Australia's National
Science Agency

Hazard characterisation of microplastics in wastewater

Effect on wastewater contaminant sorption and exposure

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A report for the NSW Environmental Protection Authority

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Acknowledgments

I would like to thank Nikita Pring, Bao Tran and Seng Gi Saw for their technical assistance, Jason Kirby for his helpful comments on the study and Sydney Water staff for assistance with collection of wastewater samples.

This project was funded by NSW EPA.

Executive summary

Plastic is a highly diverse material with many uses in modern society. The global reliance on plastics has led to its increasing presence in the environment. In Australia, wastewater treatment plants (WWTPs) are a significant pathway for plastics to enter the marine environment, especially in the form of microplastics, or plastics < 5 mm in any dimension. These microplastics are typically present due to the physical or chemical degradation of their parent products or release from consumer products, including microbeads and microfibres. It is estimated that millions of these microplastics can be released from a WWTP each day into the marine environment. The nature of microplastics, having a relatively high amount of carbon and surface area, can associate strongly with chemical contaminants already present in wastewater. There is therefore concern related to the potential for microplastics to bind with contaminants in wastewater and transport them throughout the marine environment, leading to exposure of these contaminants to marine organisms.

The aim of this study was to:

(1) Assess the degree of association (sorption) and release (desorption) of wastewater contaminants of concern with microplastics commonly found in wastewater; and

(2) Determine the potential hazard of these contaminants to marine organisms following their association with microplastics that are released through WWTP discharges into marine ecosystems

The contaminants of concern selected for this assessment were benzalkonium chloride (BAC) and triclosan (TCS), both disinfectants and bisphenol A (BPA), a plasticiser. All contaminants are widely used industrial chemicals and commonly measured at ng/L concentrations in wastewater. The microplastics used in this assessment were polyethylene (PE) in the form of microbeads, fibres and fragments, as well as polyethylene terephthalate (PET) fibres. Modified batch sorption and desorption assays, using comparatively high concentrations of contaminants and microplastics, were used to assess the degree of association with and release of the contaminants from microplastics.

The selected contaminants had a high degree of association (K_d values 160-8,800 L/kg) in the order TCS >BAC >BPA, with the contaminants generally showing the greatest affinity for PE. This was based on a 24 h sorption equilibrium period, which represented a balance between short-term sorption equilibrium and minimising degradation of each contaminant. In the case of release (or desorption) from the microplastics, desorption was assessed in seawater solutions. To simulate the potential release of contaminants from microplastics within the guts of marine organisms, seawater was also amended with a biomimetic surfactant (taurocholate) as well as taurocholate solution adjusted to pH 4 and 38^{o}C. Desorption into seawater was minimal over a 24 h period, suggesting microplastics have the potential to transport these contaminants throughout marine environments. In contrast, the release of the contaminants (especially BAC and TCS) was greatly enhanced under the simulated biological conditions, suggesting these microplastic-associated contaminants can be released once ingested.

A hazard assessment of these selected contaminants associated with a number of microplastics, however, indicated that any adverse outcomes from their exposure to marine organisms are likely to be negligible. This was based on (a) the low concentrations of contaminants in wastewater, which are further diluted following discharge into the marine environment; (b) the relatively low total loads of contaminants that are likely to associate with microplastics due to the low concentration of microplastics relative to organic natural matter and; (c) the mitigating effect of contaminants associating with microplastics, where complete desorption does not occur. Also, the relatively low loads of microplastics (compared with natural particulate matter and organic carbon) in the marine environment means that exposure to microplastics and associated contaminants is also likely to be low.

While the hazards of microplastics and associated contaminants are likely to be low in the marine environment, increasing plastic consumption by society and its presence in waste means that this current scenario has the potential to change. It is also apparent that the effectiveness of WWTPs removing microplastics from discharge streams can lead to the transfer of microplastics to the terrestrial environment and it is imperative to characterise the hazards of microplastic-associated contaminants in all receiving ecosystems.

1 Introduction

Global production of synthetic polymers, or plastics, is around 300 million tonnes per year and the marine environment can serve as the ultimate repository of discarded or end of life plastic products such as microplastics, or plastics < 5 mm in any dimension (Browne et al., 2011; Jambeck et al., 2015; Mason et al., 2016).

Microplastics in the marine environment can be found in a number of different forms, including plastic derived from fragmented plastic waste, microbeads used in personal care products and medicines and microfibres derived from clothing manufactured from synthetic polymer fibres (Browne et al., 2011; Mason et al., 2016; Mintenig et al., 2017). Wastewater treatment plants (WWTPs) are critical for reducing environmental impacts of wastewater contaminants, such as nutrients and organic/inorganic contaminants, but can also serve as a pathway into the marine environment of microplastics derived from common domestic products. For example, treated water released from WWTPs, or effluents, can contribute millions of microplastic particles per day into a receiving environment, including marine systems (Mason et al., 2016; Murphy et al., 2016). This is despite WWTPs, particularly those with a high treatment level (e.g. secondary or tertiary), being effective in removing microplastics from wastewater entering a WWTP (Murphy et al., 2016). As Australian major urban centres are located adjacent to coastal regions, the potential for WWTP effluents contributing to the load of microplastics in the marine environment is high.

There is increasing evidence that the presence of microplastics can have detrimental impacts in the marine environment through physical effects (Jemec et al., 2016; Wright et al., 2013). It has also been suggested that microplastics have the potential to facilitate the transfer of contaminants, including chemicals and pathogens, present in wastewater to organisms (Browne et al., 2013; Cole et al., 2011; Kirstein et al., 2016), including those associated with their manufacture such as additives or intrinsic contaminants (Alimi et al., 2018; Gandara e Silva et al., 2016; Hermabessiere et al., 2017; Teuten et al., 2009). This is because microplastics are composed of carbon-rich polymeric backbones that can strongly associate with organic contaminants, with the lipophilicity of a contaminant (measured by the octanol-water partition coefficient, K_{OW}) a good indicator of their likely extent of association with the microplastic (Alimi et al., 2018). The most commonly found microplastics in marine environments are polyethylene (PE), polypropylene (PP) and polyethylene terephthalate (PET), which are all carbon rich polymers (Figure 1).

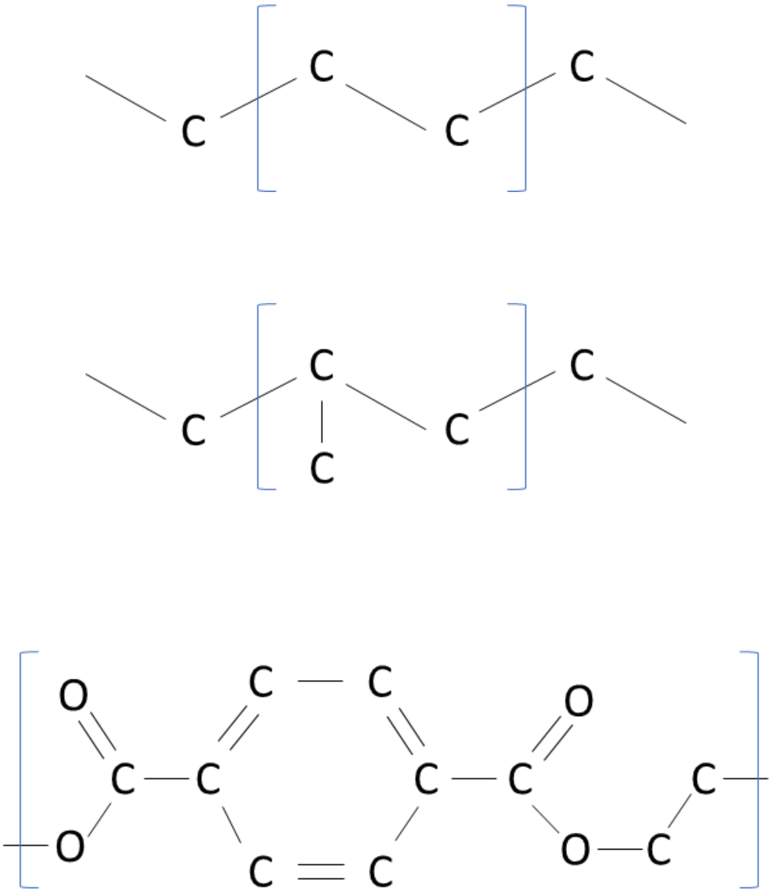


Figure 1 Repeating monomer units (within the blue brackets) of polyethylene (PE), polypropylene (PP) and polyethylene terephthalate (PET) polymers, which are commonly found in marine environments.

This highlights the carbon-rich nature of these polymers which is conducive to their interaction with organic contaminants.

While lipophilicity of a contaminants gives a good estimate of the extent of association with a microplastic, there are other factors relating to the contaminant, the microplastic and the environment that can influence the degree of association. Along with carbon and hydrogen bonds, organic contaminants can consist of a range of other elements and functional groups that can modify the lipophilicity of the contaminant. For example, pH-dependent ionisable functional groups (that can have a positive or negative charge associated with it) on contaminants can reduce the water solubility and lipophilicity of a contaminant and, subsequently, their degree of association with particulates (Delle Site, 2001; Ishikawa et al., 2005). With respect to microplastics, different polymers have varying physical and chemical structures that have a strong influence on how they associate with contaminants. For example, depending on their glass-transition temperatures, plastics are referred to as glassy or rubbery polymers, which influences the rate of diffusion of contaminant within their structure (Bakir et al., 2012; Pascall et al., 2005; Saquing et al., 2010). The polymer type of microplastics has an important influence on the extent of association with contaminants in solution, with polyethylene (PE) typically having a greater degree of association with contaminants compared with other polymer types, such as polypropylene (PP), polyvinyl chloride (PVC) or polyethylene terephthalate (PET) (Alimi et al., 2018). Also, the size of microplastics (relative to plastics of size >5 mm) means they have a greater surface area for interacting with contaminants already present in wastewater (Alimi et al., 2018). This surface area (and characteristics) can be further modified in marine environments with effects such as weathering, where roughened microplastic surfaces can enhance the extent of a contaminant associating with the microplastic (Napper et al., 2015). Environmental factors, such as temperature, salinity and pH of water, can also affect the extent of association of contaminants with microplastics (Alimi et al., 2018). For example, the high salinity of the marine environment can reduce the water solubility of organic contaminants ("salting out"), which can enhance the affinity with solid particles such as microplastics (Wu et al., 2016). These environmental variables can affect both the contaminant and microplastic in different ways, therefore making it difficult to predict the actual effect on the sorption between a contaminant and microplastic (Alimi et al., 2018).

Microplastics have been shown to transport contaminants over long distances in the marine environment, with increasing ageing of and distance travelled by the microplastics potentially increasing the contaminant load (Gorman et al., 2019; Mai et al., 2018). Based on the amount of plastic currently estimated to be in the marine environment it is still unclear as to the relative importance of sorption of contaminants to microplastics in comparison with other materials, such as naturally occurring dissolved organic matter. For example, the mass of microplastics is many orders of magnitude less than that of naturally occurring suspended matter in marine environments, which may make their contribution to transporting wastewater contaminants comparatively small (Gouin et al., 2011; Koelmans et al., 2016; Mai et al., 2018). However, the carbon-rich chemical nature of microplastics means that they may still be an important vector for transferring chemicals to marine organisms, including fish and invertebrates (Browne et al., 2013; Rochman et al., 2013).

Even where tertiary treatment technologies are used, WWTP effluents release a broad range of contaminants, including pharmaceuticals, personal care products and various industrial chemicals (Deblonde et al., 2011; Kasprzyk-Hordern et al., 2009; Launay et al., 2016). The concentrations of such contaminants are typically in the ng/L to μg/L range, although once effluents are discharged into the marine environment these concentrations can be considerably reduced through dilution and attenuation (Alygizakis et al., 2016; Vidal-Dorsch et al., 2012). Despite these low concentrations, many of these contaminants are biologically active and can have adverse impacts on exposed organisms, including behavioural modification, endocrine disruption or contributing to the development of antimicrobial resistance (Bossus et al., 2014; Kotlarska et al., 2015; Zhou et al., 2019). WWTPs can therefore be a source of not only microplastics but also of contaminants that can associate with microplastics prior to discharge into the marine environment. Based on the likely association of wastewater contaminants with microplastics prior to WWTP discharges, the potential for transport of microplastic-associated contaminants in the marine environment exists, which can ultimately lead to exposure of these contaminants to marine organisms following ingestion of the microplastics (Figure 2). The extent of transport of contaminants and their exposure to marine organisms is highly dependent on the strength of association of the contaminant with a microplastic, which will affect the contaminant's release when a microplastic is discharged into the marine environment or ingested by an organism. Determining the extent of association of a contaminant with microplastics is therefore an essential component of characterising the potential hazards of exposure of WWTP-sourced microplastics to marine organisms.

The aim of this study was therefore:

1. To determine the extent of association (sorption) and release (desorption) of a number of commonly detected wastewater contaminants with a range of microplastics. The extent of desorption was measured in seawater and seawater solutions modified to simulate biological conditions following ingestion by marine organisms.
2. To assess whether the release of these contaminants under different conditions may constitute a hazard to exposed marine organisms.

