

This is a repository copy of *Novel Approach for Characterizing pH-Dependent Uptake of Ionizable Chemicals in Aquatic Organisms*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/117208/>

Version: Accepted Version

Article:

Karlsson, Maja Viktoria, Carter, Laura Jayne, Agatz, Annika orcid.org/0000-0003-3228-8822 et al. (1 more author) (2017) Novel Approach for Characterizing pH-Dependent Uptake of Ionizable Chemicals in Aquatic Organisms. *Environmental science & technology*. pp. 6965-6971. ISSN 1520-5851

<https://doi.org/10.1021/acs.est.7b01265>

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

This document is confidential and is proprietary to the American Chemical Society and its authors. Do not copy or disclose without written permission. If you have received this item in error, notify the sender and delete all copies.

A novel approach for characterising pH-dependent uptake of ionisable chemicals in aquatic organisms

Journal:	<i>Environmental Science & Technology</i>
Manuscript ID	es-2017-012652.R2
Manuscript Type:	Article
Date Submitted by the Author:	24-May-2017
Complete List of Authors:	Karlsson, Maja; University of York, Environment Department Carter, Laura; University of York, Environment Department Agatz, Annika; University of York, Environment Department Boxall, Alistair; University of York, Environment Department

SCHOLARONE™
Manuscripts

1 **A novel approach for characterising pH-dependent uptake of ionisable chemicals in**
2 **aquatic organisms**

3 Maja V. Karlsson¹, Laura J. Carter¹, Annika Agatz¹, Alistair B.A. Boxall^{1*}

4 ¹ – Environment Department, University of York, Heslington, York, YO10 5NG, UK

5 * - Corresponding author, email: Alistair.boxall@york.ac.uk

6

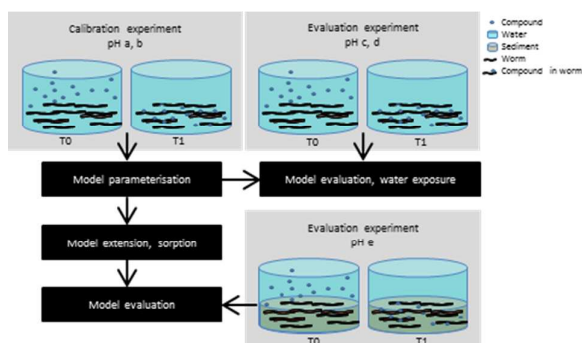
7

8 Abstract

9 Here, we present and evaluate a combined experimental and modelling approach for
10 characterising the uptake of ionisable chemicals from water and sediments into aquatic
11 organisms under different pH conditions. We illustrate and evaluate the approach for two
12 pharmaceuticals (diclofenac and fluoxetine) and one personal care product ingredient
13 (triclosan) for the oligochaete *Lumbriculus variegatus*. Initially, experimental data on the
14 uptake of the three chemicals at two pH values were fitted using a toxicokinetic model to
15 derive uptake and depuration constants for the neutral and ionised species of each
16 molecule. The derived constants were then used to predict uptake from water and sediment
17 for other pH conditions. Evaluation of predictions against corresponding experimental data
18 showed good predictions of uptake for all test chemicals from water for different pH
19 conditions and reasonable predictions of uptake of fluoxetine and diclofenac from a
20 sediment. Predictions demonstrated that the level of uptake of the study chemicals, across
21 pH ranges in European streams, could differ by up to a factor of 3035. Overall, the approach
22 could be extremely useful for assessing internal exposure of aquatic organisms across
23 landscapes with differing pH. This could help support better characterisation of the risks of
24 ionisable chemicals in the aquatic environment.

25 Keywords

26 *Lumbriculus variegatus*; toxicokinetic modelling; pH; sorption; ionisable chemicals



27

28 TOC Art

29

30 Introduction

31 A wide range of pharmaceuticals and ingredients used in personal care products has been
32 detected in natural environments across the globe.¹⁻⁵ The presence of these chemicals in the
33 environment has prompted concerns over potential toxic effects in non-target organisms. For
34 a chemical to elicit an effect in an organism, it must usually be first taken up from the
35 ambient environment. Understanding the internal exposure of a chemical can provide
36 valuable insights to inform our understanding of the effects of chemicals in organisms. The
37 information can also help in extrapolating from effects in standard laboratory studies to
38 effects across different exposure scenarios.^{6, 7} For example, for active pharmaceutical
39 ingredients (APIs), it has been suggested that by understanding the internal concentrations
40 in organisms in the natural environment and the presence/absence of the target receptors
41 and pathways for the API, it may be possible to predict potential ecological effects of
42 pharmaceuticals based on preclinical and clinical pharmacological data that are produced in
43 the drug development process.⁸

44 It has been estimated that between 85 and 95 % of APIs are ionisable⁹ and therefore there
45 is the potential that the behaviour of these chemicals in the environment may be affected by
46 changes in pH. As the pH of natural water bodies ranges from 2.2 - 9.8^{10, 11}, the fate and
47 effects of APIs could vary significantly across broad landscapes. A number of studies have
48 explored the effects of pH on the uptake and toxicity of ionisable APIs from/in water.^{12, 13}
49 Nakamura et al.¹² investigated the toxicity and bioconcentration of fluoxetine, a weak base,
50 in Japanese medaka (*Oryzias latipes*) at pH values of 7, 8 and 9. Median lethal
51 concentrations ranged from 0.2 mg/L at pH 9 to 5.5 mg/L at pH 7. The toxicological
52 observations were explained by differences in bioconcentration factors (BCF) at different pH
53 values which ranged from 13 at pH 7 to 330 at pH 9.

