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1 **BIOMONITORING OF PESTICIDES, PHARMACEUTICALS AND ILLICIT DRUGS**
2 **IN A FRESHWATER INVERTEBRATE TO ESTIMATE TOXIC OR EFFECT**
3 **PRESSURE**

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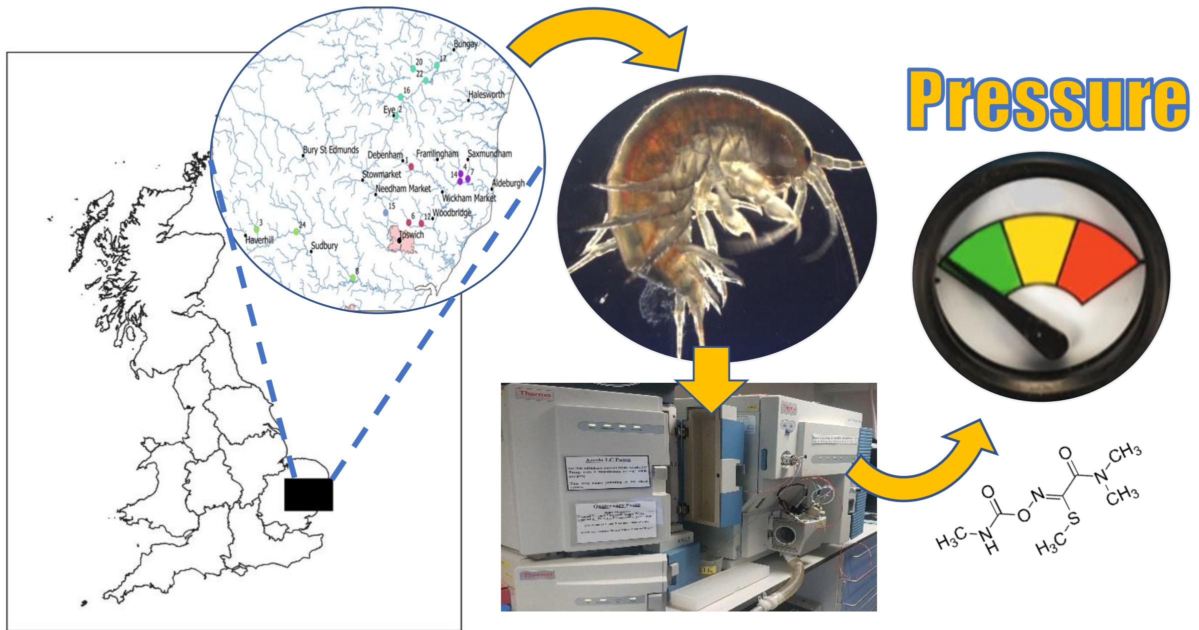
26 **GRAPHICAL ABSTRACT**



Abstract

Multiple classes of environmental contaminants have been found in aquatic environments, globally. Understanding internalised concentrations in the organism could further improve the risk assessment process. The present study is concerned with the determination of several contaminant classes (107 compounds) in *Gammarus pulex* collected from 15 sites covering 5 river catchments across Suffolk, UK. Quantitative method performance was acceptable for 67 compounds including pharmaceuticals, pesticides, illicit drugs and drugs of abuse. A total of 56 compounds were detectable and ranged from <LOQ to 45.3 ng g^{-1}, with cocaine and lidocaine being the most frequently detected compounds present in all biota samples (n=66). For surface water, 50 compounds were detectable and ranged from <LOQ to 382.2 ng L^{-1}. Additionally, some pesticides currently not approved for use were detected, including fenuron that reached a maximum of 16.1 ng g^{-1}. The internal concentrations of pesticides were used to estimate toxic pressure which showed that for the measured pesticides toxic pressure was low ranging from logTU ≤-7 to ≤-2. This methodology was extended to pharmaceuticals and drugs of abuse in a novel approach that proposed the use of pharmacological data (human therapeutic plasma concentrations) to estimate the likelihood of an effect (or effect pressure) to occur based on the internal exposure of the organism. The quantified effect pressure ranged from logEU ≤-9 to ≤1 with haloperidol showing the largest likelihood for an effect. The approach showed that several pharmaceuticals have the potential to elicit effects but further investigation surrounding thresholds for effects would be required. This new approach presented showed potential to be used to improve risk assessment for pharmaceuticals in the environment.

51 **Keywords;** Exposome, Pesticides, Pharmaceuticals**,** Environmental Risk
52 Assessment

53 **1. Introduction**

54 The contamination of the aquatic environment has been the focus of many
55 investigations and many issues have been identified with respect to a number of
56 classes of compounds including pharmaceuticals [1] and plant protection products
57 (pesticides) [2] Within each class, adverse effects of some specific contaminants on
58 biota have been well studied, although effects and/or associated risks are often
59 derived based on exposure concentration levels measured external to the organism
60 (e.g., in water or sediment). A reason for this is that the determination of trace
61 contaminants in biota has traditionally been very challenging, not only in terms of the
62 analytical selectivity required to reliably separate hundreds of different compounds but
63 to do so quantitatively at trace concentrations (e.g. pg-ng g^{-1}) [1]. However, advances
64 in analytical workflows have now enabled trace quantitative measurements in complex
65 biological matrices such that internalised contaminant concentrations can be used to
66 set thresholds for effects [3-5].

67 Arguably, routine determination of internalised concentrations of
68 pharmaceuticals in particular is still critically lacking [1]. This is also true for some other
69 contaminant classes such as illicit drugs. Additionally, neonicotinoid insecticides,
70 which are largely used on land and have rarely been targeted for measurement in
71 aquatic fauna except for a small number of recent studies in fish and invertebrates [6-
72 8]. However, other pesticides have been more routinely monitored in aquatic biota,
73 such as organochlorine insecticides, which are reported at the low to mid ng g^{-1} range
74 in both vertebrates and invertebrates [9, 10]. This is likely due to extensive regulation
75 of these types of contaminants following seminal research in the 1950s (e.g., with
76 dichlorodiphenyltrichloroethane (DDT) [11]) to the more recent Stockholm Convention
77 treaty on persistent organic pollutants which cover many other such compounds [12].

78 Previous studies have used the Species at Risk (SPEAR) index [13, 14] to
79 relate the 'toxic pressure' of pesticides in agricultural catchments to the impact on
80 invertebrate communities and is quantified in toxic units (TU) [15]. The TU is derived
81 from the ratio between the measured concentration of the contaminant in surface
82 water and known toxicity data, such as the LC_{50}. Recently, the TU approach has been
83 applied using internal pesticide concentration measurements and predicted EC_{50}
84 values [6]. Aside from pesticides, this approach could also be extended for other
85 contaminant types such as pharmaceuticals. This would prove particularly useful as it
86 would provide an estimate of risk, based on both measured concentrations and effect
87 data. This has already been performed for selected pharmaceuticals in the Antarctic
88 peninsula [16]. However, a significant barrier to wider application is that there is a
89 paucity of effect data for pharmaceuticals and reported EC_{50} data can vary
90 considerably [17]. Other approaches such as the use of critical environmental
91 concentrations (CECs) proposed by Fick et al. [18], which are based on the fish plasma
92 model [19], could be a useful alternative to the use ecotoxicity endpoint data.

93 The aim of this work was to determine the extent of contaminant occurrence
94 and to estimate the toxic pressure of pesticides and extend this approach to
95 pharmaceuticals, drugs of abuse and illicit drugs to determine an 'effect pressure'
96 across several watercourses in Suffolk. This was achieved through the development
97 of an extended analytical methodology to reliably quantify several classes of
98 contaminants in both surface waters and a freshwater invertebrate species
99 (*Gammarus pulex*). Samples were collected from 15 sites covering five river
100 catchments and used to estimate toxic/effect pressure. Internalised concentrations
101 determined herein and a previously developed model for prediction of bioconcentration
102 factors in *G. pulex* [20] along with the well-established EPISuite [21] BCF predictions

