Persistence of endocrine-disrupting chemicals in agricultural soils[1]

Angela Lorenzen, Kent Burnison, Mark Servos, and Edward Topp

Abstract: Biosolids and animal wastes can contain natural hormones or synthetic chemicals that have the potential to disrupt endocrine function in wildlife should they move offsite. The persistence in soil of estrogenic substances that could reach agricultural land via fertilization with organic amendments has been evaluated. 4-Nonylphenol, ethynylestradiol, estradiol, and estrone are rapidly dissipated in soils under a range of conditions typical of a temperate growing season with half-lives ranging from a few hours to a few days. We conclude that these chemicals are rapidly removed from aerated soils under temperate growing conditions such that application methods that minimize preferential flow or runoff of animal or human wastes should protect adjacent water from contamination. The use of recombinant yeast and cell culture estrogen receptor gene transcription bioassays were investigated as potential tools to detect non-labile estrogenic or anti-estrogenic substances in runoff from soils receiving liquid swine slurry or biosolids.

Key words: biosolids, manures, endocrine disruption, estrogen, nonylphenol, soil persistence, bioassay.

Résumé : Les biosolides et les déchets d'origine animale peuvent contenir des hormones naturelles ou des produits chimiques de synthèse qui ont un potentiel de perturber la fonction endocrine chez les animaux sauvages si ces produits devaient s'échapper du site où ils sont produits. La persistance dans le sol de substances œstrogéniques qui pourraient atteindre les terres agricoles par la fertilisation au moyen de fertilisants organiques a été évaluée. Le 4-nonylphénol, l'éthynylestradiol, l'œstradiol et l'œstrone sont rapidement dissipés dans les sols sous une plage de conditions typiques d'une saison de croissance tempérée avec des demi-vies biologiques variant de quelques heures à quelques jours. Nous avons conclu que ces produits chimiques sont rapidement éliminés de sols aérés sous des conditions de croissance tempérées; les méthodes d'application qui minimisent l'écoulement préférentiel des déchets d'origine humaine ou animale devraient permettre de protéger l'eau adjacente contre la contamination. L'utilisation de levure recombinante ainsi que les essais biologiques sur la transcription des gênes des récepteurs d'œstrogènes en culture cellulaire ont également été examinés comme outils potentiels pour détecter les substances œstrogéniques et anti-œstrogéniques non labiles dans le ruissellement provenant de sols recevant des boues liquides de lisiers de porcs ou des biosolides.

Mots clés : biosolides, fumiers, lisiers, perturbation endocrinienne, œstrogènes, nonylphénol, persistance dans le sol, essai biologique.

[Traduit par la Rédaction]

Introduction

There is growing concern about the apparently increasing incidence of reproductive disorders and abnormal development in wildlife and reduced fertility in human males, problems which may be caused by so-called endocrine disrupting chemicals (EDCs) released anthropogenically into the environment (Ashby et al. 1997; Sonnenschein and Soto 1998). Known or suspected man-made EDCs include industrial chemicals such as dioxins, PCBs, and nonylphenols, and a number of persistent pesticides, such as DDT, that are no longer in use (Safe and Gaido 1998; Kavlock et al. 1996). Endocrine disrupting chemicals possess the ability to alter or disrupt endocrine system function by mimicking, antagonizing, or interfering with the biosynthesis or biodegradation of endogenous hormones (Sonnenschein and Soto 1998). Perhaps the clearest evidence of endocrine disruption in wildlife is reproductive abnormalities observed in fish or amphibians exposed to effluents from sewage treatment plants or pulp and paper mills, and possibly agricultural runoff.

Agriculture could potentially be a source of environmental EDCs through the use of certain pesticides, land application of sewage biosolids, or land application of animal wastes containing elevated levels of hormones such as 17β-estradiol and estrone excreted by poultry or livestock (Nichols et al. 1997; Ternes et al. 1999). These hormones are found in some animal wastes, and are notably excreted by pregnant livestock or laying poultry (Peterson et al. 2000; Shore et al. 1995). Furthermore, mixtures of estrogenic compounds may act in combination to

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Written discussion of this article is welcomed and will be received by the Editor until 30 September 2006.

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produce an enhanced response, complicating the risk assessment of mixed effluents such as agricultural runoff (Daughton and Ternes 1999). 17β-Estradiol and estrone are bioactive at remarkably low environmental concentrations. For example, the induction of vitellogenin production in male fish occurs at estradiol concentrations as low as 1 ng/L (Purdom et al. 1994). A number of recent studies have revealed movement of 17β-estradiol from manured land into surface and groundwater in concentrations that could affect wildlife (Peterson et al. 2000).

The synthetic analogue of 17β-estradiol, 17α-ethynylestradiol, is a potent xenoestrogen and is a component of oral contraceptives. Excreted 17α-ethynylestradiol is incompletely degraded in sewage treatment plants, and contributes to the feminization of male fish in rivers receiving sewage outflow (Routledge et al. 1998). The efficiency of biodegradation of both natural and synthetic estrogenic substances, and the concentrations that will be exported in biosolids, varies according to sewage treatment characteristics, with aeration notably increasing efficiency (Esperanza et a. 2004; Joss et al. 2004; Lorenzen et al. 2004).

