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USE OF A SURROGATE TO EVALUATE THE IMPACT OF TILLAGE ON THE TRANSPORT OF STEROID HORMONES FROM MANURE-AMENDED AGRICULTURAL FIELDS

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ABSTRACT. *Beef feedlot manure distributed to row crop production areas is a potential surface water contaminant source of the steroid hormones commonly used in beef cattle production. This article reports on research conducted at the University of Nebraska Haskell Agricultural Laboratory near Concord, Nebraska, in July 2009. Manure, collected from beef feedlot pens, was stockpiled for ten months prior to application to a row crop field. Previous research identified that the detection frequency of steroid hormones in beef manure varies greatly. Thus, a surrogate (17α-ethynylestradiol, EE2) was applied at a rate of 75 g ha^{-1} to ensure detectable concentrations in surface runoff samples. EE2 was applied directly to beef cattle manure and to bare soil. The EE2 and manure were either incorporated using a single disk treatment (T) or left on the soil surface in a no-till practice (NT). A rainfall simulation experiment was conducted 24 h after manure and EE2 incorporation using a factorial design consisting of tillage, manure, and EE2 treatments. Runoff samples were collected at 5 min intervals during a 30 min runoff period for each plot. Results indicated 96% less EE2 mass transport from disk-tilled plots compared to no-till. The greatest loss of EE2 was 156 and 6 mg ha^{-1} from no-till and disked plots, respectively. Results of this study showed that a single-pass disk tillage treatment can limit the overland transport of steroid hormones from crop production areas.*

Keywords. *Ethynylestradiol, Manure, Steroid hormone, Surface runoff, Tillage.*

Steroid hormones in surface water have become an environmental and public health concern, as they can disrupt normal endocrine function during critical growth stages of aquatic life (Colborn et al., 1993; Guillette et al., 2000; Matthiessen, 2003; Falconer et al., 2006). Adverse effects of steroid hormone exposure to aquatic organisms include abnormal blood hormone levels, masculinization of females, feminization of males, altered sex ratios, intersexuality, and reduced fertility (Jobling and Tyler, 2003). Moreover, exposure to steroid hormones has also been linked to increased incidence of human cancers, sexual disorders, and decline in male:female ratios (Miller and Sharpe, 1998; WHO, 2002; Hood, 2005; Mackenzie et al., 2005). Although wastewater treatment plants, septic systems, rangeland grazing, aquaculture, and paper and pulp industries are common sources of natural and artificial steroid hormones in the environment, beef cattle feedlots are the only source of the synthetic steroid hormones trenbolone acetate and melengestrol acetate (Swartz et al., 2006; Kolodziej and Sedlak, 2007; Kolodziej et al., 2004; Jenkins et al., 2003; Young and Borch, 2009).

Biswas et al. (2013) provided a status report on the issues surrounding the use of steroid hormones to produce beef cattle, and they summarized studies designed to determine the environmental fate of steroid hormones. Nichols et al. (1997) evaluated the effect of broiler litter application rate on estradiol concentration in surface runoff and found that concentrations of estradiol in runoff increased with litter application rate. In a follow-up study, Nichols et al. (1998) evaluated the efficacy of grass buffer strip length in reducing the transport of estradiol in runoff from tall fescue plots amended with poultry litter. At a litter applica-

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tion rate of 5 Mg ha^{-1}, the mean runoff concentration of estradiol entering the buffer strip was 3500 ng L^{-1}, which was reduced by 58%, 81%, and 94% upon exiting buffer strips 6.1, 12.1, and 18.3 m wide, respectively. In a similar study, Finlay-Moore et al. (2000) found that runoff from grasslands amended with chicken litter reached maximum concentrations of 2530 and 1830 ng L^{-1} for estradiol and testosterone, respectively, Studies by Jenkins et al. (2009) and Dutta et al. (2010) evaluated the loss of steroid hormones in surface runoff under two tillage practices at different poultry litter application rates and found significantly less hormone loss in runoff from no-till plots due to low runoff generation when compared to a conventional or reduced tillage treatment. However, few studies have investigated the effects of beef manure management strategies and tillage on the fate and transport of steroid hormones in runoff from agricultural fields.

