Mediator solution influence on the sorption potential of sulfo-conjugated estrogenic steroid hormone and its metabolite in New Zealand dairy farm soils

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Abstract

Estrogenic steroid hormones have been shown to potentially contribute to damage to wildlife and ecosystem health. Understanding their fate and behaviour is important to assess their risk in the environment. We investigated the sorption potential of three dairy farm soils for an estrogenic conjugated steroid hormone and its metabolite employing a complex solvent extraction scheme using $CaCl_2$ and artificial urine as mediator solution. The sorption potential of E1-3S was about one order of magnitude lower than for the free counterpart, and the K_f values significantly changed between the two mediator solutions. The calculation of concentration-dependent effective distribution coefficients (K_d^{eff}) revealed that for a range of realistic exposure concentrations in grazed dairying environment, the common approach of employing $CaCl_2$ may deliver incorrect inferences for a proper risk assessment.

Key Words

Estrone, estrone-3-sulfate, concentration-dependent effective distribution coefficient, exposure scenario.

Introduction

Global concerns exist about the potential ability of estrogenic steroid hormones to interfere with the normal functioning of wildlife and human endocrine systems (Jobling *et al.* 1998). While in faeces free estrogens dominate, in mammalian urine, estrogens are primarily present in a conjugated and hydrophilic form. Estrone-3-sulfate (E1-3S) appears to be the dominant estrogen conjugate in cattle urine. Deconjugation of estrogen sulfates leads to the formation of estrone (E1), which has been quantified in agricultural soils. New Zealand's agricultural sector plays an important role in the country's overall economy, and dairy products, accounting for ~19% of the annual exports in 2007. The dairy cattle population of nearly 5.3 million outnumbers the human population by over a million heads, and most of the livestock continuously graze the pastures throughout the year. Furthermore, land application of dairy effluent has become increasingly popular. Given that approximately 80% of the defecations and urinations in a grazed dairy system occur on the paddock, potential exists for estrogens, and in particular for E1 and E1-3S, to reach receiving waters via surface runoff or leaching. Sorption of estrogens has been extensively studied over the past decade; however, the results are to some extent controversial due to the differences in the experimental protocols and the unique characteristics of the sorbents investigated (Sarmah *et al.* 2008). Estrogen sorption is also dependent on the mediator solution, but there is a dearth of information on the sorption potential from cow urine and essentially no information exists about the sorption behaviour of E1-3S to soils. Therefore the aim of this work was to conduct batch equilibration studies for E1-3S and E1 sorption onto three agricultural soils representative of the dairying region of North Island of New Zealand using 5 mM $CaCl_2$ and artificial cow urine as mediator solution.

Methods

Chemicals and soils

Estrone (>99% purity) and estrone-3-sulfate (≥95% purity) were purchased from Sigma-Aldrich, Australia. Three top soils (0–5 cm) were selected from dairy farming region of Waikato with contrasting properties.

Equilibrium batch sorption studies

A modified batch-equilibration method previously described by Sarmah et al (2008) was used to measure sorption of E1 and E1-3S to soils from a 5 mM $CaCl_2$ solution (pH 7.2, EC 1.4 dS/m), and an artificial urine solution (AU, pH 8.3, EC 30.7 dS/m) consisting of $KHCO_3$ (22.2 g/l), KCl (3.95 g/l), K_2SO_4 (6.7 g/l), $(NH_2)_2CO$ (23.5 g/l), and $C_2H_5NO_2$ (6.2 g/l). An appropriate amount of stock solution of each compound was added to the mediator solutions to yield 6 initial aqueous solution concentrations ranging from 0.25 to 5 mg/l. Air-dried soils (~2 g) were equilibrated with 30 mL of the two mediator solutions containing the single hormone at each of the 6 concentrations in 35 mL glass centrifuge tubes sealed with Teflon®-lined screw-caps, covered with aluminium foil and placed on a shaker for 2 h at 22°C (±2) in the dark. After equilibration, the tubes were centrifuged (20 min at 550 x g) and an aliquot of 5 mL supernatant solution was extracted using 5 mL dichloromethane (liquid-liquid), while soil remaining in the tube was also extracted using a combination of dichloromethane and dicyclohexylamine hydrochloride. An aliquot of 2 mL of the solvent from each phase was evaporated to dryness under nitrogen and reconstituted in 0.5 and 1.0 mL of 70% methanol in H_2O (20% methanol in H_2O for the E1-3S). Samples were analyzed by a high performance liquid chromatography and UV detector. Soil and mediator blanks were run to determine losses to the glassware and to check for interfering peaks during analysis.