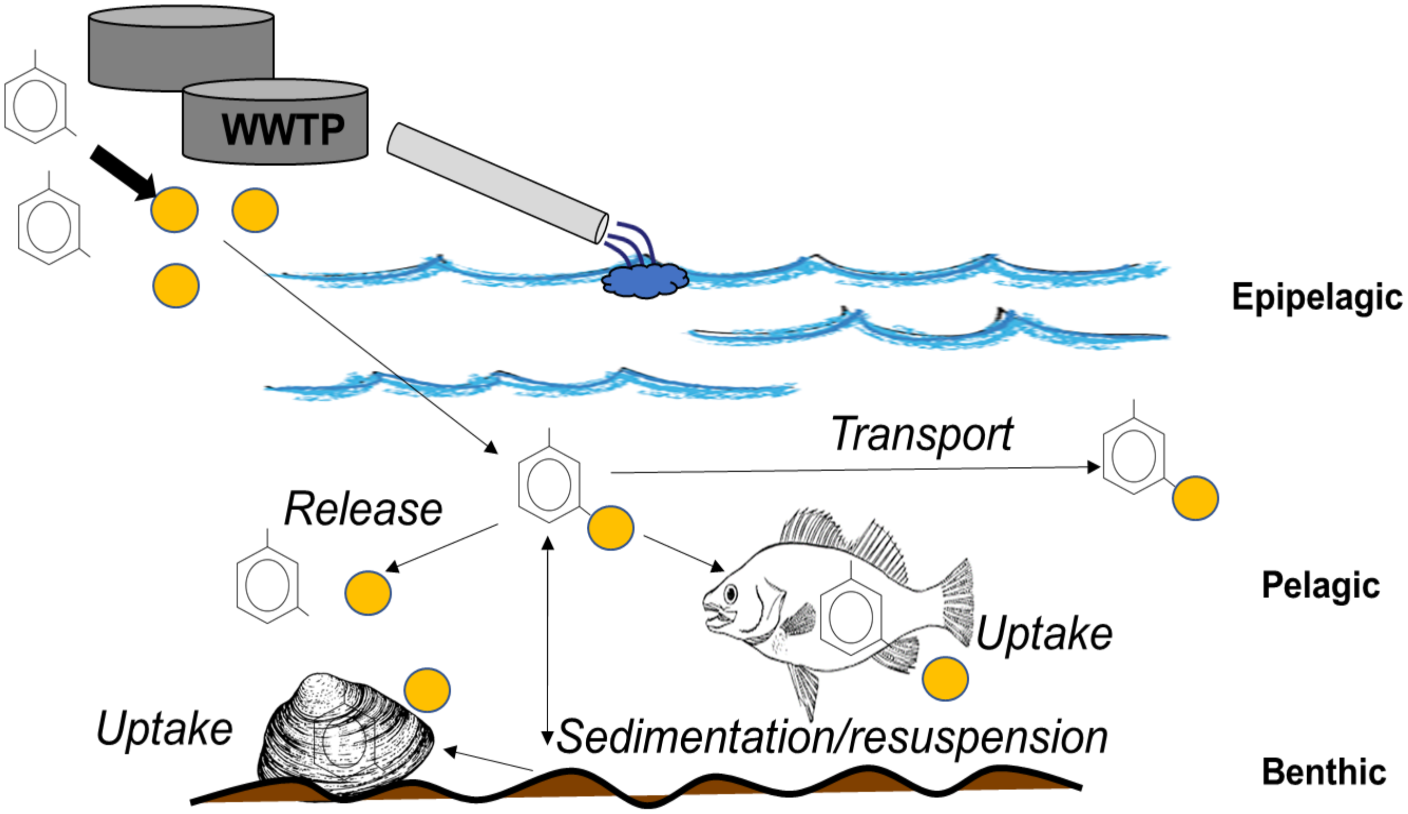


Figure 2 Overview of contaminants present in wastewater treatment plants (WWTPs) interacting with microplastics and how this can influence their exposure to marine organisms.

This can include association with the microplastic within the WWTP, transport to different marine ecosystem zones (epi-pelagic, pelagic and benthic) and exposure to organisms living within these zones. Association and release of the contaminant can occur within all of these scenarios, the extent of which is dependent on the contaminant, the microplastic that it is interacting with and the influence the environment has on the contaminant and microplastic within the different zone.

Representative contaminants were selected based on their concentrations in wastewater and their association with microplastics was assessed using a modified batch sorption method. This allowed a determination of the extent of association that may occur in wastewater, as well as the release of the contaminants in seawater and under simulated physiological conditions. From this, the potential for the microplastics to transport contaminants once released into the marine environment, as well as being released from the microplastic when ingested by marine organisms, was assessed. Following this, an estimate of the potential hazard that these contaminants may have in marine ecosystems through their association with microplastics was also undertaken.

2 Wastewater contaminant selection

For the batch sorption studies, the degree of association of wastewater contaminants with the microplastics and their subsequent release when discharged into the marine environment was assessed. Wastewater samples were initially screened for a range of contaminants to select candidates to include in batch sorption studies. The final selection of contaminants to be used in the batch sorption studies was based on their measured concentrations in wastewater, their potential to cause toxic effects at relatively low concentrations and their physicochemical properties that may be conducive to association with microplastics (Table 1).

2.1 Contaminant concentrations in wastewater

To measure the concentrations of the contaminants in wastewater, effluent samples were collected in September 2018 from Cronulla (WWTP 1) and Malabar (WWTP 2) WWTPs in the Sydney metropolitan area (Table 2). WWTP 1 treats ~50 ML/day to a tertiary level, while WWTP 2 treats ~500 ML/day to a primary level and service relatively large catchment areas of approximately 250,000 and 1.5 million people, respectively. Time-proportional composite water samples were collected over 24 h in refrigerated automated samplers, where samples were collected at time intervals to match average wastewater flow rates. Triplicate samples were transferred to 200 mL (phenol, polycyclic aromatic hydrocarbon and phthalate analysis) and 500 mL (pharmaceutical and personal care product) amber glass bottles, previously detergent and solvent washed and baked for 4 h at 350^{o}C. Wastewater samples for phenol, polycyclic aromatic hydrocarbon and phthalate analysis, transferred into 200 mL amber glass containers, were immediately sent to a NATA-accredited laboratory (National Measurement Institute) packed in chilled ice boxes.

The 500 mL amber bottles had a 0.25 mL aliquot of H_2SO_4 added to each bottle for sample preservation immediately after transfer of the wastewater samples. Following acidification, bottles were placed on ice for transportation back to the laboratory for processing within 4 h of collection. Water samples were processed by filtering (0.7 μm glass fibre filter; Whatman GF/F) and then passed through a pre-conditioned solid phase extraction (SPE) cartridge (Waters HLB; 6 mL, 200 mg) at a rate of ~1 mL/min. SPE cartridges were then stored at -18^{o}C; immediately prior to analysis, SPE cartridges were eluted with 2x3 mL of methanol and 1x3 mL of dichloromethane, which was blown down under nitrogen (N_2) until dry and reconstituted in 1 mL 90 % water:10 % methanol for analysis by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

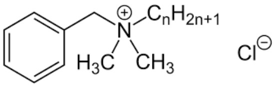
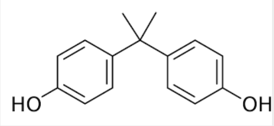
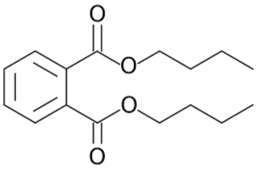
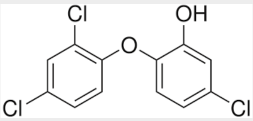
2.2 Wastewater contaminant selection

Based on the measured concentrations of the contaminants in wastewater (Table 1), their potential to cause adverse impacts and their physicochemical properties, especially high lipophilicity (K_{OW}) and low water solubility, four wastewater contaminants were selected for the batch sorption assessment. Bisphenol A (BPA), benzalkonium chloride (BAC), dibutylphthalate (DBP) and triclosan (TCS) (Table 1). Measured concentrations of the contaminants were in the high ng/L range for BPA, BAC and TCS, while mid-μg/L concentrations of DBP were measured in both WWTPs (Table 1). The moderate degree of lipophilicity for all compounds suggested that there would also be a moderate degree of association with microplastics that was also reversible (Hartmann et al., 2017; Heinrich and Braunbeck, 2019; Lee et al., 2018; Seidensticker et al., 2018).

In terms of potential for ecotoxicity, BPA concentrations were approximately ten times greater in WWTP 2 (1,111 ng/L) than WWTP 1 (196 ng/L), which were within an order of magnitude of the lower range of freshwater species sensitivity distributions (SSDs) and less than a 40-fold difference for concentrations that are protective of 95 % of marine species (HC5) (Table 1). Lowest concentrations of effects in marine organisms for TCS were generally less than that of BPA, while there was considerably less data available for BAC. Both BAC and TCS, however, are widely used antimicrobial agents that are inherently toxic and their reported effects at low μg/L concentrations on marine algae warranted their inclusion.

Concentrations of DBP in wastewater were considerably greater than the other selected contaminants, with Australia using 100s of tonnes each year in a range of consumer products and in industrial applications, mainly as a solvent or plasticiser (NICNAS, 2013). There is concern relating to phthalates as a class of contaminants, due to their potential effect as an endocrine disrupting chemical (EDC) in humans and animals (NICNAS, 2013), although there is comparatively little data available for the ecotoxicity of DBP especially in marine organisms. There is evidence, however, that adverse impacts on endocrine function in freshwater fish can occur at μg/L concentrations (Bhatia et al., 2014). On-going analysis of DBP during the sorption experiments, however, revealed a major issue with concentrations of DBP in process blanks increasing over the course of analysis and over the course of the sorption experiments, such that reliable quantification of DBP was not possible for this study. Results for DBP were therefore not included in the sorption and desorption study.

Table 1 Contaminants selected for batch sorption assessment based on their physicochemical properties, measured concentrations in wastewater and potential toxicity in marine organisms

CONTAMINANT	USE	EFFLUENT CONCENTRATION	PHYSICOCHEMICAL PROPERTIES				MARINE TOXICITY
		(ng/L)	Molecular weight (g/mol) [a]	Lipophilicity (log K_{OW}) [a]	Water solubility (mg/L) [a]	Ionisation constant (pK_a) [a]	(µg/L)
Benzalkonium chloride (BAC)  n = 8, 10, 12, 14, 16, 18	Biocide	998±228 (WWTP 1) 338±38 (WWTP 2)	304 (C_{12})	2.93	782	n.a.	30-72 (EC10)[b]
Bisphenol A (BPA) 	Plasticiser	196±34 (WWTP 1) 1111±201 (WWTP 2)	228	3.32	120	9.6	37.8 (HC5)[c] 307-664 (EC10)[d] 985-1394 (EC50)[d]
Dibutylphthalate (DBP) 	Plasticiser	11,000 (WWTP 1) 41,500±26,163 (WWTP 2)	278	4.50	11.2	n.a.	n.a.
Triclosan (TCS) 	Biocide	221±19 (WWTP 1) 164±18 (WWTP 2)	289	4.76	10	7.9	11.8 (HC5)[c] 12-17 (EC10)[b] 28-670 (EC50)[d,]

Source: [a] Toxnet (toxnet.nlm.nih.gov); [b](Tato and Beiras, 2019); [c](Durán and Beiras, 2017); [d](Tato et al., 2018); [e](Gorenoglu et al., 2018)

3 Sorption/desorption of wastewater contaminants

3.1 Sorption experiments

A modified batch sorption methodology to assess the likely degree of association and release of the selected contaminants with microplastics. A limitation of this batch approach is that sorption is assessed at elevated contaminant and microplastic concentrations compared with those found in realistic marine environments. For example, concentrations of contaminants under a realistic scenario would be in the ng/L range, while those of microplastic particles would be a few particles per litre, where quantification may lead to a high degree of variability. Elevated contaminant concentrations were used to enable their accurate detection, while elevated microplastic content was used to ensure measurable sorption could occur. The batch sorption assessment was modified from standard OECD protocols, where sorption to solid matrices such as soils or sediments are typically assessed (OECD, 2000). In this case, solutions containing the selected contaminants spiked at 1 mg/L were mixed with microplastics at a concentration of 1 g/L. The concentrations of the microplastics and contaminants in the batch sorption experiments were considerably (≥3 orders of magnitude) greater than what would be expected in wastewater (Tables 1 and 3).

3.1.1 Selection of solutions

Wastewater effluent from WWTP 2 was used to represent sorption in wastewaters, while seawater was collected from a marine nearshore location (approximately 35°31’ S 138°47’ E). The solutions were filtered through a 0.7 µm glass fibre filter and characterised for selected physical and chemical parameters (Table 2). The solutions were stored at 4°C until use in the sorption experiments, where they were brought to room temperature overnight prior to the sorption assessments. The background concentrations for the selected contaminants in the wastewater is summarised in Table 1.

Table 2 Summary of water quality parameters of the seawater and wastewater selected for the sorption/desorption experiments

PARAMETER	SEAWATER	WASTEWATER
pH	8	6.1
EC (dS/m)	53	1.1
Total carbon (mg/L)	13	82
Organic carbon (mg/L)	4	62
NO_x-N (mg/L)	0.25	<0.005
PO_4-P (mg/L)	0.009	0.1
SO_4^{2-} (mg/L)	3010	37
Cl- (mg/L)	19,200	166
Ca (mg/L)	377	28
K (mg/L)	450	20
Mg (mg/L)	1,230	12
Na (mg/L)	10,300	112
Al (mg/L)	<0.5	<0.05
B (mg/L)	4.8	0.69
Co (mg/L)	<0.5	<0.05
Cu (mg/L)	<0.5	<0.05
Fe (mg/L)	<1	0.4
Mn (mg/L)	<0.5	0.06
Si (mg/L)	<2	1
Sr (mg/L)	6.5	0.1
Zn(mg/L)	<0.5	0.47

3.1.2 Selection of microplastics

Based on previous studies, the most common microplastics present in wastewater by morphology are fragments or fibres and polymer type is typically PE or PP, although fibres are also commonly composed of PET (Table 3). Typical quantities of microplastics in wastewater are in the range of a few particles per litre, although some studies have shown hundreds of particles are possible, which equates to ng/L to low μg/L when considered on a mass per volume basis (Long et al., 2019; Simon et al., 2018). Based on the chemical similarity of the polymer subunits of PE and PP, and therefore potential for interaction with organic contaminants, PE was selected for this sorption assessment along with chemically-distinct PET (Figure 1).

For the batch sorption studies, four different microplastics were selected with different polymer type and morphology: (1) PET fibre (supplied by CSIRO Manufacturing); (2) PE fragments (prepared from shredded carry bags and sieved through a 2 mm filter); (3) PE fibres and; (4) PE microbeads. PET and PE fibres were supplied from CSIRO Manufacturing (Clayton, Australia), PE fragments were sourced from carry bags supplied by QIS Packaging (Sydney, Australia) that were passed through a high-speed shredder followed by a 2 mm mesh sieve (processed at CSIRO) and PE microbeads (53-63 μm) were supplied by Cospheric LLC (Santa Barbara, USA) (Figure 3).

All plastics were rinsed in a solution of acetone and methanol, prior to being dried at 40°C and stored in a clean glass vial before their use in the batch sorption experiments.