103 in fish were used to calculate internal toxic units (TU_{int}) and effect units (EU_{int}) for
104 pesticides and pharmaceuticals, respectively.
105
106 **2. Materials and Methods**
107 *2.1 Reagents, chemicals and consumables*
108 HPLC grade methanol, acetonitrile, and LC-MS grade (Optima™) ammonium acetate
109 were purchased from Fischer Scientific (Loughborough, UK). A total of 141
110 compounds were used in this study (see Supplementary Information (SI)). Of these,
111 85 were pharmaceuticals/illicits, 22 were pesticides and 34 were stable isotopically
112 labelled internal standards (SIL-IS). All analytical standards were of a purity of ≥ 97%.
113 Ultra-pure water was obtained from a Millipore Milli-Q water purification system with a
114 specific resistance of 18.2 MΩ cm or greater (Millipore, Bedford, MA, USA). Stock
115 solutions (1 mg mL^{-1}) were prepared in methanol or acetonitrile and stored in silanised
116 amber vials (20 mL). Working solutions were prepared daily in ultra-pure water, as
117 required. All solutions were stored at -20 °C and in the dark to reduce possible
118 degradation.
119
120 *2.2 Sample collection*
121 Samples were collected in July 2018. Locations were chosen based on previous
122 Environment Agency sampling sites in catchments of the river Alde, Waveney, Stour,
123 Gipping and Deben (Figure 1). Macroinvertebrates were collected by kick sampling
124 into a 250 µm net. *G. pulex* was present at all sites except the River Box in the Stour
125 catchment and one site on the River Waveney, where the most abundant
126 macroinvertebrate *Ephemera vulgata* (larvae) and *Asellus aquaticus* was sampled
127 instead. At the site on the river Gipping, *G.pulex* numbers were low and the caddis fly

128 *Hydropyshe pellucidula* (larvae) were also sampled. Macroinvertebrates were sorted
129 on site, excess water removed by tissue paper and immediately frozen on dry ice.
130 Samples were kept at -80 ^{o}C prior to processing. Water pH and temperature were
131 measured (Table S3) and a 500 mL water sample taken, acidified (0.1% HCl) and
132 stored at 4 ^{o}C for a maximum of 4 days prior to analysis to improve stability of analytes
133 as shown in previous studies [22, 23].
134
135 *2.3 Sample preparation*
136 Prior to extraction, frozen *G. pulex* samples were lyophilised at -50 °C under vacuum
137 for 24 h. Pooled samples of 5-6 organisms were placed into 2 mL Eppendorf tubes
138 with a 3 mm diameter tungsten carbide bead and subsequently ground into a fine
139 powder using a TissueLyser LT (Qiagen, Hilden, Germany) set at 50 Hz for 5 min.
140 Freeze-dried composite samples of *G. pulex* material (20 mg) were transferred to a
141 new 2 mL Eppendorf tube with any necessary spiking of standards or SIL-IS carried
142 out directly onto the solid matrix using a 100 µL volume of an appropriate working
143 solution before proceeding with the extraction. A 2 mL volume of 3:1 (MeCN:H_2O)
144 acidified with 0.1% (v/v) glacial acetic acid was added to the material and agitated for
145 5 min at 50 Hz in the Tissuelyser LT. The samples were then placed in an ultrasonic
146 bath for 15 min followed by centrifugation for 5 min at 14,000 rpm to pellet insoluble
147 particulate matter. Following extraction and settling, an aliquot of the supernatant (1.9
148 mL) was diluted to 100 mL with 10 mM ammonium acetate in ultra-pure water (pH
149 6.5). Tandem solid phase extraction (SPE) was then carried out on the diluted sample
150 using a Strata Alumina-N cartridge (6 mL, 1 g, Phenomenex Ltd., Cheshire, UK)
151 coupled to an Oasis HLB cartridge (6 mL, 200 mg, Waters Corp., Hertfordshire, UK).
152 Tandem SPE was utilised to remove interfering pigments and lipids (alumina) and pre-

153 concentrate target analytes (HLB). Before loading of the sample, the combined SPE
154 cartridges were first conditioned with 6 mL of methanol and 6 mL of ultra-pure water
155 with 10 mM ammonium acetate. After sample loading, both cartridges were then
156 washed with 1 mL ultra-pure water and dried for ~30 min under vacuum. Cartridges
157 were then stored at -20 °C until analysis. Cartridges were eluted with 5 mL MeOH and
158 dried under pure nitrogen (1.0 bar) at 35 °C using a TurboVap LV (Biotage, Uppsala,
159 Sweden). Extract residues were reconstituted in 0.1 mL 90:10 (v/v) 10 mM ammonium
160 acetate in H_2O:MeCN (optimised). Surface water samples were filtered through a 0.45
161 µm glass-fibre filter and split into three aliquots (100 mL). Surface water samples then
162 underwent SPE and reconstitution as described above, but without use of the Strata
163 Alumina-N cartridges (as pigments were not problematic). Any necessary spiking or
164 liquid volume measurements were carried out using positive displacement pipettes
165 (Gilson Microman, Villiers-le-Bel, France).
166
167 *2.4 Instrumental analysis and conditions*
168 Briefly, liquid chromatography (LC) was performed on a Vanquish series LC system
169 (ThermoFisher Scientific, Hemel Hempstead, UK) using a Waters SunFire C_{18} column
170 (3.5 µm, 2.1 mm × 150 mm, Waters Corp., Milford, MA, USA) with a KrudKatcher™
171 Ultra pre-filter (0.1 mm ID, 0.5 µm filter, Phenomenex, Macclesfield, UK) and a Sunfire
172 C_{18} VanGuard Cartridge (3.5 µm, 2.1 mm x 5 mm) at a flow rate of 0.3 mL min^{-1} and
173 an injection volume of 20 µL. Mobile phases were 90:10 (v/v) 10 mM ammonium
174 acetate in H_2O:MeCN (A) and 20:80 (v/v) 10 mM ammonium acetate in H_2O:MeCN
175 (B). The gradient elution profile followed a linear ramp of mobile phase B which
176 increased to 10 % at 1 min, 35 % at 5.6 min, 40 % at 7 min, 50 % at 8 min and 100 %
177 at 11 min and was held for a further 11 min before returning to initial conditions. Re-

178 equilibration time was 3 min resulting in an overall run time of 25 min. Detection and
179 quantification was carried out with a TSQ Vantage triple quadrupole mass
180 spectrometer (Thermo Fisher Scientific, Hemel Hempstead, UK) equipped with an
181 atmospheric pressure interface–heated electrospray ionisation (API-HESI-II) source.
182 Mass spectrometry (MS) was performed in selected reaction monitoring (SRM) mode
183 using positive–negative ionisation polarity switching. See the SI for full details of
184 analytical conditions and method performance testing procedures.

185

186 2.6 Estimation of toxic and effect pressure

187 Toxic pressure was calculated according to Munz et al. [6] using toxic units (TU) to
188 estimate the internal toxic pressure of pesticides. The internal toxic unit (TU_{int}) or effect
189 unit (EU_{int}) used here is defined by equations 1-3.

190

191 $$EC50_{int} = EC50 \times BCF \qquad (1)$$

192 $$TU_{int} = \frac{C_i}{EC50_{int}} \qquad (2)$$

193 $$EU_{int} = \frac{C_i}{CEC} \qquad (3)$$

194 Where, $EC50_{int}$ is the internal concentration which affects 50% of the population; EC_{50}
195 is the exposure medium concentration affecting 50% of the population; BCF is the
196 bioconcentration factor; C_i is the concentration of contaminant determined in the
197 organism. For pesticides, available EC_{50} values (48 h acute in *Daphnia magna*)
198 available from the Pesticide Properties Database [24]. The BCFs were estimated from
199 both EPI Suite BCFBAF v3.02 [21] software and our own previously developed
200 artificial neural network (ANN) for prediction of BCFs in *G. pulex* [20] (Figure 2). The
201 comparison of the predicted BCFs between both approaches showed relatively good

202 agreement for most cases (see Table S4 and Figure S1) and overall were not
203 statistically significant (*p-value* = 0.36).
204 For pharmaceuticals, drugs of abuse and illicit drugs, EC_{50} values were substituted
205 (due to lack of available data) with CECs [18]. Here, the CEC is the estimated surface
206 water concentration that will give rise to a fish plasma concentration equivalent to the
207 human therapeutic plasma concentration (Equation 4). Thus, it would be expected and
208 assumed that if drug targets are conserved, an effect would be elicited.
209 $$CEC = \frac{H_TPC}{(CR \times P_{blood:water})} \quad (4)$$
210 Where, H_TPC is the human therapeutic plasma concentration (µg mL^{-1}), CR is the
211 concentration ratio between the human therapeutic plasma concentration and the fish
212 steady-state plasma concentration (assumed to be 1 herein), $P_{blood:water}$ is the partition
213 coefficient of a compound between blood and water.
214
215 ## 3. Results and Discussion
216 ### *3.1 Method performance*
217 Method performance was assessed in *G. pulex* to ensure that the method could
218 reliably quantify targeted analytes at the low ng g^{-1} concentration level (Table 1). A
219 total of 107 compounds were assessed and 67 compounds (55 pharmaceuticals and
220 12 pesticides) were deemed acceptable for quantification purposes with the remaining
221 analytes suitable for qualitative analysis (according to ICH guidelines). A *t*-test
222 assuming unequal variances showed that there was no significant difference between
223 the performance of the method for either pharmaceuticals or pesticides in terms of
224 recovery and precision ($p > 0.05$). The method showed good sensitivity for trace-
225 analysis with LOQs ranging from 0.09 – 25.2 ng g^{-1} (median: 1.7 ng g^{-1}) dry weight
226 and LODs as low as 0.03 ng g^{-1} (median: 0.6 ng g^{-1}) dry weight. The sensitivity of the