Isotherm modelling

The sorption isotherms were modelled with the Freundlich sorption model ($C_s = K_f C_w^N$) where C_s (mg/kg), and C_w (mg/l) are the sorbed and solution phase concentrations at equilibrium, respectively, and K_f (mg^{1-N} L^N/kg) and N (unitless) are the Freundlich sorption coefficient and exponent signifying sorption magnitude and nonlinearity (N = 1 represents a linear isotherm). Given the apparent nonlinearity observed in most soil-solute combinations in the present study, we calculated the concentration-dependent effective distribution coefficient ($K_d^{eff} = K_f C_w^{N-1}$) values, and the corresponding concentration-dependent OC nomalized partition coefficients (K_{OC}, L/kg OC) for each soil and hormone.

Results and discussion

Sorption from $CaCl_2$ solution

Figure 1 (A) displays the measured sorption isotherms of E1 and E1-3S using 5 mM $CaCl_2$ solution with the corresponding Freundlich fits for the three soils. The K_d^{eff} values for E1 were 37.5, 34.2, and 71.7 mg^{1-N} L^N /kg, for the Horotiu, Hamilton, and Te Kowhai soils. The matching N values correspond well with previously reported literature values for E1 and imply linear sorption of E1 in the Hamilton soil (N~1), limitless sorption potential in the Horotiu soil (N > 1) and limited sorption potential (N < 1) in the Te Kowhai soils. In general, the sorption of estrogens in agricultural soils appears to be limited, i.e. only a limited amount of specific sorption sites exist, which are dominantly allocated within the organic matter domain of the soils. In contrast, soils with a high specific surface area have been reported to exhibit limitless sorption potential for estrogens. High organic matter (8.2%) and the high content of imogolite (30%), an allophanic clay mineral with hydrophobic features, may explain the limitless sorption of E1 in the Horotiu soil. In contrast to the Horotiu soil, the clay mineralogy of the Hamilton and Te Kowhai soils is dominated by kaolinite and halloysite, with major fractions of amorphous volcanic glass (Te Kowhai) and vermiculite (Hamilton). Kaolinite has been found to only weakly bind E2 and appears to have no sorption potential for E1.

