MULTICOMPARTMENT AND CROSS-SPECIES MONITORING OF CONTAMINANTS OF EMERGING CONCERN IN AN ESTUARINE HABITAT

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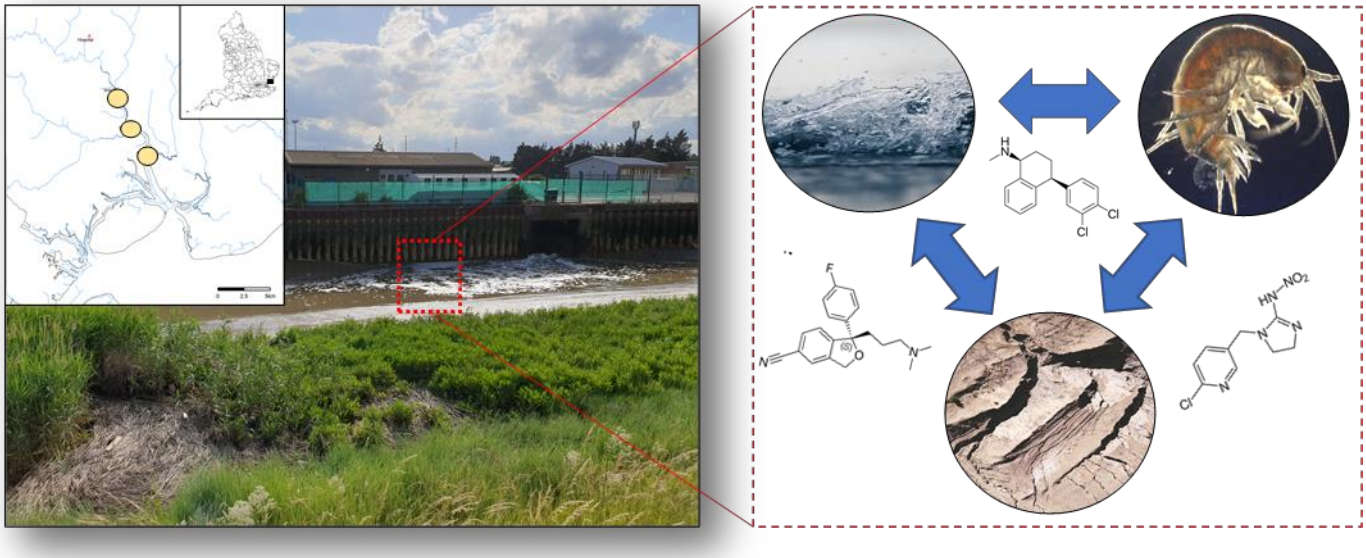
GRAPHICAL ABSTRACT



Abstract

The fate of many chemicals in the environment, particularly contaminants of emerging concern (CEC), have been characterised to a limited extent with a major focus on occurrence in water. This study presents the characterisation, distribution and fate of multiple chemicals including pharmaceuticals, recreational drugs and pesticides in surface water, sediment and fauna representing different food web endpoints in a typical UK estuary (River Colne, Essex, UK). A comparison of contaminant occurrence across different benthic macroinvertebrates was made at three sites and included two amphipods (*Gammarus pulex* & *Crangon crangon*), a polychaete worm (*Hediste diversicolor*) and a gastropod (*Peringia ulvae*). Overall, multiple contaminants were determined in all compartments and ranged from; <LOQ – 386 $ng \cdot L^{-1}$ in surface water (n=59 compounds), <LOQ – 146 $ng \cdot g^{-1}$ in sediment (n=39 compounds) and <LOQ – 91 $ng \cdot g^{-1}$ biota (n=33 compounds). *H. diversicolor* and *P. ulvae* (sediment dwellers) showed greater chemical body burden compared with the two swimming amphipod species sampled (up to 2.5 - 4-fold). The most frequently determined compounds in biota (100%, n=36 samples) included; cocaine, benzyoylecgonine, carbamazepine, sertraline and diuron. Whilst some of the highest concentrations found were in species *H. diverscolor* and *P. ulvae* for psychoactive pharmaceuticals including citalopram (91 $ng \cdot g^{-1}$), sertraline (69 $ng \cdot g^{-1}$), haloperidol (66 $ng \cdot g^{-1}$) and the neonicotinoid, imidacloprid (33 $ng \cdot g^{-1}$) Sediment was noted as an important exposure route for these benthic dwelling organisms and will be critical to monitor in future studies. Overall, the analysis of multiple species and compartments demonstrates the importance of including a range of exposure pathways in order to appropriately assess chemical fates and associated risks in the aquatic environment.

Keywords: Occurrence, Pesticides, Pharmaceuticals**,** Environmental Risk, Sediment, Invertebrate

Capsule: Multi-class contaminants of emerging concern were determined across surface water, sediment and macroinvertebrate species demonstrating widespread exposure in the aquatic environment.

1. Introduction

Anthropogenic activity is increasing pressure on both environmental and public health. One of these pressures is related to chemical contamination which has already led to some significant impacts in the environment (Desforges et al., 2018; Oaks et al., 2004). Many different classes of chemicals have now been found to occur in the environment that vary in terms of their persistence, bioaccumulation and toxicity. Two well-known classes of CEC that have been reported in the environment are pharmaceuticals and pesticides. Many studies have examined these two chemical classes for potential exposure and hazard in the environment, but there remain several knowledge gaps for reliable understanding of their potential risks (Naidu et al., 2016). Another class of chemicals often present in wastewater (that overlap with pharmaceuticals) are recreational drugs, but these have not had nearly as much focus as environmental contaminants by comparison, likely due to the obvious difficulties for regulation. Our previous study (Miller et al., 2019b) showed that several of these compounds were frequently found in surface water and biota which included cocaine, ketamine and 3,4-methylenedioxymethamphetamine (MDMA). These compounds overlap with sewage epidemiology studies that often report their occurrence in wastewater to link to public consumption (González‐Mariño et al., 2020).

