THE UNIVERSITY of EDINBURGH

Edinburgh Research Explorer

pH dependence of steroid hormone-organic matter interactions at environmental concentrations

Citation for published version:
Neale, PA, Escher, BI & Schaefer, AI 2009, 'pH dependence of steroid hormone-organic matter interactions at environmental concentrations', *Science of the Total Environment*, vol. 407, no. 3, pp. 1164-1173. https://doi.org/10.1016/j.scitotenv.2008.09.035

Digital Object Identifier (DOI):
10.1016/j.scitotenv.2008.09.035

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Science of the Total Environment

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 17. Sep. 2021

Neale, P.A. ; Escher, B.I. ; Schäfer, A.I. ; (2009) pH dependence of steroid hormone–organic matter interactions at environmental concentrations, The Science of the Total Environment, 407, 1164-1173. doi:10.1016/j.scitotenv.2008.09.035

pH dependence on steroid hormone–organic matter interactions at environmental concentrations

Peta A. Neale[a]*, Beate, I. Escher[b] and Andrea, I. Schäfer[a]

[a]School of Engineering and Electronics, The University of Edinburgh, Edinburgh, EH9 3JL, United Kingdom

[b]Swiss Federal Institute of Aquatic Science and Technology (Eawag), 8600, Dübendorf, Switzerland

Submitted to Science of the Total Environment

Date Submitted: June 2008

Date Re-Submitted: September 2008

* Corresponding author: Peta Neale, E-mail: p.neale@ed.ac.uk, Ph: +44(0)131 650 7860; Fax: +44(0)131 650 6781

Abstract

The interaction of estradiol, estrone, progesterone and testosterone with environmentally relevant concentrations of Aldrich humic acid, alginic acid and tannic acid was studied using solid-phase microextraction (SPME). Since bulk organic matter and certain hormones such as estradiol and estrone contain dissociable functional groups, the effect of pH on sorption was investigated as this will influence their fate and bioavailability. For humic acid and tannic acid, sorption was strongest at acidic pH when the bulk organic matter was in a non-dissociated form and decreased when they became partially negatively charged. At acidic and neutral pH the strength of partitioning was influenced by hormone functional groups content, with the strongest sorption observed for progesterone and estrone. At alkaline pH conditions, when the bulk organics were dissociated, sorption decreased considerably (up to a factor of 14), although the non-dissociated hormones testosterone and progesterone indicated greater sorption to humic acid at pH 10 compared to the partially deprotonated estradiol and estrone. This study demonstrates that SPME can be used to assess organic matter sorption behaviour of a selected range of micropollutants and at environmentally relevant organic matter concentrations.

Keywords: Steroid hormones, organic matter, pH, partition coefficient, solid-phase microextraction

1. Introduction

Steroidal hormones, such as 17β-estradiol, estrone, progesterone and testosterone, are naturally excreted by humans and many animals. These hormones are essential for growth and development, however, elevated concentrations in the environment can have adverse affects on organisms, such as developmental and reproductive abnormalities in fish (Jobling *et al.*, 1998). Previous studies have indicated that steroidal hormones are not completely removed during biological wastewater treatment, with estradiol, estrone and testosterone recorded in effluent in low nanogram per litre concentrations (Baronti *et al.*, 2000; Johnson *et al.*, 2005). This was further confirmed by a study of contaminants in US streams in 1999–2000 which found median concentrations of testosterone and progesterone around 100 ng/L, while estradiol and estrone were an order of magnitude less (Kolpin *et al.*, 2002). As the more potent hormones, such as estradiol, can have significant implications for

aquatic organisms at sub-nanogram concentrations (Purdom *et al.*, 1994; Tabata *et al.*, 2001), the current concentrations of hormones in natural waters is of concern. In addition to point sources such as wastewater effluent, diffuse sources such as animal agriculture can also contribute to elevated concentrations of steroidal hormones in the aquatic environment (Kolodziej *et al.*, 2004). A study by Finlay-Moore *et al.* (2000) demonstrated that land application of chicken broiler litter can lead to runoff concentrations of estradiol and testosterone of up to 2 µg/L. In addition, natural hormones such as estrogens and testosterone are used to increase growth and feeding efficiency of cattle in countries such as the US (Orlando *et al.*, 2004), therefore runoff from feedlots could potentially increase the concentration of hormones in water.

The fate and behaviour of natural hormones within the environment can be influenced by their interaction with bulk organic matter (Schwarzenbach *et al.*, 2003; Yamamoto *et al.*, 2003). Bulk organic matter can be used as a surrogate for natural organic matter (NOM) of different origins and phases (dissolved, colloidal and particulate) found in water and wastewater. The interactions of steroid hormones with humic acid (HA), alginic acid (AA) and tannic acid (TA) are important as they represent organic matter found in surface water and wastewater. The characteristics of both hormones and organic matter play an important role in the interaction. HA stems from a wide range of sources including vegetation, peat, coal and soil, and can be considered ubiquitous in most aquatic environments (Thurman and Malcolm, 1981). Due to the high content of the strong acidic functional group carboxylic acid (Table 1) the acid dissociation constant (pK_a) of HA is around 4.26 (Shin *et al.*, 1999). AA, a polysaccharide, is the main constituent of brown algae, therefore it is found in surface and wastewaters (Davis *et al.*, 2003). AA is composed of mannuronic (~60%) and guluronic (~40%) acids (De Stefano *et al.*, 2006) and the pK_a for AA is around 3.4 (Avaltroni *et al.*, 2007). TA is an abundant plant polyphenol, and is present in surface waters due to leaching from vegetation (Cruz *et al.*, 2000). TA contains a range of functional groups, including phenolic, catechol and gallic acid moieties, and due to the presence of phenolic hydroxyl groups (Table 1) the pK_a is around 8.5 (Kraal *et al.*, 2006).

The interaction of hormones with bulk organic matter can have implications for aqueous solubility (Chiou *et al.*, 1986), toxicity (Traina *et al.*, 1996), and removal of the hormones during wastewater treatment (Schäfer *et al.*, 2006). Due to ionisable functional groups pH influences such interactions and hence can affect both transport and bioavailability of hormones in the environment and engineered systems greatly. The strength of the interaction can be related to properties of both the hormones and bulk organic matter including hydrophobicity and functional groups content as well as the solution chemistry (Schlautman and Morgan, 1993a; Schlautman and Morgan, 1993b).

Therefore, the influence of pH (4, 7, 9, 10 and 12) was studied as the organic matter and more potent hormones (estradiol and estrone) have ionisable functional groups. Very few studies have considered the influence of pH on the interaction of hormones with organic matter. Only Yamamoto and Liljestrand (2003) have studied the sorption of estradiol to colloidal HA as a function of pH (5, 7 and 9). This study indicated there was no significant difference in sorption within this restricted pH range, however the interaction needs to be studied at a wider pH range to incorporate hormone dissociation. In addition, the difference in sorption of dissociating hormones (estradiol and estrone) and non-dissociating hormones (testosterone and progesterone) to organic matter has not previously been explored.