One of the trends that has emerged when conducting field studies using beef feedlot manure produced by animals receiving growth promotants is a low frequency of steroid hormone detections. For example, Gall et al. (2011) found few detections of individual steroid hormones in subsurface tile drainage from fields with land-applied beef and dairy lagoon effluent or solids. They analyzed for nine different natural and synthetic hormones (four natural estrogens, three synthetic androgens, and two natural androgens), and none were detected in more than 19% of the samples from drainage ditches and 23% of the samples from tile drains, except estrone, which was detected in 20% and 52% of samples from drainage ditches and tile drains, respectively. Research reported by Yang et al. (2012) found different detection frequencies of hormones depending on the timing of rainfall. Previous work by the authors noted less than 21% detections of steroid hormones or their metabolites in surface runoff samples collected during rainfall simulations on row crop production areas receiving stockpiled or composted beef feedlot manure (data not shown). Difficulty in detecting hormones in drainage and surface runoff waters make it difficult to identify differences among a set of treatments when using manure with hormone concentrations that are at or below chemical analysis detection limits. Thus, research conducted to investigate best management practices necessary to limit the movement of steroid hormones in the environment may be unsuccessful.

Despite the ubiquitous nature of hormones in the environment and the ability to clearly identify the concentration of specific steroid hormones in beef animal waste, Mansell et al. (2011) reported a sudden drop in androgen and progesterone concentrations in feedlot soils following a rainfall simulation study. The authors could not account for the decrease in concentration despite a rigorous soil sampling protocol. They postulated that soilborne microbes converted the hormones into forms that were not in the analysis. Others have found conversion of steroid hormones from one form to another during the course of controlled laboratory experiments (Colucci et al., 2001; Lee et al., 2003; Das et al., 2004). The work conducted by these researchers and others has documented that steroid hormones can be transformed by a range of environmental factors, making mass balance studies nearly impossible. Thus, while a mass balance study may have been preferable, the difficultly of conducting such an experiment was beyond the scope of this project.

Application of surrogate compounds has been used in a broad range of studies (Pantone et al., 1996; Hall et al., 1983), including investigation of the potential loss of veterinary antibiotics (Burkhardt et al., 2005; Dolliver and Gupta, 2008). In order to limit the issues discussed above, the purpose of using a surrogate in this study was to apply a steroid hormone that acted similarly to steroid hormones found in manure but is not contained in the manure. Surrogates are typically applied at concentrations that will ensure detection in the runoff or leachate samples. Previous research conducted by the authors of this article indicated that some steroid hormones contained in manure have a relatively short half-life, and some exhibited very strong adsorption-desorption characteristics (data not shown). The combination of these factors and the dilution of the surrogate by simulated rainfall create substantial uncertainty in the potential concentration of the surrogate in surface runoff. Consequently, the concentration needs to be great enough to ensure detection of the surrogate following dilution by water applied during a rainfall simulation study. Thus, the objective of this study was to evaluate the impact of incorporating manure with tillage on the transport of steroid hormones from cattle manure-amended agricultural field using a surrogate.

Materials and Methods

A rainfall simulation study was conducted in late July 2009 at the University of Nebraska-Lincoln Haskell Agricultural Laboratory. The soil at the site was a Nora silty clay loam (fine-silty, mixed, mesic Udic Haplustoll) with 28% sand, 48% silt, and 24% clay, and the field slope was approximately 8%. The soil has NRCS soil capability unit of IIIe-1, permeability in the range of 15 to 50 mm h^{-1}, and available water holding capacity of 0.17 to 0.22 mm mm^{-1} (USDA, 1978). The field was under no-till practice for five years prior to the study. The area receives average annual precipitation of 672 mm year^{-1}, and the average annual temperature is 8°C (HPRCC, 2011).

Ninety-six 230 to 270 kg heifers that had not received steroid hormone treatments were split into six pens: three pens contained animals that were treated with Ralgro (α-zearalanol) and Revalor-H (trenbolone acetate and 17β-estradiol benzoate) and feed containing MGA 200 Premix (melengestrol acetate), and three pens contained animals that did not receive any type of steroid hormones. The animals were placed on test until they reached a slaughtering weight of approximately 500 kg. Manure was collected from the pens containing animals that received steroid hormones in September 2008 and stockpiled on a concrete pad under a roof until April 2009, when it was transferred to the edge of the research area.