To understand risk and enable mitigation, it is critical to more fully understand exposure to different chemicals in the environment. This can be defined as the 'exposome,' a recent term that is extended from human toxicology into ecotoxicology and represents all potential exposures an organism will experience over its lifecycle (Escher et al., 2017). Surveillance of these chemicals exposures that make up the exposome can be used to prioritise chemicals of concern and/or regional areas of concern further directing mitigation and management strategies. However, within the aquatic environment, few studies have focussed on compartments beyond surface waters and fewer have determined chemicals across multiple compartments (Álvarez-Muñoz et al., 2015; Álvarez-Ruiz et al., 2015; Wang et al., 2014; Wilkinson et al., 2018), which is critical to gain a more holistic understanding of contaminant distribution and fate in the environment. Biomonitoring and measurement of internal concentrations have been recognised for many years as an important approach to understand chemical risk for biota (Connell, 2001; Connell et al., 1999; Sijm and Hermens, 2005). However, studies focussed on determination of internalised concentrations of chemicals have generally been limited (Escher et al., 2011; Miller et al., 2018). As a consequence, there have been large disparities reported in thresholds for exposure and hazard, which are based on extrapolated concentrations from exposure media. The bioavailability of chemical contaminants present in surface water and sediment will govern the uptake and accumulation by different organisms. By determining the internalised concentrations in biota, a better representative dose metric can link cause to effect and understand potential risk in the environment (Huerta et al., 2016; Margiotta-Casaluci et al., 2016; Munz et al., 2018). Thus, internal concentrations will be critical to refining current exposure thresholds that lead to toxicity.

The aim of this study was to characterise and better understand the exposure of CEC in an estuarine habitat through monitoring of multiple compartments. Pharmaceuticals, pesticides and recreational drugs were measured across multiple environmental compartments (sediment, water, biota) and multiple macroinvertebrate species at three sites along the estuarine River Colne (Essex, UK) downstream from the town of Colchester and its wastewater treatment works (e.g. Hythe). A previously validated analytical method for *Gammarus pulex* was applied to determine concentrations present across these different matrices and the selected species which included *G. pulex* (amphipod), *Peringia ulvae* (mollusc), *Hediste diversicolor* (polychaete, alternatively *Nereis diversicolor*) and *Crangon crangon* (amphipod). The characterisation of the chemical burden in multiple compartments and species will enable better understanding of chemical fate and subsequent risk to the aquatic environment.

2. Materials and Methods

2.1 Reagents, chemicals and consumables

High performance liquid chromatography (HPLC) grade methanol, acetonitrile, and Liquid chromatography-mass spectrometry (LC-MS) grade (Optima™) ammonium acetate were purchased from Fisher Scientific (Loughborough, UK). A total of 141 compounds were targeted in this study. All analytical standards were of a purity of ≥ 97%. Ultra-pure water was obtained from a Millipore Milli-Q water purification system with a specific resistance of 18.2 MΩ·cm or greater (Millipore, Bedford, MA, USA). Stock solutions (1 mg·mL^{-1}) were prepared in methanol (MeOH) or acetonitrile (MeCN) and stored in silanised amber vials (20 mL). Working solutions were prepared daily in ultra-pure water, as required. All solutions were stored at -20 °C and in the dark to reduce possible degradation.

127

128 *2.2 Sample collection*

129 Samples were collected in August 2019. Three locations were selected along the River
130 Colne estuary (Essex, UK) which included Hythe, Wivenhoe and Alresford (Fig. 1).
131 The first site Hythe was located downstream of a nearby wastewater treatment plant
132 (WWTP) discharge point. All sites, Hythe, Wivenhoe (3.5 km) and Alresford (5.5 km),
133 are tidally influenced as part of the Colne Estuary. All samples were taken on an ebb
134 tide. Surface water samples (40 mL) were collected in Nalgene bottles in triplicate at
135 each sampling site and transported in back to the lab in a cool box before subsequent
136 storage at -20ºC.

137 Four species were selected for sampling including the sediment dwelling gastropod
138 snail, *P. ulvae*, the sediment dwelling polychaete worm *H. diversicolor*, and two
139 amphipods, *G. pulex*, and *C. crangon*. The amphipod *G. pulex* replaced *C. crangon* at
140 the Hythe site due to lower salinity at this site. Amphipod species were collected by
141 five kick samples into a 250 µm net per site. These were then sorted and transported
142 back to the lab. Sediment dwelling species (*H. diversicolor* and *P. ulvae)* were
143 collected from 20 cm mud cores in replicates of 4 per site. Animals were extracted
144 through a 1 mm sieve and combined into species and sample specific replicate vials.
145 Sediment/biota separation was completed within 3 hours of sample collection to
146 alleviate potential compound excretion from biota. All combined biomass
147 macroinvertebrate replicates were stored at -20°C prior to analysis. The remaining
148 core sediment was separated into replicate specific vials (4 per site) and stored at
149 20°C prior to analysis.