The lipophilic aromatic compound nonylphenol is suspected to be a xenoestrogen, a man-made molecule that has estrogenic effects. This suspicion is based in part on evidence that nonylphenol can bind to the human estrogen receptor, stimulate human breast cancer cell growth, and induce the expression of the egg protein vitellogenin in fish (e.g., Lech et al. 1996). 4-Nonylphenol is suspected of contributing to the feminization of male fish in sewage outflows. This compound is a primary breakdown product of nonylphenol ethoxylates, non-ionic surfactants widely used in industrial, agricultural, and household applications. Large amounts of nonylphenol ethoxylates are typically processed by municipal sewage treatment plants where they are partially degraded to 4-nonylphenol. Most of the 4-nonylphenol produced during the breakdown of nonylphenol ethoxylates during sewage treatment becomes sorbed to the solids, and concentrations of this chemical in sewage sludge can be in the gram per kilogram range.

Given the significant scientific and regulatory concern regarding the potential human and environmental impacts of these chemicals, we have been evaluating the persistence and mobility of selected estrogenic compounds in agricultural soils, and estrogen receptor gene transcription activity of agricultural runoff by means of bioassays.

Materials and methods

The persistence and mineralization of estradiol, estrone, ethynylestradiol, and 4-nonylphenol were evaluated in soil microcosms in laboratory incubations by following the disposition of C^{14} or H^3 labeled parent compound. Detailed descriptions of the soils used in the experiments, laboratory microcosms, sample preparation and solvent extraction, HPLC analysis, radioisotope methods, and hormonal activity bioassays are to be found in a series of publications (Colucci et al. 2001; Colucci and Topp 2001, 2002; Lorenzen et al. 2004; Topp and Starratt 2000).

Extracts of liquid swine slurry or municipal biosolids were subjected to a toxicity identification evaluation (TIE) analysis to quantitatively identify which chemicals were contributing to estrogen receptor binding activity as described previously (Burnison et al. 2003). Properties of the aerated municipal biosolids obtained from a city with a population of 130 000, and liquid swine manure slurry obtained from a commercial farm have been described (Burnison et al. 2003; Lee and Peart 2002). Briefly, methanol extracts were fractionated by means of HPLC, and the fractions were analyzed by a recombinant yeast estrogenicity screen (YES assay) to determine estrogen receptor binding activities (Gaido et al. 1997). The identity of compounds with estrogen receptor binding activities was established on the basis of retention time in comparison with standard compounds.

To assess the ability of existing assays to provide full concentration–response data for estrogen receptor gene transcription activities of agricultural effluents, selected biosolid and liquid swine manure extracts were analyzed by two in vitro bioassays. Properties of the aerated municipal biosolids and the swine manure slurry used for the methods comparison have been described (Burnison et al. 2003; Jacobsen et al. 2005). The first method was a YES assay based on an estrogen-responsive recombinant yeast strain developed by Routledge and Sumpter (1996) as described previously (Colucci et al. 2001; Colucci and Topp 2001, 2002). The second assay utilized a recombinant estrogen-responsive human ovarian carcinoma cell line, BG1Luc4E_2 , propagated in cell culture (Rogers and Denison 2000). This cell line has been stably transfected with an estrogen responsive luciferase reporter gene. For both, the principle of the assay consists of placing a reporter enzyme under the control of genetic control elements that are activated in the presence of hormonal substances. The expression of the reporter enzyme is quantitatively detected by means of colour or luminescence, and is related to the estrogenic activity in the test extract.

Results

Toxicity identification evaluations of liquid swine slurry (Fig. 1) and municipal biosolids (Fig. 2) illustrate the variety of natural and synthetic estrogenic substances in these complex materials. For these studies, HPLC fractions were each evaluated by means of a YES assay, and the chromatographic retention time of active fractions compared to standard compounds (Burnison 2003). The estrogen receptor gene transcription activity in this manure sample was dominated by equol, a phytoestrogen derived from soybeans that are fed to the pigs. In addition, natural steroidal female hormones estradiol and estrone had significant activities. In contrast, estrogen receptor gene transcription activity in the municipal biosolid sample was dominated by alkylphenols, components of a wide variety of detergents that are released during anaerobic treatment.

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Fig. 1. Toxicity identification evaluation of liquid swine slurry. Bars indicate the estrogen receptor gene transcription activity of HPLC fractions with the indicated retention times, the dashed line indicates UV absorbance during the HPLC run. The retention time of estrogen standards are indicated by the solid line.

