-120 ppt lw. The highest blood TEQ<sub>df+pcb</sub> levels were found in the adult turtle blood (130 ppt lw, n=1). For the juvenile samples ( $n=21$ ), the mean middle bound TEQ $_{df+pcb}$  levels were 19 (range 7-39) and 33 (range 13-62) ppt lw using mammalian and avian TEFs, respectively.

For four blood samples, concentrations of some individual PCDD/F and PCB congeners could not be quantified due to analytical interferences in the chromatograms, partially due to low sample volumes. Reported TEQs would potentially be appreciably understated if these congeners were not included in the TEQ calculation. Consequently, predicted values for these congeners were determined and are reported in brackets in [Table 6,](#page-29-0) and in [Table 10](#page-87-0) and Table 11 in Appendix [8.1.](#page-86-1) A consistent relative contribution for each PCDD/F and PCB congener (i.e. congener profile) across all (complete) blood samples for juvenile green turtles was observed, and this common profile was used to predict the missing congener values.

Analytes of bioaccumulative pesticides and organotins were mostly below the limit of quantification, or were present at relatively low concentrations in blood of green turtles. Results for all analytes in these groups and for each individual turtle are provided in [Table 7,](#page-30-0) and in Table 12 and Table 13 in Appendix [8.1.](#page-86-1)

Total metal and metalloid concentrations were highly variable in blood of green turtles, often ranging over 2 (maximum 3) orders of magnitudes for individual elements [\(Table 8;](#page-31-0) [Figure 3\)](#page-32-0). For three turtles, blood, liver and kidney concentrations were determined for each individual (Ex 2, 3 and 22; [Table](#page-31-1) 9). Concentrations of most metals and metalloids were, as expected, lower in blood compared to the matched liver or kidney samples, but concentrations of arsenic (As), iron (Fe) copper (Cu), selenium (Se) and lead (Pb) in blood were similar (within the same order of magnitude) or higher compared to those present in matched kidney (and for As also liver) samples. The concentrations for all analytes in this group are summarised in [Table 8](#page-31-0) and [Table 9](#page-31-1) and are listed for each individual sample in Table 14 and 15 in Appendix [8.1.](#page-86-1)

<span id="page-29-0"></span>**Table 6** Summary (descriptive statistics) of concentrations of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F; ppt lw) and WHO-PCBs (ppt lw) in blood from individual (n=22) green turtles (*Chelonia mydas*) from Boyne River estuary near Gladstone, Queensland.



their LOQ

< Below the limit of quantification (LOQ); (<) Predicted value (see text)

<span id="page-30-0"></span>**Table 7** Summary (descriptive statistics) of concentrations of bioaccumulative pesticides (ppb ww) and organotins (ppb ww) in blood from individual (n=7) green turtles (*Chelonia mydas*) from Boyne River estuary near Gladstone, Queensland.



< Below the limit of quantification (LOQ)

NA Not applicable

<span id="page-31-0"></span>**Table 8** Summary (descriptive statistics) of concentrations of metals and metalloids (ppb ww) in blood from individual (n=40) green turtles *(Chelonia mydas)* from Boyne River estuary near Gladstone, Queensland.



ND not determined

< Below the limit of quantification (LOQ)

<span id="page-31-1"></span>**Table 9** Summary (descriptive statistics) of concentrations of metals and metalloids (ppm ww) in liver and kidney from individual (n=3) green turtles (*Chelonia mydas*) from Boyne River estuary near Gladstone, Queensland.



ND No data



<span id="page-32-0"></span>**Figure 3** Box and whisker plots for metal and metalloid concentrations (ppb ww) in blood from individual (n=40) green turtles (*Chelonia mydas*) from Boyne River estuary near Gladstone, Queensland. Box plots show the mean (red cross) and median (red line), the 25<sup>th</sup> (bottom of box) and  $75<sup>th</sup>$  (top of box) percentiles, and 1.5 times the inter quartile range (whiskers).

# <span id="page-33-0"></span>**5.0 DISCUSSION**

# <span id="page-33-1"></span>**5.1 CONTAMINANT LEVELS IN RELATION TO TURTLE BIOMETRICS, HEALTH AND SAMPLING LOCATION**

Among the forty individual green turtles included in the present study, 39 were juveniles, thus minimising variation of bioaccumulative contaminant levels due to organism age or breeding status. Across the bioaccumulative contaminant groups analysed, only dioxin concentrations were clearly higher in the adult specimen compared to juveniles (see discussion below). Other contaminant groups that were detected and known to bioaccumulate with age (PCBs, mercury, arsenic, cadmium, lead, selenium, silver), were often lower, or similar in the adult specimen compared to most juveniles. This may be due to a) different long-term feeding habitats of the adult, or b) recent increases in the exposure regimen for metals/metalloids.

A large proportion (55%) of the animals sampled presented with poor (35%) or very poor (20%) body conditions, and associated deficient nutritional and general poor health states. Despite this, no clear and consistent differences were observed in organic contaminant levels between animals with different body conditions. Although mobilisation of lipids and associated lipophilic contaminants are expected in these specimens, blood lipid content did not differ among animals of different body conditions and, although generally low compared to other sea turtles, percent lipid in blood was comparable to live captured green turtles in Moreton Bay (average 0.18; range 0.080-0.55%; n=35) [\(Hermanussen, 2009\)](#page-80-1). Concurrent with this, no significant difference was observed between the levels of lipophilic contaminants (which were mostly present at relatively low levels) in blood of Gladstone green turtles with different body conditions.

Among metal and metalloids, Cr, Fe, Mn, Ni, and Zn levels in blood were significantly (p<0.05) lower in green turtles that were classified with very poor compared to those with normal body conditions. Similar trends have previously been reported for loggerhead turtles, and were hypothesised to relate to a lack of feeding, and thus lower metal exposure, for some time prior to sampling (Day *[et al.](#page-78-3)*, [2010\)](#page-78-3). These metals (Cr, Fe, Mn, Ni, Zn) have among the fastest clearance rates from blood with halflives in the order of 2-48 hours ([\(Farheen](#page-79-2) *et al.*, 2002) for Fe; [\(ATSDR, 2008b\)](#page-77-0) for Cr; [\(ATSDR, 2005a\)](#page-76-1) for Ni; and [\(ATSDR, 2005b\)](#page-77-1) for Zn). Since the green turtles presenting with very poor body conditions are likely to have stopped or reduced feeding for prolonged periods, it is feasible to assume associated reduced metal uptake and rapid decrease in blood concentrations of metals with such short half-lives. Metals that generally have longer half-lives and relatively slow blood clearance rates in vertebrates (Hg, Cd, and V) were present at similar concentrations in turtles across all three body conditions. These results may thus reflect the varying toxicokinetics of individual element species and forms in combination with the animals' exposure levels and cessation of exposure relative to the time of sampling.

Juvenile green turtles recruit to neretic feeding grounds at around 40-50 cm CCL (Limpus and Limpus, 2003). Satellite tracking studies indicate that green turtles display high site fidelity to foraging grounds, undertaking short term movements of 2-24 km [\(Limpus, 2008\)](#page-82-3). Upon reaching maturity, adults migrate to their breeding sites, but return to the same feeding area home range (Limpus et al 1992). Considering these studies, the contaminant levels in juvenile green turtles of this study are likely associated with exposure in the local area from which they were sampled.

# <span id="page-34-0"></span>**5.2 CONTAMINANT EXPOSURE CONCENTRATIONS AND RISK EVALUATION**

Contaminant exposure and associated risks of adverse effects are ideally assessed by integrating spatial and temporal data on the exposure with detailed understanding on the mechanisms and dose-dependent effect measures. Realistically, exposure is often unknown, and effect doses, which vary among species, are only investigated for few, mostly laboratory test animals. Regardless of the complexities and existing gaps, however, evaluations can be carried out using exposure surrogates (e.g. analysis of blood) in combination with information on toxicokinetics (uptake, metabolism, elimination) and effects, which, to some degree, can be extrapolated across species. Such evaluations inherently carry uncertainties, but provide a reasonable preliminary basis to assess whether the estimated exposure may be of concern [\(Hermanussen, 2009;](#page-80-1) [Grillitsch and Schiesari,](#page-80-2)  [2010\)](#page-80-2).

While reptiles are generally underrepresented in toxicological studies [\(Grillitsch and Schiesari, 2010\)](#page-80-2) several publications are available to provide a comparative basis on tissue based exposure concentrations of many contaminant groups in sea turtles, including green turtles (reviewed in [\(Eisler, 2010;](#page-79-3) [Grillitsch and Schiesari, 2010\)](#page-80-2)). These studies indicate that, similar to vertebrates, tissue levels in sea turtles parallel the degree of environmental contamination (Day *et al.*[, 2005;](#page-78-4) [Hermanussen, 2009;](#page-80-1) [Grillitsch and Schiesari, 2010\)](#page-80-2). Particularly for metals and metalloids, however, it is difficult to establish what constitutes non-elevated, normal background levels in sea turtles due to a) the spatial variability of naturally occurring elements, b) a general bias in the literature on stranded and/or moribund sea turtles or live captured specimens from areas near agricultural, urban and industrial sources, and c) relatively few comparable blood based concentrations in combination with the rapid clearance rates of most metals and metalloids in blood. Available studies also indicate that key uptake pathways, systemic transport, distribution and elimination routes of many contaminants are similar in reptiles compared to those known for other vertebrates [\(Hermanussen,](#page-80-1)  [2009;](#page-80-1) [Grillitsch and Schiesari, 2010\)](#page-80-2), and that adverse effects are possible in sea turtles at elevated exposure levels experienced by wild specimens (Day *et al.*[, 2007;](#page-78-5) [Grillitsch and Schiesari, 2010\)](#page-80-2). In the absence of reptile specific data for most contaminants, extrapolation from across vertebrate taxa (fish, birds, mammals) is thus a commonly used approach [\(Grillitsch and Schiesari, 2010\)](#page-80-2).

For the present study, exposure was assessed using predominantly blood, and where available, matched tissue concentrations in comparison to those reported for other green turtles (Appendi[x 8.2](#page-93-0) and Section [5.3\)](#page-37-0). Where insufficient data was available, data from other sea turtles, and organisms were used to evaluate whether contaminant tissue levels may be elevated, taking into account the potential for contaminant biomagnification through the food chain. To determine whether exposure may represent a risk to the study population, blood and tissue levels or, where possible, estimated body burdens were compared to blood, tissue or body burden based effect concentrations reported for reptiles, and across other vertebrate taxa (see Section [5.3\)](#page-37-0). Note that comparisons of the measured blood or tissue concentrations to oral doses (e.g. lowest-observed adverse effect levels) was not possible, as very limited species/reptile-specific data is available in the literature, and blood or tissue concentrations are not comparable to dose effect concentrations across taxa; thus, comparisons focus on tissue based effect levels. Based on these comparisons, contaminants were classified into the following three categories:

#### <span id="page-35-0"></span>5.2.1 Contaminants of relatively low concern

Overall, the samples analysed for the present study contained relatively low levels of bioaccumulative pesticides, organotins, flame retardants (PBDEs), and perfluorinated compounds (PFOS/PFOA). The concentrations of these contaminant groups in fat, liver and/or blood samples were mostly near or below the limit of quantification (LOQ) [\(Table 3,](#page-26-0) [Table 5,](#page-27-0) [Table 7,](#page-30-0) Table 12, Table 13). Comparisons to the literature indicate that these concentrations are similar to background levels reported in other green turtles and sea turtles [\(Hermanussen](#page-80-0) *et al.*, 2008; [Swarthout](#page-85-2) *et al.*, 2010; [Malarvannan](#page-82-4) *et al.*, 2011), or other marine megafauna, and considerably lower than the levels that are considered elevated or of concern in such wildlife [\(Kannan](#page-81-0) *et al.*, 2000; Keller *et al.*[, 2004a;](#page-81-1) [Ross](#page-84-1) *et al.*[, 2007;](#page-84-1) Orós *et al.*[, 2009;](#page-83-0) [van de Merwe](#page-85-3) *et al.*, 2009). Where detected, the concentrations of these contaminants in tissue or blood therefore most likely represent background levels, and the risk of adverse effects associated with each of these compounds in the study population is considered relatively low; no further review was therefore conducted

Concentrations of aluminium (Al), iron (Fe), manganese (Mn) and zinc (Zn) were present in blood and tissues (except for Al in blood, which could not be quantified) that were comparable to those reported for many other sea turtles, including from apparently healthy specimens and locations considered to be relatively unimpacted by local urban, agricultural or industrial point sources (Appendix [8.2\)](#page-93-0). The concentrations of these elements also generally fall within normal ranges observed in other marine megafauna, and are thus considered likely to represent normal background levels of little hazard to the study population; no further review was thus conducted.

In this context, it has to be noted that the presence of low levels of complex chemical mixtures may result in chronic biochemical and/or physiological changes that can adversely affect organisms, even if individual contaminants are below their respective threshold levels. However, current scientific understanding is insufficient to quantitatively assess such mixture effects in most biota, particularly megafauna such as sea turtles.

## <span id="page-35-1"></span>5.2.2 Contaminants possibly of concern

Concentrations of silver (Ag), copper (Cu), chromium (Cr), molybdenum (Mo) and lead (Pb) in blood and for Cu also in liver and kidney were higher than those reported for most other sea turtles and other vertebrates, but comparable to the upper range reported from moribund green turtles and specimens from relatively polluted areas (Appendix 8.2). Compared to other vertebrates, these levels appear to be elevated. Toxicological information for other organisms, where available, indicates tissue-based concentrations for acute effects are considerably higher, but information on chronic effects was lacking. Thus, there was insufficient information to assess whether these elements pose a risk of effects to the turtle population at the exposure levels in Gladstone.

Dioxin and PCB toxic equivalencies (TEQ) in blood of juvenile green turtles from Gladstone were comparable to the lower ranges reported for other green turtles (Appendix 8.2) and similarly low trophic marine mammals (dugongs). However, the adult specimen contained elevated TEQ levels
(mainly due to elevated dioxins, rather than PCBs), comparable to the upper ranges reported for turtles and dugongs, and other, higher trophic marine wildlife. This may indicate chronic exposure to elevated levels of dioxins, rather than elevated recent exposure, but more adult specimens would be required to evaluate this. Risk assessment based on estimated body burdens suggests that TEQ levels in 6.6% of the juvenile green turtles are above adverse effect levels (LOAELs) where biochemical effects occur in mammals; these may or may not be harmful to the animals. Using upper bound TEQs (i.e. a worst case scenario), the estimated body burden of up to 5.0% of the juvenile green turtles are above the LOAELs for chronic developmental toxicity in avian species. While only one adult specimen was obtained, it is noteworthy that the estimated body burden in this specimen exceeds the LOAEL for immunological and developmental effects in both mammals and birds. The risk assessment and results are discussed in more detail below (Sectio[n 5.3.10\)](#page-70-0).

# 5.2.3 Contaminants of concern

For several metals/metalloids (arsenic (As), cadmium (Cd), cobalt (Co), mercury (Hg), nickel (Ni), selenium (Se), and vanadium (V)), blood and/or tissue concentrations were clearly higher in green turtles from Gladstone compared to those reported for most other green turtles (and for nonbiomagnifying compounds other sea turtles or vertebrates), or within the upper levels reported for specimens that were moribund and/or originated from areas considered relatively polluted (Appendix 8.2). In addition, these elements were present at higher levels compared to normal background concentrations known for other vertebrates, including marine megafauna. In many cases, elevated levels were present in blood, rather than in matched liver or kidney samples. Based on the relatively rapid blood clearance rates of many of these elements, this suggests that exposure may have occurred relatively recent prior to sampling (days to months, depending on the element and level of exposure), rather than via chronic accumulation. Concentrations of Hg, Cd and Se were above or within the levels where adverse effects have been suggested to occur in reptiles. The concentrations of the remaining metals/metalloids (As, Co, Ni, and V) were above, near or within tissue-based concentrations where acute effects have been observed in other vertebrates (birds and mammals). Although the sensitivity of sea turtles to these elements is unknown, this suggests that acute adverse effects from exposure of green turtles in Gladstone are possible, and chronic effects may be expected if exposure persists.

These elements are discussed individually in Section [5.3.](#page-37-0) These sections include a brief background on sources, fate and toxicokinetics, a detailed comparison of blood and tissue levels among green turtles, sea turtles and other organisms, and a review of studies on toxicology and effects, including a summary of available information on tissue based effect concentrations.

# <span id="page-37-0"></span>**5.3 REVIEW - CONTAMINANTS OF CONCERN**

# 5.3.1 Arsenic (As)

#### **SOURCES**

Arsenic is a metalloid that occurs naturally in trace quantities in rock, soil, water and air. Arsenic exists as four, but commonly only as three valency states (0 (elemental), +III (arsenite), +V (arsenate)), and as numerous species in inorganic (combined with oxygen, chlorine, sulfur) and organic (combined with carbon and hydrogen) form, which vary in their properties (e.g. water solubility) and toxicities. Mining, smelting of non-ferrous metals, and burning of fossil fuels are the key industrial processes that contribute to arsenic contamination of air, water and soil [\(Gomez-](#page-79-0)[Caminero](#page-79-0) *et al.*, 2001). Depending on the level of industrialisation, significant quantities may also be released by wastewater runoff derived from e.g. atmospheric depositions, residues from pesticide usage, phosphate detergents and industrial effluent, particularly from the metal-processing industry [\(Gomez-Caminero](#page-79-0) *et al.*, 2001). Arsenic has been widely used in wood treatment as copper chromate arsenate (CCA) and arsenic-containing pesticides were historically used primarily in cotton and orchards [\(Gomez-Caminero](#page-79-0) *et al.*, 2001; [ATSDR, 2007\)](#page-77-0). Typically, key arsenic input pathways to marine environments are river runoff and atmospheric deposition [\(Sanders, 1980;](#page-84-0) [Gomez-Caminero](#page-79-0) *et al.*[, 2001\)](#page-79-0).

#### **TOXICOKINETICS**

Organisms are exposed to many different forms of inorganic and organic arsenic species in food, water, air, soil and sediments. Approximately 25% of arsenic in human food is inorganic, but levels in fish or shellfish are low (<1%). Due to the different properties of arsenic species, including their varying bioavailability and considerable interspecies differences in metabolism, the toxicokinetics of arsenic is highly complex [\(Gomez-Caminero](#page-79-0) *et al.*, 2001).

Arsenic is rapidly cleared from blood (within hours to days, depending on the organism, arsenic forms and dose) [\(Gomez-Caminero](#page-79-0) *et al.*, 2001; [ATSDR, 2007\)](#page-77-0). Blood and urine thus serve as useful markers for very recent acute or stable, chronic, high-level exposure. Keratin rich tissues (e.g. scutes of the shell in turtles) can, in contrast, be used as indicators of past arsenic exposure [\(Gomez-](#page-79-0)[Caminero](#page-79-0) *et al.*, 2001).

In general, organisms can be exposed to arsenic via inhalation, ingestion of contaminated food or water, and dermal exposure. As(III) and As(V) are also known to readily cross the placenta in laboratory animals and humans [\(Gomez-Caminero](#page-79-0) *et al.*, 2001). Oral bioavailability in laboratory animals varies widely (5-85%), depending on the dose, matrix, arsenic form and animal species. Dermal absorption of organoarsenic chemicals are in the order of 3-40%. Soluble arsenates (arsenobetaine, monomethyl arsenic (MMA) and dimethyl arsenic (DMA)) and arsenates are rapidly and extensively (near complete) absorbed from the gastrointestinal tract in laboratory animals and most forms are excreted primarily via urine [\(Gomez-Caminero](#page-79-0) *et al.*, 2001; [ATSDR, 2007\)](#page-77-0). In general, organoarsenicals are less extensively metabolized than inorganic arsenic, but more rapidly eliminated in both laboratory animals and humans. After exposure, arsenic is transported in blood and distributed to liver, kidney, spleen and lung. Several weeks after exposure, arsenic is

translocated to ectodermal tissues (hair, nails) because of the high concentration of sulfur-containing proteins in these matrices [\(Eisler, 1988\)](#page-78-0). Aquatic organisms, particularly marine plants, can accumulate organic arsenic species, but biomagnification has not been observed in the aquatic food chain [\(Eisler, 1988;](#page-78-0) [Gomez-Caminero](#page-79-0) *et al.*, 2001[; ATSDR, 2007\)](#page-77-0).

# **EXPOSURE CONCENTRATIONS IN GLADSTONE GREEN TURTLES**

Arsenic concentrations in blood of green turtles from Gladstone were highly variable, ranging from 40 ppb to 20,000 ppb ww (mean: 2,300 ppb ww). To date, only few reports exist on arsenic in blood of sea turtles and similarly high levels (94-20,000 ppb ww; mean 4,400 ppb; SE ±1400; n=16) have been reported in green turtles from Moreton Bay or other areas in southeast Queensland [\(van de](#page-85-0)  [Merwe](#page-85-0) *et al.*, 2010). However, the latter study was focused on severely debilitated turtles which died at the SeaWorld rehabilitation program and may have originated from relatively contaminated zones [\(van de Merwe](#page-85-0) *et al.*, 2010). In contrast, arsenic levels in blood from green turtles from San Diego Bay (average  $160 \pm 26$  ppb ww; n=30) are an order of magnitude lower, despite its highly urbanised sources and routine dredging activities [\(Komoroske](#page-81-0) *et al.*, 2011). Similarly, urine (an appropriate marker for recent exposure) of green turtles from Japan contained arsenic levels of 900 ppb ww (n=2) (Agusa *et al.*[, 2011\)](#page-76-0), which is comparable to blood of clinically healthy loggerhead turtles from the Mediterranean (average 770; range 230-2,600 ppb ww; n=5) (Jerez *et al.*[, 2010\)](#page-80-0). Much lower background blood As concentrations have been reported in four Amazon river turtle species (averages 1.3, 3.5, 3.8, 4.9 ppb ww; n=60) [\(Burger](#page-77-1) *et al.*, 2009) which are similar to those considered normal for non-exposed humans, (<1 ppb) [\(ATSDR, 2007\)](#page-77-0).