The interaction of steroidal hormones with bulk organic matter was studied using solid-phase microextraction (SPME). SPME can measure the freely dissolved hormone concentration in solution as any hormones bound to organic matter cannot be extracted by the polymer SPME fibre (Figure 1) (Ramos et al., 1998; Vaes et al., 1996). In this study a full mass balance form of SPME will be used (Neale *et al.*, 2008). It is similar to negligible-depletion SPME (nd-SPME) as less than 5% of freely dissolved hormones are extracted to the polymer fibre (Heringa *et al.*, 2002). However,

Neale, P.A. ; Escher, B.I. ; Schäfer, A.I. ; (2009) pH dependence of steroid hormone–organic matter interactions at environmental concentrations, The Science of the Total Environment, 407, 1164-1173. doi:10.1016/j.scitotenv.2008.09.035

due to the low concentrations of bulk organic matter in the experiment the mass extracted by the polymer fibre was equal to mass of hormone sorbed to the organic matter. As a result the typical nd–SPME assumptions were not applicable, and a full mass balance was required. SPME has previously been used to study the interaction of phenols and 4-quinolones with HA as a function of pH (Holten Lützhøft *et al.*, 2000; Ohlenbusch *et al.*, 2000). However the application of SPME to determine the comparative partitioning of hormones to organic matter as a function of pH is novel and the measurement of these interactions is highly relevant to determine the fate of such compounds in the environment.

2. Materials and Methods

2.1. Chemicals: All chemicals were of analytical grade. The background electrolyte was 1 mM $NaHCO_3$ 20 mM NaCl, and the pH was adjusted using 1 M HCl and NaOH. The radiolabelled hormones used were [2,4,6,7–^{3}H] 17β-estradiol (3.15 TBq/mmol), [2,4,6,7–^{3}H] estrone (3.55 TBq/mmol), [1,2,6,7–^{3}H] progesterone (3.52 TBq/mmol) and [1,2,6,7–^{3}H] testosterone (2.70 TBq/mmol) (GE Healthcare, Little Chalfont, UK). The radioactive concentration of all hormones was 37 MBq/mL. Non-labelled hormones (≥98% purity) were purchased from Sigma Aldrich (Gillingham, UK). The properties of the hormones were shown in Table 2. In all experiments the concentration of hormones ranged from 100 ng/L to 100 µg/L.

2.2. Organic Matter: The bulk organic matter selected represented several types of organic matter present in the natural waters including NOM surrogates, polysaccharides and polyphenols. A wide range of NOM surrogates were studied in our previous work (Neale *et al.*, 2008) and no significant difference in the sorption of estradiol to these organics was observed. Aldrich humic acid, alginic acid (sodium salt) from *Macrocystis pyrifera* and tannic acid were all purchased from Sigma Aldrich. Aldrich HA may not be a representative natural terrestrial humic acid (Malcolm and MacCarthy, 1986), however it was selected as it was commonly studied in the literature. The concentration of organic matter in natural waters can vary from 0.5 to 100 mgC/L (Frimmel, 1998), and due to a reduction in atmospheric acidity the concentration of organic matter in surface waters has increased (Monteith *et al.*, 2007). In all experiments a concentration of 12.5 mgC/L was used. Selected properties of the studied bulk organics including molecular weight and acidic functional group content were shown in Table 1.

2.3. SPME Fibre and Protocol: Polyacrylate (PA) fibres were purchased from Polymicro Technologies (Phoenix, USA). The fibre coating thickness was 34.5 µm. In 100 mL flasks radiolabelled hormones and bulk organic matter were added to deionised water containing 1 mM $NaHCO_3$ and 20 mM NaCl as background electrolyte. The pH was adjusted to 4, 7, 9, 10 and 12 using 1 M HCl and NaOH. At a constant temperature of 25 °C the flasks were shaken for 24 hours at 200 rpm using a Certomat BS-1 incubator shaker (Göttigen, Germany). Following this 5 cm of PA fibre was added to each flask, and these were shaken for 48 hours.

2.4. Analysis: The activity of the fibres was measured using Beckman LS 6500 scintillation counter (Fullerton, USA). The fibres were placed in a vial containing 7 mL of Ultima Gold LLT (Perkin Elmer, Waltham USA) and counted for 10 minutes each. The activity, in disintegration per minute, was used to calculate mass of hormone sorbed to the fibre. The organic matter was analysed using solid-state ^{13}C cross-polarisation magic-angle spinning (CPMAS) nuclear magnetic resonance (NMR) using a Varian VNMRS spectrophotometer (Palo Alto, USA) operated at 100.56 MHz. Clean and organic matter exposed fibres were also analysed using solid-state ^{13}C CPMAS NMR.

2.5. Determination of Organic Matter Partition Coefficient (K_{OM}): The organic matter-water partition coefficient, log K_{OM} (L/kg), was calculated using a linear sorption isotherm of concentration of hormone sorbed to the bulk organic matter, C_{OM} (ng/g), as a function of the concentration of hormone freely dissolved in solution at equilibrium, C_W (ng/L). The free and sorbed hormone concentrations were calculated using a full mass balance. The complete methodology used was described in Neale *et al.* (2008). The measurement uncertainty associated with log K_{OM} was 5.4%, and this was calculated using error propagation. The measurement

uncertainty was mainly due to variations in fibre length, as well as errors associated with analytical equipment.

3. Results and Discussion

The partitioning of steroidal hormones to Aldrich HA, AA and TA was studied as a function of pH (Table 3). To consider comparative sorption of steroidal hormones to bulk organic matter the interaction was also studied at neutral pH. The pH of natural waters is generally considered to be between 6 and 9 (Ra et al., 2008), however this can vary naturally. For example, natural surface water from a volcanic crater lakes can have pH values less than 0.3 due to volcanic gases (Löhr et al., 2005). Further, the pH can also be varied during water treatment with some chemical clarification processes requiring alkaline pH conditions (Semerjian and Ayoub, 2003). As variations in pH could have implications for the charge, conformation and solubility of both organic matter and hormones it was important to understand the pH dependence of partitioning.

3.1. Comparative Sorption of Different Steroidal Hormones to Bulk Organic Matter: The partitioning of estradiol, estrone, progesterone and testosterone to bulk organic matter was studied at pH 7 (Figure 2). All hormones partitioned strongest to TA, followed by Aldrich HA and AA. The order of partitioning was not the same for all hormones. For TA and Aldrich HA estrone had the highest K_{OM} followed by progesterone, while testosterone and estradiol were lower, but had similar K_{OM} values. In contrast, for AA K_{OM} was highest for estradiol followed by progesterone and testosterone.

Sorption of hormones to bulk organic matter was dependent on a number of properties of both the sorbent and the sorbate, including acidic functional group content and charge (Yamamoto *et al.*, 2003). At pH 7 all hormones were neutrally charged, while Aldrich HA and AA were >99% dissociated and anionic, but TA was primarily in a non-dissociated form (99%). In consequence, the interaction of steroidal hormones was strongest with TA compared to the other organics.

