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# Fate and transport of the β-adrenergic agonist ractopamine hydrochloride in soil–water systems

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ABSTRACT

The feed additive ractopamine hydrochloride was fortified at four concentrations into batch vials containing soils that differed in both biological activity and organic matter (OM). Sampling of the liquid layer for 14 days demonstrated that ractopamine rapidly dissipated from the liquid layer. Less than 20% of the fortified dose remained in the liquid layer after 4 hr, and recoveries of dosed ractopamine ranged from 8 to 18% in the liquid layer at 336 hr. Sorption to soil was the major fate for ractopamine in soil:water systems, *i.e.*, 42%–51% of the dose at 14 days. The major portion of the sorbed fraction was comprised of non-extractables; a smaller fraction of the sorbed dose was extracted into water and acetone, portions which would be potentially mobile in the environment. Partitioning coefficients for all soils suggested strong sorption of ractopamine to soil which is governed by hydrophobic interactions and cation exchange complexes within the soil OM. Ractopamine degradation was observed, but to mostly non-polar compounds which had a higher potential than ractopamine to sorb to soil. The formation of volatiles was also suggested. Therefore, despite rapid and extensive soil sorption, these studies indicated a portion of ractopamine, present in manures used to fertilize soils, may be mobile in the environment via water-borne events.



## Introduction

Ractopamine hydrochloride (ractopamine) is a feed additive used to improve feed efficiency, rate of weight gain, and to increase carcass leanness in hogs (Paylean®), cattle (Optaflex®), and turkeys (Topmax®) in more than 20 countries, including the United States, Canada, Japan and Mexico. Using β-agonists as feed additives for food animals is not without controversy. Ractopamine use affects heart, thyroid, urethra, and prostate (Catalano et al., 2012), and caused cardiac toxicity in greyhounds at levels 2–4 times the recommended dietary concentrations used for swine (Yaeger et al., 2012). As a consequence of β-agonist intoxications, particularly from clenbuterol, there are approximately 160 countries that have not approved the usage of any β-agonists as growth promoters, including China, the countries of the European Union, and Russia. Recent trade restrictions of American beef and/or pork by China, Russia and Taiwan (Anonymous, 2014; Bauer, 2013; Flynn, 2013), has made the detection of this drug in meat a high priority by US trade representatives.

ADME studies with ractopamine have provided insights into metabolic pathways, but also the environmental inputs to be expected from administration to target organisms. Intestinal adsorption of ractopamine is rapid, and the metabolism/

*Abbreviations:* Ractopamine, ractopamine hydrochloride; $C/C_0$, relative concentration; $K_d$, soil–water adsorption coefficient; $K_{oc}$, soil organic carbon–water partitioning coefficient; TRR, total radioactive residues; OM, soil organic matter; NSB, non-specific binding

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excretion of ractopamine and its metabolites appears to be species-dependent. Fecal/biliary elimination is an important excretion route in rats and turkeys, where 35%–60% of a single oral dose was eliminated in the bile (Smith and Paulson, 1994), but urinary elimination was most important in dog, monkey, and swine (>80% of dose; Dalidowicz et al., 1992). In rat, the major biliary metabolite was a mixed sulfate ester, glucuronide diconjugate (27% of dose), with lesser amounts of monosulfate ester (15%) and monoglucuronide (3.5%) conjugates (Smith et al., 1995). In colostemized turkeys, where nearly 38% of the dose was glucuronidated conjugates in the urine, the favored biliary metabolite of diastereomeric ractopamine was identified as the C-10 monoglucuronide, and monoglucuronidation at the C-10′ position occurred for the (1R, 3R) and (1R, 3S) stereoisomers; sulfate conjugates were not reported in turkeys (Smith et al., 1993). Similarly, bile and urine contained mainly monoglucuronide conjugates (*ca*. 40% of dose) in bile-duct cannulated turkeys (Smith et al., 2000).

Animal waste is frequently spread onto fields, and any ractopamine borne in this waste may serve as a vehicle for entry into the environment or the food chain. Ractopamine has been detected in swine lagoon wastewater at levels ranging from 134 to 524 ng/L and in groundwater removed from a nearby well (Bartelt-Hunt et al., 2011). In addition, ractopamine has been detected in an agricultural watershed (Jaimes-Correa et al., 2015) and in effluent-dominated streams (Brown et al., 2015). The mechanism by which ractopamine may move in the environment is essentially unknown, and although generally considered biologically inactive, conjugates can be hydrolyzed to release the parent compound. The previously-mentioned metabolism studies showed that there is, therefore, a potential for this β-agonist to enter the environment and affect non-target species. Many potential non-target species, such as quail, duck, trout, bluegill, Daphnia, green algae, earthworms, and even seed germination, were evaluated in toxicological studies, and the results indicated that lethality and overt physical signs of toxicity occurred at concentrations not expected in runoff (Elanco Animal Health, 1995). However, negative consequences of ractopamine on more sensitive endpoints have recently been described in nematodes, *e.g.*, decreased brood size and locomotion behavior, reactive oxygen species production, and chronic exposure to 10 μg/L of ractopamine reduced lifespans (Zhuang et al., 2014). Conjugates are more mobile than parent compound and the possibility of long-range, water-borne transport has been demonstrated for glucuronide and sulfate conjugates of 17β-estradiol (Shrestha et al., 2012; Bai et al., 2015). If even a third (Elanco, 1995) of the 67.8 million pigs raised in the US each year were to receive the lowest recommended dose levels of ractopamine (5 ppm), and most was eliminated unchanged or as glucuronide metabolites capable of reverting to parent, nearly 16,750 kg of ractopamine would be introduced into the American environment annually. This estimate would increase significantly when the contribution of ractopamine usage in cattle and turkey, for which it is also approved in the US, is included.