150

151 *2.3 Sample preparation*

152 The analytical workflow used for determination of compounds in animal, water and
153 sediment samples followed a previous validated method for *G. pulex* (Miller et al.,
154 2019b). Macroinvertebrate samples were lyophilised at -50 °C under vacuum for 24 h.
155 Pooled samples of organisms were placed into 2 mL Eppendorf tubes with a 3 mm
156 diameter tungsten carbide bead and subsequently ground into a fine powder using a
157 TissueLyser LT (Qiagen, Hilden, Germany) set at 50 Hz for 5 min. Freeze-dried
158 composite samples homogenised material (20 mg) was transferred to a new 2 mL
159 Eppendorf with any necessary spiking carried out directly onto the solid matrix using
160 a 100 µL volume of an appropriate working solution for matrix matched calibration
161 curves.
162 A 2 mL volume of 3:1 (MeCN:H_2O) acidified with 0.1% (v/v) glacial acetic acid was
163 added to the material and agitated for 5 min at 50 Hz in the Tissuelyser LT (Qiagen,
164 Hilden, Germany). The samples were then placed in an ultrasonic bath for 15 min
165 followed by centrifugation for 5 min at 14,000 rpm to pellet insoluble particulate matter.
166 Following extraction and settling, an aliquot of the supernatant (1.9 mL) was diluted to
167 100 mL with 10 mM ammonium acetate in ultra-pure water (pH 6.5). Tandem solid
168 phase extraction (SPE) was then carried out on the diluted sample using a Strata
169 Alumina-N cartridge (6 mL, 1 g, Phenomenex Ltd., Cheshire, UK) coupled to an Oasis
170 HLB cartridge (6 mL, 200 mg, Waters Corp., Hertfordshire, UK). Before loading of the
171 sample, the combined SPE cartridges were first conditioned with 6 mL of methanol
172 and 6 mL of ultra-pure water with 10 mM ammonium acetate. After sample loading,
173 both cartridges were then washed with 1 mL ultra-pure water and dried for ~30 min
174 under vacuum. The alumina cartridge was then discarded and the HLB cartridges were
175 stored at -20 °C until analysis. Cartridges were eluted with 5 mL MeOH (2 x 2.5 mL
176 volumes) and dried under pure nitrogen (99.9%, 1.0 bar) at 35 °C using a TurboVap
177 LV (Biotage, Uppsala, Sweden).

178 For sediment, samples were lyophilised as above and then 50 mg of material was
179 weighed and placed in a 2 mL Eppendorf tube with a tungsten carbide bead (3 mm).
180 The extraction and clean-up method were the same as described above but without
181 the use of Strata Alumina-N cartridges.
182 For surface water, samples (20 mL) were filtered through a 0.45 µm glass-fibre filter
183 and were subsequently cleaned-up by the same SPE method described above without
184 the use of the Strata Alumina-N cartridges.
185 All extracts (animal, sediment and water) were reconstituted in 0.1 mL 90:10 (v/v) 10
186 mM ammonium acetate in H_2O:MeCN transferred to 200 µL silanised glass inserts,
187 held within a 2 mL amber autosampler vial. After reconstitution, samples were
188 immediately analysed using liquid chromatography tandem mass spectrometry (LC-
189 MS/MS) method described below.
190
191 *2.4 Instrumental analysis and conditions*
192 Analytical separations were performed on a Nexera X2 LC system (Shimadzu, Kyoto,
193 Japan) using a Raptor^{TM} biphenyl column (100 x 2.1 mm, 2.7 µm particle size)
194 (Thames Restek, Saunderton, UK) and a Raptor^{TM} biphenyl guard column (5.0 x 2.1
195 mm, 2.7 mm particle size) (Thames Restek, Saunderton, UK), which was housed
196 within an EXP® Direct Connect Holder (Thames Restek, Saunderton, UK). An injection
197 volume of 20 µL with 0.3 mL min^{-1} flow rate was used. Mobile phases were 90:10 (v/v)
198 10 mM ammonium acetate in H_2O:MeCN (A) and 20:80 (v/v) 10 mM ammonium
199 acetate in H_2O:MeCN (B). The gradient elution profile followed a linear ramp of mobile
200 phase B which increased to 10 % at 1 min, 35 % at 5.6 min, 40 % at 7 min, 50 % at 8
201 min and 100 % at 11 min and was held for a further 11 min before returning to initial
202 conditions. Re-equilibration time was 3 min resulting in an overall run time of 25 min.
203 Detection and quantification were performed using an 8060 triple quadrupole mass

spectrometer with electrospray ionisation (ESI) interface (Shimadzu, Kyoto, UK). Pureshield argon was used as the collision-induced dissociation gas (BOC Gases, Guildford, UK). Nitrogen and dry air were generated using Genius 1051 gas generator (Peak Scientific, Inchinnan, UK). Mass spectrometry (MS) was performed in multiple reaction monitoring (MRM) mode using positive–negative ionisation polarity switching. MRM optimisation of each precursor was performed using the LabSolutions optimisation for method (version 5.93, Shimadzu, Kyoto, Japan), where individual solutions for each analyte in methanol at 1.0 $\mu g \cdot mL^{-1}$ was injected (10 µL) at 0.5 $mL \cdot min^{-1}$ to an isocratic profile; 30% mobile phase A and 70 % mobile phase B. One MRM event was acquired for subsequent quantification with a second transition for identification, where possible. Chromatographic data was acquired by LabSolutions (version 5.93, Shimadzu, Kyoto, Japan) and processed using LabSolutions Insight (version 3.2, Shimadzu, Kyoto, Japan). See the SI for full details of analytical conditions (Table S1 & 2).

Whilst the analytical method was validated for *G. pulex*, it was applied to the other matrices where in-depth method performance assessment data is not available. Nevertheless, quantification was performed using matrix-matched calibration for each matrix type. For animal samples, a total of four calibration curves were prepared at 0.5, 1, 5, 10, 25 and 50 $ng \cdot g^{-1}$ for each individual species to be analysed (the same species were pooled across sites for the calibration). Sediment calibration curves were prepared for each site at 0.5, 1, 5, 20, 50 and 100 $ng \cdot g^{-1}$ leading to a total of three separate calibration curves. Additionally, compounds quantified in sediment samples were assessed for method repeatability at two concentrations of 20 $ng \cdot g^{-1}$ and 100 $ng \cdot g^{-1}$ (see Table S3). The surface water calibration curve was prepared by pooling individual sites (20.0 mL per site) into a composite matrix and spiking at 5, 20, and 80

