Universiteit
Antwerpen

This item is the archived peer-reviewed author-version of:

Distribution and toxicity of persistent organic pollutants and methoxylated polybrominated diphenylethers in different tissues of the green turtle Chelonia mydas

Reference:
Weltmeyer Antonia, Dogruer Gulsah, Hollert Henner, Ouellet Jacob D., Townsend Kathy, Covaci Adrian, Weijs Liesbeth.- Distribution and toxicity of persistent organic pollutants and methoxylated polybrominated diphenylethers in different tissues of the green turtle Chelonia mydas
Environmental pollution - ISSN 0269-7491 - 277(2021), 116795
Full text (Publisher's DOI): https://doi.org/10.1016/J.ENVPOL.2021.116795
To cite this reference: https://hdl.handle.net/10067/1781920151162165141

uantwerpen.be
Institutional repository IRUA

1 **Distribution and toxicity of persistent organic pollutants and methoxylated**
2 **polybrominated diphenylethers in different tissues of the green turtle *Chelonia mydas***
3
4 Antonia Weltmeyer[1,2], Gülsah Dogruer[2,3], Henner Hollert[1,4], Jacob D. Ouellet[1,4], Kathy
5 Townsend[5], Adrian Covaci[6], Liesbeth Weijs[2*]
6
7 [1]RWTH Aachen University, Institute for Environmental Research, Aachen, Germany
8 [2]School of Environment and Science, Australian Rivers Institute, Griffith University, Gold
9 Coast, Australia
10 [3]Wageningen Marine Research, Wageningen University and Research, Ijmuiden, The
11 Netherlands
12 [4]Department Evolutionary Ecology and Environmental Toxicology, Faculty Biological
13 Sciences, Goethe University Frankfurt, Frankfurt am Main, Germany
14 [5]Faculty of Science and Engineering, University of the Sunshine Coast, Hervey Bay,
15 Australia
16 [6]Toxicological Center, University of Antwerp, Belgium
17
18 *****corresponding author email:
19 Dr Liesbeth Weijs
20 Griffith University, School of Environment and Science, Australian Rivers Institute
21 Queensland, Australia
22 E-mail: l.weijs@griffith.edu.au
23
24 **Keywords:** POPs, PCBs, MeO-PBDEs, toxicity, micro-EROD assay, green turtles, Australia

25 **Highlights:**

26 - Liver, fat, kidney and muscle tissue from 30 green turtles were investigated
27 - Lipophilic compounds and potential mixture toxicity were assessed
28 - Majority of POPs were not detected or were found at low concentrations
29 - Few correlations between POPs and age, gender, location and year of death
30 - μEROD assay outcomes suggest presence of dioxin-like compounds

Abstract

Investigating environmental pollution is important to understand its impact on endangered species such as green turtles (*Chelonia mydas).* In this study, we investigated the accumulation and potential toxicity of selected persistent organic pollutants (POPs) and naturally occurring MeO-PBDEs in liver, fat, kidney and muscle of turtles (n=30) of different gender, size, year of death, location and health status. Overall, POP concentrations were low and accumulation was highest in liver and lowest in fat which is likely due to the poor health of several animals, causing a remobilization of lipids and associated compounds. PCBs and *p,p'*-DDE dominated the POP profiles, and relatively high MeO-PBDE concentrations (2'-MeO-BDE 68 up to 192 ng/g lw, 6-MeO-BDE 47 up to 79 ng/g lw) were detected in all tissues. Only few influences of factors such as age, gender and location were found. While concentrations were low compared to other marine wildlife, biological toxicity equivalences obtained by screening the tissue extracts using the micro-EROD assay ranged from 2.8 to 356 pg/g and the highest values were observed in muscle, followed by kidney and liver. This emphazises that pollutant mixtures found in the turtles have the potential to cause dioxin-like effects in these animals and that dioxin-like compounds should not be overlooked in future studies.

Capsule

Concentrations of legacy pollutants in green turtles from Queensland are relatively low but pollutant mixtures still have the potential to cause dioxin-like effects.

Introduction

With marine environments acting as sinks for a wide variety of chemicals, environmental pollution poses a great challenge to several coastal marine megafauna species and can impact on their ability to survive and thrive. During the sub-adult and adult life stages, the green turtle adopts a herbivorous lifestyle, shows high site-fidelity and inhabits mostly inshore foraging grounds (Arthur et al., 2008; Shimada et al., 2016) which brings them in close proximity to sources of manmade persistent organic pollutants (POPs), such as agriculture, industry or municipal wastewater (Gallen et al., 2019). Because of the proximity of green turtles to terrestrial sources of environmental pollution, previous studies have already shown the bioaccumulation and biomagnification of POPs in different tissues of these animals (Hermanussen et al., 2008; Oros et al., 2009; van de Merwe et al., 2010; da Silva et al., 2016; Clukey et al., 2018), however, information about the toxic effects of POPs in this species remains scarce.

In a review in 2016, Finlayson et al. reported just four studies that investigated common endpoints for exposure to POPs in green turtles (Finlayson et al., 2016). Since 2016, only a limited number of studies focusing on the same topic have been published (e.g. Dogruer et al., 2018; Finlayson et al., 2019a, 2019b) indicating that our knowledge about associations between exposure and effect in green turtles is sparse. This is partly due to the species' status: Like other marine megafauna species, sea turtles are protected species which forces exposure experiments to be done in an *in vitro* setting, either with turtle cells (e.g. Finlayson et al., 2019a, 2019b) or commercially available options (e.g. Dogruer et al., 2018). *In vitro* bioanalytical techniques have received increasing attention in the last decade due to their ability to detect adverse effects resulting from complex chemical mixtures (Eichbaum et al., 2014; Altenburger et al., 2015; Kienzler et al., 2016). The latter is very important for sea turtles or (marine) wildlife in general as these species are unlikely exposed to just one

77 compound or compound class. These concepts were considered in several recent studies.
78 Desforges et al. (2017) used extracts derived from polar bear and killer whale blubber to test
79 the effect of the extracts' known and unknown compounds on various immune functions *in*
80 *vitro*. Jin et al. (2015) and Dogruer et al. (2018) used extracts derived from green turtle blood
81 to study the effect of the pollutant mixtures in the extracts on several endpoints by using a
82 range of commercially available bioassays.
83 Following these examples, the present study aims to contribute to the toxicology of green
84 turtles in Australia by investigating the presence of various legacy POPs and MeO-PBDEs as
85 well as the toxicity of the chemical mixtures in fat, liver, kidney, muscle tissue and urine of
86 30 green turtles collected from various regions along the southeast Queensland (Australia)
87 coastline. Green turtles show high site fidelity upon adulthood when they go back to their
88 place of birth to reproduce which may have consequences for the pollutant body burdens in
89 the animals as evidenced by recent studies in Australian green turtles (Gallen et al., 2019;
90 Vijayasarathy et al., 2019; Gaus et al., 2019). In those studies, animals from three sites
91 characterized by different anthropogenic influences (i.e. site with agricultural activities, site
92 with industrial activities, control site) were investigated and distinct pollutant signatures were
93 reported. While the 2019 studies were part of a comprehensive project (Rivers to Reefs to
94 Turtles, WWF Australia and partners), several questions remained unanswered. Firstly, only
95 whole blood and scutes were examined (Gaus et al., 2019; Vijayasarathy et al., 2019).
96 Neither are ideal matrices for lipophilic pollutants such as PCBs, PBDEs and OCPs which
97 explains at least partly why only low levels of these compounds were found (Vijayasarathy et
98 al., 2019). Secondly, only animals from north Queensland (Great Barrier Reef) were
99 included. While the northern part of Queensland has regions with specific anthropogenic
100 influences, southeast Queensland has a higher degree of urbanization thereby further
101 increasing the pressure of pollution on the marine environment. In this regard, Moreton Bay

102 is of particular interest. Moreton Bay is a semi-enclosed, relatively shallow basin in southeast
103 Queensland that receives runoff from major rivers such as the Brisbane River which flows
104 through the third most populous city in Australia. Earlier studies reported the presence of
105 POPs and several DLCs in various marine wildlife species from this region (e.g.
106 Hermanussen et al., 2008; van de Merwe et al., 2010; Weijs et al., 2019). In 2011, excessive
107 rain caused the Brisbane River to overflow which triggered an increase in urban runoff into
108 Moreton Bay and threatened marine wildlife. This study complements the existing studies on
109 pollutants in green turtles in southeast Queensland and aims to fill knowledge gaps with
110 regards to tissue-specific bioaccumulation and mixture toxicity. Additionally, for the first
111 time, methoxylated PBDEs in several tissues of green turtles were investigated. In contrast to
112 PCBs and PBDEs, MeO-PBDEs are naturally-produced by (red) algae and sponges (Vetter,
113 2006). However, they have also been suggested as a potential source of metabolically derived
114 hydroxylated PBDEs, which exhibit greater toxicities than PBDEs and MeO-PBDEs for
115 several toxicity endpoints (Wiseman et al., 2011).