Table 3 Summary of global studies monitoring microplastic loads in wastewaters

MICROPLASTIC POLYMER	NUMBER OF PARTICLES	LOCATION	REFERENCE
PP fibres and fragments (23%) PE film (4%)	Influent $8x10^8$/day Effluent $2.2x10^7$/day (1-13/L)	UK (1 WWTP)	1
Undefined	Influent 180 fibres and 430 particles /L Effluent 4fibres and 9 particles/L Particles > fibres	Finland (1 WWTP)	2
Undefined	Influent 100-250/L (up to $20,000x10^6$/day) Effluent 3-25/L (100-600 $x10^6$/day) Fibres > particles	USA (3 WWTPs)	3
Various	Influent 1-7216/L Effluent: 0.05-81/L ($15x10^3$-$6.5x10^7$/day) (Australia 0.21-1.5/L)	Review of various countries	4
PP, PE, PET, PS (~90% of total)	Influent: 1-14/L (China) Effluent:0.2-1.7/L (China) Influent: 2.5-570/L (other countries + China) Effluent:0.05-1.4/L (other countries+ China) Granules/fibres/fragments equivalent in effluent (~30% each)	China (7 WWTPs)	5
Polyester, alkyd, PP, PS acrylic, PE, PET, acrylic, polyamide, PVA, PU	Effluent: 0.25-15.7/L (65-4100 $x10^6$/day) Fragments ~68% Fibres~19% Beads ~3%	UK (1 WWTP)	6
Influent (acrylate, PE, PP, PET) Effluent (PE, PET, PP, acrylate)	Influent: 5362-18285/L (61-1189μg/L) Effluent: 19-447/L (0.5-11.9 μg/L) Particles >>fibre	Denmark (10 WWTPs)	7

Source: [1](Blair et al., 2019); [2](Talvitie et al., 2015); [3](Conley et al., 2019); [4](Gatidou et al., 2019); [5](Long et al., 2019); [6](Murphy et al., 2016); [7](Simon et al., 2018)

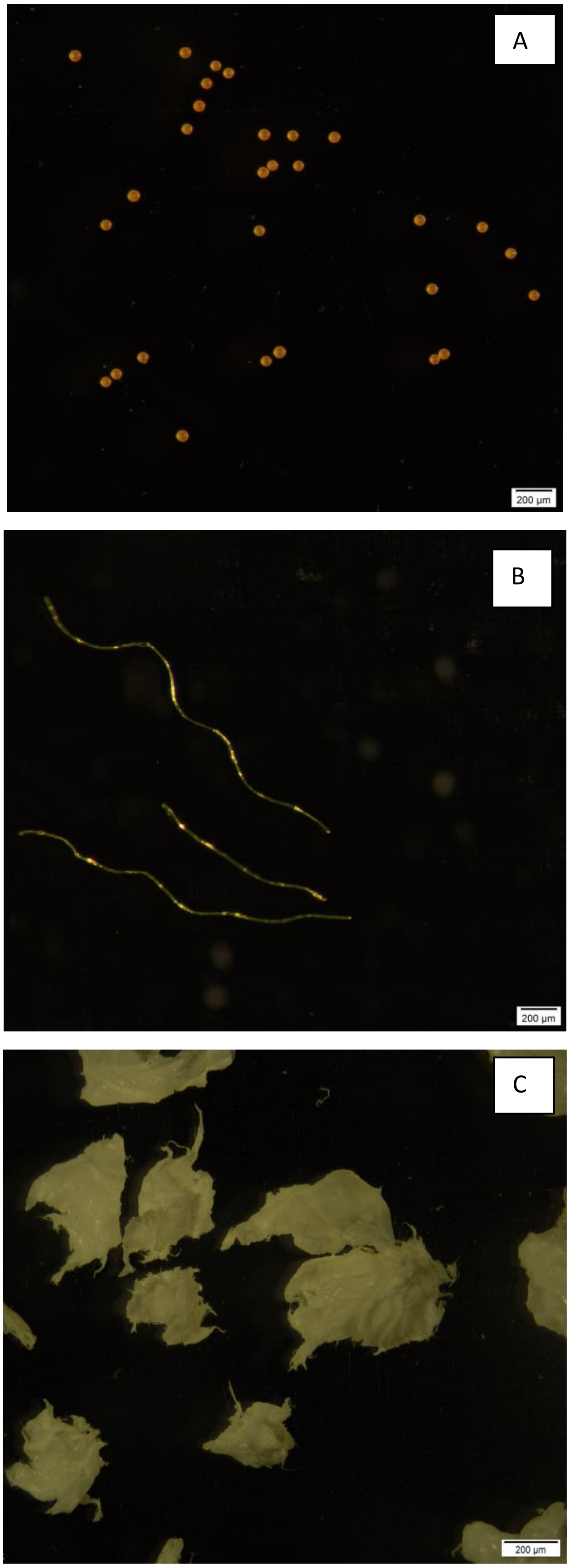


Figure 3 Microplastics, including (A) microbead, (B) microfibre and (C) microfragment, used for the batch sorption experiments

3.1.3 Batch sorption

The batch sorption study was undertaken by mixing a solution containing the contaminants with 10 mg of respective microplastics. The starting concentrations of the contaminants was 1 mg/L and was prepared by adding 0.1 mL of a 1 g/L stock solution of contaminants respectively prepared in methanol, to 100 mL of wastewater. The spiking solution, therefore, contained contaminants as a mixture with a 0.1 % final concentration of methanol. This methanol concentration is suitable to prevent undue influence on the batch sorption process (OECD, 2000).

A 10 mL aliquot of the 1 mg/L contaminant spiking solution was then added to a clean 20 mL glass tube, containing 10 mg of microplastic (either fragment, fibre or bead). Tubes were then sealed with a PTFE-lined lid and placed on a rotating shaker for a period of time determined in Section 3.1.4.. After the shaking period, 1 mL of the solution was carefully removed for quantification of the respective contaminants following LC-MS/MS methodology outlined in Section 2.1.

The concentration of contaminant remaining in solution was used to estimate the extent of sorption, which is defined by the distribution coefficient (K_d):

$$K_d = \frac{C_p}{C_s} \qquad (1)$$

which is the ratio of C_p, the concentration of the contaminant associated with the plastic, and C_s, the concentration of the contaminant remaining in solution. The concentration of C_p can be measured directly or indirectly by measuring the difference in initial solution concentration (C_i) and the final concentration, C_s:

$$C_p = \frac{(C_i \times V_i) - (C_S \times V_S)}{m_p} \qquad (2)$$

where V_i is the initial volume of solution, V_s is the final volume of solution and m_p is the mass of the plastic in solution. All treatments were prepared in triplicate.

3.1.4 Time for sorption equilibrium

The batch sorption study assumes a rapid equilibrium to form between the contaminants in solution and the microplastics. The PET and PE microfibres were selected for this experiment, to represent the two polymer types, as it has been previously suggested that particle size may only have a minor influence on the kinetics of sorption (Li et al., 2019; Wang et al., 2019). A mixture of the contaminants were added to 10 mL of both wastewater and seawater solutions and mixed on an end-over-end shaker for 4, 12, 24, 48 and 72 hours, before being removed and the solutions analysed for concentrations of the spiked contaminants to determine K_d.

3.2 Desorption of contaminants from microplastics

A desorption study was undertaken following batch sorption, using a standardised protocol (OECD, 2000). Following the sorption experiments in wastewater, batch sorption solutions were filtered on a 0.2 μm pore size (47 mm diameter) polycarbonate filter to dryness and the retained microplastics were returned to a clean 20 mL glass tube for desorption. A 10 mL aliquot of desorption solution (seawater or seawater modified with 15 mM sodium taurocholate; see Section 3.2.1) was then added to the microplastic and the solutions shaken for a 24 h period. A 0.1 mL aliquot of solution was collected for LC-MS/MS analysis.

The percent fraction of contaminant released from the microplastic was determined from the amount of contaminant adsorbed to the microplastics, quantified from the batch sorption experiments, and the concentration of contaminant measured in the desorption solutions:

$$\%\, desorption = \left(\frac{c_s \times v_s}{c_p \times m_p}\right) \times 100 \qquad (3)$$

3.2.1 Selection of desorption solutions

Microplastics isolated from the sorption studies were placed in a clean solution of either (1) seawater; (2) seawater and 15 mM sodium taurocholate (incubated at 20^{o}C); or (3) seawater and 15 mM sodium taurocholate, with pH adjusted to 4 (using 1 M HCl) and incubated at 38^{o}C. This was to represent three potential scenarios following discharge into the marine environment. The first case, using seawater only, was to assess the potential for the microplastic to release the contaminants, following discharge into the marine environment. The second and third scenario, with desorption in seawater in the presence of sodium taurocholate under two different pH and temperature values, was to assess the potential release of contaminants from microplastics under physiological conditions.

To simulate physiological conditions, sodium taurocholate (Sigma Aldrich, Australia) was added to the seawater solution with a final concentration of 15 mM. Taurocholate, a bile salt found in vertebrates, has been developed as a reagent for use in bioavailability studies that most closely reflects the solubilisation of contaminants in a benthic marine organism, *Arenicola marina* (Voparil and Mayer, 2004). Taurocholate has also been used for estimating mobilisation of contaminants from microplastics following their ingestion (Bakir et al., 2014; Coffin et al., 2019). Furthermore, reducing the pH and increasing the temperature of solution has also been used as a means of simulating a gut environment where conditions may be more favourable for the release of contaminants from microplastics (Bakir et al., 2014).

3.3 Statistical analysis

For comparison of the values obtained for the batch sorption and desorption experiments, a one-way analysis of variance (ANOVA) was used to compare all values between treatments. A Shapiro-Wilk Normality Test was applied to sets of values within each treatment to test for normality of data. Where a significant difference was found for the ANOVA, a Tukey post-hoc comparison was undertaken to determine which treatments were significantly different, at a significance level of 95%. Statistical analyses were performed by SigmaPlot v14.

4 Sorption and desorption results

4.1.1 Time for sorption equilibrium

In general, the solution concentrations of BAC, BPA and TCS in solution were found to remain reasonably constant throughout the 72 h shaking period with either the PE or PET fibres (Figures 4 and 5). For PE fibres, there was no significant difference (*p* >0.16) between the extent of sorption for each contaminant in wastewater (Figure 4). The sorption of BPA to PET fibres in wastewater was significantly less (*p* <0.002) after 24 h shaking but there was no change after this (Figure 5). In the seawater treatments, sorption of BPA was found to significantly increase (*p* <0.01) for up to 48 h after which there was no change in solution concentration (Figure 4). This indicates that sorption did not vary greatly over the 72 h shaking period, which is consistent with other studies that show equilibration can occur within a 24 h period, although a slower phase of equilibrium after this rapid equilibration can occur for up to 100 h (Bakir et al., 2012; Li et al., 2019; Liu et al., 2019; Teuten et al., 2007; Wang et al., 2019). In the case of TCS, solution concentrations after mixing with PE fibres significantly reduced (*p* <0.01) after 24 h in seawater (Figure 4), while PET fibres in seawater were found to reduce non-significantly at 72 h after remaining constant up to 48 h (Figure 5). A study by Li et al. (2019) found that TCS sorption to polystyrene particles can take up to 150 h of equilibration after an initial, rapid (<24 h) equilibration phase. The extent of sorption and the time to reach equilibrium is likely to be highly dependent on a number of factors related to the solution (such as pH, temperature, electrical conductivity, organic matter), the contaminant (such as hydrophobicity and ionisability) and the plastic (such as chemistry of polymer backbone and physical structure of polymer) that can affect the extent of interaction between the contaminant and plastic (Li et al., 2019; Seidensticker et al., 2018; Wang et al., 2018). For example, the sorption capacity of HDPE has been found to be greater for BPA, compared with PET, due to the presence of rubbery internal domains that can more readily associate with contaminants compared with rigid, glassy domains found with PET (Liu et al., 2019).

A mixing period of 24 h in wastewater was selected for this study to ensure a balance between rapid sorption reactions (equilibrium) and potential degradation of the contaminants.

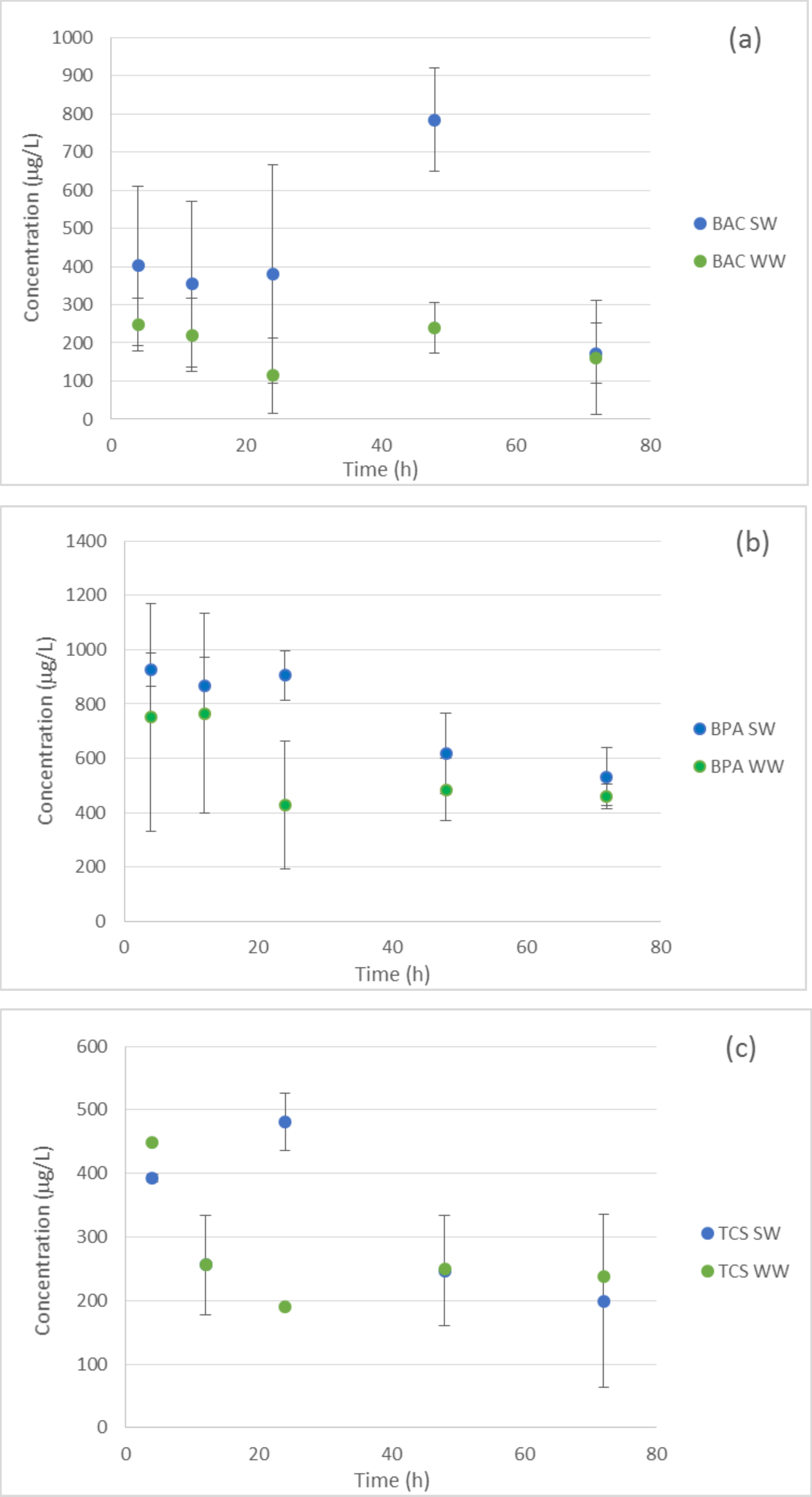


Figure 4 Concentrations of (a) benzalkonium chloride (BAC), (b) bisphenol A (BPA) and (c) triclosan (TCS) in wastewater (WW) and seawater (SW) solutions following mixing with PE fibres for 72 h. Values represent mean (±SD) of triplicate samples.