It is widely known that marine organisms, especially plants, shellfish and fish but also some higher trophic marine mammals and seabirds (Kubota *et al.*[, 2003a\)](#page-81-1) naturally accumulate higher quantities of particular arsenic compounds, and thus contain higher total arsenic levels compared to terrestrial biota. Typically, the predominant arsenic species accumulated in marine organisms, including marine mammals, are water soluble organic forms, particularly arsenosugars and arsenobetaine, respectively [\(Gomez-Caminero](#page-79-0) *et al.*, 2001; Kunito *et al.*[, 2008\)](#page-81-2), which have much lower toxic potency compared to inorganic and other organic forms. Based on arsenic speciation studies it has, however, been suggested that in addition to arsenobetaine, both green turtles and dugongs (who share common food sources and habitats) may accumulate higher proportions of lipid soluble and/or As(III) compounds such as MMA(III) and DMA(III). Arsenobetaine was for example found to be a minor constituent in dugongs (n=4/4), who contained MMA at a relatively high portion ( $\approx$ 40%) of total arsenic (Kubota *et al.*[, 2003a\)](#page-81-1). As(III) was detected in green turtles [\(Styblo](#page-84-1) *et al.*, 2000; [Kubota](#page-81-3) *et al.*[, 2003b;](#page-81-3) Kubota *et al.*[, 2003a\)](#page-81-1), including in liver and urine (Agusa *et al.*[, 2011\)](#page-76-0), and relatively high proportions of MMA(III) (1.9%; n=3/5), DMA(III) (5.2%; n=5/5), dimethylarsinic acid (6.6%; n=5/5) and tetramethylarsonium ion (1%; n=4/5) were quantified in addition to arsenobetaine in green turtle liver (Kubota *et al.*[, 2003b;](#page-81-3) Kubota *et al.*[, 2003a\)](#page-81-1). Considering such unusual accumulation patterns, adverse effects on turtle biological system are considered possible at elevated arsenic levels [\(Kubota](#page-81-4) *et al.*[, 2002\)](#page-81-4).

In addition to blood, arsenic was analysed in liver and kidney from three euthanized green turtles (EX2, EX3 and EX 22; see [Table 1\)](#page-16-0) from Gladstone. Concentrations in these tissues were 2.0, 2.0 and 2.0 ppm ww and 1.2, 1.5 and 0.88 ppm ww, respectively. These concentrations are comparable to

average levels reported from stranded or moribund green turtle liver and kidney from southeast Queensland (3.2 and 2.7 ppm ww, respectively; [\(van de Merwe](#page-85-0) *et al.*, 2010)). Similar levels have also been reported in green turtle liver and kidney from Torres Strait (1.5 and 0.42 ppm ww, respectively; [\(Gladstone, 1996\)](#page-79-1)), Turkey (2.1 and 1.7 ppm ww; (Kaska *et al.*[, 2004\)](#page-81-5)), Japan (0.38-1.2 and 1.6-2.1 ppm ww, respectively; (Agusa *et al.*[, 2008\)](#page-76-1)) and China (1.0 and 0.85 ppm ww, respectively; [\(Lam](#page-82-0) *et al.*[, 2004\)](#page-82-0)). A recent study reported relatively low (0.67 ±0.019 ppm ww; n=20) arsenic levels in eggs of flatback turtles collected on Curtis Island off Gladstone in 2006, which are considered to reflect the exposure of the laying adults [\(Ikonomopoulou](#page-80-1) *et al.*, 2011), however the transfer efficiency of arsenic to eggs is unknown. Notably, the blood arsenic concentrations in the three euthanised specimens from Gladstone were below the average (1100, 570, and 650 ppb ww, respectively), and assuming a similar relationship between blood, liver and kidney concentrations as reported in green turtles from southeast Queensland [\(van de Merwe](#page-85-0) *et al.*, 2010), liver concentrations in some live turtles from Gladstone may be several fold higher (up to 9.7 ppm ww in liver were observed in turtles with blood arsenic levels of 20,000 ppb; [\(van de Merwe](#page-85-0) *et al.*, 2010)).

For context, background arsenic levels in tissue of most other living animals are usually <1 ppm ww, including humans [\(Eisler, 1988;](#page-78-0) [Gomez-Caminero](#page-79-0) *et al.*, 2001). Low levels have also been reported for alligator and crocodile eggs (0.05-0.2 and 0.2 ppm ww, respectively; [\(Eisler, 1988\)](#page-78-0), and most marine mammals contain generally <1 ppm in liver and muscle (Muir *et al.*[, 1988;](#page-83-0) [Varanasi](#page-85-1) *et al.*, [1994;](#page-85-1) [Neff, 1997;](#page-83-1) [Gomez-Caminero](#page-79-0) *et al.*, 2001; [Stavros](#page-84-2) *et al.*, 2011; [Poulsen and Escher, 2012\)](#page-83-2). However, pinnipeds can contain arsenic up to 1.7 ppm ww [\(Eisler, 1988\)](#page-78-0). Among the highest arsenic concentration recorded in marine mammals was 2.8 ppm ww in lipid of a cetacean from Norway [\(Eisler, 1988\)](#page-78-0).

#### **TOXICITY AND EFFECTS**

Different organ systems can be affected by arsenic, including skin, respiratory, cardiovascular, immune, genitourinary, reproductive, gastrointestinal and nervous systems [\(Gomez-Caminero](#page-79-0) *et al.*, [2001\)](#page-79-0). Symptoms of toxication can include gastrointestinal disorders, hepatic and renal failure, disturbances of cardiovascular and nervous system functions, and eventually death. Chronic exposure to arsenic is linked to increased risks of cancer in the skin, lungs, bladder and kidney, as well as other skin changes such as hyperkeratosis and pigmentation changes. There is some evidence for arsenic to cause hypertension and cardiovascular disease, diabetes, reproductive effects, cerebrovascular disease, long-term neurological effects, and cancer at sites other than lung, bladder, kidney and skin [\(Gomez-Caminero](#page-79-0) *et al.*, 2001).

Both inorganic and organic forms of arsenic can cause adverse effects in organisms, however, the degree of toxicity varies with speciation and oxidation state (valency). Generally, water soluble inorganic arsenic species are more toxic than organic forms, and within these two classes, the trivalent (III) arsenites tend to be more toxic than the pentavalent (V) arsenates [\(ATSDR, 2007\)](#page-77-0). For example, the lethal dose (LD50) values (oral administration to mice) range from approximately 8 ppm (As(III), 21 ppm As(V)), 580 ppm (tetramethylarsonium chloride), 1800 ppm (MMA and DMA), to >10,000 ppm (arsenobetaine) [\(Gomez-Caminero](#page-79-0) *et al.*, 2001; [ATSDR, 2007\)](#page-77-0). However, it was also reported that dimethylarsinic acid has cytotoxicity (Ochi *et al.*[, 1999;](#page-83-3) Kubota *et al.*[, 2003b\)](#page-81-3) and genotoxicity [\(Mass and Wang, 1997;](#page-82-1) [Yamanaka](#page-85-2) *et al.*, 1997; Kubota *et al.*[, 2003b\)](#page-81-3). Furthermore,

toxicity of methylarsonous acid and di-methylarsinous acid, metabolites of methylarsonic acid and dimethylarsinic acid, respectively, is comparable or higher than that of arsenite (Styblo *et al.*[, 2000;](#page-84-1) Kubota *et al.*[, 2003b\)](#page-81-3). In addition, different biota exhibit a range of sensitivities to different arsenic species, which is modified by numerous biological and environmental factors, particularly in the aquatic environment [\(Eisler, 1988\)](#page-78-0). In marine fish, the water based LC50 (96-hours) range from 13-29 ppm for As(III). In birds, dietary LD50 were reported at 48 ppm As(III) and >2000 ppm MMA [\(Costigan](#page-78-1) *et al.*[, 2001\)](#page-78-1). In marine mammals, water doses of 0.37-3.7 ppm arsenic (as AsCl<sub>3</sub>) resulted in immunotoxic effects in lymphoma B cell lines from harbour seals (decreased lymphoproliferation, phagocytic activity and efficiency) [\(Frouin](#page-79-2) *et al.*, 2010).

No blood based effect levels are available for wildlife, but acutely toxic and fatal human cases have been reported to occur at blood As levels of ~ 1,000 ppb [\(ATSDR, 2007\)](#page-77-0). Reported tissue based effect concentrations vary widely among animal species, however, most organisms show acute effects at tissue levels in the low to mid ppm ww range. For example, lethal arsenic toxicoses in cattle, horses and deer was reported at liver concentrations of 4-22 ppm [\(Gomez-Caminero](#page-79-0) *et al.*, 2001). In birds, residues in the 2 to 10 ppm ww range in liver or kidney are considered elevated; residues >10 ppm are indicative of arsenic poisoning [\(Eisler, 1988\)](#page-78-0). Adverse effects of arsenic on aquatic organisms have been reported at concentrations of 1.3 to 5 ppm ww in tissues [\(Eisler, 1988\)](#page-78-0). However, as discussed above, many marine organisms can contain several fold higher organic arsenic levels that seem to present little hazard to the organism or its consumers [\(Gomez-Caminero](#page-79-0) *et al.*, 2001).

#### **SUMMARY**

Considering the arsenic levels reported for other sea turtles, and other marine species, As levels in green turtle blood from Gladstone are unusually elevated, but comparable to those reported for moribund green turtles. In contrast, liver and kidney As levels are within the typical ranges reported from green turtles and other marine wildlife. This suggests recent high-level exposure may have occurred. Considering the limited information on arsenic accumulation in sea turtles in general, and the lack of information on arsenic species present in Gladstone green turtles, it is not possible to conclude whether the levels observed present a hazard to these wildlife. However, available speciation studies indicate that sea turtles can contain inorganic and other highly toxic arsenic species. Considering this, in combination with blood arsenic levels (up to 20 ppm ww) in the range of those where effects can be elicited in other organisms based on blood or tissue levels, adverse effects due to arsenic exposure may be possible in Gladstone green turtles. Speciation of arsenic, which is essential in understanding the toxicity, is thus recommended to improve evaluation of risks to green turtles in Gladstone.

# 5.3.2 Cadmium (Cd)

#### **SOURCES**

Cadmium is a rare heavy metal and typically present in small amounts in zinc ores. It commonly exists only in one oxidation state (+2) and does not undergo oxidation-reduction reactions. It is typically obtained as an industrial by-product of the production of zinc, copper and lead. Cd is used in electroplating, pigment production, the manufacture of plastic stabilisers and batteries [\(ATSDR,](#page-77-2)  [2008a\)](#page-77-2). Major anthropogenic sources of Cd include smelter fumes and dusts, non-ferrous metal mining and refining, incineration and disposal of Cd containing waste and fossil fuels, fertilisers, and municipal as well as sludge discharges [\(Eisler, 1985a\)](#page-78-2). Cd contamination can be especially severe in the vicinity of smelters and urban industrialised areas, both from historical or current operations [\(Eisler, 1985a;](#page-78-2) [ATSDR, 2008a\)](#page-77-2).

The fate of Cd in the environment and its availability to organisms depends on numerous factors, including its chemical speciation, adsorption and desorption rates from soil/sediments, the concentration of complexing ligands, pH, and the redox potential of the surroundings [\(Eisler, 1985a\)](#page-78-2). Elemental Cd is mostly insoluble in water and deposits and absorbs to sediments, but its chloride and sulphate salts are freely soluble (and can also travel long distances in air a particles or vapours) [\(Eisler, 1985a;](#page-78-2) [ATSDR, 2008a\)](#page-77-2). Changes in physico-chemical conditions, particularly pH and redox potential may increase chemical mobility, and therefore bioavailability of sediment-bound Cd [\(Eisler,](#page-78-2)  [1985a\)](#page-78-2). It is also possible that Cd contaminated sediments are a source for root uptake by aquatic plants, and Cd in plants growing in contaminated soils can contain very high levels that may be detrimental not only to the plants but also to their consumers [\(Eisler, 1985a\)](#page-78-2).

#### **TOXICOKINETICS**

The major pathway for exposure to Cd are food consumption, particularly plants grown in contaminated grounds, and soil/sediment ingestion, although inhalation of significant Cd levels can occur near cadmium emitting industries [\(ATSDR, 2008a\)](#page-77-2). Dermal exposure to Cd is considered negligible (<1%) in humans and laboratory animals, but may increase over prolonged exposure [\(ATSDR, 2008a\)](#page-77-2). Aquatic plants can accumulate Cd from sediments, and very high levels can be present in contaminated areas, presenting a key source of exposure for herbivorous animals and the food chain [\(ATSDR, 2008a\)](#page-77-2). Various seagrass species have, however, been shown to contain relatively low Cd concentrations [\(Denton](#page-78-3) *et al.*, 1980; [Talavera-Saenz](#page-85-3) *et al.*, 2007). After oral exposure, only <10% of Cd in the digestive tract enters the body in humans and laboratory animals, although, absorption can be higher under iron and other nutrition deficient states [\(ATSDR, 2008a\)](#page-77-2). The biological half times of Cd are relatively long (in the order of years to decades) in humans [\(ATSDR,](#page-77-2)  [2008a\)](#page-77-2); birds showed similarly long biological half-lives of 99 days [\(Eisler, 1985a\)](#page-78-2).

Cd tends to accumulate preferentially in kidneys and liver of mammals, and only small amounts are eliminated via urine and faeces [\(ATSDR, 2008a\)](#page-77-2). Cd has also been observed to accumulate readily in sea turtle liver and kidneys, with the latter typically containing significantly higher levels (e.g. [\(Sakai](#page-84-3) *et al.*[, 2000b;](#page-84-3) Anan *et al.*[, 2001;](#page-76-2) Kaska *et al.*[, 2004;](#page-81-5) [Storelli](#page-84-4) *et al.*, 2005[; Andreani](#page-76-3) *et al.*, 2008[; Barbieri,](#page-77-3)  [2009;](#page-77-3) Agusa *et al.*[, 2011\)](#page-76-0)). Cd accumulation in these tissues is mainly due to the binding of metal ions by metallothionein, a low molecular weight metal binding protein implicated in the detoxification of toxic heavy metals and homeostasis of essential elements in humans and animals [\(ATSDR, 2008a\)](#page-77-2), including sea turtles [\(Andreani](#page-76-3) *et al.*, 2008). As metallothionein is synthesised in the liver and then transported in the bloodstream to the kidney in mammals, higher Cd concentrations in the kidney compared to the liver are considered indicative of long-term exposure, while both tissues contain similar levels after short-term exposure (except at very high levels) [\(Andreani](#page-76-3) *et al.*, 2008; [ATSDR,](#page-77-2)  [2008a;](#page-77-2) [Barbieri, 2009\)](#page-77-3). Related to this, kidney Cd levels also tend to rise slower than in the liver immediately after exposure; Cd half-lives for kidneys and liver have been estimated at 4-19 and 6-38 years, respectively [\(ATSDR, 2008a\)](#page-77-2).

Cd tends to bioaccumulate with age in organisms, particularly in carnivores and marine vertebrates [\(Eisler, 1985a\)](#page-78-2). In sea turtles, including green turtles, significantly higher Cd levels have been reported for older/larger specimens [\(Godley](#page-79-3) *et al.*, 1999; [Storelli](#page-84-4) *et al.*, 2005; [Barbieri, 2009\)](#page-77-3), although opposite trends are also reported [\(Gordon](#page-79-4) *et al.*, 1998; Sakai *et al.*[, 2000a;](#page-84-5) [Komoroske](#page-81-0) *et al.*[, 2011;](#page-81-0) [Labrada-Martagón](#page-81-6) *et al.*, 2011). It remains unknown whether this is due to the exposure history, the ontogenetic shift and associated lower Cd intake in the herbivorous life stages [\(Sakai](#page-84-5) *et al.*[, 2000a;](#page-84-5) [Labrada-Martagón](#page-81-6) *et al.*, 2011), or an associated change in physiology/metallothionein production capacity [\(Caurant](#page-78-4) *et al.*, 1999). Apart from turtles, studies on other animals also indicate that younger organisms may absorb more Cd (and have higher sensitivity to Cd) than adults [\(ATSDR,](#page-77-2)  [2008a\)](#page-77-2).

Biomagnification of Cd through the food chain is not considered significant [\(ATSDR, 2008a\)](#page-77-2), and contradictory studies in different species of sea turtles do not provide evidence of biomagnification through the food chain. Higher Cd concentrations in green turtles compared to loggerheads were reported by [\(Andreani](#page-76-3) *et al.*, 2008), while the opposite was observed in (Kaska *et al.*[, 2004\)](#page-81-5). While Cd can be transferred to offspring via mother milk, studies on turtles indicate that excretion via eggs may not be important, with only <0.5% of Cd burden being eliminated by the mother [\(Sakai](#page-84-6) *et al.*, [1995\)](#page-84-6).

Blood is an appropriate marker for Cd exposure and may reflect both recent and cumulative exposures over time; the half-life of Cd in blood of laboratory mammals (mice) is estimated at 291 days [\(ATSDR, 2008a\)](#page-77-2). Urine, which reflects kidney concentrations at chronic intakes, is also used to inform on both recent and past exposure, while kidney Cd levels are generally considered the most important indicator for toxicology [\(ATSDR, 2008a\)](#page-77-2).

### **EXPOSURE CONCENTRATIONS IN GLADSTONE GREEN TURTLES**

Cd concentrations in blood of green turtles from Moreton Bay ranged from 40 to 110 (average 40) ppb ww. Similar blood Cd levels were reported from moribund green turtles in southeast Queensland (11-122; average 35 ppb ww; n=16), but also from green turtles caught live at two sites in Mexico (10-50; average 30 and 8.0-120, average 60 ppb ww; n=30 and 60, respectively [\(Labrada-Martagón](#page-81-6) *et al.*[, 2011\)](#page-81-6)). The latter is considered generally a relatively pristine area, although agricultural and urban discharges occur, and Cd, as well as a number of other metals (Zn, Cu and Pb) in sediments were reported above those in many industrial regions, possibly due to upwelling and/or historical mining activities [\(Talavera-Saenz](#page-85-3) *et al.*, 2007; [Labrada-Martagón](#page-81-6) *et al.*, 2011). In contrast, blood Cd levels in green turtles from the highly urbanised and generally contaminated San Diego bay were several fold lower (13 ±4.2 ppb ww; n=19), and were below the limit of quantification in flatback turtles off the coast of Gladstone (<0.1 ppb ww; n=20) [\(Ikonomopoulou](#page-80-1) *et al.*, 2011). Average Cd levels in blood of Florida manatees were 1.0 (range 1.0-3.0) ppb ww (in [\(Eisler, 2010\)](#page-79-5)). Mean blood Cd in humans are typically around 0.47 ppb ww with slightly higher levels in older age groups, and females [\(ATSDR, 2008a\)](#page-77-2).

In liver and kidney of green turtles from Gladstone, Cd concentrations (average 17, 13-24 and average 47, 17-90 ppm ww, respectively) are also comparable with elevated levels reported from elsewhere. For example, green turtle liver and kidney from Moreton Bay contained what the authors considered among the highest Cd levels recorded for marine vertebrates (average 38, 2.5-57 and average 38, 1.7-76 ppm ww, respectively) [\(Gordon](#page-79-4) *et al.*, 1998). Moribund green turtles from southeast Queensland contained similarly high levels (average 14, 4.3-32 and 46, 13-100 ppm ww, respectively) [\(van de Merwe](#page-85-0) *et al.*, 2010). In the Torres Strait, Cd levels in liver and kidney of green turtles (average 11, 6.0-17 and average 26, 12-42 ppm ww) were also comparably high [\(Gladstone,](#page-79-1)  [1996\)](#page-79-1). Similar Cd levels in kidneys (average 28, 4-56 ppm ww) of green turtles were also reported from Japan, and were considered extremely high, although liver contained lower Cd concentrations (average 5.6, 1.1-12 ppm ww) compared to Gladstone turtles (Anan *et al.*[, 2001\)](#page-76-2). Similar Cd kidney concentrations were reported from moribund green turtles with severe fibropapilloma (average 42, 16-70 ppm ww) compared to a captive (22 ppm ww) and stranded specimens (average 7.6, 4.7-10 ppm ww) [\(Aguirre](#page-76-4) *et al.*, 1994). Liver Cd levels in these same specimens averaged 16 (5-26), 3.1 and 2.7 (0.39-5.4), respectively [\(Aguirre](#page-76-4) *et al.*, 1994). Apart from these studies, Cd levels in green turtles are typically one or two magnitudes lower. These include for example average kidney samples of adults and juveniles from Brazil (0.26 and 0.12 ppm ww, respectively; [\(Barbieri, 2009\)](#page-77-3)), Costa Rica (4.7 ppm ww; [\(Andreani](#page-76-3) *et al.*, 2008)), Cyprus (1.0 ppm ww; [\(Godley](#page-79-3) *et al.*, 1999)), Turkey (1.9 ppm ww; (Kaska *et al.*[, 2004\)](#page-81-5)), Hong Kong (0.30 ppm ww; (Lam *et al.*[, 2004\)](#page-82-0)) and Mexico (1.6 ppm ww; [\(Talavera-Saenz](#page-85-3) *et al.*, 2007)); liver tissue analysed in these latter studies contained similarly low or lower Cd concentrations.

In seabirds, Cd levels in liver and kidney are typically <15 ppm ww and often much lower, but high concentrations >50 ppm ww have been reported from various areas and species [\(Eisler, 2010\)](#page-79-5). Similarly, studies on marine mammals from Australia, indicate Cd concentrations typically range from <LOQ to <15 ppm ww in liver and <LOQ to <30 ppm ww in kidney, but high concentrations up to 76 ppm ww and 106 ppm ww, respectively have been identified in some individuals across several species [\(Kemper](#page-81-7) *et al.*, 1994).

It has been suggested that the sometimes very high (up to 80 ppm ww) Cd levels in green turtles may be a result of high accumulation in seagrass [\(Talavera-Saenz](#page-85-3) *et al.*, 2007) and/or incidental ingestion of sediment [\(Gladstone, 1996\)](#page-79-1), although seagrass and sediment Cd concentrations have been found to be relatively low in areas [\(Denton](#page-78-3) *et al.*, 1980; [Talavera-Saenz](#page-85-3) *et al.*, 2007) [\(Gladstone, 1996\)](#page-79-1). Similar to green turtles, loggerhead and other higher trophic sea turtles appear to be able to accumulate comparably high Cd levels which may be taken up via benthic food sources (e.g. crustaceans, muscles) [\(Caurant](#page-78-4) *et al.*, 1999). The elevated levels sometimes observed in sea turtles may also be consequence turtle specific metabolic capacities [\(Caurant](#page-78-4) *et al.*, 1999). Despite the unknown reasons for the high accumulation efficiencies, there is strong evidence that higher concentrations of Cd in individuals of a given species collected at different locations is almost always

associated with proximity to industrial and urbanised areas or to point source discharges of Cd containing waters [\(Eisler, 1985a\)](#page-78-2).

#### **TOXICITY AND EFFECTS**

There is no evidence that Cd performs a beneficial role in biological systems, but it is known to be one of the most toxic elements and exerts toxic effects including nephrotoxicity, carcinogenicity, mutagenicity and reproductive toxicity [\(Eisler, 1985a\)](#page-78-2). Cd has been implicated in severe deleterious effects on wildlife, as well as deaths in humans [\(Eisler, 1985a\)](#page-78-2). Long term exposure can lead to accumulation of Cd in the kidneys and a range of effects (e.g. decreased growth, respiratory disruption, altered enzyme levels, and abnormal muscular contractions [\(Eisler, 1985a\)](#page-78-2)) and eventually causing kidney damage and result in debilitating bone disease (Itai-Itai disease), particularly in individuals with poor nutrition [\(ATSDR, 2008a\)](#page-77-2). The various clinical symptoms from chronic exposure are thought to result from the degeneration and atrophy of the proximal tubules or, in the worse cases, interstitial fibrosis of the kidney [\(ATSDR, 2008a\)](#page-77-2).