The field experiment was a 2 × 2 × 2 factorial arrangement of tillage, manure, and surrogate application in a split-plot design with three replications. Two tillage practices were applied randomly to whole plots: single disking in the upslope and downslope direction to a depth of 150 mm (T), and no-tillage (NT). Subplots were randomly allocated to with manure and without manure, and with and without surrogate treatment. The dimensions of a whole plot were 5 m × 18 m, and each subplot was 2.2 m × 3.4 m with a 3 m border between the subplots.

Manure was weighed for each experimental unit and hand spread to field plots at a rate of 193 Mg ha^{-1}. The application rate was equivalent to 170 kg N ha^{-1} of plant-available nitrogen, which was the calculated N requirement for dryland corn in northeast Nebraska based on a 0.15 availability factor for the organic N contained in the manure (Koelsch and Shapiro, 2006).

A synthetic estrogen, 17α-ethynylestradiol (EE2), was chosen as the surrogate for the study because it has very similar chemical properties to some natural estrogens generated by beef cattle (table 1). In addition, EE2 is widely used as a human oral contraceptive and frequently observed in wastewater effluents. Consequently, several studies have characterized its physical and chemical properties and its biological behavior in the environment (Yamamoto et al., 2003; Lee et al., 2003; Young and Borch, 2009). EE2 has a sorption affinity to soil in the range between that of 17β-estradiol and estrone (table 1), which make it a good surrogate for estrogens excreted by female beef cattle. For the field study, EE2 was applied to the field surface after manure was spread and to the bare soil at an application rate of 75 g ha^{-1} or a concentration equivalent to approximately 390 ng g^{-1} in the manure. The EE2 was dissolved in 1 L of water prior to application using a 3 m boom sprayer with nozzle pressure of 207 kPa. The EE2 application was followed by tillage approximately 24 h prior to the rainfall simulations.

Artificial rainfall was applied at a precipitation rate of 70 mm h^{-1} using a rainfall simulator described by Humphry et al. (2002). Within each experimental unit (subplot), the rainfall simulator covered 2.2 m × 3.4 m areas, and the runoff plot was 0.75 m wide × 2 m long underneath. Each runoff plot was isolated on three sides by galvanized steel borders. A 150 mm i.d. PVC pipe with a 100 mm wide × 2.6 m long slot, cut lengthwise, was used to collect runoff. To prevent bypass flow around the collection pipe, a piece of galvanized steel, bent into an "L" shape, was pressed into the soil at the downstream end of the plot and directed runoff water into the PVC pipe. Rainfall was applied until runoff was initiated plus an additional 30 min during which runoff samples were collected. The application rate was equivalent to a rainfall intensity index (EI) of a single storm event expected to occur once every two years in the study area (Wischmeier and Smith, 1978).

The total volume of applied water was recorded using a water meter installed in the water distribution pipeline between a portable water supply tank and the rainfall simulator from the start of the rainfall until the last runoff sample was collected. Runoff samples were collected in 267 mL amber jars at 5, 10, 15, 20, 25, and 30 min after runoff initiation. The time to fill each jar was recorded and used to determine runoff flow rate. The total volume of runoff from the 30 min runoff event was determined by integrating the area under the flow rate curve. A total of 144 runoff samples were collected (8 treatments × 3 replications × 6 sampling times) and maintained at -20°C until analysis in the laboratory.

Runoff samples were analyzed using on-line solid-phase extraction (SPE) liquid chromatography and tandem mass spectrometry (LC/MS/MS) with a Spark Holland Symbiosys Environ automated extraction system and a Waters Quattro Micro liquid chromatograph tandem mass spectrometer system with an atmospheric pressure photoionization (APPI) source. Further detail of the analytical method with quality control procedures can be found elsewhere (Bartelt-Hunt et al., 2012; Snow et al., 2012). The instrument detection limit for EE2 was 57 pg, the method detection limit was 4.7 ng L^{-1}, and the average recovery rate for the on-line SPE method was 169%.

Runoff initiation times, rainfall:runoff ratios, flow-weighted concentrations, and mass transports were calculated to determine the impact of tillage, manure, and EE2 treatments. Runoff initiation times were determined as the difference in time between the beginning of rainfall simulation and the beginning of runoff. The beginning of runoff was taken as the time when discernible amounts of water began exiting the runoff collection tube. The rainfall:runoff ratio was determined as the total water applied as rainfall recorded during the rainfall simulation on each treatment (mm) divided by the total depth of runoff recorded during the 30 min runoff event (mm). The flow-weighted concentration from each plot was calculated as the summation of the EE2 concentration (ng L^{-1}) at each 5 min interval multiplied by the corresponding 5 min runoff volume (L) divided by the cumulative runoff volume recorded during the 30 min runoff sampling period (L). Mass transport of EE2 was determined by multiplying the flow-weighted concentration by the total volume of runoff recorded for each plot.