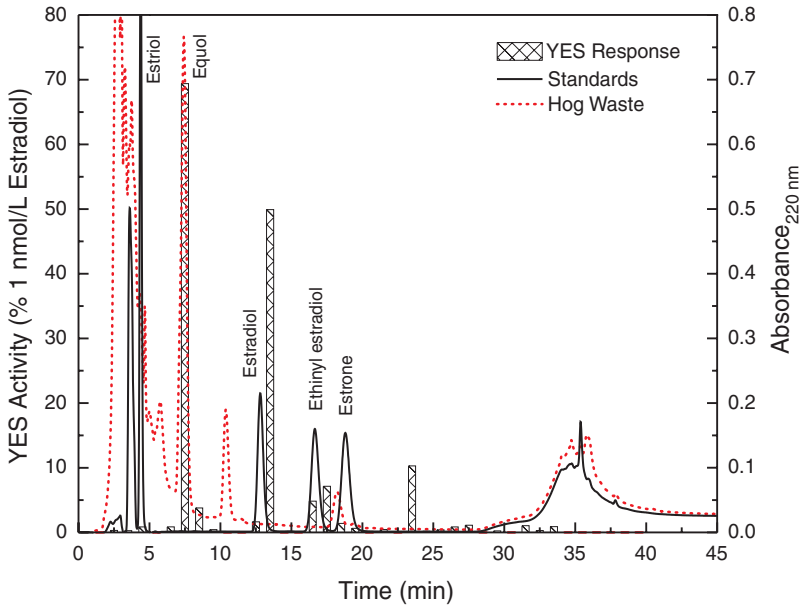
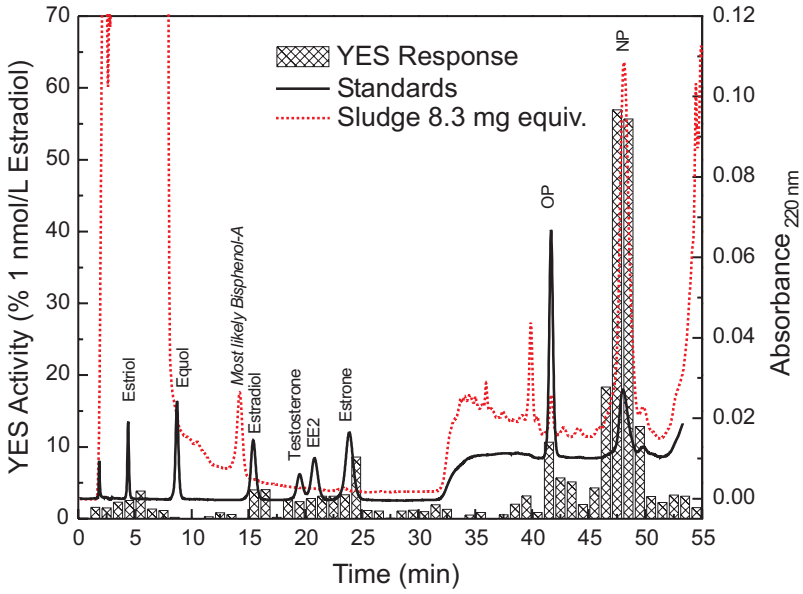


Fig. 2. Toxicity identification evaluation of a municipal biosolid sample. Bars indicate the estrogen receptor gene transcription activity of HPLC fractions with the indicated retention times, the dashed line indicates UV absorbance during the HPLC analysis of the sample, the solid line indicates absorbance during the HPLC analysis of a mixture of estrogen standards. The retention times of the estrogen standards are indicated. EE2, ethynylestradiol; OP, octylphenol; NP, nonylphenol.



A series of laboratory soil microcosm experiments were undertaken to elucidate the kinetics and mechanisms of removal of estrogenic substances from soils (Colucci et al. 2001; Colucci and Topp 2001, 2002; Topp and Starratt 2000). Figures 3 and 4 illustrate typical results with, in this case, persistence of ethynylestradiol. The removal of ethynylestradiol was closely paralleled by the loss of estrogen receptor gene transcription activity detected by the YES assay, indicating that there were no extractable intermediates produced during the biodegradation of this compound capable of binding to and activating estrogen receptors (Fig. 3). In a separate experiment, the loss of HPLC-detectable H^3-labeled ethynylestradiol in three soils varying in texture was closely accompanied by the loss of extractable radioactivity, indicating that residues became bound to the soil

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Fig. 3. Removal of ethynylestradiol (EE2) measured by HPLC (open triangles), and removal of estrogen receptor gene transcription activity (expressed as estradiol equivalents, closed triangles) in a loam soil receiving 1 mg ethynylestradiol/kg soil.