Ractopamine contains two phenolic groups, an amino functional group, and a benzylic hydroxyl group, each of which can influence the fate of the whole molecule under various environmental conditions (Fig. 1). Possessing two chiral centers ractopamine can exist as four stereoisomers, *i.e.*, RR, SS, RS, and SR. Although the commercial product is synthesized as a racemic mixture, isomer-specific treatment studies in rats have demonstrated that the RR isomer is the most biologically active (Ricke et al., 1999). No data exist in the published literature related to the behavior (sorption, leaching, and degradation) of β-agonists such as ractopamine in soil. We hypothesized that free ractopamine, which is reasonably water soluble (31 g/L; log$K_{ow}$ 2.4; pKa 9.4), and/or its highly water soluble glucuronide metabolites, can be water-borne from animal wastes and mobile in the environment. The purpose of this study was to use laboratory-scale batch studies to investigate the potential for ractopamine hydrochloride to be mobile in the environment and/or to sorb to and persist in soil. Radiolabeled ractopamine was utilized to facilitate the detection and(or) analysis of putative metabolites in soil compartments (*e.g.*, soil-bound, aqueous). Soils were selected based on organic matter, particle size, and pH differences and their relevance to agronomic practices in the Midwest region of North America. The soil–water adsorption coefficient ($K_d$), soil organic carbon–water partitioning coefficient ($K_{oc}$), and potential degradation products of ractopamine were determined.

## 1. Experimental

### 1.1. Radiochemical

[$^{14}$C]Ractopamine HCl (Elanco, Indianapolis, IN; 0.153 μCi/μmol; a mixture of all four stereoisomers; log$K_{ow}$ = 2.41 (est.) uniformly labeled in the phenethanolamine ring) was dissolved as a 1:1 water:methanol solution from which four spiking solutions were prepared. The purity of the [$^{14}$C]ractopamine dose was >98% as determined by reversed-phase HPLC (for conditions, see below). Each spiking solution was delivered as a 50 μL portion into triplicate batch vials for each dose level. The level of [$^{14}$C]ractopamine delivered to the batch vials was 0.50 μCi (High dose), 0.15 μCi (Med1), 0.07 μCi (Med2), and 0.03 μCi (Low), which corresponded to 363, 109, 51, and 22 μmol/L of ractopamine HCl.

### 1.2. Soils

Hamar series (Sandy, mixed, frigid Typic Endoaquolls) soil was collected from a cultivated field near a swine rearing facility in southeastern North Dakota, USA. Samples were collected from the top 15 cm (topsoil) and from 46 to 61 cm (subsoil) and stored at 4°C immediately upon arrival at the laboratory. The soils were collected from a location that had not received animal manure in over five years, and were the same soils used in previous laboratory (Fan et al., 2007; Zitnick et al., 2011; Shrestha et al., 2012; Bai et al., 2015) and field studies (Thompson et al., 2009; Schuh et al., 2011a; Schuh et al., 2011b) on estrogen fate and transport. Selected soil properties are given in Table 1 and were determined by the Soil Testing Laboratory at North Dakota State University. Prior to conducting the batch experiments, the soils were air dried for 48 hr, large clods were gently broken, and particles >2 mm were removed using a 2-mm sieve.

### 1.3. Experimental design

Batch absorption studies were conducted in 10 mL clear glass vials, each containing 1.6 g of soil suspended in 8 mL of

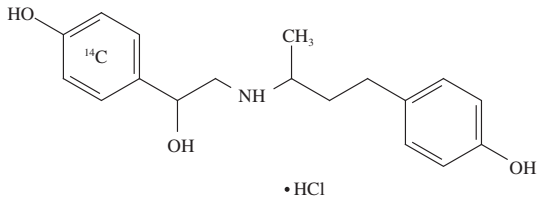


**Fig. 1 – Chemical structure of ractopamine hydrochloride. The [$^{14}$C]ractopamine used in these batch studies was labeled on the phenethanolamine ring, as indicated.**

0.01 mol/L $CaCl_2$; all vials were sealed with a crimp cap and septum. The batch studies were conducted under either natural or sterile conditions. Vials were sterilized in a Cesium-137 Irradiator (M38-4 Gammator, Radiation Machinery Corp., Parsippany, NJ) for 16 hr at 7.6 kGy. [$^{14}$C]Ractopamine was added to vials ($n$ = 3) with sterilized syringes to make the High (363 μmol/L), Med1 (109 μmol/L), Med2 (51 μmol/L), and Low (22 μmol/L) concentrations. In addition, triplicate matrix blank and control vials were prepared. Matrix blanks contained no [$^{14}$C]ractopamine and were used to measure and background radioactivity; control vials contained no soil, and were spiked at the Low dose level (22 μmol/L) with [$^{14}$C]ractopamine and were used to determine non-specific binding (NSB) of radioactivity to the experimental apparatus. All vials were placed into a rotating drum which continuously stirred the mixtures (12 revolutions per min). No attempts were made to measure headspace radioactive residues. Ractopamine concentrations utilized in this study were higher than free ractopamine concentrations in swine wastewater from a hog feeding facility in North Dakota (0.12 μmol/L) that we surveyed, but necessary in order to use radioactive tracing methods. The dissipation of total radioactive residues (TRR) from the aqueous phase was measured by sampling the liquid layer (100 μL) of the batch vials at 4, 8, 24, 48, 72, 168, and 336 hr. Aliquots were assayed for radioactivity for 10 min on a liquid scintillation counter (LSC; Packard Model 1900 CA, Meridan, CT) in Ecolite liquid scintillation cocktail (MP Biomedicals, Santa Ana, CA). Aliquots (100 μL) of the aqueous phase were also removed for HPLC analyses (see below). At the conclusion of the study, all vials were centrifuged, decanted, and both the aqueous and soil layers were sterilized with 2.7% (V/V or V/w) formaldehyde, and stored at –20°C until analyzed.