227 method was comparable to others that have determined pharmaceuticals and
228 pesticides in invertebrates. For example, Inostroza et al., had method quantification
229 limits (MQLs) of 0.01-2.13 ng g^{-1} wet weight [4], Althakafy et al., reported detection
230 limits ranging 0.04 – 2.38 ng g^{-1} wet weight [25] and Munz et al., achieved LOQs of
231 0.1 to 9 ng g^{-1} wet weight [6]. Linearity was acceptable (R^2 >0.98) and the
232 chromatographic separation showed good reproducibility with an average standard
233 deviation in retention time of ±0.015 min (n = 5). The repeatability of the method was
234 also acceptable with average intra-day imprecision of 9±5%, 9±4% and 8±4% at three
235 different concentrations of 25, 50 and 100 ng g^{-1} dry weight. Inter-day precision
236 determined at 50 ng g^{-1} across three days showed slightly lower precision but was still
237 considered acceptable (average 14±4%) and was perhaps due to the inhomogeneity
238 of such small samples and different operators between days. Absolute recoveries of
239 the method ranged from 26 – 100 % (average: 74 %) and is in line with a recent study
240 that focussed on quantification of both pharmaceuticals and pesticides in *G. pulex*
241 where recovery ranged from 9 – 70% [6]. Method accuracy averaged 92 ±10 %, 97
242 ±12 % and 104 ±9 % compared to the expected nominal concentration at 25, 50 and
243 100 ng g^{-1}.
244

245 *3.2 Biomonitoring of emerging contaminants across Suffolk catchments*

246 Occurrence studies are often focussed on the determination of contaminant
247 concentrations in surface water samples and other abiotic matrices such as
248 wastewater and sediment. The limitation of this is approach is that for spot sampling
249 of water, for example, temporal and spatial fluctuations can be considerable and are
250 unlikely to be representative of a chronic exposure scenario. Alternatively, passive
251 sampling that represents a time-weighted average concentration is generally

252 considered semi-quantitative [26]. Furthermore, these measurements do not
253 accurately represent the real risk to aquatic wildlife as they do not account for
254 bioavailability and it is the internalised xenobiotic concentration that will be the initiating
255 event for any adverse effects. As such, biomonitoring campaigns are now receiving
256 more attention for their importance in determining exposure and hazard [6, 27].

257 Both water and biota samples were collected across 15 sites in the county of
258 Suffolk. The 15 sites covered 5 different river catchments including Gipping, Alde,
259 Deben, Stour and Waveney. Across the 67 compounds determined, concentrations of
260 compounds were generally very low in both biota samples (parts per billion range) and
261 water samples (parts per trillion range). For biota samples (n = 66), the average
262 concentration determined was 4.3 ±5.2 ng g^{-1}, with maximum and minimum
263 concentrations of 45.5 ng g^{-1} (propranolol) and 0.2 ng g^{-1} (acetamiprid), respectively
264 (Figure 3). In comparison to surface water samples, concentrations averaged 23.8
265 ±54.9 ng L^{-1}, with the maximum and minimum concentrations of 382.2 ng L^{-1} (tramadol)
266 and 0.1 ng L^{-1} (nordiazepam), respectively (Figure 4). In general, Site 1 in the Deben
267 catchment showed increased concentrations of compounds such as ketamine,
268 carbamazepine and citalopram compared to the other sites within the same catchment
269 and between the remaining catchments. These higher concentrations also coincide
270 with higher concentrations in surface water for compounds such as ketamine,
271 carbamazepine and tramadol, the source of which is unclear but for these compounds
272 their removal at WWTPs is low [28]. Debenham is a large village of 2200 inhabitants
273 (Figure 1) served by a small WWTP upstream of the sample site. The sources for
274 these contaminants are likely to be related to public consumption and output through
275 WWTP effluents (for pharmaceuticals, drugs of abuse and illicits). A previous study
276 that has quantified related compounds in influent and effluent samples from a WWTP

277 in London showed that the concentrations in the surface water determined here are in
278 the range of those determined in effluent (~10 – 50 ng L^{-1}) [28]. Additionally, spread of
279 sludge and bio-solids including [29] reclaimed wastewater for irrigation from WWTPs
280 onto agricultural land could lead to further surface run-off or leaching of
281 pharmaceuticals and controlled substances into surface waters [30]. For pesticides,
282 run-off and leaching (including possible re-mobilisation) are the potential sources
283 relating the compounds detected herein [31].
284
285 *3.2.1 Illicit drugs, drugs of abuse and life-style related compounds*
286 Interestingly, the most frequently detected and highest concentration compounds in
287 biolgoical samples were illicit drugs and/or drugs of abuse, such as cocaine, ketamine,
288 alprazolam and diazepam. Cocaine was detected and quantified in all biota samples
289 across all 15 sites at an average of 5.9 ± 4.3 ng g^{-1} (max. 30.8 ng g^{-1}). Average
290 concentrations of cocaine between different catchments did not vary significantly
291 showing widespread contamination (Alde = 6.9, Deben = 6.9, Gipping = 6.8, Stour =
292 6.2 & Waveney = 4.2 ng g^{-1}). Lidocaine was the second most frequently detected
293 compound in the biota samples that can be used as an adulterant to 'cut' cocaine due
294 to its synergistic effects [32] or is used as local anaesthetic. Another commonly used
295 adulterant for cocaine use is levamisole. This compound, however, was not frequently
296 detected in either biota or surface water samples. However, illicit compounds are
297 rarely monitored in aquatic fauna, with only one previous occurrence study in the
298 literature that determined cocaine at an average concentration of 0.28 ng g^{-1} dw in
299 *Mytilus spp* [3]. A separate investigation into the bioaccumulation potential of cocaine
300 in European eels (*Anguilla anguilla*) in Italy revealed tissue concentrations ranging
301 from 0.47 – 30.5 pg g^{-1} ww depending on tissue type at an exposure concentration of

302 20 ng L^{-1} [33]. However, eels were not studied as part of this or previous works in our
303 laboratory. The source of the widespread cocaine contamination is unclear. Scattered
304 throughout the catchments of these Suffolk rivers are small wastewater treatment
305 plants that will discharge into the water courses. However, secondary wastewater
306 treatment with activated sludge are efficient at removing cocaine (~90% [34]), whereas
307 trickling filters are less efficient (35-37% removal [34]). The dispersal of deactivated
308 sewage sludge onto farmland as a fertiliser is unlikely to be a primary source and
309 concentrations of cocaine in sludge have been reported as low, at ~3 ng g^{-1} [35]. The
310 primary metabolite of cocaine, benzoylecgonine (BZE) was also frequently detected,
311 but often below the LOQ in both water and biological extracts. The concentration of
312 cocaine determined in surface water samples was also below the LOQ for all sites and
313 previous studies in the UK have often determined cocaine at ~1-10 ng L^{-1} in surface
314 water [28, 36]. The ratio between cocaine to BZE is also important to consider and
315 may potentially indicate the source of input into the environment. For example, in
316 wastewater analysed from London in 2014, the ratio between cocaine and BZE was
317 0.51 ±0.09 in influent, but was very different and more variable in effluents measured
318 on the same days (2.60 ±1.46) [28]. Therefore, it is expected that the ratio between
319 cocaine and BZE in river water catchments should be similar to effluent ratios but this
320 was not the case for London, where the ratio for cocaine:BZE over six weeks of daily
321 monitoring was 0.21 ±0.1 (similar to influent ratios) [28]. Thus, it is proposed that the
322 input of cocaine into surface waters in the UK is likely due to combined sewer overflow
323 events or leakage from sewer misconnections and cesspit overflow. Interestingly, the
324 ratio in the biota samples measured here (mean: 5.00) indicated that cocaine had
325 preferential accumulation over its demethylated metabolite, BZE.