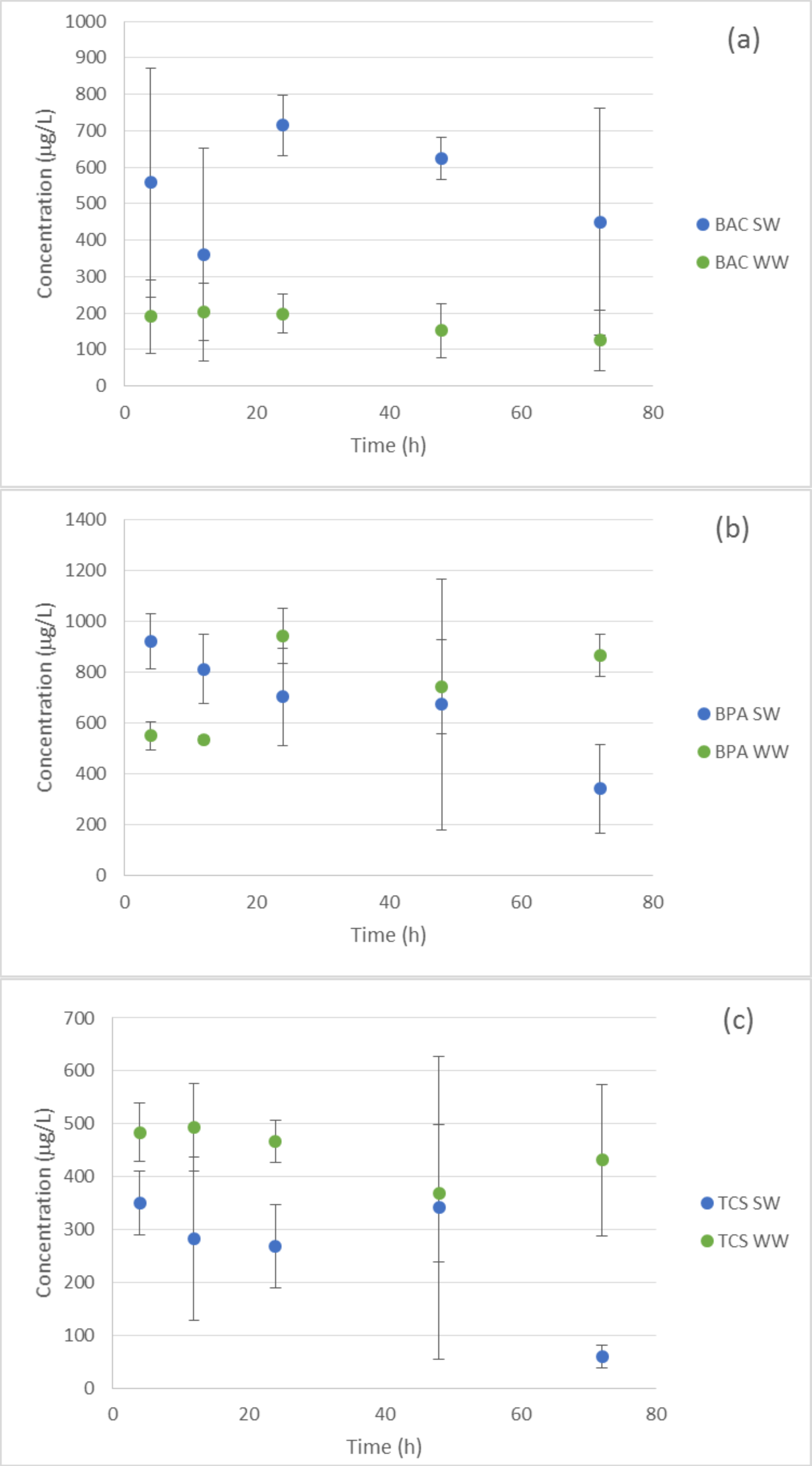


Figure 5 Concentrations of (a) benzalkonium chloride (BAC), (b) bisphenol A (BPA) and (c) triclosan (TCS) in wastewater (WW) and seawater (SW) solutions following mixing with PET fibres for 72 h. Values represent mean (±SD) of triplicate samples.

4.1.2 Batch sorption of selected contaminants to microplastics

The association of contaminants to the various microplastics after 24 h of mixing in wastewater, based on K_d values, was found to be greatest for TCS (2,700-8,800 L/kg), followed by BAC (660-2,050 L/kg) and BPA (161-1,060 L/kg) (Table 4). This was statistically significant ($p < 0.001$) for PE fibres and PET fibres for all contaminants, as well as for TCS for PE beads ($p = 0.012$). There was no significant difference ($p = 0.056$) between K_d values of all contaminants for PE fragments. In all cases, association was greatest with PE microplastics, compared with PET, while there was no clear relationship with the morphology of the microplastic. The enhanced association with PE in this study is consistent with its rubbery polymer structure, which allows a greater extent of diffusion into the polymer matrix compared with more glassy polymers such as PET (Alimi et al., 2018; Rochman et al., 2013). In the case of microplastic morphology, TCS had significantly greater ($p < 0.001$) sorption to PE beads, while BAC had significantly greater ($p < 0.002$) sorption to PE beads relative to PET fibres. However, there was no significant difference between sorption values of different microplastic morphologies for the respective contaminants.

There is limited published information relating to the extent of association of the selected contaminants with microplastics. A batch sorption study by Wu et al. (2016) found a similar degree of association of TCS to PE particles, with a K_d value of 5140±290 L/kg. In the same study it was further demonstrated that increasing salinity led to a greater degree of association of TCS with PE, which is a phenomena widely described in literature on organic chemicals and known as “salting out” (Turner and Millward, 2002; Wu et al., 2016). Additionally, TCS partitioning to PE microparticles has been estimated to be 1,100 L/kg (Seidensticker et al., 2018), which is within an order of magnitude of the K_d values for PE in this study (Table 4).

The more favourable association of TCS found in the present study may be expected based on its relatively higher K_{OW} value, although the slightly higher K_{OW} value of BPA did not lead to greater association than BAC (Table 4). All three contaminants are subject to ionisation at the pH of seawater (8), including BAC which is always a cation due to its quaternary amine group. Deviation from the expected relationship between sorption and log K_{OW} might suggest that ionisation of the contaminants can have a role in the extent of association with microplastics. In the study by Seidensticker et al. (2018), partitioning of TCS (and other ionisable contaminants) to PE was considered to be largely due to the unionised species. At the pH of the seawater in the sorption experiments, however, 50% of TCS would have been ionised, while the K_d values of TCS would suggest that more than 50% of initially added TCS was associated with the microplastics.

For a number of other unionised contaminants, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and personal care products, association with microplastics is expected to be largely driven by K_{OW} values and plastic type (Alimi et al., 2018; Bakir et al., 2012; Rochman et al., 2013; Seidensticker et al., 2018).

Table 4 Summary of sorption coefficient (K_d) values for BAC, BPA and TCS in wastewater. Values are mean (±SD) of triplicate samples.

CONTAMINANT	MICROPLASTIC	WASTEWATER K_D (L/kg)
Benzalkonium chloride (BAC)	PET fibre	656±118
	PE fibre	978±65
	PE fragment	1001±695
	PE bead	2048±666
Bisphenol A (BPA)	PET fibre	161±38
	PE fibre	175±104
	PE fragment	1063±976
	PE bead	955
Triclosan (TCS)	PET fibre	2733±542
	PE fibre	3640±149
	PE fragment	3126±976
	PE bead	8793±1548

4.1.3 Desorption of selected contaminants from microplastics

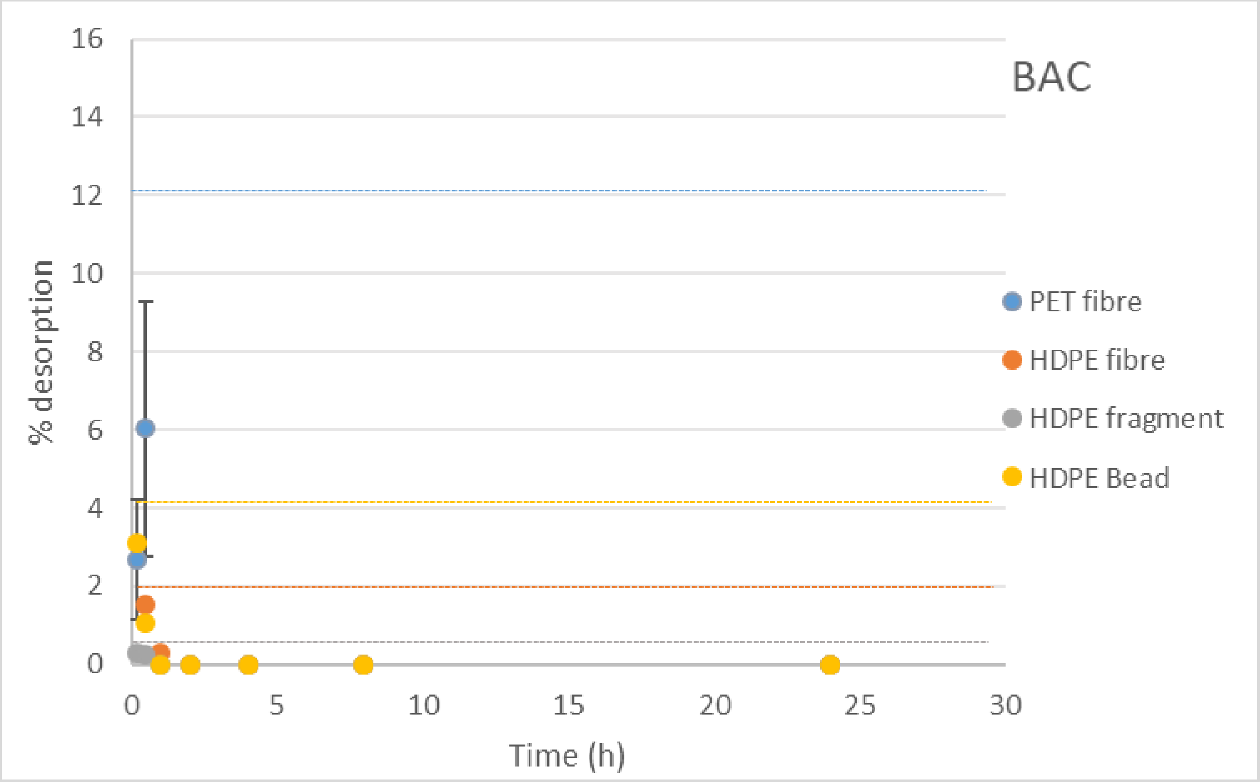
Desorption of the selected contaminants from the microplastics was generally greater in the simulated gut solution, containing 15 mM of the surfactant taurocholate, compared with seawater (Figures 6, 7 and 8). For example, the majority of TCS was desorbed in the two taurocholate solutions (for both temperature/pH values) for PET and PE fibres (Figures 7 and 8), while this was the case for all plastics in the pH 4 taurocholate solution (Figure 8). For BAC, a higher proportion was also desorbed in the pH 4 taurocholate solution, with 99±45% desorption in the case of PET fibres and ~50 % (45±24% and 47±33%, respectively) for the PE fibres and fragments. In the other solutions, the extent of desorption of BAC was <12 %. There was no apparent effect on desorption based on morphology. The only significant difference in morphology was found for BAC between PET fibres and PE fibres (*p*=0.016) and PE fragments (*p*=0.03) for n seawater and PET fibres and PE bead (*p*=0.03) in the simulated gut solution (Figures 6 and 8). Also, desorption was significantly greater for PET fibre compared with all other PE morphologies (*p* <0.047) for TCS in seawater solution

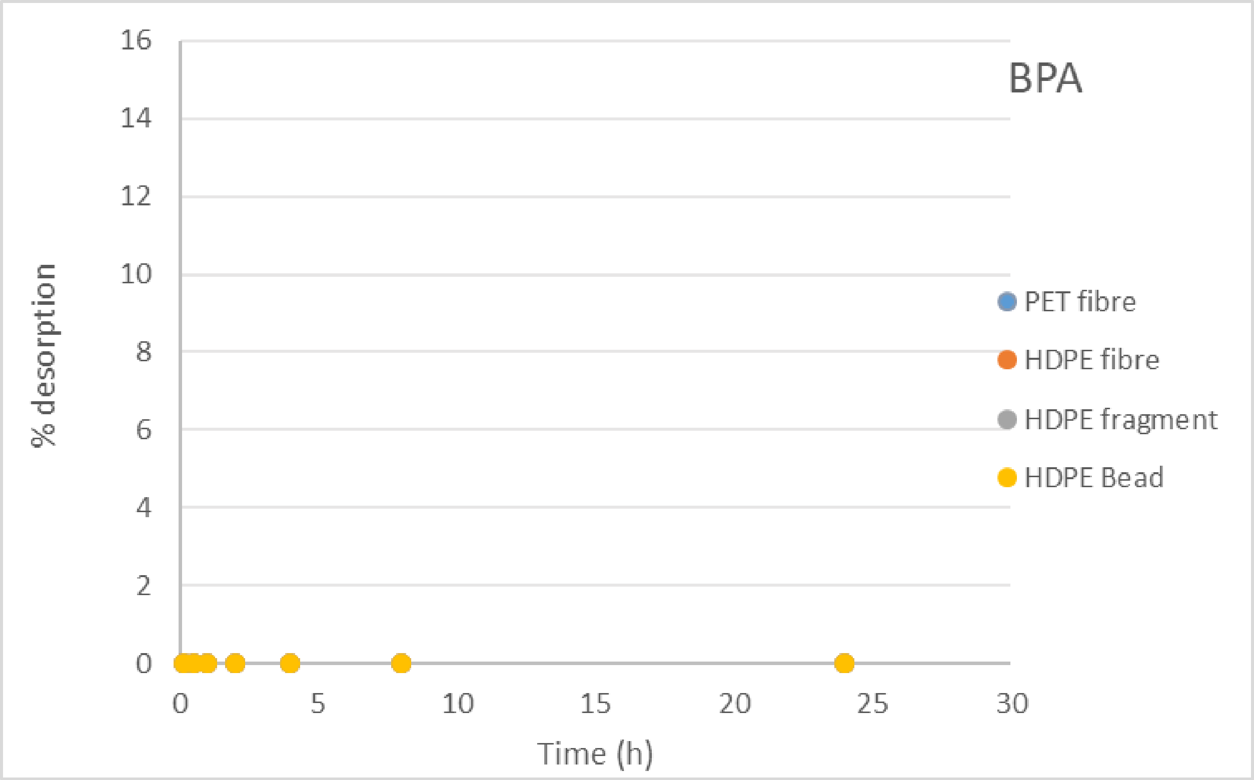
(Figure 6). This would suggest the polymer type was more important than morphology for these two contaminants in respective solutions.