Previous studies have indicated that hormones primarily interact with bulk organic matter through hydrogen bonding (Yamamoto *et al.*, 2003) which is a specific interaction that occurs between hydrogen donors and acceptors. The bulk organics studied all contained bipolar function groups (both hydrogen donor and acceptors) such as carboxylic, phenolic and hydroxyl groups. The hormones were also bipolar due to hydroxy substituents, except for progesterone which contained monopolar ketone groups only.

The sorption of estrone and progesterone to HA and TA was 2.3 to 7.9 times greater than estradiol and testosterone respectively. This was due to the ketone functional group in C-17 position of estrone and C-20 position of progesterone (refer to Table 2). Ketone groups are strong hydrogen acceptors, and a study by Le Questel *et al.* (2000) demonstrated that the C-20 ketone moiety in progesterone is a triple hydrogen acceptor. The structure of estradiol and estrone is very similar as both have a phenolic hydroxyl group in C-3, however estradiol has a hydroxyl group in C-17 instead of a ketone group. In addition, there are similarities between testosterone and progesterone as both have a ketone group in C-3, but like estradiol, testosterone also has a hydroxyl group in C-17. The order of partitioning was not observed for alginic acid, with the highest partitioning observed for estradiol. TA and HA both contain carboxylic and phenolic hydroxyl groups, while AA primarily contains carboxylic groups (Table 1). This suggests that carboxylic groups do not interact as strongly with ketone groups as the phenolic hydroxyl moieties found in HA and TA. In addition, no relationship was observed between log K_{OM} and log K_{OW} (Table 2). While such a correlation was often seen for hydrophobic contaminants such as polycyclic aromatic hydrocarbons (Kopinke *et al.*, 1995), no relationship between log K_{OM} and log K_{OW} has been observed for steroid hormones in the literature (Holbrook *et al.*, 2004; Liu *et al.*, 2005; Yamamoto *et al.*, 2003).

Neale, P.A. ; Escher, B.I. ; Schäfer, A.I. ; (2009) pH dependence of steroid hormone–organic matter interactions at environmental concentrations, The Science of the Total Environment, 407, 1164-1173. doi:10.1016/j.scitotenv.2008.09.035

3.2. Hormone Partitioning as a Function of pH: The interaction of natural steroidal hormones with Aldrich HA, AA and TA was studied at pH 4, 7, 9, 10 and 12 (Figure 3). Tentative dissociation of the bulk organics as a function of pH was also shown in Figure 3. A limitation of SPME is a reduced capacity to extract charged micropollutants (Escher *et al.*, 2002). Progesterone and testosterone do not contain ionisable functional groups, therefore they remain in a non-dissociated form over the studied pH range, however as estradiol and estrone had pK_a values of 10.23 and 10.34 respectively (Kwon *et al.*, 2006), they began dissociating around pH 9. The fraction of neutral species ($f_{neutral}$) in the dissociating hormones can be calculated using Equation 1. Calibration experiments for estradiol and estrone without organic matter showed significantly reduced hormone extraction at alkaline pH (one order of magnitude less at pH 12). Therefore, as sorption of charged species to the fibre at high pH was negligible it was not possible to measure log K_{OM} for estradiol and estrone above pH 10.

$$f_{neutral} = \frac{1}{1+10^{pH-pK_a}} \quad \textbf{(1)}$$

Another limitation of SPME is the potential for fibre fouling by organic matter as this could lead to an overestimation of partitioning due to altered micropollutant sorption. Previous studies have reported fouling of SPME fibre by protein (Heringa *et al.*, 2006) and humic acid (Zhang *et al.*, 1996). The solid-state ^{13}C NMR spectra for clean polyacrylate fibre and polyacrylate fibres exposed to HA and AA for 48 hours are shown in Figure 4. No difference in spectra was observed in the presence of Aldrich HA or AA. Further, no colour change of fibre which can indicate fouling was detected. No organic matter fouling of the polyacrylate fibres was detectable.

The Aldrich HA – water sorption isotherms are shown in Figure 5. The results indicate that partitioning was slightly higher at pH 4 for most hormones, when Aldrich HA was only 30-40% dissociated. However, HA was already dissociated at pH 7 (99 %) and at higher pH there was little difference in partitioning.

Solid-state ^{13}C NMR spectra (Figure 6a) indicated Aldrich HA primarily contained aliphatic function groups. This spectra was similar to that published by Shin *et al.*, (1999), and was because Aldrich HA used in this study was not purified or fractionated. However, the total surface acidity measurements for Aldrich HA indicated it contained both carboxylic (4.80 meq/g) and phenolic hydroxyl (2.26 meq/g) functional groups (Kim *et al.*, 1990). Zeta potential measurements of Aldrich HA by Yan and Bai (2005) indicated an increased negative charge from pH 4 to 7 (-27 to -38 mV) due to the dissociation of the carboxylic functional groups. The charge was constant from pH 7 to 12 despite the presence of phenolic hydroxyl groups which dissociate around pH 9.9 (pK_a for unsubstituted phenol moieties). However, based on zeta potential measurements, it appeared that phenolic groups did not have a significant effect on the charge of Aldrich HA (Yan and Bai, 2005). Therefore, the small difference in log K_{OM}, particularly for the non dissociating hormones from neutral to alkaline pH was related to HA charge consistency from pH 7 to 12.

The decrease in hormone sorption from acidic to neutral conditions was not only influenced by speciation of HA but also conformational changes. A study by Ghosh and Schnitzer (1980) demonstrated that HA changed from a rigid and coiled structure at low pH to flexible, linear polyelectrolytes at neutral and alkaline pH due to charge repulsion. The uncoiling was thought to reduce the hydrophobicity of Aldrich HA (Ra et al., 2008), which may reduce partitioning. An alternate view to the polyelectrolyte theory suggests that HA is a supramolecular structure formed by weak bonding of HA molecules (Piccolo, 2001). Studies have indicated that the molecular mass of HA increased at acidic pH (around 4 – 5) due to the formation of hydrogen bonds between the HA molecules (Cozzolino and Piccolo, 2001). Previous studies have indicated stronger sorption to

higher molecular weight organics (Chin *et al.*, 1997), and this may also account for the increased hormone partitioning in acidic solutions.

At pH 10, Aldrich HA was the only bulk organic where a partition coefficient for the partially dissociated estradiol and estrone could be measured. This was due to functional group content of Aldrich HA compared to AA and TA. While the charge of Aldrich HA is dominated by strong acidic functional groups, it also contains other functional groups, such as carbonyl and hydroxyl moieties which are not dissociated at pH 10 (Sparks *et al.*, 1997). Progesterone and testosterone sorbed stronger to Aldrich HA compared to estradiol and estrone at pH 10, as neutrally charged hormones were more likely to partition to organic matter compared to partly dissociated hormones (31-37% for estrone and estradiol respectively) (Ra et al., 2008). In addition, in line with pH dependent octanol-water distribution ratios (Log D_{OW}), the hydrophobicity of estrone and estradiol was reduced from 3.13 to 2.97 and 4.01 to 3.81 at pH 10 respectively (Table 2) and this may have decreased partitioning of charged species to bulk organic matter.