326 Tramadol was frequently detected in surface water and reached the highest
327 measured concentration across the sites of 382.2 ng L^{-1}. This compound has
328 previously been detected in UK rivers ranging from <30 ng L^{-1} to 5970 ng L^{-1} [28, 36].
329 Effect assessments studies demonstrate lowest observed effects concentrations
330 (LOEC) of 10 µg L^{-1} in fish embryo tests [37]. Occurrence of this compound here was
331 infrequent with a maximum measured concentration of 7.5 ng g^{-1}. Field-derived
332 bioaccumulation studies have suggested that bioaccumulation is low with BAFs <5
333 and tissue concentrations in fish were <6 ng g^{-1} [38]. Ketamine was also frequently
334 detected in biolgocal and surface water samples here, with concentrations reaching
335 up to 22.5 ng g^{-1} and 205 ng L^{-1}. However, to the authors' knowledge, ketamine has
336 not been previously reported in aquatic fauna, but surface water concentrations have
337 been measured at 12 ng L^{-1} [28].

338 The benzodiazepines are a class of compounds used for medicinal purposes
339 but are also misused/abused. Alprazolam, diazepam and temazepam was determined
340 at 2.7 ±1.3 ng g^{-1}, 1.5 ±1.4 ng g^{-1} and 2.4 ±2.3 ng g^{-1}, respectively. Lorazepam,
341 oxazepam and nordiazepam were infrequently detected. Our previous work has
342 shown that diazepam and temazepam have a low potential to accumulate in *G. pulex*
343 and which are capable of rapid biotransformation and elimination of these compounds
344 [39]. In surface water samples, diazepam was infrequently detected and often
345 occurred at <1 ng L^{-1}. Alprazolam was also infrequently detected and below the LOQ.
346 The average concentrations of the remaining benzodiazepines were 9.0 ±9.4 ng L^{-1}
347 (temazepam), 5.2 ±3.5 ng L^{-1} (oxazepam), 4.8 ±3.3 ng L^{-1} (lorazepam) and 2.2 ±0.8
348 ng L^{-1} (nordiazepam).

349 Synthetic cathinones including methedrone, mephedrone, methcathinone and
350 4-fluoromethcathinone were not detected at any site in the biota samples. However,

351 methcathinone was detected below the LOQ at a small number of sites in surface
352 water samples from the river catchments of Waveney, Deben and Alde. Cathinones
353 are psychoactive substances and their consumption across the UK and Europe formed
354 the basis of several occurrence studies in surface water and wastewater [40]. Nicotine
355 was determined in surface water samples up to 342.8 ng L^{-1} and was also detected in
356 38 % of the biota samples ranging from <LOQ to 16.5 ng g^{-1}. Its primary metabolite,
357 cotinine, was also detected in biota and surface water samples, but less frequently
358 and at lower concentrations. Based on human metabolism, the expected ratio of
359 nicotine to cotinine would range between 0.65 – 1.00 [41]. However, for surface water
360 samples the average ratio of nicotine:cotinine was 7.61 and in biota samples was 2.39.
361 The higher concentration of nicotine to cotinine has been reported previously for
362 effluent wastewater [42] and a similar ratio to surface water can be estimated (6.3)
363 from reported concentrations in influent wastewater samples [43]. These types of
364 compounds are useful to monitor in the environment as they can serve as indicators
365 of population health and lifestyle choices. Previous studies have identified markers of
366 alcohol consumption such as ethyl sulfate [44]. Whilst other sewage epidemiology
367 studies have used drug concentrations in wastewater to relate back to recreational
368 drug use of the population [45]. In addition to the association with human health, these
369 drugs are often not monitored in biota and so any potential risk from exposed aquatic
370 wildlife is poorly understood. The reason for poor exposure and hazard assessment is
371 likely to stem from that many of these substances are also medicines and therefore
372 will be considered ‘legacy’ products, which do not require ERA. Interestingly, seven of
373 the top ten most frequently detected compounds in biota samples are related to illicit
374 drugs/drugs of abuse. The risk of these compounds is not well understood due to the

375 lack of literature, but as these compounds are all psychoactive, any effects on fauna
376 may be elicited through behavioural changes [46, 47].
377
378 *3.2.2 Pharmaceuticals*
379 The most frequently detected pharmaceutical in both biota and surface water samples
380 was carbamazepine. This compound has been shown to occur in *G. pulex*, surface
381 water and sludges samples [5, 29]. Measured concentrations in the biota samples
382 ranged from <LOQ to 31.5 ng g^{-1} and in surface water, the concentrations ranged from
383 <LOQ to 272 ng L^{-1}. The highest surface water concentrations were measured at Site
384 1 (average: 225 ng L^{-1}) which also corresponded to relatively high concentrations
385 measured in *G. pulex* with an average of 16.3 ng g^{-1}. Higher concentrations of
386 carbamazepine were determined at site 6 and 8 for the *Ephemera vulgata* and *Asellus*
387 *aquaticus* samples. Site 8 surface water concentration of carbamazepine were below
388 the LOQ and site 16 averaged 92.6 ng L^{-1}. This may suggest that *E. vulgata* and *A.*
389 *aquaticus* are more sensitive than *G. pulex* to the accumulation of carbamazepine.
390 However, surface water concentrations often do not translate well into internal
391 concentrations for several reasons such as temporal variation, spatial variation and
392 migration behaviour of aquatic fauna among other influences. Additionally, the main
393 human metabolite of carbamazepine, CBZ-epoxide, was detected across 30% of the
394 biota samples. This metabolite has been detected and measured in invertebrate
395 species including *G. pulex* and *Mytilus galloprovincialis* showing conservation of
396 biotransformation pathways [39, 48]. The increased concentration of carbamazepine
397 at Site 1 *G. pulex* samples also coincided with increased detection of the epoxide
398 metabolite. However, the metabolite was not detected in *E. vulgata* larvae and was
399 minimal in *A. aquaticus* despite higher concentrations of carbamazepine measured in

400 these species. This may indicate a different sensitivity of these organisms to
401 carbamazepine through toxicokinetics, where biotransformation and elimination routes
402 are different. The mean ratio of carbamazepine to the epoxide metabolite was 8.9 in
403 the biota samples, which is closer to observed human therapeutic ratios of ~5 [49].

404 The highest measured pharmaceutical concentration across the biota samples
405 alone was for the beta-blocker propranolol (45.5 ng g^{-1} at Site 4). The concentrations
406 of propranolol in surface water ranged from <LOQ to a maximum of 27 ng L^{-1}, which
407 is significantly below (two orders of magnitude) the reported no-observed effects
408 (NOEC) and lowest-observed effects (LOEC) in fish and invertebrates [50, 51]. Other
409 beta-blockers were detected at lower concentrations and less frequently which
410 included betaxolol, salbutamol and metoprolol. The remaining beta-blockers included
411 in this method, were not detected at any site for the biota samples (timolol, nadolol
412 and bisoprolol). However, for surface water samples, bisoprolol was detected
413 frequently across all river catchments, with metoprolol and the beta-agonist salbutamol
414 less frequently detected.

415 The selective serotonin reuptake inhibitor citalopram was frequently detected
416 in biota samples at Site 7, Site 1 and Site 20, with concentrations ranging from 3.8 to
417 36.6 ng g^{-1}. The maximum concentration was determined to be 42.4 ng g^{-1} at Site 14.
418 Surface water concentrations of citalopram were often below the LOQ but were
419 determined at higher average concentrations of 14.7±10.6 ng L^{-1} for Site 1, Site 7 and
420 Site 20. Citalopram has been previously determined up to concentrations of 20.6 ng
421 g^{-1} in bivalves (*Mytilus spp.*) [52], 0.212 ng g^{-1} in fish brain tissue (*Catostomus*
422 *commersonii*) [53] and more recently was reported to reach concentrations of ~6000
423 ng g^{-1} in *Hydropsyche spp* [54]. From the literature, citalopram has been observed to
424 have low accumulation factors ranging from less <7 to 47 [38, 55]. Based on

425 occurrence data presented here, it would also likely have a low bioaccumulation factor.
426 Furthermore, the analytical method here could not distinguish between the
427 enantiomeric forms of citalopram with the S-enantiomer responsible for the
428 pharmacological action where it has also been suggested that R-enantiomer inhibits
429 this therapeutic effect. Other researchers have shown that racemic mixtures of
430 pharmaceuticals can often be enriched by either human or microbial biotransformation
431 or may remain as racemates if biodegradation does not occur [56]. Many of the
432 pharmaceuticals reported here display stereoisomerism, which is poorly understood
433 in terms of environmental risk, and is often overlooked in both fate and effect-based
434 studies [56]. The most frequently detected antibiotic was trimethoprim with measured
435 concentrations ranging from 1.5 – 4.6 ng g^{-1}. Other antibiotics detected included three
436 sulphonamides: sulfamethazine; sulfapyridine; and sulfadimethoxine. However,
437 sulfamethazine was not quantifiable in any sample and sulfadimethoxine was only
438 measured once reaching 1.7 ng g^{-1}. Bioconcentration studies for sulfamethazine in
439 *Oryzias melastigma* have ranged from <1 – 145 depending on tissue and biological
440 sex indicating that there is no or little potential for bioaccumulation [39, 57]. The low
441 bioaccumulation is likely to stem from the polarity (logP = 0.44, $logD_8$ = 0.1) and
442 ionisation state of the drug which has been shown to influence uptake in fish and
443 invertebrates [20, 58, 59]. Sulfapyridine, was also infrequently detected except at Site
444 1, with an average concentration of 4.8 ng g^{-1}. The low occurrence of the
445 sulphonamides in biota is likely due to the high polarity and metabolism of these
446 compounds.
447
448 *3.2.3 Pesticides*