116 **Materials & Methods**

117 *Sample information*

118 Tissue samples were collected from 30 green turtles (*Chelonia mydas*) from Queensland
119 (QLD), Australia, from 2008 to 2017. Fat (F, n=22), liver (L, n=28), kidney (K, n=22) and
120 muscle (M, n=15) were analysed. All tissue samples were collected post-mortem: most
121 animals were found dead, but two individuals were euthanized by authorities due to poor
122 health. Available information on age, gender, body size, location, year of death, and
123 pathology is provided in the Supplementary Information (SI; Table S1). Three urine samples
124 were also included in the analyses. Details about the sample preparation and analysis of the
125 urine samples can be found in SI, while details about the sample preparation and analysis of
126 liver, kidney, muscle and fat are given below.

127

128 *Sample preparation and analysis*

129 Organ tissues were wrapped in aluminium foil in plastic ziplock bags and stored frozen at -
130 20°C until preparation. Tissues were defrosted and subsamples were taken from the core
131 where possible to avoid any cross contamination from the foil or bags. Some tissues were
132 freeze-dried prior to sample preparation - these are indicated in the graphs where appropriate.
133 The sample clean-up and extraction method for the liver, kidney, muscle, and fat samples
134 followed in the present study has been published previously in Weijs et al. (2019) and is
135 briefly described below. Approximately 1.5 g (liver, kidney, muscle) or 0.2 g (fat) was
136 homogenized in anhydrous Na_2SO_4 and spiked with 75 µL internal standard consisting of
137 PCB 143 (200 pg/µL), ^{13}C-HCB (25 pg/µL), ε-HCH (25 pg/µL) and BDE 77 (25 pg/µL) in
138 iso-octane. The subsamples were extracted three times (>2h, overnight, >2h) with ~20 mL
139 hexane:acetone (*v:v*, 3:1), followed by gravimetrical lipid determination. Subsequently, the
140 samples were cleaned up with approximately 8 g of acidified silica (44% sulphuric acid, w/w)

141 and extracted with 20 mL hexane and 15 mL dichloromethane. The extracts were then
142 evaporated to near dryness and reconstituted in 50 µL iso-octane and 50 µL recovery
143 standard consisting of PCB 207 (50 pg/µL). All solvents used were SupraSolve grade.
144 In all samples, 29 polychlorinated biphenyl congeners (PCBs; IUPAC numbers: 28, 47, 49,
145 52, 66, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 177, 180, 183,
146 187, 194, 196/203, 199, 206, 209), 7 polybrominated diphenyl ethers (PBDEs; IUPAC
147 numbers: 28, 47, 99, 100, 153, 154, 183), 3 pesticides (DDXs; *p,p'*-DDE, *p,p'*-DDD, *p,p'*-
148 DDT), 5 chlordanes (CHLs; oxychlordane - OxC, *trans*-nonachlor - TN, *cis*-nonachlor - CN,
149 *trans*-chlordane - TC, *cis*-chlordane - CC), hexachlorobenzene (HCB), 3
150 hexachlorocyclohexanes (HCHs; α, β, γ) and 2 methoxylated PBDEs (2'-MeO-BDE 68 and
151 6-MeO-BDE 47) were targeted for analysis.
152 An Agilent 6890 gas chromatograph equipped with a 30 m × 0.25 mm × 0.25 µm DB-5
153 capillary column was coupled with a 5973 mass spectrometer (MS) used in electron capture
154 negative ionisation (ECNI) mode. Individually selected samples (CM-14 (L, K), CM-16 (M),
155 CM-27 (K), CM-28 (L, K), CM-41 (L, F, K), CM-42 (L, K, M) and both reference samples)
156 with significant PCB contamination and abundance were additionally measured with electron
157 impact ionization (EI) mode to screen for lower halogenated PCBs (IUPAC numbers: 28, 47,
158 49, 52, 66, 74, 95, 110, and 149) which have lower sensitivity in the ECNI mode. Both MS
159 modes were used in selected ion-monitoring (SIM) mode with two ions monitored for each
160 analyte or homologue group.
161
162 *Quality Assurance/Quality Control (QA/QC)*
163 QA/QC was accomplished by procedural blanks and quality control samples. These quality
164 control samples were taken from a large blubber sample of a humpback dolphin that was
165 analysed previously by several different laboratories and groups (Table S2). For each analyte,

166 the mean procedural blank value was subtracted, and the recovery of the respective internal
167 standard was used to calculate the analyte concentrations. Recoveries (standard deviation) for
168 the internal standards across all matrices were 102 (18), 78 (13), 100 (21) and 91 (16) % for
169 PCB 143, ^{13}C-HCB, ε-HCH and BDE 77, respectively. For compounds present in the
170 procedural blanks, the limit of quantification (LOQ) was set at 3 times the standard deviation
171 of the procedural blanks, which ensures >99% certainty that the reported value is originating
172 from the sample. For compounds not present in the blanks, LOQ was defined as the
173 concentration giving an instrumental S/N=10. LOQs ranged from 0.1-10 ng/g lipid weight
174 (lw) (median: 0.4 ng/g lw) depending on the matrix and analyte. QC was performed by
175 regular analyses of procedural blanks, process control samples, calibration standards and
176 solvent blanks.
177
178 *Cell culture*
179 H4IIE cells (ATCC, cat. no. CRL 1548) were grown at 95% humidity, 5% CO_2 and 37°C in
180 Dulbecco's modified eagle medium (DMEM; low glucose, Life Technologies GmbH)
181 supplemented with fetal bovine serum (BioWest), 1M HEPES buffer (Sigma-Aldrich) and a
182 200 mM GlutaMAX solution (Life Technologies) according to Schiwy, et al. (2015). The
183 cells were passaged every 3-4 days with a splitting factor of 1:10. Cells were passaged no
184 more than 50 times.
185
186 *Micro-EROD assay*
187 Dioxin-like compounds (DLCs) are known to bind to the aryl hydrocarbon receptor (AhR),
188 which leads to the induction of cytochrome P450 (CYP1A1) (Whyte et al., 2000).
189 Importantly, CYP1A1 metabolizes DLCs and can be quantified by the fluorometric
190 determination of the artificial substrate 7-ethoxyresorufin within the micro-EROD assay

191 (Schiwy et al., 2015). The results from the micro-EROD assay are generally expressed as
192 biological equivalence quotients (BEQ), which characterize the toxic potencies of the DLCs
193 (measured by effect concentrations [EC]) compared to the benchmark substance 2,3,7,8-
194 TCDD (also measured by EC) (reviewed in Van den Berg et al. (1998)). The micro-EROD
195 assay followed the protocol of Schiwy et al. (2015) and is briefly presented below. Before
196 testing, the extracts were transferred from iso-octane to the respective amount of dimethyl
197 sulfoxide (DMSO, >99.5%, Roth) by evaporation until dryness and resuspension.
198 Approximately 10,000 cells in 50 µL medium were seeded in transparent 96 well plates
199 (CytoOne), except for the first row which was later used for the protein standard and blank,
200 and left to attach for at least 2 h (95% humidity, 5% CO_2, 37°C). Subsequently the cells were
201 exposed to the extracts across a serial dilution range for 68-72 h. A negative control (DMSO
202 + medium) and standard (2,3,7,8-TCDD) were also included on each plate. All sample
203 dilutions and the TCDD standard were exposed in final concentrations of 1% DMSO to
204 prevent variable substance uptake into the cells. After incubation, 100 µL of 100-fold dilution
205 of ethoxyresorufin (49 mL PBS with $MgCl_2$ and $CaCl_2$, 500 µL of 8 µM ethoxyresorufin
206 (ETX) and 500 µL of 1 mM Dicumarol) was added to the wells and incubated (37 °C, 5%
207 CO_2, and 95% humidity) for exactly 30 min. The reaction was stopped by the addition of 75
208 µL of cold methanol and placed on a shaker for 10 min at 300 rpm. Fluorescence was
209 measured with a TECAN M200 Infinite using an excitation and emission wavelength of 530
210 nm and 590 nm, respectively.
211 Following the measurement of fluorescence, the bicinchoninic acid assay was conducted to
212 assess the protein concentration in each well. A calibration curve containing bovine serum
213 albumin:ETX solution (*v:v*, 1:4) with a two-fold serial dilution was applied to the first row of
214 each plate. All wells were then filled with 100 µL of a solution of 40 mL BCA reagent A and
215 800 µL BCA reagent B and incubated for 40 min at room temperature. The protein

