Partitioning of persistent hydrophobic contaminants to different storage lipid classes

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HIGHLIGHTS

- POPs showed a slightly higher affinity for wax esters than for triacylglycerol.
- Climate change possibly leads to changes in lipid composition of plankton communities.
- Results may suggest altered future POP accumulation dynamics in the food web.
- Differences may not be substantial and within existing natural variability.
- Suggested differences in absorptive capacities need further scrutiny.

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ABSTRACT

Lipids generally represent the major matrix contributing to the absorptive capacity for hydrophobic organic contaminants in aquatic ecosystems. The aim of the present study was to determine whether contaminants partition to a different degree to the different storage lipid classes: wax ester (WE) and triacylglycerol (TAG). This was undertaken by studying experimentally the partitioning of organochlorine compounds between lipids (WE or TAG) and silicone rubber phase. Our results indicate that hydrophobic compounds have a slightly higher affinity for WE than for TAG. The findings thus corroborate earlier suggestions that contaminants accumulate to a greater extent in food webs with a higher reliance on WE, such as in the Arctic. This knowledge is of interest since it implies that possible changes in planktonic community species composition, and thereby possible changes in the lipid composition, may have consequences for accumulation of hydrophobic contaminants in apex predators. However, the magnitude of these consequences remains unknown, and there may well be other factors of importance for previously observed higher accumulation of contaminants in Arctic systems. Thus, we have here identified aspects regarding partitioning of contaminants to lipids that need further scrutiny, and there is a need for further quantitative estimates of the suggested difference in absorptive capacities for hydrophobic contaminants between WE and TAG.

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1. Introduction

Lipids generally represent the major matrix contributing to the absorptive capacity for nonpolar non-ionized organic contaminants

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in aquatic ecosystems, because of the lipophilic and hydrophobic nature of these compounds (Mackay and Fraser, 2000; Arnot and Gobas, 2006). Thus, contaminant concentrations in biological matrices are often reported normalized to the lipid content (total extractable lipids). This allows for comparison between organisms, e.g. to quantify biomagnification in a food web (Ruus et al., 2002).

Obviously, high lipid content represents a high storage capacity for hydrophobic contaminants such as hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs; with octanol-water partition coefficients, \( K_{\text{OW}} > 10^2 \)). If changes in food availability and metabolism affect the energy allocation and lipid storage in organisms, subsequent alterations in hydrophobic contaminant bioaccumulation and lipid class distribution is likely. In addition, the probability of toxicological effects may increase, as increased lipid turnover results in increased contaminant mobilization to the blood stream that can transport them to target organs (Bustnes et al., 2010).

The lipid dynamics of aquatic organisms are of particular importance for Arctic ecosystems because of the high seasonal amplitude in food availability and use of lipids as an energy buffer (Sobek et al., 2010). Arctic marine zooplankton differs in winter activity, which is reflected in energy storage as wax esters (WE) or triacylglycerols (TAG). Wax esters are more energy dense and are formed, e.g. by copepods as a response to short periods with excess of food, followed by long periods of food scarcity (Scott et al., 2000; Wold et al., 2007). TAG is used as short-term storage for some activity during winter. TAG is the main storage lipid class in vertebrates, and there is also a difference in use of these lipid classes between temperate and arctic zooplankton, with a dominance of WE in arctic copepods, and TAG in temperate and sub-Arctic copepods. Hydrophobic organic contaminants dissolve into neutral storage lipids. There is limited knowledge regarding possible differences in the contaminant storage capacity among neutral lipid classes (Geisler et al., 2012). Available data, however, suggest that differences in sorption capacity between different types of lipids are limited for PCBs (Jahnke et al., 2008; Smedes et al., 2017). More detailed knowledge regarding lipid class partitioning of organic contaminants is important since species composition of planktonic communities may change. Such change may alter the lipid composition at the base of the food web, with consequences for apex predators, as the food web biomagnification of contaminants is exponential (Ruus et al., 2002).