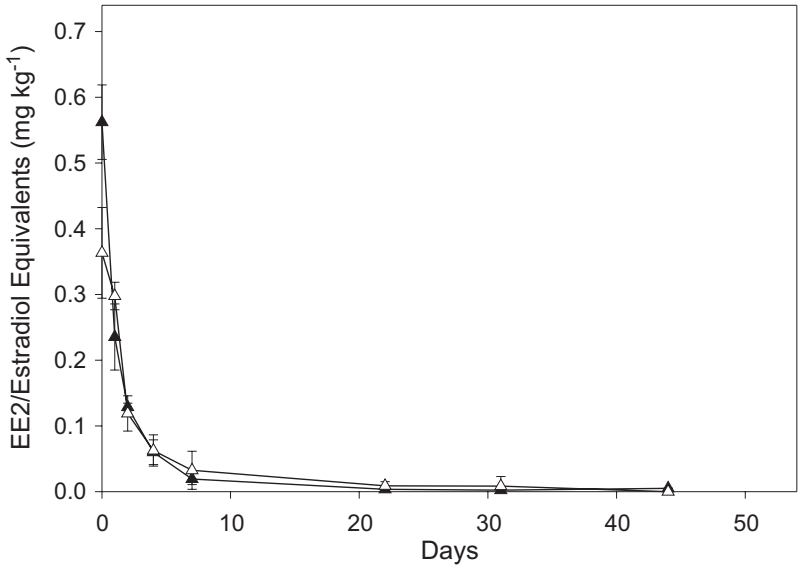

Fig. 4. Removal of ethynylestradiol (EE2) measured by HPLC (top panel), and removal of extractable radioactivity (bottom panel) in three agricultural soils varying in texture. The initial ethynylestradiol concentration was 120 ng/kg soil. The HPLC detection limit was 12 ng/kg soil, indicated by the dotted line on the top panel (Colucci and Topp 2002).

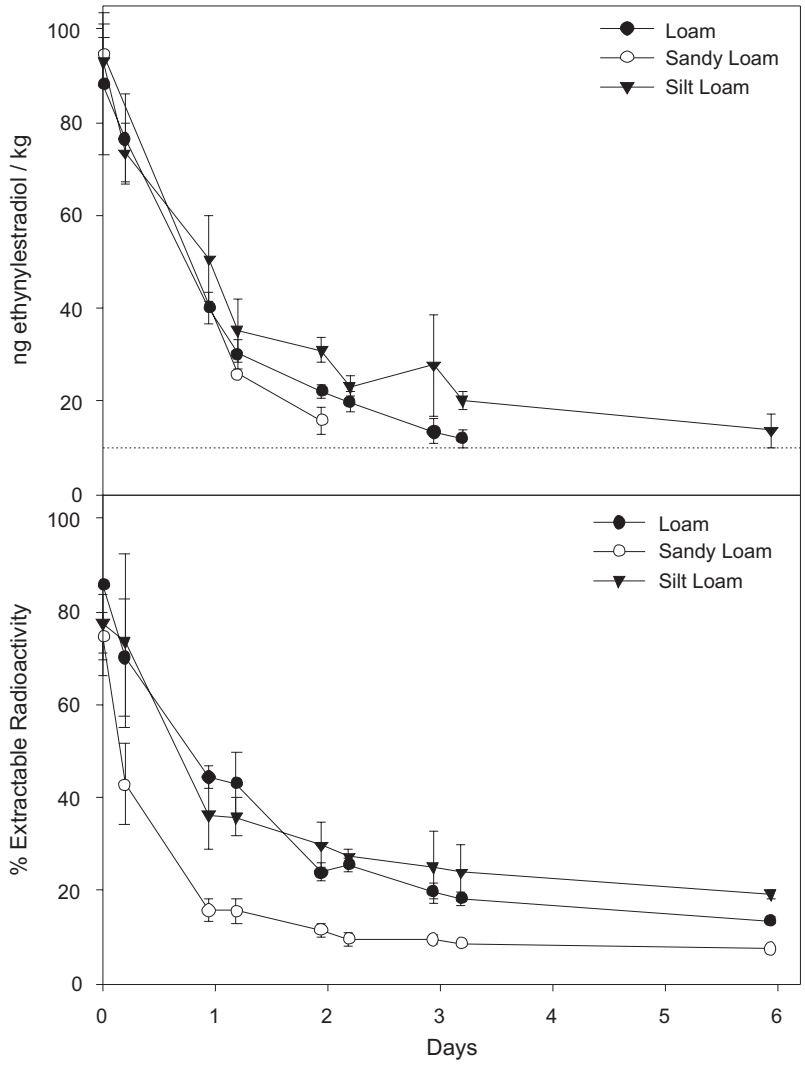

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Fig. 5. Concentration–response data for estrogen receptor gene transcription activity of estradiol in BG1Luc4E$_2$ cells (filled circles) and recombinant yeast (open circles). Bars represent standard error; $n = 5$ for BG1Luc4E$_2$ cells; $n = 224$ for YES assay.