216 measurement was carried out with the TECAN reader at an absorbance measurement
217 wavelength of 550 nm.
218
219 *Statistical analysis*
220 Non-parametric statistical analyses were performed as the data were not normally distributed
221 and sample sizes were often relatively small. For all compounds that were detected in more
222 than 50% of the tissue samples (Table S3), concentrations below LOQ were replaced with f
223 (frequency of detection) * LOQ according to James et al. (2002). Compounds that were
224 detected in less than 50% of the tissue samples were excluded from further calculations and
225 statistics. The influences of gender, age group and location on concentrations in liver were
226 tested using Kruskal-Wallis tests while correlations between concentrations and CCL (curved
227 carapace length) were tested with Spearman's test. Temporal differences were only
228 investigated in the liver of animals from Moreton Bay (Kruskal-Wallis). Statistical tests were
229 performed with GraphPad Prism (Version 8) or SPSS (IBM SPSS Statistics version 25) and
230 the level of statistical significance was set at $\alpha = 0.05$. Graphs were produced with GraphPad
231 Prism (Version 8).
232 For the micro-EROD analysis, EC_{50} values were calculated with a four-parameter variable
233 slope model in GraphPad Prism (Version 8). BEQs were calculated by dividing EC_{50} of the
234 respective TCDD standard by EC_{50} of the respective sample. T-tests were used to evaluate
235 whether there were differences between the biological replicates (n=2).

Results

Detection frequency, non-detects and lipid percentages

In general, compounds CB 47, CB 49, CB 95, CB 110, *p,p'*-DDT, BDE 28 and BDE 183 were not detected in any sample, while 36 compounds were only detected in < 50% of the tissue samples (Table S3). 2'-MeO-BDE 68 had the highest detection frequency (average of 91% in all tissues; 96% in liver, 100% in fat, 91% in kidney, 73% in muscle) followed by CB 153 (average of 71% in all tissues; 100% in liver, 27% in fat, 77% in kidney, 40% in muscle). CB 138 (51%) and 180 (66%), TN (51%), HCB (57%) and 6-MeO-BDE 47 (65%) were further found in > 50% of all tissue samples (Table S3). Statistics and calculations were only done with compounds detected in > 50% of the samples, i.e. CB 153, 138, and 180 (with sum of PCBs being the sum of these three congeners), 2'-MeO-BDE 68 and 6-MeO-BDE 47 (with sum of MeO-PBDEs being the sum of these two compounds), HCB, and TN (Table S3). The average lipid percentages (± SD) were 63 ± 16 % for fat, 6.5 ± 6.3 % for liver, 3.1 ± 1.1 % for kidney, and 0.5 ± 0.5 % for muscle (Table S4). Fat samples with too low lipid percentages (range: 0 - 3.7%) were excluded from any calculations. There were no statistically significant differences between lipid percentages in males, females, and individuals of unknown gender ($p > 0.05$ for all comparisons). There were also no statistically significant correlations between lipid percentages of any tissue and concentrations of sum PCBs, sum MeO-PBDEs, HCB, or TN ($0.0663 < p\text{-values} < 0.8761$; Fig S1). No POPs or MeO-PBDEs were found in the urine samples.

Tissue distribution: profiles and concentrations

Overall, the highest concentrations of PCBs (CB 138, 153 and 180) and TN were detected in the liver (max concentrations: 306 ng/g lw, 975 ng/g lw, 290 ng/g lw, 208 ng/g lw for CB 138, 153, 180 and TN, respectively) while the highest concentrations of HCB were found in

261 muscle (max: 63 ng/g lw; Table 1). Regarding the naturally occurring MeO-PBDEs, the
262 highest levels were reported either in fat (2'-MeO-BDE 68; max: 192 ng/g lw) or muscle (6-
263 MeO-BDE 47; max: 79 ng/g lw) (Table 1). On an individual level, however, these profiles
264 were inconsistent: for animals for which all tissues were available (n = 10; liver, kidney,
265 muscle, and fat), liver was more dominant followed by muscle, fat, and kidney regardless of
266 the compound (Table 2).
267

268 *Influence of gender, age, and location*

269 Statistical tests were only done for the liver as this was the tissue with the highest sample size
270 (n = 23; a total of 28 liver samples were analysed, however, either gender, age or location
271 was not available for four animals and one animal was identified as outlier as identified
272 through boxplots) and detection frequencies for most compounds (Table S3). The only
273 statistically significant differences were found for CB 138, 153, and 180 between animals
274 from the Coral Sea (n = 3) and coastal animals (Moreton Bay (n = 14) and Sunshine Coast (n
275 = 6)) (p = 0.013, 0.007, and 0.042 for CB 138, 153 and 180, respectively) with the coastal
276 animals having higher PCB concentrations compared to the animals from the Coral Sea. As
277 there were only three animals from the Coral Sea, these results should be investigated with a
278 larger sample size and could be a statistical artifact. Nevertheless, as animals from the Coral
279 Sea were all immature females, these differences could be caused by age and/or gender. For
280 this reason, interactions between the variables were also tested. The spatial differences were
281 found to be driving the statistics when combined with the age and gender of the animals for
282 CB 153 (p = 0.043 for location and age, p = 0.017 for location and gender) and for CB 138 (p
283 = 0.034 for location and gender). However, no statistically significant differences were found
284 for TN ($0.297 < p < 0.820$), HCB ($0.397 < p < 0.609$) or any of the MeO-PBDEs ($0.227 < p <$
285 0.995) when testing the single variables (age, gender, location) or interactions between the

286 variables. As there were more immature and juvenile animals compared to adults,
287 correlations with the individual body sizes (curved carapace length; CCL) were also
288 explored. In contrast to the results using the age groups, no correlations between PCB, TN, or
289 6-MeO-BDE 47 concentrations and CCL were detected for animals from either location. A
290 statistically significant and positive correlation was found between HCB concentrations and
291 CCL of animals from the Sunshine Coast (Spearman's $r = 0.7827$, $p = 0.0389$), while a
292 statistically significant and negative correlation was found between 2'-MeO-BDE 68 and
293 CCL of animals from the Sunshine Coast (Spearman's $r = -0.8857$, $p = 0.0167$) (Fig S2).
294

295 *Temporal changes in Moreton Bay*

296 To assess potential changes in pollutant body burdens as a result of the 2011 floods in
297 Brisbane (Moreton Bay), animals from Moreton Bay were divided into three groups: pre-
298 floods (2008-2010; n = 3), during floods (2011; n = 8), and after floods (2013-2016; n = 3).
299 For CB 138, TN, HCB, and 2'-MeO-BDE 68, median concentrations were higher in 2011
300 during the floods than in the years before and after the floods (Fig 1). The opposite was
301 observed for CB 153 and 180 which were lowest in 2011 compared to the years before and
302 after (Fig 1). However, none of these observations were statistically significant ($0.060 < p <$
303 0.973).
304

305 *EROD activity*

306 A total of 24 samples, including 22 turtle and 2 reference samples (i.e. humpback dolphin
307 blubber samples also used as quality controls for POPs, see Table S2), were examined with
308 the micro-EROD assay. Two biological replicates were analysed for all samples, each
309 containing 4 technical replicates per concentration. The mean for the 2,3,7,8-TCDD EC_{50}
310 value in this study was 1.3 ± 0.4 pg/mL. Of all 24 investigated samples, only 7 did not result

311 in EROD-induction with one sample (CM-1 (kidney)) having ambiguous results between

312 replicates, resulting in 17 EC_{50} values (including 2 references) ranging from 2.8 to 356 pg

313 TCDD/g lw (Table 3).

314

Discussion

Tissue distribution, pollutant concentrations and profiles.