In reptiles, ovo-exposure to toxic elements including Cd and As has been shown to affect hatchling growth, foraging efficiency, mortality, thyroid function or later reproductions [\(Hopkins](#page-80-2) *et al.*, 1999; [Brasfield](#page-77-4) *et al.*, 2004; Marco *et al.*[, 2004;](#page-82-2) [Guirlet](#page-80-3) *et al.*, 2008). A correlation between reduced vitellogenic capacity and increased hepatic Cd concentrations was also reported for freshwater turtles [\(Storelli](#page-84-4) *et al.*, 2005).

The sensitivity of mammalian kidneys to Cd is related to Cd distribution in the body and the production of metallothionein (a metal binding protein) in the kidney. Similarly, metallothionein has been suggested to be involved in the regulation of Cd in sea turtles (Anan *et al.*[, 2001\)](#page-76-2). Binding of Cd to metallothionein decreases the toxicity of Cd [\(ATSDR, 2008a\)](#page-77-2). When total Cd content in the renal cortex reaches between 50-300 ppm ww, however, the amount of Cd not bound to metallothionein becomes sufficiently high to cause tubural damage [\(ATSDR, 2008a\)](#page-77-2).

Sublethal effects in most marine animals occur at Cd levels of 0.5-10 ppb in water [\(Eisler, 1985a\)](#page-78-2). Cd concentrations exceeding 10 ppm ww in liver or kidney of vertebrates, or 2 ppm ww whole body are considered evidence of probably contamination, while elevated levels of 13-15 ppm ww in tissue may represent a significant hazard to animals of higher trophic levels, and residues of 200 ppm ww or 5 ppm ww whole body, are probably life-threatening to most organisms [\(Eisler, 1985a\)](#page-78-2).

A recent study on freshwater turtles showed that relatively low Cd levels (7 ppm) in yolk could impact on gonadal development and may impact the animals by disrupting reproductive process and lowering fertility [\(Guirlet](#page-80-3) *et al.*, 2008[; Kitana and Callard, 2008\)](#page-81-8). Blood Cd levels (average 13 ±4.2 ppb ww) were also correlated with several health markers in green turtles however interpretation was confounded by covariance with turtle size [\(Komoroske](#page-81-0) *et al.*, 2011). Cd levels of 8.3 and 3.3 ppm ww in liver of loggerhead turtles were considered high enough to potentially affect the health of these organisms [\(Storelli](#page-84-4) *et al.*, 2005).

In occupationally exposed humans, chronic blood Cd levels of 5.6 and 10 ppb were associated with a 10% prevalence of abnormal biomarkers of tubular damage (β2-microglobulin) and renal dysfunction, respectively, and 33% had signs of glomerular damage at blood Cd levels of 5.6-<8.4 ppb [\(ATSDR, 2008a\)](#page-77-2). Kidney Cd burdens >50 ppm cortex are associated with renal damage in humans,

and blood Cd levels of >1.5 ppb are significantly correlated with reduced sperm count in humans, and showed weak correlations with defective sperm [\(ATSDR, 2008a\)](#page-77-2).

### **SUMMARY**

Cd levels in green turtle blood, liver and kidney from Gladstone are comparable to the upper concentrations reported from green and other sea turtles from elsewhere, and are relatively high compared to average concentrations typically found in marine mammals and seabirds. Lowest levels reported in green and higher trophic sea turtle species are 1-2 orders of magnitude below these concentrations; however, several other studies have reported high levels of Cd in different species of sea turtles. While various hypotheses have been proposed to explain such elevated Cd levels in turtles, to date these observations cannot be explained, and it remains unknown whether the associated individuals or populations are adversely affected. In accord with other studies, the elevated Cd levels observed in Gladstone green turtles are near or above tissue based concentrations where significant adverse effects are observed in other animals, and higher compared to levels where sublethal and biochemical effects were implicated, also for sea turtles. While the sensitivity of turtles to Cd is unknown, these studies suggest that adverse effects are possible at the observed Cd exposure levels, although sea turtle specific information is sparse.

# 5.3.3 Cobalt (Co)

# **SOURCES**

Cobalt is a naturally occurring element present at relatively low concentrations in the environment. It commonly occurs in three valence states (0, +2 and +3) [\(ATSDR, 2004\)](#page-76-5). It is an essential element required in trace amounts to maintain health in animals and humans. In the environment, it is usually combined with other elements (e.g. oxygen, sulfur, and arsenic). Co is used in the form of alloys in a range of industrial, medical and agricultural applications. It may be released from a number of anthropogenic activities, including coal-fired power plants and incinerators, vehicle exhaust, industrial activities related to mining and processing of cobalt-containing ores, smelting facilities and the production and use of cobalt alloys and chemicals [\(ATSDR, 2004\)](#page-76-5).

The fate of Co in the environment depends on many factors, such as the release route, the chemistry of the water and sediment. In general, Co compounds are non-volatile and most have a high affinity for particles [\(ATSDR, 2004\)](#page-76-5). Such forms are thus strongly associated with soils and sediments, but ionic forms can also remain in the water column and the amount of Co that is mobile increases under more acidic conditions [\(ATSDR, 2004\)](#page-76-5). Plants can accumulate the cobalt from their surroundings and animals can accumulate Co in their body, but biomagnification through the food chain has not been observed [\(ATSDR, 2004\)](#page-76-5).

# **TOXICOKINETICS**

Generally, exposure to Co may occur via air or food and water, but Co can also readily enter an organism via abraded parts of the skin, and has been shown to cross the placenta in animal studies [\(ATSDR, 2004\)](#page-76-5). Based on its fate in the environment, the predominant exposure route for turtles would be expected to be contaminated sediments and seagrass, however, exposure via water may also occur. The proportion of Co that enters the body from the gastrointestinal tract varies considerably (18-97% in humans, 13-34% in rats, 1-2% in cows), based on the animal species, type and dose of Co, and the nutritional status of the subjects, with higher absorption under iron deficient nutritional states [\(ATSDR, 2004\)](#page-76-5). Dermal exposure through abraded skin has been observed to be ~80% in guinea pigs, but is low (<1%) through intact skin [\(ATSDR, 2004\)](#page-76-5).

After exposure, Co distributes via the blood to all tissues, predominantly the liver, kidney and bones [\(ATSDR, 2004\)](#page-76-5). Absorbed Co is eliminated from the body within days to weeks in humans and laboratory animals, with the main route of excretion via urine [\(ATSDR, 2004\)](#page-76-5). Blood is thus an appropriate and commonly used marker for relatively recent (in the order of days to weeks) exposure to Co [\(ATSDR, 2004\)](#page-76-5).

# **EXPOSURE CONCENTRATIONS IN GLADSTONE GREEN TURTLES**

Blood Co levels in Gladstone green turtles ranged from 28-440 (average 150) ppb ww. Average concentrations (36 ±6.7 ppb ww) were considerably lower in moribund green turtles from southeast Queensland [\(van de Merwe](#page-85-0) *et al.*, 2010). In adult nesting flatback turtles off the coast of Gladstone (Curtis Island), blood Co levels were below the LOQ (<0.1 ppb ww) [\(Ikonomopoulou](#page-80-1) *et al.*, 2011). No other information could be identified for Co blood concentrations in turtles. Normal Co blood levels in humans range from 0.05-2.7 ppb [\(Catalani](#page-78-5) *et al.*, 2011), while Co levels as high as 57-187 ppb ww

have been observed in occupationally exposed cohorts [\(ATSDR, 2004\)](#page-76-5), and a medical human case study reported extremely high Co levels of 549 ppb ww [\(Catalani](#page-78-5) *et al.*, 2011).

In liver and kidney of Gladstone turtles, average Co levels detected were 1.4 (range 0.93-2.3) and 2.1 (range 0.99-3.2) ppm ww, respectively. These concentrations are higher compared to those reported from most other green turtles, particularly in liver. In liver of green turtles from Japan for example, average Co levels were <0.030 (n=2; (Sakai *et al.*[, 2000a\)](#page-84-5)), 0.077 (n=25; (Anan *et al.*[, 2001\)](#page-76-2)), and 0.067 ppm ww (n=1; (Sakai *et al.*[, 2000b\)](#page-84-3)). Approximately one order of magnitude lower levels were also reported in liver of green turtles from Hong Kong (0.13; n=2; (Lam *et al.*[, 2004\)](#page-82-0)) and southeast Queensland (0.61; n=16; [\(van de Merwe](#page-85-0) *et al.*, 2010)). Average kidney Co levels were also lower in green turtles from Japan (0.3 (Sakai *et al.*[, 2000a\)](#page-84-5), 0.51 (Anan *et al.*[, 2001\)](#page-76-2), 0.81 (Sakai *et al.*[, 2000b\)](#page-84-3)), however the maximum Co levels in these studies reached the average concentration of green turtles from Gladstone. Similar Co kidney concentrations compared to Gladstone were reported in green turtles from Hong Kong (1.4 ppm ww; n=2) (Lam *et al.*[, 2004\)](#page-82-0) and southeast Queensland (1.5 ppm ww; n=x) [\(van de Merwe](#page-85-0) *et al.*, 2010); both studies show similarly elevated levels of other metals and metalloids.

Co in livers of various seabird species are typically very low ranging from 0.0011 to 0.024 ppm ww [\(ATSDR, 2004\)](#page-76-5). Similarly, in livers of cetaceans from Hong Kong, Co levels ranged from 0.0015-0.016 ppm ww (n=33) [\(Lam, 2009\)](#page-82-3) and Co levels in marine mammals are usually less than 0.13 ppm ww [\(Eisler, 2010\)](#page-79-5). Similarly low levels have been reported for human liver (0.017 ppm ww) from Japan [\(ATSDR, 2004\)](#page-76-5).

### **TOXICITY AND EFFECTS**

Co is part of the vitamin B12, and is (at trace levels) essential to the growth and development of various organisms. On the other hand, Co may also elicit harmful effects in organisms if exposure is sufficiently high. These include developmental and behavioural effects, and effects on the blood, liver, kidneys and heart. After dermal exposure, the most commonly observed effect is dermatitis, possibly caused by an allergic reaction [\(ATSDR, 2004\)](#page-76-5). After inhalation, a range of effects on the respiratory system are also observed (e.g. decreased pulmonary function, asthma, lung disease, dyspnea), as well effects on thyroid and allergic dermatitis [\(ATSDR, 2004\)](#page-76-5). Co has also been classified as possibly carcinogenic by IARC [\(ATSDR, 2004\)](#page-76-5).

Adequate chronic studies on the oral toxicity of Co in humans and animals are currently not available [\(ATSDR, 2004\)](#page-76-5). A human case study reported very high cobalt levels (549 ppb ww; whole blood) in a subject that presented with cranial nerve impairment and mild distal sensory-motor disturbances, followed by blindness, deafness and severe limbs motor weakness. The blood Co dropped to ~100 ppb within 10 days and remained elevated (33.9 ppb ww) above background (0.05-2.7 ppb ww) 14 months after exposure [\(ATSDR, 2004\)](#page-76-5).

The doses for oral LD50 in rats range from 42 ppm body weight as cobalt chloride to 317 ppm body weight as cobalt carbonate [\(ATSDR, 2004\)](#page-76-5). Box turtles that were subcutaneously injected with 5 ppm body weight 5 times per week died within 14 to 147 days [\(Altland and Thompson, 1958\)](#page-76-6).

#### **SUMMARY**

Based on the available studies on turtles and other organisms, blood Co levels appear to be relatively high in Gladstone green turtles, however, comparative data are sparse. Co concentrations determined in tissue samples also support that elevated exposure may have occurred, but while both liver and kidney concentrations are higher compared those reported for most other sea turtles, they are within the upper ranges of previously reported levels. Based on the general toxicokinetics of Co in other organisms, this suggests exposure to elevated Co may have occurred relatively recent (days, weeks to months) prior to sample collection. There is no information on the toxic effects of Co in reptiles and it is unknown whether the observed levels may be associated with effects. However, the blood Co levels are in the range of those where acute effects have been described in humans.

# 5.3.4 Mercury (Hg)

# **SOURCES**

Mercury occurs naturally in the environment and exists in several (elemental, inorganic, and organic) forms. There are numerous anthropogenic sources of Hg to the environment, including fossil fuel combustion, mining, smelting, steel mills, chloralkali plants, solid waste incineration, as well as via fertilisers, fungicides and municipal waste, cement production, uncontrolled industrial releases and from industrial wastewater [\(ATSDR, 1999\)](#page-76-7). After release to air, the fate of mercury depends on its speciation. For example, gaseous elemental Hg can undergo global-scale transport, or particulate and reactive gaseous Hg is primarily deposited within the vicinity of the source. Hg can also be released directly to the marine environment (e.g. via wastewater) or indirectly, via contaminated soil. In aquatic systems, mercury is mostly bound to particles where it is relatively stable. Inorganic Hg is microbially transformed to methylmercury (MeHg), a potent neurotoxin with strong tendency to biomagnify in the aquatic food chain [\(ATSDR, 1999;](#page-76-7) [Kampalath](#page-80-4) *et al.*, 2006).

# **TOXICOKINETICS**

The toxicokinetics of Hg species in organisms are complex and depend on the Hg form. Aquatic organisms are exposed to mercury mainly via food (and ingested sediments), but exposure via the water, air and skin may also occur, with bioavailability depending on the Hg form [\(ATSDR, 1999\)](#page-76-7). In addition, Hg can transfer to offspring readily in humans [\(ATSDR, 1999\)](#page-76-7), however, it has been suggested that maternal transfer is not a major elimination pathway for turtles, with only <5% of the maternal Hg burden transferred per clutch (Sakai *et al.*[, 1995;](#page-84-6) [Godley](#page-79-3) *et al.*, 1999).

Generally, ingested MeHg is absorbed (almost completely) into the bloodstream within hours, which is the primary transport mechanism of mercury through the body. In blood, the cellular component (e.g. red blood cells) has the highest affinity for Hg, and can contain 10-200 times higher concentrations compared to plasma (Day *et al.*[, 2007\)](#page-78-6). However, blood Hg concentrations decline within weeks after exposure ceases, as the dose is distributed to organs and tissues [\(ATSDR, 1999\)](#page-76-7). This is followed by a slower elimination phase, which may last several months (Day *et al.*[, 2007\)](#page-78-6). Organic Hg compounds are mainly excreted via the faeces in humans and animals, and predominantly in the inorganic form [\(ATSDR, 1999\)](#page-76-7).

Blood is therefore a biomarker for measuring relatively recent (within days to weeks) exposure to Hg, but is affected by short-term changes in Hg levels (Day *et al.*[, 2005\)](#page-78-7). Particularly the onset of debilitated conditions in animals, including turtles, and the cessation of feeding may create artificially low Hg levels in blood that are no longer representative of the burden during the beginning of their health decline (Day *et al.*[, 2010\)](#page-78-8). Despite this, blood Hg levels are often correlated to those in less dynamic tissues suggesting a proportion of blood Hg may also reflect longer term exposure [\(Day](#page-78-7) *et al.*, [2005\)](#page-78-7). Exposure assessment of mercury via blood has the added advantage that the majority of Hg present is in its most toxic methylated form (MeHg) thus reducing the need for complex speciation (Day *et al.*[, 2005;](#page-78-7) Day *et al.*[, 2007\)](#page-78-6).

In contrast to blood, keratinised tissues are commonly used biomarkers of long-term exposure to Hg due to its strong binding of keratin proteins, and relative persistence in these (Day *et al.*[, 2005\)](#page-78-7). Liver and kidney typically contain larger proportions of inorganic Hg forms, due to Hg demethylation in these organs (Day *et al.*[, 2005;](#page-78-7) Day *et al.*[, 2007\)](#page-78-6). This is valid for green turtles, where liver MeHg was shown to contribute approximately 9-19% of total mercury [\(Kampalath](#page-80-4) *et al.*, 2006).

Hg has been shown repeatedly to bioaccumulate in a variety of organisms [\(ATSDR, 1999\)](#page-76-7). Seemingly contradictory to this, studies indicate that green turtles contain higher Hg levels in their juvenile, rather than adult life stages [\(Gordon](#page-79-4) *et al.*, 1998; [Kampalath](#page-80-4) *et al.*, 2006; [Komoroske](#page-81-0) *et al.*, 2011). This has been suggested to be related to their ontogenetic shift in diet from a higher to low trophic level and an associated growth dilution of Hg body burdens [\(McKenzie](#page-82-4) *et al.*, 1999; [Kampalath](#page-80-4) *et al.*, [2006;](#page-80-4) [Komoroske](#page-81-0) *et al.*, 2011). As the opposite trend is observed for other metals (e.g. lead) alternative hypotheses for negative correlations between Hg levels and green turtle size may be a change in physiological biotransformation and elimination, or up-regulation of metallothionein in adult specimens [\(Komoroske](#page-81-0) *et al.*, 2011). Despite this, juvenile green turtles typically contain considerably (order of magnitude) lower Hg levels compared to loggerheads when collected from the same area (e.g. [\(Godley](#page-79-3) *et al.*, 1999; Anan *et al.*[, 2001;](#page-76-2) [Kampalath](#page-80-4) *et al.*, 2006)). This is consistent with the strong tendency of Hg to biomagnify through the food chain.

# **EXPOSURE CONCENTRATIONS IN GLADSTONE GREEN TURTLES**

Hg concentrations in blood of live green turtles from Gladstone ranged from <0.22 to 38 ppb ww (average 9.3 ppb ww). Approximately 4 and 9 times lower concentrations were reported in blood of moribund green turtles from southeast Queensland (0.25-7.1; average 2.5 ppb ww; n=16; [\(van de](#page-85-0)  [Merwe](#page-85-0) *et al.*, 2010)), and a highly urbanised estuary in San Diego (1.0 ppb ±0.16; n=30; [\(Komoroske](#page-81-0) *et al.*[, 2011\)](#page-81-0)), respectively. While Hg exposure in turtles has been investigated in a number of other studies, including in blood, these studies focus mostly on higher trophic species. Notwithstanding the expected higher Hg levels in higher trophic species [\(Kampalath](#page-80-4) *et al.*, 2006) (even in blood, as discussed above), Hg was below the limit of quantification  $\leq 0.01$  ppb ww) in blood of nesting flatback turtles collected on Curtis Island, off Gladstone in 2006 [\(Ikonomopoulou](#page-80-1) *et al.*, 2011). Relatively low levels were also reported in blood of nesting females of carnivorous olive ridley turtles in Mexico (0.6 ppb dw or approx. 0.15 ppb ww using the reported conversion; n=25; [\(Páez-Osuna](#page-83-4) *et al.*[, 2011\)](#page-83-4)).

In contrast to the above mentioned literature, which possibly reflect low background exposure, higher concentrations of Hg were reported in blood of live (higher trophic) loggerhead turtles from the USA (6-77 ppb ww; average 29; n=60; (Day *et al.*[, 2007\)](#page-78-6) and 5-188 ppb ww (Day *et al.*[, 2005\)](#page-78-7)). Similar levels were detected in live loggerhead turtles from the same area collected 3 years earlier (average 29 ppb; n=34; (Day *et al.*[, 2005\)](#page-78-7), with one severely and chronically emaciated animal containing 188 ppb ww (Day *et al.*[, 2005\)](#page-78-7). Mercury levels in these individuals were correlated with the distance to the nearest major industrial river mouth. Blood from stranded loggerhead turtles, collected in conjunction with the latter study, contained an average of 99 ppb ww and the highest concentrations (306 ppm ww) was present in an individual that was severely and chronically emaciated and exhibited extreme muscle atrophy as well as an empty gastrointestinal tract [\(Day](#page-78-7) *et al.*[, 2005\)](#page-78-7). These studies suggest a link between the observed blood Hg levels and negative impacts on loggerhead turtle immune function (Day *et al.*[, 2007\)](#page-78-6), as further discussed below. For context, whole blood Hg levels of <5-20 ppb are considered normal in humans [\(ATSDR, 1999\)](#page-76-7).

Similar to blood, Hg liver (1.3; 0.86-1.6 ppm ww) and kidney (0.42; 0.39-0.72 ppm ww) levels in the three euthanized green turtles from Gladstone were several fold to several magnitudes higher compared to those reported for other green turtles from Australia, and elsewhere. Stranded green turtles from Moreton Bay contained an average of 0.021 (<LOQ-0.052) and 0.020 (<LOQ-0.049) ppm ww (n=23), respectively [\(Gordon](#page-79-4) *et al.*, 1998). Liver and kidney of failed rehabilitation green turtles from southeast Queensland also contained lower concentrations (0.19; <LOQ-0.54 and 0.06; <LOQ-0.20 ppm ww; n=16; [\(van de Merwe](#page-85-0) *et al.*, 2010), and Hg levels were below the limit of quantification (<0.05 ppm ww) in eggs of the higher trophic flatback turtles collected off Gladstone on Curtis Island [\(Ikonomopoulou](#page-80-1) *et al.*, 2011). Similarly low levels were reported from Torres Strait (0.08; 0.02-0.17 ppm ww and 0.02; 0.01-0.04 ppm ww, n=12, respectively) [\(Gladstone, 1996\)](#page-79-1). Similarly, liver and kidney Hg levels in Gladstone turtles were higher compared to those from other areas around the world, including e.g. Japan (0.29; 0.053-0.64 and 0.13; 0.029-0.25 ppm ww; n=46; [\(Sakai](#page-84-5) *et al.*, [2000a\)](#page-84-5)), Hong Kong (0.17 and 0.04 ppm ww; (Lam *et al.*[, 2004\)](#page-82-0)) and the Mediterranean (0.12 and <LOQ ppm ww; [\(Godley](#page-79-3) *et al.*, 1999)). In this respect it is noteworthy that liver and kidney samples were only analysed from three specimens from Gladstone, which contained blood mercury levels similar to the average (n=1) and 10-42 times lower (n=2) than the average of all turtles sampled. Assuming a relationship exists between Hg blood, liver and kidney concentrations [\(van de Merwe](#page-85-0) *et al.*[, 2010\)](#page-85-0), considerably higher levels may be present in live turtles with high blood Hg levels (estimated at 9-17 ppm ww, or more). Considering the dose-dependent demethylation of Hg in the liver, prediction of Hg in liver from blood will be more suitable at low concentrations, and may underestimate total Hg in this organ (and to a lesser extent in the kidney and brain) when higher concentrations are present (Day *et al.*[, 2005\)](#page-78-7).