The Intergovernmental Panel on Climate Change (IPCC) predicts climatic changes and consequences for the ecosystem that will occur fastest and with largest magnitude in Polar Regions (IPCC, 2001). Atlantic water transport towards the Fram Strait (between Greenland in the West and Svalbard in the East) has increased, and such advection is known to influence zooplankton community composition, by transport of species, in the Arctic (Willis et al., 2006, 2008; Wassmann, 2011). It has e.g. been proposed that the lipid rich Arctic Calanus species may decrease in abundance (Karnovsky et al., 2003). In addition to direct effect on the Arctic environment and ecosystem, climate change is expected to alter the distribution, uptake and effects of contaminants (due to e.g. changes in transport, partitioning, carbon pathways, and bioaccumulation process rates) in ecosystems (Borga et al., 2010). An increase in temperature, will for instance decrease lipid water partitioning, potentially leading to reduced bioaccumulation (Borga et al., 2010). These prospective ecological and physiological changes render knowledge regarding the affinity of pollutants to different storage lipids used by different organisms important.

The aim of the present study was to determine experimentally whether there is a difference in the partitioning of HCB, PCBs and PBDEs to two storage lipid classes, namely wax ester (WE) and triacylglycerol (TAG). Our hypothesis was that there may be partitioning to a larger extent to WE, since WE is less polar than TAG (Graeve and Janssen, 2009). Similarly to estimates of bio-concentration factor (BCF; the steady state ratio of the chemical concentration in an organism to the concentration in water, when exposed to a solely waterborne chemical), measurements of lipid-water partition coefficients of substances with log \( K_{\text{OW}} > 5–6 \) suffer from large uncertainties related to the very large differences in concentrations between lipid and water (Jonker and van der Heijden, 2007). Therefore, we evaluated partitioning of HCB, PCBs and PBDEs to the two classes of lipids, using silicone rubber polymer as a reference phase (Jahnke et al., 2008). Measurements are improved and show less variability as a result of relatively low lipid-silicone rubber partitioning coefficients \( K_{\text{Lipid-Silicone}} \), e.g. in the range 10–50 g/g for PCBs in olive, fish and seal oils, all mainly consisting of TAG (Jahnke et al., 2008). Olive oil differ, however, from marine oils concerning fatty acid composition, generally containing higher levels of saturated, monoene and n-6 fatty acids (Jahnke et al., 2008). In the present study, a seven-day long TAG- and WE-silicone rubber partition experiment was conducted to assess the relative contaminant partitioning between the lipids and silicone rubber.

2. Material and methods

2.1. Chemicals, glassware and solvents

Subjects of this study were persistent organohalogen compounds, more specifically HCB and PCB-28, -52, -101, -118, -138, -153, and -180, and PBDE-28, -47, -85, -99, -100, -153, -154, -183 and -209. PBDE-209 was, however, associated with logistical intractabilities (likely pertaining to its much higher lipophilicity than the other compounds; Table S2, Supplemental data) and was thus omitted from further processing. Solvents and other chemicals used are listed in Supplemental data. Glassware was baked in a muffle furnace at 540 °C before use. Ultrapure water (Option 3, Elga™) was used for diluting solutions and rinsing equipment. Glass vials (capped with an aluminum foil seal) used in the experiments were silanized using dichlorodimethylsilane.

2.2. Lipids

Wax Esters (WE) were obtained as Calanus® Oil from Calanus AS (Tromsø, Norway), while Tri Acyl Glycerol (TAG) was obtained as commercial cod liver oil (Tran), from Möllers (Oslo, Norway). The WE and TAG were stored sealed at −20 °C and 4 °C, respectively, until analysis/start of the experiment (the latter was obtained sealed in a glass bottle with a protective atmosphere). Triacylglycerols were polished/purified prior to arrival and needed no treatment. TAG spiked with organohalogenahogens was prepared as follows: a stock batch (25 mL) was made where HCB/PCB and PBDE standards were added (standards were added, solvent was then evaporated using a gentle stream of N₂-gas before the TAG was added) and the mixture was shaken on an orbital shaker (KSS501, IKA Labortechnik, Saufen, Germany; ~100 rpm) for 24 h. Wax esters were thawed and centrifuged (4500 rpm for 30 min, 10 °C) to remove any solids and WE spiked with organohalogenahogens (a stock batch of 25 mL) was prepared as for TAG, above. The resulting analyte concentrations measured in the lipids ranged from 5.6 to 184.0 ng/g, depending on compound (Table 1). Relative standard deviation (n = 5) of the amounts of chemicals spiked to the lipids were in the range 3.5–21.5% and on average 10.0% for all chemicals.
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Table 1