The lipid content of the tissues is one of the drivers of accumulation of lipophilic compounds and usually follows the order of fat > liver > kidney > muscle in marine turtles according to the literature (e.g van de Merwe et al., 2010; da Silva et al., 2016). This order in lipid percentage was also found in the present study but could not explain the pollutant profiles in the tissues. In marine mammals, blubber typically contains the highest levels of lipophilic compounds. In addition, lower PCB concentrations in liver compared to fat tissue in marine turtles were also reported by Miao et al (2001), McKenzi et al (1999) and da Silva et al (2016).

In the present study, however, the highest levels of pollutants were often detected in liver with fat being the preferred target in only a limited number of cases (Table 2). This may be a consequence of green turtles being cold-blooded reptiles that rely less on blubber or fat to maintain body temperature compared to marine mammals. At the same time, it may also reflect the animals' health condition, a different lipid composition or the complexity behind the distribution and fate of pollutants in the body of a species for which metabolic capacities and toxicokinetics in general are poorly known. Barco et al. (2016) observed that the amount of lipids in fat tissue differed between turtles who died either from fishing incidents or other causes, indicating that those who died through the former cause were in better condition with higher lipid amounts. In the present study, the causes of death could not be identified for all individuals, however, the majority of individuals had infections, serious flesh wounds or considerable quantities of plastic debris in their stomachs. As for other marine megafauna species, sea turtles mobilize their lipids and associated pollutants when debilitated. Re-circulated pollutants are then more likely to accumulate in the liver as a highly perfused and

340 lipid rich tissue (van de Merwe et al., 2010; Keller et al., 2004; Oros et al., 2009) which may
341 (partly) explain the overall higher levels of pollutants found in the liver compared to the fat.
342 Our data suggest a higher dominance of MeO-PBDEs in fat compared to all other tissues with
343 anthropogenic lipophilic compounds having a higher presence in liver (Table 2) which is not
344 entirely similar as what was found for dugongs from the same area (Weijs et al., 2019).
345 Although dugongs and green turtles have a comparable diet and share the same habitat, they
346 are completely different species that use fat or blubber in different ways and likely have
347 different energetic requirements as well as metabolic and absorption capacities. The latter is
348 also evident when comparing the pollutant profiles of HCB, TN, CB 138, CB 153, CB 180
349 and 2'-MeO-BDE 68 in liver of both species (Fig 2). According to Figure 2, green turtles
350 have much lower proportions of CB 138 and 153 compared to dugongs while dugongs have
351 much lower proportions of 2'-MeO-BDE 68 than green turtles. As both animals share the
352 same habitat and food, this may be attributed to species specific differences in metabolizing
353 pollutants. This is supported by the fact that *p,p'*-DDE can be found in considerable levels in
354 dugongs (Weijs et al., 2019), while it was not even found in half the samples (any tissue) of
355 green turtles (Table S3). However, other explanations such as differences in age (most green
356 turtles were immature/juvenile, all dugongs were adult males) or in ingestion/absorption, may
357 also be valid. In addition, maternal transfer of pollutants primarily via the lipid-rich milk has
358 been reported numerous times in various marine mammal species, resulting in relatively high
359 loads of pollutants in their young. In contrast, reproduction in green turtles occurs through
360 egg-laying and hatchlings live in the open ocean for the first 15 years of their lives which
361 gives a lower body burden or 'starting point' for green turtles.
362
363 Out of approximately 50 targeted compounds, none of these compounds were detected in the
364 urine samples and only 7 were detected in > 50% of the liver, fat, muscle, and kidney

365 samples. The absence of compounds in the urine samples can be explained by the
366 hydrophobicity or lipophilicity of the targeted compounds as well as their overall low levels
367 in the other tissues. Rehabilitated green turtles from southeast Queensland have previously
368 been investigated by van de Merwe et al. (2010) and POPs were generally detected at higher
369 levels compared to the median values found in the present study. However, maximum values
370 from the present study mostly exceeded the POP concentrations reported by van de Merwe et
371 al. (2010). POPs and especially PCBs are some of the most investigated compounds in
372 marine wildlife and have been shown to influence population dynamics on a long-term scale
373 (Jepson et al., 2016; Desforges et al., 2018). In our study, only three PCB congeners were
374 detected in > 50% of the samples with CB 153 being the most dominant one. CB 153 was
375 followed by CB 180 or CB 138. Many studies with green turtles (Miao et al., 2001; da Silva
376 et al., 2016; Richardson et al., 2010; Swarthout et al., 2010) and other marine turtle species
377 (Corsolini et al., 2000; Keller et al., 2004; Oros et al., 2009) have reported similar patterns
378 although with greater concentrations of CB 138 compared to CB 180. In the present study,
379 CB 180 dominated over CB 138 in 53% of all tissue samples, especially in liver in which CB
380 180 was greater than CB 138 in 64% of the samples, which may be attributed to different
381 PCB congener compositions in the environment. In contrast to the dugongs from Moreton
382 Bay, DDE was not even found in half the samples and was therefore not included in any
383 statistics. However, *p,p'*-DDE had the highest concentrations among DDXs and was, except
384 for one single detection of *p,p'*-DDD, the only detected compound in the present study. As it
385 is a metabolite of DDT and DDD (Hellou et al., 2013), these results indicate that there are no
386 new sources of DDXs in this region despite an illegal pesticide application in Dunwich
387 (Stradbroke Island, Moreton Bay), which has been linked to the death of many nearby soldier
388 crabs and stingrays (Kathy Townsend, personal communication).

389 Among CHLs, profiles in liver resembled previously detected ones for chlordanes with
390 highest abundance of *trans*-nonachlor and oxychlordane followed by *cis*-chlordane (van de
391 Merwe et al., 2010; Malarvannan et al., 2011). *Cis*-chlordane was found to dominate
392 primarily in kidney (this study: 0.1-1.2 ng/g lw), and appears to be more persistent than other
393 CHLs as it was detected in animals that did not accumulate any other CHLs. HCHs were only
394 sporadically detected and were mostly in the order of β->α->γ-HCH. This is also in
395 accordance with previous observations from sea turtles (McKenzie et al., 1999; van de
396 Merwe et al., 2010; Malarvannan et al., 2011).
397 Although none of the PBDE congeners were found in more than 50% of the samples, BDE 47
398 was detected more frequent than all other PBDEs while no clear pattern for other detected
399 PBDEs could be observed as a result of low detection frequencies (Table S3). This
400 predominance of BDE 47 is fairly common in marine wildlife (Losada et al., 2009;
401 Hermanussen et al., 2008). In Moreton Bay and Brisbane river sediments, which represent
402 preferential sinks for pollutants because of their close proximity to pollutant sources, BDE
403 209 was found to dominate the PBDE profile in 87% (Toms et al., 2008) and 100% (Anim et
404 al., 2017) of all samples. BDE 209 was only found in one turtle in the present study, however,
405 it is possible that it could have degraded in other samples as it is photosensitive
406 (Christiansson et al., 2009).
407 Methoxylated PBDEs are naturally occurring analogues of PBDEs that are produced by
408 sponges and algae (Vetter, 2006; Haraguchi et al., 2010). With the exception of two kidney
409 samples, levels of 2'-MeO-BDE-68 (ranging from <LOQ-188 ng/g lw) were greater than
410 levels of 6-MeO-BDE-47 (ranging from <LOQ-79 ng/g lw), which is consistent with
411 previous findings from Australia in dugongs and several other marine species in various
412 positions in the marine food chain (Losada et al., 2009; Weijs et al., 2019). Both compounds
413 were detected frequently; the detection frequency averaged 91% for 2'-MeO-BDE-68 and

65% for 6-MeO-BDE-47 throughout all tissues which is consistent with studies in dugongs (Weijs et al., 2019; 90% and <50%, respectively) and various other marine species from Sydney harbor (Losada et al., 2009).

Influence of gender, age, year of death and location.

In the present study, samples from turtles with different gender, age, and location were examined, however, only few statistically significant differences were found. Significant differences were detected for PCBs between animals from the Coral Sea and animals from coastal regions (Moreton Bay and Sunshine Coast) with animals from coastal regions having higher levels compared to the offshore ones. This corresponds with the results from the Rivers to Reefs to Turtles project in which animals from a remote region were less contaminated to animals from regions with different anthropogenic stressors (industrial, agricultural) (Dogruer et al., 2018). Gender and age did not play a significant role in pollutant bioaccumulation in the present study, possibly due to limited sample size, a higher number of immature and juvenile animals compared to adults, or the higher number of females compared to males.