Soils (336 hr) at the High dose level (363 μmol/L; for ease of radioactive detection) were sequentially extracted 3× by sonication for 30 min with 4 mL of water, centrifuged at 380 ×$g$ for 20 min, and supernatant decanted. The soil was then extracted in the same manner using acetone. Within replicate, extracts were pooled by solvent and assayed for [$^{14}$C] by LSC. Quintuplicate aliquots (0.1 g) of the residual soil were combusted with a Packard Model 307 tissue oxidizer (Meridan, CT) to determine non-extractable [$^{14}$C]. Water and acetone extracts of soil and aqueous layer aliquots were chromatographed by reversed phase HPLC, eluant was trapped every minute, and radiochromatograms were constructed after assaying an aliquot of each eluant fraction for [$^{14}$C] by LSC. The HPLC system consisted of a single Gilson 306 pump (Middleton, WI), a Waters Resolve C18 column (5 μm, 250 × 4.6 mm), and a Gilson FC 204 fraction collector. Mobile phase was 72:28:2 water:acetonitrile:acetic acid with 1.08 g/L of 1-octanesulfonic acid (to promote peak sharpness). Isocratic conditions were used at a flow rate of 0.75 mL/min.

**Table 1 – Selected properties of soil samples as determined from the Soil Testing Laboratory at North Dakota State University.**

| | Topsoil | Subsoil |
|---|---|---|
| Depth (cm) | 0–15 | 46–61 |
| Organic matter (OM) (%) | 1.70 | 0.50 |
| Organic carbon (OC) (%) | 1.29 | 0.26 |
| Inorganic carbon (IC) (%) | 0.00 | 0.00 |
| pH | 7.0 | 7.4 |
| Cation exchange capacity (CEC) (cmolc/Kg) | 9.3 | 9.8 |
| Sand:silt:clay (%) | 83:10:7 | 90:4:6 |
| Mn (μg/g) | 292 | 223 |

### 1.4. LC–MS/MS analyses

Soil water and acetone extracts from 336 hr were filtered, evaporated to dryness, reconstituted with 80:20 water/methanol and submitted for LC–MS/MS analysis to identify ractopamine and/or its metabolites present in soil extracts, LC–MS/MS was performed with a Waters Acquity UPLC (BEH-C18, 1.7 μm, 2.1 × 50 mm) connected to a Waters Acquity TQD in ES+ mode (Waters Corporation, Milford, MA). For the UPLC portion of the LC–MS/MS, solvent A was 95:5 water/methanol plus 0.01% formic acid, and solvent B was methanol. A gradient from 5% B to 100% B was used over 1.25 min followed by a 1 min hold after which B returned to 5% and was held for 1.75 min. The flow rate was 0.6 mL/min. The source temperature was 150°C, desolvation temperature was 500°C, cone voltage was 30 V, capillary voltage was 3400 V. Collision energy was individually optimized for each fragment ion (302 > 284, 302 > 164, and 302 > 107), and quantitation included all three transitions. Data were acquired with MassLynx® software and quantitation software was TargetLynx® (Waters Corporation, Milford, MA).

### 1.5. Statistical analyses

Three-way full factorial statistical analyses were used to determine the main effects of biological activity (*i.e.*, natural vs. sterile), soil depth (*i.e.*, topsoil vs. subsoil), and ractopamine dose level (High, Med1, Med2 and Low) and interactions on liquid layer $C/C_0$ at 72 hr, 168 hr and 336 hr (Table S4). Significance was determined at α values of 0.05. Statistical software employed was JMP Statistical Software (ver. 11.2.0; Cary, NC).

## 2. Results and discussion

### 2.1. Soil and liquid layer distribution and mass balances

The TRR (*i.e.*, the parent [$^{14}$C]ractopamine and/or its radioactive degradates) was measured in the liquid layer and sorbed fraction of both natural and sterile topsoils and subsoils. The TRR levels at 336 hr were highest in the sorbed fraction for all experimental conditions, and the extent of the sorbed fraction binding did not vary much among the various experimental

conditions at 336 hr (*e.g.*, 42.5%–51.6% in Table 2). These values are lower than previously reported where 59%–83% soil sorption was observed in clay loam, loam or sandy loam soils (Elanco, 1995). However, soil sorption differences between studies may have been due to higher ractopamine concentrations, which were 7–122 fold higher in the present study and many have affected the degree of sorption, or the differences in soil textures, which were not fully reported by Elanco (1995). The liquid layer TRR measured at 336 hr never fell to background levels within the 2-week limitations of the study (8.0%–18.0% dose; Table 2), indicating that ractopamine-derived residues remain water soluble for long periods of time, putatively contributing to its mobility in the environment. Time-series data from control samples (radiochemical and $CaCl_2$ solution, but no soil) indicated increasing non-specific binding (NSB) of radioactivity to the glassware and/or the cap until steady-state conditions were reached at approximately 72 hr (Fig. 2). The amount of TRR in the control group reached 22.8% ± 1.2% of the applied radioactivity (Fig. 2). Total radioactivity recovered in the Low dose portion of the study ranged from 84.5%–91.2%, including NSB (Table 1). The partitioning results for the Med2, Med1 and High doses are presented in Supplemental Information (Table S1–S3), respectively.