(a.) Concentrations (ng/g) of hexachlorobenzene (HCB) and polychlorinated biphenyls (PCBs) in lipids (wax esters, WE, or triacylglycerols, TAG), after partitioning to silicone, and mass (g) of silicone (pre/post experiment) and lipid (pre experiment). *(b.) Content (ng/silicone rod) of HCB and PCBs in silicone, after partitioning from lipid (WE or TAG), and mass (g) of silicone (pre/post experiment) and lipid (pre experiment). *(c.) Concentrations (ng/g) of PCBs in lipids (WE or TAG), without partitioning to silicone (pre experiment). *(d.) Concentrations (ng/g) of polybrominated diphenylethers (PBDEs) in lipids (WE or TAG), after partitioning to silicone, and mass (g) of silicone (pre/post experiment) and lipid (pre experiment). *(e.) Content (ng/silicone rod) of PBDEs in silicone, after partitioning from lipid (WE or TAG), and mass (g) of silicone (pre/post experiment) and lipid (pre experiment). *(f.) Concentrations (ng/g) of PBDEs in lipids (WE or TAG), without partitioning to silicone (pre experiment). *(g.) Recovery (%) of HCB and PCBs added to the experimental units. Mean and (standard deviation). n = 5 for all.

| (a.) | Mass (g) | Concentrations (ng/g) |
|------|----------|-----------------------|
| Lipid class | Silicone (pre exp.) | Silicone (post exp.) | Lipid (pre exp.) |
| WE | 2.23 | 2.29 | 1.75 |
| | (0.05) | (0.05) | (0.03) |
| TAG | 2.22 | 2.23 | 1.80 |
| | (0.05) | (0.05) | (0.03) |

| (b.) | Mass (g) | Content (ng/silicone rod) |
|------|----------|--------------------------|
| Lipid class | Silicone (pre exp.) | Silicone (post exp.) | Lipid (pre exp.) |
| WE | 2.23 | 2.29 | 1.75 |
| | (0.05) | (0.05) | (0.03) |
| TAG | 2.22 | 2.23 | 1.80 |
| | (0.05) | (0.05) | (0.03) |

2.3. Silicone rubber

Silicone rubber rods were prepared from AlteSil™ silicone rubber sheets (average thickness 4 mm), purchased from Altec Ltd. (Bude, UK). The silicone rubber was cut into rods (4.5 cm long, 1 cm wide and 0.4 cm thick) with an average weight of 2.21 g (±0.06 standard deviation). Prior to their application in the experiments, they were rinsed with ultra-pure water and air-dried, before Soxhlet extraction at low temperature in a 50:50 methanol:petane mixture for 8 h. Silicone rods were then air-dried and further cleaned in methanol prior to use. The silicone rods were stored at –20 °C until the start of the experiment.
2.4. Experimental setup

Ten silicone rods were placed in separate vials and weighed. WE and TAG spiked with organohalogen compounds were each added to 5 of the vials (2 ml in each) containing the silicone rods, plus 5 vials (2 ml in each) for chemical analysis of organohalogen concentrations at zero-time. The amount of lipid added was weighed.

Additionally, 3 vials each of pristine (not spiked) TAG and WE were stored (−80 °C) for analysis of lipid class and fatty acid composition prior to the experiment, and 3 vials each of pristine TAG and WE added a silicone rod were prepared to undergo experimental procedure for analysis of lipid class and fatty acid composition after termination of the experiment.

To avoid oxidation of the lipids during the experiments, oxygen (O₂) was dispelled from the vials by a gentle flow of nitrogen (N₂) gas and the vials were capped to sustain a N₂ atmosphere.