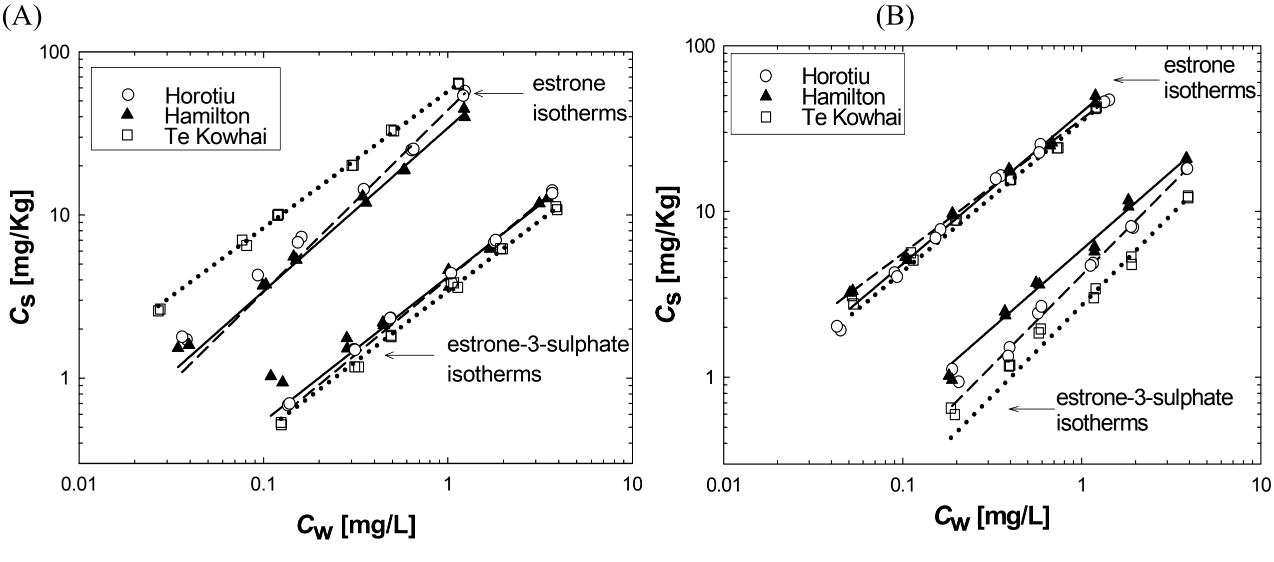

Figure 1. Sorption isotherms of estrone and estrone-3-sulfate in three soils from 5 mM $CaCl_2$ (A), and artificial urine solution (B). Lines indicate Freundlich model fits.

The sorption capacity for E1-3S was about one order of magnitude lower than for E1 (Figure 1 (A) and Table 1). The K_d^{eff} values accounted for 4.5, 4.9, and 4.0 mg^{1-N} L^N/kg, for the Horotiu, Hamilton, and Te Kowhai soils. The equivalent N values indicate more limited sorption of E1-3S as opposed to E1 in the Horotiu

(0.932 vs 1.115) and Hamilton (0.887 vs 1.001) soils, and a slightly higher sorption capacity in the Te Kowhai (0.886 vs 0.837) soil. Given the ionic and rather hydrophilic nature of E1-3S, it is expected to exhibit lower sorption affinity to the organic matter domain of soils than its free counterpart. At a pKa of −3.0, the E1-3S molecule is always negatively charged, and hence electrostatic interaction which governs anion sorption depends on the net charge of the clay minerals and organic matter constituents in the soils. However, under the given conditions with a solution pH of 7.2, the eligible clay minerals present in the soils would not be charged positively thereby excluding the possibility of significant anion retention by the clay minerals. Concentration-dependent log K_{OC} values for E1 based on the lowest equilibrium concentration of 0.25 mg/L ranged from 2.6 to 3.2 ($CaCl_2$) and 2.7 to 3.0 (artificial urine) among the soils. While there were no literature data available to compare our findings on K_{OC} values for E1-3S, log K_{OC} values for E1-3S were ~ 1 log unit lower than E1 in the three soils.

Table 1. Freundlich isotherm parameters for E1 and E1-3S from $CaCl_2$ and AU urine solution. Parameters are illustrated with standard error (SE). Regressions were all significant at $p < 0.001$. Differences in sorption parameters were calculated by means of a paired t test (two tailed).

Soil/ Mediator sol	K_d^{eff} (L/kg)	K_f ± SE ($mg^{1-N} L^N /kg$)	N ± SE	R^2 adj	log K_{OC}# $C_w = 0.25$ mg/L
E1- $CaCl_2$					
Horotiu	37.5	44.0 ± 0.7	1.115 ± 0.045	0.995	2.66
Hamilton	34.2	34.2 ± 0.7	1.001 ± 0.043	0.991	2.93
Te Kowhai	71.7	57.2 ± 0.4	0.837 ± 0.013	0.999	3.15
E1-AU					
Horotiu	46.8	36.0* ± 0.5	0.81* ± 0.023	0.995	2.75
Hamilton	44.8	39.8* ± 0.8	0.915 ± 0.041	0.99	3.04
Te Kowhai	39.8	34.9* ± 0.6	0.905 ± 0.034	0.993	2.9
E1-3S-$CaCl_2$					
Horotiu	4.5	4.08 ± 0.09	0.932 ± 0.020	0.997	1.73
Hamilton	4.9	4.18 ± 0.15	0.887 ± 0.034	0.992	2.08
Te Kowhai	4	3.42 ± 0.07	0.886 ± 0.019	0.998	1.9
E1-3S-AU					
Horotiu	3.7	4.13 ± 0.10	1.089* ± 0.021	0.998	1.64
Hamilton	6.4	5.89* ± 0.24	0.943 ± 0.035	0.993	2.2
Te Kowhai	2.4	2.72* ± 0.13	1.094* ± 0.038	0.994	1.67