As a quaternary ammonium chemical (QAC), BAC is often found to have a strong degree of interaction with solids in the environment due to both ionic and hydrophobic interactions (Zhang et al., 2015). This can also reduce the extent of desorption from natural materials, although increasing pH (>10) can also have an effect in reducing desorption (Flores et al., 2016; Ismail et al., 2010). Due to the ionised nature of BAC, however, it has previously been found to have minimal interaction with plastic materials (including low density PE) used in healthcare, where BAC has an important role as an antimicrobial agent (Amin et al., 2012; Bahal and Romansky, 2002). In the present study, reducing the pH increased the amount of BAC released and because of the permanent cationic charge of QACs, the observed pH effect would likely be due to altering the properties of the sorbent material rather than QAC. Furthermore, the greater temperature (38^{o}C) of the pH 4 solution may have enhanced the extent of desorption of BAC (Bakir et al., 2014).

In contrast, desorption of BPA was not enhanced in the pH 4, 38^{o}C taurocholate solution (Figure 8) and, despite its comparatively low K_d values and high water solubility (Tables 1 and 4), the degree of BPA desorption was low (<12 %) relative to the other contaminants in all solutions. In previous studies, desorption of BPA has also been found to be comparatively low in seawater solutions (Liu et al., 2019), while a 78 day desorption study demonstrated only around 4 % of initial concentrations of BPA leach from flexible polyvinyl chloride (PVC) in seawater solutions (Suhrhoff and Scholz-Böttcher, 2016). A study looking at the leaching of BPA from polycarbonate (PC), however, showed that its rates of leaching were greater in seawater, compared with river water and deionised water, and at higher temperatures (37^{o}C compared with 20^{o}C) (Sajiki and Yonekubo, 2003). These studies also demonstrate that the release of BPA from plastics will tend to be greater at higher pH, especially close to the pH at BPA's pK_a value (~10), where it becomes 50% ionised (Liu et al., 2019; Sajiki and Yonekubo, 2003). In the present study, however, BPA would have been primarily in its unionised form at the pH value (8) of the seawater.

TCS also contains a functional phenolic group that is 50 % ionised at pH 8.1 (Table 1), which would be expected to affect its lipophilicity and, therefore, degree of release into solution. For example, the association of TCS with PE was found to increase in lower pH solutions where it is in its neutral, unionised (and more lipophilic) form (Seidensticker et al., 2018). Desorption of TCS in both taurocholate solutions, however, was high with the majority of TCS being released into solution even in the lower pH solution, whereas a much smaller fraction was released in the pH 8 seawater-only solution (Figure 6). In this case, the relatively high concentration of taurocholate (~8 g/L) may have made the extent of ionisation of TCS less important than the formation of taurocholate micelles, which could have the effect of increasing the solubility of the relatively hydrophobic TCS in the seawater solution (Bakir et al., 2014). For example, the desorption rate of neutral and highly hydrophobic chemicals from PE in 15 mM taurocholate and seawater solutions is greatly enhanced compared with seawater alone, with the greatest rates occurring at 38°C compared with 18°C (Bakir et al., 2014; Teuten et al., 2007).





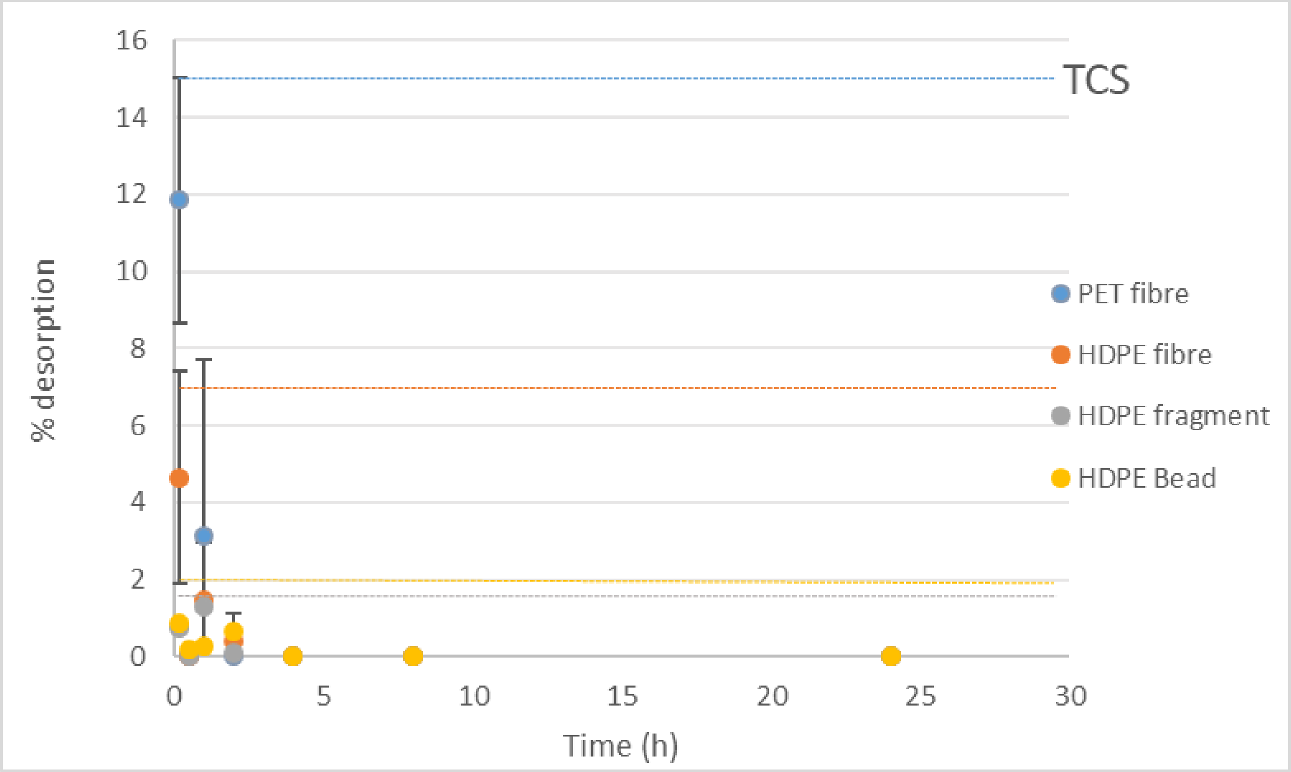
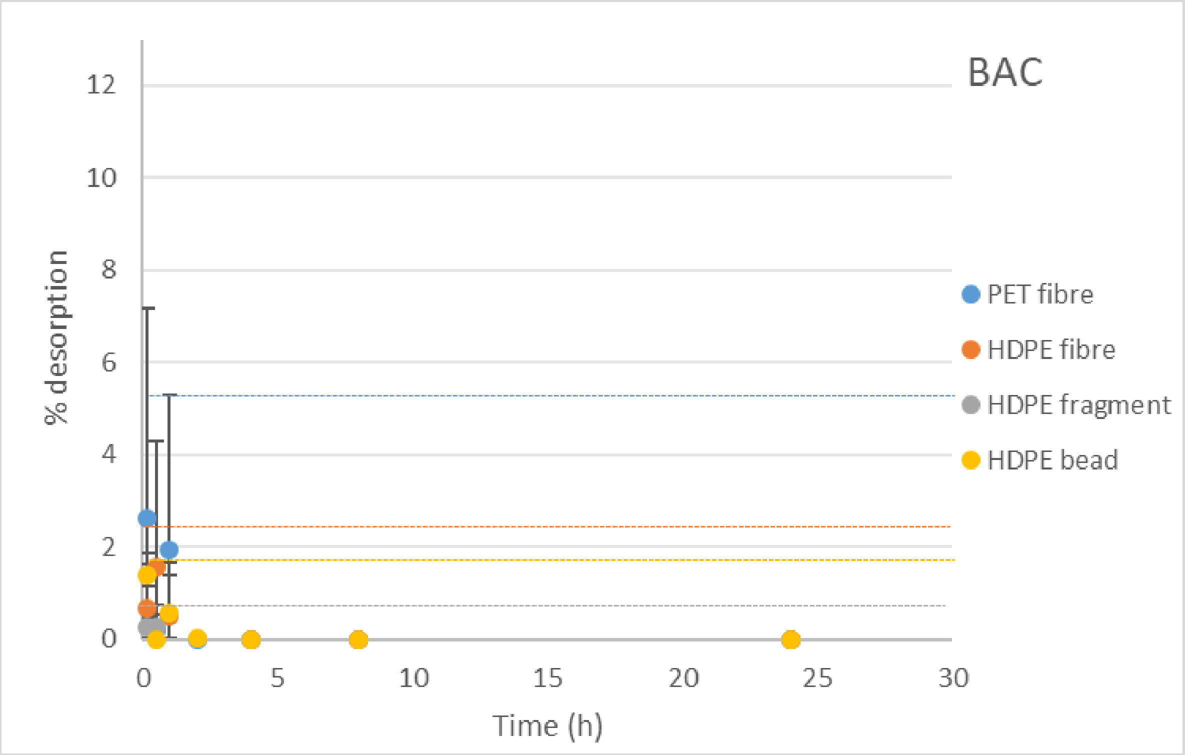
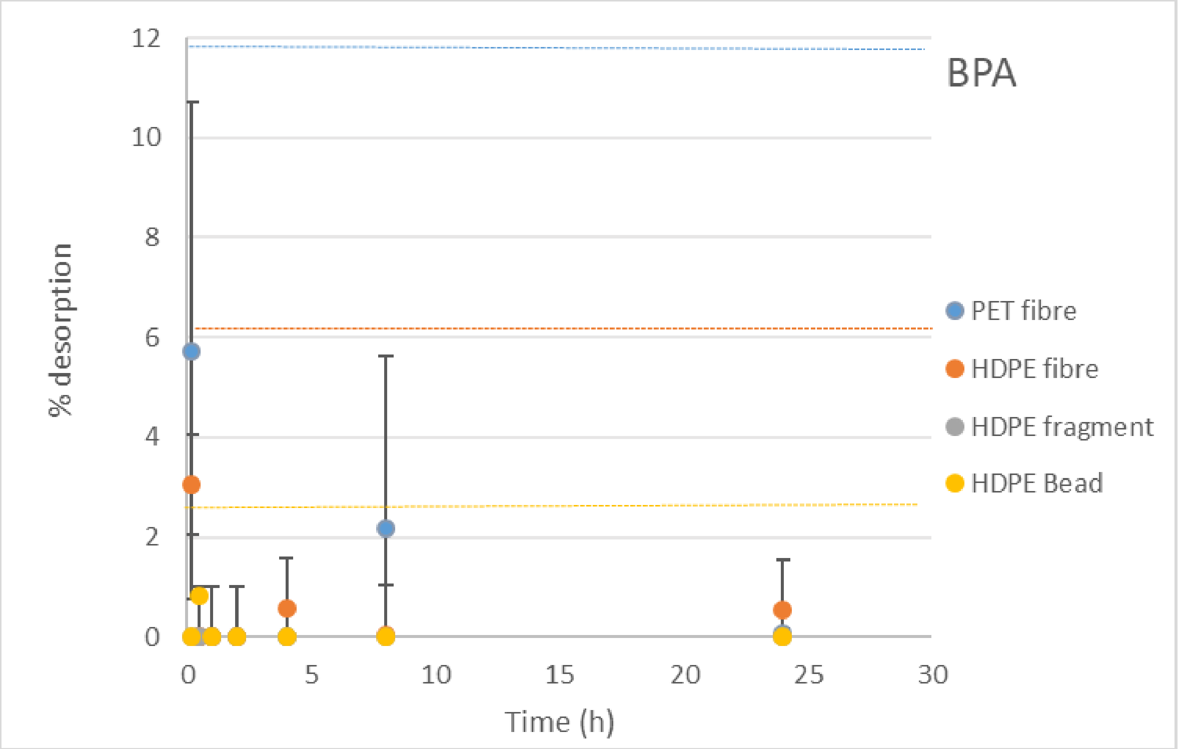


Figure 6 The percentage desorption of the total amount of benzalkonium chloride (BAC), bisphenol A (BPA) and triclosan (TCS) sorbed to the plastics in seawater.