54 Modelling approaches have been proposed to estimate the effects of environmental pH on
55 accumulation¹⁴⁻¹⁶ and effects¹⁷ of ionisable chemicals, including APIs, in organisms. For
56 example, Meredith-Williams, et al.¹⁴ suggested that the uptake of APIs into invertebrates

57 (*Gammarus pulex* and *Notonecta glauca*) could be based on a chemical's pH-corrected
58 liposome-water partition coefficient ($\text{Log } D_{\text{lip-water}}$). Nichols, et al.¹⁵ modelled the uptake of the
59 weak base, diphenhydramine, into fish plasma using a model that accounts of acidification at
60 the gill surface. The model assumed that the undissociated form of the molecule diffuses
61 freely across the branchial epithelium. The membrane transport of the cation is estimated in
62 a relation to the neutral form using a term varying from 0 to 1. Fu, et al.¹⁶ proposed
63 regression equations that estimate bioconcentration factors of acids and bases based on the
64 octanol-water partition coefficient (K_{ow}) and the logarithmic acid dissociation constant (pKa)
65 of a molecule.

66 These previous studies have focused on the situation where the external and internal
67 concentrations of the ionisable chemical are in equilibrium and have typically taken an overly
68 simplistic approach to dealing with the ionised form of the molecules. By using a more
69 mechanistic approach that considers the rate of uptake of the ionised and neutral species of
70 a molecule, it may be possible to better estimate the internal exposure of an organism over
71 time for varying pH conditions typically found in the natural environment. This could be
72 invaluable for assessing the degree of risk of these chemicals.

73 Here, we present a new combined experimental and modelling approach for characterising
74 the uptake of ionisable chemicals, such as APIs, in aquatic invertebrates over time for
75 different pH conditions. A schematic of the model underlying the approach is shown in
76 Figure 1. The approach characterises the uptake of an ionisable chemical into an organism
77 over time based on the fraction of the ionised and non-ionised species of a molecule in water
78 for the pH of interest and uptake rate constants for the neutral and ionised form of the
79 molecule which are derived from experimental uptake studies performed at two pH values. It
80 is assumed that the internal pH of the organism is constant, and unaffected, by the external
81 pH so a constant depuration rate constant for the ion and neutral form is used. If the
82 invertebrate is a sediment-dwelling organism then uptake can be characterised based on the
83 concentration of the ionisable chemical in the pore water, which is derived from the

84 concentration of the compound in the sediment and the sediment sorption coefficient (K_d).
85 This is based on the assumption that uptake is occurring only from the pore water. We
86 illustrate and evaluate the approach for two ionisable APIs and one ionisable personal care
87 product ingredient and the oligochaete *Lumbriculus variegatus* for water-only exposures at a
88 range of pH values and using previously published data on uptake of the chemicals from one
89 sediment type.

90 **Methods**

91 *Underlying model*

92 The kinetic model used is based on a first order one compartment toxicokinetic model that is
93 used to describe the internal concentrations within an organism over time (dC_{int}/dt)
94 [mass/volume] based on exposure medium concentrations and uptake and depuration rates
95 (Eq. 1). Here we extend this toxicokinetic model to account for differences in uptake of the
96 neutral and ionised forms over time (Eq. 2).

97

$$98 \quad dC_{int} / dt = k_{in} * C_w - k_{out} * C_{int} \quad \text{Equation 1}$$

99

$$100 \quad dC_{int} / dt = (k_{in-ion} * (f_{ion} * C_w) + k_{in-neut} * (f_{neut} * C_w)) - k_{out} * C_{int} \quad \text{Equation 2}$$

101

102 Where: C_w [mol/mL] is the concentration of the ionisable chemical in the water; C_{int} [mol/g] is
103 the concentration of the chemical in the organism; k_{in-ion} [d^{-1}] is the uptake rate constant of
104 the ionic species of the chemical; $k_{in-neut}$ [d^{-1}] is the uptake rate constant for the neutral form
105 of the chemical; f_{ion} [-] is the fraction of the chemical in the ionised form at the test pH; f_{neut} [-]
106 is the fraction of the neutral form of the chemical at the test pH; and k_{out} [d^{-1}] is the
107 depuration rate constant. The depuration rate constant is not altered in comparison to the
108 original model because the pH within the organism is assumed to be independent of the

109 external pH and thus fractioning of the chemical into the ionised and neutral form is constant
110 within the organism and the depuration rate constant is independent of pH alterations once
111 derived. This assumption had to be made because, to our knowledge, no information is
112 available on the internal pH of *L. variegatus* and the circumstances on if, when and to what
113 extent this pH changes over the life span or is independent of external pH.

114 The uptake and depuration rates needed to parameterise the model are obtained from
115 uptake and depuration experiments on the organism of interest. The tests need to be
116 performed at a minimum of two pH values within the naturally occurring environmental pH
117 ranges. One of the pH values needs to be chosen so that the test chemical is either fully or
118 not ionised to allow the parameterisation of k_{in_ion} and k_{in_neut} in a two-point calibration. The
119 Henderson Hasselbach Equation is used to estimate the fraction of dissociation of the study
120 chemicals at each of the test pH values. By fitting Equation 2 to experimental data for both
121 pH conditions, k_{in_neut} , k_{in_ion} and k_{out} can be derived. These rate constants can then be used,
122 in conjunction with ionisation predictions from the Henderson Hasselbach Equation, in the
123 toxicokinetic equation to predict uptake from water for other pH conditions.

124 If the organism of interest is a sediment-dwelling organism then concentrations in the
125 organism can also be estimated according to concentrations in pore water of a known pH
126 based on the sediment-water distribution coefficient K_d (L/Kg) of the chemical for the
127 sediment of interest which can be obtained from batch sorption studies based on the OECD
128 106 Batch Equilibrium Method.¹⁸ Concentrations of test chemicals in sediment over time are
129 used, alongside the sorption coefficient, to estimate concentrations in the sediment pore
130 water over time using Equation 3.

131

$$132 \quad C_{pw} = C_{sed} / ((K_d * (\%sed / \%water) * bulk\ density) + 1) \quad \text{Equation 3}$$

133

134 Where C_{pw} and C_{sed} are the concentrations of the chemical in pore water [mol/mL] and
135 sediment [mol/g ww], respectively, and %*sed* and %*water* are calculated based on the
136 moisture content of the sediment.

