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# Uptake of Natural and Synthetic Estrogens by Maize Seedlings

Marcella L. Card,[†,∥] Jerald L. Schnoor,[§] and Yu-Ping Chin*[,‡]

[†]Environmental Science Graduate Program, and [‡]School of Earth Sciences, The Ohio State University, 125 South Oval Mall, Columbus, Ohio 43210, United States

[§]Department of Civil and Environmental Engineering, College of Engineering, University of Iowa, 4119 Seamans Center, Iowa City, Iowa 52242, United States

Supporting Information

**ABSTRACT:** Runoff from manure-fertilized crop fields constitutes a significant source of natural estrogens (e.g., estradiol [17$\beta$-E2] and estrone [E1]) and synthetic estrogen mimics (e.g., zeranol [$\alpha$-ZAL] and zearalanone [ZAN]) in the environment. However, processes such as sorption to and uptake by plants may inhibit the environmental mobility of hormonally active compounds. Sorption to dried root tissue was assessed in batch sorption tests, and resulting sorption isotherms were nonlinear at aqueous concentrations below 0.1 $\mu$M and linear above that limit. To evaluate the role of crop plants in the environmental fate of such compounds, we exposed hydroponic solutions containing 2 $\mu$M 17$\beta$-E2, E1, $\alpha$-ZAL, or ZAN to maize seedlings. After 22 days of exposure, $\alpha$-ZAL and ZAN concentrations decreased by more than 96%, and 17$\beta$-E2 and E1 were undetectable. The decrease in $\alpha$-ZAL and ZAN concentrations in maize-exposed solutions was initially slow, but the observed uptake exceeded that predicted by sorption alone within 3 d. All four estrogens were detected in root tissues at concentrations up to 0.19 $\mu$mol g$^{-1}$, with concentrations peaking after 1−3 days of exposure. Only 17$\beta$-E2 and $\alpha$-ZAL were detected in shoots, and maximum concentrations were measured after 2 days for 17$\beta$-E2 (0.02 $\mu$mol g$^{-1}$) and 16 days for $\alpha$-ZAL (0.8 nmol g$^{-1}$). Concentrations measured in root and shoot tissues were 82% or less than those predicted by a partition-limited uptake model, which is attributed to transformation and possibly irreversible binding processes.

**KEYWORDS:** *estrogens, maize, manure fertilization, zeranol, zearalanone, 17ß-estradiol*

## INTRODUCTION

It is estimated that two-thirds of beef cattle raised in the United States are given hormonally active growth promoters to accelerate muscle gain and increase feed efficiency.[1] Livestock growth promoters approved for use in the U.S. include both natural hormones and synthetic mimics.[2] Hormonal growth promoters and endogenous hormones are excreted in livestock wastes and may enter the environment via pathways such as spillover from waste lagoons and runoff from manure-fertilized crop fields. These hormonally active compounds may have significant deleterious effects in the environment. For example, estrogens can feminize male fish and reduce fertility and fecundity in wild fish populations at concentrations as low as 10 ng L$^{-1}$.[3]

Among the growth promoters that are approved for use in the U.S. are a natural estrogen, 17$\beta$-estradiol (17$\beta$-E2), and its synthetic mimic, zeranol ($\alpha$-zearalanol, $\alpha$-ZAL). In mammals and aerobic microbes, the major metabolites of 17$\beta$-E2 and $\alpha$-ZAL are estrone (E1) and zearalanone (ZAN; Figure 1), respectively.[4,5] Because they are endogenous hormones, 17$\beta$-E2 and E1 are present in the excreta of all livestock (5−10 ng of estrogens per grams of manure of untreated cattle), regardless of whether the animals are treated with 17$\beta$-E2. Conversely, $\alpha$-ZAL and ZAN have only been detected in the wastes of treated animals ($\alpha$-ZAL at up to 5.81 ng g$^{-1}$ with 36 mg dose, before 72 mg doses were approved; e.g., refs 5,6). 17$\beta$-E2, E1, and $\alpha$-ZAL have been detected in surface waters impacted by livestock agriculture (e.g., refs 7−10), and 17$\beta$-E2 has been measured in agricultural runoff at concentrations high enough to impact aquatic organisms 3 months after application of manure fertilizer.[7]

The environmental mobility and fate of manure-borne estrogens can be attenuated by processes such as soil sorption[11] and uptake by plants and soil- and plant-associated microbes.[12] Field studies have demonstrated that grass buffer strips reduce the concentration of 17$\beta$-E2 in agricultural runoff,[13] and uptake of 17$\beta$-E2 and E1 by aquatic and terrestrial plants has been observed.[14−17] However, the uptake of natural and synthetic estrogens by crop seedlings, the first plants with which manure-borne contaminants interact, has not been previously studied.

The aim of this work was to evaluate the role that maize seedlings serve in the environmental fate of 17$\beta$-E2, E1, $\alpha$-ZAL, and ZAN. Specifically, we examined the uptake of estrogens from hydroponic solutions by maize seedlings and translocation of estrogens into root and shoot tissues. We further characterized the sorption behavior of the estrogens to root tissues to estimate the role of sorption in the observed removal of estrogens from hydroponic solution by fitting the experimental uptake data to a sorption-limited uptake model. These results will contribute to a complete understanding of the role of crop seedlings in the environmental fate of manure-borne estrogens and estrogen-mimicking compounds.