The coloured lines correspond with the total percentage of contaminant that desorbed over the 24 h period. Values are means (±SD) of triplicate samples


BAC
% desorption
0
2
4
6
8
10
12
Time (h)
0
5
10
15
20
25
30
● PET fibre
● HDPE fibre
● HDPE fragment
● HDPE bead


BPA
% desorption
0
2
4
6
8
10
12
Time (h)
0
5
10
15
20
25
30
● PET fibre
● HDPE fibre
● HDPE fragment
● HDPE Bead

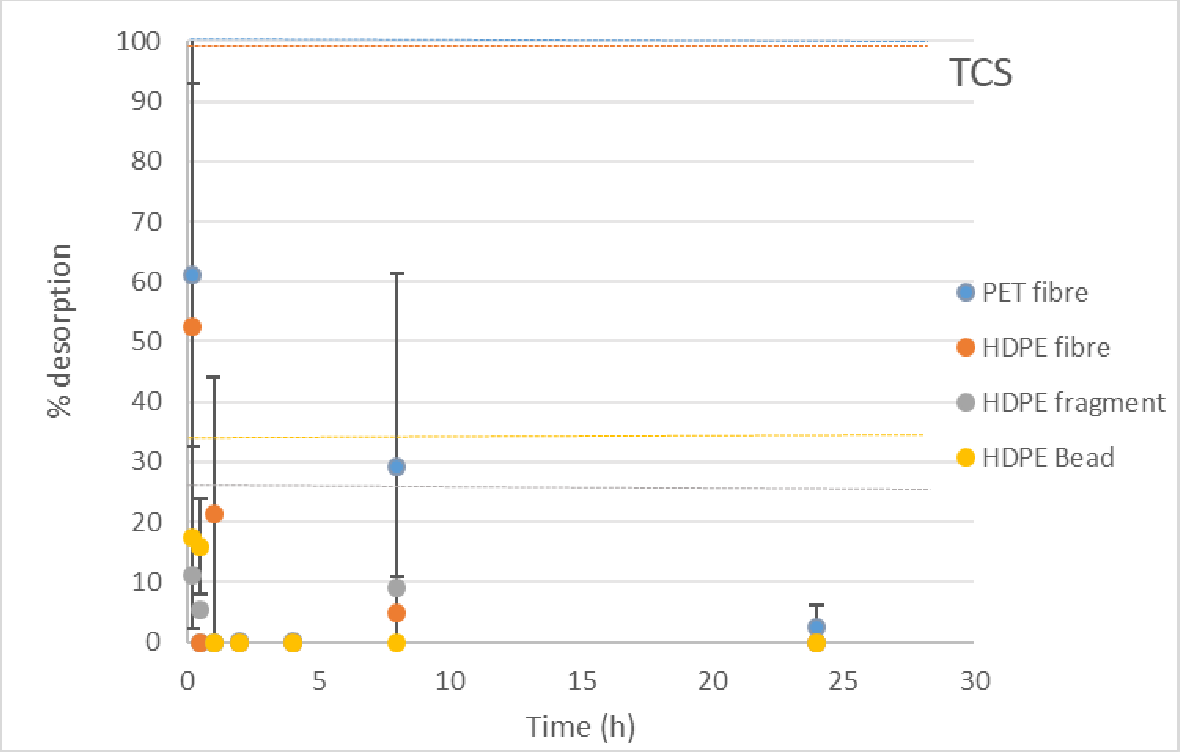
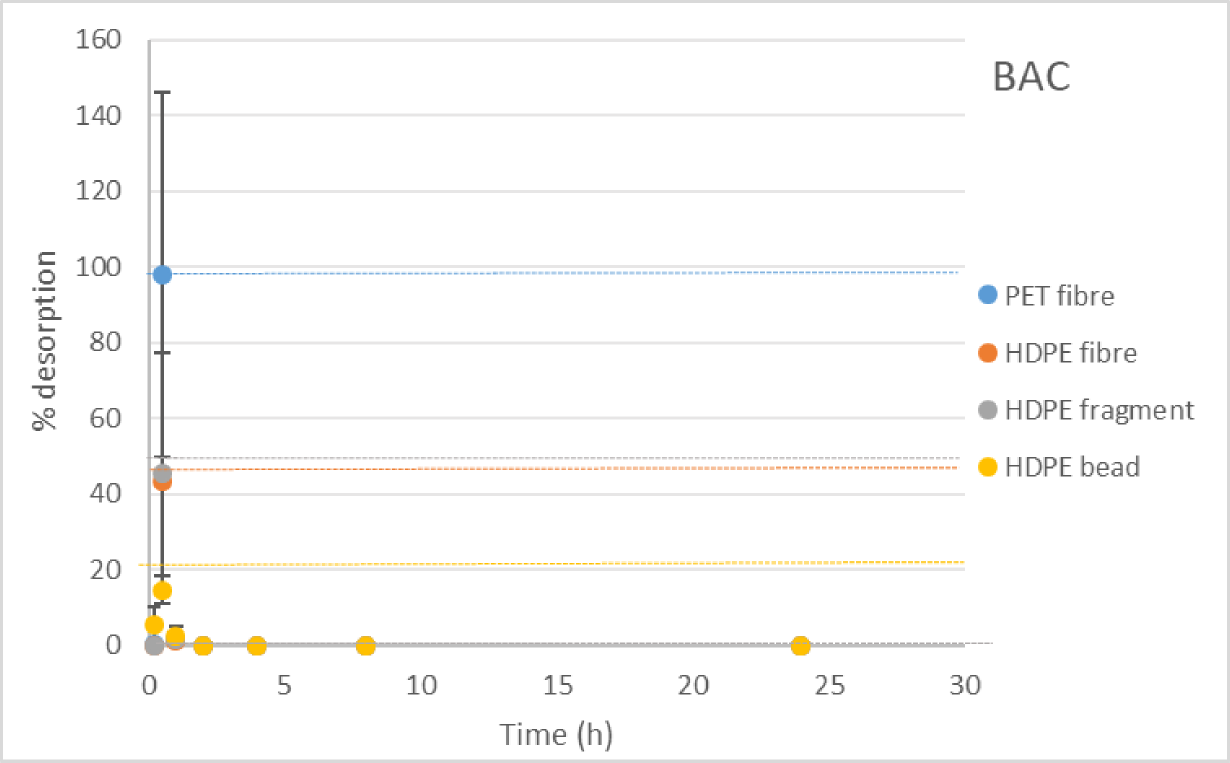
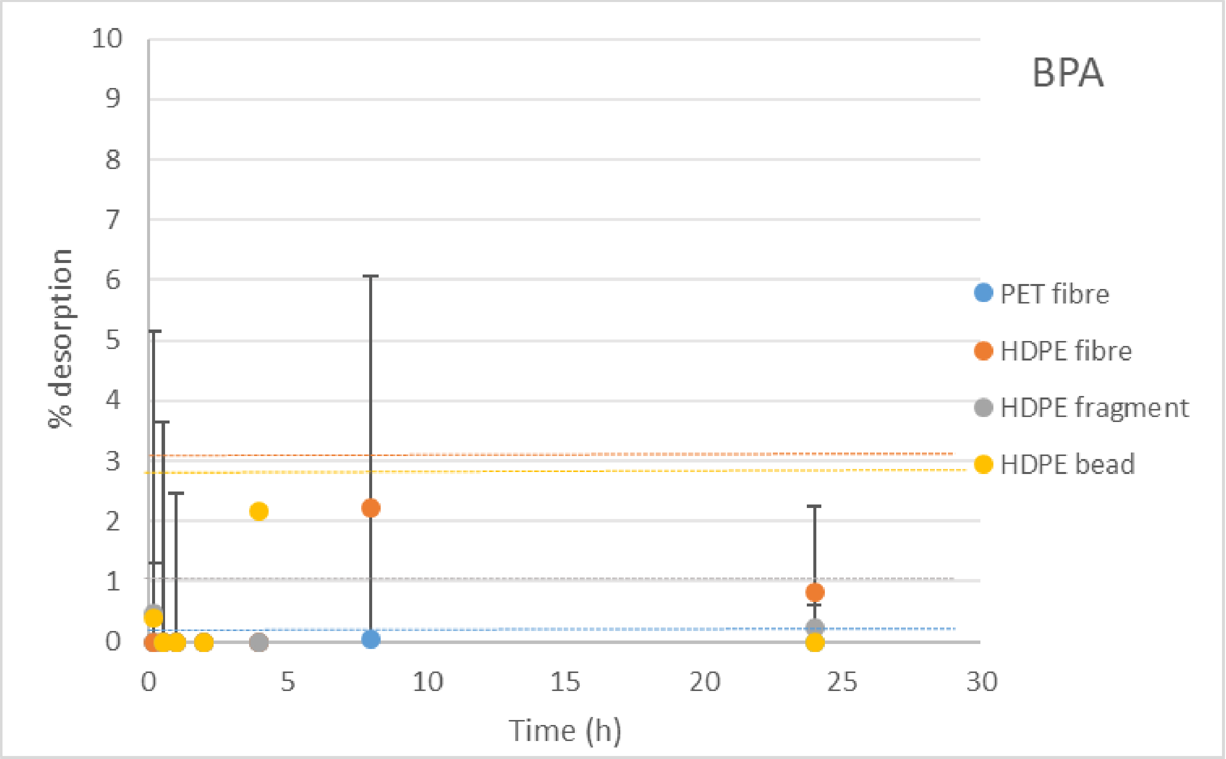


Figure 7 The percentage desorption of the total amount of benzalkonium chloride (BAC), bisphenol A (BPA) and triclosan (TCS) sorbed to the plastics in seawater containing 15 µm taurocholate.

The coloured lines correspond with the total percentage of contaminant that desorbed over the 24 h period. Values are means (±SD) of triplicate samples


BAC
% desorption
160
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (h)
PET fibre
HDPE fibre
HDPE fragment
HDPE bead


BPA
% desorption
10
9
8
7
6
5
4
3
2
1
0
0
5
10
15
20
25
30
Time (h)
PET fibre
HDPE fibre
HDPE fragment
HDPE bead

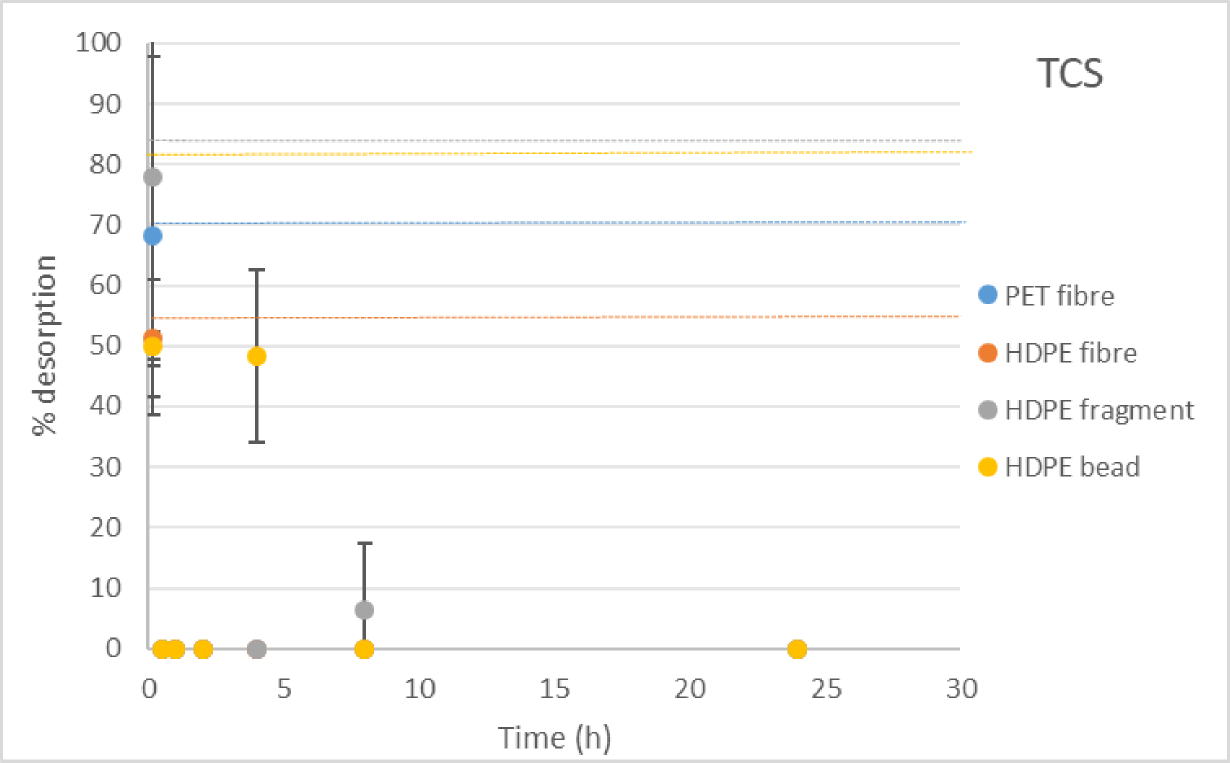


Figure 8 The percentage desorption of the total amount of benzalkonium chloride (BAC), bisphenol A (BPA) and triclosan (TCS) sorbed to the plastics in seawater containing 15 µm taurocholate (pH 4) for desorption.

The coloured lines correspond with the total percentage of contaminant that desorbed over the 24 h period. Values are means (±SD) of triplicate samples

The desorption of these three contaminants was therefore found to be negligible after microplastics were added to seawater. This would indicate that following their association with microplastics in wastewater the changed conditions of the marine environment (for example, increased salinity and pH) are unlikely to promote the release of these contaminants back into solution. Under these circumstances, microplastics composed of common polymer types could potentially play a key role in the transport of associated contaminants once released into the marine environment in wastewater discharges. Conversely, under simulated gut physiological conditions, a large proportion of TCS and BAC (around 100% in some cases) were released into solution, which suggests uptake of microplastics by marine organisms could represent an exposure pathway to associated contaminants. Characterising the potential hazards of contaminants associated with microplastics for marine organisms, however, needs to consider not only the potential for the release of the contaminants but also the exposure pathways and concentrations in environmental compartments or accumulated within a marine organism and how these concentrations correspond with toxicity in the organism. Exposure concentrations of contaminants will be related to the number of microplastics that are released into the environment from WWTPs, the load of contaminants that are likely to be associated with the microplastics and the likelihood of the microplastics (and associated contaminants) being incorporated into an organism.

The next section will outline an assessment of the inputs of microplastics and contaminants into the marine environment, the potential toxicity of these exposure concentrations and whether this may constitute a hazard to marine organisms that requires further investigation.

5 Transport and exposure of contaminants associated with microplastics

5.1 Contaminant and microplastic loads in WWTP effluent

The extent of the contaminant transport through association with microplastics in the marine environment depends on the degree of their association with the microplastics, the irreversibility of the association and how far the microplastic is expected to be carried throughout the marine environment. The three contaminants selected for this study, BAC, BPA and TCS, had a strong association with the microplastics and a low degree of desorption from the microplastic when transferred to seawater solutions. This is consistent with other sorption assessments that showed the nature of microplastics makes them amenable to adsorption or absorption of organic contaminants present in wastewater streams. Based on the high degree of sorption and low degree of desorption under conditions reflecting discharge of microplastics from wastewater into the marine environment, there is potential for the contaminants to be transported throughout the marine environment with the microplastics.

The number of contaminants that have been included in these sorption assessments is limited, based on the broad diversity of organic contaminants that are present in wastewater. The degree of association of the contaminant with a microplastic, however, has been demonstrated as being highly dependent on the physicochemical characteristics of the contaminant, the microplastic and the receiving environment. In this case, BPA had a relatively low degree of association with PE and PET microplastics, compared with the more hydrophobic TCS and BAC. It is also likely that factors apart from hydrophobicity, such as ionisation of the contaminant or polymer structure and surface area of the microplastic, will also have a strong influence on the degree of association in different environments (Hartmann et al., 2017).

It is apparent from the present study, and others, that contaminants can have a strong affinity for microplastics present in wastewater. For example, the K_d value of chemicals partitioning to PE, PET and PVC can range from low (K_d <100 L/kg) for ionic chemicals with a low degree of hydrophobicity to very high (Kd >1000), where the plastic has a very high concentration of contaminant relative to surrounding water (Bakir et al., 2012; Hale et al., 2010; Seidensticker et al., 2018; Smedes et al., 2009; Teuten et al., 2007).