In other marine megafauna such as marine mammals, there are usually distinct differences in POPs between males and females due to the maternal transfer of pollutants to the offspring during gestation and lactation. As the reproductive strategy of sea turtles is different to marine mammals (e.g. egg laying, no maternal care), large gender differences were not expected or observed in the present study. This is in agreement with several other studies that have also not observed any differences with respect to gender associated differences in POP concentrations of marine turtles (Clukey et al., 2018; Keller et al., 2004; Storelli and Zizzo, 2014). As for potential changes of pollutants in the environment over time: no consistent temporal trends could be found for the animals within this study. In addition, no impacts of

439 the 2011 floods in Moreton Bay were found (Fig 1). The absence of temporal trends in the
440 turtles of Moreton Bay is consistent with results from a previous study examining POP
441 concentrations in dugongs from Moreton Bay between 2002 and 2012 (Weijs et al., 2019).
442
443 *POP level comparisons*
444 Overall, POP levels were relatively low compared to previous studies. Compared to
445 humpback dolphins and dugongs from Moreton Bay, green turtles from the same region
446 accumulate a lower number of POPs as well as lower concentrations of POPs in their tissues
447 (Weijs et al., 2016; Weijs et al., 2019). The green turtles' herbivorous diet plays a role in this;
448 however, the diet is not solely responsible as also dugongs accumulate more POPs, both in
449 terms of number and concentrations, compared to the green turtles. Factors causing the
450 differences between green turtles and dugongs are therefore likely on the level of the species
451 or even class (i.e. Reptilia vs Mammalia) such as egg-laying vs. live bearing, variation in
452 metabolic capacities, permeability of membranes for POPs, among others. Many of these
453 potential factors are poorly studied or not studied at all with respect to environmental
454 pollutants which makes it hard to draw conclusions. Compared to green turtles across
455 Australia and globally, there are no consistent trends (Table S9). The highest PCB levels
456 detected in the present study in liver (2,634 ng/g lw) and kidney (1,221 ng/g lw) were up to
457 20 times higher than the highest PCB levels measured in green turtles from Queensland from
458 2006-2007 (130 ng/g lw in liver, 99 ng/g lw in kidney; Van de Merwe et al., 2010). This may
459 indicate an increase in PCBs over time in this region, however, temporal trends in the present
460 study were not statistically significant. The same PCB levels in liver and kidney were also
461 considerably higher compared to the PCB levels in green turtles from various locations across
462 the globe (Table S9). Looking at PBDE levels in liver of green turtles from Queensland, there
463 is an increase over time: from 1.6 ng/g lw in 2004-2006 (Hermanussen et al., 2008) to a

464 maximum of 10 ng/g lw in 2006-2007 (Van de Merwe et al., 2010) and a maximum of 49
465 ng/g lw from 2008-2017 (present study) (Table S9). A similar increase was not found for
466 PBDEs in dugongs from the same area which also had lower levels compared to the green
467 turtles in the present study (max of 9.43 ng/g lw in liver; Weijs et al., 2019). DDX
468 concentrations in the green turtles from the present study ranged from <LOQ to maximum
469 concentrations of 18 ng/g lw (kidney), 20 ng/g lw (fat), 29 ng/g lw (liver), and 648 ng/g lw
470 (muscle). The result in the liver was more than 6 times higher than the hepatic concentration
471 of DDXs in green turtles from 2006-2007 from Queensland (Van de Merwe et al., 2010).
472 Overall, however, DDX concentrations were generally lower than results reported in other
473 studies (Table S9). Compared to those studies, hepatic CHL concentrations detected in our
474 study were the highest but this was only true for liver (Table S9). The highest level of HCB
475 was found in muscle in the present study (63 ng/g lw) which was only surpassed by the
476 maximum concentration of 286 ng/g lw reported in green turtles from Baja California
477 (Gardner et al., 2003; Table S9). HCHs have not been investigated in many studies but were
478 low overall and comparable to results reported by Malarvannan et al. (2011).
479
480 *Dioxin-like activity*
481 Bioanalytical screening of wildlife tissues has not been done frequently, despite the mostly
482 unknown synergistic effects of chemicals mixtures in the environment which are difficult to
483 predict with conventional chemical analytic methods alone. In the present study, results
484 indicate that EROD induction occurred from exposure to most (70.8%, 17 out of 24) of the
485 sample extracts. EROD induction was similar (BEQ ranging from 2.8 to 356 pg/g, median:
486 24.6 pg/g) in all type of samples (Fig 3). The highest BEQ value was found for the fat extract
487 of animal CM-41 which is also the animal with the highest concentrations of pollutants,
488 especially PCBs. Despite this, pollutant concentrations and BEQ values are not linearly

489 correlated as the BEQ values of the humpback dolphin fat extracts (Reference 1 and 2 in
490 Table 3) are moderate (Fig 3), while the POP levels are several orders of magnitude higher
491 compared to the turtles (Table S2). The most likely explanation for this would be that the
492 majority of the POPs investigated are not known for inducing EROD activity which means
493 that there are other compounds present in the extracts responsible for the cell responses.
494 These compounds may be known compounds that were not targeted in this study, such as
495 dioxins (PCDDs), however, they may also be yet unknown compounds.
496 Dogruer et al. (2018) and Jin et al. (2015) investigated green turtle blood samples from
497 animals from the Australian east coast and both studies observed similar BEQ values
498 compared to the green turtle tissues investigated in the present study (Fig 3). This consistency
499 in BEQ values between blood and tissues such as liver and fat is remarkable considering the
500 different tissue extraction methods used as well as the different bioassays. Dogruer et al
501 (2018) used non-target QuEChERS extraction, Jin et al (2015) used a semi-targeted passive
502 sampling approach with PDMS polymers, while this study used an SPE extraction with
503 hexane and acetone. Bioassays with varying degrees of sensitivity were also used with
504 µEROD assay employed in the present study and CA(F)LUX in other studies. This suggests
505 that AhR responses in green turtles are conserved and can reliably be investigated utilizing
506 various techniques in several tissues.
507 Overall, although the BEQs of the extracts from the turtle tissues in the present study were
508 lower than BEQs observed in other studies from species of higher trophic levels (Fig 3),
509 EROD induction and subsequent BEQs of the turtle extracts suggests that DLCs are present
510 in some of the turtle tissues in levels high enough to cause a cell response.
511
512

513 **Conclusions**

514 The tissue distribution of POPs was found to vary for different pollutants, with most
515 compounds having the highest levels in the liver. While blubber is usually the preferred target
516 for lipophilic pollutants, the fat tissue of the turtles in the present study had relatively low
517 concentrations of pollutants, assumingly due to the poor health status of several animals.
518 Despite previous findings, a limited number of correlations were found between chemical
519 concentrations and factors such as gender, carapace length, location and year of death,
520 potentially indicating that tissue accumulation is multifactorial and requires more biological,
521 ecological and pathological information available for each animal as well as larger sample
522 sizes. The overall lack of correlations could also be attributed to a relatively stable
523 background level of legacy pollutants for coastal sea turtles in Australia. This latter is
524 supported by the low number of pollutants and the low concentrations of pollutants that could
525 be found in the animals. However, despite the absence or presence in low concentrations of
526 pollutants, the assay results still indicated the presence of dioxin-like compounds which
527 suggest that these legacy pollutants continue to be harmful to the animals' well-being. As this
528 knowledge cannot be gained from chemical analysis alone, the combination of chemical and
529 effect-based methods forms a more comprehensive approach to increase our understanding of
530 athropogenic impacts on wildlife.

531

532 **Acknowledgements**

533 The authors would like to thank all parties that were involved in sampling. Special thanks to
534 K Townsend and C Gaus for the collection and preservation of the turtle tissues. L Weijs
535 acknowledges financial support from the Australian Research Council (Discovery Early
536 Career Researcher Award (DECRA); project DE160100468).