The body of literature on Hg concentrations in tissues of other aquatic organisms is extensive, mainly from higher trophic marine mammals (~0.3-300 ppm ww in liver) and seabirds (~0.1-100 ppm in liver) (Sakai *et al.*[, 2000a\)](#page-84-5), however, marine birds and mammals appear to have either lower proportions of organic Hg, or lower susceptibility to Hg (reviewed in [\(NJDEP, 2001\)](#page-83-5). Compared to these levels, Hg levels in green turtles are low, likely due to their low trophic level status, but it has been suggested that sea turtles may be substantially more sensitive to Hg toxicity (Day *et al.*[, 2007\)](#page-78-6). Alligators from the Everglades contained approximately 10 ppm ww in kidney, which exceeded the chronic risk threshold [\(Yanochko](#page-85-4) *et al.*, 1997; [Duvall and Barron, 2000;](#page-78-9) [NJDEP, 2001\)](#page-83-5).

### **TOXICITY AND EFFECTS**

Toxic effects of various mercuric forms include neurotoxicity, impaired growth and development, reproductive effects, liver and kidney damage and immunotoxicity [\(ATSDR, 1999;](#page-76-7) Day *et al.*[, 2007\)](#page-78-6). Such effects have been shown to occur in mammals, birds and fish, and in contrast to many other metals and metalloids, are also increasingly being investigated in turtles (Day *et al.*[, 2007\)](#page-78-6). The nervous system is highly sensitive to mercury [\(ATSDR, 1999\)](#page-76-7). It has also been shown that Hg elicits immunosuppressive effects for most lymphocyte functions, which is often accompanied by an increase in the susceptibility to infectious agents (e.g. herpes virus; [\(Ellermann-Eriksen](#page-79-6) *et al.*, 1994) or tumour cells ([Moszczyński, 1997](#page-83-6); Day *et al.*[, 2007\)](#page-78-6)). In aquatic organisms, MeHg is the most toxic and physiologically important portion of the Hg burden.

Blood Hg levels in green turtles (average 1 ppb ww) were correlated with several clinical health markers from San Diego estuary [\(Komoroske](#page-81-0) *et al.*, 2011), and similar results were reported in Kemp's ridley sea turtles (Day *et al.*[, 2007\)](#page-78-6) as well as loggerhead turtles (average 29 ppb ww) from southeast USA (Day *et al.*[, 2007\)](#page-78-6), however, confounding factors could not be ruled out in these field studies. Nevertheless, these findings, together with ex-vivo results showing negative correlations with lymphocyte numbers and B-cell proliferative responses (in a population with average blood mercury of 29 ppb ww) and in-vitro immunosuppressive responses (e.g. suppression of B-cell proliferation with a no-observed-effect-level (NOEL) of 50 ppb ww in blood), indicate that adverse effects on sea turtle immune function are possible from elevated exposure to mercury (Day *[et al.](#page-78-6)*, [2007\)](#page-78-6). These studies also suggest that effects in sea turtles occur at substantially lower concentrations compared to other vertebrates, including rats and humans; and thus, the sea turtle immune system may be highly sensitive to Hg toxicity (Day *et al.*[, 2007\)](#page-78-6).

Tissue based concentrations of 0.5-6 ppm in various bird eggs are associated with decreased egg weight, malformations, lowered hatchability, and /or altered behaviour in various species (reviewed in [\(NJDEP, 2001\)](#page-83-5)), while acutely poisoned birds usually have whole body mercury levels >20 ppb ww [\(UNEP, 2002\)](#page-85-5). In contrast, lethal or harmful effects in marine and terrestrial mammals are reported at Hg concentrations >25 to 60 ppm ww in kidneys and liver [\(UNEP, 2002\)](#page-85-5), while sublethal adverse effects in harp seals were observed at tissue residue concentrations of 47-83 ppm ww (reviewed in [\(NJDEP, 2001\)](#page-83-5)). In this respect it is however interesting to note that significantly higher levels of liver Hg (20 ppm ww) levels were reported in harbour porpoises that died from infectious diseases compared to uninfected animals (2.3 ppm ww) (reviewed in [\(Poulsen and Escher, 2012\)](#page-83-2)).

For protection of human consumers, maximum allowed or recommended levels of Hg in fish by various countries (including Australia) and WHO/FAO range from 0.5 ppm (fish, crustaceans, molluscs) ww to 1 ppm ww (high trophic fish).

### **SUMMARY**

Overall, these comparisons suggest that green turtles from Gladstone were exposed to elevated levels of mercury that resulted in blood and tissue levels mostly exceed those reported from green turtles in Australia or elsewhere. As both blood and tissues are consistent in these results, the data indicate mercury levels may be chronically elevated, although short term high-level exposure may also have occurred. While the levels in low trophic, including juvenile, green turtles are expected to be considerably lower compared to higher trophic species, levels detected in Gladstone specimens are comparable to those reported from higher trophic loggerhead turtles foraging near known point sources or in relatively polluted areas. Sensitivity to mercury toxicity is species specific, making it difficult to predict toxic thresholds for green turtles from the limited available data. Nevertheless, previous studies suggests that sea turtles may be particularly sensitive to mercury exposure, and specimens from Gladstone, particularly those with higher mercury blood levels are within the range of those associated with abnormal haematological markers of health in other green and loggerhead turtles. These upper concentrations are also within the order of NOEL for immunosuppressive responses determined ex-vivo for loggerhead turtles. Compared to other relatively sensitive species (e.g. birds), tissue mercury levels in green turtles from Gladstone are also within the determined effect concentrations. These results suggest Hg levels in Gladstone green turtles are sufficiently high to pose a potential risk of adverse effects in the study population.

# 5.3.5 Nickel (Ni)

# **SOURCES**

Nickel is a natural element that occurs at very low levels in the environment, and is essential for the normal growth of many organisms [\(Eisler, 1998a;](#page-79-7) [ATSDR, 2005a\)](#page-76-8). It occurs as five stable isotopes, most commonly in 0 and +2 oxidation states, and interacts with numerous inorganic and organic compounds. The dominant species in water is  $Ni<sup>2+</sup>$  in the form of octahedral hexahydrate ion (Ni(H<sub>2</sub>O)<sub>6</sub>)<sup>2+</sup>) as soluble salts (Ni chloride hexahydrate, and Ni sulphate hexahydrate) and adsorbed to organic matter (Ni nitrate, Ni hydroxide and Ni carbonate). The fate of Ni in marine and other aquatic systems is strongly affected by its speciated form, as well as the pH, redox potential, ionic strength, type and concentration of ligands [\(Eisler, 1998a\)](#page-79-7). Anthropogenic sources of nickel include ore and mineral mining, smelting, refining, fossil fuel and waste combustion, processing of iron, steel, nonferrous metals, and timber products, electroplating, sludge disposal or application, effluents, and other industries that use, process or manufacture chemicals, gum and wood or carbon black [\(Eisler,](#page-79-7)  [1998a\)](#page-79-7)

# **TOXICOKINETICS**

Organisms are typically exposed to Ni via ingestion of food or sediments/soils, inhalation or dermal absorption [\(Eisler, 1998a\)](#page-79-7). The absorption of Ni is governed by the quantity of exposure and the forms of Ni. Absorption from the gastrointestinal tract is in the order of 1-10% in humans and laboratory animals, but higher absorption rates have been found when Ni is taken up via water, in the absence of food [\(ATSDR, 2005a\)](#page-76-8). Unabsorbed Ni is rapidly excreted in the faeces, while absorbed Ni is primarily eliminated via urine [\(Eisler, 1998a;](#page-79-7) [ATSDR, 2005a\)](#page-76-8). Absorption via the skin has been observed with an efficiency of 55-77% within 24 hours [\(Eisler, 1998a;](#page-79-7) [ATSDR, 2005a\)](#page-76-8). Ni retention is relatively low in mammals with a rapid half-life of only several days [\(Eisler, 1998a\)](#page-79-7). After absorption, Ni enters the bloodstream where it is present as free hydrated  $Ni<sup>2+</sup>$  ions, small and protein complexes, and as Ni bound to blood cells in mammals. The partitioning among these compartments varies according to the metal-binding properties of serum albumin, which is highly variable among species [\(Eisler, 1998a\)](#page-79-7). Via the bloodstream, Ni is distributed to all organs, but is typically found at highest levels in the kidneys, although significant levels can also be deposited in liver, heart, lungs and fat [\(Eisler, 1998a;](#page-79-7) [ATSDR, 2004\)](#page-76-5).

Nickel does not bioaccumulate to a great extent in animals [\(ATSDR, 2005a\)](#page-76-8), but accumulation may occur in some species, and Ni has been observed to increase in various organs with age of terrestrial and marine mammals [\(Eisler, 1998b\)](#page-79-8). In mammals, Ni can cross the placental barrier, although this transfer route may be limited, and trophic position in the food chain, sex and reproductive state typically do not significantly influence the Ni body burdens [\(Eisler, 1998a\)](#page-79-7).

Ni levels in blood, as well as serum, plasma and urine provide the most appropriate indices of Ni exposure; blood rapidly reflects current exposure, peaking within hours after oral exposure but Ni is rapidly cleared with mean serum half-time of 30-60 hours [\(Eisler, 1998a;](#page-79-7) [ATSDR, 2005a\)](#page-76-8); thus blood only reflects the most recent exposure before sampling (within hours to days).

#### **EXPOSURE CONCENTRATIONS IN GLADSTONE GREEN TURTLES**

Ni concentrations in blood of green turtles from Gladstone averaged 5.2 ppb ww (range 0.67-17 ppb ww). Concentrations of Ni in blood were below the LOQ (<0.1 ppb ww) in adult flatback turtles collected in 2006 from Port Curtis [\(Ikonomopoulou](#page-80-1) *et al.*, 2011), but surprisingly high levels were reported in blood of live captured and apparently healthy, higher trophic Olive Ridley turtles from Mexico (average 76 ±35 ppb ww). Normal serum levels for most mammalian animals are in the range of 2.0-5.3 ppb ww [\(Eisler, 1998a\)](#page-79-7), while reference Ni levels in human serum are 0.20 ppb ww [\(ATSDR,](#page-76-8)  [2005a\)](#page-76-8), although values of 3-7 ppb ww have been reported in whole blood [\(Eisler, 1998a\)](#page-79-7), and occupationally exposed humans plasma levels can reach >11 ppb ww, but decrease rapidly [\(Eisler,](#page-79-7)  [1998a\)](#page-79-7).

Kidney of green turtles from Gladstone contained average Ni levels of 9.0 ppm ww (range 0.42-26 ppm ww). These concentrations are 1-2 orders of magnitude higher compared to kidney Ni concentrations reported from other green turtles [\(Aguirre](#page-76-4) *et al.*, 1994; Sakai *et al.*[, 2000a;](#page-84-5) [Sakai](#page-84-3) *et al.*[, 2000b;](#page-84-3) Lam *et al.*[, 2004{Barbieri, 2009 #103;](#page-82-0) [Barbieri, 2009\)](#page-77-3), including Mexico [\(Talavera-Saenz](#page-85-3) *et al.*[, 2007\)](#page-85-3), and several fold higher compared to the maximum reported levels (average 1.2; range 0.51-1.7 ppm ww; n=14) from green turtles in the Mediterranean off Turkey (Kaska *et al.*[, 2004\)](#page-81-5). Kidney Ni levels in green turtles from Gladstone are similar to levels considered high in loggerhead turtles from the Mediterranean off Spain (Torrent et al 2004) while the levels reported for other loggerhead turtles from Japan (Sakai *et al.*[, 1995;](#page-84-6) Sakai *et al.*[, 2000b\)](#page-84-3) and the Mediterranean off Spain (Kaska *et al.*[, 2004\)](#page-81-5) are considerably lower. In contrast to kidney, liver Ni levels in green turtles from Gladstone (average 0.20; range 0.17-0.23 ppm ww) are an order of magnitude lower compared to those reported in liver of green turtles from Turkey (Kaska *et al.*[, 2004\)](#page-81-5) and Mexico (range <LOQ-6.8 ppm ww; [\(Talavera-Saenz](#page-85-3) *et al.*, 2007)) and similar or slightly higher compared to those reported for other green turtles from a range of areas, including Oman (average 0.090 ppm ww; [\(Al-Rawahy](#page-76-9) *et al.*[, 2007\)](#page-76-9)), Japan (average 0.065; range 0.059-0.071 ppm ww; (Sakai *et al.*[, 2000b\)](#page-84-3) and range 0.60- 0.31 ppm ww (Sakai *et al.*[, 2000a\)](#page-84-5)), Hong Kong (average 0.059 ppm ww; (Lam *et al.*[, 2004\)](#page-82-0)) and Brazil (average 0.029 ppm ww; [\(Barbieri, 2009\)](#page-77-3)).

Mammalian wildlife from uncontaminated habitats usually contain less than 0.1 to about 0.5 ppm dw (or approx. 0.025-0.125 ppm ww) in tissues, while these levels can reach up to 10 ppm dw (or approx. 2.5 ppm ww) in Ni contaminated areas [\(Eisler, 1998a\)](#page-79-7). Similar levels are reported from birds (0.1-2.5 ppm ww in liver from contaminated areas) [\(Eisler, 1998a\)](#page-79-7) and lower levels are found in unexposed humans (0.062 ppm dw (or approx. 0.0155 ppm ww) in kidney and 0.005 ppm dw (or approx. 0.0125 ppm ww) in liver) [\(ATSDR, 2005a\)](#page-76-8).

#### **TOXICITY AND EFFECTS**

Ni is reportedly an essential micronutrient for maintaining health in plants, invertebrates, birds and mammals, including humans [\(Eisler, 1998a\)](#page-79-7), although the functional important of Ni has not been clearly demonstrated [\(ATSDR, 2005a\)](#page-76-8). Ni deficiency is primarily manifested in the liver with effects including abnormal liver morphology, oxidative and lipid metabolism, delayed gestation periods and fewer offspring, decreased growth, anaemia, and dermatitis, [\(Eisler, 1998a;](#page-79-7) [ATSDR, 2005a\)](#page-76-8).

The toxicity of Ni is strongly dependent on its chemical and physical forms. Soluble Ni forms are more toxic (e.g. rat single oral dose LD50 = 39 and 136 ppm body weight for Ni sulphate and Ni acetate, respectively) than less soluble forms (e.g. rat single oral dose LD50 >3,930 ppm body weight for Ni oxide) [\(ATSDR, 2005a\)](#page-76-8). Generally, hazards to human health are lower when ingested, but can be severe when inhaled with dust, and for some aquatic crustaceans and fish, Ni is more potent at higher pH [\(Eisler, 1998a\)](#page-79-7). In addition, mixtures of metals (As, Cd, Cu, Cr, Hg, Pb, Zn) containing Ni salts have been shown to be more toxic than predicted on the basis of individual components [\(Eisler,](#page-79-7)  [1998a\)](#page-79-7). The toxic and carcinogenic effects of Ni compounds are associated with Ni mediated oxidative damage to DNA and proteins and the inhibition of cellular antioxidant defences [\(Eisler,](#page-79-7)  [1998a\)](#page-79-7). At the cellular levels, Ni interferes with enzymatic functions of calcium, iron, magnesium, and zinc [\(Eisler, 1998a\)](#page-79-7). Toxic effects of Ni to humans and laboratory animals are documented for respiratory, cardiovascular, gastrointestinal, haematological, musculoskeletal, hepatic, renal, dermal, ocular, immunological, developmental, neurological, and reproductive systems [\(Eisler, 1998a\)](#page-79-7). The WHO classifies nickel compounds as Group 1 carcinogens and metallic nickel as Group 2B (possible human carcinogens) [\(Eisler, 1998b\)](#page-79-8). The carcinogenicity of Ni compounds, however, varies significantly with the chemical form, route and duration of exposure and species [\(Eisler, 1998a\)](#page-79-7). Ni carbonyl is a potent animal teratogen [\(Eisler, 1998a\)](#page-79-7).

In birds, adverse effects are expected in most species at kidney and liver concentrations of >10 ppm dw (approx. 2.5 ppm ww) and >3 ppm dw (approx. 0.75 ppm ww), respectively [\(Eisler, 1998a\)](#page-79-7). Liver and kidney of birds fed very high Ni containing diets contained <1.0 ppm ww in survivors, but up to 22.7 ppm ww in liver and 74.4 ppm ww in kidney in those that died [\(Eisler, 1998a\)](#page-79-7). Reduced growth rate in chickens fed with high Ni containing diets produced elevated kidney levels of 4.2 ppm ww versus 0.13 ppm ww in controls [\(Eisler, 1998a\)](#page-79-7).

In humans, serum Ni levels >4.6 ppb ww and plasma Ni levels >11.9 ppb ww are considered elevated while less than 2.6 ppb are considered normal [\(Eisler, 1998a\)](#page-79-7). Workers accidentally exposed to high Ni levels contained serum concentrations of up to 286 ppb ww one day after exposure in individuals with symptoms, and 50 ppb in those without symptoms [\(Eisler, 1998a\)](#page-79-7). However, Ni tissue levels do not always accurately predict potential health effects from exposure [\(ATSDR, 2005a\)](#page-76-8).

Rats exposed to high Ni doses, and showing depressed growth, low hematocrit and haemoglobin, and low tissue cytochrome oxidase had elevated Ni concentrations in kidney (40.7 ppm dw, or approx. 10 ppm ww) and liver (4.0 ppm dw, or approx. 1 ppm ww) [\(Eisler, 1998a\)](#page-79-7).

#### **SUMMARY**

The Ni concentrations in blood of green turtles from Gladstone are lower compared to levels reported for other, albeit higher trophic sea turtle species. Considering the rapid clearance rates of Ni from blood in other organisms blood Ni levels provide information on exposure during the last hours to days prior to sampling, while kidneys and liver represent Ni exposure days to weeks, or longer prior to sampling, respectively. Liver Ni levels appear slightly elevated, but are within the levels observed for green turtles from several other areas. In contrast, kidney Ni concentrations are 1-2 orders of magnitude higher compared to those reported for other green turtles. While there are no toxicological data for sea turtles, the levels observed in kidney are within the tissue based concentrations where adverse effects have been reported for birds and rats.

# 5.3.6 Selenium (Se)

#### **SOURCES**

Se is widely but unevenly distributed in the environment and particularly abundant in sulphide minerals of various metals, including iron, lead and copper [\(Eisler, 1985b\)](#page-78-10). It exists as six stable isotopes, three allotropic forms and five valence states (-2 (selenide), 0 (elemental Se), +2 (selenium), +4 (selenite) and +6 (selenate), which are commonly combined with other substances. Key anthropogenic sources are combustion of coal, various industries, municipal wastes, as well as mining and smelting operations [\(ATSDR, 2003\)](#page-76-10). Se is primarily obtained as a byproduct of copper refining, was used as a pesticide to control plant pests, and is today extensively used in the manufacture and production of e.g. glass, rubber, metal alloys, and petroleum. However, aside from highly localised contamination, the major source of Se is weathering of natural rock [\(ATSDR, 2003\)](#page-76-10). Se tends to be present in large amounts where soils have been derived from cretaceous rocks [\(Eisler,](#page-78-10)  [1985b\)](#page-78-10).

The fate of Se is highly complex and depends largely on its form and the conditions of the environment. In the absence of oxygen and in acidic soils, only low amounts of Se enter plants [\(ATSDR, 2003\)](#page-76-10). Elemental Se and selenides are insoluble and largely unavailable to the biosphere, hydrogen selenide is highly toxic and unstable, while soluble selenates occur in alkaline soils, which are slowly reduced to selenites and may be taken up by plants. Selenites are less soluble and easily reduced to elemental Se; they are often the dominant chemical species in seawater [\(Eisler, 1985b\)](#page-78-10)

#### **TOXICOKINETICS**

Se is taken up as essential nutrient with food, both as organic (mainly selenomethionine and selenocysteine) and inorganic (mainly selenate and selenite) forms, but higher than normal levels of Se can also be taken up via soil/sediment, associated plants or water at naturally high Se sites or anthropogenically contaminated areas [\(ATSDR, 2003\)](#page-76-10). Dermal exposure to selenomethionine has been observed, but there is limited information for other forms [\(ATSDR, 2003\)](#page-76-10). Se is readily absorbed in the gastrointestinal tract of humans and laboratory animals, often to >80% [\(ATSDR, 2003\)](#page-76-10). After absorption, Se is distributed by the circulatory system to all body organs, the concentrations being often highest in liver and kidney of mammals [\(ATSDR, 2003\)](#page-76-10), as well as sea turtles (Anan *et al.*[, 2001;](#page-76-2) [Storelli](#page-84-4) *et al.*, 2005). However, accumulation depends on the chemical form and exposure levels, and build up of Se can also occur in blood, lungs, heart, testes, and hair [\(ATSDR, 2003\)](#page-76-10), as well as carapace in sea turtles [\(Komoroske](#page-81-0) *et al.*, 2011). Se has a relatively short biological life (in the order of hours or days to weeks) in various organisms [\(Eisler, 1985b\)](#page-78-10), and elimination occurs primarily in the urine, but also the faeces, depending on exposure time and level [\(ATSDR, 2003\)](#page-76-10). However, Se metabolism is significantly modified by interaction with various heavy metals, other chemicals, and numerous physico-chemical factors, and it is thus difficult to meaningfully interpret Se residues in various tissues [\(Eisler, 1985b\)](#page-78-10).

Se exposure can be measured in blood and urine to provide information on recent exposure to high levels. The time of exposure reflected by Se blood levels depends on renewal of red blood cells, which is approximately 120 days in humans [\(ATSDR, 2003\)](#page-76-10). In sea turtles, Se concentrations in different tissues are often correlated, for example, blood Se levels were significantly correlated with

those in liver, kidney and muscle [\(van de Merwe](#page-85-0) *et al.*, 2010) as well as with those in eggs [\(Guirlet](#page-80-3) *et al.*[, 2008\)](#page-80-3).

Se concentrations in mammals are often also correlated with those of other metals, particularly Hg, As and Cd. Similar results have been observed for sea turtles [\(Komoroske](#page-81-0) *et al.*, 2011), including in green turtles of this study (p<0.05 for As, Cd, Co, Hg, Mo). This may be the result of Se playing a role in various metal detoxification processes, as has been observed in other species.

While Se is typically eliminated rapidly from the body, it can accumulate with age to elevated levels under long or high exposure regimes [\(ATSDR, 2003\)](#page-76-10), particularly in higher trophic, long-lived, marine vertebrate species [\(Eisler, 1985b\)](#page-78-10). However, chronically ill and older people have been shown to have lower organ concentrations of selenium than healthy individuals, although it is not clear if this is a cause or consequence of aging or illness [\(ATSDR, 2003\)](#page-76-10). In green turtles, Se has been observed to be significantly negatively correlated with size [\(Komoroske](#page-81-0) *et al.*, 2011), but positive correlations have been reported for hawksbill turtles (Anan *et al.*[, 2001\)](#page-76-2). There is evidence that Se biomagnifies in the food chain and maternal transfer has been demonstrated for Se in humans and various animals [\(ATSDR, 2003\)](#page-76-10) including turtles [\(Guirlet](#page-80-3) *et al.*, 2008).