Several studies have investigated the effect of pH on the sorption of various micropollutants, such as PAH and tributyltin (Arnold *et al.*, 1998; Schlautman and Morgan, 1993b) to HA using techniques such as equilibrium dialysis and fluorescence quenching. These studies have indicated a decrease in sorption in alkaline conditions due to deprotontation of HA (Schlautman and Morgan, 1993b). In addition, Yamamoto and Liljestrand (2003) studied the partitioning of estradiol to colloidal Suwannee River HA at pH 5, 7 and 9 using fluorescence quenching. This study indicated no significant difference in partitioning as a function of pH. This differed from the findings of the present study, however this may be related to the restricted pH range in their study. Due to the high carboxylic acid content of Suwannee River HA (Ritchie and Perdue, 2003) it would be significantly more dissociated at pH 5 compared to pH 4. As estradiol remains undissociated in the studied range, pH would not be expected to have a significant effect on the interaction.

The AA – water sorption isotherms for estradiol, progesterone and testosterone are shown in Figure 7. Log K_{OM} for AA were generally lower for all hormones in the studied pH range compared to the other bulk organic matter studied. The solid-state ^{13}C NMR spectrum for AA (Figure 6b) indicated carboxylic and ringed carbon functional groups which are common for polysaccharides. Based on titration data, the total acidity of AA was predominately due to carboxylic functional groups (7.02 meq/g) (Jeon *et al.*, 2002), and therefore it began to dissociate from pH 3. Similar to HA, the conformation of AA was affected by solution chemistry. A study by Avaltroni *et al.* (2007) demonstrated that the structure of AA changed with increasing pH. At acidic pH the structure was coiled and rigid due to intramolecular hydrogen bonding. As the pH increases the structure becomes more linear due to charge repulsion as a result of carboxylic dissociation, and above pH 8 depolymerisation can occur which reduced AA to small, flexible fragments (Avaltroni *et al*., 2007).

From the available data, one could observe a slight decrease in partitioning for estradiol and progesterone as pH increased, however this was not statistically significant in the case of progesterone. In contrast, log K_{OM} for testosterone increased significantly as pH increased from pH 7 to 9, which corresponded to the depolymerisation of AA.

With the exception of Neale *et al.* (2008) who investigated estradiol, the interaction of AA with micropollutants has not been studied as a function of pH. Yamamoto *et al.* (2004; 2003) determined log K_{OM} for AA for a range of estrogenic micropollutants, including estradiol at pH 7. Log K_{OM} for estradiol calculated using solubility enhancement was remarkably similar to results from this study, log K_{OM} 3.75 (Yamamoto *et al.*, 2003) compared to 3.96.

TA – water sorption isotherms for all hormones were shown in Figure 8. The effect of pH on partitioning was strongest for TA. Partitioning decreased over one order of magnitude from acidic

Neale, P.A. ; Escher, B.I. ; Schäfer, A.I. ; (2009) pH dependence of steroid hormone–organic matter interactions at environmental concentrations, The Science of the Total Environment, 407, 1164-1173. doi:10.1016/j.scitotenv.2008.09.035

to alkaline conditions for the majority of the hormones studied. Total surface acidity studies indicated TA contains a high content of phenolic hydroxyl moieties (9.55 meq/g) (Flores-Céspedes *et al.*, 2006), and started to dissociate around neutral pH conditions (pK_a 8.5). The solid-state ^{13}C NMR spectrum for TA (Figure 6c) confirmed the total acidity results, as it predominately contained polyphenolic functional groups. The strong sorption to TA was due to hydrogen bonding with the high concentration of phenolic groups present in TA (Jin *et al.*, 2007; Yamamoto *et al.*, 2003).

The strongest partitioning was observed at pH 4 when TA was protonated, and this decreased slightly at pH 7, as TA began to dissociate and undergo hydrolysis to gallic acid (Osawa and Walsh, 1993). For all hormones there was a significant decrease in partitioning (factor of 4 to 14) from pH 7 to pH 9 due to dissociation of TA. Similar to Aldrich HA, there was no further significant decline in sorption of non-dissociating hormones at pH 10 or 12.

Similar to AA, the partitioning of micropollutants to TA has not been studied as a function of pH. Previously, the interaction of estradiol with TA was studied at pH 7 using fluorescence quenching and solubility enhancement (Yamamoto *et al.*, 2004; Yamamoto *et al.*, 2003). Partitioning of estradiol to TA was higher than SPME when using fluorescence quenching (log K_{OM} 5.28 (Yamamoto and Liljestrand, 2003; Yamamoto *et al.*, 2003) compared to 4.86), but similar when using solubility enhancement (log K_{OM} 4.94 (Yamamoto *et al.*, 2003)).

4. Conclusions

While many studies have focused on aqueous organic matter–water partition coefficients for estradiol (Bowman *et al.*, 2002; Holbrook *et al.*, 2004; Liu *et al.*, 2005; Neale *et al.*, 2008; Yamamoto and Liljestrand, 2003; Yamamoto *et al.*, 2003), there were less studies on estrone (Bowman *et al.*, 2002; Liu *et al.*, 2005) and progesterone (López de Alda *et al.*, 2002), and to the authors' knowledge, none for testosterone. In addition, very few studies have looked at partitioning of hormones in acidic or alkaline solutions, or at environmentally relevant (low) concentrations of bulk organic matter. This study indicated that sorption of hormones to organic matter was generally strongest in acidic solutions, due to the non-dissociated form of the organic matter. Partitioning decreases significantly (up to a factor of 14) in alkaline solutions when the organic matter was negatively charged. Knowledge of log K_{OM} is required for environmental models, such as level III fugacity models (MacKay and Paterson, 1991) to determine the fate of contaminants in the aquatic environment. Therefore, the mass balance form of SPME will improve the understanding of hormones in the aquatic environment and can take into account the variations in log K_{OM} with pH, which is a natural variable.

Acknowledgements

The authors thank Alan Simm, University of Edinburgh, and Mathias Ulbricht, Universität Duisburg-Essen, for helpful discussions. Dr David Apperley, Durham University, is thanked for solid state ^{13}C NMR analysis of organic matter and fibres.

References

Arnold CG, Ciani A, Müller SR, Amirbahman A, Schwarzenbach RP. Association of triorganotin compounds with dissolved humic acids. Environ. Sci. Technol. 1998; 32: 2976-2983.

Avaltroni F, Seijo M, Ulrich S, Stoll S, Wilkinson KJ. Conformational changes and aggregation of alginic acid as determined by fluorescence correlation spectroscopy. Biomacromolecules 2007; 8: 106-112.

Baronti C, Curini R, D'Ascenzo G, Di Corcia A, Gentili A, Samperi R. Monitoring natural and synthetic estrogens in activated sludge sewage treatment plants and in a receiving river water. Environ. Sci. Technol. 2000; 34: 5059-5066.