229 ng·L^{-1}. Pre-extraction spikes were added using 100 µL of an appropriate working
230 solution containing the full mixture of analytes (stored in MeCN). Neat samples (i.e.
231 containing no spikes) were run in triplicate to background correct when performing
232 quantifications. Calibration curves, where necessary, were normalised against stable
233 isotopically labelled internal standards (SIL-IS) that were spiked at a constant
234 concentration (50 ng·g^{-1} for solids samples, 100 ng·L^{-1} for surface water) between
235 calibration points and in the environmental samples (see SI for details). Quantifications
236 were only performed where linearity was acceptable (R^2 ≥0.98). Analytes were
237 reported below the limit of detection or quantification when the corresponding peak
238 was below a signal-to-noise threshold of 3:1 to 10:1, respectively.
239
240 **3. Results and Discussion**
241
242 *Contamination across different macroinvertebrate species*
243 Four species of macroinvertebrate representing three potential food web routes were
244 sampled in this study including amphipods (e.g. *G. pulex* or *C. crangon*), a gastropod
245 (*P. ulvae*) and a polychaete (*H. diversicolor*). The macroinvertebrate species sampled
246 occupy different ecological niches. In order to understand the implication of CEC, it is
247 important to understand whether exposure in these organisms vary and what role they
248 have in estuarine food-webs. For example *P.ulvae* is a sediment dwelling biofilm
249 grazer and is a large dietary component of estuarine birds (Burton, 1974; Patterson,
250 1982). *H.diversicolor* is a predatory polychaete and is a large dietary component of
251 estuarine birds and fish (Burton, 1974; Green et al., 2009). A comparison between
252 average total body burden in each species for the three sites showed that *H.*
253 *diversicolor* had higher average concentrations of contaminants measured at both
254 Hythe (8.0 ng·g^{-1}) and Wivenhoe (4.2 ng·g^{-1}). In contrast, *P. ulvae* showed a higher

255 average body burden of 4.1 ng·g^{-1} at Alresford but contaminants were more evenly
256 distributed when compared to *H. diversicolor* (3.1 ng·g^{-1}) and *C. crangon* (3.0 ng·g^{-1}).
257 The reduction in average body burden for these species at Wivenhoe and Alresford
258 as mentioned above is due to the sites being located further downstream from the
259 discharge point of the WWTP. Overall, the data shows that *H. diversicolor* had higher
260 accumulation of the targeted chemical contaminants. Studies in the lab have shown
261 that species life stage and traits can affect uptake and elimination processes (Rubach
262 et al., 2010a, 2010b). This species burrows into sediments, is a generalist including
263 predatory behaviours and might represent an important exposure route as this
264 compartment was also shown to have highest average burden across all
265 compartments measured in this study.

266 Amphipods have been a commonly used organism for biomonitoring studies
267 with many authors using gammarids to determine concentrations of CEC (Miller *et al.*,
268 2015; Inostroza *et al.*, 2016; Sordet *et al.*, 2016; Munz *et al.*, 2018; Miller, *et al.*, 2019).
269 These are generally seen as an ecologically important species for their role in nutrient
270 cycling. However, in this study this *Gammarus* showed the lowest body burden at
271 Hythe and was also the case for *C. crangon* at Wivenhoe and Alresford. The lower
272 concentrations determined in the amphipods suggest that these organisms might be
273 a more conservative bioindicator to assess exposure in the environment. However,
274 few studies have looked at chemical contamination across different macroinvertebrate
275 species in the field. An investigation into occurrence of estrogenic compounds in Taihu
276 Lake, China compared concentrations of E1, E2, E3, EE2 and BPA in a fish, clam and
277 snail species (Wang et al., 2014). Concentrations detected in the species were
278 dependent on the site, but high concentrations determined in the sediment led to
279 largest concentrations observed in the snails reaching up to ~1 mg·kg^{-1}. This reiterates
280 the observation here, that sediment can be an important exposure route for benthic

dwelling organisms and is not often investigated in occurrence studies. In marine bivalves collected from the Ebro Delta in Spain, the oyster *Crassostrea gigas* was shown to have higher measured concentrations of several pharmaceuticals when compared to two mussel species including *Mytilus* spp., and *Chamalea gallina* (Álvarez-Muñoz et al., 2015). A third study (Wilkinson et al., 2018) that investigated multiple contaminant classes in two benthic invertebrates (*G. pulex* and *Bithynia tentaculata*) showed similar measured concentrations for pharmaceuticals and recreational drugs but varied more widely for plasticisers and perfluorinated compounds, with *B. tentaculata* accumulating up ~10-fold more than *G. pulex* . This previous study also estimated sediment bioaccumulation factors (BSAF) which indicated that sediment was a more significant exposure route than surface water.

Pharmaceutical Exposure

Pharmaceuticals detected at high concentrations included haloperidol, sertraline and imidacloprid. Haloperidol was detected at higher concentrations in *H. diversicolor* at both Hythe and Wivenhoe with mean concentrations of 35.5 $ng \cdot g^{-1}$ and 23.3 $ng \cdot g^{-1}$, respectively. However, haloperidol and sertraline were again determined at lower concentrations in *G. pulex* than when compared with *P. ulvae* and *H. diversicolor*. Haloperidol was determined in *G. pulex* in our previous study (Miller, *et al.*, 2019) but was only measured once at 5.3 $ng \cdot g^{-1}$ in a comparatively rural catchment. *G. pulex* showed low measured concentrations of haloperidol in the present study and was also low in *C. crangon*. This compound was determined to have a high likelihood of an effect occurring in the environment due to the low human therapeutic plasma concentration to elicit pharmacological effect (1 $ng \cdot mL^{-1}$) (Fick et al., 2010). However,

this compound has not been previously reported in the literature and further investigation into potential effects would be necessary.