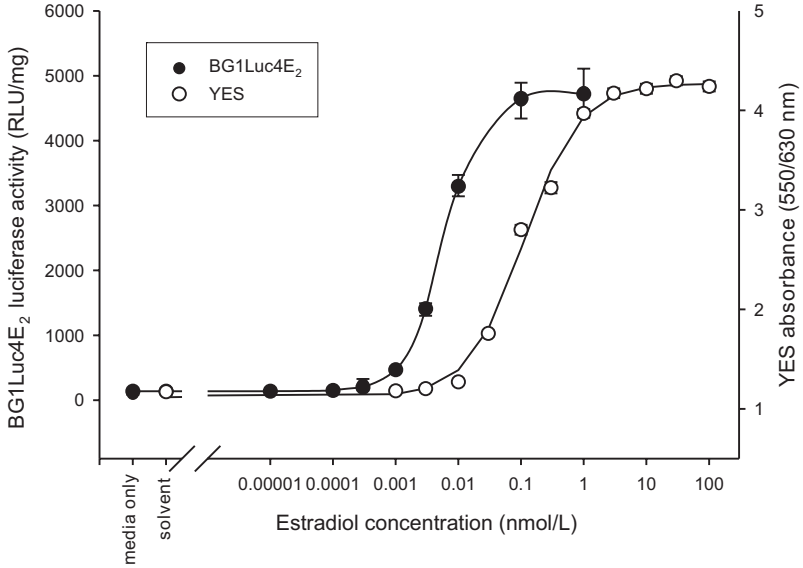
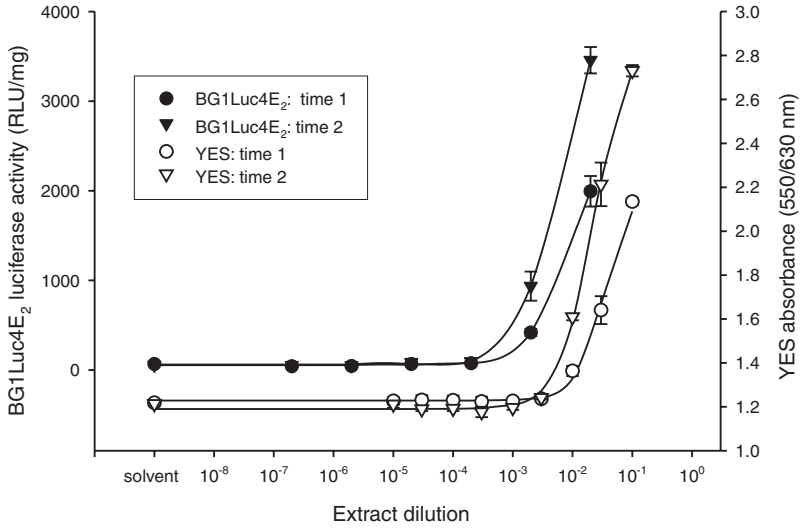


Fig. 6. Concentration–response data for estrogen receptor gene transcription activity of two biosolid extracts in BG1Luc4E$_2$ cells (filled circles) and recombinant yeast (open circles). Bars represent standard deviation; $n = 3$ for BG1Luc4E$_2$ cells; $n = 2$ for YES assay.



(Fig. 4). Ethynylestradiol was reduced to the extremely low HPLC-radioactivity detector detection limit of 12 ng/kg soil (i.e., 12 parts per trillion).

Concentration–response data for estrogen receptor gene transcription activity of estradiol are shown in Fig. 5. Similar curves were obtained for both the YES assay and the BG1Luc4E$_2$ cells. However, the recombinant yeast were approximately 10–15 times less sensitive to estradiol than the BG1Luc4E$_2$ cells. Typical EC50 (concentration of estradiol required to achieve 50% of the maximal amount of activity) and EC20 (concentration of estradiol required to achieve 20% of the maximal amount of activity) values were 0.0058 nmol/L and 0.0023 nmol/L, respectively, for BG1Luc4E$_2$ cells and 0.11 nmol/L and 0.027 nmol/L, respectively, for the YES assay.

Serial dilutions of biosolid extracts obtained from the same site at two different time points were analyzed for estrogen receptor gene transcription activities in both BG1Luc4E$_2$ cells and the YES assay. Similar concentration–response curves were obtained for both assays and, as for estradiol, the recombinant yeast were approximately 10–15 times less sensitive to the extracts than the BG1Luc4E$_2$ cells (Fig. 6). Relative potencies of the extracts were determined by dividing the EC20 of estradiol by the dilution of extract required to attain a response equal to 20% of the maximum response observed for estradiol. For

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Fig. 7. Concentration–response data for estrogen receptor gene transcription activity of a liquid swine manure extract in BG1Luc4E$_2$ cells (filled triangles) and recombinant yeast (open triangles). Bars represent standard deviation; $n = 3$ for BG1Luc4E$_2$ cells; $n = 2$ for YES assay.