Bowman JC, Zhou JL, Readman JW. Sediment - water interactions of natural oestrogens under estuarine conditions. Mar. Chem. 2002; 77: 263-276.

Chin Y-P, Aiken G, Danielsen KM. Binding of pyrene to aquatic and commercial humic substances: The role of molecular weight and aromaticity. Environ. Sci. Technol. 1997; 31: 1630-1635.

Chiou C, Malcolm RL, Brinton TI, Kile DE. Water solubility enhancement of some organic pollutants and pesticides by dissolved humic and fulvic acids. Environ. Sci. Technol. 1986; 20: 502-508.

Cozzolino A, Piccolo A. Polymerization of dissolved humic substances catalyzed by peroxidase. Effects of pH and humic composition. Org. Geochem. 2001; 33: 281-294.

Cruz BH, Díaz-Cruz JM, Ariño C, Esteban M. Heavy metal binding by tannic acid: A voltammetric study. Electroanalysis 2000; 12: 1130-1137.

Davis TA, Volesky B, Mucci A. A review of the biochemistry of heavy metal biosorption by brown algae. Water Res. 2003; 37: 4311 - 4330.

De Stefano C, Gianguzza A, Piazzese D, Porcino N, Sammartano S. Sequestration of biogenic amines by alginic and fulvic acids. Biophys. Chem. 2006; 122: 221–231.

Escher BI, Berg M, Mühlemann J, Schwarz MAA, Hermens JLM, Vaes WHJ, Schwarzenbach RP. Determination of liposome/water partition coefficients of organic acids and bases by solid-phase microextraction. The Analyst 2002; 127: 42 - 48.

Finlay-Moore O, Hartel PG, L CM. 17β-estradiol and testosterone in soil and runoff from grasslands amended with broiler litter. J. Environ. Qual. 2000; 29: 1604-1611.

Flores-Céspedes F, Fernández-Pérez M, Villafranca-Sánchez M, González-Pradas E. Cosorption study of organic pollutants and dissolved organic matter in a soil. Environ. Pollut. 2006; 142: 449-456.

Frimmel FH. Characterization of natural organic matter as major constituents in aquatic systems. J. Contam. Hydrol. 1998; 35: 201-216.

Ghosh K, Schnitzer M. Macromolecular structures of humic substances. Soil Sci. 1980; 129: 266-276.

Hansch C, Leo A, Hoekman D. Exploring QSAR: Hydrophobic, Electronic, and Steric Constants. Washington, DC: American Chemical Society, 1995.

Heringa MB, Hogevonder C, Busser F, Hermens JLM. Measurement of the free concentration of octylphenol in biological samples with negligible depletion-solid phase microextraction (nd-SPME): Analysis of matrix effects. J. Chromatogr. B 2006; 834: 35-41.

Heringa MB, Pastor D, Algra J, Vaes WHJ, Hermens JLM. Negligible depletion solid-phase microextraction with radiolabeled analytes to study free concentrations and protein binding: an example with [^{3}H]estradiol. Anal. Chem. 2002; 74: 5993-5997.

Holbrook RD, Love NG, Novak JT. Sorption of 17β-estradiol and 17α-ethinylestradiol by colloidal organic carbon derived from biological wastewater treatment systems. Environ. Sci. Technol. 2004; 38: 3322-3329.

Holten Lützhøft H-C, Vaes WHJ, Freidig AP, Halling-Sørensen B, Hermens JLM. Influence of pH and other modifying factors on the distribution behavior of 4 - quinolones to solid phases and humic acids studied by "negligible - depletion" SPME - HPLC. Environ. Sci. Technol. 2000; 34: 4989-4994.

Jeon C, Park JY, Yoo YJ. Characteristics of metal removal using carboxylated alginic acid. Water Res. 2002; 36: 1814-1824.

Jin X, Hu J, Ong SL. Influence of dissolved organic matter on estrone removal by NF membranes and the role of their structures. J. Membr. Sci. 2007; 41: 3077-3088.

Jobling S, Nolan M, Tyler CR, Brighty G, Sumpter JP. Widespread sexual disruption in wild fish. Environ. Sci. Technol. 1998; 32: 2498-2506.

Johnson AC, Aerni H-R, Gerritsen A, Gibert M, Giger W, Hylland K, Jürgens M, Nakari T, Pickering A, Suter MJ-F, Svenson A, Wettstein FE. Comparing steroid estrogen, and nonylphenol content across a range of European sewage plants with different treatment and management practices. Water Res. 2005; 39: 47-58.

Kim JI, Buckau G, Li GH, Duschner H, Psarros N. Characterization of humic and fulvic acids from Gorleben groundwater. Fresenius J. Anal. Chem. 1990; 338: 245-252.

Kolodziej EP, Harter T, Sedlak DL. Dairy wastewater, aquaculture, and spawning fish as sources of steroid hormones in the aquatic environment. Environ. Sci. Technol. 2004; 38: 6377-6384.

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. Environ. Sci. Technol. 2002; 36: 1202-1211.

Kopinke FD, Pörschmann J, Stottmeister U. Sorption of organic pollutants on anthropogenic humic matter. Environ. Sci. Technol. 1995; 29: 941-950.

Kraal P, Jansen B, Nierop KGJ, Verstraten JM. Copper complexation by tannic acid in aqueous solution. Chemosphere 2006; 65: 2193-2198.

Kwon J-H, Liljestrand HM, Katz LE. Partitioning of moderately hydrophobic endocrine disruptors between water and synthetic membrane vesicles. Environ. Toxicol. Chem. 2006; 25: 1984-1992.

Le Questel JY, Boquet G, Berthelot M, Laurence C. Hydrogen bonding of progesterone: A combined theoretical, spectroscopic, thermodynamic, and crystallographic database study. J. Phys. Chem. B 2000; 104: 11816-11823.

Liu R, Wilding A, Hibberd A, Zhoa JL. Partition of endocrine-disrupting chemicals between colloids and dissolved phase as determined by cross-flow ultrafiltration. Environ. Sci. Technol. 2005; 39: 2753-2761.

Löhr A, Bogaard T, Heikens A, Hendriks M, Sumarti S, van Bergen M, van Gestel KCAM, van Straalen N, Vroon P, Widianarko B. Natural pollution caused by the extremely acidic crater lake Kawah Ijen, East Java, Indonesia. Environ. Sci. Pollut. Res. 2005; 12: 89-95.

López de Alda MJ, Gil A, Paz E, Barceló D. Occurrence and analysis of estrogens and progestogens in river sediments by liquid chromatography-electrospray-mass spectrometry. The Analyst 2002; 127: 1299-1304.

MacKay D, Paterson S. Evaluating the multimedia fate of organic chemicals: A level III fugacity model. Environ. Sci. Technol. 1991; 25: 427-436.

Malcolm RL, MacCarthy P. Limitations in the use of commercial humic acids in water and soil research. Environ. Sci. Technol. 1986; 20: 904-911.