Analysis of variance (ANOVA) was performed to evaluate the impact of tillage, manure, and EE2 application on the total rainfall, rainfall:runoff ratio, flow-weighted concentration, and mass transport using PROC GLIMMIX in SAS (Littell et al., 1996). An alpha level of $\alpha = 0.10$ was used to identify significant treatment differences. Tillage, manure, and EE2 application were fixed effects. Replication and interaction between replication and tillage were random effects.

Table 1. Chemical properties of EE2 and other common natural estrogens (Lee et al., 2003).

Chemical Structure	Formula	Mole Weight (g mol^{-1})	Aqueous Solubility (mg L^{-1})	LogK_{oc}	K_d (L kg^{-1})	Half-Life (days)
17α-ethynylestradiol (EE2)	$C_{20}H_{24}O_2$	296.4	4.8	3.67	23.4	3.1 to 3.9
17β-estradiol	$C_{18}H_{24}O_2$	272.4	13	4.01	83.2	0.8 to 1.1
Estrone	$C_{18}H_{22}O_2$	270.4	13	3.13	48.1	2.8 to 4.9
Estriol	$C_{18}H_{24}O_3$	288.4	32	2.81	-	0.7 to 1.7

Table 2. Analysis of variance (ANOVA) and mean values of total rainfall application, runoff initiation time, rainfall:runoff ratio, flow-weighted concentration, and mass transport of EE2 according to treatment effects (NT = no-till, T = single-disked plots, S = bare soil without manure or EE2, M = manure only, EM = EE2 applied on manure, ES = EE2 applied on soil).

		Total Rainfall (mm)	Runoff Initiation Time (min)	Rainfall:Runoff Ratio[a] (mm mm^{-1})	Flow-Weighted Concentration[b] (ng L^{-1})	Mass Transport[b] (mg ha^{-1})
Treatment effects (Pr > F)	Tillage	0.0958	0.2712	0.5251	0.0003	0.0033
	Manure	0.2436	0.1901	0.2279	0.0504	0.1107
	EE2	0.8068	0.8577	0.8022	-	-
	Tillage × Manure	0.7013	0.5727	0.9947	0.8348	0.8936
	Tillage × EE2	0.3112	0.4176	0.4902	-	-
	Manure × EE2	0.0660	0.1492	0.2889	-	-
	Tillage × Manure × EE2	0.2537	0.1947	0.3948	-	-
Mean values[c]	NT	100 b	53	28	3123 a	156 a
	T	133 a	81	36	141 b	6 b
	S	105	59	27	2351 a	106
	M	127	74	37	913 b	57
	Without EE2	115	68	33	ND	ND
	With EE2	118	66	31	1632	81
	NT-S	97	55	29	ND	ND
	NT-M	88	44	24	ND	ND
	NT-ES	74	30	17	4482	202
	NT-EM	139	83	43	1765	110
	T-S	135	83	36	ND	ND
	T-M	140	90	44	ND	ND
	T-ES	116	70	26	221	10
	T-EM	141	80	38	62	3

[a] Rainfall:runoff ratio is the depth of water applied per millimeter of surface runoff.
[b] Analysis of variance included flow-weighted concentrations and mass transport rates for treatments only where EE2 was applied. For mean values, ND indicates that the concentration of EE2 was below the method detection level.
[c] Treatments followed by the same letter are not statistically different at $p \leq 0.10$.

RESULTS AND DISCUSSION

RAINFALL AND RUNOFF

Total water application ranged from 74 mm for the NT-ES treatment to 141 mm for the T-EM treatment (table 2). Significant differences in the total water applied were identified between the T and NT tillage treatments, with the T treatment requiring 33 mm more water application (Pr > F, 0.0958). This fact was anticipated, since newly disturbed or tilled soils exhibit greater porosity and initial soil water infiltration rates than untilled soils, while these factors are often reversed for later water applications (Wilson et al., 2004). Significant differences were also identified for the manure × EE2 interaction (Pr > F, 0.0660). This appears to be the result of outliers that increased the difference between EM and ES treatment means.