537 **References**

538

539 Altenburger R, Ait-Aissa S, Antczak P, Backhaus T, Barcelo D, Seiler TB, Brion F, Busch
540 W, Chipman K, de Alda ML, de Aragao Umbuzeiro G, Escher BI, Falciani F, Faust M,
541 Focks A, Hilscherova K, Hollender J, Hollert H, Jager F, Jahnke A, Kortenkamp A,
542 Krauss M, Lemkine GF, Munthe J, Neumann S, Schymanski EL, Scrimshaw M, Segner H,
543 Slobodnik J, Smedes F, Kughathas S, Teodorovic I, Tindall AJ, Tollefsen KE, Walz KH,
544 Williams TD, Van den Brink PJ, van Gils J, Vrana B, Zhang X, Brack W, 2015. Future
545 water quality monitoring-adapting tools to deal with mixtures of pollutants in water
546 resource management. Science of the Total Environment, 512-513, 540-551.

547

548 Anim AK, Drage DS, Goonetilleke A, Mueller JF, Ayoko GA, 2017. Distribution of PBDEs,
549 HBCDs and PCBs in the Brisbane River estuary sediment. Marine Pollution Bulletin, 120,
550 165-173.

551

552 Arthur KE, Boyle MC, Limpus CJ, 2008. Ontogenetic changes in diet and habitat use in
553 green sea turtle (*Chelonia mydas*) life history. Marine Ecology Progress Series, 362, 303-
554 311.

555

556 Barco S, Law M, Drummond B, Koopman H, Trapani C, Reinheimer S, Rose S, Swingle
557 WM, Williard A, 2016. Loggerhead turtles killed by vessel and fishery interaction in
558 Virginia, USA, are healthy prior to death. Marine Ecology Progress Series, 555, 221-234.

559

560 Clukey KE, Lepczyk CA, Balazs GH, Work TM, Li QX, Bachman MJ, Lynch JM, 2018.
561 Persistent organic pollutants in fat of three species of Pacific pelagic longline caught sea
562 turtles: Accumulation in relation to ingested plastic marine debris. Science of the Total
563 Environment, 610-611, 402-411.

564

565 Christiansson A, Eriksson J, Teclechiel D, Bergman A, 2009. Identification and quatification
566 of products formed via photolysis of decabromodiphenyl ether. Environmental Science
567 and Pollution Research, 16, 312-321.

568

569 Corsolini S, Aurigi S, Focardi S, 2000. Presence of Polychlorobiphenyls (PCBs) and
570 Coplanar Congeners in the Tissues of the Mediterranean Loggerhead Turtle *Caretta*
571 *caretta*. Marine Pollution Bulletin, 40, 952-960.

572

573 da Silva J, Taniguchi S, Becker JH, Werneck MR, Montone RC, 2016. Occurrence of
574 organochlorines in the green sea turtle (*Chelonia mydas*) on the northern coast of the state
575 of Sao Paulo, Brazil. Marine Pollution Bulletin, 112, 411-414.

576

577 Desforges JP, Levin M, Jasperse L, De Guise S, Eulaers I, Letcher RJ, Acquarone M, Nordoy
578 E, Folkow LP, Jensen TH, Grondahl C, Bertelsen MF, Leger JS, Almunia J, Sonne C,
579 Dietz R, 2017. Effects of polar bear and killer whale derived contaminant cocktails on
580 marine mammal immunity. Environmental Science and Technology, 51, 11431-11439.

581

582 Desforges JP, Hall A, McConnell B, Rosing-Asvid A, Barber JL, Brownlow A, De Guise S,
583 Eulaers I, Jepson PD, Letcher RJ, Levin M, Ross PS, Samarra F, Víkingson G, Sonne C,
584 Dietz R, 2018. Predicting global killer whale population collapse from PCB pollution.
585 Science, 361, 1373-1376.

586

587 Dogruer G, Weijs L, Tang JY, Hollert H, Kock M, Bell I, Madden Hof CA, Gaus C, 2018.
588 Effect-based approach for screening of chemical mixtures in whole blood of green turtles
589 from the Great Barrier Reef. Science of the Total Environment, 612, 321-329.
590
591 Eichbaum K, Brinkmann M, Buchinger S, Reifferscheid G, Hecker M, Giesy JP, Engwall M,
592 van Bavel B, Hollert H, 2014. *In vitro* bioassays for detecting dioxin-like activity--
593 application potentials and limits of detection, a review. Science of the Total Environment,
594 487, 37-48.
595
596 Erdmann SE, Dietz R, Sonne C, Bechshoft TO, Vorkamp K, Letcher RJ, Long M, Bonefeld-
597 Jorgensen EC, 2013. Xenoestrogenic and dioxin-like activity in blood of East Greenland
598 polar bears (*Ursus maritimus*). Chemosphere, 92, 583-91.
599
600 Finlayson K, Leusch FDL, van de Merwe JP, 2016. The current state and future directions of
601 marine turtle toxicology research. Environment International, 94, 113-123.
602
603 Finlayson K, Leusch F, Limpus CJ, van de Merwe JP, 2019. Towards the development of
604 standardized sea turtle primary cell cultures for toxicity testing. Ecotoxicology and
605 Environmental Safety, 173, 63-70.
606
607 Finlayson K, Leusch F, van de Merwe JP, 2019. Cytotoxicity of organic and inorganic
608 compounds to primary cell cultures established from internal tissues of *Chelonia mydas*.
609 Science of the Total Environment, 664, 958-967.
610
611 Gallen C, Heffernan AL, Kaserzon S, Dogruer G, Samanipour S, Gomez-Ramos MJ, Mueller
612 JF, 2019. Integrated chemical exposure assessment of coastal green turtle foraging
613 grounds on the Great Barrier Reef. Science of the Total Environment, 657, 401-409.
614
615 Gaus C, Villa CA, Dogruer G, Heffernan A, Vijayasarathy S, Lin CY, Flint M, Madden Hof
616 C, Bell I, 2019. Evaluating internal exposure of sea turtles as model species for identifying
617 regional chemical threats in nearshore habitats of the Great Barrier Reef. Science of the
618 Total Environment, 658, 732-743.
619
620 Haraguchi K, Kotaki Y, Relox JR, Romero MLJ, Terada R, 2010. Monitoring of naturally
621 produced brominated phenoxyphenols and phenoxyanisoles in aquatic plants from the
622 Philippines. Journal of Agricultural and Food Chemistry, 58, 12385-12391.
623
624 Hellou J, Lebeuf M, Rudi M, 2013. Review on DDT and metabolites in birds and mammals
625 of aquatic ecosystems. Environmental Reviews, 21, 53-69.
626
627 Hermanussen S, Matthews V, Papke O, Limpus CJ, Gaus C, 2008. Flame retardants (PBDEs)
628 in marine turtles, dugongs and seafood from Queensland, Australia. Marine Pollution
629 Bulletin, 57, 409-18.
630
631 Hirai H, Takada H, Ogata Y, Yamashita R, Mizukawa K, Saha M, Kwan C, Moore C, Gray
632 H, Laursen D, Zettler ER, Farrington JW, Reddy CM, Peacock EE, Ward MW, 2011.
633 Organic micropollutants in marine plastics debris from the open ocean and remote and
634 urban beaches. Marine Pollution Bulletin, 62, 1683-1692.
635