Monteith DT, Stoddard JL, Evans CD, de Wit HA, Forsius M, Hogasen T, Wilander A, Skjelkvale BL, Jeffries DS, Vuorenmaa J, Keller B, Kopacek J, Vesely J. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. Nature 2007; 450: 537-540.

Neale PA, Escher BI, Schäfer AI. Quantification of solute-solute interactions using negligible-depletion solid-phase microextraction: Measuring the affinity of estradiol to bulk organic matter. Environ. Sci. Technol. 2008; 42: 2886-2892.

Ohlenbusch G, Kumke MU, Frimmel FH. Sorption of phenols to dissolved organic matter investigated by solid phase microextraction. Sci. Total Environ. 2000; 253: 63-74.

Orlando EF, Kolok AS, Binzcik GA, Gates JL, Horton MK, Lambright CS, Gray LE, Soto AM, Guillette LJ. Endocrine-disrupting effects of cattle feedlot effluent on an aquatic sentinel species, the fathead minnow. Environ. Health Perspect. 2004; 112: 353-358

Osawa R, Walsh TP. Effects of acidic and alkaline treatments on tannic acid and its binding property to protein. J. Agric. Food Chem. 1993; 41: 704-707.

Piccolo A. The supramolecular structure of humic substances. Soil Sci. 2001; 166: 810-832.

Purdom CE, Hardiman PA, Bye VJ, Eno NC, Tyler CR, Sumpter J. Estrogenic effects of effluents from sewage treatment works. Chem. Ecol. 1994; 8: 275-285.

Ra JS, Oh S-Y, Lee BC, Kim SD. The effect of suspended particles coated by humic acid on the toxicity of pharmaceuticals, estrogens, and phenolic compounds. Environ. Int. 2008; 34: 184-192.

Ramos EU, Meijer SN, Vaes WHJ, Verhaar HJM, Hermens JLM. Using solid-phase microextraction to determine partition coefficients to humic acids and bioavailable concentrations of hydrophobic chemicals. Environ. Sci. Technol. 1998; 32: 3430-3435.

Ritchie JD, Perdue EM. Proton-binding study of standard and reference fulvic acids, humic acids, and natural organic matter. Geochim. Cosmochim. Acta 2003; 67: 85-96.

Schäfer AI, Nghiem LD, Oschmann N. Bisphenol A retention in the direct ultrafiltration of greywater. J. Membr. Sci. 2006; 283: 233-243.

Schlautman MA, Morgan JJ. Binding of a fluorescent hydrophobic organic probe by dissolved humic substances and organically-coated aluminum oxide surfaces. Environ. Sci. Technol. 1993a; 27: 2523-2532.

Schlautman MA, Morgan JJ. Effects of aqueous chemistry on the binding of polycyclic aromatic hydrocarbons by dissolved humic materials. Environ. Sci. Technol. 1993b; 27: 961-969.

Schwarzenbach RP, Gschwend PW, Imboden DM. Environmental Organic Chemistry. Hobeken: John Wiley and Sons, 2003.

Semerjian L, Ayoub GM. High-pH-magnesium coagulation-flocculation in wastewater treatment. Adv. Environ. Res. 2003; 7: 389-403.

Shin H-S, Monsallier JM, Choppin GR. Spectroscopic and chemical characterizations of molecular size fractionated humic acid. Talanta 1999; 50: 641-647.

Simpson AJ. Determining the molecular weight, aggregation, structures and interactions of natural organic matter using diffusion ordered spectroscopy. Magn. Reson. Chem. 2002; 40: S72-S82.

Sparks KM, Wells JD, Johnson BB. The interaction of humic acid with heavy metals. Aust. J. Soil Res. 1997; 35: 89-101.

Tabata A, Kashiwada S, Ohnishi Y, Ishikawa H, Miyamoto N, Itoh M, Magara Y. Estrogenic influences of estradiol-17β, p-nonylphenol and bis-phenol-A on Japanese Medaka (*Oryzias latipes*) at detected environmental concentrations. Water Sci. Technol. 2001; 43: 109-116.

Thurman EM, Malcolm RL. Preparative isolation of aquatic humic substances. Environ. Sci. Technol. 1981; 15: 463-466.

Traina SJ, McAvoy DC, Versteeg DJ. Association of linear alkylbenzenesulfonates with dissolved humic substances and its effect on bioavailability. Environ. Sci. Technol. 1996; 30: 1300-1309.

Vaes WHJ, Ramos EU, Verhaar HJM, Seinen W, Hermens JLM. Measurement of the free concentration using solid-phase microextraction: Binding to protein. Anal. Chem. 1996; 68: 4463-4467.

Yamamoto H, Liljestrand HM. The fate of estrogenic compounds in the aquatic environment: Sorption onto organic colloids. Water Sci. Technol. 2003; 47: 77-84.

Yamamoto H, Liljestrand HM, Shimizu Y. Effects of dissolved organic matter surrogates on the partitioning of 17β-estradiol and p-nonylphenol between synthetic membrane vesicles and water. Environ. Sci. Technol. 2004; 38: 2351-2358.

Yamamoto H, Liljestrand HM, Shimizu Y, Morita M. Effects of physical-chemical characteristics on the sorption of selected endocrine disruptors by dissolved organic matter surrogates. Environ. Sci. Technol. 2003; 37: 2646-2657.

Yan WL, Bai R. Adsorption of lead and humic acid on chitosan hydrogel beads. Water Res. 2005; 39: 688-698.

Zhang Z, Poerschmann J, Pawliszyn J. Direct solid phase microextraction of complex aqueous samples with hollow fibre membrane protection. Anal. Commun. 1996; 33: 219-221.

Neale, P.A. ; Escher, B.I. ; Schäfer, A.I. ; (2009) pH dependence of steroid hormone–organic matter interactions at environmental concentrations, The Science of the Total Environment, 407, 1164-1173. doi:10.1016/j.scitotenv.2008.09.035

List of Tables

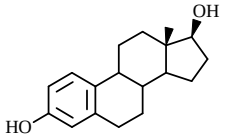
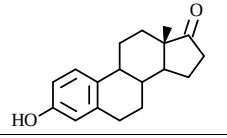
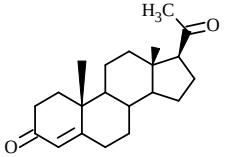
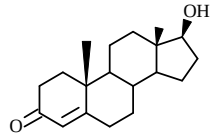
Table 1

	Molecular weight (g/mol)	pK_a	Total acidity (meq/g)	Carboxylic groups (meq/g)	Hydroxyl groups (meq/g)	Conformation changes with pH
Aldrich humic acid	600 – 60000[a]	4.3[b]	7.06[e]	4.80[e]	2.26[e]	Low pH: Coiled and rigid High pH: Linear and flexible [h]
Alginic acid	210000	3.4[c]	8.65[f]	7.02[f]	1.63[f]	Low pH: Coiled at pH 3, but size and flexibility increases from pH 4 High pH: Depolymerises above pH 8 [I]
Tannic acid	1701	8.5[d]	11.4[g]	1.88[g]	9.55[g]	pH > 6.5: Hydrolysis to gallic acid which increases with pH [j]

[a]Simpson, 2002; [b]Shin *et al*. 1999; [c]Davis *et al.* 2003; [d]Kraal *et al.* 2006; [e]Kim *et al.* 1990; [f]Jeon *et al.* 2002; [g]Flores-Céspedes *et al*. 2006; [h]Ghosh and Schnitzer, 1980; [i]Avaltroni *et al*. 2007; [j]Osawa and Walsh, 1993.