Over half of the pharmaceuticals detected in biota samples were psychoactive drugs including carbamazepine, amitriptyline, memantine, diazepam, citalopram, nordiazepam, venlafaxine, clozapine, temazepam, sertraline, haloperidol and risperidone. These types of contaminants have recently been gaining attention for their potential for sub-lethal effects on behaviour which current risk assessments do not account for (Bláha et al., 2019; Brodin et al., 2013; Huerta et al., 2016). These compounds often have low therapeutic doses and typically designed to be more hydrophobic to permeate the blood brain barrier (Iyer et al., 2002; Tanoue et al., 2019). Thus, accumulation and the potential for effects might be increased for these compounds in the environment. Several authors have shown various effects for SSRI compounds including sertraline, citalopram, fluoxetine and benzodiazepines such as diazepam, oxazepam and temazepam (Bossus et al., 2014; Martin et al., 2017; Valenti et al., 2012). It has been demonstrated that non-target exposure to many of these compounds can produce inimical effects in the form anxiolytical, physiological and behavioural responses. For example, environmentally relevant exposure to SSRI's has been found to reduce locomotor activity and feeding efforts in the three-spine stickleback (Kellner et al., 2018), decrease body size in juvenile brown trout (Ziegler et al., 2020), and promote premature larval release in freshwater mussels (Hazelton et al., 2013). These compounds have also been found to elicit adverse effects at a population level in vertebrate species including effects on sexual selection in the male Mosquitofish *Gambuisi holbrooki* (Bertram et al., 2018), and the modification of courtship behaviour in the male Starling *Sturnus vulgaris* (Whitlock et al., 2018).

The highest measured concentration of single contaminant in the macroinvertebrates reached 90.8 $ng \cdot g^{-1}$ for the SSRI citalopram in *P. ulvae* at Hythe

followed by sertraline (69.2 ng·g^{-1}) and haloperidol (65.6 ng·g^{-1}) in H. diversicolor (Fig. 2). The compound citalopram was also determined at high concentrations in *H. diversicolor* and while not detected in *G. pulex,* it was in *C.crangon*. *P. ulvae* can make up 89.5% of the diet of estuarine birds, which also consume sediment while foraging upon them. Shelduck have been found with up to 3000 individuals ingested (Anders et al., 2009), whilst acknowledging that dietary transfer has not been demonstrated here, there is a potential risk to foraging vertebrates in estuaries that are most often both internationally protected for shorebirds and universally exposed to WWTP effluent containing biologically active behavioural modifying drugs. The focus of these behavioural compounds in the literature likely stems from their high consumption, but future studies should aim to measure exposure to other psychoactive drugs to ensure non-bias of targeted analyte lists (i.e. the Matthews Effect (Daughton, 2014)). As such, exposomics is a developing field that is benefitting from established workflows developed for metabolomic studies that focus on untargeted analysis. By utilising these approaches, characterisation of exposure in the environment will be improved.

Pesticide Exposure

The neonicotinoid pesticide imidacloprid was not detected in *P. ulvae* but measured on average 24±8 ng·g^{-1} in *H. diversicolor* and 8±3 ng·g^{-1} in *G. pulex*. This is similar to concentrations that have been previously reported in the literature that measured up to 21 ng·g^{-1} in *G. pulex* (Munz et al., 2018). However, very few other studies have measured neonicotinoid presence in biota and so more general trends are difficult to establish. Only one other neonicotinoid was detected in biota and was present at relatively lower concentrations (thiacloprid), that was <1 ng·g^{-1} in *G. pulex* and ranged from 2 – 7 ng.g^{-1} in *H. diversicolor*. Three neonicotinoids were subject to

356 regulations published May 2018 (European Commission, 2016a, 2016b) (with a grace
357 period up to December 2018) by the European Commission which banned all outdoor
358 uses and seed treatments of imidacloprid, clothianidin and thiamethoxam, with the
359 exception of greenhouse use (European Commission). Thiacloprid while not subject
360 to these regulations, is a potential endocrine disruptor and has been recommended
361 as a candidate for substitution(European Commission). Acetamiprid was evaluated by
362 EFSA to present a low hazard to pollinators and no further restrictions were applied
363 (European Food Standards Agency, 2016). Pesticide usage in the UK is estimated by
364 the Department of Environment, Food and Rural Affairs (Defra)(Department of
365 environment food and rural affairs, 2020). Interestingly, 2018 surveys revealed that
366 compared to 2016 levels, acetamiprid use for arable crop growth decreased by 59%,
367 whereas thiacloprid usage has increased by 58%. Additionally, no usage of
368 imidacloprid has been reported in these surveys. In terms of measured occurrence, it
369 is possible that persistence of these compounds in soils could lead to continued
370 exposure in waters through leaching. Imidacloprid and clothianidin have been shown
371 to have the longest soil half-lives of 191 and 545 days, respectively(University of
372 Hertfordshire, 2007). However, clothianidin whilst more persistent was not detected in
373 any of the compartments sampled and was used in larger quantities than imidacloprid
374 (79.2 tonnes compared with 0.2 tonnes in 2016). Thus, it difficult to pinpoint the source
375 of imidacloprid in the aquatic environment. However, neonicotinoids are also used in
376 veterinary medicine (e.g. tick/flea control) and might represent an additional route of
377 exposure these substances to enter the aquatic environment beyond agricultural use.
378 Other detected pesticides included propamocarb, azoxystrobin and diuron. These
379 three pesticides are all approved under current European Commission regulations.
380 However, whilst azoxystrobin and propamocarb are currently used in the UK, diuron
381 usage has not been reported since 2016 (0.2 tonnes) (Department of environment

382 food and rural affairs, 2020). The detection in biota might be related to the persistence
383 in sediment as it was detected in this compartment but not in surface water.
384
385 *Recreational drug exposure*
386 Cocaine and its metabolite benzoylecgonine were detected in every animal,
387 sediment and surface water sample, demonstrating high frequency occurrence that
388 was also reported in our previous study for rural Suffolk for both biota and surface
389 water(Miller et al., 2019b). Surprisingly, average concentrations for cocaine in this
390 study were 2.5±2.6 $ng \cdot g^{-1}$, in contrast to the average concentrations determined in
391 Suffolk (5.9±4.3 $ng \cdot g^{-1}$). Land use near the sampling sites in Suffolk were much less
392 urbanised areas with considerably smaller populations thus contamination was
393 expected to be higher in the present study. Cocaine is the second most used
394 recreational drug in the UK (below cannabis) (Home Office, 2019). Ketamine was only
395 detected in *G. pulex* at Hythe at low concentrations (≤1.1 $ng \cdot g^{-1}$). This compound
396 however was detected in all surface water and sediment samples.. Whilst this
397 compound is used in veterinary medicine, its misuse has increased with larger rises
398 recorded between 2016 – 2018(Home Office, 2019). Other potential recreational drugs
399 included tramadol, diazepam and temazepam but these also have medical uses.
400 Tramadol was determined at higher concentrations in *P. ulvae* at both Hythe and
401 Wivenhoe when compared with the other macroinvertebrate species.
402 Very few studies exist that have looked at the potential effects of recreational
403 drugs in animals outside captivity and as with pharmaceutical pollution this requires
404 further consideration for potential environmental risk. Regulation on use may not be
405 possible with recreational drugs and so other innovative solutions would be needed.
406 For example, schemes that aim to treat drug abuse as a public health issue rather
407 than using traditional law and order approaches(Mold, 2018; Volkow et al., 2017) could

have additional benefits in this scenario by reducing the number of users and subsequently reducing input into the environment.