All vials were placed on an orbital shaker (K5001; IKA Labor-technik, Saufen, Germany; −100 rpm) in a climate room (15 °C, at temperatures ≤10 °C) the viscosity of the WE was judged too high to facilitate proper equilibrium with the silicone phase) for one week. Experimental lipids were in liquid form during the experiment. The duration of the experiment was decided as a compromise between allowing sufficient time to achieve equilibrium, and avoidance of oxidation (alteration/rancidity) of the lipids. The vials were inspected and manually turned once every second day. Upon termination of the experiment, all silicone rods were removed from the vials, wiped clean with a clean lint-free tissue, weighed and stored (−20 °C) for extraction (described in Supplemental data) and chemical analysis.

The lipid fraction (TAG or WE) in the vials were stored for extraction (described in Supplemental data) and organohalogen analysis (−20 °C), and analysis of lipid class and fatty acid composition (−80 °C).

2.5. Validation experiment

One crucial aspect of the measurement is to ensure that the measurement of partitioning is done at or very close to equilibrium. To validate the partitioning of organohalogen between lipids and silicone, a second validation experiment was set up, identical to that described above, however, with the organohalogen initially spiked to the silicone rubber phase. The rods were spiked with the organohalogen using a 50:50 methanol-water solution containing HCB, PCBs and PBDEs using a procedure similar to that described previously (Booij et al., 2002; Allan et al., 2010) (see Supplemental data for details and results).

The assays with organohalogen spiked to the silicone rubber have almost reached equilibrium confirming that the assays with organohalogen spiked to the lipids are close to or at equilibrium for all compounds of interests. However, the difference in total sorption capacity of the lipids phase relative to the silicone rubber phase means that despite being close to equilibrium in the assays with organohalogen spiked to the silicone rubber, apparent $K_{lipid-Silicone}$ for these experiments deviate from the final expected value. While these $K_{lipid-Silicone}$ values remain relatively far from target values, these assays do confirm that the data from the experiments with organohalogen spiked to lipid phase are valid. The total amount of chemical needing to transfer to the silicone rubber from the lipids is substantially smaller than the amount that needs to transfer to the lipids from the silicone rubber, so for the same exposure time, $K_{lipid-Silicone}$ from these assays will be closest to the true values. Time to equilibrium is expected to be shorter for organohalogen spiked to the lipids, as explained in Booij and Tucca (2015) and in Booij et al. (2017). This experimental issue has also been observed for performance reference compounds used in other similar studies (Smedes et al., 2017).

2.6. Organohalogen analysis

Extracts from lipids and silicone rods were analyzed by gas chromatography with electron capture detection (GC-EC) and with negative chemical ionization-mass spectrometry (GC–NCI–MS; PBDEs), largely as previously described (Ruus et al., 2005), at NIVA’s laboratory. The lab is accredited by the Norwegian Accreditation as a testing laboratory according to the requirements of NS-EN ISO/IEC 17025 (further analytical details in Supplemental data). Analytical quality of the laboratory is also ensured by the participation in international calibration tests, including QUASIMEME twice a year (see Supplemental data).

2.7. Lipid class and fatty acid analysis

Lipids (TAG and WE) were analyzed for the composition of lipid classes and fatty acids at Unilab Analyse AS (Tromsø, Norway) by high performance liquid chromatography and evaporative light scattering detection (HPLC-ELSD) (Graeve and Janssen, 2005), and acidic derivation and gas chromatography with flame ionization detection (GC-FID) (Christie, 1982), respectively. The analysis of fatty acids is accredited according to the requirements of NS-EN ISO/IEC 17025 (see Supplemental data for description of analytes).

2.8. Data treatment and statistical methods

Statistical analyses (linear regression and factorial ANOVA) were performed using Statistica software (Ver 13; Dell Inc./Statsoft). A significance level of α = 0.05 was chosen. Octanol-water partition coefficients ($K_{OW}$) for HCB, PCBs and PBDEs were from Mackay et al. (1992), Hawker and Connell (1988) and Braekvoll et al. (2003) (Table S2, Supplemental data), respectively. Silicone-water partition coefficients ($K_{Silicone-Water}$) used were from Smedes et al. (2009) (same silicone as in the present study, AltesSil™; available for PCBs only; Table S2, Supplemental data).