Neale, P.A. ; Escher, B.I. ; Schäfer, A.I. ; (2009) pH dependence of steroid hormone–organic matter interactions at environmental concentrations, The Science of the Total Environment, 407, 1164-1173. doi:10.1016/j.scitotenv.2008.09.035

Table 2

	Structure	Formula	Molecular Weight (g/mol)	pK_a[a]	Log K_{OW} at pH 7[b]	pH dependent octanol-water distribution ratio[c]	
						pH	Log D_{OW}
Estradiol		$C_{18}H_{24}O_2$	272.4	10.23	4.01	9	3.99
						10	3.81
						11	3.17
						12	2.23
Estrone		$C_{18}H_{22}O_2$	270.4	10.34	3.13	9	3.11
						10	2.97
						11	2.38
						12	1.46
Progesterone		$C_{21}H_{30}O_2$	314.5	-	3.87	-	
Testosterone		$C_{19}H_{28}O_2$	288.4	-	3.32	-	

[a]Kwon *et al.* 2006; [b]Hansch *et al.* 1995; [c]D_{OW} is pH dependent octanol-water distribution ratio

$D_{OW} = f_{neutral} \cdot K_{OW}$ (Schwarzenbach *et al*., 2003).

Table 3

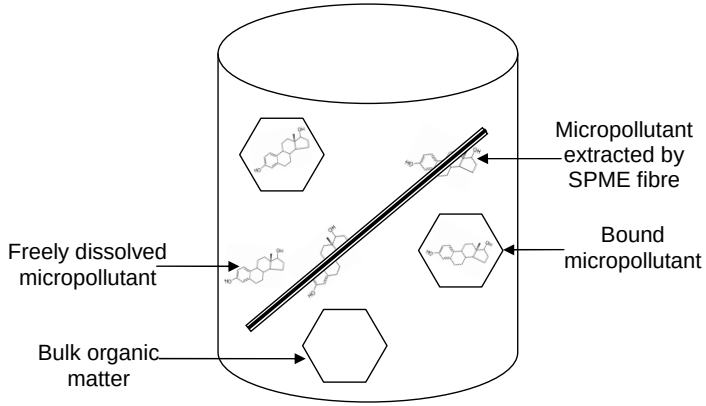
		pH 4 ± M.U*	pH 7 ± M.U*	pH 9 ± M.U*	pH 10 ± M.U*	pH 12 ± M.U*
Estradiol	Aldrich HA	4.18±0.23	4.21±0.23	3.95±0.21	3.83±0.21	-
	Alginic Acid	3.88±0.21	3.96±0.21	3.42±0.18	-	-
	Tannic Acid	5.11±0.28	4.86±0.26	4.02±0.22	-	-
Estrone	Aldrich HA	5.27±0.28	4.82±0.26	4.75±0.26	4.52±0.24	-
	Tannic Acid	6.02±0.33	5.51±0.30	4.69±0.25	-	-
Progesterone	Aldrich HA	4.73±0.26	4.59±0.25	4.58±0.25	4.48±0.24	4.54±0.25
	Alginic Acid	3.89±0.21	3.57±0.19	3.40±0.18	3.24±0.17	-
	Tannic Acid	5.87±0.32	5.53±0.30	4.38±0.24	3.79±0.20	4.27±0.23
Testosterone	Aldrich HA	4.13±0.22	4.04±0.22	4.17±0.23	4.12±0.22	4.45±0.24
	Alginic Acid	-	3.16±0.17	3.67±0.20	3.77±0.20	-
	Tannic Acid	4.88±0.26	4.74±0.26	4.14±0.22	3.87±0.21	4.40±0.24

* M.U is used to indicate the measurement uncertainty associated with the partition coefficients.

Neale, P.A. ; Escher, B.I. ; Schäfer, A.I. ; (2009) pH dependence of steroid hormone–organic matter interactions at environmental concentrations, The Science of the Total Environment, 407, 1164-1173. doi:10.1016/j.scitotenv.2008.09.035

List of Figures

Figure 1



Neale, P.A. ; Escher, B.I. ; Schäfer, A.I. ; (2009) pH dependence of steroid hormone–organic matter interactions at environmental concentrations, The Science of the Total Environment, 407, 1164-1173. doi:10.1016/j.scitotenv.2008.09.035

Figure 2

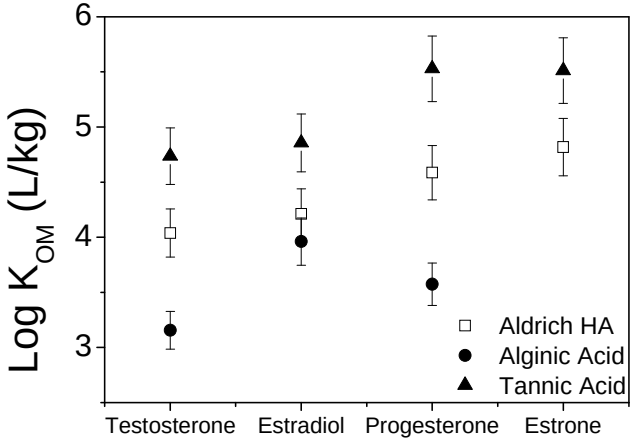
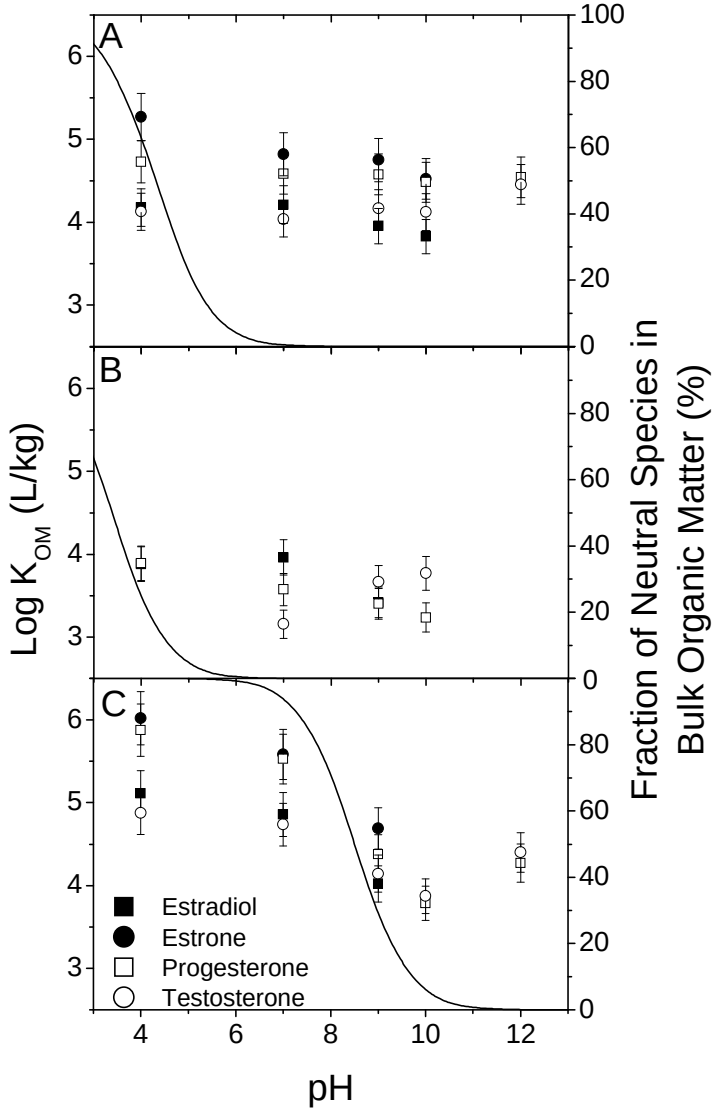