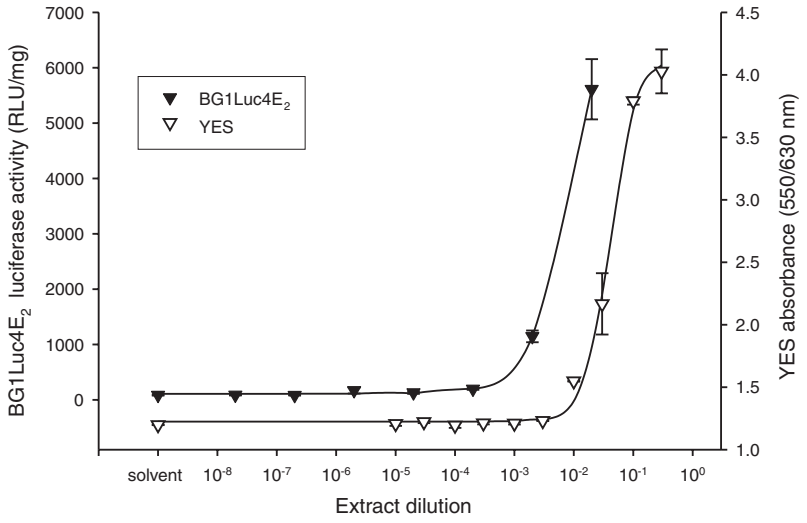
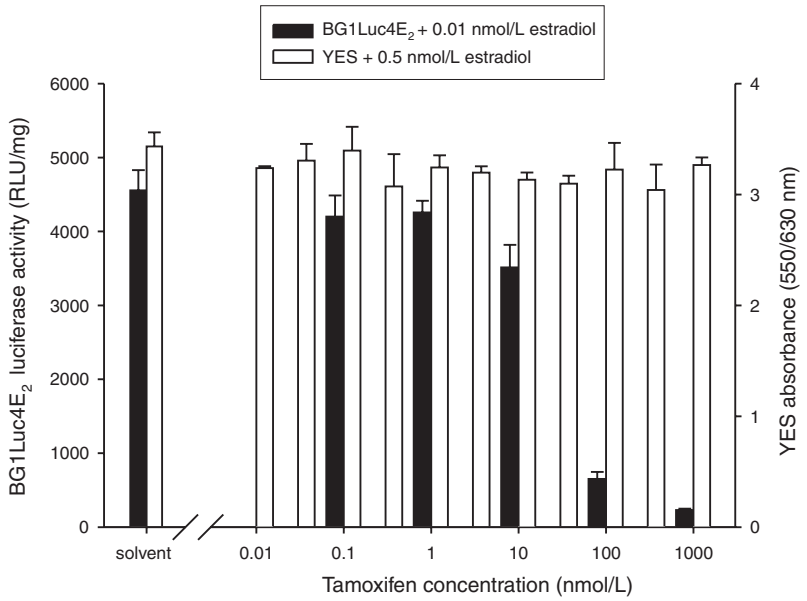


Fig. 8. Concentration–response data for estrogen receptor gene transcription activity of tamoxifen in BG1Luc4E$_2$ cells (solid bars) and recombinant yeast (open bars) co-treated with estradiol. Errors represent standard deviation; $n = 3$ for BG1Luc4E$_2$ cells; $n = 2$ for YES assay.



the extract collected at time 1, the relative potencies were 0.40 for the BG1Luc4E$_2$ cells and 0.63 for the YES assay and for the extract collected at time 2 were 1.03 for the BG1Luc4E$_2$ cells and 1.94 for the YES assay. The estrogen receptor gene transcription assays were also used to obtain concentration–response data for a liquid swine manure extract. Data obtained for this extract are shown in Fig. 7. Relative potencies of the extract were calculated to be 1.03 for the BG1Luc4E$_2$ cells and 1.44 for the YES assay.

To assess whether the BG1Luc4E$_2$ cells or the YES assay could be used to discriminate estrogenic (receptor agonist) and anti-estrogenic (receptor antagonist) activities, cells were treated with a concentration of estradiol that produced sub-maximal receptor binding activities (0.01 nmol/L for BG1Luc4E$_2$ cells, 0.5 nmol/L for YES assay) plus various concentrations of the pharmacological anti-estrogen, tamoxifen. As shown in Fig. 8, tamoxifen effectively inhibited estrogen receptor gene transcription activity in a concentration-dependent

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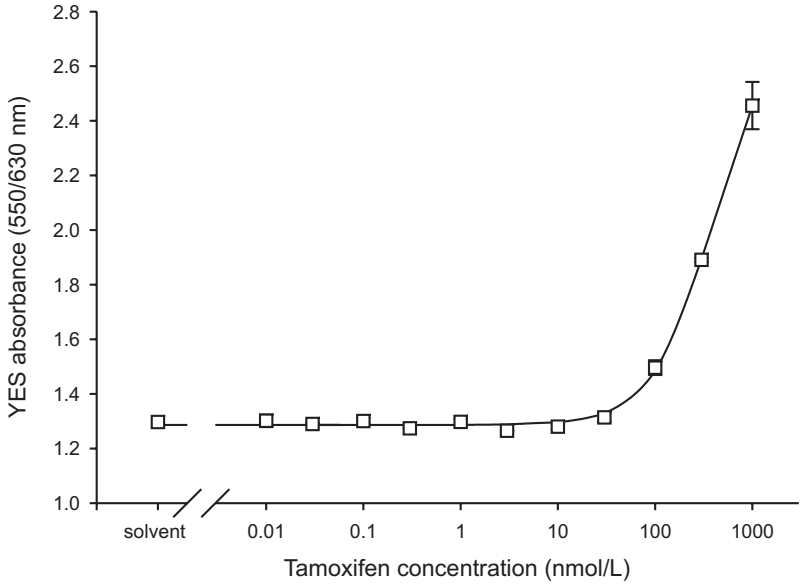
Fig. 9. Concentration–response data for estrogen receptor gene transcription activity of tamoxifen in recombinant yeast. Bars represent standard deviation; $n = 2$.



manner in the BG1Luc4E$_2$ cells, but not in the YES assay. When the recombinant yeast were treated with tamoxifen alone, receptor gene transcription activity was observed (Fig. 9).