137 *Illustration and evaluation of the approach for diclofenac, fluoxetine and triclosan*

138 To illustrate and test the approach, we performed studies into the uptake and depuration of
139 diclofenac (a non-steroidal anti-inflammatory compound), fluoxetine (an antidepressant) and
140 triclosan (an antimicrobial compound). Studies were done using water at four pH values –
141 two of these being used to derive uptake and depuration constants for the neutral and ionic
142 species of each molecule and two being used to test the predictive power of the approach
143 for other test conditions. A previous dataset on uptake of the study chemicals from sediment
144 was used to evaluate whether the method can be extended to predict uptake from the
145 sediment compartment.

146 Test chemicals

147 Experimental studies were done using ^{14}C -labelled versions of the test chemicals, ranging in
148 specific activity from 2.04 and 2.43 GBq mmol⁻¹. Diclofenac was obtained from Perkin Elmer
149 (Boston, USA), fluoxetine was obtained from American Radiolabelled Chemicals (St Louis,
150 USA), and triclosan was obtained from Unilever (Colworth, UK).

151 Test organism

152 Animals were initially reared in 20 L glass aquaria containing artificial pond water (APW,
153 Naylor et al. 1989), at 20 ±2 °C, using a 16:8 h light:dark cycle. Shredded unbleached tissue
154 paper was used as a substrate and the culture water was renewed once a week. The
155 cultures were fed with ground fish food (Tetramin, Tetra Werke, Melle, Germany) twice a
156 week.

157 Uptake and depuration studies

158 Uptake and depuration rates of the study chemicals into/from *L. variegatus* were determined
159 in artificial pond water (APW) and soft standard reference water (SRW) adjusted to either pH

160 5.5, 7 and 8.5 based on an approach recommended by the USEPA¹⁹. The pH was not
161 buffered or manipulated in the APW (pH 7.4) treatment. For the SRW treatments, a
162 NaH_2PO_4 was used and the pH was maintained for the duration of the uptake and
163 depuration phase through the addition of either 0.1 M HCl or NaOH.

164 Prior to the studies, the *L. variegatus* were acclimatized to the test conditions for 18 h. For
165 the uptake studies, animals were then exposed in groups of 10 animals, contained in 40 ml
166 of either APW or the SRW at the different pH values, to between 3 – 12 nmol l^{-1} of test
167 chemical for 3, 6, 12, 24 or 48 h. All test concentrations were below toxicological thresholds.
168 For the depuration studies, groups of animals were exposed to the test chemical for 48 h
169 after which time they were transferred to either APW or pH-adjusted SRW for 3, 6, 12, 24 or
170 48 h. Three replicates per time point and pH treatment were used. The study temperature
171 was 20 ± 2 °C and the beakers were kept in the dark throughout the test to minimize
172 potential photodegradation of the test chemical. Control beakers containing SRW and radio-
173 labelled test chemical were used to monitor sorption to the jars. At the end of the exposure,
174 samples of the test media were taken for chemical analysis. Exposed worms were rinsed
175 with distilled water, blotted dry on tissue paper, weighed and then analysed.

176 Chemical analysis

177 Concentrations of the study chemicals in test media and worm extracts were determined
178 using Liquid Scintillation Counting (LSC) using a Beckman LS 6500 LSC counter (Beckman
179 Coulter Inc., Fullerton, USA). For the analysis of test media, 1 ml of sample was taken and
180 placed into a 20 ml scintillation vial and 10 ml Ecoscint A scintillation cocktail (National
181 Diagnostics) was added. For the analysis of worm samples, animals were placed in 20 ml
182 scintillation vials, 2 ml of tissue solubilizer (Soluene®-350, Perkin Elmer, Waltham,
183 Massachusetts) was then added and the vials were left for 24 h to allow the worm tissue to
184 dissolve completely. Prior to scintillation counting, 10 ml of Hionic Fluor scintillation cocktail
185 (Perkin Elmer) was added to the vials.

186 Samples were counted three times for 5 min. Counts were corrected for background activity
187 by using blank controls. Counting efficiency and colour quenching were corrected using the
188 external standard ratio method. A mass balance was performed to account for all
189 radioactivities present in the experiments by summing the mass of chemical contained in the
190 organism and in the test media for each treatment and timepoint.

191 Parameterisation and testing of the model against experimental data

192 For the water-only studies, for each chemical tested, there was one experiment that was
193 conducted at a pH where the molecule was almost fully dissociated (diclofenac, pH 8.5;
194 fluoxetine, pH 5.5) or non-dissociated (triclosan, pH 5.5). These experiments were used to fit
195 the depuration rate constant and the uptake rate constant (k_{in_ion} for diclofenac and fluoxetine
196 and k_{in_neut} for triclosan) for the chemical to measured internal concentrations by fixing the
197 fraction of ionisation to either 0 or 1. The experiment with the most deviating pH (i.e., the
198 highest variation in the fractioning of ionisation) was then used to fit the 2nd uptake rate
199 constant keeping the prior fitted uptake rate constant and the depuration rate constant fixed
200 and adjusting the fraction of ionisation and neutralisation. Measured pH values were used in
201 these calculations. Modelling was conducted in OpenModel V 2.4.2. (<http://openmodel.info/>)
202 using the Runge-Kulta (4th Order) ordinary differential equation method (with Monte Carlo
203 simulations to obtain the 95% confidence interval and the Nash–Sutcliffe Efficiency
204 calculation for goodness of fit indication where a value ≥ 0 shows an acceptable fit/prediction
205 and a value < 0 indicates an unacceptable fit) using the full data set and also using the
206 minimised design method described in Carter et al.²⁰

207 These fitted rate constants and the fractions of ionisation, derived from the pH
208 measurements, in the other two experiments were then used to predict the internal
209 concentration over time in the uptake studies performed at the other pH values of 7.0 (SRW)
210 and 7.4 (APW) using both the full and minimised methods.