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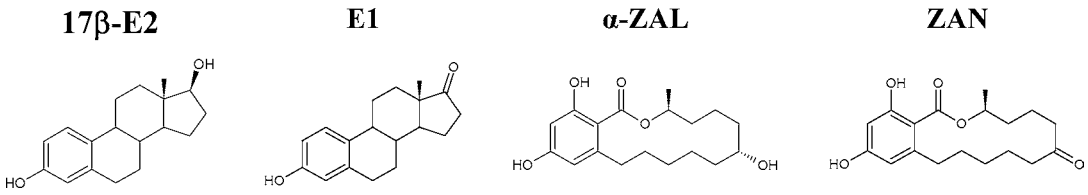


**Figure 1.** Structures of estrogens and estrogen mimics used in this study.

## MATERIALS AND METHODS

**Chemicals.** 17$\beta$-E2 (98%), E1 (99%), and ZAN (98%) were obtained from Sigma-Aldrich, Inc. (St. Louis, MO). $\alpha$-ZAL was extracted and purified from Ralgro Magnum (Schering-Plough Animal Health Corp., Union, NJ) (see the Supporting Information). Other chemicals, including HPLC solvents, were purchased from VWR International (Radnor, PA).

**Hydroponic Uptake.** Uptake of 17$\beta$-E2, E1, $\alpha$-ZAL, and ZAN was evaluated with maize seedlings (*Zea mays*, Golden Cross Bantam [Hybrid]; Ferry-Morse Seed Co, Fulton, KY). Seeds were surface-sterilized by a 5 min soak in Milli-Q water (Millipore, Billerica, MA), two rinses in Milli-Q water, 1 min soak in hydrogen peroxide (30%), and three rinses in Milli-Q water. The seeds were then placed onto dampened filter paper in glass Petri dishes, which were sterilized by autoclave (Tuttnauer USA, Hauppauge, NY), and germinated in the dark for 4 d.

Uptake experiments were conducted by growing maize seedlings in hydroponic solutions of estrogens. Solutions of target estrogens were prepared from methanol stock solutions in half-strength Hoagland's nutrient solution (pH 6.8),[18] for an initial concentration of 2 $\mu$M 17$\beta$-E2, E1, $\alpha$-ZAL, or ZAN. Autoclaved 9 mL glass vials were filled with estrogen or blank control solutions and capped with plastic open-top caps containing Teflon septa (National Scientific, Rockwood, TN) with 3 mm holes. One seedling was then placed into the hole in each cap such that the roots were in the solution and the kernel was situated on top of the septum (Figure SI-2). There were four replicates per treatment, including blank controls containing no estrogens and glassware controls containing no plant. All vials were wrapped in foil, with the cap and plant exposed, and placed under plant growth lights (Ecolux Plant and Aquarium, GE, Louisville, KY; a similar setup yields 150−300 $\mu$mol $s^{-1}$ $m^{-2}$) with a 16 h photoperiod. To maintain water levels, vials were refilled daily with Milli-Q water to not concentrate salts in the solutions (dilution was taken into account in mass balances). Plants were grown at room temperature for up to 18 d.

Plants were destructively sampled at given time points. The aqueous phases were filtered using Gelman type A/E glass fiber filters with 0.6 $\mu$m pore size (Pall Corp.) and collected for analysis by reverse-phase high-pressure liquid chromatography (RP-HPLC). Roots were rinsed in Milli-Q and blotted dry, and then roots and shoots (including the kernels) were weighed, ground in liquid nitrogen, and extracted in 1.5 mL of methanol at room temperature overnight. Methanol extracts were filtered, and estrogen concentrations were analyzed by RP-HPLC.

**Sorption to Root Tissues.** The role of sorption to root tissues in estrogen removal from hydroponic solutions was assessed in batch sorption tests similar to those conducted by Li et al.[19] Sorption was assessed as a function of time in kinetics studies and as a function of concentration in isotherm studies. Dried plant tissue was used as a sorbent to isolate the effects of abiotic (i.e., sorption) processes in reducing estrogen concentrations from the biotransformation processes that occur in live tissue. The roots of approximately 40 (18-d old) maize seedlings were rinsed in Milli-Q water, blotted dry, and dried overnight at 90 °C. Dried roots were then cut into approximately 2 mm pieces, and 10 mg of dried tissues was placed into each of a series of 50 mL Corex centrifuge tubes. Estrogen solutions were prepared in half-strength Hoagland's solution (pH 6.8) with initial concentrations between 3 and 0.01 $\mu$M, and 25 mL aliquots of estrogen solutions were added to each tube. Blank and glassware controls were also prepared, and all samples were prepared in duplicate. Samples were shaken at room temperature on a reciprocating shaker (Elderbach Corp., Ann Arbor, MI) on "low" setting, and, at given time points, the aqueous phases were filtered as described above and analyzed by RP-HPLC. Isotherm samples were collected after 27 h. Because of the very low root masses used for each isotherm, we were unable to completely remove all of the associated aqueous phase without also compromising the sorbent and solute such as accidental loss of sorbent or loss of solute through the drying process for extraction and mass balance purposes. Further, estrogen concentrations did not change significantly in glassware controls, and no transformation products were detected in any sample in the sorption experiments. Thus, sorption to root tissues is the only significant factor in changing estrogen concentrations, so the mass of estrogen sorbed was assumed to be the difference between initial and measured masses of estrogens in the aqueous phase.

**RP-HPLC Analysis of Estrogen Concentrations.** Estrogen concentrations were quantified using RP-HPLC (1515 isocratic pump and 717plus autosampler, Waters Corp., Milford, MA) with UV−vis detection (Waters 2487 dual $\lambda$ absorbance detector) and a Waters Sunfire C18 column. Injection volumes were 150 $\mu$L, and the mobile phase was 50:50 v/v acetonitrile:water with 1 mL $min^{-1}$ flow rate. Detection wavelengths were 280 nm (17$\beta$-E2), 265 nm (E1), and 220 nm ($\alpha$-ZAL and ZAN). Under these conditions, the limits of detection and quantification (LOQ) were 0.001 $\mu$M (0.27−0.32 ng $mL^{-1}$).