The number of contaminants determined across four species of macroinvertebrates representing three unique food web routes is concerning, but without better understanding of potential for effects it is beyond the scope of this study to link to risk. Nevertheless, this study does demonstrate that there are cross-species differences in exposure which might lead to some species being more susceptible to effects of environmental contaminants. As a final consideration we should focus on characterising exposure in biota as this represents the at-risk group from the potential hazards of chemical contaminants in the environment. Particularly, as it is challenging to link exposure between different compartments without further mechanistic studies on bioavailability and accumulation.

Characterising contamination of sediment and surface water

The measurement of contaminants in abiotic compartments has been prioritised in previous monitoring studies with surface waters often accounting for most measurements. For example, with pharmaceuticals, only 2% of measured data (up to October 2013) was determined in sediment compared with surface waters which accounted for 55% of data (total of 123, 761 measured datapoints)(aus der Beek et al., 2016) . Regarding, recreational drugs there have been a limited number of studies focusing on occurrence beyond wastewater and surface water. Very few researchers have characterised recreational drugs in biota (Klosterhaus *et al.*, 2013; Wilkinson *et al.*, 2018; Miller,*et al.*, 2019) and sediment (Álvarez-Ruiz et al., 2015; Klosterhaus et al., 2013; Langford et al., 2011; Wilkinson et al., 2018).

Sediment

Sediment acts as an additional route of exposure for benthic-dwelling organisms and potential re-mobilisation of adsorbed chemical contaminants (especially at periods of high flow or tidally influenced rivers). The highest measured concentration was for the compound citalopram at Hythe that reached up to 145.8 $ng \cdot g^{-1}$ (mean: 120.5 $ng \cdot g^{-1}$) (Fig. 3). Other compounds that reached higher concentrations at Hythe included propranolol (mean: 49.4 $ng \cdot g^{-1}$), amitriptyline (mean: 44.6 $ng \cdot g^{-1}$), sertraline (mean: 35.5 $ng \cdot g^{-1}$), diphenhydramine (mean: 31.4 $ng \cdot g^{-1}$), verapamil (mean: 22.0 $ng \cdot g^{-1}$), oxazepam (mean: 20.3 $ng \cdot g^{-1}$), diuron (mean: 19.0 $ng \cdot g^{-1}$) and bezafibrate (mean: 13.6 $ng \cdot g^{-1}$). These compounds were also present at higher concentrations at Wivenhoe and Alresford in comparison to the remaining contaminants detected. The sediment at all three sites were broadly similar estuarine muds (silt & clay) with 99% of particles <1 mm by mass. Sorption of chemical contaminants is via several different mechanisms including cation exchange, hydrophobic interaction, hydrogen bonding and surface complexation (Tolls, 2001). The higher occurrence of propranolol might be related to its high partition coefficient (K_d) which was shown to be the largest compared with several other beta-blockers for two different sediment types (Ramil et al., 2010). In addition to non-polar interactions, propranolol will likely interact via hydrogen bonding between hydroxyl groups with free silanol in sediment. Previous studies have noted that pH and sediment type/composition can have significant influence on the dominant mechanism of sorption (Jones et al., 2006; Schaffer et al., 2012). Furthermore, hydrophobicity is unreliable for prediction of the fate of pharmaceuticals, which has also been noted for uptake in biota (Chang et al., 2019; Miller et al., 2019a; Schaffer et al., 2012). A potentially important route for sorption of pharmaceuticals is cation exchange as sediment surfaces often have an associated negative charge(Martínez-Hernández et al., 2014). A total of three pesticides were

459 determined in the sediment samples with the remaining compounds being
460 pharmaceuticals and recreational drugs. Whilst most drugs are either basic or acidic,
461 a higher proportion are basic (57% basic, 29% acidic from a 582 compound dataset)
462 and this trend is more pronounced for CNS drugs where the distribution of basic
463 compounds increased to 75% (Manallack, 2007). The higher proportion of basic drugs
464 for CNS treatments is related to penetration of the blood brain barrier (BBB) where
465 functional amines can favour transport across this membrane. This may further
466 suggest that sediments are an important compartment regarding drug transport and
467 fate in freshwater systems, particularly for psychoactive drugs.
468
469 *Surface water*
470 Surface water was the most contaminated compartment in terms of number of unique
471 compounds determined, but the data further demonstrate that occurrence in water
472 does not translate well into concentrations present in biota. For example, of the 70
473 unique compounds determined across all three aquatic compartments, 24 compounds
474 were determined in surface water only and a further 9 compounds were determined in
475 both surface water and sediment, but not present in biota (Fig S1). Another 7
476 compounds were not detected in surface water but were present in biota only or both
477 biota and sediment. The issue when compared to sediment and biota samples, is that
478 surface water samples normally represent a single “snapshot” in time, unless using
479 passive samplers or high frequency composite samplers. Multiple compounds
480 measured in the surface water of this current study showed large variations in
481 measured concentrations and is likely due to the high spatiotemporal variability
482 associated with surface water.
483 The highest concentration determined in surface water was 386 $ng \cdot L^{-1}$ corresponding
484 to risperidone which is an antipsychotic medication (Fig 4). Additional compounds