211 To evaluate the approach for chemical uptake from sediment, we used data from a
212 previously reported study into the uptake and depuration of diclofenac, fluoxetine and

213 triclosan into *L. variegatus* from a sediment obtained from Buttercrambe in Yorkshire ²¹ (See
214 Supporting Information Section 1 for more details). The pH of the sediment was 7.67 and the
215 bulk density of the test sediment was estimated to be 1.16 g/mL using the approach
216 proposed by Avnimelech, et al. ²². The sorption coefficient between water and the sediment
217 was obtained using a batch sorption test and were found to be 4.2, 422.5 and 241.2 for
218 diclofenac, fluoxetine and triclosan respectively. A full description of the methods used to
219 calculate the sediment sorption coefficients is provided in the Supporting Information
220 (Section 2). The sorption data were used to estimate concentrations of the study chemicals
221 in pore water over the duration of the study (Eq. 3) and internal concentrations in the
222 organisms were then estimated from the pore water concentration using the same approach
223 as used in the water only studies (Eq. 2). Experimental observations were then compared to
224 the predictions.

225 **Results and discussion**

226 *Uptake and depuration experiments in Lumbriculus variegatus*

227 The pH in the APW treatments ranged from 7.5 – 8.3. For the SRW, the pH of the pH 5.5
228 and 7 treatments remained within ± 0.3 pH units of the nominal value. For the pH 8.5
229 treatment, measured pH decreased by up to 1.1 pH unit during the experiment. Radioactivity
230 in the media in the chemical controls, containing test chemical and aqueous media only, was
231 stable for the duration of the study indicating that there was no sorption to the vessels.
232 Losses of activity from the water phase in the beakers with organisms could be explained by
233 uptake into the study organisms. Mass balance calculations showed recoveries of greater
234 than 89% of the applied radioactivity in the different treatments (Supporting Information,
235 Section 3, Table S1). No mortality was observed either in the treatments or in the controls
236 during the uptake or depuration phase.

237 The uptake and depuration studies at different pH values demonstrate the importance of
238 exposure medium pH for predicting ionisable chemical uptake into non-target organisms. At

239 the end of the 48 h uptake phase 47 and 37 fold differences were seen between the internal
240 concentrations in *L. variegatus* in the highest and lowest pH treatments for diclofenac and
241 fluoxetine, respectively whereas the fraction of ionisation only changed by 3 and 2%,
242 respectively. These experimental results demonstrate that the uptake of ionisable chemicals
243 can, in certain circumstances, be extremely sensitive to changes in exposure medium pH
244 where the pKa value of a chemical falls within the environmentally relevant pH range of 2.2 –
245 9.8.¹⁰ The hydrophobicity and value of the chemical pKa in relation to environmental pH
246 ranges is important – for diclofenac which is an acid with a pKa towards the lower end of
247 typical environmental pH, large differences in uptake and toxicity might be expected across
248 environmental pH values whereas for triclosan, which is a hydrophobic acid with a pKa
249 towards the upper end of the environmental pH range, lower variability in uptake might be
250 expected as the chemical will not be as extensively ionised and the neutral form would be
251 the dominant species.

252 *Evaluation of the modelling approach*

253 The first order one compartment model was successfully fitted (success being indicated by
254 Nash-Sutcliffe Efficiency values well above 0 (See Supporting Information Section 4 Table
255 S2) to the uptake and depuration measurements for the diclofenac, fluoxetine and triclosan
256 treatments for pH 5.5 and 8.5 (Figure 2). Resulting uptake parameters for the neutral and
257 ionised species and the combined depuration rates of the chemicals, obtained using both the
258 full and minimised methods, are provided in Table 1. For diclofenac and fluoxetine the
259 uptake rate constants for the ionised form of the molecules were more than three orders of
260 magnitude lower than the corresponding neutral form. Despite the fact that it is typically
261 assumed that the uptake of the ionic form of a molecule is lower than the neutral form (as
262 observed for diclofenac and fluoxetine), for triclosan, this was found not to be the case with
263 the uptake rates for the neutral and ionised forms of the molecule being similar. The triclosan
264 findings are similar to previous observations into the uptake of chlorinated phenols at fish

265 gills where uptake at different pH values was similar even though the degree of ionisation of
266 the chlorinated phenols at the pH values studied was very different.²³

267 The full and the minimised methods provided similar predictions demonstrating that the
268 minimised approach can be used to derive uptake and depuration rate constants for the
269 neutral and ionic species of a molecule and thus reduce the amount of experimental effort
270 (by a factor of four) needed in studies of this type. Predictions of concentrations in *L.*
271 *variegatus* based on the derived $K_{in-neut}$ and K_{in-ion} values to estimate uptake from water for
272 the other pH conditions (i.e. pH 7 and 7.4) are shown in Figure 2. Overall the model
273 performed best for triclosan followed by fluoxetine and diclofenac. Whilst predictions, using
274 rate constants derived using both the full and minimised approaches, accurately matched
275 observations for triclosan (Nash-Sutcliffe Efficiency > 0.56) the model significantly
276 underestimated internal concentrations of fluoxetine and diclofenac at pH 7.4 (Nash-Sutcliffe
277 Efficiency < 0) and fluoxetine at pH 7.0 when rate constants using the full approach were
278 used and significantly underestimated internal concentrations of diclofenac and fluoxetine at
279 pH 7.4 when rate constants obtained using the minimised approach were used. Even so,
280 predicted internal concentrations of fluoxetine concentrations, obtained using the model,
281 were within a factor of two of experimental values while internal concentration predictions for
282 diclofenac were within a factor of four of experimental values. Given the large observed
283 range seen in the uptake experiments for fluoxetine (a 37 fold difference between pH 5.5
284 and pH 8.50) and diclofenac (a 47 fold difference between pH 5.5 and 8.5), the model
285 predictions seem reasonable and useful for use in spatial environmental risk assessments.