Discussion

Using a TIE approach, a wide variety of estrogenic compounds was detected in both animal wastes and municipal biosolids. In general, animal manure was shown to contain both natural estrogens and phytoestrogens derived from animal feeds. The predominate compounds with estrogen receptor gene transcription activities in municipal biosolids were the alkylphenols — particularly nonylphenol and octylphenol.

Based on our findings concerning the soil persistence of these natural and synthetic estrogenic substances a number of general comments can be made. All of the compounds we have studied to date are non-persistent, with half-lives of a few hours or days when soil is incubated at moderate temperature and moisture. Since these compounds are much more stable in sterilized soils, their degradation is apparently microbially-mediated (data not shown). Finally, oxygen greatly enhances degradation — these compounds are much more persistent in anaerobic soils (data not shown).

Several experiments were conducted to assess the utility of two in vitro assays for the determination of concentration–response data for estrogen receptor gene transcription activities of biosolids and liquid swine manure extracts. The first assay, based on an estrogen-responsive recombinant yeast strain developed by Routledge and Sumpter (1996) was for the most part able to provide sufficient concentration–response data to obtain relative potencies for the extracts tested. However, cytotoxicity (cell lysis) was observed for almost all of the higher concentrations of the extracts tested (data not shown). Because cell lysis influenced the absorbance readings, data from any dilutions of the extracts exhibiting lysis were not included in the concentration–response analysis. For some extracts, this resulted in truncated concentration–response curves that did not achieve a response equal to or greater than 20% of that observed for estradiol and relative potencies could not be determined. As cell lysis may be an important endpoint for the assessment of the toxicology of the extracts, efforts are currently underway to more accurately quantitate cytotoxicity in this recombinant yeast strain.

The second estrogen receptor bioassay investigated utilized a recombinant estrogen-responsive human ovarian carcinoma cell line BG1Luc4E$_2$ (Rogers and Denison 2000). The BG1Luc4E$_2$ cells were approximately 10–15 times more sensitive to estradiol and the extracts tested than the recombinant yeast, yet the potencies of the extracts tested relative to estradiol were very similar. No overt cytotoxicity (cell detachment) was observed at the highest concentrations of extracts tested in the BG1Luc4E$_2$ cells. However, due to method differences, the actual concentration of the stock extract and dilutions tested were 50 times lower than those that were tested concurrently in the YES assay.

Previous reports have suggested that known receptor antagonists exhibit "positive" estrogen receptor gene transcription activity in YES assays (reviewed in Gaido et al. 1997). These findings were confirmed in the present study with the pharmacological anti-estrogen tamoxifen. Therefore, the YES assay can only be used to detect all chemicals that bind to and activate the estrogen receptor including both receptor agonists (estrogenic chemicals) and antagonists (anti-estrogenic chemicals). On the other hand, the BG1Luc4E$_2$ cells appear to be able to discriminate receptor agonists and antagonists. When various concentrations of tamoxifen were added to cultures of BG1Luc4E$_2$

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cells that had been pre-treated with estradiol, a reduction in luciferase activity was observed, indicating that tamoxifen was anti-estrogenic. Previous studies have shown that treatment of BG1Luc4E$_2$ cells with tamoxifen alone (3 μmol/L) resulted in no luciferase induction (Rogers and Denison 2000).

Although the BG1Luc4E$_2$ cell assay is more cumbersome than the YES assay, the ability of this cell line to discriminate estrogenic and anti-estrogenic chemicals is important for a complete endocrine activity assessment of agricultural effluents. Further studies utilizing the YES assay as a primary screen to detect estrogen receptor binding compounds in complex matrices, such as amended soil, are underway. Those samples that exhibit receptor binding can then be screened using the BG1Luc4E$_2$ cell assay to determine whether the sample contains estrogenic or anti-estrogenic (or both) activities.

Conclusions

All of the estrogenic substances we have examined to date are rapidly dissipated in aerated soils at temperatures typical of a growing season. We conclude that the risk to water from these chemicals is low, if manures or biosolids are applied appropriately to prevent preferential flow to depth, or runoff to adjacent waters. We would predict that these compounds will be significantly more stable in cooler oxygen-limited environmental matrices such as anaerobic sediments. We are currently further evaluating the hormonal activity of manures and biosolids, and the persistence of this activity in soils, by means of bioassays.