449 Neonicotinoids have gained much attention recently, with the EU now enforcing a near
450 total ban on their use [60]. Few studies have determined the presence of these
451 compounds in aquatic fauna [6, 27]. Other studies have targeted these pesticides in
452 fish, but ultimately were not detected [7, 8]. However, these compounds do occur in
453 surface water and averaged at 130 ng L^{-1} across 19 studies [61]. The compounds
454 thiacloprid and acetamiprid were infrequently detected in surface water samples
455 across all sites here and remained below the LOQ. Imidacloprid was not detected at
456 any site. This agreed with a recent report on neonicotinoid contamination in UK surface
457 waters [62], which summarised that thiacloprid and acetamiprid showed low
458 contamination which is likely related to their low use as opposed to other
459 neonicotinoids such as clothiandin and thiamethoxam. The qualitative data showed
460 thiamethoxam was not detected across any sites and clothiandin was infrequently
461 detected. This contrasts data reported for thiamethoxam in the river Waveney which
462 showed concentrations reaching up to 1.03 µg L^{-1} and an average concentration of
463 ~60 ng L^{-1}. A possible reason for the disparity between the data reported here is that
464 the previous report was from a monitoring campaign in 2016. The samples collected
465 in the present study were from July 2018, following the driest period record with no
466 rain in the previous 55 days [63] suggesting that input from surface run-off and
467 leaching was likely to be minimal. Furthermore, thiamethoxam use (area treated of
468 arable crops) peaked in 2012 and has been followed by a decrease up to 2016 [62].
469 For the biota samples, acetamiprid was infrequently detected in the Waveney, but
470 consistently detected in the catchments of Alde, Deben, Gipping and Stour. However,
471 this compound was often below the LOQ and upon quantification showed
472 concentrations ranging from 0.2 – 0.7 ng g^{-1}. Thiacloprid was frequently measured in
473 the river Waveney and Deben with average concentrations of 3.3 ± 1.6 ng g^{-1} and 1.6

474 ± 1.7 ng g^{-1}. With so little data available, meaningful comparisons of neonicotinoid
475 concentrations with other pesticides in biota samples is difficult. Nonetheless,
476 concentrations measured here were in the range to that of a previous investigation
477 with thiacloprid ranging from LOQ – 21 ng g^{-1}. Out of 10 pesticides that no longer have
478 approval in the EU [64], a total of seven were detected in biota samples here (ametryn,
479 dimethametryn, fenuron, propazine, aclonifen and oxycarboxine), including three that
480 were quantifiable (ametryn, dimethametryn, fenuron). The most widespread
481 occurrence corresponded to fenuron (0.7 – 16.1 ng g^{-1}), oxycarboxine (qualitative) and
482 ametryn (LOQ – 1.9 ng g^{-1}). The compound oxycarboxine was detected with 100 %
483 frequency (Table S5) and fenuron with 86 % frequency in biota samples. Detection of
484 banned pesticides has recently been reported with atrazine (banned since 2003)
485 quantified in 63 % of samples [65]. However, there is little occurrence data available
486 for the banned pesticides detected here, but several banned pesticides including
487 fenuron, atrazine and simazine have been found to occur in UK groundwaters [66].
488 The detection of these compounds in the environment might be explained by
489 persistence and subsequent release of these compounds in sediments and/or soil [65].
490

491 *3.3 Estimating the toxic or effect pressure of contaminants in the aquatic environment*

492 It has been suggested that internalised concentrations of contaminants are more
493 appropriate for the assessment of potential risk in the environment than effect
494 thresholds based on external exposure (i.e. in the water) [1]. From the data here, we
495 estimated the internal toxic pressure (pesticides) or 'effect pressure'
496 (pharmaceuticals/drugs of abuse) [6] using predicted bioconcentration data [20, 21]
497 and the available effect data (EC_{50} or CEC) [18, 24]. This approach is analogous to
498 risk quotients (RQ) estimated from predicted environmental concentrations and

499 predicted no effect concentration (PEC/PNEC). The $logTU_{int}$ for the pesticides
500 determined ranged from approximately -7 to -2 (Figure 5a), where previous studies
501 have indicated that a logTU threshold based on water concentrations for pesticides of
502 -3 and higher can elicit adverse effects [13-15], Only one compound (oxamyl) was
503 above the threshold of logTU ≥ -3. This compound is still approved for use in the EU
504 and may indicate the potential for risk at the concentrations measured in the biota
505 samples. The EC_{50} was based on *D. magna* acute toxicity studies which have been
506 shown to be the most sensitive across all aquatic organisms that were tested.
507 However, the risk based on available evidence was concluded to be low [67]. The
508 neonicotinoids acetamiprid and thiacloprid showed low $logTU_{int}$ values of less than -
509 4.6. In comparison, Munz et al. [6] estimated thiacloprid to have a higher $logTU_{int}$ in *G.*
510 *pulex* than reported here and exceeded the threshold for several of the measured
511 samples. The disparity between the estimation of toxic pressure is that concentrations
512 of thiacloprid determined here in *G. pulex*, were relatively lower. In addition, the EC_{50}
513 value used in this study was ~10-fold larger than in the previous study. For this
514 approach EC_{50} data is often not well distributed and can vary depending on the end
515 point, experimental conditions and species used. For these reasons, it may be more
516 appropriate to include a range of the EC_{50} data available or review the quality of the
517 available literature data to give more reliable estimation of toxic pressure [68].

518 The logTU threshold value is not likely to be directly applicable to
519 pharmaceuticals, which are likely to be less toxic than pesticides by nature of their
520 design. Thus, for this work we use the term 'effect units' (EU_{int}) for pharmaceuticals,
521 as thresholds that might be associated to toxicity are unknown. Instead, CEC data are
522 used instead of $EC50_{int}$, but in themselves are not a toxicity endpoint. Substantial
523 further work would be needed to determine possible thresholds associated with TU for

different contaminant classes and for internalised concentrations, as opposed to surface water concentrations. Larger effect pressures were mainly associated with pharmaceuticals such as haloperidol that showed the highest EU_{int} (Figure 5b). The reason haloperidol has high EU_{int} values is due to the low CEC of 6.5 ng L^{-1} based on human therapeutic plasma concentrations of 1 ng mL^{-1}. Additional antipsychotic drugs including chloropromazine (CEC = 36 ng L^{-1}) and risperidone (CEC = 129 ng L^{-1}) were also estimated to have a high toxic pressure. Other neuroactive pharmaceuticals including antidepressants and anxiolytics such as alprazolam, lorazepam, citalopram and busipirone also showed higher EU_{int} which may indicate that these types of contaminants have a greater risk in the environment which has been previously suggested from surface water risk assessments [69]. This may be particularly apparent when focussing on sub-lethal endpoints such as altered behaviour phenotypes [70]. Despite its widespread occurrence, cocaine showed a low potential for an effect based on its CEC and BCF. The benefits of using CECs for pharmaceuticals is that the availability of data for human therapeutic values is greater than ecotoxicological data. In particular, EC_{50} data for 'legacy' pharmaceuticals is critically lacking. However, the use of CECs has some limitations in that a therapeutic effect does not necessarily correspond to an adverse effect and that the onset of pharmacological action may differ between humans and non-target organisms [18, 71]. Furthermore, molecular targets of pharmacological action are not always conserved between species and bioavailability may also differ between them [19, 71].

4. Conclusion

Cocaine was the most widespread contaminant found in both surface water and biota samples, but no conclusions can be drawn about the potential for adverse effects of

this compound without further work. Out of 67 compounds that could be quantitatively determined 56 were measured with the higher frequencies of detection for cocaine (100%), lidocaine (95%), alprazolam (88%), fenuron (86%) and ketamine (76%) in biota samples. In comparison for surface water samples, 50 compounds were measured including cocaine, carbamazepine, fenuron, ketamine and lidocaine, propranolol and tramadol that all had 100% detection frequency. The detection of several pesticides that no longer have approval in the EU warrants further investigation, as the sources for their input into the environment remain unclear. The total body burden of the contaminants determined in the biota samples ranged from 6.5 ng g^{-1} to 163.5 ng g^{-1} dw depending on the site. The total body burden is also an underestimate when accounting for the qualitative data, in addition to contaminants that were not targeted for in this study (including biotransformation products). Overall, whilst toxic pressure and effect pressure estimates were low in this study, the contribution of total body burden, the variability in effect data available (including lack of internal effect data) and thresholds for toxic/effect pressure are limitations to improving environmental risk assessment based on this approach. Nevertheless, the approach does support prioritisation of contaminants in the environment through the use of biomonitoring to reveal both the exposure, hazard and, ultimately, risk.