**Estimation of Equilibrium.** Uptake and root sorption data were fitted to the partition-limited model proposed by Chiou et al.[20] The approach to equilibrium partitioning between the hydroponic solution and plant tissue ($\alpha_{pt}$) is expressed as a ratio of the concentration in the sap water to the aqueous concentration, and was estimated using the equation:

$$\alpha_{pt} = \frac{C_{pt}}{C_w(f_{pom}K_{pom} + f_{pw})} \quad (1)$$

where $C_{pt}$ is the estrogen concentration in the plant ($\mu$mol kg wet weight$^{-1}$; calculated by summing the concentrations of estrogens measured separately in the roots and shoots); $C_w$ is the concentration in the hydroponic solution ($\mu$M); $f_{pom}$ is the weight fraction of organic matter in the plant; $f_{pw}$ is the whole-plant weight fraction of water (where $f_{pom}$ and $f_{pw}$ sum to 1.0); and $K_{pom}$ is the partition coefficient between plant organic matter and water determined from root tissue sorption isotherms.[20]

## RESULTS AND DISCUSSION

**Uptake from Hydroponic Media.** It was predicted that 17$\beta$-E2, E1, $\alpha$-ZAL, and ZAN would be bioavailable to plants because they are moderately hydrophobic (octanol−water partition coefficient [log $K_{ow}$] values between 3.5 and 4.1) (see refs 11,12, Table 1, Table SI-1). Indeed, these natural and synthetic estrogens were rapidly removed from hydroponic solutions exposed to maize seedlings (Figure 2; Table SI-2). After 22 d of exposure to maize seedlings, $\alpha$-ZAL and ZAN decreased by a respective 99% and 96%, while 17$\beta$-E2 and E1 were undetectable after 12 and 18 d of exposure, respectively. Aerobic conditions were maintained in the hydroponic solutions throughout the study period (Figure SI-3, Table SI-3). The corn seedlings appeared healthy throughout the


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**Table 1. Log $K_{ow}$ Values, Apparent Rate Constants, and Linear Fit for Rate Constant Calculation for the First 5 days of Hydroponic Uptake by Maize Seedlings**

| | log $K_{ow}$ | rate constant ($d^{-1}$) | $R^2$ |
|---|---|---|---|
| 17$\beta$-E2 | 3.70 | 0.549 | 0.99 |
| E1 | 3.55 | 0.480 | 0.97 |
| $\alpha$-ZAL | 3.88 | 0.237 | 0.90 |
| ZAN | 4.08 | 0.158 | 0.89 |

experimental period, and plant health was demonstrated by continual increases in biomass (reported in the Supporting Information) and transpiration. Transpiration was not quantified but was apparent due to the volume of hydroponic solution lost on a daily basis from vials containing plants, as compared to the negligible volume lost on a daily basis from glassware controls. Glassware controls showed little loss of estrogens, and estrogens were not detected in any system blanks.

Because uptake rates were measured with hydroponic solutions, they are anticipated to be higher than similar uptake in natural systems where sorption to manure and soil particles will inhibit uptake by plants. This study was designed to elucidate the role of maize in the fate of the target estrogens, and thus the uptake rates from hydroponic systems are seen as the maximum rate at which maize may remove the estrogens from solution. Soil-based studies will be completed in the future to more accurately simulate agricultural systems, including rhizosphere and sorption processes. In soil, sorption will also inhibit the bioavailability to other organisms and the environmental mobility of these estrogens, but microbial and rhizosphere processes will contribute to the overall degradation rate of estrogens. Our system therefore likely represents the upper limit of the contribution from maize seedlings to uptake rates of these compounds.

Uptake resembled pseudo-first-order kinetics for the first 3–5 d of exposure, and we determined apparent rate constants based on those data (Table 1; Figure SI-4). The observed removal of parent estrogens from solution likely represents a series of transformation reactions, with the rate constants reported here representing the first step in that series. However, the apparent rate constants should only be considered as phenomenological values because uptake can be attributed to many mechanisms (e.g., sorption, transformation, and irreversible binding to plant tissues and conjugating enzymes). The apparent rate constants demonstrate that 17$\beta$-E2 and E1 are taken up more quickly than $\alpha$-ZAL and ZAN, with uptake rates of the natural estrogens roughly 2–4 times greater than those of the synthetic estrogens over the first 5 d of exposure. This difference in removal rates may be attributed to differences in rates of partitioning into plant tissues or degradation by plant or microbial enzymes. Uptake was not constant throughout the entire sampling period, and rates slowed beyond 5 d as aqueous phase estrogen concentrations decreased.

The rates were inversely related to hydrophobicity (manifested as aqueous activity coefficient or log $K_{ow}$), insofar as 17$\beta$-E2 and E1 as a pair have lower log $K_{ow}$ values but larger rate constants than $\alpha$-ZAL and ZAN. If removal of estrogens from solution were dominated by sorption, the rate constants would be positively related to hydrophobicity. However, the rates reported here also reflect active uptake by the plant and loss to transformation, binding, and degradation.

**Sorption to Root Tissues.** To estimate the role of sorption in the observed removal of estrogens from the aqueous phase, partitioning between hydroponic solution and root tissues was assessed. All four estrogens reached pseudo-equilibrium between the two phases within approximately 13 h (Figure SI-5, Table SI-4), but we extended our sorption isotherm experiments for an additional 14 h (27 h total). Sorption isotherms were linear at relatively high aqueous concentrations and nonlinear at low concentrations (e.g., Figure 3; see Figure SI-6 and Table SI-5 for full data set). As was observed with the apparent uptake rate constants, the measured $K_{pom}$ values were not correlated to the respective log $K_{ow}$ values.