The relative amount of contaminants being removed from wastewater through association with microplastics, however, will be highly dependent on the amount of microplastics present in wastewater.

Even in the case of extremely high partitioning (K_d >10,000 L/kg), where the microplastics have a high capacity to remove contaminants from solution, the relative amount of other sorptive material present in wastewater also needs to be considered. This will allow an assessment of the relative importance that microplastics may have in transporting contaminants within the marine environment. Previous studies that have measured the concentration of microplastics present in WWTP discharges into the environment (typically reported as number of microplastics per volume) show they are highly dependent on the level of wastewater treatment, with secondary and tertiary treatment being very effective (usually >90 %) in removing microplastics (Gatidou et al., 2019). These surveys of microplastics in WWTP effluents show that discharges can contain from <1 up to 447 microplastic particles/L (Table 3). Although this can represent thousands to millions of microplastic particles being released per day the concentration of microplastics, in terms of mass, is likely to be relatively low. An analysis by Simon et al (2018) showed that the mass of microplastics in 10 different WWTP effluents ranged from 0.5-11.9 μg/L. Although the numbers of microplastics detected in the study by Simon et al (2018) was high (19-447 microplastic particles/L effluent) compared with other studies (typically < 80 microplastic particles /L), this high value could be used to represent a worse-case scenario for discharges into the marine environment.

While it has been previously noted that tonnes of microplastics are discharged into the marine environment contributing to thousands of tonnes being present in oceans, this represents only a very small fraction of the mass of naturally occurring organic carbon (Eriksen et al., 2014; Gouin et al., 2011; Koelmans et al., 2016). The dissolved organic carbon (DOC) content of wastewater selected for this assessment was 62 mg/L (Table 2). This is orders of magnitude greater than even the higher range of microplastic concentrations reported in wastewater effluent (low μg/L but typically much less)(Simon et al., 2018), which would suggest that the presence of organic carbon is likely to be the main factor driving the transport of contaminants through WWTP discharges (Gouin et al., 2011; Koelmans et al., 2016). For example, a scenario that represents a high degree of microplastics contamination in wastewater could be where microplastics are present at 12 μg/L (Simon et al. 2018), in conjunction with the concentrations measured in the present study for TCS, BAC and BPA, which were in the high ng/L to low μg/L range (Table 1). PE, one of the most common polymer types for microplastics present in WWTP discharges (Gatidou et al., 2019; Long et al., 2019;

Simon et al., 2018), also had the highest sorption capacity for the contaminants, which can give an approximation of the higher amounts of the contaminants that could become associated with the microplastic particles (Table 4). From this, it can be estimated that only a very small fraction (< 0.01%) of the total amount of contaminant being released in WWTP effluents are likely to become associated with the microplastics (Table 5).

Table 5 Estimate of percentage of BAC, BPA and TCS measured in wastewater effluent that could become associated with microplastics present in wastewater at a concentration of 12 μg/L

CONTAMINANT	SORPTION CAPACITY RANGE	WASTEWATER CONCENTRATION	AMOUNT SORBED TO PLASTIC	% TOTAL SORBED TO PLASTIC
	K_d (L/kg)[a]	(ng/L)	(ng)[b]	
BAC	316±63-2048±666[c]	388±38-998±228[c]	0.001-0.025	0.0004-0.0025
BPA	134±84-3150±3104	196±34-1111±201	0.0003-0.04	0.0002-0.004
TCS	1429±451-8793±1548	164±18-221±19	0.003-0.023	0.002-0.01

[a]based on K_d values for PE particles (Table 4); [b]based on 12 μg/L microplastic in effluent (Simon et al. 2018), mean effluent contaminant concentration range (Table 1) and mean K_d value range for PE particles (Table 4); [c]value represents mean value ± standard deviation of 3 replicates

5.2 Transport and uptake of microplastics in the marine environment

Following the discharge of microplastics into the marine environment, their transport can be extensive based on the recovery of microplastics in areas of low anthropogenic activity (Gorman et al., 2019). However, the extent of this transport will be dependent on a number of physicochemical factors related to the microplastic and receiving environment that can influence whether a microplastic stays on the surface (epipelagic zone), settles into sediments or is cycled between the two (pelagic zone). These can include physical (such as wave action, tides, nearshore currents, microplastic size, water temperatures), chemical (polymer type, nutrient interactions, particulate matter, salinity) and biological (ingestion, colonisation) factors in the ocean (Akdogan and Guven, 2019; Reisser et al., 2013). Although higher concentrations of surface microplastics have been identified near large population centres in Australia, there is still potential for microplastics to be transported over longer distances (Reisser et al., 2013). Longer range transport has been observed in other studies that demonstrate the potential for widespread transport of microplastics in the marine environment (Akdogan and Guven, 2019; Sebille et al., 2019). This is especially the case for microplastics deriving from high production PP and PE polymers, which also have a lower density than seawater and are less likely to undergo vertical movement (Schwarz et al., 2019). Studies in various aquatic systems, however, suggest that high concentrations of naturally occurring particulate matter are effective in removing microplastics primarily through aggregation processes leading to sedimentation and accumulation within sediments (Besseling et al., 2017b; Schwarz et al., 2019). Particulate matter can therefore have a strong influence on the amount of microplastic remaining in the epipelagic zone, with aggregation or biofouling causing the majority of microplastics to sink below the epipelagic zone into either benthic or pelagic zones, especially near the point of release (Burns and Boxall, 2018; Erni-Cassola et al., 2019; Koelmans et al., 2017; Schwarz et al., 2019). Although large numbers of microplastics are released in WWTP discharges, there is likely to be a considerable degree of dilution once they enter the marine environment. Previous work has indicated that nearshore concentrations of microplastics are typically <1 particle/L, while concentrations in sediments can be much greater (Burns and Boxall, 2018; Erni-Cassola et al., 2019).

Organisms feeding on suspended particulates and sediments therefore have potentially greater exposure to microplastics, although there are comparatively few studies that have determined amounts of microplastics in organisms exposed in the environment relative to those exposed under laboratory conditions (Lusher, 2015). The uptake of microplastics is also species dependent, with feeding and respiration mechanisms and behaviour playing an important role in the extent of microplastics that are ingested. Microplastics may be inadvertently consumed during feeding where marine organisms are unable to distinguish them from food but also where microplastics are incorporated in food sources, leading to trophic transfer (Lusher, 2015). The size of microplastics has an important influence on whether they are ingested and the length of time prior to egestion. For example, plastics > 5 mm or in the nanoparticle range (< 1 μm) are likely to be retained within an organism for longer than particles in the microplastic (1 μm-2 mm) range (Lusher, 2015).

The uptake of microplastics into marine organisms can also depend on the niche within which a particular species inhabits a marine ecosystem. For example, a survey of fish showed that around two thirds of benthic fish species had microplastic particles in their gut compared with around one third of pelagic species, although only around 20% of all fish surveyed (of 263) had microplastics in their guts (Neves et al., 2015). Furthermore, the type of plastic present in the gut differed dependent on where the fish was collected, with benthic species having more fibres compared with more particles present in guts of pelagic fish (Neves et al., 2015). Marine organisms, including invertebrates, fish and mammals, also have the ability to excrete microplastics in their faeces following uptake, while invertebrates can incorporate microplastics in their pseudo-faeces, where microplastics are rejected prior to ingestion (Lusher, 2015). Overall, surveys of a range of marine organisms show corresponding particle loads in organisms to be reasonably low (typically <5 particles/organism), with the majority of surveyed organisms (i.e. >50%) typically not containing microplastics (Burns and Boxall, 2018; Jamieson et al., 2019; Lusher, 2015).

6 Hazards assessment of contaminants associated with microplastics

Along with characterising the potential exposure of marine organisms to microplastics and associated contaminants, the hazard associated with these contaminants is related to concentrations of contaminants that an organism will be exposed to. This is typically associated with unbound concentrations of a contaminant, or those which are released from the microplastic, as these are the most available for uptake into an organism (Besseling et al., 2017a; Koelmans et al., 2016). This is particularly the case with contaminants bound to microplastics, where there is less potential for polymers to be solubilised (Beckingham and Ghosh, 2017). Along with uptake into an organism, the potential for the contaminants to cause effects in marine organisms once they are taken up by an organism needs to be accounted for. From this relationship, the hazard of the contaminant is expressed as a hazard quotient (H):

$$H=\frac{EC}{PNEC} \qquad (4)$$

where *EC* is the environmental concentration of a contaminant and, while *PNEC* is the predicted no effect concentration and relates to a corresponding concentration where no effect of a contaminant is predicted to occur. A value of H >1 indicates it is likely an adverse effect will occur, while a value of H <1 indicates no adverse effects are likely.

6.1 Exposure concentrations and uptake of contaminants associated with microplastics

Generally, WWTP discharges would be substantially diluted soon after release into the marine environment, also substantially reducing exposure concentrations (Muhammetoglu et al., 2012; Stark et al., 2016; Vidal-Dorsch et al., 2012). If a marine organism occupied an ecological niche close to WWTP discharges it is possible that dilution of effluent, and therefore microplastics, could be minimal. Additionally, a worst-case scenario for determining *H* would be where contaminants associated with microplastics are completely released following ingestion of microplastics by a marine organism. Based on the desorption assessment, this may be feasible for a contaminant such as BAC and TCS but for other contaminants such as BPA this release may not be complete within a 24 h period. As well as contaminant-specific release from a microplastic, the residence time of the microplastic within the digestive tract of an organism, which can range from minutes to days, must also be considered (Bakir et al., 2014; Gouin et al., 2011; Koelmans, 2015).

When microplastics are ingested by organisms, contaminants associated with the microplastics can be released within the gut. Based on the extent of desorption of BAC and TCS in simulated gut fluid in this study, this could be the majority of the fraction sorbed to the microplastics (Figures 7 and 8). This is in accordance with other desorption studies, that indicate rapid and complete desorption of contaminants can occur under simulated gut conditions (Bakir et al., 2014; Coffin et al., 2019; Lee et al., 2019; Mohamed Nor and Koelmans, 2019). Exposure of organisms to microplastics loaded with contaminants have further demonstrated the ability of microplastics to transfer contaminants to an organism following ingestion (Browne et al., 2013; Gomiero et al., 2018; Khan et al., 2017; Rochman et al., 2013). However, it has been demonstrated that release of contaminants following ingestion of microplastics under environmentally realistic scenarios is likely to be highly dependent on concentration gradients of contaminants in the environment not associated with microplastics (Bakir et al., 2016; Gouin et al., 2011; Koelmans, 2015). For example, if the concentrations of contaminants are relatively greater in the organism or solution, then the contaminants are more likely to become associated with the microplastics, rather than desorbing, having the effect of cleaning the organism of the contaminants (Koelmans et al., 2016). This is especially the case for microplastics that have a greater affinity for contaminants, such as polyethylene, whereas microplastics with lower contaminant affinity, such as polystyrene, is unlikely to exhibit such an effect (Koelmans et al., 2013). With the majority of microplastics released into the environment being polyethylene or polypropylene, this cleaning effect is more likely to occur.

An exception to this cleaning effect may be found where contaminants are present at relatively high concentrations in the microplastics due to, for example, contamination or from their use in the manufacture of the plastic (Koelmans et al., 2016; Mohamed Nor and Koelmans, 2019). BPA is widely used as plasticiser or monomer in the manufacture of a range of plastics, while TCS is increasingly being used as an antimicrobial agent in the manufacture of plastics and fibres due to its stability (Dann and Hontela, 2011). TCS is incorporated into plastic packaging at concentrations of low g/kg (0.1-1% w/w) and BPA concentrations are potentially lower (Biles et al., 1997; Hirai et al., 2011; Koelmans et al., 2014; Teuten et al., 2009), while there is scarce data relating to BAC.

In terms of what this may represent for loads of TCS or BPA in microplastics from their manufacture, the median mass of microplastic particles has been estimated to be around 12 ng, although larger particles could potentially have a mass of up 4 μg (Simon et al., 2018). In the study by Simon et al. (2018), which quantified microplastics at comparably very high loads in wastewater, the mass of microplastics in 10 WWTPs was estimated to be up to 1200 μg/L in untreated wastewater and up to 12 μg/L in treated wastewater. If a 1 % w/w of contaminant in plastic is taken as a worst-case load of plastic additives, however, this would be equivalent to approximately 10 g contaminant/kg plastic or 12 μg contaminant/1200 μg plastic per litre of wastewater, for the untreated wastewater scenario. The present study and others (e.g. Biles et al., 1997; Koelmans et al., 2014; Staniszewska et al., 2016) suggests release of BPA into solution is potentially low, although estimating the release under biological conditions should also include rates of microplastic ingestion and egestion and the rate of release of a contaminant once ingested. This rate of release can be dependent on the physicochemical properties of the microplastic and contaminant, the amount of co-ingested food (and its physicochemical properties and contaminant load), the lipid content in an organism and the concentration of the contaminant in the organism (Koelmans et al., 2013).

A detailed analysis of microplastic ingestion and transfer of associated contaminants (including BPA) to an invertebrate (*Arenicola marina*) and fish (*Gadus morhua*), conservatively used high concentrations of microplastics (a few μg to 100s of mg ingested/day) and contaminants (0.1 mg/kg for BPA) and found comparatively negligible uptake of contaminants from microplastics (Koelmans et al., 2014). The concentration estimated for BPA in the study by Koelmans et al. (2014) was in the range estimated for a high loading of microplastics in wastewater as summarised in Table 5, where concentrations of BPA in microplastics were between 25 and 3300 μg/kg. This analysis by Koelmans et al. (2014) took into account ingestion of microplastics relative to food intake, excretion rates of ingested microplastics and partitioning of contaminants between microplastics and body lipids but not uptake from solution. A further assessment of the importance of uptake for BPA concentrations of up to 330 ng/L, however, estimated that uptake of BPA from microplastics would be around three orders of magnitude less than that from uptake from solution (Koelmans et al., 2014). This is comparable with undiluted wastewater concentrations of BPA measured in this study, of 196-1,111 ng/L (Table 5). Futhermore, the study by Koelmans et al. (2014) also included a more lipophilic contaminant, nonylphenol, which has a log K_{OW} (4.5) comparable with TCS. Once again, the uptake of nonylphenol from microplastics was estimated to be orders of magnitude less than uptake from solution.