286 Comparison of the model predictions of internal concentrations of the chemicals for the
287 sediment studies with measured concentrations in *L. variegatus* (Figure 3) showed that the
288 approach worked reasonably well for diclofenac and fluoxetine with predictions being less
289 than a factor of five lower than empirical observations. For triclosan, however, predicted
290 concentrations in the worms were 10-15 times lower than the experimental observations.
291 The mismatch for all three chemicals might be explained by differences between the

292 physico-chemical characteristics of the sediment pore water and the media used in the
293 water-only studies. Previous studies with neutral organic chemicals have shown that
294 dissolved organic carbon, which will be present in the sediment water, can enhance the
295 diffusive mass transfer of compounds.²⁴ It is also possible that the sorption coefficients used,
296 which were obtained using OECD-type batch sorption studies using one concentration, do
297 not reflect the actual sorption behaviour of the chemical in the sediment-worm system. For
298 example, sorption isotherms may deviate from linearity at high concentrations.
299 Concentrations used in the sorption experiments were however low (15 – 22.6 nM) and
300 within an order of magnitude of concentrations observed in the aqueous phase of the
301 sediment uptake studies (diclofenac 21.5-23.5 nM; fluoxetine 5.30-5.82 nM; and triclosan
302 1.64-3.5 nM). The differences for triclosan may also be partly explained by the fact that
303 sediment ingestion has been shown to play a role in the uptake of this chemical by *L.*
304 *variegatus* from this sediment²⁰ while this has been shown to not be an important uptake
305 route for diclofenac and fluoxetine. The impacts of pore water chemistry on both sorption
306 and uptake and, for selected chemicals, ingestion may therefore need to be considered in
307 the future in order to develop approaches to better assess uptake from sediments across a
308 landscape. Finally, it is important to recognise that the studies presented here measured
309 levels of radioactivity in the different components of the system over time. It is possible, that
310 once accumulated, the study chemicals were metabolised to some degree. Differences in
311 properties of the transformation products compared to the parent chemical may also
312 contribute to the mismatch between predictions and experimental observations. Previous
313 work we have performed into the uptake and metabolism of unlabelled compounds indicates
314 that diclofenac is non-metabolised by the worms which fluoxetine and triclosan may be
315 metabolised to some degree.²⁵

316 *Implications for environmental risk assessment*

317 The application of the combined experimental and modelling approach to characterise
318 internal concentrations worked reasonably well for all chemicals in the water-only studies

319 and for diclofenac and fluoxetine in the sediment studies. The results indicate that, if data
320 are available on exposure medium pH and concentrations in sediment for a landscape, then
321 by deriving uptake and depuration rate constants for two pH conditions, it will be possible to
322 establish the internal concentration of ionisable chemicals to within a factor of 4.0 in water
323 and sediment dwelling invertebrates across the landscape over time. Further work on a
324 wider variety of ionisable chemicals and sediments is however needed in order to test the
325 general applicability of the approach. The generation of a more extensive dataset on uptake
326 and depuration constants of neutral and ionic species of molecules could, in the longer term,
327 result in the development of models that allow prediction of uptake across a landscape
328 based on chemical structure alone.

329 Incorporation of our approach into current risk assessment practices offers a move towards
330 making risk assessment more representative of the natural environment. For example in the
331 UK, typical stream water pH ranges from 5.2 – 8.4.¹⁰ This increase of over 3 pH units will
332 result in changes in the ionised fraction of chemicals. For example at pH 5.2, diclofenac will
333 be 93.9 % ionised in comparison to complete ionisation (100%) at pH 8.4. Based on the
334 relationship between uptake rates for ionised and neutral diclofenac presented in this study,
335 if lumbricids occur in sediments across these pH ranges, the uptake of diclofenac in *L.*
336 *variegatus* across UK streams could vary by up to a factor of 168. For fluoxetine the
337 differences in uptake would differ by up to a factor of 68, whereas for triclosan only small
338 differences in uptake might be expected across UK streams (factor 1.15). Taking into
339 account the increased pH variation across European streams (pH range of 2.2 – 9.8) these
340 factors dramatically increase for diclofenac (3035) and fluoxetine (749), but stay almost
341 constant for triclosan (1.20). Establishing which sites are of greatest concern based on pH
342 data will allow for targeting monitoring and a more comprehensive evaluation of the risks.
343 The combined experimental and modelling approach can be used to predict the internal
344 concentration of ionisable chemicals across a wide spatial scale in water-sediment systems
345 covering a broad range of pH values and sediment sorption coefficients.

346 Even with extensive monitoring data demonstrating the presence of ionisable chemicals in
347 the aquatic environment, very little data currently exists with regards to measurements of
348 these chemicals in biota, and even fewer studies have demonstrated the uptake of ionisable
349 chemicals in water-sediment systems. There is a real need to understand the uptake of
350 ionisable chemicals in water and sediment systems to fully understand the risks these
351 chemicals may pose to non-target organisms.²⁶ The model and experimental model
352 parameterisation approach presented in this paper offers a way to fill this knowledge gap by
353 generating data on the internal concentration of selected ionisable chemicals in invertebrates
354 such as *L. variegatus* and other aquatic species. The results clearly demonstrate that
355 ionisable chemical uptake is sensitive to changes in exposure medium pH and this needs to
356 be considered when evaluating the risk of such chemicals in aquatic systems. The modelling
357 approach presented could be a very useful tool for assessing the risks of ionisable
358 compounds to benthic organisms at the landscape scale in the future.

359 **Acknowledgements**

360 We would like to thank Unilever who funded MVK's work on the project. LJC's contribution to
361 the study received support from the EU/EFPIA Innovative Medicines Initiative Joint
362 Undertaking (iPiE Grant Number: 115735). AAs contribution to the study received support
363 from the Innovate UK-funded VFETL project. We would also like to thank two anonymous
364 reviewers for very useful insights on an earlier version of this manuscript.

365 **Supporting Information**

366 Detailed information on sorption of study compounds to sediment, mass balance calculations
367 are provided in the Supporting Information. This material is available free of charge at
368 <http://pubs.acs.org>.

369 **Table legends**

370 Table 1: Physico-chemical properties of the compounds tested, range of measured pH
371 values during each study, and derived uptake and depuration rate constants. Standard
372 deviations are shown in the parentheses.