3. Results and discussion

3.1. General observations

Of the compounds added to the experimental units, 88%–110% of the organochlorine compounds (HCB and PCBs), and 91%–136% of the PBDEs were recovered (136% for BDE-183 in TAG, otherwise recoveries were 91%–115%; Table 1). All compounds were below the limit of detection (see Supplemental data) in blanks.

There was a small increase in the weight of the silicone rods during the experiment. This increase was larger for those that equilibrated with WE (−2.8%) than for those that equilibrated with TAG (−0.4%; significantly different; ANOVA; Fig. S2; Table S3, Supplemental data). If assumed that the silicone rod weight increase is remnants of lipids entered into the silicone, and that these remnants have identical properties to the bulk lipids in terms of organohalogen compound content/absorptive capacity, correcting the organohalogen concentrations observed in the silicone phase would produce negative concentrations for some compounds. It is therefore assumed that the silicone rod weight increase may be attributed to fractions of the lipids (especially WE) with lower capacity for hydrophobic compounds, than the bulk lipid phase. Thus, no corrections have been made to the concentrations measured in the silicone rods. This is in accordance with Jahnke et al. (2008), Ossianander et al. (2008) and Smedes et al. (2017) who found no significant measurement bias from lipid phase components in/on the silicone phase. In case this assumption is erroneous, $K_{lipid-Silicone}$ partition coefficients may be somewhat underestimated, and more so for WE than for TAG (as the silicone rod weight increased
more in the experimental units with WE than with TAG). Therefore, a difference in partitioning of organochlorines to the two lipid classes, with higher KLipid-Silicone partition coefficients for WE than for TAG (see below), represents a conservative estimate as the difference in KLipid-Silicone in reality may be higher. If possible, characterizing the composition of the fraction of the lipids associating with the silicone phase could be an option for future studies.

The lipid class and fatty acid composition did not show noteworthy changes during the experiment. The analyses showed that TAG samples were 100% TAG, whereas the WE samples consisted of 98% WE and 2% TAG (Table S4; Fig. S3, Supplemental data). Thus, the lipid class analyses confirmed high quality and purity of the WE and TAG lipids (Table S4, Supplemental data).

### 3.2. Partition coefficients

Lipid-silicone partition coefficients (KLipid-Silicone (g/g); Fig. 1) were calculated as:

\[
KLipid - Silicone = \frac{C_{lipid}}{C_{Silicone}}
\]

where \(C_{lipid}\) was the concentration of the organohalogen in question in lipid (WE or TAG), and \(C_{Silicone}\) was the concentration in the silicone phase, at the end of the experiment. In general, the KLipid-Silicone increased with degree of halogenation, depicted by a positive relationship with \(K_{OW}\) (Fig. 1), mainly for compounds with log \(K_{OW}\) > 6.

The results indicate that the organohalogenes have higher affinity for WE than for TAG, this is despite the possible above-mentioned underestimation of KLipid-Silicone for WE in particular. A Factorial ANOVA for \(\log KLipid-Silicone\) with lipid (WE or TAG) and compound (each compound/congener) as predictors (as well as the lipid × compound interaction) showed both lipid and compound significant (see Table S5, Supplemental data, for details). Thus, the KLipid-Silicone is dependent on lipid class and differs among compounds. Furthermore, the lipid × compound interaction was significant (Table S5, Supplemental data), indicating that the differences among compounds are not the same between the lipid classes.

More specifically, KLipid-Silicone for PCB-28 and PCB-52 were approximately the same in the two lipid classes (Fig. 1; Table S6, Supplemental data). For BDE-183 KLipid-Silicone appeared 20% lower in WE than in TAG (Fig. 1; Table S6, Supplemental data). Otherwise, KLipid-Silicone appeared −10% to −115% higher for WE than for TAG (Fig. 1; Table S6, Supplemental data).

The importance of differences in storage lipid quality for organohalogen accumulation was suggested by Severinsen et al. (2000), who found different concentrations of organochlorine compounds in different sections of pinniped blubber. They attributed this partly to different biochemical compositions of the blubber layer sections as a probable consequence of functional differences. Several recent articles also emphasize that the possibility of different sorptive capacities among different lipids and this is not sufficiently considered in partitioning models (Geisler et al., 2012; Jonker, 2012; Endo et al., 2013).