The concentrations of these three contaminants measured in WWTP influent were in the high ng/L to low μg/L concentrations (Table 5). Previous monitoring studies from various other countries measured concentrations of BPA (Corrales et al., 2015; Staples et al., 2018; Tato et al., 2018) and TCS (Bayen et al., 2013; Tato et al., 2018) at ng/L concentrations. There is considerably less information relating to BAC in the marine environment, although concentrations in wastewater effluents have also been previously measured at ng/L concentrations (Östman et al., 2017; Zhu et al., 2018). The physicochemical properties of BAC mean it has a strong affinity for particulates in the environment (Zhang et al., 2015) and very high concentrations (mg/kg) have been measured in estuarine sediments (Li and Brownawell, 2009). This would indicate concentrations of BAC in WWTP effluents would be considerably reduced once discharged into marine systems through both dilution and sorption to sediments.

6.2 Effects of contaminants and microplastics in marine organisms

One method for deriving PNEC to calculate *H* from laboratory exposures for a number of species is to use a species sensitivity distribution (SSD) and PNEC corresponds with the concentration where 5% of species (HC5) have an observed adverse effect (Wheeler et al., 2002). The concentrations of expected effects of BAC, BPA and TCS in a range of marine organisms are summarised in Table 1. An HC5 value needs to be derived from a SSD curve, where the concentration that causes an effect in 5 % of species is used as a protective value (Wheeler et al., 2002). A study by Duran and Beiras (2017), derived water quality criteria, including an HC5 value, for diverse marine organisms with HC5 values at μg/L concentrations for both TCS (11.8 μg/L) and BPA (37.8 μg/L). This value for BPA is comparable with other studies that derived either EC10 (where effects occur in 10% of organisms) for other marine organisms (Tato et al., 2018) or HC5 values for freshwater species (Staples et al., 2008) (Table 1). Although there are comparatively few ecotoxicity studies for BAC, it is a broad spectrum antimicrobial chemical like TCS and available EC10 values from literature for BAC are in the μg/L range and comparable with TCS (Table 1). From Table 1, using the highest EC and lowest PNEC (from HC5 or EC10) values, the worst-case values of H for BPA (0.03), BAC (0.03) and TCS (0.02) are all considerably less than 1.

Based on the relative importance of uptake of contaminants from microplastics being orders of magnitude less than that of solution due to higher concentrations in solution, these worst-case H values would suggest that the potential for toxicity from the contaminants released from microplastics under conservative scenarios would also be negligible.

Studies where contaminants and microplastics were exposed separately in toxicity exposures indicate microplastics may both potentiate (Syberg et al., 2017) or reduce the effects of the sorbed contaminants (Magara et al., 2018; Rehse et al., 2018; Zhu et al., 2019). The exposure scenarios in these toxicity studies, however, had unrealistically high concentrations of microplastics and contaminants and realistic exposure scenarios are desirable to determine whether microplastics may enhance the effect of contaminants. Microplastics have also been demonstrated to cause a toxic response due to physical impacts or biological responses. These effects may be due to blocking access to, and digestion of, normal sources of nutrition, damage to gill surfaces and alimentary tracts, which may lead to variations in molecular signalling through to effects on growth and fecundity (Burns and Boxall, 2018; Rochman et al., 2014). These observed effects are often dependent on the exposed organism, as well as the size and type of microplastic used for the exposure. As with the studies comparing the effect of the microplastics and contaminants separately, direct microplastic exposures have been laboratory-based at very high loads of microplastics, relative to what is typically found in the environment, to elicit an adverse response. Leachates of microplastics have also been demonstrated to cause toxicity, although the leachates that caused effects were derived from concentrations of microplastics in the g/L, which is highly unrealistic in marine environments (Hermabessiere et al., 2017). This highlights the need for realistic ecotoxicological exposures for hazard assessments of contaminants associated with microplastics. Based on current evidence, however, microplastics are unlikely to elicit significant biological impacts in marine organisms based on their present loadings in marine environments (Burns and Boxall, 2018).

7 Conclusions

The sorption of the three wastewater contaminants, BAC, BPA and TCS, to the PE and PET fibres and PE beads and fragments was moderate to strong in wastewater. The corresponding desorption of all contaminants into seawater was minimal, while the presence of a biomimetic surfactant enhanced the desorption of the contaminants, especially BAC and TCS. The wastewater contaminants having a high degree of association with microplastics is also supported in a number of previous studies, where various contaminants have been demonstrated to associate with a range of polymers. The extent of this association is generally related to the lipophilicity of the contaminant, although ionisation of a contaminant's functional group(s) can also affect the degree of association. In terms of desorption, the variable extent of desorption seen in this study was also evident in other previous studies. The variability of desorption can be related to the properties of the contaminant and the polymer type of the microplastic and the environment in which the microplastics and contaminants are present. For example, desorption of contaminants from microplastics in seawater solution is typically minimal, especially for more hydrophobic contaminants, but mimicking physiological conditions can enhance the extent of desorption. This can be in the form of increased temperatures and acidity or the presence of chemicals that are present in the digestive tracts of marine organisms.

The findings from this study suggest microplastics can transport contaminants when wastewater discharges enter the marine environment and expose marine organisms to contaminants associated with microplastics when they are ingested. There are several factors, however, that need to be considered when determining what the potential hazards to marine organisms of these microplastic-associated contaminants may be. The hazard of a contaminant is related to its predicted or measured concentration in the environment compared with the concentration where an effect on the organism is expected. The concentration of the contaminant, and therefore the hazard, can be modified through its association with microplastics, in terms of (a) the quantity of contaminants associated with microplastics; and (b) the ability of the contaminant to desorb from the microplastic once ingested.

With respect to (a), the amount of contaminants that associate with microplastics on a weight (w/w) basis is quite high, due to a high degree of association of contaminants with the microplastic based on sorption assessments, as well as extractions of microplastics collected from marine environments. Microplastics have an extremely low mass for their size, however, and the quantity of contaminants associated with microplastics by mass is correspondingly small. This means that even in a worst-case scenario where the total amount of contaminant associated with a microplastic is released when ingested by an organism, this is likely to represent only a fraction of a contaminant present in wastewater discharges or in contaminated sediments. Despite the large numbers of microplastics reported to be released in wastewaters, the relative proportion of this compared with organic matter present in wastewaters is low. Furthermore, measured concentrations of contaminants like BAC, BPA and TCS in WWTP discharges are typically in ng/L concentrations. This is in comparison with concentrations in the μg/L range where effects have been reported, or predicted to occur, which are generally protective of a high number of species (e.g. corresponding to HC5). Even with the contribution of contaminants associated with microplastics, toxicity to organisms from BAC, BPA and TCS is likely to be low in the marine environment, based on their measured concentrations in wastewater. Mitigation of toxicity will be further enhanced when dilution of contaminant loads occurs through mixing of WWTP effluents once discharged.

With respect to (b), both this study and previous assessments of desorption of contaminants from microplastics show that this is variable but usually not a complete process. This variability is associated with a range of factors, related to the polymer, the organism and the environment, that can enhance or reduce the amount and rate of desorption of contaminants. Enhanced desorption can occur under physiological conditions although, unless this desorption is complete, association with the microplastic must be considered as a means of mitigating exposure of contaminants to an organism.

Characterising the risk of contaminants associated with microplastics needs to also consider the likelihood of exposure of the organism to the microplastic. Although this was not done here, several factors were considered that can reduce the exposure. For example, once released into the marine environment, microplastics can distribute both vertically and horizontally depending on the density of the polymer, weathering of microplastics, their degree of association with other suspended materials or colonisation with biofilms and the extent of water and air circulation through wind, tide, currents and salinity or temperature gradients. All of these factors have the potential to distribute contaminants associated with microplastics widely throughout the epipelagic, pelagic and benthic zones of the marine environment, particularly where desorption into seawater is likely to be unfavourable. This has the potential to expose a wide range of marine organisms inhabiting these zones but it also has the effect of dispersing (and diluting) the microplastics. Surveys of marine organisms have revealed that the majority (>50 %) do not contain microplastics and, where they do, loads of microplastics (and associated contaminants) are also low (<5/organism). Based on this available evidence, exposure to contaminants associated with microplastics is therefore likely to be low.

Despite the potential low hazard or risk of contaminants associated with microplastics, the presence of microplastics in the marine environment is highly undesirable due to other potential adverse outcomes, including accumulation within certain environments (such as benthic zones) or organisms. This is likely to occur to a greater extent in future as global production and use of plastics, which are sources of microplastics in wastewater, continues to increase. Based on current evidence relating to the degree of association of contaminants with microplastics and relative loads of microplastic particulate matter, however, it is unlikely that microplastic transport of contaminants will constitute a hazard for marine organisms. WWTP discharges are likely to remain a major pathway for microplastics entering the marine environment, although WWTPs have been shown to remove a considerable proportion of microplastics (>90%) from wastewater into sludges produced during wastewater treatment. This removal effect is more pronounced at higher treatment levels. Depending on the final use of these sludges this has the potential to transfer microplastics from aquatic systems to terrestrial systems (Gatidou et al., 2019; Li et al., 2018; Mahon et al., 2017). For example, beneficial (and desirable) reuse of these sludges in agriculture is undertaken in a number of countries, including Australia. Microplastics have been measured in reused sludges (or biosolids) at quantities of up to 171,000/kg (Gatidou et al., 2019) and with thousands of tonnes of sludges being produced each year, this represents a substantial amount of microplastics being transferred to the terrestrial environment. It has been previously demonstrated, however,, this quantity may not necessarily translate into adverse outcomes for terrestrial organisms, especially where there is a considerably higher degree of organic matter, for example, that reduce the potential hazard and exposure of contaminants (Huerta Lwanga et al., 2016; Judy et al., 2019). A characterisation of hazards of microplastics (and associated contaminants) in terrestrial environments would therefore be desirable to ensure that beneficial reuse of wastewater sludges has greater benefits compared with terrestrial risks.

Appendix A Analysis of wastewater contaminants

Apx Table A.1 Concentrations of organic contaminants measured in wastewater treatment plant (WWTP) effluent at WWTP1 and WWTP2.

CONTAMINANT	WWTP 1	WWTP 2
Pharmaceuticals	**(ng/L)**	**(ng/L)**
Amitriptyline	<5	<5
Carbamazepine	23±1	12±1
Diclofenac	<5	<5
Ketoprofen	<5	<5
Trimethoprim	26±1	14±1
Venlafaxine	259±37	45±12
Personal Care Products		
Benzalkonium chloride (BAC)	998±228	338±38
Benzotriazole	1209±64	340±203
Bisphenol A (BPA)	196±34	1111±201
Caffeine	55±3.9	3812±244
DEET	33±13	3303±609
Methyl benztriazole	256±11	199±12
Methyl paraben	2446±990	1715±236
Propyl paraben	<50	<50
Triclosan (TCS)	221±19	164±18
Phthalates		
Bis(2-ethylhexyl) phthalate	<20,000	<20,000
Butyl benzyl phthalate	<10,000	<10,000
Diethyl phthalate	<10,000	29,000±1,410
Dimethyl phthalate	<10,000	28,000±4,240
Di-n-butyl phthalate (DBP)	11,000	41,500±26,163
Di-n-octyl phthalate	<10,000	<10,000
Phenols		
2-Chlorophenol	<10,000	<10,000
4-Chloro-3-methylphenol	<10,000	<10,000
2,4-Dichlorophenol	<10,000	<10,000
2,4-Dimethylphenol	<10,000	<10,000
2,6-Dichlorophenol	<10,000	<10,000
2-Methylphenol	<10,000	<10,000

CONTAMINANT	WWTP 1	WWTP 2
Phenols cont.	**(ng/L)**	**(ng/L)**
3&4-Methylphenol	<20,000	205,000±107,000
Phenol	<10,000	79,500±43,130
Pesticides	<20,000	<20,000
Aldrin	<20,000	<20,000
Atrazine	<5	<5
a-BHC	<20,000	<20,000
b-BHC	<20,000	<20,000
d-BHC	<20,000	<20,000
Carbaryl	<20,000	<20,000
Chlorpyrifos	<20,000	<20,000
2,4-D	<20	<20
4,4,'-DDD	<20,000	<20,000
4,4,'-DDE	<20,000	<20,000
4,4,'-DDT	<20,000	<20,000
Diazinon	<5	<5
Dieldrin	<20,000	<20,000
Difenconazole	<5	<5
Dimethoate	<20,000	<20,000
Diuron	<5	<5
Endosulfan sulphate	<20,000	<20,000
Endosulphan I	<20,000	<20,000
Endosulphan II	<20,000	<20,000
Endrin	<20,000	<20,000
Endrin Aldehyde	<20,000	<20,000
Fenitrothion	<20,000	<20,000
Heptachlor	<20,000	<20,000
Heptachlorepoxide	<20,000	<20,000
Imidacloprid	<5	<5
Indoxacarb	<5	<5
Lindane	<20,000	<20,000
Malathion	<20,000	<20,000
MCPA	<20	113
Metalaxyl	<5	<5
Pirimicarb	<5	<5
Prochloraz	<5	<5

CONTAMINANT	WWTP 1	WWTP 2
Pesticides cont.	**(ng/L)**	**(ng/L)**
Pyraclostrobin	<5	<5
Pyrimethanil	<5	<5
Simazine	<5	<5
Trifloxystrobin	<5	<5
PAHs		
Acenaphthene	<10,000	<10,000
Acenaphthylene	<20,000	<20,000
Anthracene	<10,000	<10,000
Benz(a)anthracene	<10,000	<10,000
Benzo(a)pyrene	<10,000	<10,000
Benzo(b,k)fluoranthene	<20,000	<20,000
Benzo(g,h,i)perylene	<10,000	<10,000
Chrysene	<10,000	<10,000
Dibenz(a,h)anthracene	<10,000	<10,000
Fluoranthene	<10,000	<10,000
Fluorene	<10,000	<10,000
Indeno(1,2,3-cd)pyrene	<10,000	<10,000
2-Methylnaphthalene	<10,000	<10,000
Naphthalene	<20,000	<20,000
Phenanthrene	<10,000	<10,000
Pyrene	<10,000	<10,000
Chlorinated hydrocarbons		
2-Chloronaphthalene	<20,000	<20,000
1,2-Dichlorobenzene	<20,000	<20,000
1,3-Dichlorobenzene	<20,000	<20,000
1,4-Dichlorobenzene	<20,000	<20,000
Hexachloro-1,3-butadiene	<20,000	<20,000
Hexachlorobenzene	<20,000	<20,000
Hexachlorocyclopentadiene	<20,000	<20,000
Hexachloroethane	<20,000	<20,000
1,2,4-Trichlorobenzene	<20,000	<20,000
Ethers		
4-Bromophenyl phenyl ether	<20,000	<20,000
4-Chlorophenyl phenyl ether	<20,000	<20,000
Bis(2-chloroethyl)ether	<20,000	<20,000

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