485 detected at relatively higher concentrations included venlafaxine (antidepressant),
486 acetamiprid (neonicotinoid), imidacloprid (neonicotinoid) and trimethoprim (antibiotic).
487 Of the compounds determined at Hythe many of these compounds were reduced
488 below the LOQ by the second site Wivenhoe. Other studies have shown similar trends
489 where concentrations further downstream of WWTPs are significantly reduced (Baker
490 and Kasprzyk-Hordern, 2013; Munro et al., 2019). Cocaine and its metabolite BZE
491 were also detected at a mean value of 3.2 $ng \cdot L^{-1}$ and 19.7 $ng \cdot L^{-1}$, respectively. The
492 ratio of cocaine:BZE is 0.16 which is similar to ratios found in other surface waters
493 (Munro et al., 2019). This ratio indicates that the input into the river is likely to stem
494 from untreated sewage (influent) (Baker et al., 2014) entering the River Colne from
495 combined sewer overflows. In 2019, the Colchester storm overflow upstream of the
496 Hythe sampling point, spilled 342 times totalling 7,248 hours (302 days) of untreated
497 wasted entering the River Colne (The Rivers Trust). Other recreational drugs
498 determined at higher concentrations included ketamine and MDMA and two
499 benzodiazepines (temazepam and oxazepam). Diazepam was not quantifiable, but
500 this compound is extensively metabolised (>90%) to temazepam and oxazepam.
501 Therefore, measured levels of these two compounds will also be related to diazepam
502 consumption.
503 Of the pharmaceuticals detected, cardiovascular drugs were frequently detected
504 across all sites and reached higher concentrations at Hythe which included the
505 anticoagulant warfarin (11 $ng \cdot L^{-1}$) and the beta-blockers; propranolol (59 $ng \cdot L^{-1}$),
506 metoprolol (26 $ng \cdot L^{-1}$), bisoprolol (13 $ng \cdot L^{-1}$) and timolol (<LOQ). Carbamazepine and
507 its metabolite carbazmepine-10,11-epoxide were both detected reaching an average
508 of 31 $ng \cdot L^{-1}$ and 68 $ng \cdot L^{-1}$ at Hythe, respectively. This drug is extensively metabolised,
509 with the major route to the epoxide form (and is the pharmaceutically active species

being a pro-drug). However, the drug is considered persistent due to it limited removal during wastewater treatment and presence across multiple environmental compartments(Zhang et al., 2008).

Four of the seven neonicotinoids available on the market were detected here. Acetamiprid and imidacloprid averaged 181.5 $ng \cdot L^{-1}$ and 119.2 $ng \cdot L^{-1}$, respectively, whereas thiamethoxam measured 15.3 $ng \cdot L^{-1}$ and thiacloprid was only detected below LOQ. However, all neonicotinoids were measured below the LOQ at Wivenhoe and Alresford. This suggests that the source of input may come from the discharge point of the WWTP as opposed to run-off or leaching. As mentioned previously, it is difficult to identify the source of neonicotinoid presence in this study, but it is a possibility that the occurrence is related to wastewater from indoor agricultural practices or potentially from veterinary use. Wastewater from greenhouses have been recognised as a contributor to pollution related to high nutrient content such as phosphate (Dunets and Zheng, 2014; European Commission, 2015) and thus could be have the potential to be further linked as a source for micropollutants. Further investigation is warranted into this aspect as few studies have looked at indoor agriculture practices regarding pollution and therefore regulations surrounding indoor pesticides may need revision to be protective of the environment.

To summarise, increasing our surveillance of the aquatic environment by monitoring multiple compartments and species will increase our characterisation of the exposome, whilst overcoming limitations with different sampling approaches, further improving our understanding of environmental exposures.

Occurrence of pharmaceuticals, recreational drugs and pesticides across multiple compartments

Research efforts for the determination of pharmaceuticals and pesticides in the environment have primarily been placed on the measurement of these chemicals in abiotic compartments such as surface waters, ground waters and marine waters (aus der Beek et al., 2016). A holistic understanding of exposure requires consideration of live biota exposed to these abiotic compartments. Consideration of multiple species is relevant as species that occupy different ecological niches and roles within food-webs are likely to receive and transport contaminants in unique ways. The compounds detected in this study included pharmaceuticals, pesticides and recreational drugs, with pharmaceuticals being the most frequently determined compounds and present at higher concentrations relative to the detected pesticides and recreational drugs.

Overall and out of 141 compounds included in the analytical method; a total of 33 compounds were detected in the macroinvertebrates sampled, 39 compounds detected in sediment samples and 59 compounds detected in surface water samples. The most contaminated site was Hythe (Fig 1), closely located downstream of a WWTP and accounts for both the elevated concentrations and diversity of detected chemicals in comparison to Wivenhoe and Alresford. All three sites are tidally influenced and have periods of ebb and flow, where the tide moves inland (flow) and then drains outward (ebb). A previous investigation into the River Thames (Munro et al., 2019) showed that tidal cycles led to homogeneity across a majority of quantified pharmaceuticals and recreational drugs due to mixing from multiple combined sewer overflow and treated effluent influx points in the Central London region. The WWTP located upstream of the Hythe sampling site, serves a population equivalent of 131,413 with primary, secondary and UV treatment stages in place (Office International de l'Eau).

Concentrations of the chemical contaminants generally decreased further downstream from the WWTP for all sample types, but this was most apparent for

surface water samples (Fig 5). Previous studies have also shown that WWTP discharges are a significant source of contamination and for sites located inland, concentrations are generally lower due to dilution by coastal waters (Biel-Maeso et al., 2018; Čelić et al., 2019). Decreases in concentrations in sediment and biota samples were less apparent between sites particularly for Wivenhoe and Alresford as the ebb and flood of the tide will affect the mixing of sediment and surface water between the two sites.