636 James RA, Hertz-Picciotto I, Willman E, Keller JA, Charles MJ, 2002. Determinants of
637 serum polychlorinated biphenyls and organochlorine pesticides measured in women from
638 the child health and development study cohort, 1963-1967. Environmental Health
639 Perspectives, 110, 617-624.
640
641 Jepson PD, Deaville R, Barber JL, Aguilar A, Borrell A, Murphy S, Barry J, Brownlow A,
642 Barnett J, Berrow S, Cunningham AA, Davison NJ, Ten Doeschate M, Esteban R, Ferreira
643 M, Foote AD, Genov T, Gimenez J, Loveridge J, Llavona A, Martin V, Maxwell DL,
644 Papachlimitzou A, Penrose R, Perkins MW, Smith B, de Stephanis R, Tregenza N,
645 Verborgh P, Fernandez A, Law RJ, 2016. PCB pollution continues to impact populations
646 of orcas and other dolphins in European waters. Scientific Reports, 6, 18573.
647
648 Jin L, Escher BI, Limpus CJ, Gaus C, 2015. Coupling passive sampling with *in vitro*
649 bioassays and chemical analysis to understand combined effects of bioaccumulative
650 chemicals in blood of marine turtles. Chemosphere, 138, 292-299.
651
652 Keller JM, Kucklick JR, Harms CA, McClellan-Green PD, 2004. Organochlorine
653 contaminants in sea turtles: Correlations between whole blood and fat. Environmental
654 Toxicology and Chemistry, 23, 726-738.
655
656 Kienzler A, Bopp SK, van der Linden S, Berggren E, Worth A, 2016. Regulatory assessment
657 of chemical mixtures: Requirements, current approaches and future perspectives.
658 Regulatory Toxicology and Pharmacology, 80, 321-34.
659
660 Losada S, Roach A, Roosens L, Santos FJ, Galceran MT, Vetter W, Neels H, Covaci A,
661 2009. Biomagnification of anthropogenic and naturally-produced organobrominated
662 compounds in a marine food web from Sydney Harbour, Australia. Environment
663 International, 35, 1142-1149.
664
665 Malarvannan G, Takahashi S, Isobe T, Kunisue T, Sudaryanto A, Miyagi T, Nakamura M,
666 Yasumura S, Tanabe S, 2011. Levels and distribution of polybrominated diphenyl ethers
667 and organochlorine compounds in sea turtles from Japan. Marine Pollution Bulletin, 63,
668 172-178.
669
670 McKenzie C, Godley BJ, Furness RW, Wells DE, 1999. Concentrations and patterns of
671 organochlorine contaminants in marine turtles from Mediterranean and Atlantic waters.
672 Marine Environmental Research, 47, 117-135.
673
674 Miao XS, Balazs GH, Murakawa SKK, Li QX, 2001. Congener-specific profile and toxicity
675 assessment of PCBs in green turtles (*Chelonia mydas*) from the Hawaiian Islands. Science
676 of The Total Environment, 281, 247-253.
677
678 Nor NHM, Koelmans AA, 2019. Transfer of PCBs from microplastics under simulated gut
679 fluid conditions is biphasic and reversible. Environmental Science and Technology, 53,
680 1874-1883.
681
682 Oros J, Gonzalez-Diaz OM, Monagas P, 2009. High levels of polychlorinated biphenyls in
683 tissues of Atlantic turtles stranded in the Canary Islands, Spain. Chemosphere, 74, 473-
684 478.
685

686 Richardson KL, Lopez Castro M, Gardner SC, Schlenk D, 2010. Polychlorinated biphenyls
687 and biotransformation enzymes in three species of sea turtles from the Baja California
688 peninsula of Mexico. Archives of Environmental Contamination and Toxicology, 58, 183-
689 193.
690
691 Schiwy A, Brinkmann M, Thiem I, Guder G, Winkens K, Eichbaum K, Nüßer L, Thalmann
692 B, Buchinger S, Reifferscheid G, Seiler TB, Thoms B, Hollert H, 2015. Determination of
693 the CYP1A-inducing potential of single substances, mixtures and extracts of samples in
694 the micro-EROD assay with H4IIE cells. Nature Protocols, 10, 1728.
695
696 Shimada T, Limpus C, Jones R, Hazel J, Groom R, Hamann M, 2016. Sea turtles return home
697 after intentional displacement from coastal foraging areas. Marine Biology, 163(1).
698
699 Storelli MM, Zizzo N, 2014. Occurrence of organochlorine contaminants (PCBs, PCDDs and
700 PCDFs) and pathologic findings in loggerhead sea turtles, *Caretta caretta*, from the
701 Adriatic Sea (Mediterranean Sea). Science of the Total Environment, 472, 855-861.
702
703 Suzuki G, Tue NM, van der Linden S, Brouwer A, van der Burg B, van Velzen M, Lamoree
704 M, Someya M, Takahashi S, Isobe T, Tajima Y, Yamada TK, Takigami H, Tanabe S,
705 2011. Identification of major dioxin-like compounds and androgen receptor antagonist in
706 acid-treated tissue extracts of high trophic-level animals. Environmental Science and
707 Technology, 45, 10203-10211.
708
709 Swarthout RF, Keller JM, Peden-Adams M, Landry AM, Fair PA, Kucklick JR, 2010.
710 Organohalogen contaminants in blood of Kemp's ridley (*Lepidochelys kempii*) and green
711 sea turtles (*Chelonia mydas*) from the Gulf of Mexico. Chemosphere, 78, 731-741.
712
713 Toms LM, Mortimer M, Symons RK, Paepke O, Mueller JF, 2008. Polybrominated diphenyl
714 ethers (PBDEs) in sediment by salinity and land-use type from Australia. Environment
715 International, 34, 58-66.
716
717 van de Merwe JP, Hodge M, Olszowy HA, Whittier JM, Lee SY, 2010. Using blood samples
718 to estimate persistent organic pollutants and metals in green sea turtles (*Chelonia mydas*).
719 Marine Pollution Bulletin, 60, 579-588.
720
721 Van den Berg M, Birnbaum L, Bosveld AT, Brunström B, Cook P, Feeley M, Giesy JP,
722 Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FX, Liem
723 AK, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes
724 M, Waern F, Zacharewski T, 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs,
725 PCDFs for humans and wildlife. Environmental Health Perspectives, 106, 775-792.
726
727 Velzeboer I, Kwadij CJAF, Koelmans AA, 2014. Strong sorption of PCBs to nanoplastics,
728 microplastics, carbon nanotubes, and fullerenes. Environmental Science and Technology,
729 48, 4869-4876.
730
731 Vetter W, 2006. Marine Halogenated Natural Products of Environmental Relevance. Reviews
732 of Environmental Contamination and Toxicology, 188, 1-57.
733

734 Vijayasarathy S, Weijs L, Grant S, Gallen M, Gaus C, 2019. PCDD/F and PCB levels in
735 different tissues from dugongs (*Dugong dugon*) inhabiting the Queensland coastline.
736 Marine Pollution Bulletin, 139, 23-31.
737
738 Weijs L, Vijayasarathy S, Villa CA, Neugebauer F, Meager JJ, Gaus C, 2016. Screening of
739 organic and metal contaminants in Australian humpback dolphins (*Sousa sahulensis*)
740 inhabiting an urbanized embayment. Chemosphere, 151, 253-262.
741
742 Weijs L, Leusch F, Covaci A, 2019. Concentrations of legacy persistent organic pollutants
743 and naturally produced MeO-PBDEs in dugongs (*Dugong dugon*) from Moreton Bay,
744 Australia. Chemosphere, 229, 500-508.
745
746 Whyte JJ, Jung RE, Schmitt CJ, Tillitt DE, 2000. Ethoxyresorufin-O-deethylase (EROD)
747 activity in fish as a biomarker of chemical exposure. Critical Reviews in Toxicology, 30,
748 347-570.
749
750 Wilcox C, Puckridge M, Schuyler Q, Townsend KA, Hardesty BD, 2018. A quantitative
751 analysis linking sea turtle mortality and plastic debris ingestion. Scientific Reports, 8,
752 12536.
753
754 Wiseman SB, Wan Y, Chang H, Zhang X, Hecker M, Jones PD, Giesy JP, 2011.
755 Polybrominated diphenyl ethers and their hydroxylated/methoxylated analogs:
756 environmental sources, metabolic relationships, and relative toxicities. Marine Pollution
757 Bulletin, 63, 179-188.

758 **Tables**
759
760 Table 1: Median concentrations and range (ng/g lipid weight) of contaminants detected in different tissues. Only results for compounds detected
761 in > 50% of the samples are given. Individual results of all targeted compounds can be found in Supporting Information: Table S5 (Fat), S6
762 (Liver), S7 (Kidney), and S8 (Muscle).
763

	Liver n = 28			**Fat n = 10**			**Kidney n = 22**			**Muscle n = 15**		
	Median	**Min**	**Max**	**Median**	**Min**	**Max**	**Median**	**Min**	**Max**	**Median**	**Min**	**Max**
CB 138	1	< LOQ	306	6	< LOQ	9	1	< LOQ	152	16	< LOQ	133
CB 153	3	0	975	16	< LOQ	45	2	< LOQ	453	44	< LOQ	339
CB 180	1	< LOQ	290	10	< LOQ	17	1	< LOQ	131	17	< LOQ	121
TN	1	< LOQ	208	1	< LOQ	1	0	< LOQ	69	33	< LOQ	44
HCB	1	< LOQ	25	2	< LOQ	2	1	< LOQ	28	11	< LOQ	63
2‘-MeO-BDE68	6	< LOQ	86	22	3	192	6	< LOQ	39	6	< LOQ	188
6-MeO-BDE47	0	< LOQ	5	2	< LOQ	8	1	< LOQ	3	7	< LOQ	79

