Fate and Transport of Sex Hormones from Poultry Litter Applied to Till and No-Till Cropping Systems

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INTRODU CTION

The U.S. poultry industry produces millions of tons broiler litter annually. Most of it is applied to agricultural fields as a source of fertilizer. Poultry litter is also a source of the sex hormones estradiol and testosterone, both of which have been detected in surface waters. The question arises whether the continuous application of large quantities of litter to agricultural fields is a threat to contaminate our water resources with these sex hormones? Because no-till or conventional tillage can change the hydrologic character of a field we hypothesized that differences in drainage and runoff and in the flow weighted concentration of the hormones would be observed.

OBJECTIVES

Determine if the concentrations of estradiol and testosterone in runoff and drainage from conventional-till and no-till soils receiving broiler litter are significantly greater than in runoff and drainage from control plots receiving mineral fertilizer. Determine the transport characteristics of the two hormones through intact soil columns and isotherms.

MATERIALS AND METHODS

Experimental design:

Six no-till and six tilled 10 X 30 m plots were in a completely random block design (Fig. 1). The treatments, with replicates of three, were the following: conventional till-conventional fertilizer (CTCF), conventional till-poultry litter (CTPL), no-till-conventional fertilizer (NTCF), and no-till-poultry litter (NTPL).

Figure 1: Experimental plots setup showing the Winter cover crop rye (A) and the Summer crop corn (B).

MATERIALS AND METHODS

Experiments:

1. For a winter cover crop, rye was planted. Three no-till and three tilled plots received 7,500 kg of litter ha^{-1} (equivalent to 130 kg N ha^{-1} and recommended level for rye), and the other six plots received mineral N, P, K at equivalent levels. Three days after planting in November 2001, the plots were irrigated. The quantity of water applied was measured with an array of rain gauges. Approximately 10 mm of irrigation water h^{-1} was applied for seven hours on the first day for a total of 68.8 mm, and 5 hours on the second day for a total of 54.9 mm of water $plot^{-1}$. The total amount of estradiol and testosterone from the litter application for each plot was 6 mg and 75 µg respectively.

Figure 2: Applying poultry litter.

MATERIALS AND METHODS

Experiments:

2. For a summer corn crop, 22,420 kg of litter ha^{-1} (equivalent to 336 kg N ha^{-1} and twice the recommended level for corn) (Fig. 1B) was applied to the plots receiving litter in June of 2003. The other six plots received mineral N, P, K at equivalent levels. A rain event for a total of 54.7 mm occurred soon after planting that negated the need for irrigation. The total amount of estradiol and testosterone from the litter application for each plot was 1.62 mg, and 0.22 mg respectively.

MATERIALS AND METHODS

Drainage and runoff:

Drainage was measured and subsampled with tipping buckets. Runoff was measured with a pressure transducer (0 to 5 psi), and subsampled with a Coschocton wheel. Both drainage and runoff samples were collected in refrigerated ISCO samplers (Fig. 3). Each sample represented a particular known volume of either drainage or runoff.

Soil samples:

Five soil samples were taken at depths of 0-10, 10-20, and 20-30 cm plot^{-1}; they were composited by depth, air-dried, sieved, and analyzed for the hormones. Samples were taken soon after litter application, and several weeks after application.

Runoff

Drainage

Figure 3: ISCO samplers showing runoff and drainage collected.

MATERIALS AND METHODS

Hormone analysis:

Twenty milliliters of water sample or 1 g of litter or 1 g of soil were extracted with ethylacetate, which was evaporated under a stream of dinitrogen. The residue was analyzed for the hormones with competitive enzyme immunoassay kits (Cayman Chemical Company, Ann Arbor, MI). For the tissue culture assay, solid phase extraction with an Oasis HLB cartridge was used on 250 ml of aqueous sample; cartridges were eluted with a series of solvents, and the residue resuspended in nanopure water and tested against MCF-7 cells.

MATERIALS AND METHODS

Sorption isotherm experiments:

Surface (0-10 cm) and subsurface (10-20 cm) soils were tested. A mix of radiolabeled and non-radiolabeled ^{3}H-estradiol and ^{14}C-testosterone was added to the soils at concentrations of 1, 1 0, 100, 500, and 1000 ng ml^{-1}. Kinetics were determined at 2, 24, 48,72,120, and 144 h.

Column study:

Intact soil columns (15 cm-dia. by 35 cm-length) were removed from the study site, and taken to the lab. A pulse containing $CaCl_2$ and both radiolabeled and non-radiolabeled estradiol and testosterone was followed by hormone-free solution. Fractions of leachate were collected and analyzed. At the end of the experiment, the columns were divided into 1 cm increments, and 2-g samples of soil were oxidized to determine radioactivity.