It has frequently been observed that more polar solutes (defined here as those compounds with log $K_{ow}$ < 4.0), such as the analytes used in the present study, exhibit nonlinearity at low concentrations relative to nonpolar solutes (e.g., refs

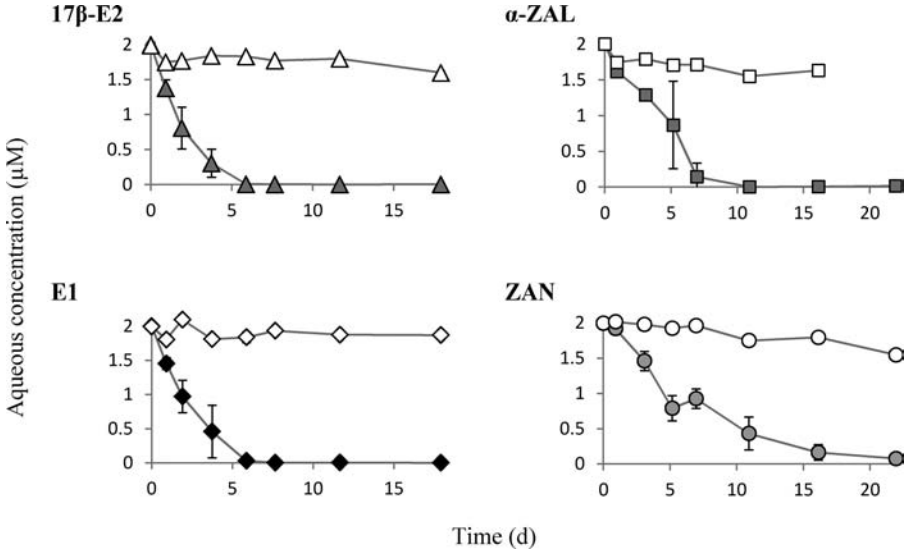


**Figure 2.** Decrease of aqueous estrogen concentrations in maize-exposed solutions. Error bars represent one standard deviation (SD). Closed symbols represent maize-exposed samples, and open symbols represent glassware controls of 17$\beta$-E2 (▲, △), E1 (◆, ◇), $\alpha$-ZAL (■, □), and ZAN (●, ○).

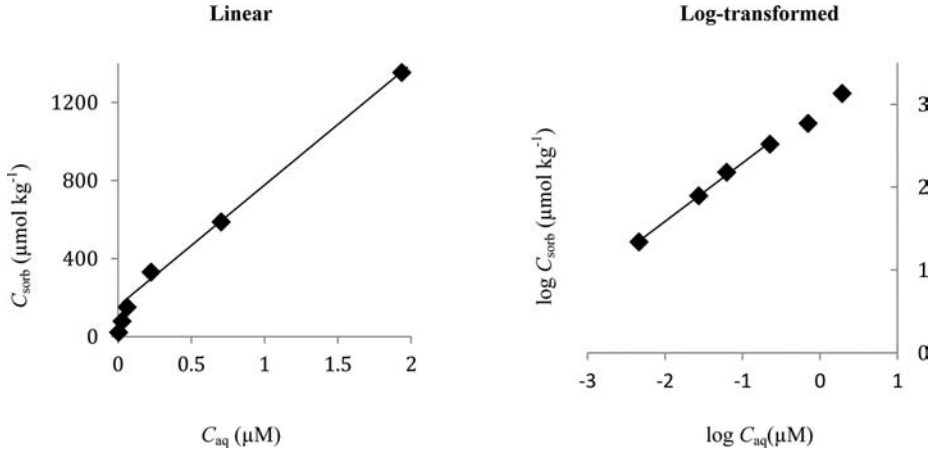


**Figure 3.** Linear and log-transformed isotherms representing the sorption of E1 to dried maize root tissue. Error bars indicate SD. Trendlines are drawn through the portions of the data for which $K_{pom}$ was modeled as linear (four highest concentrations) and concentration-dependent (four lowest concentrations).