K_d^{eff} is the concentration-dependent effective distribution sorption coefficient calculated using $K_d^{eff} = K_f C_w^{N-1}$ at $C_w =$ 0.25 mg /L; the asterisk (*) indicates significant difference ($p<0.01$) between $CaCl_2$ and artificial urine; # concentration-dependent organic carbon normalized sorption coefficient calculated using $K_{OC} = K_f C_w^{N-1}/f_{oc}$, where f_{oc} is the fraction of organic carbon in soil.

Sorption from artificial urine solution

Figure 2 (B) displays the measured sorption isotherms along with the Freundlich fits for E1 and E1-3S sorption from AU for the three soils. The K_d^{eff} values for E1 increased to 46.8 and 44.8 $mg^{1-N} L^N/kg$ in the Horotiu and Hamilton soils, while the value for the Te Kowhai soil decreased to 39.8 $mg^{1-N} L^N/kg$ relative to sorption from the $CaCl_2$ solution (Table 1), with a significant change in the N value only noticeable for the Horotiu soil (Table 3). Similarly, the Freundlich sorption parameters changed for E1-3S in the soils with an increase in the K_d^{eff} for the Hamilton soil. In contrast, the K_d^{eff} for the Horotiu and Te Kowhai soil decreased. The matching N values slightly increased, indicating a more linear sorption isotherm for the Hamilton and Te Kowhai soils. There was at least one log unit difference in the concentration-dependent log K_{OC} values for E1 and E1-3S under AU (Table 1) similar to the values observed under $CaCl_2$ solution. Since the AU had a higher conductivity than the $CaCl_2$ solution, the increased K_d^{ef} values for the sorption of E1 from AU in a few instances (Horotiu and Hamilton soil) could therefore result from a salting-out effect. Increased sorption in the presence of increasing soluble ions has also been reported earlier for other hydrophobic organic chemicals (polycylic aromatic hydrocarbons), and was attributed to a combination of the salting-out effect and changes in the net charge of the organic matter rendering its overall charge toward neutral and eventually enhancing the compound sorption. While the aqueous solubility of E1-3S was plausibly decreased in the AU, the higher ionic strength may have facilitated the sorption of the ionic sulfate part of E1-3S, for instance by alteration of clay minerals. The presence of potassium in the AU can cause the contraction of vermiculite to illite, during which temporary stronger intercalation of E1-3S could have occurred resulting in slightly higher K_d^{eff} values in the Hamilton clay loam. In contrast, the clay mineralogy of the Te Kowhai soil indicates very low potential for sorption of E1-3S, which lends further support to our assumption that the observed decrease in K_d^{eff} is a result of conformational changes in the organic matter domain due to the slightly higher pH and the high concentrations of hydrated ions in the AU.

Table 2. Isotherm parameters for the sorption of E1 formed during E1-3S from $CaCl_2$ solution. Parameters are illustrated with standard error (SE). Regressions were all significant at $p < 0.001$. Differences in sorption parameters were calculated by means of a paired t test (two tailed).[a]

Soil/	K_d^{eff} (L/kg)	$K_f \pm SE$ ($mg^{1-N} L^N$ /kg)	$N \pm SE$	R^2 adj	log K_{OC}# $C_w = 0.25$ mg/L
Horotiu	37.7	27.3* ± 3.0	0.766* ± 0.031	0.991	2.66
Hamilton	40.7	37.8 ± 4.6	0.946 ± 0.041	0.993	3.01
Te Kowhai	39.9	28.7* ± 3.6	0.762 ± 0.040	0.987	2.9