# **EXPOSURE CONCENTRATIONS IN GLADSTONE GREEN TURTLES**

Blood Se levels in Gladstone green turtles averaged 1900 (range 84-8600) ppb ww. These concentrations are high compared to those considered normal in other animals or humans, but similar levels (average 2400; range 68-9100 ppb ww) have been reported from moribund green turtles in southeast Queensland [\(van de Merwe](#page-85-0) *et al.*, 2010), as well as apparently healthy green turtles from two areas in Mexico (average 1600 and 1800; range 30-5700 and 150-4700 ppb ww, respectively) [\(Labrada-Martagón](#page-81-6) *et al.*, 2011). Lower Se concentrations were reported in green turtles from San Diego bay in the USA (average 780 ±250 ppb ww) [\(Komoroske](#page-81-0) *et al.*, 2011).

In blood of herbivorous and omnivorous Amazon river turtles, average Se levels ranged from 164 to 538 ppb ww [\(Burger](#page-77-1) *et al.*, 2009). Despite their higher trophic levels, typical Se blood levels in humans range from 59 (New Zealand) to 210 (USA) ppb ww [\(ATSDR, 2003\)](#page-76-10), and blood Se levels of >40-50 ppb are recommended for cattle and sheep to avoid Se deficiency [\(Eisler, 2007\)](#page-79-9).

In liver and kidney from three necropsied green turtles from Gladstone, Se concentrations averaged 5.4 (range 4.0-7.2) and 1.3 (range 0.62-2.4) ppm ww, respectively. Respective blood Se levels in these animals were below the average in two of these specimens (850 ppb ww) and above the average in the third individual (3,300 ppb ww). Similar to blood, Se levels in kidney and liver are comparable to the upper ranges observed in other green turtles. For example, similar or higher Se concentrations have been reported in kidney and liver of moribund green turtles from southeast Queensland (average 1.7, range 0.29-5.1 and average 4.0, range 0.52-10 ppm ww, respectively) [\(van de Merwe](#page-85-0) *et al.*[, 2010\)](#page-85-0), stranded specimens from Japan (average 1.0, range 0.41-2.1 and average 1.6, range 0.62- 3.1 ppm ww, respectively) (Anan *et al.*[, 2001\)](#page-76-2) and stranded specimens from the Mediterranean (average 0.94, range 0.31-1.4 and average 2.3, range 0.22-4.2 ppm ww, respectively) [\(Kaska](#page-81-5) *et al.*, [2004\)](#page-81-5), as well as Hong Kong (average 0.71 and 5.6 ppm ww, respectively) (Lam *et al.*[, 2004\)](#page-82-0). The remaining reported average Se levels in kidney and liver of green turtles are, however, 2-3 (kidney) and 3-24 (liver) fold lower. These include samples collected from Australia (e.g. Moreton Bay [\(Gordon](#page-79-4)

*et al.*[, 1998\)](#page-79-4) and Torres Strait [\(Gladstone, 1996\)](#page-79-1)), Hawaii [\(Aguirre](#page-76-4) *et al.*, 1994), and Oman [\(Al-Rawahy](#page-76-9) *et al.*[, 2007\)](#page-76-9).

Corresponding with the potential for Se to biomagnify through the food chain, higher Se levels have been reported from higher trophic hawksbill turtles from Japan (average of 5.5 and 15 ppm ww in liver and kidney, respectively) (Anan *et al.*[, 2001\)](#page-76-2), however, hawksbill turtles from Moreton Bay in Australia contained Se levels comparable to green turtles from Gladstone (maximum 2.5 and 3.7 ppm ww, respectively) [\(Gordon](#page-79-4) *et al.*, 1998). Similarly, Se levels in kidney and liver of carnivorous loggerhead turtles from Moreton Bay and the Mediterranean were similar or lower compared to green turtles from Gladstone (e.g. average kidney Se levels: 1.5 and 0.93, average liver Se levels: 2.2 and 2.8, respectively). Mean concentrations of Se in kidneys of coastal birds from highly industrialised areas in Texas, usually vary between 1.7 and 5.6 ppm ww; these concentrations are considered sufficient to possibly impair reproduction in shorebirds [\(Eisler, 1985b\)](#page-78-10), but levels higher than 2 ppm ww have often been recorded in liver and kidney from higher trophic marine and coastal vertebrates, including birds and mammals [\(Eisler, 1985b\)](#page-78-10), and Se appears to readily accumulate to elevated levels in reptiles [\(Grillitsch and Schiesari, 2010\)](#page-80-5).

# **TOXICITY AND EFFECTS**

Se is an essential micronutrient for humans and many animals, but can be harmful at levels not much higher than those considered beneficial [\(Eisler, 1985b\)](#page-78-10). It constitutes an integral part of important proteins involved in antioxidant defense mechanisms (e.g. glutathione peroxidases), the thyroid hormone metabolism and redox control of intracellular reactions [\(ATSDR, 2003\)](#page-76-10). Similar to vertebrates, it has been suggested that Se similarly plays a pivotal role at the beginning of embryonic development in reptiles, whereby Se might affect the activation, synthesis and release of thyroid hormones [\(Guirlet](#page-80-3) *et al.*, 2008).

Se deficiency may in part underlie susceptibility to cancer, arthritis, hypertension, heart disease, and possibly other diseases, including high embryonic mortality, anemia, poor growth and reproduction, hepatic necrosis, hair loss and sterility [\(Eisler, 1985b;](#page-78-10) [ATSDR, 2003\)](#page-76-10). On the other hand, exposure to Se above its beneficial levels can affect growth and reproduction in various organisms [\(Eisler, 1985b\)](#page-78-10), and may cause cancer [\(ATSDR, 2003\)](#page-76-10). Acute poisoning can result in nausea, vomiting and diarrhea in humans [\(ATSDR, 2003\)](#page-76-10), and a range of symptoms have been observed in livestock (e.g. abnormal movements, laboured breathing, bloating, lethargy and death), with post-mortems indicating many pathological changes in the heart, lungs, rumen, liver, kidney and other organs [\(Eisler, 1985b\)](#page-78-10). Chronic selenosis may be induced by dietary Se levels 10-20 times the norm [\(ATSDR, 2003\)](#page-76-10); signs include skin lesions, lymph channel inflammation, loss of hair and nails, anaemia, enlarged organs, fatigue, and dizziness [\(Eisler, 1985b\)](#page-78-10). Chronic doses around 5 times higher the norm may cause cardiovascular, gastrointestinal, haematological, hepatic, dermal, immunological, neurological and reproductive effects [\(Eisler, 1985b\)](#page-78-10).

A wide variety of interactions of Se have been demonstrated with essential and nonessential elements, vitamins, and xenobiotics, including reduction of toxicity of many metals such as Hg, Cd, Pb, Ag and to some extent, Cu. The degree to which Se is toxic, however, can be influenced by these interactions, but they are complex and still poorly understood [\(ATSDR, 2003\)](#page-76-10).

Chicken embryos are among the most sensitive to Se, and deformed embryos are observed at concentrations of 6-9 ppm in feeds [\(Eisler, 1985b\)](#page-78-10). A field study on wild birds (n=347) showed high incidences (40 and 20%, respectively) of dead embryos and chicks with severe external anomalies in animals from ponds with very high Se levels in water (300 ppb). The liver of these birds contained 19- 130 ppm dw (approx. 4-29 ppm ww). It was concluded that Se was the probable cause of poor reproduction and developmental abnormalities in these animals, due to interference with their reproductive processes [\(Eisler, 1985b\)](#page-78-10).

For snakes, it was estimated that individuals with >1700 ppb ww Se in blood would exceed liver toxicity thresholds recommended for other oviparous vertebrates and be at risk of reduced reproductive success (Hopkins et al 2005). In green turtles, Se in blood (average 780 ppb ww) was found to be correlated with several health markers, however, interpretation was confounded by covariance with turtle size [\(Komoroske](#page-81-0) *et al.*, 2011).

In humans, Se blood levels of 55-200 ppb were correlated with grasping power, blood pressure, serum cholesterol, triglycerides, and lipoproteins in humans [\(ATSDR, 2003\)](#page-76-10). The NOEL for chronic selenosis in humans is based on a blood Se level of 1054 ppb ww [\(ATSDR, 2003\)](#page-76-10). In cows, an association of cystic ovaries with blood selenium concentrations >108 ppb was reported [\(ATSDR,](#page-76-10)  [2003\)](#page-76-10) and other adverse effects in mammals, including body weight loss, were associated with concentrations in erythrocyte >2300 ppb ww and plasma >2800 ppb ww [\(Eisler, 2007\)](#page-79-9).

# **SUMMARY**

Selenium appears to be readily accumulated to elevated levels in many reptile species, including sea turtles. Blood and tissue Se concentrations in green turtles from Gladstone are, however, among the upper ranges reported from other green turtles and thus appear to be elevated. In addition, the Se levels in green turtles from Gladstone (as well as green turtles from elsewhere) are above those considered harmful in many vertebrates, including reptiles, although reptile specific data are limited.

# 5.3.7 Silver (Ag)

### **SOURCES**

Silver is a naturally but relatively rare occurring element. It exists in several oxidation states, most commonly as elemental Ag (0) and monovalent ion (+1). Silver is extracted mainly from argentite ore (by cyanide, zinc reduction, or electrolytic processes), and is often recovered as byproduct from smelting of nickel ores, lead-zinc and porphyry copper ores, platinum and gold deposits [\(Eisler, 1996\)](#page-79-10). Secondary sources include scrap generated in the manufacture of silver containing products and electrical products, old film and photoprocessing wastes or batteries. Elevated silver concentrations in biota can occur in the vicinity of sewage outfalls, mine waste sites, smelting operations, manufacture and disposal of photographic and electrical supplies and coal combustion [\(Eisler, 1996;](#page-79-10) [Howe and Dobson, 2002\)](#page-80-6). Major anthropogenic releases to the aquatic environment include mining tails, soil erosion, urban runoff, sewage treatment plants and electroplating industries [\(Eisler, 1996\)](#page-79-10).

The fate of Ag in soils, sediments and water is controlled mainly via sorption processes, and sediments may be a significant source of Ag to the water column. Ag can be highly persistent in sediments under high pH and salinity conditions [\(Howe and Dobson, 2002\)](#page-80-6). In water, Ag exists mainly as metallo-organic complexes or adsorbed to organic materials, including marine algae, which have been shown to have high bioconcentration factors (commonly up to 66,000) [\(Eisler, 1996\)](#page-79-10). With increasing salinity in brackish and marine waters, sorption to particles decreases and concentrations of chloro complexes (e.g. silver chloride (AgCl), silver chloride ion (AgCl<sub>2</sub>)) increases, which retain some silver in the dissolved form. Thus, relatively small inputs can substantially increase dissolved Ag loads in these environments [\(Eisler, 1996\)](#page-79-10).

### **TOXICOKINETICS**

Organisms can be exposed to Ag via inhalation and ingestion, but Ag can also move across mucous membranes and broken skin [\(Eisler, 1996\)](#page-79-10). After exposure, Ag is mainly transported in the protein fraction of blood plasma as silver albuminate or silver chloride [\(Eisler, 1996\)](#page-79-10). Accumulation, retention and elimination of Ag differ widely among species. In general, the majority of Ag is excreted rapidly (in the order of hours to days/weeks) in faeces with <1% of intake absorbed and retained in tissues, primarily the liver, via precipitation of insoluble silver salts. But Ag may also accumulate in the spleen, muscles, kidney, skin and brain [\(Eisler, 1996\)](#page-79-10). Tissue concentrations of Ag are related to the dose, chemical form and route of exposure. Intestinal absorption in rodents, canids and primates range from 10-50% [\(ATSDR, 1990\)](#page-76-11).

Blood is an appropriate marker for recent Ag exposure (over days to weeks) prior to sampling [\(ATSDR, 1990\)](#page-76-11). Silver has been shown to bioaccumulate in mammalian tissues [\(Eisler, 1996\)](#page-79-10), but food chain biomagnification in aquatic systems is not considered likely at background concentrations [\(Eisler, 1996\)](#page-79-10).

### **EXPOSURE CONCENTRATIONS IN GLADSTONE GREEN TURTLES**

Ag concentrations in blood of green turtles from Gladstone ranged from 0.011 to 7.1 ppb ww (average 0.66 ppb ww), although the majority of blood samples contained Ag levels <2.0 ppb ww. The only identified published data on Ag in blood of green turtles reported approximately two times higher average Ag levels (1.6 ppb ww ( $\pm$ 0.53 SE; n=30)) from a highly urbanised and generally contaminated estuary in San Diego [\(Komoroske](#page-81-0) *et al.*, 2011). In Kemp's ridley sea turtles, however, average blood Ag levels were reported at 0.94 (range 0.042-2.7) ppb ww [\(Kenyon](#page-81-9) *et al.*, 2001). In humans, blood Ag levels in unexposed humans are very low (<0.1-0.2 ppb [\(Armitage](#page-76-12) *et al.*, 1996)). In highly and chronically exposed humans (chemical manufacturing workers) average blood Ag levels were 11 ppb ww [\(ATSDR, 1990\)](#page-76-11) and 0.1-23 ppb ww [\(Armitage](#page-76-12) *et al.*, 1996).

Relatively high concentrations of Ag were observed in liver of green turtles from Japan (0.99 ppm ww; 0.21-2.9 ppm ww); kidney contained considerably lower levels (0.0057 ppm ww; 0.00059-0.023 ppm ww) (Anan *et al.*[, 2001\)](#page-76-2). Similar levels were reported from liver and kidney of green turtles from South China (0.78 ±0.65 SE and 0.0070 ±0.0030 SE ppm ww, respectively) (Lam *et al.*[, 2004\)](#page-82-0). In the present study, Ag could not be analysed in tissues of green turtles due to matrix interferences.

Normal background levels in liver of most organisms are generally several orders of magnitude lower compared to those reported for green turtles from Japan and China (e.g. 0.0044-0.14 ppm ww in various birds, 0.006 ppm ww in humans, 0.16-0.21 in seals and polar bear ppm ww) [\(Eisler, 1996\)](#page-79-10). Maximum concentrations, collected from contaminated areas, range from approximately 0.33 ppm ww in liver of marine mammals, 0.44 ppm ww in trout liver, 9.7 ppm ww in bird liver [\(Eisler, 1996;](#page-79-10) [Howe and Dobson, 2002\)](#page-80-6).

# **TOXICITY AND EFFECTS**

Silver has no known biological function in the body of mammals, and is (as  $Ag<sup>+</sup>$ ) one of the toxicologically most potent metals to aquatic organisms [\(Eisler, 1996\)](#page-79-10). The acute toxicity to aquatic species varies depending on the chemical form and correlates with the availability of free ionic silver Ag+, which is the most toxic species [\(Eisler, 1996\)](#page-79-10). Soluble silver salts are in general more toxic than insoluble salts, and in water, the soluble ion  $Ag<sup>+</sup>$  is the form of most concern. More recently toxicity of silver nanoparticles has become an issue of concern because nano silver is widely a applied antibacterial in consumer products. The toxic species of nano silver is also the  $Ag^*$ , which is set free intracellularly from the ingested nanoparticles.

Long-term, chronic exposure to silver and its compounds by birds and mammals have been associated with induction of sarcomas, cardiac enlargement, vascular hypertension, hepatic necrosis, anemia, lowered immunological activity, altered membrane permeability, kidney pathology, enzyme inhibition, and growth retardation [\(Eisler, 1996\)](#page-79-10). In aquatic environments ionic or free Ag have been shown to interfere with calcium metabolism in frogs (resulting in deterioration of muscle fibres) and with sodium and chloride uptake in gills of fish [\(Eisler, 1996\)](#page-79-10).

There is limited data on the toxic threshold of Ag in avian or mammalian wildlife, and no information for reptiles. Adverse effects of Ag on poultry occur at 1.8 ppm ww (whole egg), 10 ppm ww in copper-deficient diets and 200 ppm ww in copper adequate diets [\(Eisler, 1996\)](#page-79-10). Death in sensitive mammalian species occurs at 14-20 ppm body weight (intraperitoneal injection).

### **SUMMARY**

The average blood Ag levels in Gladstone green turtles are similar or lower compared to other green turtles (from relatively contaminated areas) and sea turtles. However, some individuals from Gladstone contained higher levels of Ag. Considering data from other organisms, the blood Ag concentrations in some green turtles from Gladstone may be above background, however, normal background levels in sea turtles are unknown. No blood based Ag effect concentrations were identified in the literature and only few tissue based effect levels are available. However, Ag could not be quantified in tissues of the present study. Considering the limited comparable data for blood in marine turtles, and lack of blood based effect levels, it is not possible to assess whether exposure to Ag presents a hazard to the study population, but some individuals may contain levels that are elevated.

# 5.3.8 Vanadium (V)

### **SOURCES**

Vanadium is a naturally occurring element present in soil, water and air [\(ATSDR, 2009\)](#page-77-5). It most commonly exists in the oxidation states of +3, +4 and +5 and various inorganic forms, e.g., vanadium pentoxide (V<sub>2</sub>O<sub>5</sub>), sodium metavanadate (NaVO<sub>3</sub>), sodium orthovanadate (Na<sub>3</sub>VO<sub>4</sub>), vanadyl sulphate (VOSO<sub>4</sub>), and ammonium vanadates (NH<sub>4</sub>VO<sub>3</sub>). V is found mostly in fossil fuel such as coal, oil shale, tar sands and crude oil and in numerous minerals such as bauxite and magnetite. Releases of V to the environment are primarily associated with oil refineries and power plants via combustion of petroleum crude oils and coal, and may also occur from mining, clay and metallurgical industries, municipal sewage and fertilisers [\(ATSDR, 2009\)](#page-77-5). Upon entering the marine environment the main fraction of V is deposited and adsorbed to sediments and only a small fraction (0.001%) is estimated to persist in soluble form [\(Byerrum](#page-77-6) *et al.*, 1974).

### **TOXICOKINETICS**

Key pathways for marine organism exposure to vanadium are ingestion of soil/sediments and food. After oral exposure, only a small fraction of vanadium is absorbed through the gastrointestinal tract of animals (up to 17% in laboratory animals; approx. 3-20% in humans) [\(Costigan](#page-78-1) *et al.*, 2001). Dermal adsorption is thought to be minimal due to the low lipid/water solubility of vanadium. Vanadium is distributed in blood and primarily to bone tissue with lesser amounts to kidney and liver [\(Costigan](#page-78-1) *et al.*, 2001). Blood, as well as organ concentrations have been found to decline rapidly to trace levels within days upon cessation of exposure in mammals [\(ATSDR, 2009\)](#page-77-5), via urine as the primary elimination route [\(Costigan](#page-78-1) *et al.*, 2001). Reported elimination half-lives in various tissues and organisms are in the order of 1-14 days [\(ATSDR, 2009\)](#page-77-5) [\(Costigan](#page-78-1) *et al.*, 2001) [\(Miramand](#page-82-5) *et al.*, [1992\)](#page-82-5). There is no evidence of long-term accumulation in humans or marine organisms and food chains [\(Costigan](#page-78-1) *et al.*, 2001). Bioconcentration factors for primary consumers in the marine food chain have been reported to range from 40 to 150 [\(Miramand](#page-82-5) *et al.*, 1992[; Costigan](#page-78-1) *et al.*, 2001).

Considering the toxicokinetics of vanadium in organisms, blood and urine are the most reliable indicators on the level of exposure in mammals and fish [\(Costigan](#page-78-1) *et al.*, 2001). Based on the relatively rapid clearance of V from blood (in the order of days), V blood levels inform on recent exposure regimens.

#### **EXPOSURE CONCENTRATIONS IN GLADSTONE GREEN TURTLES**

Vanadium concentrations in blood of green turtles from Gladstone ranged from 3.5 to 38 ppb ww with an average concentration of 12 ppb ww (SD 9.1). No published data is available on vanadium in blood of any sea turtle species, however, average levels are approximately 4 times lower in blood from live captured green turtles in Moreton Bay (average 2.8; 0.29-8.5 ppb) (unpublished data from 2011; n=9). Compared to levels in human blood (average background: <0.05 ppb ww; [\(Byrne and](#page-77-7)  [Kosta, 1978;](#page-77-7) [Sabbioni](#page-84-7) *et al.*, 1996; Nixon *et al.*[, 2002\)](#page-83-7), these concentrations would be considered elevated, and are in the order of those observed for occupationally exposed cohorts (average 33 ppb ww) (Lin *et al.*[, 2004\)](#page-82-6). However, marine organisms, including plants, invertebrates and seafood generally contain higher levels of vanadium than their terrestrial counterparts [\(Costigan](#page-78-1) *et al.*, 2001), although reports on concentrations in blood from marine species are limited and background levels

for turtles are unknown. The maximum levels present in green turtles from Gladstone compare to the maximum V levels reported in blood from ospreys from relatively polluted Chesapeake Bay and Delaware Bay (range <ND to 0.49 ppm dw or approx. 54 ppb ww) [\(Rattner](#page-83-8) *et al.*, 2008; [Eisler, 2010\)](#page-79-5).

To facilitate further comparisons, liver and kidney (in addition to blood) were analysed from three euthanized green turtles from Gladstone. Vanadium levels in these tissues (0.23-0.79 ppm ww in liver and 0.23-0.34 ppm ww in kidney) are comparable to those in stranded or moribund green turtle liver and kidney from Hawaii and Japan, while approximately one order of magnitude lower V levels were reported in liver and kidney of stranded green turtles from Hong Kong and hawksbill turtles from Japan. These levels are several orders of magnitude higher than typical levels in meat, poultry and fish (around 0.1 ppt; range 0-11.9 ppt)[\(ATSDR, 2009\)](#page-77-5) as well as those typically found in most marine organisms (generally in the order of ppb [\(Michibata,](#page-82-7) 2012), <0.01 ppm ww in marine mammals [\(Mackey](#page-82-8) *et al.*, 1996) or fish (2.9-74 ppb ww; (Sepe *et al.*[, 2003;](#page-84-8) [ATSDR, 2009\)](#page-77-5). Liver tissue concentrations in turtles are, however, comparable to chronically elevated liver concentrations in common dolphins affected by high vanadium release via the Erika oil spill off the coast of France in 2000 (average 0.11; range 0.01-0.32 ppm ww, [\(Ridoux](#page-84-9) *et al.*, 2004)), and approach the highest V concentrations recorded in some marine mammals (up to 1.6 ppm ww in liver of harbour seal [\(Saeki](#page-84-10) *et al.*[, 1999;](#page-84-10) [Eisler, 2010\)](#page-79-5).