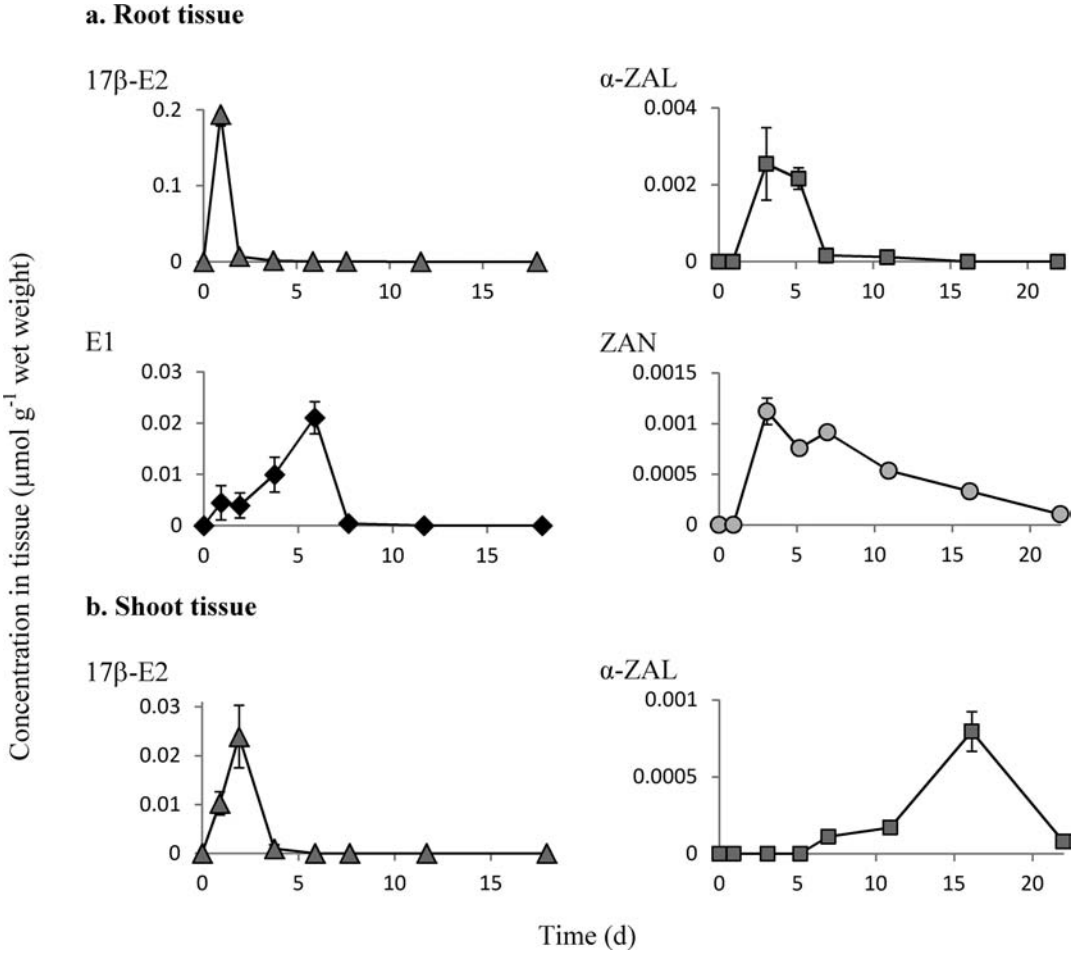


**Figure 4.** Concentrations of 17$\beta$-E2, E1, $\alpha$-ZAL, and ZAN in root and shoot tissues in exposed maize seedlings. Error bars represent one SD.

21,22). These patterns are related to both sorbent and solute properties. For example, the pattern of linearity at mid to high concentrations and nonlinearity at low concentrations is consistent with dual-phase soil-based sorption models, which posit the existence of a low abundance high affinity sorbent and a much more abundant lower affinity fraction[21,23] through which partitioning can occur. The low abundance high affinity sorbent in soils has been identified by others as black carbon[22,24,25] and/or "glassy" carbon phases[26,27] where true adsorption can occur. Once these limited sites are saturated, the solutes partition linearly into the more abundant "soft" soil organic matter phases.

Unlike soil sorbents, recently germinated plants lack black or glassy carbon phases. Nonetheless, nonlinear sorption to plant cuticles (which are rich in lipids and waxes) has previously been reported for organic solutes with physicochemical properties

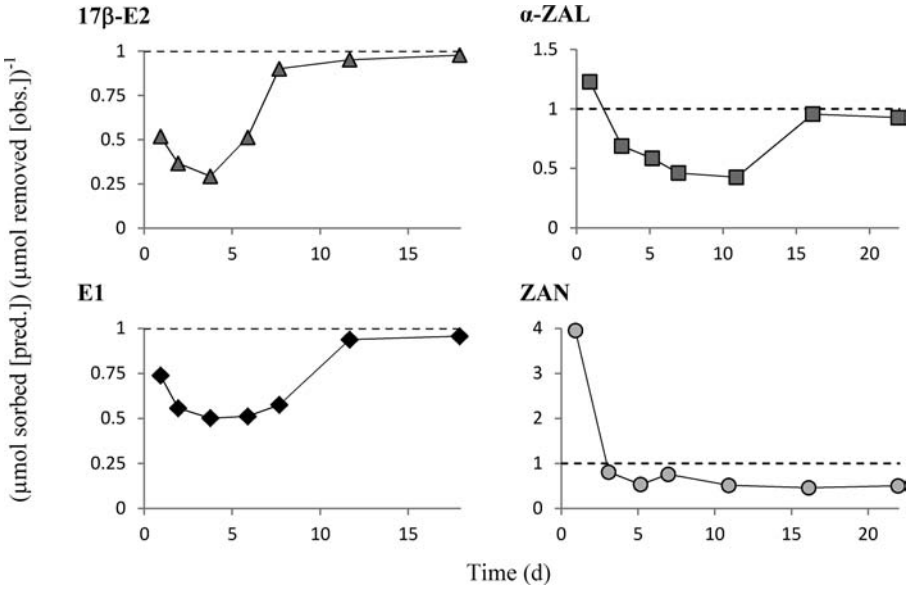


**Figure 5.** Change over time of the ratio of estrogen removal from the aqueous phase predicted by sorption to observed estrogen removal. Dashed line indicates 1:1 ratio.

similar to those of our compounds.[28] These investigators reported both linear and nonlinear sorption to different organic matter fractions isolated from green bell pepper cuticle. The "non-saponifiable and non-hydrolyzable" cuticle fraction exhibited the most nonlinear behavior, and these investigators characterize this component as comprised of aromatic moieties and "crystalline" aliphatic molecules. We suspect that similar components in the root tissue (i.e., a very low abundance, high affinity sorbent) could explain the observed nonlinear sorption for our samples at the lowest analyte concentrations.[28]

Given that lipids compose approximately 2.2% by dry weight (dw) corn root mass,[29] we estimate that roughly 5−12% of the estrogen load was sorbed to root lipids in the batch tissue sorption tests (refs 30,31; Table SI-6; see Supporting Information section 7 for calculations). Conversely, cellulose accounts for 22.6%[32] and starch comprises 6.0% of root mass (dw).[33] Although these carbohydrates comprise a large portion of root mass, sorption to carbohydrates is estimated to sorb less than 0.2% of the aqueous estrogen content in sorption tests. Although these are only coarse approximations, a difference of 1−2 orders of magnitude (0.2% versus 5−12%) demonstrates that lipids likely play a disproportionately large role in sorption of estrogens to corn root tissues. The remainder of the organic mass in the roots is comprised of other components (e.g., lignin and proteins) that can interact favorably with solutes through linear partitioning mechanisms.

**Translocation into Tissues.** 17$\beta$-E2, E1, $\alpha$-ZAL, and ZAN were all detected in root tissues, while only the more reduced form from each pair (i.e., 17$\beta$-E2 and $\alpha$-ZAL) was detected in shoot tissues (Figure 4, Table SI-7) at lower concentrations than in root tissues. The disparity in root and shoot estrogen concentrations may be attributed to limited translocation or transformation and irreversible binding of 17$\beta$-E2 and $\alpha$-ZAL in root tissues. Concentrations of 17$\beta$-E2 in root tissue were 1 order of magnitude greater than E1 concentrations, and 2 orders of magnitude greater than $\alpha$-ZAL and ZAN concentrations. In shoot tissues, 17$\beta$-E2 concentrations were 1 order of magnitude greater than $\alpha$-ZAL concentrations. The lower tissue concentrations of $\alpha$-ZAL and ZAN may, in part, explain the slower uptake rates of these estrogens.