373

374 **Figure legends**

375 Figure 1: Schematic diagramme of the modelling approach for estimating uptake of an
376 ionisable chemical, AB, into an aquatic invertebrate. $K_{in-neut}$ = uptake rate constant for the
377 neutral species; K_{in-ion} = uptake rate constant for the ionised species; K_{out} = elimination rate
378 constant for the combination of the neutral and ionised species; pH_M = pH of the external
379 media; pH_O = internal pH of the organism.

380 Figure 2: Measured (points) and simulated (lines) internal concentration of three ionisable
381 chemicals in *Lumbriculus variegatus* in water at different pH values. Dashed lines indicate
382 the 95 % confidence interval of the simulations following the parameter estimation with the
383 full data set (black) or according to the minimised design method (blue).

384 Figure 3: Comparison of measured (points) and predicted (lines) internal chemical
385 concentrations over time in sediment-exposed *Lumbriculus variegatus*. Dashed lines indicate
386 the 95 % confidence interval of the simulations following the parameter estimation with the
387 full data set (black) or according to the minimised design method (blue).

388

Table 1

		Diclofenac	Fluoxetine	Triclosan
Log Kow		4.06	4.09	5.17
pKa		4.01	9.62	7.90
Acid/Base		Acid	Base	Acid
Measured pH	Water pH 5.5	5.5-5.7	5.4-5.6	5.4-5.6
	Water pH 7.0	7.0-7.2	6.8-7.9	7.0-7.2
	Water pH 7.4	7.6-8.3	7.5-7.6	7.9-8.3
	Water pH 8.5	7.4-8.9	7.7-8.7	7.7-8.6
	Sediment	7.67	7.67	7.67
f_{ion} used for modelling	Water pH 5.5	0.9731 (0.0043)	1 (0.0004)	0 (0.0004)
	Water pH 7.0	0.9993 (0.0001)	0.9959 (0.0053)	0.1445 (0.0146)
	Water pH 7.4	0.9998 (0.0001)	0.9915 (0.0014)	0.6076 (0.1522)
	Water pH 8.5	1 (0.0001)	0.9458 (0.0278)	0.7213 (0.1055)
	Sediment pH 7.64	0.9999 (0.001)	0.9889 (0.001)	0.3706 (0.001)
$K_{in-neut}$ (L kg⁻¹ d⁻¹)	Full data set	17811 (585)	14203 (747)	1119 (76.4)
K_{in-ion} (L kg⁻¹ d⁻¹)		5.75 (0.97)	11.43 (1.42)	1181 (50.9)
K_{out} (L kg⁻¹ d⁻¹)		0.86 (0.18)	0.25 (0.09)	0.01 (0.035)
$K_{in-neut}$ (L kg⁻¹ d⁻¹)	Minimised design	14342 (1007)	9735 (1130)	1084 (162)
K_{in-ion} (L kg⁻¹ d⁻¹)		6.82 (0.27)	8.54 (0.99)	1302 (156)
K_{out} (L kg⁻¹ d⁻¹)		0.634 (0.04)	0.039 (0.06)	0.00001 (0.059)