Analysis found no significant impact due to treatments or the interactions among treatments for runoff initiation time (table 2), where the mean values for the disk and no-till treatments were 81 and 53 min, respectively. We had some expectation that the disk treatment would increase runoff initiation time based on the results described by Wilson et al. (2004), who reported significantly greater runoff initiation times from freshly tilled soil due to an increase in soil permeability. They observed that the runoff initiation time from no-till plots was 71% less than from conventionally tilled plots. Our project found a similar trend but with no significant difference (35% greater runoff initiation time for the disk treatment). Another factor that would tend to delay runoff from freshly tilled soils is a greater level of random roughness, which would provide small storage areas on the soil surface of the disked plots that would be much less pronounced in the no-till treatment (Gilley and Finkner, 1991).

The rainfall:runoff ratio provides an indication of the mean depth of applied rainfall required to produce 1 mm of surface runoff. Greater values can indicate increased infiltration rate or increased soil surface storage. No significant differences were identified based on tillage or manure application treatments (Pr > F, tillage = 0.5251; manure = 0.2279). While the treatment means were not statistically different, the mean values indicated a trend toward greater runoff rates for the NT plots and for plots not receiving manure application (table 2).

The combination of differences in water applied, runoff initiation time, and rainfall:runoff ratio showed that disking aerates the soil in the tillage zone, resulting in an increase in infiltration rate. Wilson et al. (2004) noted that a tilled soil reconsolidated rapidly during the first rainfall event post-tillage, and subsequent rainfall events produced more runoff. Disking also likely increased surface roughness, leading to additional soil surface storage when compared to the NT treatment. Thus, fields recently tilled to incorporate the applied manure provide a means of reducing surface runoff and the transport of contaminants that might be dissolved in surface runoff, at least initially. Incorporation places the manure mostly below the soil surface, where interaction with surface runoff water is greatly reduced. However, if the average surface water runoff rate was decreased in the long term by tillage, then EE2 could come into contact with surface runoff at a later date due to soil erosion, unless soil microbial activity degraded or converted the steroid hormone into less toxic metabolites.

EFFECT OF INCORPORATION ON EE2 IN RUNOFF

Analysis of the flow-weighted concentrations of EE2 found a significant impact of tillage and manure application on flow-weighted concentrations of EE2 in runoff samples (Pr < F, 0.0003) (table 2). The T treatment reduced the flow-weighted concentration of EE2 in surface runoff samples by 95%, from 3123 ng L^{-1} for the NT treatment to 141 ng L^{-1} for the T treatment. The addition of manure also impacted the flow-weighted concentrations of EE2 (Pr < F, 0.0504) (table 2), which were reduced by 61%, from 2351 to 913 ng L^{-1}, when compared to bare soil conditions. Incorporating the manure and EE2 via tillage is a standard best management practice for maximizing the use of nitrogen contained in the manure resource; thus, producers are accustomed to incorporating manure from various animal sources.

Based on the hydrophobic sorption characteristics of EE2, sorption increases and mobility decreases with increasing organic carbon content and decreases with particle size (Loffredo and Senesi, 2002; Lee et al., 2003; Hildebrand et al., 2006). According to Hildebrand et al. (2006), EE2 follows a two-phase sorption in which a rapid phase occurs within the first few hours in soil followed by a slower phase requiring 24 h to reach sorption equilibrium. Thus, faster sorption kinetics should lead to less transport of EE2, and the time of rainfall relative to application may be a major factor resulting in differences across studies, in addition to differences in soil textural properties that affect sorption. Additionally, the measured organic carbon partition coefficient for EE2 ($\log K_{oc}$) ranged from 2.91 to 3.04 (table 1), which supports the notion that a large fraction of EE2 could be sorbed to the organic fraction of beef manure (Lee et al., 2003). Further, the aqueous solubility for EE2 (table 1) is less than that of other steroid hormones, which would support less EE2 dissolved in surface runoff. However, Yang et al. (2012) found that 95% of progesterone was identified in the dissolved phase rather than the particle-associated phase. Hildebrand et al. (2006) found that EE2 did not exhibit a specific trend of desorption across different initial EE2 concentrations. These factors suggest that EE2 and other steroid hormones could exhibit different characteristics depending on the environmental conditions in which the hormones are placed.