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Table 1: Method performance assessment for *G. pulex* covering all stages of the analytical workflow. Repeatability was assessed by intra-day (3 concentrations) and inter-day precision (1 concentration) and is expressed by relative standard deviation (RSD). Matrix effects were assessed at 50 ng g^{-1} (n=5) by comparing post-extraction spiked matrix matched standards to a pure analytical standard and negative values indicate suppression effects.

	Matrix Effect				Recovery	Intra-day Precision (%RSD)			Inter-day	Accuracy (%)								LOQ	LOD
	(%)		t_R (min)		(%)	25 ng g^{-1}	50 ng g^{-1}	100 ng g^{-1}	Precision (%RSD)	25 ng g^{-1}	SD	50 ng g^{-1}	SD	100 ng g^{-1}	SD	Linearity	Range	ng g^{-1}	ng g^{-1}
Compound	(n=5)	SD	(n=5)	SD	(n=5)	(n=3)	(n=5)	(n=3)	(n=3)	(n=3)		(n=3)		(n=3)		R^2	ng g^{-1}	(n=6)	(n=6)
4-fluoromethcathinone	-44	± 5	6.92	± 0.014	45	14	13	14	19	75	± 12	92	± 30	117	± 15	0.9930	3.7-500	3.7	1.2
Acetamiprid	-57	± 8	6.92	± 0.001	92	1	15	7	9	102	± 1	98	± 6	96	± 7	0.9997	0.2-500	0.2	0.08
Alprazolam	-46	± 10	10.58	± 0.011	71	8	7	6	13	80	± 7	80	± 12	90	± 6	0.9976	1.2-250	1.2	0.4
Ametryn	-62	± 8	11.76	± 0.002	80	12	8	8	7	72	± 9	111	± 8	96	± 12	0.9950	1.3-250	1.3	0.4
Antipyrin	-52	± 6	5.43	± 0.001	72	1*	10	7	16	84	± 11	125	± 17	126	± 9	0.9973	6.8-500	6.8	1.4
Benzotropine	-70	± 8	11.89	± 0.002	65	9	5	6	15	74	± 7	106	± 12	97	± 5	0.9901	0.6-250	0.6	0.2
Benzoylecgonine	-58	± 2	4.65	± 0.017	71	4	7	8	15	102	± 9	95	± 11	116	± 0	0.9982	0.6-500	0.6	0.2
Betaxolol	-53	± 8	10.29	± 0.014	94	3	11	3	22	100	± 18	88	± 18	80	± 4	0.9944	0.5-250	0.5	0.2
Bezafibrate	-61	± 6	6.96	± 0.016	63	8	13	6	15	89	± 12	97	± 7	108	± 6	0.9984	9.2-500	9.2	3.0
Bisoprolol	-28	± 17	9.54	± 0.066	93	5	6	10	13	85	± 1	101	± 12	86	± 8	0.9898	0.9-250	0.9	0.3
Busipirone	-50	± 9	11.26	± 0.002	76	7	3	6	11	105	± 14	97	± 11	102	± 12	0.9956	1.1-500	1.1	0.4
Carbamazepine	-48	± 8	9.59	± 0.001	67	15	5	8	14	92	± 11	86	± 11	101	± 4	0.9971	0.9-500	0.9	0.3
CBZ_epoxide	-38	± 3	7.70	± 0.001	85	2	12	5	16	92	± 2	113	± 13	89	± 6	0.9988	0.6-500	0.6	0.2
Chloropromazine	-82	± 5	12.66	± 0.008	91	11	5	12	*12	99	± 11	98	± 28	140	± 8	0.9928	1.8-500	1.8	5.5
Citalopram	-71	± 5	10.62	± 0.001	76	11	10	13	24	82	± 15	107	± 15	78	± 11	0.9974	2.6-250	2.6	0.9
Cocaine	-37	± 7	9.97	± 0.014	80	8	2	1	9	96	± 7	90	± 11	114	± 12	0.9973	0.5-500	0.5	0.2
Cotinine	30	± 8	3.78	± 0.011	62	15	8	3	13	93	± 21	78	± 19	109	± 3	0.9986	2.6-500	2.6	0.8
Cycluron	-34	± 13	10.73	± 0.002	77	2	5	7	8	109	± 2	100	± 8	104	± 7	0.9997	1.5-500	1.5	0.5
Diazepam	-60	± 10	11.95	± 0.002	87	9	11	8	13	86	± 6	87	± 10	88	± 7	0.9963	0.3-250	0.3	0.1
Dimethmetryn	-50	± 10	13.02	± 0.011	76	5	10	7	7	86	± 3	89	± 2	121	± 8	0.9958	0.1-250	0.1	0.03
Diphenydramine	-51	± 9	10.94	± 0.011	76	12	12	10	16	94	± 11	111	± 16	92	± 8	0.9936	1.8-250	1.8	0.6
Ethirimol	-81	± 4	8.93	± 0.015	81	10	9	11	11	89	± 6	87	± 7	94	± 9	0.9935	1.7-500	1.7	0.6
Fenuron	-56	± 9	6.25	± 0.016	79	9	10	3	11	105	± 12	117	± 10	94	± 3	0.9991	0.6-500	0.6	0.2
Flutamide	-42	± 16	12.27	± 0.002	80	9	7	2	16	90	± 5	96	± 11	75	± 1	0.9942	0.2-250	0.2	0.1
Haloperidol	-79	± 7	11.26	± 0.002	83	2	5	6	16	104	± 2	92	± 17	113	± 8	0.9935	5.3-250	5.3	1.8
Hyrochlorothiazide	-77	± 2	3.90	± 0.011	77	5*	13	2	19	100	± 33	85	± 16	118	± 13	0.9952	2.1-500	2.1	0.7
Ketamine	-56	± 6	11.00	± 0.002	54	10	5	7	9	85	± 8	94	± 2	110	± 13	0.9949	1-500	1.0	0.3
Ketoprofen	10	± 11	6.21	± 0.001	69	15	9	10	14	106	± 12	90	± 8	117	± 11	0.9970	15.3-500	15.3	5.0
Ketotifen	-55	± 11	10.74	± 0.002	70	11	8	10	16	81	± 6	104	± 16	99	± 10	0.9955	3.9-250	3.9	1.3
Levamisole	-58	± 4	7.76	± 0.014	61	12	8	6	13	116	± 13	83	± 6	118	± 7	0.9894	4.0-500	4.0	1.3
Levocabastine	-42	± 8	8.16	± 0.015	97	16	10	6	15	71	± 8	97	± 6	113	± 6	0.9934	0.3-250	0.3	0.1
Lidocaine	-46	± 6	11.46	± 0.002	67	1	7	7	13	104	± 1	91	± 8	115	± 8	0.9956	0.7-500	0.7	0.2
Lincomycin	-18	± 5	7.80	± 0.009	82	5	4	9	11	112	± 9	93	± 8	100	± 10	0.9968	4.5-500	4.5	1.5
Lorazepam	-26	± 14	10.44	± 0.013	71	12	10	8	22	81	± 6	85	± 9	118	± 8	0.9895	1.9-250	1.9	0.6
MDMA	-59	± 6	6.10	± 0.042	64	6	9	10	16	111	± 7	101	± 9	97	± 9	0.9995	1.9-500	1.9	0.6
Mephedrone	-12	± 6	7.65	± 0.031	69	12	6	9	14	95	± 8	72	± 13	91	± 3	0.9943	10.5-500	10.5	3.5
Mephosfolan	-49	± 12	11.52	± 0.002	69	7	9	12	7	91	± 14	91	± 3	98	± 11	0.9934	1.4-500	1.4	0.4
Methamphetamine	-61	± 5	6.25	± 0.061	65	4	12	1	12	109	± 16	123	± 17	115	± 10	0.9981	1.7-500	1.7	0.6
Methcathinone	-56	± 1	6.24	± 0.016	43	9	13	2	15	83	± 6	83	± 10	117	± 19	0.9856	3.9-250	3.9	1.3
Methedrone	-63	± 3	6.56	± 0.015	73	1	9	15	11	70	± 1	122	± 8	93	± 15	0.9991	2.9-500	2.9	1.0
Methylphenidate	-74	± 3	9.31	± 0.055	84	15	5	10	11	89	± 11	100	± 15	88	± 8	0.9944	0.2-250	0.2	0.05
Metoprolol	-81	± 12	7.64	± 0.051	84	13	14	5	20	81	± 11	89	± 9	100	± 5	0.9929	2.8-500	2.8	0.9
Nicotine	59	± 17	6.18	± 0.032	51	10	8	13	13	73	± 12	115	± 23	91	± 21	0.9859	2.6-250	2.6	0.9
Nadolol	-20	± 10	5.13	± 0.022	77	5	12	13	12	106	± 18	99	± 2	112	± 10	0.9949	2.2-500	2.2	0.7
Nordiazepam	-13	± 24	11.18	± 0.016	78	7	13	15	15	95	± 5	83	± 9	108	± 5	0.9972	2.4-250	2.4	0.8
Oxamyl	-20	± 23	6.17	± 0.001	90	11	14	18	16	124	± 33	113	± 25	100	± 19	0.9956	1.9-500	1.9	0.6
Oxazepam	-10	± 19	10.19	± 0.012	86	15	12	1	18	81	± 15	122	± 14	76	± 1	0.9948	3.2-250	3.2	1.1
Pirenzipine	-41	± 2	5.33	± 0.016	74	5	3	7	17	82	± 7	97	± 11	119	± 13	0.9917	0.4-500	0.4	0.1
Prometon	-51	± 11	11.22	± 0.002	71	5	2	9	7	92	± 4	104	± 7	100	± 9	0.9971	0.9-500	0.9	0.3
Propamocarb	-65	± 8	5.20	± 0.022	47	3*	8	1	8	64	± 5	63	± 5	92	± 13	0.9934	0.6-500	0.6	0.2
Propazine	-43	± 8	11.85	± 0.015	70	7	12	6	13	102	± 9	80	± 3	93	± 19	0.9919	3.5-250	3.5	1.2
Propranolol	-56	± 12	9.96	± 0.015	76	19	15	5	19	74	± 5	74	± 10	107	± 6	0.9990	7.1-250	7.1	2.4
Risperidone	-76	± 4	10.28	± 0.001	73	1	8	5	15	101	± 5	104	± 12	100	± 1	0.9924	0.4-500	0.4	0.1
Rizatriptan	-47	± 5	4.69	± 0.021	55	14	14	8	13	101	± 13	118	± 6	122	± 9	0.9955	3-500	3.0	1.0
Salbutamol	7	± 10	3.30	± 0.016	26	13	10	15	24	88	± 16	96	± 13	117	± 16	0.9987	7-500	7.0	2.0
Sulfadimethoxine	-80	± 3	4.37	± 0.016	76	15	7	6	17	87	± 20	87	± 6	124	± 30	0.9986	0.9-500	0.9	0.3
Sulfamethazine	-75	± 3	4.70	± 0.011	78	6	12	10	23	90	± 13	76	± 15	118	± 12	0.9981	1-500	1.0	2.9
Sulfapyridine	-61	± 7	4.27	± 0.014	77	6	15	12	17	108	± 3	92	± 15	125	± 3	0.9873	3-500	3.0	1.0
Tacrine	-72	± 4	6.51	± 0.060	70	5	12	11	15	93	± 5	104	± 20	85	± 10	0.9912	1.6-500	1.6	0.5
Tamsulosin	-39	± 14	9.91	± 0.001	78	1	15	16	17	72	± 4	121	± 12	84	± 12	0.9976	2.6-500	2.6	0.8
Temazepam	-33	± 12	11.13	± 0.002	81	9	6	9	13	109	± 7	88	± 10	100	± 9	0.9936	0.5-500	0.5	0.2
Thiacloprid	-	-	7.09	± 0.015	100	7	11	3	9	119	± 9	83	± 7	117	± 3	0.9993	0.2-500	0.2	0.07
Timolol	-45	± 3	7.24	± 0.050	72	15	12	10	16	73	± 14	98	± 21	107	± 10	0.9940	25.2-250	25.2	8.3
Tramadol	-67	± 9	8.53	± 0.078	91	10	15	12	16	109	± 12	105	± 14	82	± 10	0.9957	3.1-500	3.1	1.0
Trimethoprim	-67	± 5	6.06	± 0.001	73	20	8	3	16	57	± 20	92	± 11	121	± 15	0.9915	0.1-500	0.1	0.04
Verapamil	-67	± 8	12.03	± 0.002	67	13	8	8	15	82	± 10	109	± 19	117	± 9	0.9904	2.4-250	2.4	0.8
Warfarin	-71	± 4	6.17	± 0.012	75	4	14	9	18	74	± 3	109	± 16	96	± 8	0.9959	0.9-500	0.9	0.3