Figure 1

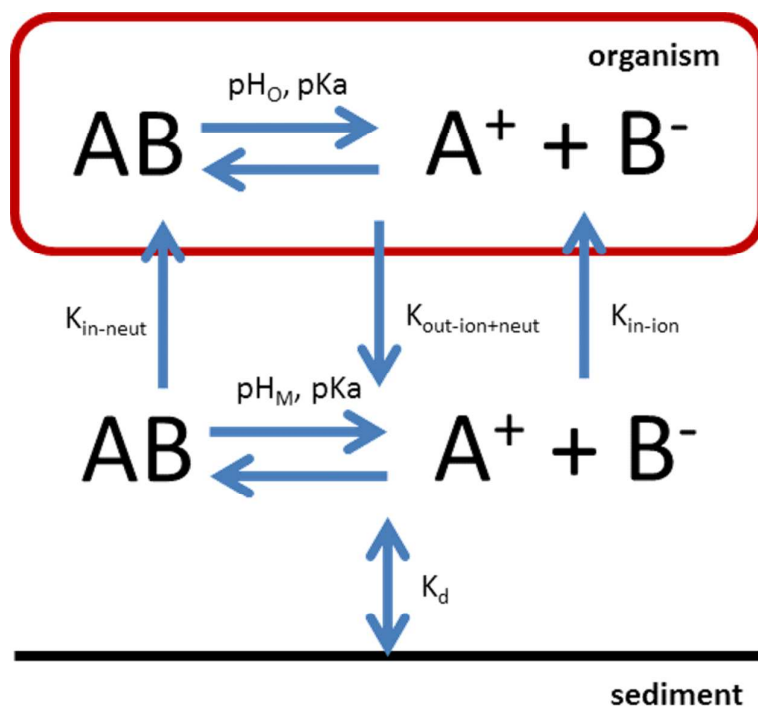


Figure 2

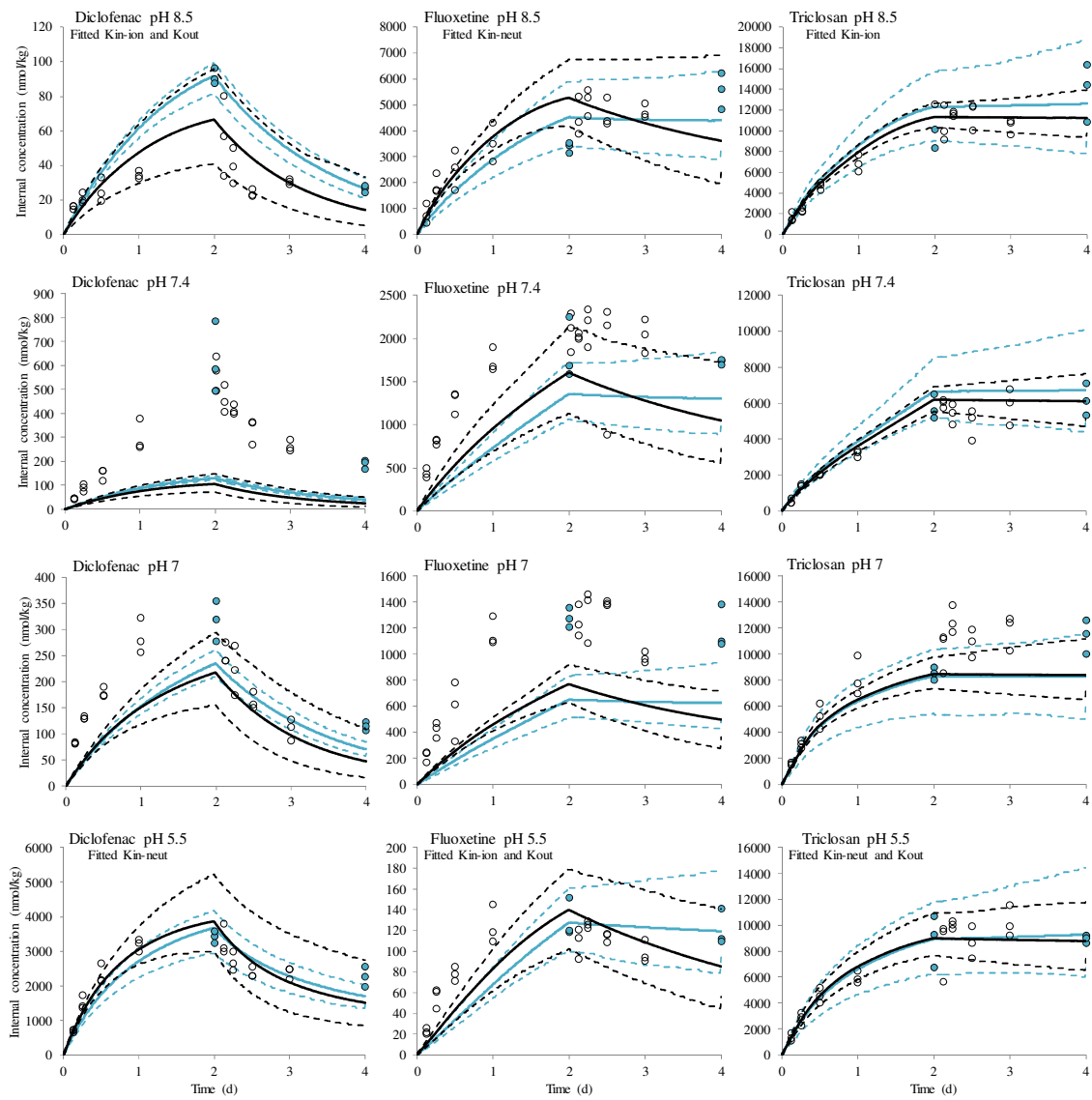
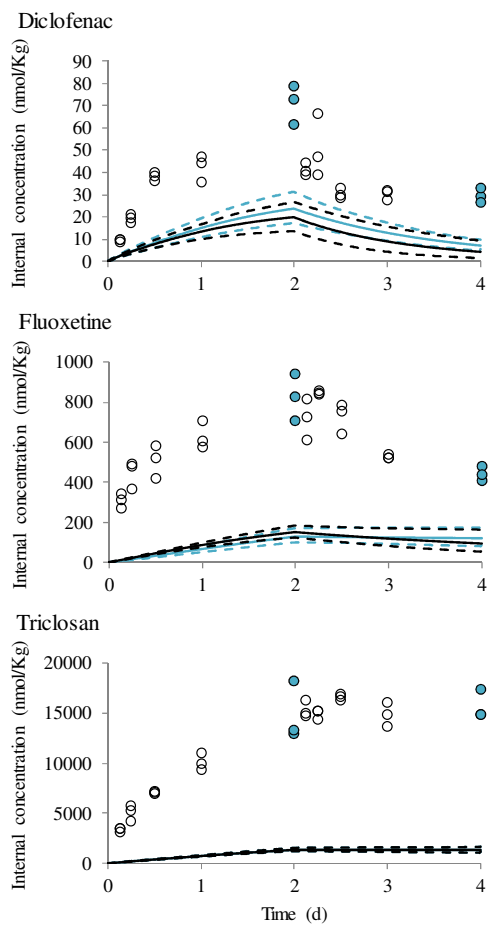