RESULTS

Results of the first experiment (Tables 1 and 2) indicated that agronomic rates of application of the poultry litter applied to the plots had no impact on the flow weighted concentration of the two hormones in runoff or drainage. No differences were observed between plots to which litter was applied and to plots to which mineral fertilizer was applied. We observed the same results in the second experiment (Tables 3 and 4). The soil analysis (Tables 5 and 6) indicated that the litter added a negligible amount of both hormones to the soil profile, and that background levels of both estradiol and testosterone appeared to exist. Results of the tissue culture assay for estrogenicity (data not shown) indicated levels of bioactivity in drainage from both litter and non-litter applied plots.

RESULTS

The isotherm experiments (Fig. 4) indicated that estradiol sorbed greater to surface (K_d = 36.3 cm^3 g^{-1}) rather than to subsurface soil (K_d = 26.3 cm_3 g^{-1}) for both conventional and no-till soil. Although testosterone sorbed (average K_d = 21.3 cm^3 g^{-1}) less than estradiol, sorption patters were similar (data not shown). Detectable concentrations of estradiol and testosterone broke through no-till soil columns simultaneously with the chloride tracer (Fig. 5). Breakthrough curves for conventional till soil columns were similar (data not shown). Forty to 50% of estradiol and testosterone were located in the 0 – 10 cm depth of the soil column.

Table 1. Mean value of flow-weighted concentrations of estradiol and testosterone in drainage from irrigation.

Treatment	Estradiol	Testosterone
	ng/l	
CTCF	23.3a	6.7a
CTPL	36.6a	7.9a
NTCF	5.7a	4.7a
NTPL	9.7a	8.6a

Values in each column followed by a different letter are significantly different at P = 0.05.

Table 2. Mean value of flow-weighted concentrations of estradiol and testosterone in runoff from irrigation.

Treatment	Estradiol	Testosterone
	ng/l	
CTCF	27.8a	7.6a
CTPL	26.9a	8.4a
NTCF	35.2a	7.4a
NTPL	20.5a	5.6a

Values in each column followed by a different letter are significantly different at P = 0.05.
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Table 3. Mean value of flow-weighted concentrations of estradiol and testosterone in drainage from a rain event.

Treatment	Estradiol	Testosterone
	ng/l	
CTCF	25.2a	5.67a
CTPL	10.0a	32.2a
NTCF	10.7a	15.9a
NTPL	8.2a	99.5a

Values in each column followed by a different letter are significantly different at P = 0.05.

Table 4. Mean value of flow-weighted concentrations of estradiol and testosterone in runoff from a rain event.

Treatment	Estradiol	Testosterone
	ng/l	
CTCF	8.7a	16.8a
CTPL	24.2a	17.6a
NTCF	16.5a	13.6a
NTPL	43.7a	18.9a

Values in each column followed by a different letter are significantly different at $P = 0.05$.

Table 5. Mean concentrations of estradiol extracted from composited soil samples taken at three depths, 0 - 10, 10 - 20, and 20 - 30 cm.

Date	Treatment	Depth (cm)		
		0 - 10	10 - 20 pg/g	20 - 30
6/11/03	CTCF	38.8a	17.3a	46.6a
	CTPL	58.9a	58.7a	42.2a
	NTCF	29.7a	32.9a	3.5a
	NTPL	26.1a	38.3a	27.8a
7/25/03	CTCF	29.9a	22.8a	35.4a
	CTPL	40.5a	34.8a	74.9a
	NTCF	35.1a	19.1a	49.0a
	NTPL	38.6a	26.7a	25.6a

Values in each column followed by a different letter are significantly different at $P = 0.05$.

Table 6. Mean concentrations of testosterone extracted from composited soil samples taken at three depths, 0 - 10, 10 - 20, and 20 - 30 cm.

Date	Treatment	Depth (cm)		
		0 - 10	10 - 20	20 - 30
			pg/g	
6/11/03	CTCF	86.5a	7.0b	138.7a
	CTPL	82.4a	80.5a	92.5a
	NTCF	144.0a	107.2a	85.3a
	NTPL	164.6a	177.0a	125.0a
7/25/03	CTCF	76.3a	32.3a	5.7a
	CTPL	61.9a	45.7a	59.4a
	NTCF	71.6a	15.0a	56.2a
	NTPL	75.2a	47.2a	94.8a

Values in each column followed by a different letter are significantly different at $P = 0.05$.