Multiplying the volume of runoff in mm by the flow-weighted concentration provides an estimate of the total mass transport of EE2 (steroid hormone) from an agricultural field. Table 2 indicates that there was a significant impact of the T treatment on the mass transport of EE2 (Pr > F, 0.033). The NT treatment resulted in a total mass transport of 156 mg ha^{-1}, compared to 6 mg ha^{-1} for the T treatment. Thus, incorporation of EE2 reduced transport by over 96% compared to leaving the EE2/manure on the soil surface.

The concentration of EE2 in runoff for each of the four treatments receiving EE2 is presented in figure 1. Following an initial spike in EE2 concentration at the 5 min sampling time, the concentration of EE2 (left vertical axis) began to decrease and continued its downward trend for the remainder of the simulation events. This was expected because the runoff rate (right vertical axis) was on an upward trend (fig. 1) for all of the simulation events. Regardless of tillage treatment, the results showed that the mass of EE2 transported with surface water approached a constant rate,

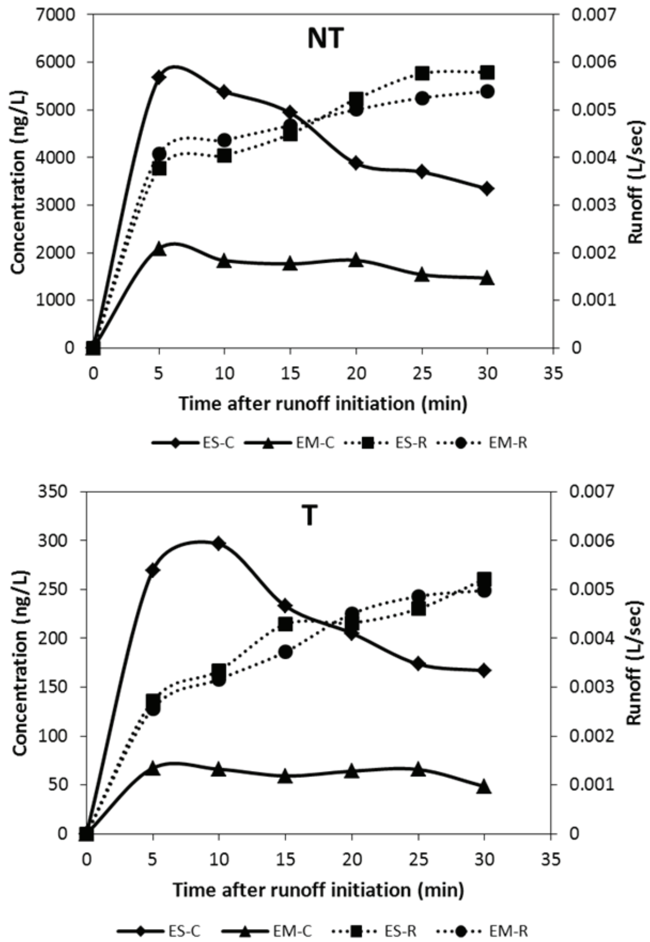
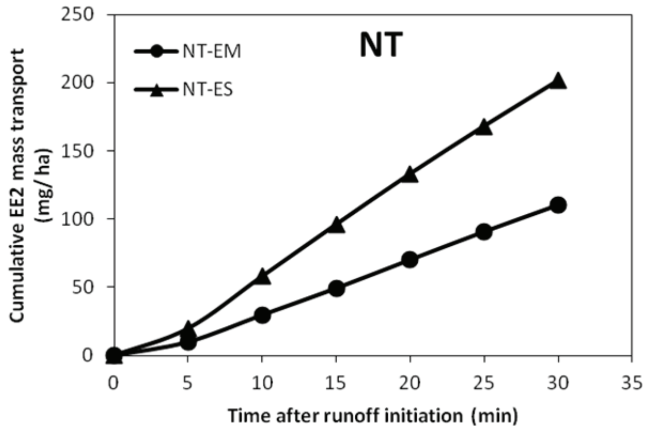


Figure 1. EE2 concentration and surface runoff rate recorded at 5 min intervals during a 30 min runoff collection period from no-till (NT) and single-disked (T) plots: ES-C = EE2 concentration when EE2 is applied to the soil (ng L^{-1}), ES-R = runoff rate when EE2 is applied to the soil (L s^{-1}), EM-C = EE2 concentration when EE2 is applied to manure on the soil surface (ng L^{-1}), and EM-R = runoff rate when EE2 is applied to the soil (L s^{-1}).

with the total mass of EE2 lost dependent on the field treatment. This was reinforced by NT concentrations that were 10 times greater than detected in the T treatment.