[a] The asterisk (*) indicates significant difference ($p < 0.01$) to E1 sorption as parent compound; concentration dependent organic carbon normalized sorption coefficient calculated using $K_{oc} = k_f C_w^{N-1}/f_{oc}$

Sorption of E1 as a metabolite of E1-3S

In the AU treatment the formation of E1 accounted for < 0.1% of the initial mass in all three soils and the data obtained were too scattered to construct feasible isotherms. However, in the $CaCl_2$ treatment E1 was formed with values of 4.6, 4.8, and 6.7% of the initial mass of E1-3S in the Horotiu, Hamilton, and Te Kowhai soils, respectively. Construction of additional metabolite isotherms for E1 was therefore warranted and sorption parameters are summarized in Table 2. The K_d^{eff} values for the Hamilton and Te Kowhai soils were 6.5 and 31.8 units lower for the metabolite isotherms and no change was observed in the K_d^{eff} for the Horotiu soil. While the corresponding N values decreased for all soils, a significant ($p < 0.01$) change was observed only for the Horotiu soil (Table 2). The range of log K_{OC} values for E1 as a metabolite of E1-3S in the three soils was similar to the values obtained when E1 sorption was performed as a parent compound. Sarmah *et al.* (2008) reported an N value of 0.75 for E1 in a similar soil from Horotiu series from an isotherm constructed using measured sorbed and solution phase concentrations during equilibrium of E2 with the soil. The N value of 0.766 in the present study for the Horotiu soil is thus comparable to the earlier value for a similar concentration range. The parent compound (E2) isotherm gave an N value > 1 for the Horotiu soil in Sarmah *et al.*'s (2008) study, which is in agreement with the N value for E1 as a parent compound in the present study (Table 2), confirming limitless sorption capacity for both E1 and E2 in the Horotiu soils. The differences in the N values observed in the literature are likely due to the different concentration ranges, since N is sensitive to the experimental concentration range.

Environmental implication

The common simplification of normalizing partition coefficients to the OC content of soils is not useful for inferring environmental implications and assessing risk assessment for estrogens and estrogen sulfates. Given many risk assessment models often require values for partitioning coefficients, the concentration-dependent effective distribution coefficient (K_d^{eff}) may serve as an alternative. Based on the exposure scenarios, we calculated the effective distribution coefficient ($K_d^{eff} = K_f\, C_w^{N-1}$) for a concentration range of 0.0001 to 10 mg/L for both compounds and treatments. When plots were made for the K_d^{eff} as a function of the aqueous hormone concentration for E1 and E1-3S, we observed that E1 sorption from AU would be considerably higher than from $CaCl_2$ at aqueous concentrations < 0.1 mg/L for the Hamilton and Horotiu soils, while the opposite applies to the Te Kowhai soil. The difference becomes more distinct at lower C_w, implying that, by using the common $CaCl_2$ isotherm, one would underestimate E1 sorption especially at low exposure concentrations in a grazed system for the Horotiu and Hamilton soils. For the Te Kowhai soil an overestimation is likely to occur by using the $CaCl_2$ isotherm. Differences were also observed for the K_d^{eff} of E1-3S at possible exposure concentrations, with pronounced effect at lower exposure concentrations, thus emphasizing that $CaCl_2$ isotherm would overestimate E1-3S sorption in the Horotiu and Te Kowhai soils. In contrast, sorption of E1-3S would be higher in the Hamilton soil in the given exposure concentration range. More work is needed to clarify the sorption mechanisms of these compounds under field conditions.

References

Jobling S, Nolan M, Tyler C, Brighty G, Sumpter J (1998) Widespread sexual disruption in wild fish. *Environmental Science & Technology* **32,** 2498–2506.

Sarmah AK, Northcott GL, Scherr FF (2008) Retention of estrogenic steroid hormones by selected New Zealand soils. *Environment International* **34**, 749–755.