The extent of NSB from an aqueous solution should be related to a compound's octanol:water partition coefficient ($\log K_{ow}$). Relatively low $\log K_{ow}$ values are reported for ractopamine (Elanco, 1995), and are 1.75 at pH 5.0, 1.02 at pH 7.0, and 17.4 at pH 9.0 in buffered solution. The pH of the present experiments was near 7 (data not shown); therefore, log $K_{ow}$ considerations would predict reasonably high water solubility and low NSB. However, when taking into consideration the p*K*a of ractopamine (p*K*a = 9.4), more than 99% of it is predicted to be ionized at pH 7.4 (calculations not shown), perhaps favoring ionic interactions with the cap or glass. Sorption of [$^{14}$C] did occur to the cap, as determined by combustion, but the amount detected (~1% of dose, data not shown) could not adequately account for the balance of radiocarbon not recovered.

The lack of full radiochemical mass balance (Table 1; Tables S1–S3), even after NSB was accounted for, suggests either (a) volatilization of radiochemical or (b) under-reporting of liquid layer and/or sorbed phase radiochemical assays. A review of the published literature did not mention conversion of ractopamine into volatile metabolites within animal systems, but rather to water-soluble glucuronide conjugates (Smith et al., 1995; Prezelj et al., 2003). However, Elanco reported that 7%–9% of 10.6 ppm $^{14}$C-ractopamine was mineralized to $CO_2$ over 64 days, and another 1% was converted to uncharacterized volatiles (Elanco, 1995). No provisions were made in the present study design to capture volatile radioactivity in the vial headspace; therefore, it was not possible to prove if volatile metabolites of ractopamine formed in our soils. Additional incubation studies utilizing the study design outlined in Fan et al. (2007) would address any putative mineralization of ractopamine. However, if an equivalent level of $CO_2$ and volatile formation had occurred in the present study, it would satisfactorily explain the radiochemical mass balance shortfalls (Tables 1; S1–S3), improving the radiochemical recoveries to between 93% and 99%. Incomplete combustion of complex matrices like soil may also have contributed to the low mass balance recoveries. Incomplete combustion of radioactivity fortified as chlorinated dioxins into dried feces has been observed by Hakk et al. (2009), but unlike ractopamine, dioxins are compounds which require high temperatures to completely oxidize. Additional sources of incomplete combustion may be ascribed to strong soil adsorption (Berry and Boyd, 1985), ionic binding (Gevao et al., 2000), or sequestration by macromolecules (Pignatello and Xing, 1996).

**Table 2 – Fractionation of radiolabel derived from fortification of a Low dose of [$^{14}$C]ractopamine hydrochloride (22 μmol/L) in batch vials after incubation with 8 mL of 0.01 mol/L $CaCl_2$ and 1.6 g of natural topsoil at 336 hr.**

| | Natural topsoil | Sterile topsoil | Natural subsoil | Sterile subsoil |
|---|---|---|---|---|
| Liquid layer | 9.7 ± 0.3 | 8.0 ± 0.2 | 16.9 ± 1.7 | 18.0 ± 0.3 |
| Aliquots removed | 2.8 ± 0.2 | 3.2 ± 0.2 | 5.5 ± 0.1 | 6.6 ± 0.1 |
| Sorbed layer | 49.2 ± 5.1 | 51.6 ± 6.8 | 46.0 ± 6.7 | 42.5 ± 4.4 |
| %Recovery (±SD) | 84.5 ± 5.3[a] | 85.6 ± 6.8[a] | 91.2 ± 7.7[a] | 89.9 ± 4.6[a] |

[a] Reported recoveries include non-specific binding (22.8% ± 1.2% of dose), which was also determined at the low dose level (*i.e.*, 22 μmol/L).

### 2.2. Liquid layer residues

Ractopamine had a high affinity for soil as evidenced by the rapid declines in liquid layer [$^{14}$C] for all experimental conditions. The relative aqueous concentration ($C/C_0$) values reached ~0.20 at 4 hr regardless of initial ractopamine concentration (Fig. 3). More frequent liquid layer sampling during the first 4 hr would have been beneficial to better define the rapid sorption of ractopamine; however, interpretation of the data during this period would have been complicated by competition with NSB. Thus, data analyses were conducted after 72 hr when steady-state conditions for NSB were achieved. The ractopamine liquid layer levels for the natural topsoil (0–15 cm soil depth) reached steady-state $C/C_0$ values of 0.07–0.13, which were lower ($p < 0.0001$) compared to the $C/C_0$ values of 0.10–0.19 for the natural subsoil (46–61 cm soil depth; Fig. 3). Similar results were observed for the pair of sterile soils, where topsoils $C/C_0$ values were lower compared to the subsoil values ($p < 0.0001$). The higher liquid layer TRR levels in the subsoils being inversely related to OM, suggested hydrophobic sorption of the ractopamine with soil OM. The topsoil consisted of 1.70% organic matter, while subsoil contained 0.50% (Table 2).