The majority of compounds determined were pharmaceuticals with fewer pesticides and recreational drugs detected. For example, in the biota samples 33 compounds were detected; 25 were pharmaceuticals (76%), 4 pesticides (diuron, propamocarb, thiacloprid & imidacloprid), 2 recreational drugs (cocaine & ketamine) and 2 metabolites (benzoylecgonine & carbamazepine-10,11-epoxide). Within the pharmaceutical class, 13 compounds (52%) were psychoactive drugs including antidepressants and antipsychotics. There are a range of potential sources of behavioural drugs in this urban WWTW catchment, from well documented increases of use in the general population to a main regional hospital. In the UK, most direct emissions from hospitals into sewerage is prohibited (Water UK, 2014) and so occurrence in the environment is most likely to come from domestic wastewater after human consumption which may explain the relatively low contributions of hospital effluent to WWTP influent contaminant loads in previous works (Verlicchi et al., 2012).

A principal component analysis of the chemical monitoring data explained 41% of the variance across the sampled species and sites (Fig 6a). The analysis showed that *P. ulvae* and *H. diversicolor* were most impacted in terms of chemical body burden at Hythe with *G. pulex* clustering more closely with the site clusters for Wivenhoe and Alresford. The closer association of *G. pulex* with these two downstream sites is attributed to the lower chemical burden in biota that were sampled from them.

587 Interestingly, the confidence ellipses are clustered within each other and indicate that
588 variance in occurrence data is smaller the further samples are taken from the point
589 source. Contamination was generally low with (semi)solid samples (i.e., sediment and
590 biota) on the parts per billion scale and surface water samples in parts per trillion.

591 Comparing the average contamination for each compartment (sediment, water
592 & biota) showed that sediment had a higher contaminant burden followed by biota and
593 then surface water which is an order of magnitude lower (Fig 6b). The mean chemical
594 burden for sediment samples was 13±24, 6±10 and 5±6 $ng \cdot g^{-1}$. In comparison, the
595 average chemical burden in biota samples was 6±11, 3±5 and 3±4 $ng \cdot g^{-1}$ and in
596 surface water samples was 52±73, 8±12, 7±12 $ng \cdot L^{-1}$ for Hythe, Wivenhoe and
597 Alresford, respectively. Reduction in the mean burden from the first site (Hythe) to
598 second site (Wivenhoe) for sediment was 2.2-fold, 1.6-fold in biota and 6.2-fold in
599 surface water. The greater reduction in surface water is likely to arise from multiple
600 processes occurring including dilution (rainfall/tide), degradation, transformation,
601 sorption and accumulation. Interestingly, fewer compounds were determined in both
602 sediment and biota samples when compared with surface water which has been seen
603 in previous multi-compartmental studies (Inostroza et al., 2017; Wilkinson et al., 2018).
604 Therefore, it is more useful to measure multiple compartments in an aquatic habitat to
605 gain a holistic understanding of exposure and arguably biomonitoring should be of
606 primary focus in terms of relating pollutants to their potential risk in the wider
607 environment.

608

609 **4. Conclusion**

610 A total of 70 unique compounds were determined across surface water, sediment and
611 biota samples collected from the three sites along the estuarine River Colne (Essex,
612 UK). The most frequently detected chemicals belonged to pharmaceuticals and

recreational drugs. Of these, psychoactive pharmaceuticals showed the highest concentrations across all compartments including multiple macroinvertebrate species that are unique and important resources for estuarine birds and fishes. The data suggest that sediment is an important exposure route with benthic-dwelling organisms typically showing higher contaminant concentrations. Amphipods showed lower contamination and may indicate that these are a more conservative indicator species for environmental exposure. The neonicotinoid, imidacloprid was determined at higher concentrations in sediment and biota samples despite the recent EU-wide ban, although the source of contamination was unclear. Additional pesticides that no longer have approval for use in the EU were also detected which included fenuron, atrazine, pymetrozine and simazine. The detection of these banned substances is a cause for concern and further investigations should look to understand the source of the input. The mixture of chemicals present in the different compartments may be associated with potential hazards for organisms exposed to them. With this in mind, it is important to increase our surveillance in the environment so that we can identify areas and chemicals that are of higher concern. As a final consideration whilst broad targeted analytical methods are useful to quantitatively determine chemical contaminants the bias of these lists is problematic for characterising the full extent of the exposome. Thus, future studies should consider non-target exposomics-type strategies where possible and across multiple compartments to give greater coverage of the contaminant space in the aquatic environment.

Figure Captions

Figure 1: Location of sites for sample collection along the Colne Estuary. Hythe, Wivenhoe and Alresford were situated downstream of the major WWTP and are tidally

638 influenced. Grey areas indicate buildings and green areas indicate woodland. Inset
639 shows relative position to the rest of England.
640 **Figure 2**: Heatmap showing concentrations (ng.g^{-1}) of CEC in the four
641 macroinvertebrate species collected from each sampling site. Grey tiles indicate
642 samples were below the limit of quantification.
643 **Figure 3**: Heatmap showing concentrations (ng.g^{-1}) of CECdetermined in the sediment
644 cores collected from each sampling site. Grey tiles indicate samples were below the
645 limit of quantification.
646 **Figure 4**: Heatmap showing concentrations (ng.L^{-1}) of CECdetermined in surface
647 water collected from each sampling site. Grey tiles indicate samples were below the
648 limit of quantification.
649 **Figure 5**: The concentration ranges of chemical contaminants determined across each
650 of the three sites and the three compartments (e.g. surface water, sediment and biota).
651 Concentrations for solid samples are based on dry weight.
652 **Figure 6**: Comparison of chemical burden across sampling sites, compartments and
653 species. **(a)** Principal component analysis showing the variance in the chemical
654 burden in biota between sites with ellipses representing the 95% confidence interval.
655 **(b)** Mean chemical burden for each compartment sampled in the Colne Estuary, **(c)**
656 mean chemical burden determined in macroinvertebrates collected from Hythe, **(d)**
657 mean chemical burden determined in macroinvertebrates collected from Wivenhoe
658 and **(e)** mean chemical burden determined in macroinvertebrates from Alresford. All
659 radar plots are based on a part per billion (ppb) scale.
660

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668

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