# **TOXICITY AND EFFECTS**

Although there is some evidence to suggest that vanadium is an essential nutrient, a functional role has not been established. It acts as phosphate analogue and as such interferes with various ATPases, phosphatises and phosphate-transfer enzymes; additionally, it has been shown to have insulinmimicking properties and the ability to induce cell proliferation, and IARC classifies vanadium pentoxide as possibly carcinogenic (Group 2B) [\(IARC, 2009\)](#page-80-7). Primary targets of toxicity following oral vanadium exposure include the gastrointestinal tract, haematological system and developing organism. Depending on the dose, effects in humans and laboratory animals exposed to vanadium can include decreased number of red blood cells, increased blood pressure, diarrhoea, neurological effects (e.g. decreased fetal growth, skeletal malformations) and lung cancer (through vanadium pentoxide). Clinical signs of toxicity include lethargic behaviour, paralysis, lacrimation and diarrhoea, and histological examination revealed necrosis of liver cells and cloudy swelling of renal tubules [\(Yao](#page-85-6)  [and Zhang, 1986;](#page-85-6) [Costigan](#page-78-1) *et al.*, 2001).

In birds, liver V levels approaching 0.5 ppm ww have been suggested to alter lipid metabolism in laying females (White *et al.*[, 1980;](#page-85-7) [Eisler, 2010\)](#page-79-5). Similarly, V concentrations of around 0.4 ppm ww (whole body) have been reported to elicit effects in fish (reduced growth in juvenile rainbow trout) [\(Hilton and Bettger, 1988\)](#page-80-8). In mammalian species, Lethal Doses (LD50) for sodium metavanadate range from 10-137 (rats) to 23-31 (mice) ppm/day (oral; 14 days) [\(Llobet and Domingo, 1984;](#page-82-9) [Sun,](#page-84-11)  [1987;](#page-84-11) [Costigan](#page-78-1) *et al.*, 2001; [ATSDR, 2009\)](#page-77-5). Minimal risk levels (MRLs) have been established for oral exposure to vanadium (e.g. intermedium duration (15-364 days) exposure oral MRL: 0.01 ppm/day).

### **SUMMARY**

Considering V levels reported for sea turtles, and other wildlife and organisms, the concentrations detected in green turtles from Gladstone appear to be relatively high in blood, although information on baseline levels in sea turtles are lacking. Tissue levels of green turtles from Gladstone are within or near the upper ranges reported from other green turtles and other marine wildlife. The levels present in blood from green turtles in Gladstone may indicate relatively recent exposure to elevated levels in food/sediment and/or water, but species specific toxicokinetics (e.g. absorption, long halflives, bioaccumulation) are unknown. While limited information exists on tissue based effect concentrations across any species, and none is available for reptiles, the V tissue levels in green turtles from Gladstone are similar to those shown to elicit adverse effects in other wildlife (birds, fish).

# 5.3.9 Zinc (Zn)

# **SOURCES**

Zinc is among the most common elements and is naturally present in air, soil, water and food. It occurs as two common oxidation states (Zn(0) and Zn(+2)). A large proportion of zinc also enters the environment as a result of mining, purification of zinc, lead, and cadmium ores, steel production, coal burning, and burning of wastes [\(ATSDR, 2005b\)](#page-77-8). Waste streams from metal manufacturing and zinc chemical industries, domestic waste-water and run-off from soil can discharge zinc into waterways. Sludge and fertilisers can also contribute to increased zinc levels in soil [\(ATSDR, 2005b\)](#page-77-8).

Zinc can combine with other elements, such as chlorine, oxygen, and sulfur to form organic or inorganic zinc compounds. In the aquatic environment, zinc occurs primarily in the +2 oxidation state, as the hydrated form of the divalent cation. Sorption is the dominant reaction, resulting in enrichment of zinc in suspended and bed sediments. However, a small proportion may remain either dissolved in the water or suspended with sediments. The levels of dissolved zinc in water can increase with the acidity of the water [\(ATSDR, 2005b\)](#page-77-8).

# **TOXICOKINETICS**

The major zinc exposure pathways for organisms are ingestion of food and contaminated soils/sediments, although exposure via water is a key pathway for fish and other organisms may be exposed via drinking water; inhalation exposure may also occur in contaminated areas [\(ATSDR,](#page-77-8)  [2005b\)](#page-77-8). Dermal exposure can occur, but absorption studies are limited [\(ATSDR, 2005b\)](#page-77-8). Absorption of zinc from the gastrointestinal tract is homostatically regulated and ranges from 20 to 30% under normal physiological conditions [\(ATSDR, 2005b\)](#page-77-8). A number of factors can influence the absorption, including the chemical form of zinc, the presence of inhibitors (e.g. calcium, phosphorus, dietary fiber) and enhancers (amino acids, picolinic acid) in the diet. After absorption, zinc increases most rapidly in blood (peaking within hours) and bone after exposure. In an initial phase after absorption, zinc is concentrated in the liver, and subsequently distributed throughout the body with major storage sites being the liver, pancreas, bone, kidney and muscle [\(ATSDR, 2005b\)](#page-77-8). Highest concentrations are typically present in muscle, bone, gastrointestinal tract, kidney, brain, and skin. Elimination is predominantly via the urine and feces.

Zinc concentrations in humans increase in several organs with age, including the liver and kidney, although levels in the kidneys peak at approximately 40-50 years of age and then decline. Zinc does not concentrate in fish tissues with exposure to elevated concentrations [\(ATSDR, 2005b\)](#page-77-8) and has not been observed to biomagnify in reptiles [\(Grillitsch and Schiesari, 2010\)](#page-80-5).

Blood is a commonly used marker for recent zinc exposure [\(ATSDR, 2005b\)](#page-77-8). In mammals, approximately two-thirds of zinc in plasma is loosely bound to albumin, which represents the metabolically active pool of zinc [\(ATSDR, 2005b\)](#page-77-8). However, since zinc levels can be affected by dietary deficiency and cell stress, these result may not be directly related to current zinc exposure [\(ATSDR, 2005b;](#page-77-8) [Eisler, 2010\)](#page-79-5).

#### **EXPOSURE CONCENTRATIONS IN GLADSTONE GREEN TURTLES**

Zn blood levels in turtles from Gladstone averaged 8,400 ppb ww (range 3,800-12,000 ppb ww). Similar, or higher levels have been observed in blood of green turtles from Mexico (average 14,000; range 490-20,000 ppb ww) [\(Labrada-Martagón](#page-81-6) *et al.*, 2011), moribund specimens from southeast Queensland (average 7,900; range 3,500-12,000 ppb ww) [\(van de Merwe](#page-85-0) *et al.*, 2010) and leatherback turtles from French Guiana (average 11,000 ppb ww) [\(Guirlet](#page-80-3) *et al.*, 2008). Similarly high Zn levels were also reported in blood of Kep's ridley sea turtles from the Gulf of Mexico (average 7,500; range 3,280-18,900 ppb ww) [\(Grillitsch and Schiesari, 2010\)](#page-80-5). In contrast, Zn levels in blood of flatback turtles from Curtis Island were considerably lower (average 150; range 98-210 ppb ww) [\(Ikonomopoulou](#page-80-1) *et al.*, 2011).

Substantially lower mean Zn concentrations are typically reported in whole blood of humans from regions with low pollution (6.0-7.0 ppb ww), while up to 4,000 ppb ww were have been detected in whole blood of children in highly industrialised urban areas of India [\(ATSDR, 2005b\)](#page-77-8).

In kidney and liver of turtles from Gladstone, Zn concentrations averaged 33 and 46 ppm ww (range 20-40 and 41-51 ppm ww), respectively. While these levels lay within the upper ranges of those reported previously from sea turtles, they are comparable to stranded specimens in Moreton Bay (average 21 and 40 ppm ww in kidney and liver, respectively) [\(Gordon](#page-79-4) *et al.*, 1998), and moribund specimens from southeast Queensland (average 29 and 36 ppm ww, respectively) [\(van de Merwe](#page-85-0) *et al.*[, 2010\)](#page-85-0). Similar Zn levels in kidney and liver have also been reported for several other green turtles around the world, e.g. Japan (average 34 and 58 ppm ww, respectively) (Sakai *et al.*[, 2000a\)](#page-84-5), a captive specimen from Hawaii (32 and 38 ppm ww, respectively) [\(Aguirre](#page-76-4) *et al.*, 1994), and specimens from Hong Kong (average 17 and 28 ppm ww, respectively ) (Lam *et al.*[, 2004\)](#page-82-0).

Zn concentrations in marine birds from New Zealand are typically around 88 ppm ww in liver. Elevated Zn liver levels of 890 ppm dw (approx. 220 ppm ww) have been reported in liver of heron from Rhode Island [\(Eisler, 2010\)](#page-79-5). Mallards exposed to high levels of zinc 450 ppm body weight contained 217 ppm dw (approx. 54 ppm ww) in liver and 79 ppm dw (approx. 20 ppm ww) in kidney [\(Eisler, 2010\)](#page-79-5). Zn concentrations in tissues of marine mammals are usually less than 210 ppm dw (approx. 53 ppm ww), but can range from 1.5-1390 ppm dw (or approx. 0.38-348 ppm ww). In human kidney and liver, background zinc levels are typically around 47 and 23 ppm ww, respectively. In exposed people, kidney and liver levels of 60 and 30 ppm ww, respectively, have been reported.

#### **TOXICITY AND EFFECTS**

Zinc is a trace mineral nutrient, and required in all animals for the function of several metalloenzymes, and as such is required for normal nucleic acid, protein, and membrane metabolism, as well as cell growth and division. Zinc deficiency can cause dermatitis, anorexia, growth retardation, impaired reproductive capacity, impaired immune function, and depressed mental function [\(ATSDR, 2005b\)](#page-77-8).

Chronic exposure to zinc has been shown to decrease the absorption of copper from the diet, resulting in development of copper deficiency. At low doses and intermediate exposure durations, subclinical changes in copper-sensitive enzymes can occur. Higher exposure levels result in more severe symptoms of copper deficiency, including anaemia, and lesions in liver, pancreas and kidneys, infertility, developmental effects and skin irritations [\(ATSDR, 2005b\)](#page-77-8). Oral exposure to zinc may also impair immune and inflammatory responses [\(ATSDR, 2005b\)](#page-77-8).

The oral LD50 in rats and mice for several zinc compounds range from 186 to 623 ppm/day [\(ATSDR,](#page-77-8)  [2005b\)](#page-77-8). Zinc acetate was the most lethal compound in these laboratory animals, respectively.

Zn blood serum levels of 45,000 ppb were reported in a crocodile diagnosed with zinc poisoning; blood Zn levels dropped to 30,000 ppb after 18, and to 4,000 ppb after 39 days of treatment [\(Eisler,](#page-79-5)  [2010\)](#page-79-5). Similarly, Zn poisoned birds frequently contain 16,000 ppb in plasma and 75-156 ppm dw (approx. 19-39 ppm ww) in liver, versus <2 ppb and 21-33 ppm dw (approx. 5.3-8.3 ppm ww) in controls, respectively [\(Eisler, 2010\)](#page-79-5). Tissue residues of Zn are not yet reliable indicators of contamination in mammals, although Zn intoxication is documented in terrestrial mammals when Zn exceeds 274 ppm dw (approx. 68 ppm ww) in kidney, and 465 ppm dw (appox 116 ppm ww) in liver [\(Eisler, 2010\)](#page-79-5). Comparable data for marine mammals could not be identified, and zinc concentrations in marine mammals frequencly exceed 100 ppm ww without apparent damage to the animal [\(Eisler,](#page-79-5)  [2010\)](#page-79-5)

# **SUMMARY**

The Zn levels in green turtles from Gladstone generally lay within the upper ranges, but are comparable to those reported for several other sea turtles from around the world. On the other hand, levels associated with Zn poisoning in crocodiles, birds or mammals are only slightly higher compared to the maximum concentrations detected in Gladstone green turtles, and chronic effect levels are unknown. However, it appears that sea turtles frequently accumulate elevated levels of Zn, and it is not possible to assess whether this may be a concern to these populations.

# <span id="page-70-0"></span>5.3.10 Dioxins and PCBs

# **SOURCES**

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) comprise two different groups of tricyclic, aromatic ethers with 210 possible congeners. PCDD/Fs are produced as unintentional by-products of various combustion and industrial processes, e.g. during pesticide manufacture, bleaching of paper pulp and waste incineration. In addition, current-use pesticides may contain elevated levels of dioxins as impurities (Holt *et al.*[, 2010\)](#page-80-9). Dioxins are released from these sources as complex mixtures and while each congener has slightly different physico-chemical properties, all PCDD/Fs display high lipophilicity and chemical stability [\(Mackay](#page-82-10) *et al.*, 2006).

Polychlorinated biphenyls (PCBs) are thermally stable, good insulators, and are relatively inflammable; hence they have been used widely as flame retardants, lubricants, coolants, and as dielectric fluids [\(NAS, 2001;](#page-83-9) [EC, 2006\)](#page-78-11). Intentional manufacture of PCBs has ceased in many parts of the world; however, PCBs remaining within stock piles still have the potential to enter the environment. Commercial PCB sources consist of complex mixtures of individual PCBs (up to 209 congeners), resulting in complex mixtures of these lipophilic compounds in the environment.

The spatial distribution of PCCD/Fs and PCBs is related to the source location, type of emission source, physico-chemical properties and environmental processes. These compounds have the potential for dispersal throughout the environment, usually in association with mobile particles such as organic matter, for example via atmospheric transport or with river systems [\(Eitzer, 1993;](#page-79-11) [Pearson](#page-83-10) *et al.*[, 1997;](#page-83-10) Gaus *et al.*[, 2001\)](#page-79-12). Consequently, dioxins and PCBs can be found at trace levels in most environmental matrices (air, soil and water) [\(Wagrowski and Hites, 2000\)](#page-85-8), and in particular, are known to accumulate in the marine system.

# **TOXICOKINETICS**

Generally, exposure to PCDD/Fs and PCBs occurs mainly via ingestion. For herbivorous marine turtles, contaminated seagrasses, as well as incidental consumption of sediment bound PCDD/Fs and PCBs, represent the dominant uptake routes [\(Haynes](#page-80-10) *et al.*, 1999; Gaus *et al.*[, 2004\)](#page-79-13). After exposure, these highly lipophilic compounds can be found in most tissues with the highest quantities in the liver and fat (adipose tissue). Although some elimination can occur via faeces and to a lesser extent urine, body fat and possibly the liver can store PCDD/Fs and PCBs for years to decades [\(ATSDR, 1998,](#page-76-13) [2000\)](#page-76-14).

Absorption efficiency of PCDD/Fs and PCBs across the gastrointestinal tract vary depending on the physico-chemical properties of the congeners and generally increases with decreasing degree of chlorination [\(Niimi, 1996\)](#page-83-11). The smaller congeners tend to be the more toxic congeners, and their accumulation can lead to increased tissue toxicity levels compared to surrounding sediments [\(Broman](#page-77-9) *et al.*, 1992). Concentrations of mixtures of PCDD/Fs and PCBs are commonly reported on a toxic equivalency (TEQ) basis. Toxic equivalence factors (TEF), relative to the toxicity of tetra-dioxin (TCDD), have been assigned to 17 PCDD/F congeners and 12 PCBs. TEFs are not available for reptiles, and assessments for turtles rely on TEFs determined for mammalian and avian species.

Due to their lipophilicity and resistance to metabolism, PCDD/Fs and PCBs can bioaccumulate in biota, biomagnify through the food web, and transfer to offspring via gestation and/or lactation (Borgå *et al.*[, 2001;](#page-77-10) Boon *et al.*[, 2002;](#page-77-11) [Falandysz](#page-79-14) *et al.*, 2002).

Lipid-normalised concentrations of PCBs in marine turtle blood have been shown to significantly correlate to levels found in matched fat samples, indicating blood to be a suitable marker for exposure to PCDD/Fs and PCBs (Keller *et al.*[, 2004a\)](#page-81-10). Lipid-normalised PCDD/F and PCB concentrations in green turtles have been shown to closely reflect sediment contamination [\(Hermanussen](#page-80-11) *et al.*, 2006).

### **EXPOSURE CONCENTRATIONS IN GLADSTONE GREEN TURTLES**

Middle bound TEQs in blood of juvenile green turtles from Gladstone averaged 19 ppt lw (range <7.1- 39 ppt lw). These concentrations are comparable to those reported for juvenile green turtles from Shoalwater (average 27; range 24-29 ppt lw; n=2) and eastern Moreton Bays (average 17; range 6.0- 22 ppt lw) [\(Hermanussen, 2009\)](#page-80-12). Higher TEQ levels have been reported from juvenile green turtles in Hervey Bay (average 33; range 9.6-71) and western Moreton Bay (average 78; range 37-120 ppt lw) [\(Hermanussen, 2009\)](#page-80-12).

Blood from the adult specimen in Gladstone contained considerably higher TEQ levels compared to juveniles (130 ppt lw), which is comparable to the upper concentrations reported from Hervey Bay and Western Moreton Bay [\(Hermanussen, 2009\)](#page-80-12). It is interesting to note that the major proportion of this TEQ was derived from PCDD/Fs (120 ppt lw) while PCBs only contributed a minor fraction (7.9 ppt lw). As dioxins and PCBs bioaccumulate in organisms over their lifespan, these results suggest adult turtles may be exposed to chronically elevated levels of dioxins; however, further data from adult turtles would be required to evaluate chronic exposure in this region. No other TEQ levels have been reported for sea turtle blood or tissues. In dugongs from Queensland, TEQ levels were surprisingly elevated compared to many other marine biota, even higher trophic animals, ranging from 5-140 and 0.92-55 ppt lw in adult males and females, respectively (Gaus *et al.*[, 2004\)](#page-79-13)

### **TOXICITY AND EFFECTS**

PCDD/Fs and dioxin-like PCBs primarily exert toxic effects in animals by binding to the aryl hydrocarbon receptor (Ah receptor), and the ligand-activated Ah receptor acts as a transcription factor for the regulation of genes [\(Hahn, 1998\)](#page-80-13). Limited information is available on the effect of dioxins and PCBs on reptiles; although field based epidemiological studies have indicated reproductive and developmental effects in freshwater turtles [\(Bishop](#page-77-12) *et al.*, 1998; [De Solla](#page-78-12) *et al.*, [1998\)](#page-78-12), and possible immune suppression in marine species (Keller *et al.*[, 2004b;](#page-81-11) Keller *et al.*[, 2006\)](#page-81-12).

The effects of PCDD/Fs and dioxin-like PCBs on humans and other mammals, including marine mammals, are well established. Effects include chloracne, liver and kidney damage, behavioural alterations, reproductive and developmental abnormalities, reduced fertility, tetragenicity, endocrine disruption and immune system suppression [\(ATSDR, 1998,](#page-76-13) [2000\)](#page-76-14). TCDD has also been classified as a Group 1 carcinogen by IARC. Lowest observed adverse effect levels (LOAELs) of dioxins and PCBs are reported on a body burden basis – TEQ per kilogram of body weight (bw). LOAEL thresholds in mammals range from biochemical effects at 3 ng  $kg^{-1}$  bw which may or may not result in adverse health effects, immunological effects leading to increased viral sensitivity at 10 ng  $kg^{-1}$  bw,
developmental neurotoxicity at 21 ng  $\text{kg}^{-1}$  bw, and reproductive toxicity resulting in reduced sperm count at 28 ng kg<sup>-1</sup> bw [\(WHO, 1998;](#page-85-0) [USEPA, 2003\)](#page-85-1). For birds, one of the most sensitive species is the domesticated chicken with a LOAEL of 9 ng  $kg^{-1}$  bw for developmental toxicity resulting in cardiac malformation [\(USEPA, 2003\)](#page-85-1).

#### **PROBABILISTIC RISK ASSESSMENT**

A probabilistic approach can be used to estimate the proportion of the Gladstone turtle population at risk of adverse effects based on the reported PCDD/F and PCB blood levels. TEQ data (on a lipid basis in blood) for the turtle samples were transformed to body burdens by multiplying TEQ by the expected total body lipid percentage. The expected population distribution of TEQ body burdens was then determined using risk modelling software (Crystalball 2000 Decisioneering Inc.) and compared to LOAELs in mammals and avian species (in the absence of reptile-specific dose-response toxicological information). Total green turtle lipid percentage was assumed to vary uniformly between 4 and 12% for foraging benthic-phase animals not undergoing breeding migration, consistent with previously reported exposure assessments [\(Hermanussen, 2009\)](#page-80-0). As only one adult blood sample was obtained, data from the 21 juvenile turtles were assessed separately to the adult. Lognormal frequency distributions are commonly observed for environmental pollutant concentrations in animals [\(Ott, 1990\)](#page-83-0), and therefore lognormal distributions were fitted to lipid normalised TEQ concentrations for juveniles using mammalian and avian TEFs separately, and on both a middle and upper bound basis.

Assuming that the juvenile turtles sampled in this study are representative of the Gladstone juvenile turtle population, the likelihood (% of population) of TEQ body burdens at or above levels where effects have been observed were determined. At middle bound TEQ, up to 6.6% of the juvenile population may be above the LOAELs where biochemical effects are expected in mammals; when considering the more conservative upper bound TEQ basis, this percentage increases to 29% (Figure 3). On an upper bound TEQ basis, up to 5% of the population may also be above the LOAEL for developmental toxicity effects in avian species (Figure 3).

In contrast to juveniles, higher TEQs were observed in the adult blood sample. Probabilistic assessments cannot be performed; however, based on the expected total body lipid percentage (4 – 12%) the estimated body burden is in the range of 5.0-15 ng  $kg^{-1}$  bw (middle bound) and 5.6-17 ng  $\mathsf{kg}^\mathsf{-1}$  bw (upper bound) assuming mammalian TEFs. Using avian TEFs, the comparable ranges would be 4.7-14 ng kg<sup>-1</sup> bw (middle bound) and 5.6-17 ng kg<sup>-1</sup> bw (upper bound). This adult's predicted body burden is in excess of the LOAEL for biochemical effects in mammals, and may exceed the LOAELs for immunological effects and developmental effects in mammals and birds, respectively.

It is important to note that this risk assessment does not incorporate reptile-specific sensitivity to PCDD/Fs and PCBs. When the toxicity threshold for a different species (but from the same class) is used for risk assessment, the uncertainty is usually offset by dividing the LOAEL by a safety factor of 10 [\(WHO, 1998\)](#page-85-0). In the case of this study, the uncertainty may be higher due to the class-difference between the measured LOAELs (mammalian and avian) and species of interest (reptilian). Safety factors have not been applied in the above assessment.



Figure 4 Probabilistic distributions of body burden (ng kg<sup>-1</sup> bw (x-axis)) in juvenile green turtles from Gladstone; A) derived using mammalian TEFs and B) derived using avian TEFs. The blue portion of the graph depicts the fraction of the juvenile population at or above the LOAEL of A) 3 ng kg<sup>-1</sup> bw for biochemical effects in mammals (29%) and B) 9 ng kg<sup>-1</sup> bw for developmental toxicity in chickens (5.0%).