Despite the rapid, initial decline in TRR, the liquid layer TRR never dropped to 0% of the applied dose (*e.g.*, Fig. 3). It can be hypothesized that: (a) ractopamine TRR remained in solution by associations with dissolved organic matter, (b) ractopamine TRR may have associated with buoyant colloids suspended in the liquid layer, (c) ractopamine water solubility was sufficient to keep it solubilized, and/or (d) ractopamine was degraded to polar, water-soluble metabolites. Previous batch studies demonstrated steroid hormones and environmental contaminants can remain suspended in the liquid layer of saturated soil:water systems to a similar extent observed in the present study (Casey et al., 2003;

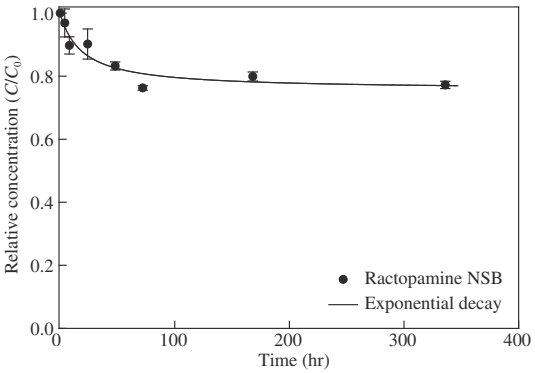


**Fig. 2 – Average (±s.d.) non-specific binding (NSB) of radioactivity to the experimental apparatus in control vials ($n$ = 3) fortified with 22 μM [$^{14}$C]ractopamine hydrochloride and 8 mL of 0.01 mol/L $CaCl_2$ and no soil ($n$ = 3).**

Casey et al., 2004; Fan et al., 2006). Subsequent studies confirmed the presence of both dissolved organic matter (Chambers et al., 2014) and colloids (Zitnick et al., 2011) as the agents for keeping 17β-estradiol in the liquid layer of batch studies at equilibrium. These findings have important environmental implications in that such associations may contribute to the water-based mobility of estrogens in the environment. Water solubility of ractopamine is 31.0 g/L at pH 7.0 (Elanco, 1995), and concentrations of fortified ractopamine in the liquid layer of even the high dose treatments at equilibrium was approximately 133 mg/L (calculations not shown). Evidence for the metabolism/degradation of ractopamine to polar by-products was not observed in RP-HPLC chromatograms, instead occasional detections of non-polar metabolites were observed in both the liquid layers and soil extracts (Fig. S1A; see Section 2.5). In vertebrates, ractopamine is converted mainly into glucuronide conjugates, with small fractions of sulfate conjugates, that are more water soluble compared to the parent compound (Smith et al., 1993;