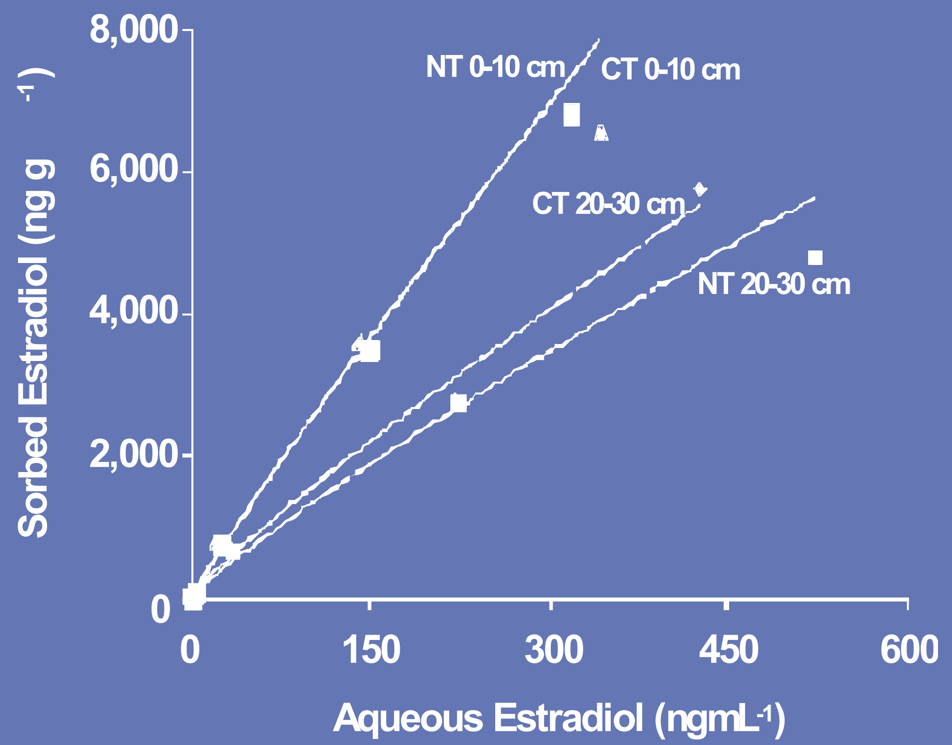

Fig. 4. Freundlich Isotherms from batch experiments using estradiol and soil from conventionally tilled and no-till plots at two different depths after 72 h of shaking.

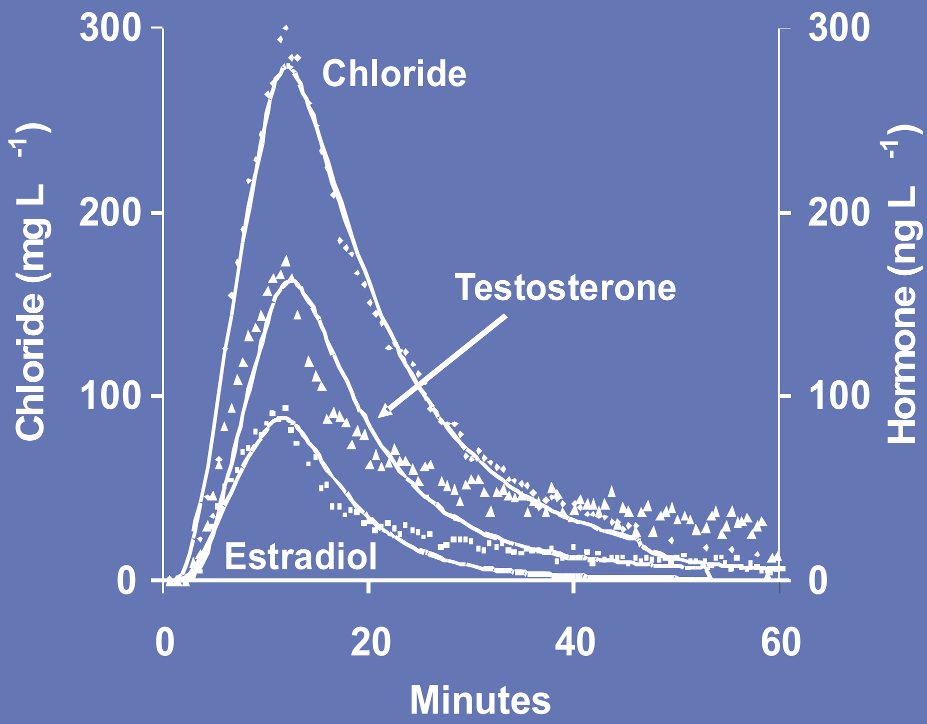

Fig. 5. Breakthrough curves for estradiol, testosterone, and chloride from a no-till soil column. Solid lines indicate breakthrough curve predicted by HYDRUS-1D.

DISCUSSION

- The two field experiments indicated that the application of poultry litter to conventional till and no-till cropping systems at recommended and even twice recommended agronomic rates does not increase the level of the sex hormones in runoff or drainage that can occur from either irrigation or rain events soon after application. Background levels of the hormones appear to exist as confirmed by the tissue culture assay for estradiol. The litter we applied came from two different commercial poultry houses. The concentrations of estradiol and testosterone from other houses may be significantly greater than our sources of litter and could impact surface and ground water.

DISCUSSION

Although both estradiol and testosterone sorb to soil, the column experiments demonstrated that they can move through a soil profile by preferential flow. Conventional till and no-till practices were not a factor in the transport or fate of these hormones.

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