It has previously been reported that compounds with log $K_{ow}$ greater than 3.5 are too hydrophobic to move through vascular tissue,[12] but all of the estrogens in this study have log $K_{ow}$ values above this limit (Table 1). Indeed, the log $K_{ow}$ of E1 is the lowest of all of the estrogens in this study, but it was not detected in shoot tissues. The difference in translocation between the reduced (17$\beta$-E2 and $\alpha$-ZAL) and oxidized forms (E1 and ZAN) is also not correlated to log $K_{ow}$. Whereas the log $K_{ow}$ of 17$\beta$-E2 is greater than that of E1, $\alpha$-ZAL is less hydrophobic than ZAN. There have been efforts to relate translocation to molecular connectivity indices,[34] but while the resulting quantitative structure−activity relationships (QSARs) may explain differential translocation of the alcohols or ketones, they do not explain our observations for E1 or ZAN. Thus, enzymatic activity and/or active exclusion of E1 and ZAN from the shoots are more likely explanations for this observation.

**Estimation of Approach to Equilibrium Partitioning.** We calculated the ratio of estrogen loss predicted by sorption to observed removal from the aqueous phase (Figure 5; Table SI-8) based on the measured root mass at each time point and the calculated linear log $K_{pom}$ (aqueous concentrations greater than 0.1 $\mu$M) or concentration-dependent log $K_{pom}$ based upon the Freundlich equation (aqueous concentrations below 0.1 $\mu$M; see Supporting Information section 9). For $\alpha$-ZAL and ZAN, the ratio of predicted sorption to observed loss was initially greater than 1.0, meaning that uptake from the aqueous phase was below that which would be expected from sorption. We attribute this disparity to a system that is not well mixed, in contrast to the root sorption isotherms, which were determined in continuously mixed samples. In the initial days of exposure, the roots extended less than 1 cm into the 6 cm water columns, and the samples were not stirred or shaken after the plants had been placed. After the initial days of exposure to $\alpha$-ZAL and ZAN, the ratio decreased to below 1.0 (to approximately 0.5), indicating that sorption accounted for only part of the observed removal, while processes such as active translocation, trans-

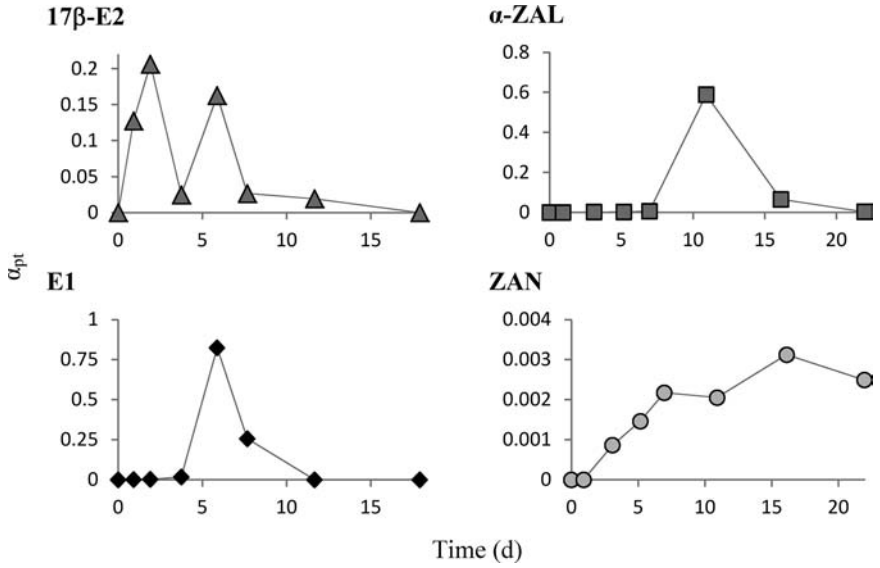


**Figure 6.** Calculated estrogen $\alpha_{pt}$ values over time of exposure to maize seedlings.

formation, and binding caused further removal of our analytes from the system.

The ratios of 17$\beta$-E2 and E1 removed by sorption to the observed removal were below one at every time point, and their uptake rates were faster than those of $\alpha$-ZAL and ZAN. This suggests that the natural estrogens are transformed or mineralized by maize seedlings or the local microbial consortia more readily than the synthetic estrogen mimics. As 17$\beta$-E2, E1, and $\alpha$-ZAL concentrations decreased to levels at which sorption behavior is concentration-dependent (i.e., fitted to the Freundlich model), $K_{pom}$ values increased approximately 1 order of magnitude, and the observed mass of estrogens removed from the aqueous phase approached the values predicted by sorption alone (i.e., the ratio of predicted to observed removal from solution approached 1.0).

Using the partition-limited model developed by Chiou et al.,[20] we calculated the approach to equilibrium partitioning between the hydroponic solution and plant tissue ($\alpha_{pt}$) for each estrogen over time (Figure 6; Table SI-9), taking into account the estrogen concentrations in root and shoot tissues, aqueous estrogen concentrations, and conditional changes in log $K_{pom}$ as calculated from root sorption isotherms. Calculation of $\alpha_{pt}$ values for each time point, including the data used in the model (e.g., plant biomass at each time point), is available in the Supporting Information. Over the first several days of exposure, the change in $\alpha_{pt}$ closely mirrored the estrogen concentrations in roots and shoots because $C_{pt}$ is the numerator of the $\alpha_{pt}$ calculation (eq 1). At longer exposure times, decreasing $C_w$ led to larger $\alpha_{pt}$ values for 17$\beta$-E2, $\alpha$-ZAL, and ZAN, but this effect was not observed with E1 because $C_{pt}$ decreased to zero. The largest $\alpha_{pt}$ was calculated for E1 (82% of equilibrium after 6 d of exposure), followed by $\alpha$-ZAL (58% of equilibrium after 11 d of exposure). Conversely, $\alpha_{pt}$ values for 17$\beta$-E2 only reached 26% of equilibrium. Although 17$\beta$-E2 concentrations in roots and shoots were significantly higher than tissue concentrations of the other estrogens, the 17$\beta$-E2 tissue concentrations peaked very early in the study time. Subsequently, 17$\beta$-E2 aqueous concentrations decreased in the tissues to relatively low concentrations, and $\alpha_{pt}$ never exceeded 26%. Finally, ZAN $\alpha_{pt}$ values were 2 orders of magnitude lower throughout the 22 d of exposure, due to its slower uptake rate and lower root tissue sorption.