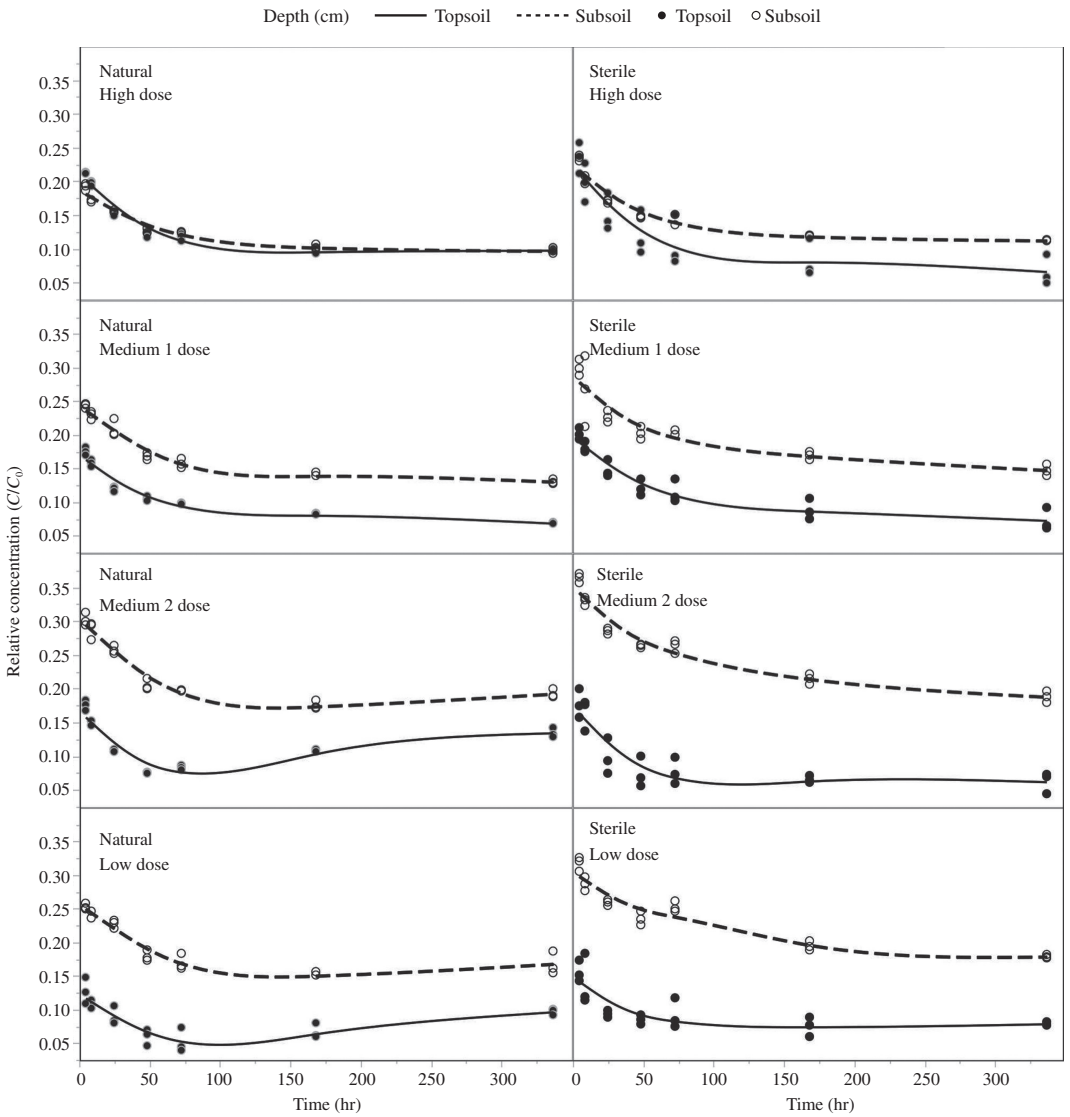


**Fig. 3 – Dissipation of [$^{14}$C]ractopamine hydrochloride from the liquid layers ($n$ = 3) of batch vials containing 8 mL of 0.01 mol/L $CaCl_2$ and 1.6 g of natural or sterile topsoil or subsoil (as described in Experimental).**

Smith and Paulson (1994) as cited in JECFA 2004; Smith et al., 1995); however, the present study was unlikely to have duplicated conditions necessary to yield conjugates, *e.g.*, presence of UDP-glucuronosyl transferase or sulfotransferase enzymes. Ractopamine does degrade in soil maintained at a moisture content of 70% of field capacity (Elanco, 1995). A 71% decline in soil-incorporated ractopamine occurred rapidly (2 days) while a slower decline of an additional 19% of dose occurred over another 15 weeks. No attempt to characterize degradation products was performed, however (Elanco, 1995).

### 2.3. Soil sorption

The distribution coefficients ($K_d$) for ractopamine were determined from the slopes of the linear regressions between sorbed phase concentrations and the liquid layer concentrations with intercepts forced to zero. These $K_d$ values increased with increasing incubation time, which indicated sorption was still approaching steady-state conditions (Table 3). Additionally, there were no significant differences (*t*-test p value was 0.15) between the $K_d$ values at steady-state (*i.e.*, after 72 hr) for the natural and sterile soils, suggesting that the biological activity of natural soils played a small role in the sorption of ractopamine (Table 3). Higher $K_d$ values are indicative of lower leaching potential, while low values are correlated to environmental mobility. The reported $K_d$ for ractopamine from sandy loam soils was 14.5 and 36.0 L/kg for clay loam soil (Elanco, 1995), which compared reasonably well to the range found in this study, *i.e.*, 19.8–47.2 in natural topsoil; 20.7–43.2 in natural subsoil (Table 3). The soil organic carbon–water partitioning coefficients ($\log K_{oc}$) for ractopamine were determined by normalizing the $K_d$ values for organic content and performing a $\log_{10}$ transform (Table 3). Identical to $K_d$ values, higher $\log K_{oc}$ values are indicative of low environmental mobility of an organic compound. When comparing the steady-state $\log K_{oc}$ values, the natural topsoil values (1.88–2.26) were significantly greater (*t*-test $p < 0.0001$) than subsoil (1.21–1.52; Table 3). The $\log K_{oc}$ values were normalized for OC, and should be the very similar for both topsoil and subsoil if hydrophobic sorption alone were occurring. Since they differed, these data suggest that another sorption process must be operating. As mentioned, ractopamine (pKa 9.6) was cationic under the experimental conditions selected (pH 7). We hypothesize that topsoil $K_d$ and $\log K_{oc}$ values were greater than subsoil values because more cation exchange complexes were present in higher OM soils (Astera, 2010). Elanco reported (1995) decreasing $K_d$ values for clay loam, to loam, to sandy loam soils, suggesting a correlation between ractopamine sorption and clay content, where greater clay would result in more ractopamine sorption. Elanco's (1995) data supports the hypothesis that cationic ractopamine binds in soils with greater amounts of cation exchange complexes, *i.e.*, soils with greater clay contents. These data may have consequences for crops grown in ractopamine-contaminated soils. Both alfalfa and wheat can take up ractopamine from ractopamine-contaminated soils, and uptake was directly correlated to ractopamine fortification and inversely related to soil OM (Shelver and DeSutter, 2015). Contaminated alfalfa used in animal feed could present problems to producers seeking to market ractopamine-free animals.

### 2.4. Main effects

The main effect of soil activity was not significant (at 72 hr, $p = 0.546$, at 168 hr and $p = 0.8552$, and at 336 hr and $p = 0.1706$; reported in Table S4) in explaining $C/C_0$ for any time, indicating soil biological activity had little effect on liquid layer ractopamine concentrations. In general, liquid layer TRRs from topsoil were not affected by biological status regardless of soil depth (Fig. 4). The main effect of depth was significant in explaining $C/C_0$, where mean values of $C/C_0$ in the subsoil were higher than topsoil. This effect of depth indicates the importance of hydrophobic sorption, where the higher OM of the topsoil would have greater capacity to sorb ractopamine and result in low $C/C_0$ values in the liquid layer compared to the lower OM subsoil. The main effect of initial dose level was significant in explaining $C/C_0$, where mean values of $C/C_0$ were higher for lower dose initial concentrations compared to high dose initial concentrations. This effect of dose level would indicate a concentration gradient driven sorption process, where greater concentrations would drive more ractopamine to be sorbed to the soil. This result implies that the lower concentrations of ractopamine present in the environment would be present in higher liquid layer concentrations compared to the high initial dose levels used in this study.