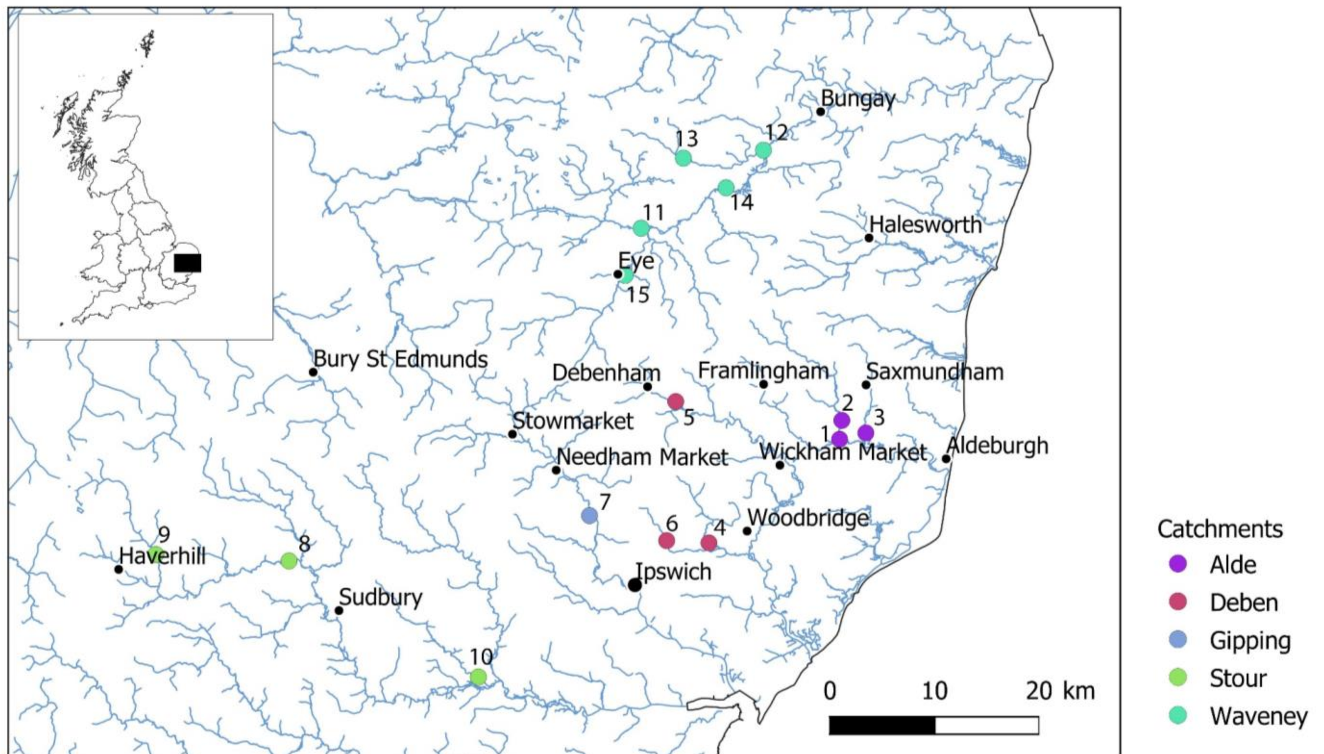

Figure 1: Sampling locations of collected biota and surface water samples within the respective river catchments of Suffolk. Black dots indicate urbanised areas.