Figure 3



Neale, P.A. ; Escher, B.I. ; Schäfer, A.I. ; (2009) pH dependence of steroid hormone–organic matter interactions at environmental concentrations, The Science of the Total Environment, 407, 1164-1173. doi:10.1016/j.scitotenv.2008.09.035

Figure 4

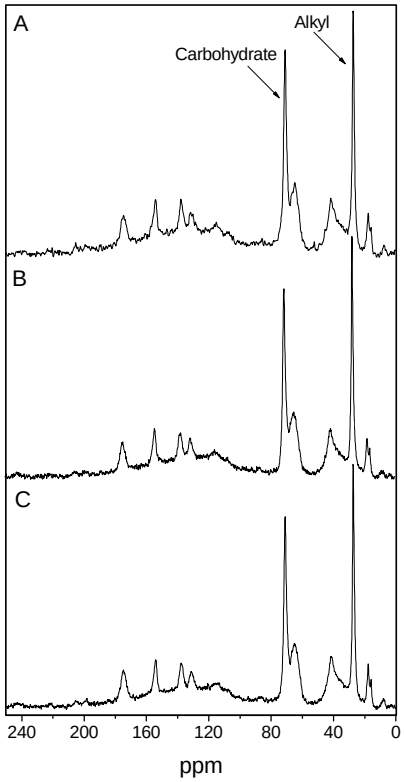
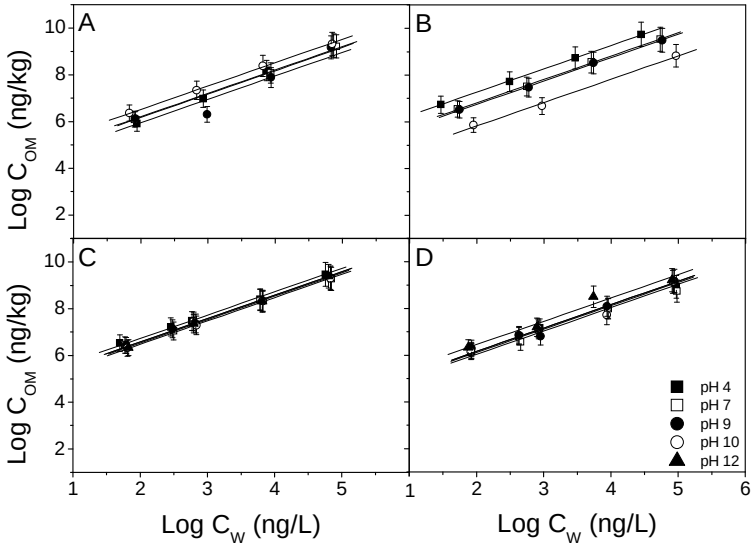


Figure 5



Neale, P.A. ; Escher, B.I. ; Schäfer, A.I. ; (2009) pH dependence of steroid hormone–organic matter interactions at environmental concentrations, The Science of the Total Environment, 407, 1164-1173. doi:10.1016/j.scitotenv.2008.09.035

Figure 6

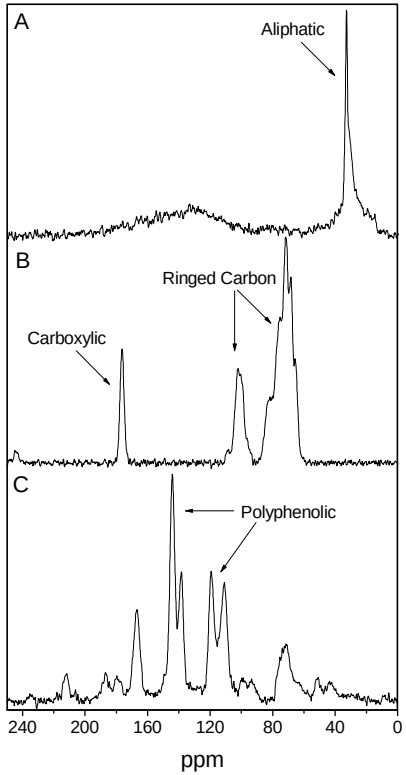
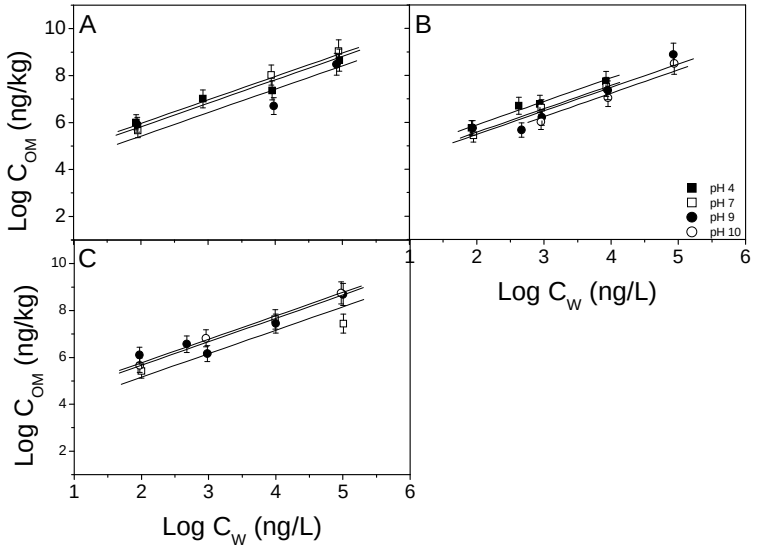


Figure 7



Neale, P.A. ; Escher, B.I. ; Schäfer, A.I. ; (2009) pH dependence of steroid hormone–organic matter interactions at environmental concentrations, The Science of the Total Environment, 407, 1164-1173. doi:10.1016/j.scitotenv.2008.09.035

Figure 8