Continual rapid transformation and irreversible binding of estrogens in plant tissues prevented us from observing true equilibrium partitioning of 17$\beta$-E2, E1, $\alpha$-ZAL, and ZAN in these systems. Even without the confounding factors of transformation and binding, however, equilibrium partitioning would not be expected using this experimental design. Whereas a constant aqueous estrogen concentration would be required to approach steady-state, near-equilibrium partitioning,[19] the seedlings in the present study were exposed to only one initial pulse of estrogens. Further, the seedlings grew throughout the experiments, and their biomass changed rapidly over the 3 weeks of sampling. For compounds that are slowly taken up, such as ZAN, this continual increase in biomass prevents the system from achieving equilibrium with respect to sorption because the mass of sorbent is no longer constant. Despite these challenges facing our experimental design, we believe that the use of seedlings and a single dose of estrogens (as opposed to maintaining a constant level of analyte in the aqueous phase) more accurately reflects dosing regimes in manure-fertilized fields.

In conclusion, our results demonstrate that maize may have a significant impact on the environmental fate and mobility of natural and synthetic estrogens in manure fertilizer by rapidly removing estrogens from runoff. Given these findings, we suggest that further study is warranted on the effects of maize on the environmental fate of estrogens, including in soil-based systems. The effects of maize or other plants, such as buffer-strip or riparian species, may be exploited to attenuate the environmental mobility of these compounds and, perhaps, other biomolecules present in manure fertilizer.

## ■ ASSOCIATED CONTENT

### Supporting Information

Extraction of zeranol from Ralgro, setup of hydroponic uptake experiments, log $K_{ow}$ measurements, uptake data, initial uptake rate constants, root sorption data, contribution of root components to sorption behavior, concentrations of estrogens in tissues, predicted sorption to roots, and estimation of

approach to equilibrium partitioning. This material is available free of charge via the Internet at http://pubs.acs.org.

## AUTHOR INFORMATION


### Corresponding Author

*Tel.: (614) 292-6953. E-mail: chin.15@osu.edu.

### Present Address

[‖]National Research Centre for Environmental Toxicology, 39 Kessels Road, Brisbane QLD 4108, Australia.



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### Notes

The authors declare no competing financial interest.

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## ABBREVIATIONS USED

17$\beta$-E2, 17$\beta$-estradiol; $\alpha$-ZAL, $\alpha$-zearalanol or zeranol; $\alpha_{pt}$, approach to equilibrium partitioning between the hydroponic solution and plant tissue; $C_{pt}$, concentration in the plant; $C_w$, aqueous concentration; E1, estrone; $f_{pom}$, weight fraction of plant organic matter; $f_{pw}$, whole-plant weight fraction of water; $K_{ow}$, octanol–water partition coefficient; $K_{pom}$, plant organic matter–water partition coefficient; LOQ, limit of quantification; RP-HPLC, reverse-phase high-pressure liquid chromatography; ZAN, zearalanone

## REFERENCES


(1) Willingham, E. J. Trenbolone and other cattle growth promoters: Need for a new risk-assessment framework. *Environ. Pract.* **2006**, *8*, 58–65.

(2) *Animal drugs @ FDA*; U.S. Food and Drug Administration: Washington, DC; www.accessdata.fda.gov/scripts/animaldrugsatfda/.

(3) Jobling, S.; Williams, R.; Johnson, A.; Taylor, A.; Gross-Sorokin, M.; Nolan, M.; Tyler, C. R.; van Aerle, R.; Santos, E.; Brighty, G. Predicted exposures to steroid estrogens in UK rivers correlate with widespread sexual disruption in wild fish populations. *Environ. Health Perspect.* **2006**, *114*, 32–39.

(4) Ternes, T.; Kreckel, P.; Mueller, J. Behaviour and occurrence of estrogens in municipal sewage treatment plants - II. Aerobic batch experiments with activated sludge. *Sci. Total Environ.* **1999**, *225*, 91–99.

(5) Bories, G.; Sutra, J.; Tulliez, J. Metabolism and disposition of [$^3$H]zeranol implanted in the pig. *J. Agric. Food Chem.* **1992**, *40*, 284–288.

(6) Dixon, S. N.; Mallinson, C. B. Radioimmunoassay of the anabolic agent zeranol. 3. Zeranol concentrations in the feces of steers implanted with zeranol (Ralgro). *J. Vet. Pharmacol. Ther.* **1986**, *9*, 88–93.

(7) Kjær, J.; Olsen, P.; Bach, K.; Barlebo, H. C.; Ingerslev, F.; Hansen, M.; Halling, B. Leaching of estrogenic hormones from manure-treated structured soils. *Environ. Sci. Technol.* **2007**, *41*, 3911–3917.

(8) Kinani, S.; Bouchonnet, S.; Bourcier, S.; Porcher, J.; Ait-Aissa, S. Study of the chemical derivatization of zearalenone and its metabolites for gas chromatography-mass spectrometry analysis of environmental samples. *J. Chromatogr., A* **2008**, *1190*, 307–315.