#### **SUMMARY**

Based on limited comparable data for PCDD/Fs and PCBs in turtles, blood levels in juvenile turtles from Gladstone appear to be similar compared to green turtles from relatively low impacted areas in Queensland. The adult specimen, however, contained elevated TEQ levels, comparable to the highest concentrations identified for green turtles and dugongs; however, only one adult specimen was sampled from Gladstone. Probabilistic risk assessment for the juvenile population suggests that low proportions of the population may have body burdens in excess of LOAELs for biochemical effects in mammals, which may or may not result in adverse health effects (6.6-29%) and developmental toxicity in birds (0-5.0%). It should be noted, however, that no reptile-specific LOAELs are available and no uncertainty factors have been applied to the mammalian and avian LOAELs used. For the one adult turtle sampled, its estimated body burden was in excess of the LOAEL for biochemical effects in mammals, and exceeds the LOAELs for immunological effects and developmental effects in mammals and birds, respectively.

### **6.0 CONCLUSIONS AND RECOMMENDATIONS**

The results of this study show that exposure concentrations for several organic contaminant groups and a range of metals are relatively low and unlikely to present a substantial hazard to the study population; these include bioaccumulative pesticides, organotins, perfluorinated compounds, brominated flame retardants, aluminium (Al), iron (Fe), manganese (Mn), and zinc (Zn).

A number of contaminant groups were detected at levels that suggest elevated exposure may have occurred for a proportion of the green turtles from Boyne River estuary. These include dioxins and dioxin-like PCBs, silver (Ag), copper (Cu), chromium (Cr), molybdenum (Mo), and lead (Pb). Effects associated with exposure to these compounds may be possible, and may present a concern to the health of the green turtle population in Gladstone. Where available, tissue based concentrations for acute effects across vertebrate taxa are, however, considerably higher.

Levels of the metals/metalloids arsenic (As), cadmium (Cd), cobalt (Co), mercury (Hg), nickel (Ni), selenium (Se), and vanadium (V) were clearly elevated in turtles from Gladstone and near or above tissue based effect concentrations were acute adverse effects have been reported across different vertebrate taxa. In the absence of information regarding the sensitivity of green turtles to such elements, these results suggest they should be considered of concern to the health of the population.

Based on these results, monitoring of the health and contaminant levels in juvenile green turtle population is strongly recommended. As some of the contaminants investigated in this study are known to have tendencies to bioaccumulate with age of organisms, and biomagnify through the food chain it is additionally recommended to investigate the contaminant levels in adult sea turtles as well as higher trophic level marine organisms. This would additionally provide more information on whether acute high level, rather than chronic exposure occurred in this area. Analyses of varying storage tissues (e.g. carapace) may further assist evaluation of exposure duration.

It is further recommended to identify and investigate suitable control populations, to provide a better understanding on typical baseline levels for metals/metalloids in green turtle populations from the wider Gladstone region, as the levels of some metals and metalloids may vary naturally across different locations.

Since the toxic potency of many metals/metalloids are known to differ depending on chemical forms, speciation of metals/metalloids in turtle blood and tissues should be considered to provide a better understanding on the possible risks associated with elevated exposure.

#### **7.0 REFERENCES**

- Aguirre, A.A., Balazs, G.H., Zimmerman, B., Galey, F.D., 1994. Organic contaminants and trace metals in the tissues of green turtles *(Chelonia mydas)* afflicted with fibropapillomas in the Hawaiian islands. Marine Pollution Bulletin 28, 109-114.
- Agusa, T., Takagi, K., Kubota, R., Anan, Y., Iwata, H., Tanabe, S., 2008. Specific accumulation of arsenic compounds in green turtles *(Chelonia mydas)* and hawksbill turtles *(Eretmochelys imbricata)* from Ishigaki Island, Japan. Environmental Pollution 153, 127-136.
- Agusa, T., Takagi, K., Miller, T.W., Kubota, R., Anan, Y., Iwata, H., Tanabe, S., 2011. Intake and excretion of arsenicals in green *(Chelonia mydas)* and hawksbill turtles *(Eretmochelys imbricata)*. Environmental Chemistry 8, 19-29.
- Al-Rawahy, S.H., Al Kindi, A.Y., Elshafie, A., Ibrahim, M., Al Bahry, S.N., Al Siyabi, S.S., Mansour, M.H., Al Kiyumi, A.A., 2007. Accumulation of Metals in the Egg Yolk and Liver of Hatchling of Green Turtles *Chelonia mydas* at Ras Al Hadd, Sultante of Oman. Journal of Biological Sciences 7, 918- 924.
- Altland, P.D., Thompson, E.C., 1958. Some Factors Affecting Blood Formation in Turtles. Proceedings of the Society for Experimental Biology and Medicine 99, 456-459.
- Anan, Y., Kunito, T., Watanabe, I., Sakai, H., Tanabe, S., 2001. Trace element accumulation in hawksbill turtles *(Eretmochelys imbricata)* and green turtles *(Chelonia mydas)* from Yaeyama Islands, Japan. Environmental Toxicology and Chemistry 20, 2802-2814.
- Andreani, G., Santoro, M., Cottignoli, S., Fabbri, M., Carpenè, E., Isani, G., 2008. Metal distribution and metallothionein in loggerhead *(Caretta caretta)* and green *(Chelonia mydas)* sea turtles. Science of the Total Environment 390, 287-294.
- Armitage, S.A., White, M.A., Wilson, H.K., 1996. The determination of silver in whole blood and its application to biological monitoring of occupationally exposed groups. Annals of Occupational Hygiene 40, 331-338.
- ATSDR, 1990. Toxicological profile for Silver. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, Georgia.
- ATSDR, 1998. Toxicological profile for Chlorinated Dibenzo-p-dioxins (CDDs). Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, Georgia.
- ATSDR, 1999. Toxicological profile for Mercury. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, Georgia.
- ATSDR, 2000. Toxicological profile for Polychlorinated Biphenyls (PCBs). Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, Georgia.
- ATSDR, 2003. Toxicological profile for Selenium. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, Georgia.
- ATSDR, 2004. Toxicological profile for Cobalt. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, Georgia.
- ATSDR, 2005a. Toxicological profile for Nickel. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, Georgia.
- ATSDR, 2005b. Toxicological profile for Zinc. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, Georgia.
- ATSDR, 2007. Toxicological profile for Arsenic. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, Georgia.
- ATSDR, 2008a. Toxicological profile for Cadmium: Draft for Public Comment. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, Georgia, p. 454.
- ATSDR, 2008b. Toxicological profile for Chromium: Draft for Public Comment. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, Georgia, p. 520.
- ATSDR, 2009. Toxicological profile for Vanadium: Draft for Public Comment. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, Georgia.
- Barbieri, E., 2009. Concentration of heavy metals in tissues of green turtles *(Chelonia mydas)* sampled in the Cananéia estuary, Brazil. Brazilian Journal of Oceanography 57, 243-248.
- Bishop, C.A., Ng, P., Pettit, K.E., Kennedy, S.W., Stegeman, J.J., Norstrom, R.J., Brooks, R.J., 1998. Environmental contamination and developmental abnormalities in eggs and hatchlings of the common snapping turtle *(Chelydra serpentina serpentina)* from the Great Lakes-St Lawrence River basin (1989-91). Environmental Pollution 101, 143-156.
- Boon, J.P., Lewis, W.E., Tjoen-A-Choy, M.R., Allchin, C.R., Law, R.J., de Boer, J., ten Hallers-Tjabbes, C.C., Zegers, B.N., 2002. Levels of Polybrominated Diphenyl Ether (PBDE) Flame Retardants in Animals Representing Different Trophic Levels of the North Sea Food Web. Environmental Science & Technology 36, 4025-4032.
- Borgå, K., Gabrielsen, G.W., Skaare, J.U., 2001. Biomagnification of organochlorines along a Barents Sea food chain. Environmental Pollution 113, 187-198.
- Brasfield, S.M., Bradham, K., Wells, J.B., Talent, L.G., Lanno, R.P., Janz, D.M., 2004. Development of a terrestrial vertebrate model for assessing bioavailability of cadmium in the fence lizard *(Sceloporus undulatus)* and in ovo effects on hatchling size and thyroid function. Chemosphere 54, 1643-1651.
- Broman, D., Naef, C., Rolff, C., Zebuehr, Y., Fry, B., Hobbie, J., 1992. Using ratios of stable nitrogen isotopes to estimate bioaccumulation and flux of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in two food chains from the Northern Baltic. Environmental Toxicology and Chemistry 11, 331-345.
- Burger, J., Jeitner, C., Schneider, L., Vogt, R., Gochfeld, M., 2009. Arsenic, Cadmium, Chromium, Lead, Mercury, and Selenium Levels in Blood of Four Species of Turtles from the Amazon in Brazil. Journal of Toxicology and Environmental Health, Part A 73, 33-40.
- Byerrum, R.U., Eckardt, R.E., Hopkins, L.L., Libsh, J.F., Rostoker, W., Zenz, C., 1974. Vanadium. Washington, DC.
- Byrne, A.R., Kosta, L., 1978. Vanadium in foods and in human body fluids and tissues. Science of The Total Environment 10, 17-30.
- Catalani, S., Leone, R., Rizzetti, M.C., Padovani, A., Apostoli, P., 2011. The Role of Albumin in Human Toxicology of Cobalt: Contribution from a Clinical Case. ISRN Hematology 2011.
- Caurant, F., Bustamante, P., Bordes, M., Miramand, P., 1999. Bioaccumulation of Cadmium, Copper and Zinc in some Tissues of Three Species of Marine Turtles Stranded Along the French Atlantic Coasts. Marine Pollution Bulletin 38, 1085-1091.
- Costigan, M., Cary, R., Dobson, S., 2001. Vanadium pentoxide and other inorganic vanadium compounds. World Health Organization, Geneva.
- Day, R.D., Christopher, S.J., Becker, P.R., Whitaker, D.W., 2005. Monitoring Mercury in the Loggerhead Sea Turtle, *Caretta caretta*. Environmental Science & Technology 39, 437-446.
- Day, R.D., Keller, J.M., Harms, C.A., Segars, A.L., Cluse, W.M., Godfrey, M.H., Lee, A.M., Peden-Adams, M., Thorvalson, K., Dodd, M., Norton, T., 2010. Comparison of mercury burdens in chronically debilitated and healthy loggerhead sea turtles *(Caretta caretta)*. Journal of Wildlife Diseases 46, 111-117.
- Day, R.D., Segars, A.L., Arendt, M.D., Lee, A.M., Peden-Adams, M.M., 2007. Relationship of Blood Mercury Levels to Health Parameters in the Loggerhead Sea Turtle *(Caretta caretta)*. Environmental Health Perspectives 115, 1421-1428.
- De Solla, S.R., Bishop, C.A., Van der Kraak, G., Brooks, R.J., 1998. Impact of organochlorine contamination on levels of sex hormones and external morphology of common snapping turtles *(Chelydra serpentina serpentina)* in Ontario, Canada. Environmental Health Perspectives 106, 253-260.
- DEEDI, 2011. Fish Health Sampling Reports. Gladstone Harbour. 3rd November 2011. Brisbane: Department of Employment, Economic Development and Innovation, Queensland.
- Denton, G.R.W., Marsh, H., Heinsohn, G.E., Burdon-Jones, C., 1980. The unusual metal status of the dugong *Dugon dugon*. Marine Biology 57, 201-219.
- DERM, 2012. Strandings hot spots. Department of Environment and Resource Management, Queensland Government.
- Duvall, S.E., Barron, M.G., 2000. A Screening Level Probabilistic Risk Assessment of Mercury in Florida Everglades Food Webs. Ecotoxicology and Environmental Safety 47, 298-305.
- EC, 2006. Polychlorinated Biphenyls (PCBs) Fate and Effects in the Canadian Environment. Available at: [http://www.ec.gc.ca/Publications/default.asp?lang=En&xml=261E1245-FB9E-4A01-91FC-](http://www.ec.gc.ca/Publications/default.asp?lang=En&xml=261E1245-FB9E-4A01-91FC-BF3D4EED546E)[BF3D4EED546E.](http://www.ec.gc.ca/Publications/default.asp?lang=En&xml=261E1245-FB9E-4A01-91FC-BF3D4EED546E)
- Eden, P., Flint, M., Mills, P., Owen, H., 2011. Health assessment of green sea turtles from Gladstone Harbour: July to October 2011. Veterinary Marine Animal Research, Teaching and Investigation (Vet-MARTI), Gatton, Queensland, Australia, p. 23.
- Eisler, R., 1985a. Cadmium hazards to fish, wildlife, and invertebrates: a synoptic review. US Fish and Wildlife Service, Laurel, p. 30.
- Eisler, R., 1985b. Selenium hazards to fish, wildlife, and invertebrates: a synoptic review. US Fish and Wildlife Service, Laurel, p. 41.
- Eisler, R., 1988. Arsenic hazards to fish, wildlife, and invertebrates: a synoptic review. US Fish and Wildlife Service, Laurel, p. 65.
- Eisler, R., 1996. Silver hazards to fish, wildlife, and invertebrates: a synoptic review. National Biological Service, US Department of the Interior, Laurel, p. 48.
- Eisler, R., 1998a. Copper hazards to fish, wildlife, and invertebrates: a synoptic review. Biological Resources Division, US Geological Survey, Laurel, p. 98.
- Eisler, R., 1998b. Nickel hazards to fish, wildlife, and invertebrates: a synoptic review. Biological Resources Division, US Geological Survey, Laurel, p. 76.
- Eisler, R., 2007. Eisler's Encyclopedia of Environmentally Hazardous Priority Chemicals. Elsevier, Oxford.
- Eisler, R., 2010. Compendium of trace metals and marine biota. Elsevier, Oxford.
- Eitzer, B.D., 1993. Comparison of point and nonpoint sources of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans to sediments of the Housatonic River. Environmental Science and Technology 27, 1632-1637.
- Ellermann-Eriksen, S., Martiny Christensen, M., Mogensen, S.C., 1994. Effect of mercuric chloride on macrophage-mediated resistance mechanisms against infection with herpes simplex virus type 2. Toxicology 93, 269-287.
- Falandysz, J., Wyrzykowska, B., Strandberg, L., Puzyn, T., Strandberg, B., Rappe, C., 2002. Multivariate analysis of the bioaccumulation of polychlorinated biphenyls (PCBs) in the marine pelagic food web from the southern part of the Baltic Sea, Poland. Journal of Environmental Monitoring 4, 929-941.
- Farheen, A., Aslam, T., Almas, K., Bhatty, N., Nawaz, M., 2002. Biokinetics of inorganic iron in female volunteers. Pakistan Journal of Medical Research 41, 32-35.
- Frouin, H., Fortier, M., Fournier, M., 2010. Toxic effects of various pollutants in 11B7501 lymphoma B cell line from harbour seal *(Phoca vitulina)*. Toxicology 270, 66-76.
- Gaus, C., Brunskill, G., Weber, R., Päpke, O., Müller, J., 2001. Historical PCDD inputs and their source implications from dated sediment cores in Queensland (Australia). Environmental Science and Technology 35, 4597-4603.
- Gaus, C., O'Donohue, M., Connell, D., Müller, J., Haynes, D., Päpke, O., 2004. Exposure and potential risks of dioxins to the marine mammal dugong. Organohalogen Compounds 66, 1559-1566.
- Gladstone Ports Corporation, 2011. Briefing Western basin dredging and disposal project environmental impacts. Gladstone Ports Corporation, Gladstone, Queensland.
- Gladstone, W., 1996. Trace metals in sediments, indicator organisms and traditional seafoods of the Torres Strait. Great Barrier Reef Marine Park Authority, Townsville, Australia.
- Godley, B.J., Thompson, D.R., Furness, R.W., 1999. Do Heavy Metal Concentrations Pose a Threat to Marine Turtles from the Mediterranean Sea? Marine Pollution Bulletin 38, 497-502.
- Gomez-Caminero, A., Howe, P., Hughes, M., Kenyon, E., Lewis, D., Moore, M., Ng, J., Aitio, A., Becking, G., 2001. Arsenic and Arsenic Compounds. World Health Organization, Geneva.
- Gordon, A.N., Pople, A.R., Ng, J., 1998. Trace metal concentrations in livers and kidneys of sea turtles from south-eastern Queensland, Australia. Marine and Freshwater Research 49, 409-414.
- Grillitsch, B., Schiesari, L., 2010. Chapter 12. The Ecotoxicology of Metals in Reptiles. In: Sparling, D.W., Linder, G., Bishop, C.A., Krest, S.K. (Eds.), Ecotoxicology of Amphibians and Reptiles, Second Edition. CRC Press, pp. 337–448.
- Guirlet, E., Das, K., Girondot, M., 2008. Maternal transfer of trace elements in leatherback turtles (Dermochelys coriacea) of French Guiana. Aquatic Toxicology 88, 267-276.
- Hahn, M.E., 1998. The aryl hydrocarbon receptor: A comparative perspective. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 121, 23-53.
- Haynes, D., Mueller, J.F., McLachlan, M.S., 1999. Polychlorinated dibenzo-p-dioxins and dibenzofurans in Great Barrier Reef (Australia) dugongs *(Dugong dugon)*. Chemosphere 38, 255-262.
- Hermanussen, S., Limpus, C., Paepke, O., Connell, D.W., Gaus, C., 2006. Foraging habitat contamination determines green sea turtle PCDD/F exposure. Organohalogen Compounds 68, 592-595.
- Hermanussen, S., Matthews, V., Päpke, O., Limpus, C.J., Gaus, C., 2008. Flame retardants (PBDEs) in marine turtles, dugongs and seafood from Queensland, Australia. Marine Pollution Bulletin 57, 409-418.
- <span id="page-80-0"></span>Hermanussen, S.F., 2009. Distribution and fate of persistent organic pollutants in nearshore marine turtle habitats of Queensland, Australia. The University of Queensland, St. Lucia, p. 218.
- Hilton, J.W., Bettger, W.J., 1988. Dietary vanadium toxicity in juvenile rainbow trout: a preliminary study. Aquatic Toxicology 12, 63-71.
- Holt, E., Weber, R., Stevenson, G., Gaus, C., 2010. Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans (PCDD/Fs) Impurities in Pesticides: A Neglected Source of Contemporary Relevance. Environmental Science & Technology 44, 5409-5415.
- Hopkins, W.A., Rowe, C.L., Congdon, J.D., 1999. Elevated trace element concentrations and standard metabolic rate in banded water snakes *(Nerodia fasciata)* exposed to coal combustion wastes. Environmental Toxicology and Chemistry 18, 1258-1263.
- Howe, P., Dobson, S., 2002. Silver and Silver Compounds: Enviornmental Aspects (Draft Report). The Concise International Chemical Assessment Document Series. World Health Organization, Geneva, p. 36.
- IARC, 2009. Agents Classified by the IARC Monographs, Volumes 1–103. 1.
- Ikonomopoulou, M.P., Olszowy, H., Limpus, C., Francis, R., Whittier, J., 2011. Trace element concentrations in nesting flatback turtles (Natator depressus) from Curtis Island, Queensland, Australia. Marine Environmental Research 71, 10-16.
- Jerez, S., Motas, M., Cánovas, R.Á., Talavera, J., Almela, R.M., del Río, A.B., 2010. Accumulation and tissue distribution of heavy metals and essential elements in loggerhead turtles *(Caretta caretta)* from Spanish Mediterranean coastline of Murcia. Chemosphere 78, 256-264.
- Kampalath, R., Gardner, S.C., Méndez-Rodríguez, L., Jay, J.A., 2006. Total and methylmercury in three species of sea turtles of Baja California Sur. Marine Pollution Bulletin 52, 1816-1823.
- Kannan, K., Blankenship, A.L., Jones, P.D., Giesy, J.P., 2000. Toxicity Reference Values for the Toxic Effects of Polychlorinated Biphenyls to Aquatic Mammals. Human and Ecological Risk Assessment 6, 181-201.
- Kaska, Y., Celik, A., Bag, H., Aureggi, M., Oezel, K., Elci, A., Kaska, A., Elci, L., 2004. Heavy metal monitoring in stranded sea turtles along the Mediterranean coast of Turkey. Fresenius Environmental Bulletin 13, 769-776.
- Keller, J., McClellan-Green, P., Kucklick, J., Keil, D., Peden-Adams, M., 2006. Effects of organochlorine contaminants on loggerhead sea turtle immunity: Comparison of a correlative field study and in vitro exposure experiments. Environmental Health Perspectives 114, 70-76.
- Keller, J.M., Kucklick, J.R., Harms, C.A., McClellan-Green, P.D., 2004a. Organochlorine contaminants in sea turtles: Correlations between whole blood and fat. Environmental Toxicology and Chemistry 23, 726-738.
- Keller, J.M., Kucklick, J.R., Stamper, M.A., Harms, C.A., McClellan-Green, P.D., 2004b. Associations between Organochlorine Contaminant Concentrations and Clinical Health Parameters in Loggerhead Sea Turtles from North Carolina, USA. Environmental Health Perspectives 112, 1074-1079.
- Kemper, C., Gibbs, P., Obendorf, D., Marvanek, S., Lenghaus, C., 1994. A review of heavy metal and organochlorine levels in marine mammals in Australia. Science of The Total Environment 154, 129-139.
- Kenyon, L.O., Landry, A.M.J., Gill, G.A., 2001. Trace Metal Concentrations in Blood of the Kemp's Ridley Sea Turtle *(Lepidochelys kempii)*. Chelonian Conservation and Biology 4, 128-135.
- Kitana, N., Callard, I.P., 2008. Effect of cadmium on gonadal development in freshwater turtle *(Trachemys scripta, Chrysemys picta)* embryos. Journal of Environmental Science and Health, Part A 43, 262-271.
- Komoroske, L.M., Lewison, R.L., Seminoff, J.A., Deheyn, D.D., Dutton, P.H., 2011. Pollutants and the health of green sea turtles resident to an urbanized estuary in San Diego, CA. Chemosphere 84, 544-552.
- Kubota, R., Kunito, T., Tanabe, S., 2002. Chemical speciation of arsenic in the livers of higher trophic marine animals. Marine Pollution Bulletin 45, 218-223.
- Kubota, R., Kunito, T., Tanabe, S., 2003a. Is arsenobetaine the major arsenic compound in the liver of birds, marine mammals, and sea turtles? Journal de Physique IV 107, 707-710.
- Kubota, R., Kunito, T., Tanabe, S., 2003b. Occurrence of several arsenic compounds in the liver of birds, cetaceans, pinnipeds, and sea turtles. Environmental Toxicology and Chemistry 22, 1200- 1207.
- Kunito, T., Kubota, R., Fujihara, J., Agusa, T., Tanabe, S., 2008. Arsenic in marine mammals, seabirds, and sea turtles. In: Whitacre, D.M. (Ed.), Reviews of Environmental Contamination and Toxicology, Vol 195, pp. 31-69.
- Labrada-Martagón, V., Tenorio Rodríguez, P.A., Méndez-Rodríguez, L.C., Zenteno-Savín, T., 2011. Oxidative stress indicators and chemical contaminants in East Pacific green turtles *(Chelonia mydas)* inhabiting two foraging coastal lagoons in the Baja California peninsula. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 154, 65-75.
- Lam, J.C.W., Tanabe, S., Chan, S.K.F., Yuen, E.K.W., Lam, M.H.W., Lam, P.K.S., 2004. Trace element residues in tissues of green turtles *(Chelonia mydas)* from South China Waters. Marine Pollution Bulletin 48, 174-182.
- Lam, P.K.S., 2009. Tissue Analyses and Ecological Risk Assessment for Marine Mammals in Hong Kong. Final report submitted to the Agriculture, Fisheries and Conservation Department of the Government of the Hong Kong Special Administrative Region, China, p. 251.
- Limpus, C., 1978. The Reef: uncertain land of plenty. In: Lavery, H.J. (Ed.), Exploration North a natural history of Queensland. Lloyd O'Neill Pty Ltd, Sydney.
- Limpus, C.J., 2008. A biological review of Australian Marine Turtles. 2. Green turtle *Chelonia mydas* (Linnaeus). Queensland Environmental Protection Agency, Queensland, Australia, pp. 1-96.
- Limpus, C.J., Chalaupka, M., 1997. Non-power metric regression modelling of green sea turtle growth rates (Southern Great Barrier Reef). Marine Ecology - Progress Series 149, 27-34.
- Limpus, C.J., Cooper, P.J., Read, M.A., 1994. The green turtle, *Chelonia mydas*, in Queensland; population structure in a warm tempered feeding area. Memoires of the Queensland Museum 35, 139-154.
- Lin, T.S., Chang, C.L., Shen, F.M., 2004. Whole Blood Vanadium in Taiwanese College Students. Bulletin of Environmental Contamination and Toxicology 73, 781-786.
- Llobet, J.M., Domingo, J.L., 1984. Acute toxicity of vanadium compounds in rats and mice. Toxicology Letters 23, 227-231.
- Mackay, D., Shiu, W.Y., Ma, K.C., Lee, S.C., 2006. Handbook of physical-chemical properties and environmental fate for organic chemicals. CRC Press, Boca Raton, FL.
- Mackey, E.A., Becker, P.R., Demiralp, R., Greenberg, R.R., Koster, B.J., Wise, S.A., 1996. Bioaccumulation of vanadium and other trace metals in livers of Alaskan cetaceans and pinnipeds. Archives of Environmental Contamination and Toxicology 30, 503-512.
- Malarvannan, G., Takahashi, S., Isobe, T., Kunisue, T., Sudaryanto, A., Miyagi, T., Nakamura, M., Yasumura, S., Tanabe, S., 2011. Levels and distribution of polybrominated diphenyl ethers and organochlorine compounds in sea turtles from Japan. Marine Pollution Bulletin 63, 172-178.
- Marco, A., López-Vicente, M., Pérez-Mellado, V., 2004. Arsenic Uptake by Reptile Flexible-Shelled Eggs from Contaminated Nest Substrates and Toxic Effect on Embryos. Bulletin of Environmental Contamination and Toxicology 72, 983-990.
- Mass, M.J., Wang, L., 1997. Arsenic alters cytosine methylation patterns of the promoter of the tumor suppressor gene p53 in human lung cells: a model for a mechanism of carcinogenesis. Mutation Research - Reviews in Mutation Research 386, 263-277.
- McKenzie, C., Godley, B.J., Furness, R.W., Wells, D.E., 1999. Concentrations and patterns of organochlorine contaminants in marine turtles from Mediterranean and Atlantic waters. Marine Environmental Research 47, 117-135.
- Michibata, H., 2012. Vanadium: biochemical and molecular biological approaches. Springer, New York.
- Miramand, P., Fowler, S., Guary, J., 1992. Experimental study on vanadium transfer in the benthic fish *Gobius minutus*. Marine Biology 114, 349-353.
- Moszczyński, P., 1997. Mercury compounds and the immune system: a review. International journal of occupational medicine and environmental health 10, 247-258.
- Muir, D.C.G., Wagemann, R., Grift, N.P., Norstrom, R.J., Simon, M., Lien, J., 1988. Organochlorine chemical and heavy metal contaminants in white-beaked dolphins *(Lagenorhynchus albirostris)* and pilot whales *(Globicephala melaena)* from the coast of Newfoundland, Canada. Archives of Environmental Contamination and Toxicology 17, 613-629.
- NAS, 2001. National Academy of Science: A Risk Management Strategy for PCB-Contaminated Sediments. . Committee on Remediation of PCB-Contaminated Sediments, Board on Environmental Studies and Toxicology, National Research Council. The National Academies Press.
- Neff, J.M., 1997. Ecotoxicology of arsenic in the marine environment. Environmental Toxicology and Chemistry 16, 917-927.
- Niimi, A.J., 1996. Evaluation of PCBs and PCDD/Fs retention by aquatic organisms. The Science of the Total Environment 192, 123-150.
- Nixon, D.E., Neubauer, K.R., Eckdahl, S.J., Butz, J.A., Burritt, M.F., 2002. Evaluation of a tunable bandpass reaction cell for an inductively coupled plasma mass spectrometer for the determination of chromium and vanadium in serum and urine. Spectrochimica Acta Part B: Atomic Spectroscopy 57, 951-966.
- NJDEP, 2001. Chapter 6: Ecological Effects of Mercury. In: Gochfeld, M. (Ed.), New Jersey Mercury Task Force Report, Volume 2: Exposure and Impacts. New Jersey Department of Environmental Protection, Environmental and Occupational Health Sciences Institute, New Jersey, pp. 55-69.
- Ochi, T., Nakajima, F., Nasui, M., 1999. Distribution of *γ*-tubulin in multipolar spindles and multinucleated cells induced by dimethylarsinic acid, a methylated derivative of inorganic arsenics, in Chinese hamster V79 cells. Toxicology 136, 79-88.
- Orós, J., González-Díaz, O.M., Monagas, P., 2009. High levels of polychlorinated biphenyls in tissues of Atlantic turtles stranded in the Canary Islands, Spain. Chemosphere 74, 473-478.
- <span id="page-83-0"></span>Ott, W.R., 1990. Physical explanation of the lognormality of pollutant concentrations. Journal of the Air & Waste Management Association 40, 1378-1383.
- Páez-Osuna, F., Calderón-Campuzano, M.F., Soto-Jiménez, M.F., Ruelas-Inzunza, J., 2011. Mercury in blood and eggs of the sea turtle Lepidochelys olivacea from a nesting colony in Oaxaca, Mexico. Marine Pollution Bulletin 62, 1320-1323.
- Pearson, R.F., Swackhamer, D.L., Eisenreich, S.J., Long, D.T., 1997. Concentrations, accumulations, and inventories of polychlorinated dibenzo-p-dioxins and dibenzofurans in sediments of the Great Lakes. Environmental Science and Technology 31, 2903-2909.
- Poulsen, A., Escher, B., 2012. Chemically induced immunosuppression and disease susceptibility in marine wildlife: A literature review. National Research Centre for Environmental Toxicology, St. Lucia, p. 110.
- Rattner, B., Golden, N., Toschik, P., McGowan, P., Custer, T., 2008. Concentrations of metals in blood and feathers of nestling ospreys (Pandion haliaetus) in Chesapeake and Delaware Bays. Archives of Environmental Contamination and Toxicology 54, 114-122.
- Ridoux, V., Lafontaine, L., Bustamante, P., Caurant, F., Dabin, W., Delcroix, C., Hassani, S., Meynier, L., Pereira da Silva, V., Simonin, S., 2004. The impact of the "Erika" oil spill on pelagic and coastal marine mammals: Combining demographic, ecological, trace metals and biomarker evidences. Aquatic Living Resources 17, 379-387.
- Ross, P.S., Stern, G., Lebeuf, M., Institute of Ocean Sciences, P.B., 2007. Trouble at the Top of the Food Chain: Environmental Contaminants and Health Risks in Marine Mammals: a White Paper on Research Priorities for Fisheries and Oceans Canada. Canadian Technical Report of Fisheries and Aquatic Sciences 2734. Fisheries and Oceans Canada, Institute of Ocean Sciences.
- Sabbioni, E., Kuèera, J., Pietra, R., Vesterberg, O., 1996. A critical review on normal concentrations of vanadium in human blood, serum, and urine. Science of The Total Environment 188, 49-58.
- Saeki, K., Nakajima, M., Noda, K., Loughlin, T., Baba, N., Kiyota, M., Tatsukawa, R., Calkins, D., 1999. Vanadium accumulation in pinnipeds. Archives of Environmental Contamination and Toxicology 36, 81-86.
- Sakai, H., Ichihashi, H., Suganuma, H., Tatsukawa, R., 1995. Heavy metal monitoring in sea turtles using eggs. Marine Pollution Bulletin 30, 347-353.
- Sakai, H., Saeki, K., Ichihashi, H., Kamezaki, N., Tanabe, S., Tatsukawa, R., 2000a. Growth-Related Changes in Heavy Metal Accumulation in Green Turtle *(Chelonia mydas)* from Yaeyama Islands, Okinawa, Japan. Archives of Environmental Contamination and Toxicology 39, 378-385.
- Sakai, H., Saeki, K., Ichihashi, H., Suganuma, H., Tanabe, S., Tatsukawa, R., 2000b. Species-Specific Distribution of Heavy Metals in Tissues and Organs of Loggerhead Turtle *(Caretta caretta)* and Green Turtle *(Chelonia mydas)* from Japanese Coastal Waters. Marine Pollution Bulletin 40, 701-709.
- Sanders, J.G., 1980. Arsenic cycling in marine systems. Marine Environmental Research 3, 257-266.
- Sankey, T.L., Hedge, S.A., McKenzie, L.J., McCormack, C.V., Rasheed, M.A., 2011. Gladstone Permanent Transect Seagrass Monitoring – April 2011 Update. Department of Employment, Economic Development and Innovation (DEEDI), Fisheries Queensland, Cairns, p. 30.
- Sepe, A., Ciaralli, L., Ciprotti, M., Giordano, R., Funari, E., Costantini, S., 2003. Determination of cadmium, chromium, lead and vanadium in six fish species from the Adriatic Sea. Food Additives and Contaminants 20, 543-552.
- Stavros, H.-C.W., Stolen, M., Durden, W.N., McFee, W., Bossart, G.D., Fair, P.A., 2011. Correlation and toxicological inference of trace elements in tissues from stranded and free-ranging bottlenose dolphins *(Tursiops truncatus)*. Chemosphere 82, 1649-1661.
- Storelli, M.M., Storelli, A., D'Addabbo, R., Marano, C., Bruno, R., Marcotrigiano, G.O., 2005. Trace elements in loggerhead turtles *(Caretta caretta)* from the eastern Mediterranean Sea: overview and evaluation. Environmental Pollution 135, 163-170.
- Styblo, M., Del Razo, L.M., Vega, L., Germolec, D.R., LeCluyse, E.L., Hamilton, G.A., Reed, W., Wang, C., Cullen, W.R., Thomas, D.J., 2000. Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. Archives of Toxicology 74, 289-299.
- Sun, M., 1987. Toxicity of vanadium and its environmental health standard. Changdu, West China University of Medical Sciences, China, [cited in Costigan et al., 2001].
- Swarthout, R.F., Keller, J.M., Peden-Adams, M., Landry, A.M., Fair, P.A., Kucklick, J.R., 2010. Organohalogen contaminants in blood of Kemp's ridley *(Lepidochelys kempii)* and green sea turtles *(Chelonia mydas)* from the Gulf of Mexico. Chemosphere 78, 731-741.
- Talavera-Saenz, A., Gardner, S.C., Riosmena Rodriquez, R., Acosta Vargas, B., 2007. Metal profiles used as environmental markers of Green Turtle *(Chelonia mydas)* foraging resources. Science of The Total Environment 373, 94-102.
- UNEP, 2002. Global mercury assessment. United Nations Environment Programme, UNEP Chemicals, Geneva.
- <span id="page-85-1"></span>USEPA, 2003. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds - Part II[. http://www.epa.gov/ncea/pdfs/dioxin/nas-review/.](http://www.epa.gov/ncea/pdfs/dioxin/nas-review/)
- van de Merwe, J.P., Hodge, M., Olszowy, H.A., Whittier, J.M., Lee, S.Y., 2010. Using blood samples to estimate persistent organic pollutants and metals in green sea turtles *(Chelonia mydas)*. Marine Pollution Bulletin 60, 579-588.
- van de Merwe, J.P.V., Hodge, M., Olszowy, H.A., Whittier, J.M., Ibrahim, K., Lee, S.Y., 2009. Chemical Contamination of Green Turtle *(Chelonia mydas)* Eggs in Peninsular Malaysia: Implications for Conservation and Public Health. Environmental Health Perspectives 117, 1397-1401.
- van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E., 2006. The 2005 World Health Organization Reevaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-Like Compounds. Toxicological Sciences 93, 223-241.
- Varanasi, U., Stein, J.E., Tilbury, K.L., Meador, J.P., Sloan, C.A., Clark, R.C., Chan, S.-L., 1994. Chemical contaminants in gray whales *(Eschrichtius robustus)* stranded along the west coast of North America. Science of The Total Environment 145, 29-53.
- Wagrowski, D., Hites, R., 2000. Insights into the Global distribution of Polychlorinated Dibenzo-pdioxins and Dibenzofurans. Environ. Sci. Technol. 34, 2952-2958.
- White, D.H., King, K.A., Prouty, R.M., 1980. Significance of organochlorine and heavy metal residues in wintering shorebirds at Corpus Christi, Texas, 1976-77. Pesticides Monitoring Journal 14, 58- 63.
- <span id="page-85-0"></span>WHO, 1998. Executive summary: assessment of the health risk of dioxins: re-evaluatin of the Tolerable Daily Intake (TDI). European Centre for Environment and Health - International Programme on Chemical Safety, Geneva, Switzerland.
- Yamanaka, K., Hayashi, H., Tachikawa, M., Kato, K., Hasegawa, A., Oku, N., Okada, S., 1997. Metabolic methylation is a possible genotoxicity-enhancing process of inorganic arsenics. Mutation Research - Genetic Toxicology and Environmental Mutagenesis 394, 95-101.
- Yanochko, G.M., Jagoe, C.H., Brisbin Jr, I.L., 1997. Tissue Mercury Concentrations in Alligators *(Alligator mississippiensis)* from the Florida Everglades and the Savannah River Site, South Carolina. Archives of Environmental Contamination and Toxicology 32, 323-328.
- Yao, D., Zhang, B., 1986. Study on the acute and subchronic toxicity of vanadium pentoxide. Dukou Sanitary and Anti-Epidemic Station [cited in Sun, 1987].