764
765

766 **Table 2.** Table representing the tissue where the respective compounds dominated. Only the
767 animals for which all tissues (L(iver), K(idney), F(at), and M(uscle)) were investigated, are
768 included.
769

770

	HCB	TN	CB 138	CB 153	CB 180	2'-MeO-BDE 68	6-MeO-BDE 47
CM-06	L	L	L	L	L	L	L
CM-07	M	L	L	L	L	L	L
CM-11	L	L	L	L	L	F	M
CM-14	L	L	M	L	L	L	L
CM-16	M	M	M	M	M	M	M
CM-17	M	L	L	L	L	F	M
CM-18	L	L	L/K	L	L	F	F
CM-28	L	L	M	L	M	F	F/K
CM-29	M	L	M	L	M	M	K
CM-41	K	F	K	F	F	L	K

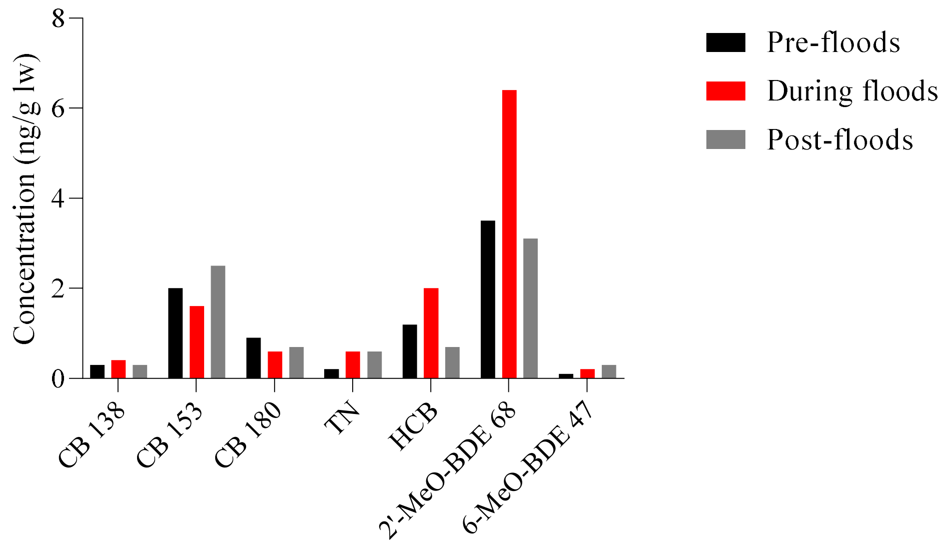
771
772
773
774 **Table 3.** Biological equivalence quotients (BEQs) in pg TCDD/g lw with a coefficient of
775 variation in percent for dioxin-like activity in selected tissue samples. NA = not analysed, NI
776 = no induction.
777
778
779

Sample ID	Liver	Fat	Kidney	Muscle
CM-01	21.6 (98.1)	NA	NI	NA
CM-14	14.3 (84.7)	NI	32.3 (96.3)	NI
CM-16	NA	NA	NA	44.7 (46.5)
CM-27	NI	NA	34.9 (12.6)	NA
CM-28	53.5 (6.8)	NA	47.2 (99.0)	NA
CM-29	NI	NA	NA	NA
CM-32	4.8 (32.9)	NA	NA	NA
CM-40	7.7 (90.8)	NA	NI	NA
CM-41	3.8 (77.9)	356.0 (11.2)	2.8 (33.5)	NI
CM-42	24.6 (0.79)	NA	24.6 (1.2)	45.6 (88.9)
Reference 1	NA	37.2 (61.3)	NA	NA
Reference 2	NA	9.6 (82.4)	NA	NA

780

781

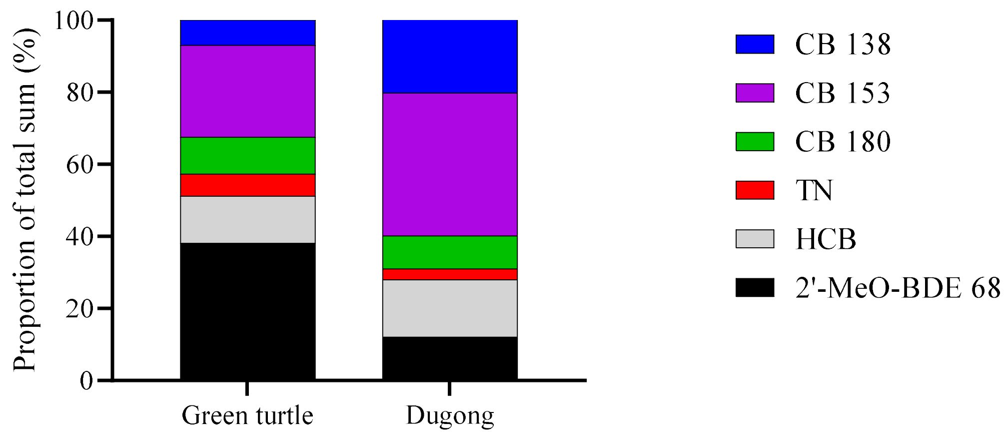
782 **Figures**
783



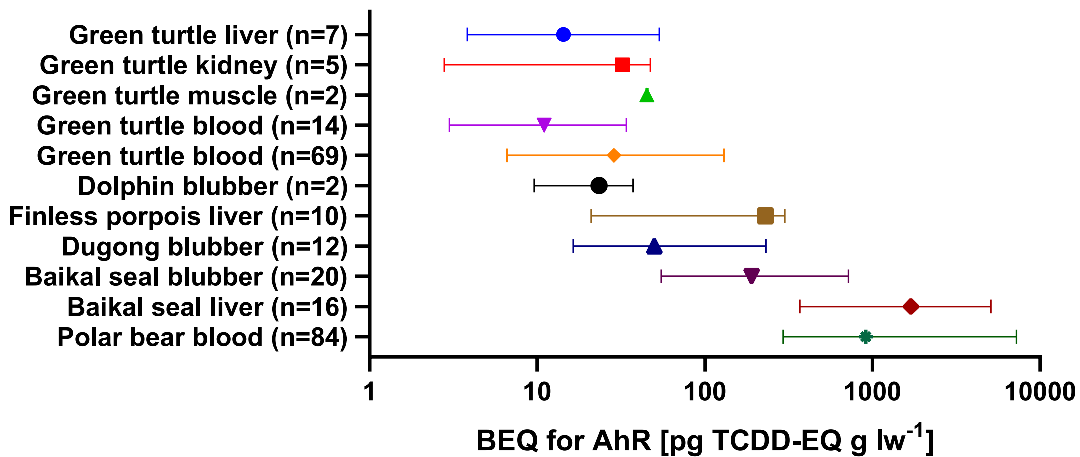
784

785 **Figure 1.** Temporal changes in compound concentrations (based on median values) before,
786 during and after the 2011 floods in the Moreton Bay region. Pre-floods refers to animals from
787 2008-2010 (n = 3), During floods refers to animals from 2011 (n = 8), and Post-floods refers
788 to animals from 2013-2016 (n = 3). None of the observed differences were statistically
789 significant ($0.060 < p < 0.973$).

790



791
792
793 **Figure 2.** Pollutant profiles in liver of green turtles (n = 29) and dugongs (n = 5) from
794 Moreton Bay, Queensland. Each compound was normalized against the total sum of the 6
795 compounds. Dugong results were taken from Weijs et al. (2019) and included only data from
796 males as offloading of POPs from females to offspring influences the profiles in both females
797 and offspring.
798
799
800



801
802 **Figure 3**. Comparison of biological equivalence factors from animal derived samples tested
803 through activation of the Ah-Receptor. Given are the ranges with median in pg TCDD-EQ
804 g/lw. Included are the following tissues from marine wildlife species: Green turtle liver,
805 kidney and muscle as well as humpback dolphin (used as reference material) blubber from
806 this study (µEROD-assay), green turtle blood (Jin et al., 2015; Dogruer et al., 2018;
807 CAFLUX-assay) and dugong blubber from Australia (Jin et al., 2015; CAFLUX-assay),
808 finless porpoise liver and baltic seal blubber and liver from Japan (Suzuki et al., 2011;
809 CALUX-assay) and polar bear blood from East Greenland (Erdmann et al., 2013; CALUX-
810 assay).
811