### 2.5. Degradation of ractopamine

In the liquid layers more than 75% of the radiochemical was present as parent compound as suggested by HPLC and confirmed by LC–MS/MS (Fig. 4), but parent ractopamine in the liquid layers did not achieve steady state conditions until 336 hr, suggesting both degradation and adsorption/desorption were simultaneously occurring (Fig. 4a). Less polar radioactive residues were observed by reversed-phase HPLC (Fig. S1A), but were not characterized. Polar radioactive compounds were not observed. Quantitatively, the degradation products in the liquid layer displayed no general trends, but instead suggested active adsorption/desorption was occurring (Fig. 4b). Ractopamine is normally stable towards hydrolysis in buffered water (pH 7) at temperatures up to 52°C (Elanco, 1995).

Similarly, soil extracts displayed a mixture of parent ractopamine and degradates (Fig. 5). High dose soils at 336 hr were partitioned into water-extractables, acetone-extractables, and a fraction that could not be extracted (non-extractables). Parent ractopamine was more abundant than any degradates in the water extractables (Fig. S1B), often the only compound detectable; in acetone-extracts, the parent compound was at least 2.5 times as abundant as the degradates (Fig. 5). These data indicated that the majority of the removable radioactivity was parent ractopamine, and that it would be the most abundant species in soil leachates. An estimate of the amount of parent ractopamine remaining at 336 hr ranged from 16.5% of the applied dose for sterile topsoil to 35.2% for natural subsoil. This matched well with measurements of the soil-incorporated decline in ractopamine previously reported (29% ractopamine remained at 2 day, which declined to 6% at 15 weeks; Elanco, 1995). The data presented in Fig. 5 also indicates that there is a difference in the extractable fraction of parent ractopamine when comparing the higher OM topsoil versus the lower OM

**Table 3 – Calculated partitioning coefficients $K_d$ from the ratio of a chemical's sorbed concentration to the dissolved concentration, and the soil organic carbon–water partitioning coefficient ($\log K_{oc}$) calculated from the normalization of the $K_d$ for total soil organic content.**

| Time (hr) | Topsoil natural $K_d$ (L/kg) | Topsoil sterile $K_d$ (L/kg) | Subsoil natural $K_d$ (L/kg) | Subsoil sterile $K_d$ (L/kg) | Topsoil natural ($\log K_{oc}$) | Topsoil sterile ($\log K_{oc}$) | Subsoil natural ($\log K_{oc}$) | Subsoil sterile ($\log K_{oc}$) |
|---|---|---|---|---|---|---|---|---|
| 72 | 38.9 | 39.0 | 34.0 | 27.5 | 2.17 | 2.18 | 1.42 | 1.33 |
| 168 | 47.4 | 51.7 | 41.1 | 34.5 | 2.26 | 2.30 | 1.50 | 1.43 |
| 336 | 47.2 | 64.8 | 43.2 | 37.6 | 2.26 | 2.40 | 1.52 | 1.46 |

Values were derived only for time points after equilibrium was achieved, *i.e.*, ≥72 hr.

subsoil. Greater amounts of extractable ractopamine in the subsoil suggested a decreasing influence of organic matter on sorption, presumably a result of more cation exchange capacity and/or hydrophobic sorption. Because it was not possible to obtain speciation data on the non-extractable radioactivity in soil, conclusions about the ultimate fate of this portion of the dose were not possible. Presumably, non-extractables would be environmentally immobile, although there are known cases where re-release of environmental contaminants (polycyclic aromatic hydrocarbons) may occur in response to soil turnover (Mahro and Kästner, 1993).

Because more parent ractopamine than degradate was detected in water and acetone extracts of subsoils than in extracts of topsoils, the degradation of ractopamine was likely governed by the preponderance of OM in topsoils. This conclusion is reasonable since biological activity is typically associated with topsoils rather than subsoils (Sradnick et al., 2014; Djajadi et al., 2012). The parent-to-degradate ratio for acetone extracts of soil was approximately 40 for natural and sterile subsoils (Fig. 5b, d), but <18 for topsoils (Fig. 5a, c). The acetone degradates were less polar than parent ractopamine, as determined by RP-HPLC (data not shown), and as such, would be more likely to sorb to soil organic matter than parent. The identity of these degradates were not elucidated, however, it could be hypothesized that dehydration of the phenethanolamine hydroxyl group would yield a stable by-product consisting of extended conjugation with the aromatic ring, and lower polarity. However, soil non-extractables, often assumed to be the products of metabolism/degradation, were present in sterile soils (Fig. 5c, d). Therefore, soil OM may be a more important factor in producing the non-extractables than biological activity. Ractopamine hydrochloride, being cationic, putatively can associate with the negatively-charged exchange complexes present on clay particles (Astera, 2010) to possibly become sequestered in the pores of soil. Ractopamine sorption previously showed a positive correlation in soils with higher clay content (Elanco, 1995), but the clay surface is not a particularly good substrate for sorbing organic compounds dissolved in water. However, the organic matter of soil also possesses cation exchange complexes (Astera, 2010) so that soils with similar clay contents but higher organic matter content, *i.e.*, topsoil vs. subsoil, will have higher amounts of negatively charged exchange complexes, presumably favoring (positively-charged) ractopamine sorption. Previously, organic cations were used to replace a portion of the inorganic cations normally present in a soil exchange complex, which resulted in an organic-clay complex capable of increased hydrophobic bonding to organic molecules from water. For example, treatment of soils with large hydrophobic cations to form organo-clays has been shown to increase the soil sorption of pentachlorophenol (Boyd et al., 1988).