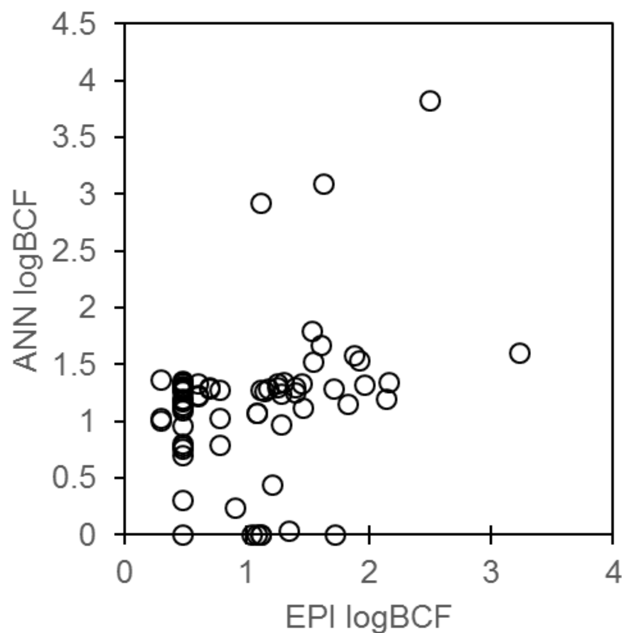

Figure 2**:** Comparison of predicted logBCF data from EPI suite and ANN model, for individual raw values please see SI Table S5.

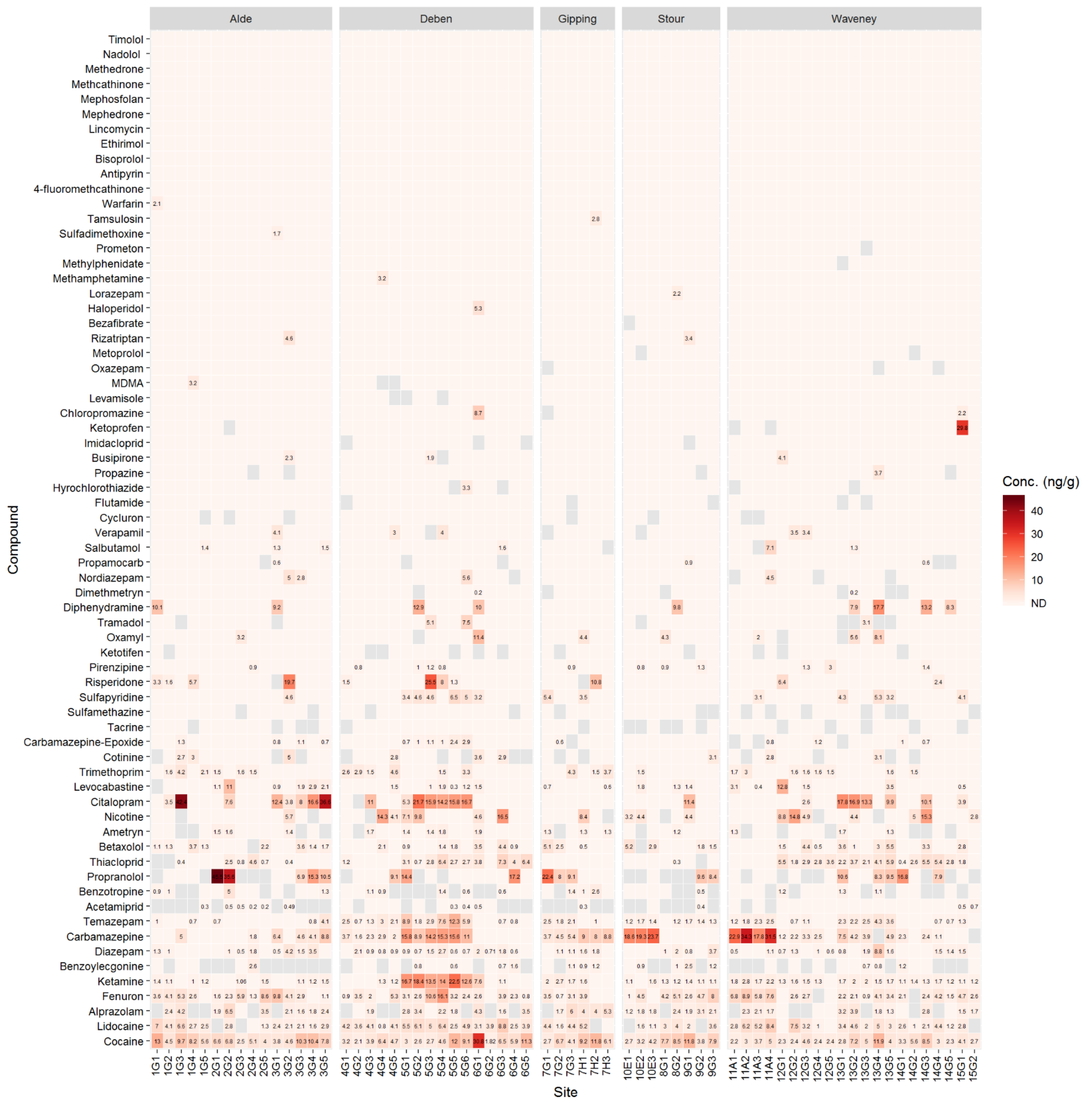

Figure 3: Heatmap of compounds determined in the biolgoical samples that showed acceptable method performance. G, H, A or E indicate the sampled species *G. pulex*, *H. pellucidula*, *A. aquaticus* or *E. vulgata*, respectively. Grey tiles indicate compounds that were detected but below the limits of quantification.

Figure 4: Heatmap of compounds determined in the surface water samples. All sites were samples in triplicate except for Site 10 (n=2). Grey tiles indicate compounds that were detected but below the limits of quantification, decimal points indicate site replicates.

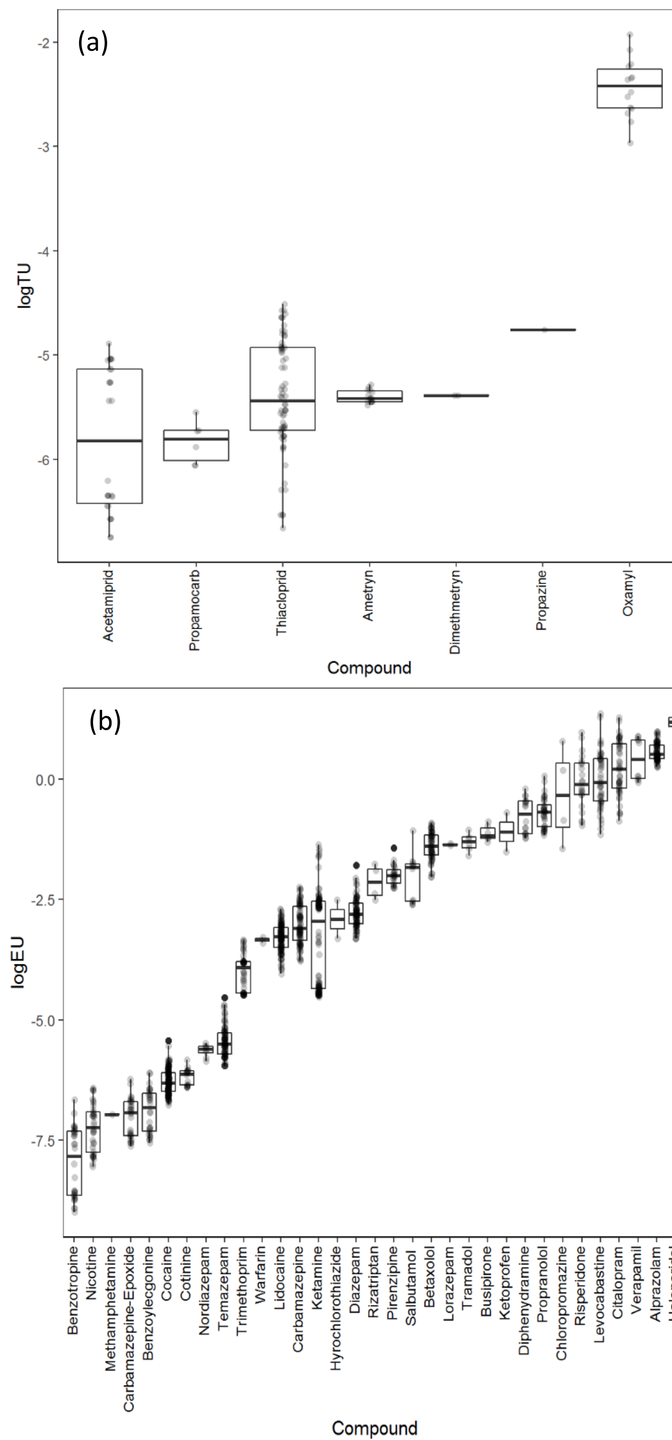

Figure 5: (a) Toxic pressure analysis of measured pesticides quantified by internal toxic units (logTU) (b) effect pressure analysis of measured pharmaceuticals and illicit drugs quantified by internal effect units (logEU)