# **8.0 APPENDICES**

### **8.1 RESULTS FOR INDIVIDUAL TURTLE SAMPLES**

**Table 10** Concentrations of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs; ppt lw) in individual (n=22) blood samples of green turtles (*Chelonia mydas*) from Boyne River estuary near Gladstone, Queensland.



< Below the limit of detection (LOD) - the values given are the LOD; note: LOD is sometimes high, this is due to low volumes available for analysis and matrix problems

(<) Values that could not be determined analytically mostly due to interferances in the chromatogram. Predicted values are shown, deduced from similar PCDD/F composition profiles observed across all other blood samples

 $^\dagger$  Middlebound TEQ reported: TEQ values (pg TEQ/g lw) are calculated by including the non-quantified congeners at half the value of their LOQ or predicted value (indicated by <) ND No data

EX ID	Lipid (%)	77	81	126	169	105	114	$118*$	123	156	157	167	189	$^{+}$ $TEQ_{05}$ $\Sigma$ PCBs
	<b>Non-ortho PCBs</b>						<b>Mono-ortho PCBs</b>							
	Juveniles that were euthanised and necropsied													
2	0.14	<170	< 55	< 110	<170	<660		< 220 < 3300 < 220		<660	$<$ 330	$<$ 440	220	8.1
3	0.22	< 97	$33$	<65	< 97	$390$		< 130 < 2000	< 130	$390$	< 200	< 260	< 130	4.8
22		$0.086$ (<200)	(< 78)	< 140	< 210	< 860		$<$ 290 $<$ 4300 $<$ 290		< 860	< 430	< 570	< 290	(511)
Live captured juveniles														
7		$0.062$ (<290) (<110) (<210)			$300$	< 1200		< 620 < 6000		< 400 < 1200	< 600	< 800	< 400	(515)
9	0.20	< 75	$25$	50<	< 75	< 300		< 100 < 1500	< 100	$300$	< 150	250	< 100	3.7
13	0.12	< 160	< 54	< 110	< 160	3300		$<$ 220 11000	390	2000	1300	1800	440	8.4
20	0.11	<170	56	< 180	<170	< 670		< 220 < 3300	< 220	< 670	$<$ 330	$<$ 440	220	11
25	0.23	$90$	<49	<60	$90$	$<$ 360		< 120 < 1800	< 120	$360$	< 180	290	< 120	4.4
30	0.13	< 200	$65$	< 130	< 200	<780		< 260 < 3900	< 260	< 780	$390$	< 520	< 260	9.5
32	0.20	< 100	$35$	< 69	< 100	460		$< 140$ $< 2100$	< 140	< 410	< 210	< 280	< 140	5.1
34	0.070	< 230	<78	< 160	< 230	< 940		$<$ 310 $<$ 4700	< 310	< 940	$<$ 470	800	$310$	12
38	0.15	< 130	<71	<86	< 130	< 510		< 170 < 2600	< 170	< 510	< 260	$340$	<170	6.3
41	0.18	< 100	57	$67$	< 100	440		< 130 < 2000	< 130	< 400	< 200	270	< 130	4.9
42	0.23	< 100	<44	< 69	< 100	$<$ 420		$< 140$ $< 2100$	< 140	< 420	< 210	< 280	< 140	5.1
43	0.11	< 140	$<$ 47	< 94	< 140	810	< 190	2900	440	1000	400	1400	200	7.0
45	0.17	< 110	$36$	< 150	< 110	430		< 150 < 2100	< 140	< 430	< 210	360	< 140	9.0
46	0.17	< 94	$31$	< 63	< 94	$380$		< 130 < 1900	< 130	$380$	$190$	430	< 130	4.6
47	0.14	< 150	< 51	< 100	< 150	< 610		< 200 < 3100	< 200	< 610	$<$ 310	500	< 200	7.5
48	0.11	< 200	< 65	< 130	< 200	<780		< 260 < 3900	< 260	<780	$390$	< 520	< 260	9.5
50		$0.12$ (<140)	(<55)	( < 110)	<150	< 600		$<$ 360 $<$ 3000	< 210	< 600	330	590	230	(5.6)
53		$0.087$ (<200)	(< 78)	< 290	< 210	< 860		< 290 < 4300	$<$ 290	< 860	< 430	< 570	< 290	( < 18)
		Live captured adult												
36	0.15	< 130	<42	< 110	< 130	1900	180	5700	270	2600	1600	1800	520	7.9
		a Delacción lingüístic de desastien (LOD), sheguestro					$L_{\text{max}}$ (OD) $\frac{1}{2}$ and $\frac{1}{2}$ OD) $\frac{1}{2}$ and			ورزاحا والمستنج الحاجا حاربا والمتحاط الماحات الحاجم ومعاطف				

**Table 11** Concentrations of polychlorinated biphenyls (WHO-PCBs; ppt lw) in individual (n=22) blood samples of green turtles (*Chelonia mydas*) from Boyne River estuary near Gladstone, Queensland.

< Below the limit of detection (LOD) - the values given are the LOD; note: LOD is sometimes high, this is due to low volumes available for analysis and matrix problems

( ) values that could not be determined analytically mostly due to interferances in the chromatogram. Predicted values are shown,

deduced from similar PCB composition profiles observed across all other blood samples

\* Indicator PCB

 $^\dagger$  Middlebound TEQ reported: TEQ values (pg TEQ/g lw) are calculated by including the non-quantified congeners at half the value of their LOQ (indicated by <)

**Table 12** Concentrations of organotins (ppb ww) in individual (n=7) blood samples of green turtles (*Chelonia mydas*) from Boyne River estuary near Gladstone, Queensland.



< Below the limit of detection (LOD) - the values given are the LOD; note: LOD is sometimes high, this is due to low volumes available for analysis and matrix problems





< Below the limit of quantification (LOQ)



**Table 14** Concentrations of metals and metalloids (ppb ww) in individual (n=40) blood samples of green turtles (*Chelonia mydas*) from Boyne River estuary near Gladstone, Queensland.

< Below the limit of detection (LOD), the values given are the LOD; note LOD is sometimes high, this is due to low volumes available for analysis and matrix problems

ND No data





ND No data

# **8.2 COMPARISONS OF CONTAMINANT CONCENTRATIONS IN SEA TURTLES**

# 8.2.1 Aluminium (Al)





# 8.2.2 Arsenic (As)



# *Kidney (ppm ww)*











# 8.2.3 Cadmium (Cd)











*Liver (ppm ww)*








# 8.2.4 Chromium (Cr)







## 8.2.5 Cobalt (Co)





China of Se







## 8.2.6 Copper (Cu)















## 8.2.7 Iron (Fe)











## 8.2.8 Lead (Pb)

















## 8.2.9 Manganese (Mn)





Loggerhead 11 Italy Cesenatico & Sicily Island, Mediterranean Cd considered background Stranded ND Pooled sample 1.6 ND ND † Andreani et al 2008 Loggerhead 7 Japan Cape Ashizuri, Kochi Highest Cd in specimens with symptoms of congestion kidney Fishing net entanglement **Adults** (SCL 76-92 cm) Females (n=6) and n=1 male 2.1 1.4 2.9 Sakai et al 1995 Sakai et al 2000a

es















# 8.2.11 Molybdenum (Mo)



**Prefecture** 



# 8.2.12 Nickel (Ni)



#### *Blood (ppb ww)*





#### considered elevated








## 8.2.13 Selenium (Se)

Kaneohe, HI

considered "normal"



with severe

and





and Hervey Bays)



## 8.2.14 Silver (Ag)



#### *Liver (ppm ww)*





# 8.2.15 Vanadium (V)



*Liver (ppm ww)*



## 8.2.16 Zinc (Zn)



## *Kidney (ppm ww)*











subadults (n=67)



#### 8.2.17 Dioxins



- † Converted x0.12 (kidney) x0.22 (liver) according to values of this study
- ‡ Converted x0.195 (kidney) x0.309 (liver) according to Anan et al 2001
- # Converted x0.027 (blood) according to Paez-Osuna et al 2010
- \* Converted x0.28 (kidney) x0.22 (liver) according to Godley et al 1998
- ! Converted x0.251 (blood) according to Paez-Osuna et al 2011
- ˄ Converted from ppm (originally reported on mass basis)

ND No data