Jahnke et al. (2008) measured lipid-silicone partition coefficients of organochlorines, including HCB and PCBs, for three types of lipids found at different trophic levels: olive oil, fish oil and seal oil. The authors found generally similar or higher KLipid-Silicone for all lipids, than we found for TAG and WE. Furthermore, the same authors applied silicone films for sampling of PCBs in intact fish tissues and compared the results with those obtained by classic exhaustive total extraction (Jahnke et al., 2011). The similarity in the results obtained by the different methods indicated the validity of the silicone phase technique and confirmed that the fugacity capacity of lipid rich fish tissues for PCBs was dominated by the lipid fraction (Jahnke et al., 2011). The silicone/polydimethylsiloxane used in these studies differs from that used here and partitioning properties of the different silicones may differ. This may induce additional uncertainty when comparing lipopolymer partition coefficients measured with silicone or PDMS from different sources.

Since silicone-water partition coefficients (KSilicone-Water) are available for PCBs (Smedes et al., 2009) (Table S2, Supplemental data), KLipid-water may be deduced:

\[
KLipid – Water = KLipid – Silicone \times KSilicone – Water
\]

As for KLipid-Silicone, KLipid-Water were significantly higher for WE than for TAG. A Factorial ANOVA for \(\log KLipid-Water\) with lipid (WE or TAG) and compound (each PCB-congener) as predictors (as well as the lipid × compound interaction) showed both lipid and compound significant (see Table S7, Supplemental data, for details), thus the KLipid-Water is dependent on lipid class and differs among compounds.

More specifically, KLipid-Water for PCB-28 and PCB-52 were approximately the same in the two lipid classes (Fig. 2). For the other PCB-compounds, KLipid-Water appeared −10% to −19% higher for WE than for TAG (Fig. 2).

Furthermore, a significant positive double-logarithmic linear relationship was observed between \(K_{OW}\) and KLipid-Water for both WE and TAG (\(R^2 = 0.98\) and \(R^2 = 0.97\), respectively; not shown). Higher sorptive capacities of the lipids, than of octanol, were indicated (i.e. higher KLipid-Water than \(K_{OW}\), a factor 8–17 higher for

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Fig. 1. Measured lipid-silicone partition coefficients (KLipid-Silicone) for organochlorine compounds (OCs; HCB and PCB-28, -52, -101, -118, -138, -153 and -180) (a.) and polybrominated diphenylethers (PBDE-28, -47, -85, -99, -100, -153, -154 and -183) (b.) in wax esters (WE) and triacylglycerols (TAG) against octanol-water partition coefficients (log \(K_{OW}\); Hawker and Connell, 1988; Mackay et al., 1992; Braekeveldt et al., 2003). Mean (n = 5) and standard deviation are depicted. Note: different scales on axes.
Silicones were measured in the present study and \( K_{\text{Silicone-Water}} \) were from Smedes et al. (2009). Mean, standard error (box) and standard deviation (whiskers) are depicted, \( n = 2009 \). Octanol-water partition coefficients (\( K_{\text{OW}} \)) for the compounds are also given (Hawker and Connell, 1988; Mackay et al., 1992). Our results suggest that a higher reliance on WE than TAG may contribute to explaining the higher Arctic bioaccumulation. Additionally, the results suggest that any prospective alterations in the food web composition, induced by e.g. a changing Arctic climate, may have consequences for the amount of hydrophobic contaminants (either decreasing or increasing) accumulating in the food web, merely based on partitioning theory. The present findings points at aspects regarding partitioning of contaminants to lipids that need further scrutiny, and there is a need for further quantitative estimates of the suggested difference in absorptive capacities for hydrophobic contaminants between WE and TAG.

**Credit author statement**

Anders Ruus, Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft. Ian J Allan, Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft. Kine Bæk, Methodology, Investigation, Resources, Writing - original draft. Katrine Borgå, Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Appendix A. Supplementary data**

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