Based on this research, incorporation of manures containing steroid hormones would clearly be a major step in preventing EE2 and other steroids from being immediately transported from agricultural cropland into streams, rivers, and lakes. Once incorporated, the long-term key to prevention of steroid hormone transport into surface waters is the conversion of steroid hormones into less toxic metabolites or into their fundamental chemical components of carbon dioxide and water by soil bacteria. Given the high sorption intensity of steroid hormones in soil and their subsequent desorption characteristics (Ma, 2009), the potential exists for transport in runoff to occur months or years after initial manure application. Conversely, manure containing steroid hormones that is applied to the soil surface in a no-till system will remain readily accessible to losses in runoff.

The cumulative mass transport of EE2 for the T and NT treatments following the application of 75 g ha^{-1} is depicted in figure 2. Again, we note the difference in magnitude (*y*-



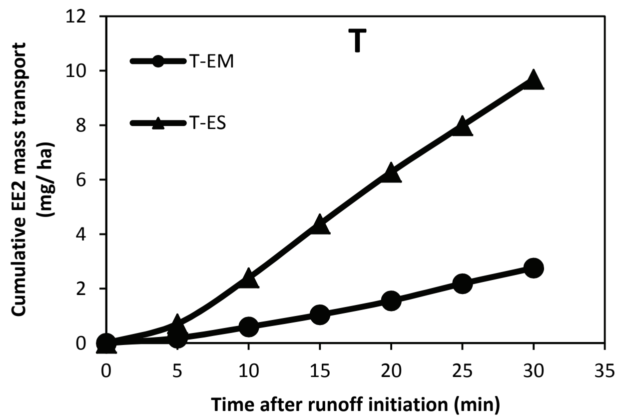


Figure 2. Cumulative EE2 mass transport recorded at 5 min intervals after runoff initiation during a 30 min runoff collection period from no-till (NT) and single-disked (T) plots: NT-EM = mass of EE2 from no-till plots when EE2 was applied directly to manure (mg ha^{-1}), NT-ES = mass of EE2 loss from no-till plots when EE2 was applied directly to the soil surface (mg ha^{-1}), T-EM = mass of EE2 loss from disked plots when EE2 was applied directly to manure (mg ha^{-1}), and T-ES = mass of EE2 loss from disked plots when EE2 was applied directly to the soil surface (mg ha^{-1}).

axis) for the T treatment (incorporated) compared to the NT treatment. In both cases, the EE2 transport resulting from EE2 application directly to bare soil was greater than when manure was present. Less transport loss when manure was present on the soil surface supports the assertion that the sorption of EE2 to organic carbon tends to influence its transport in surface runoff. Analysis of the total mass transport (table 2) indicates a significant impact of tillage on EE2 movement. This was anticipated, since it follows the same trend that was discussed previously for the reduction in EE2 flow-weighted concentrations. The lack of interaction between tillage and manure application suggests that tillage is the major factor, even though the cumulative mass transport curves show a trend of reduced EE2 loss when manure was applied prior to adding EE2.

Conclusion

The effect of rainfall one day after application of manure and subsequent tillage incorporating manure containing a steroid hormone determined that incorporation of manure significantly reduced the flow-weighted concentration of EE2 in the dissolved phase by 96% when compared to a no-till treatment. Total EE2 mass transport was reduced from 156 to 6 mg ha^{-1} due to a single-pass disking to incorporate the hormone. Application of manure and the two tillage treatments did not affect the hydrologic parameters. Use of the surrogate allowed quantification of these effects, since it could be supplied at sufficient quantity (20 times natural occurrence) to allow detection. Manure addition significantly decreased steroid hormone transport, apparently due to the increased organic matter associated with its application. The addition of manure reduced the flow-weighted concentration of EE2 by 61%, from 2351 to 913 ng L^{-1}, when compared to bare soil conditions. The implication of these findings is that the two goals of reducing erosion by reduced tillage and increased surface residue are not the management choices for decreasing the overland transport of steroid hormones contained in field-applied manure. Rather, immediate incorporation of the manure represents one way to minimize the overland transport of steroid hormones contained in beef cattle manure. Thus, tillage practices are needed that can achieve a balance between soil erosion and hormone transport in runoff.

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