(9) Kolpin, D.; Furlong, E.; Meyer, M.; Thurman, E.; Zaugg, S.; Barber, L. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: A national reconnaissance. *Environ. Sci. Technol.* **2002**, *36*, 1202–1211.

(10) Lagana, A.; Bacaloni, A.; De Leva, I.; Faberi, A.; Fago, G.; Marino, A. Analytical methodologies for determining the occurrence of endocrine disrupting chemicals in sewage treatment plants and natural waters. *Anal. Chim. Acta* **2004**, *501*, 79–88.

(11) Card, M. L.; Chin, Y.-P.; Lee, L. S.; Khan, B. Prediction and experimental evaluation of soil sorption by natural hormones and hormone mimics. *J. Agric. Food Chem.* **2012**, *60*, 1480–1487.

(12) Dietz, A.; Schnoor, J. Advances in phytoremediation. *Environ. Health Perspect.* **2001**, *109*, 163–168.

(13) Nichols, D.; Daniel, T.; Edwards, D.; Moore, P.; Pote, D. Use of grass filter strips to reduce 17$\beta$-estradiol in runoff from fescue-applied poultry litter. *J. Soil Water Conserv.* **1998**, *53*, 74–77.

(14) Lai, K.; Scrimshaw, M.; Lester, J. Biotransformation and bioconcentration of steroid estrogens by *Chlorella vulgaris*. *Appl. Environ. Microbiol.* **2002**, *68*, 859–864.

(15) Shi, W.; Wang, L.; Rousseau, D. P. L.; Lens, P. N. L. Removal of estrone, 17$\alpha$-ethinylestradiol, and 17$\beta$-estradiol in algae and duckweed-based wastewater treatment systems. *Environ. Sci. Pollut. Res.* **2010**, *17*, 824–833.

(16) Young, I. J.; Knights, B. A.; Hillman, J. R. The metabolism of estrogens *in vivo* and *in vitro* by *Phaseolus vulgaris*. *Z. Pflanzenphysiol.* **1979**, *94*, 307–316.

(17) Bircher, S. Phytoremediation of natural and synthetic steroid growth promoters used in livestock production by riparian buffer zone plants. M.S. Thesis, The University of Iowa, Iowa City, IA, 2011.

(18) Epstein, E. *Mineral Nutrition of Plants: Principles and Perspectives*; John Wiley & Sons: New York, 1972.

(19) Li, H.; Sheng, G.; Chiou, C. T.; Xu, O. Relation of organic contaminant equilibrium sorption and kinetic uptake in plants. *Environ. Sci. Technol.* **2005**, *39*, 4864–4870.

(20) Chiou, C. T.; Sheng, G.; Manes, M. A partition-limited model for the plant uptake of organic contaminants from soil and water. *Environ. Sci. Technol.* **2001**, *35*, 1437–1444.

(21) Chiou, C. T. *Partition and Adsorption of Organic Contaminants in Environmental Systems*; John Wiley & Sons: Hoboken, NJ, 2002.

(22) Chiou, C. T.; McGroddy, S. E.; Kile, D. E. Partition characteristics of polycyclic aromatic hydrocarbons on soils and sediments. *Environ. Sci. Technol.* **1998**, *32*, 264–269.

(23) Chiou, C. T.; Kile, D. E.; Rutherford, D. W. Sorption of selected organic compounds from water to a peat soil and its humic-acid and humin fractions: Potential sources of the sorption nonlinearity. *Environ. Sci. Technol.* **2000**, *34*, 1254–1258.

(24) Chiou, C. T. Comment on thermodynamics of organic chemical partition in soils. *Environ. Sci. Technol.* **1995**, *29*, 1421–1422.

(25) Gustafsson, Ö.; Haghseta, F.; Chan, C.; MacFarland, J.; Gschwend, P. M. Quantification of the dilute sedimentary soot phase: implications for PAH speciation and bioavailability. *Environ. Sci. Technol.* **1997**, *31*, 203–209.

(26) Weber, W. J.; Huang, W. A distributed reactivity model for sorption by soils and sediments. 4. Intraparticle heterogeneity and phase-distribution relationships under nonequilibrium conditions. *Environ. Sci. Technol.* **1996**, *30*, 881–888.

(27) Young, T. M.; Weber, W. J. A distributed reactivity model for sorption by soils and sediments. 3. Effects of diagenetic processes on sorption energetics. *Environ. Sci. Technol.* **1995**, *29*, 92–97.

(28) Chen, B.; Johnson, E. J.; Chefetz, B.; Zhu, L.; Xing, B. Sorption of polar and nonpolar aromatic organic contaminants by plant cuticular materials: Role of polarity and accessibility. *Environ. Sci. Technol.* **2005**, *39*, 6138–6146.

(29) Ndon, B. A.; Harvey, R. G. Effects of seed and root lipids on the susceptibility of plants to Trifluralin and Oryzalin. *Weed Sci.* **1981**, *29*, 420–425.

(30) Lin, T.; Chiou, C. Partition coefficients of organic contaminants with carbohydrates. *Environ. Sci. Technol.* **2010**, *44*, 5430–5436.

(31) Chiou, C. T. Partition coefficients of organic compounds in lipid-water systems and correlations with fish bioconcentration factors. *Environ. Sci. Technol.* **1985**, *19*, 57–62.

(32) Pilet, P.-E.; Blaschek, W.; Senn, A.; Franz, G. Comparison between maize root cells and their respective regenerating protoplasts: wall polysaccharides. *Planta* **1984**, *161*, 465−469.
(33) Muller, B.; Stosser, M.; Tardieu, F. Spatial distributions of tissue expansion and cell division rates are related to irradiance and to sugar content in the growing zone of maize roots. *Plant, Cell Environ.* **1998**, *21*, 149−158.
(34) Dowdy, D.; McKone, T. E. Predicting plant uptake of organic chemicals from soil or air using octanol/water partition ratios and a molecular connectivity index. *Environ. Toxicol. Chem.* **1997**, *16*, 2448−2456.