## 3. Conclusions

Despite its high water solubility ractopamine hydrochloride was strongly and rapidly sorbed to soil in saturated soil:water systems. Sorption was dominated by hydrophobic interactions

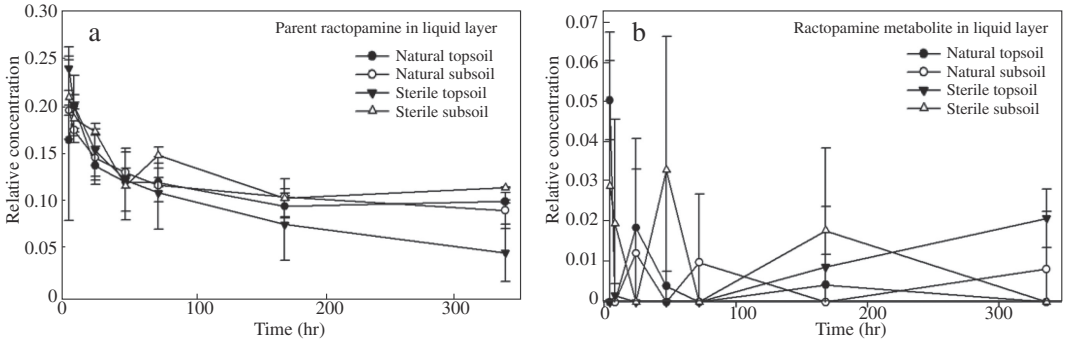


**Fig. 4 – Average percent composition of [$^{14}$C]ractopamine (a) or ractopamine degradates (b) in the liquid layer of batch vials across time as a percentage of applied dose in natural or sterile soils either with topsoil or subsoil ($n$ = 3). Data were based on the high dose fortification (363 μmol/L).**

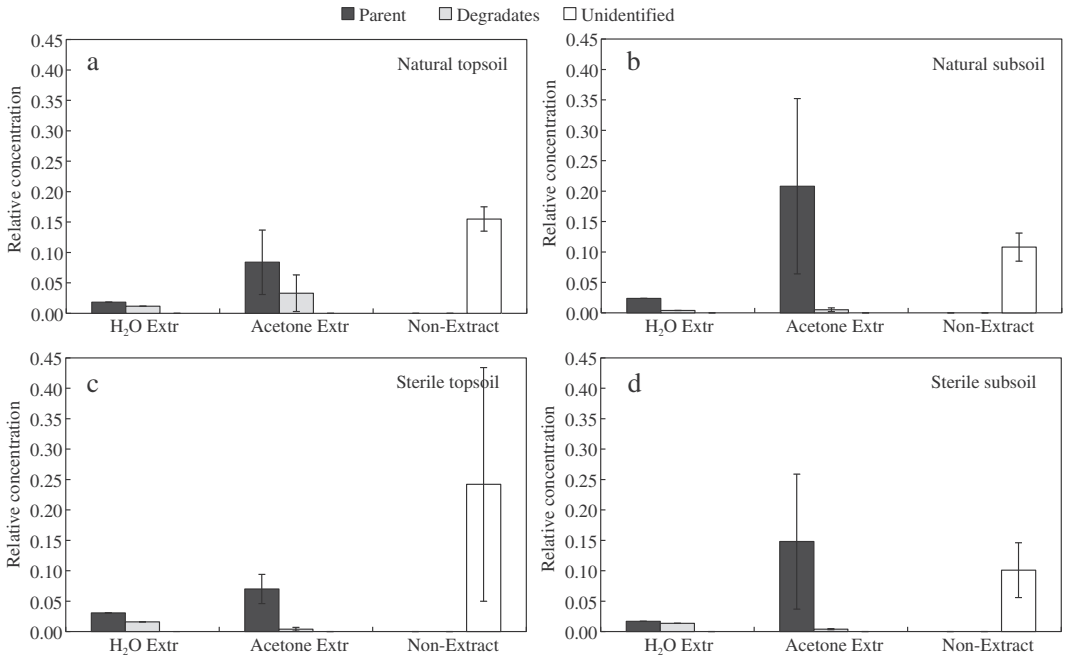


**Fig. 5 – Characterization of total radioactive residues at 336 hr in soil extracts of [$^{14}$C]ractopamine fortified natural or sterile soils either with topsoil and subsoils. Data were based on the High dose fortification (363 μmol/L).**

with soil organic matter, although sorption via cation exchange complexes was also possible. Biological activity plays only a minor role in ractopamine degradation. There were initial dose effects observed for liquid layer TRR, which may have environmental implications in that low ractopamine levels would be more prone to be water-borne (mobile) than higher levels. Ractopamine was degraded to less polar compounds in water-saturated soils, but the majority of the removable material was parent compound. Within the restrictions imposed by the study design, these data suggest that despite being sorbed quickly and/or degraded to a significant extent, ractopamine could continue to leach out of soils amended with animals wastes containing ractopamine at an estimate of 8% to 18% of applied dose. These results also indicated the mobility and risk of ractopamine to the environment is dependent on the soil type, which is highly variable, and may include other mitigating factors that might result in higher than expected concentrations, such as facilitated transport caused by macropore bypass or colloids, or lower concentrations, such as low conductivity soils and high organic fractions. However, the present research has provided some of the first insights into the fate and transport of such an environmental burden of ractopamine.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2015.11.026.

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