Figure 3



References

1. Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T., Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. *Environ. Sci. Technol.* **2002**, *36*, (6), 1202-1211.
2. Lindqvist, N.; Tuhkanen, T.; Kronberg, L., Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters. *Water Res.* **2005**, *39*, (11), 2219-2228.
3. Buser, H. R.; Poiger, T.; Muller, M. D., Occurrence and environmental behavior of the chiral pharmaceutical drug ibuprofen in surface waters and in wastewater. *Environ. Sci. Technol.* **1999**, *33*, (15), 2529-2535.
4. Kim, S. C.; Carlson, K., Temporal and spatial trends in the occurrence of human and veterinary antibiotics in aqueous and river sediment matrices. *Environ. Sci. Technol.* **2007**, *41*, (1), 50-57.
5. Jelic, A.; Petrovic, M.; Barcelo, D., Multi-residue method for trace level determination of pharmaceuticals in solid samples using pressurized liquid extraction followed by liquid chromatography/quadrupole-linear ion trap mass spectrometry. *Talanta* **2009**, *80*, (1), 363-371.
6. Van Wezel, A.; Devries, D. A. M.; Kostense, S.; Sijm, D.; Opperhuizen, A., Intraspecies variation in lethal body burdens of narcotic compounds. *Aquatic Toxicology* **1995**, *33*, (3-4), 325-342.
7. Escher, B. I.; Hermens, J. L. M., Internal exposure: Linking bioavailability to effects. *Environ. Sci. Technol.* **2004**, *38*, (23), 455A-462A.
8. Huggett, D. B.; Cook, J. C.; Ericson, J. F.; Williams, R. T., A theoretical model for utilizing mammalian pharmacology and safety data to prioritize potential impacts of human pharmaceuticals to fish. *Human and Ecological Risk Assessment* **2003**, *9*, (7), 1789-1799.
9. Manallack, D. T., The acid-base profile of a contemporary set of drugs: implications for drug discovery. *Sar and Qsar in Environmental Research* **2009**, *20*, (7-8), 611-655.
10. BGS *British Geological Survey. pH in stream waters: Great Britain. G-BASE Geochemical Map.*; Keyworth, Nottingham, UK., 2009.
11. Bundschuh, M.; Weyers, A.; Ebeling, M.; Elsaesser, D.; Schulz, R., Narrow pH Range of Surface Water Bodies Receiving Pesticide Input in Europe. *Bull Environ Contam Toxicol* **2016**, *96*, (1), 3-8.
12. Nakamura, Y.; Yamamoto, H.; Sekizawa, J.; Kondo, T.; Hirai, N.; Tatarazako, N., The effects of pH on fluoxetine in Japanese medaka (*Oryzias latipes*): Acute toxicity in fish larvae and bioaccumulation in juvenile fish. *Chemosphere* **2008**, *70*, (5), 865-873.
13. Valenti, T. W., Jr.; Perez-Hurtado, P.; Chambliss, C. K.; Brooks, B. W., Aquatic toxicity of sertraline to pimephales promelas at environmentally relevant surface water pH. *Environ. Toxicol. Chem.* **2009**, *28*, (12), 2685-2694.
14. Meredith-Williams, M.; Carter, L. J.; Fussell, R.; Raffaelli, D.; Ashauer, R.; Boxall, A. B. A., Uptake and depuration of pharmaceuticals in aquatic invertebrates. *Environmental Pollution* **2012**, *165*, 250-258.
15. Nichols, J. W.; Du, B.; Berninger, J. P.; Connors, K. A.; Chambliss, C. K.; Erickson, R. J.; Hoffman, A. D.; Brooks, B. W., Observed and modeled effects of pH on bioconcentration of diphenhydramine, a weakly basic pharmaceutical, in fathead minnows. *Environmental toxicology and chemistry / SETAC* **2015**, *34*, (6), 1425-35.

16. Fu, W.; Franco, A.; Trapp, S., Methods for estimating the bioconcentration factor of ionizable organic chemicals. *Environ. Toxicol. Chem.* **2009**, *28*, (7), 1372-1379.
17. Neuwoehner, J.; Escher, B. I., The pH-dependent toxicity of basic pharmaceuticals in the green algae *Scenedesmus vacuolatus* can be explained with a toxicokinetic ion-trapping model. *Aquatic Toxicology* **2011**, *101*, (1), 266-275.
18. OECD, *Test No. 106: Adsorption -- Desorption Using a Batch Equilibrium Method*. OECD Publishing: 2000.
19. USEPA *U.S. Environmental Protection Agency: Methods for acute toxicity testing with fish, macroinvertebrates and amphibians*. EPA-660/3-75-009; National Technical Information Service: Springfield, VA, 1975.
20. Carter, L. J.; Ashauer, R.; Ryan, J. J.; Boxall, A. B. A. Minimised Bioconcentration Tests: A Useful Tool for Assessing Chemical Uptake into Terrestrial and Aquatic Invertebrates? *Environmental Science & Technology* **2014**, *48*, (22), 13497-13503.
21. Karlsson, M. V.; Marshall, S.; Gouin, T.; Boxall, A. B. A., Routes of uptake of diclofenac, fluoxetine, and triclosan into sediment-dwelling worms. *Environ. Toxicol. Chem.* **2016**, *35*, (4), 836-842.
22. Avnimelech, Y.; Ritvo, G.; Meijer, L. E.; Kochba, M., Water content, organic carbon and dry bulk density in flooded sediments. *Aquacultural Engineering* **2001**, *25*, (1), 25-33.
23. Erickson, R.J.; McKim, J.M.; Lien, G.J.; Hoffman, A.D.; Batterman, S.L., Uptake and elimination of ionizable organic chemicals at fish gills: II. Observed and predicted effects of pH, alkalinity, and chemical properties. *Environ. Toxicol. Chem.* 2006 *25*, (6), 1522-1532.
24. Mayer, P.; Fernqvist, M. M.; Christensen, P. S.; Karlson, U.; Trapp, S., Enhanced Diffusion of Polycyclic Aromatic Hydrocarbons in Artificial and Natural Aqueous Solutions. *Environmental Science & Technology* **2007**, *41*, (17), 6148-6155
25. Karlsson, M. Uptake of pharmaceuticals and personal care products from sediments into aquatic organisms. PhD Thesis submitted to the University of York, York, UK, 2013.
26. Boxall, A. B. A.; Rudd, M. A.; Brooks, B. W.; Caldwell, D. J.; Choi, K.; Hickmann, S.; Innes, E.; Ostapyk, K.; Staveley, J. P.; Verslycke, T.; Ankley, G. T.; Beazley, K. F.; Belanger, S. E.; Berninger, J. P.; Carriquiriborde, P.; Coors, A.; DeLeo, P. C.; Dyer, S. D.; Ericson, J. F.; Gagne, F.; Giesy, J. P.; Gouin, T.; Hallstrom, L.; Karlsson, M. V.; Larsson, D. G. J.; Lazorchak, J. M.; Mastrocco, F.; McLaughlin, A.; McMaster, M. E.; Meyerhoff, R. D.; Moore, R.; Parrott, J. L.; Snape, J. R.; Murray-Smith, R.; Servos, M. R.; Sibley, P. K.; Straub, J. O.; Szabo, N. D.; Topp, E.; Tetreault, G. R.; Trudeau, V. L.; Van Der Kraak, G., Pharmaceuticals and Personal Care Products in the Environment: What Are the Big Questions? *Environmental Health Perspectives* **2012**, *120*, (9), 1221-1229.