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References

Ashby, J., Houthoff, E., Kennedy, S.J., Stevens, J., Bars, R., Jekat, F.W., Campbell, P., Van Miller, J., Carpanini, F.M., and Randall, G.L.P. 1997. The challenge posed by endocrine-disrupting chemicals. Environ. Health Perspect. **105**: 164–169.

Burnison, B.K., Hartmann, A., Lister, A., Servos, M.R., Ternes,T., and Van Der Kraak, G. 2003. A toxicity identification evaluation approach to studying estrogenic substances in hog manure and agricultural runoff. Environ. Toxciol. Chem. **22**: 2243–2250.

Colucci, M., and Topp, E. 2001. Persistence of estrogenic hormones in agricultural soils II: 17α-Ethynylestradiol. J. Environ. Qual. **30**: 2077–2080.

Colucci, M., and Topp, E. 2002. Dissipation of part per trillion concentrations of estrogenic hormones from agricultural soils. Can. J. Soil Sci. **82**: 335–340.

Colucci, M., Bork, H., and Topp, E. 2001. Persistence of estrogenic hormones in agricultural soils I: 17β-Estradiol and estrone. J. Environ. Qual. **30**: 2070–2076.

Daughton, C.G., and Ternes, T.A. 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ. Health Perspect. **107**: 907–938.

Esperanza, M., Suidan, M.T., Nishimura, F., Wang, Z.-M., and Sorial, G.A. 2004. Determination of sex hormones and nonylphenol ethoxylates in the aqueous matrixes of two pilot-scale municipal wastewater treatment plants. Environ. Sci. Technol. **38**: 3028–3035.

Gaido, K.W., Leonard, L.S., Lovell, S., Gould, J.C., Babai, D., Portier, C.J., and McDonnell, D.P. 1997. Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. Toxicol. Appl. Pharmacol. **143**: 205–212.

Jacobsen, A.-M., Lorenzen, A., Chapman, R., and Topp, E. 2005. Persistence of testosterone and 17β-estradiol in soils receiving swine manure or municipal biosolids. J. Environ. Qual. **34**: 861–871.

Joss, A., Andersen, H., Ternes, T., Richle, P.R., and Siegrist, H. 2004. Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: consequences for plant optimization. Environ. Sci. Technol. **38**: 3047–3055.

Kavlock, R.J., Daston, G.P., DeRosa, C., Fenner-Crisp, P., Gray, L.E., Kaattari, S., Lucier, G., Luster, M., Mac, M.J., Maczka, C., Miller, R., Moore, J., Rolland, R., Scott, G., Sheehan, D.M., Sinks, T., and Tilson, H.A. 1996. Research needs for the risk assessment of health and environmental effects of endocrine disrupters: a report of the US EPA-sponsored workshop. Environ. Health Perspect. **104**: 715–740.

Lech, J.J., Lewis S.K., Ren, L. 1996. In vivo estrogenic activity of nonylphenol in rainbow trout. Fund. Appl. Toxicol. **30**: 229–232.

Lee, H.-B., and Peart, T.E. 2002. Organic contaminants in Canadian municipal sewage sludge. Part I. Toxic or endocrine-disrupting phenolic compounds. Water Qual. Res. J. Can. **37**: 681–696.

Lorenzen, A., Hendel, J.G., Conn, K.L., Bittman, S., Kwabiah, A.B., Lazarovits, G., Masse, D., McAllister, T.A., and Topp, E. 2004. Survey of hormone activities in municipal biosolids and animal manures. Environ. Toxicol. **19**: 216–225.

Nichols, D.J., Daniel, T.C., Moore, P.A. Jr., Edwards, D.R., and Pote, P.H. 1997. Runoff of estrogen hormone 17β-estradiol from poultry litter applied to pasture. J. Environ. Qual. **26**: 1002–1006.

Peterson, E.W., Davis, R.K., and Orndorff, H.A. 2000. 17β-Estradiol as an indicator of animal waste contamination in mantled karst aquifers. J. Environ. Qual. **29**: 826–834.

Purdom, C.E., Hardiman, P.A., Bye, V.J., Eno, N.C., Tyler, C.R., and Sumpter, J.P. 1994. Estrogenic effects of effluents from sewage treatment works. Chem. Ecol. **8**: 275–285.

Rogers, J.M., and Denison, M.S. 2000. Recombinant cell bioassays for endocrine disruptors: development of a stably transfected human ovarian cell line for the detection of estrogenic and anti-estrogenic chemicals. In Vitro Molec. Toxicol. **13**: 67–82.

Routledge, E.J., and Sumpter, J.P. 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environ. Toxicol. Chem. **15**: 241–248.

Routledge, E.J., Sheahan, D., Desbrow, C., Brighty, G., Waldock, M., and Sumpter, J. 1998. Identification of estrogenic chemicals in STW effluent. 2. In vivo responses in trout and roach. Environ. Sci. Technol. **32**: 1559–1565.

Safe, S.H., and Gaido, K. 1998. Phytoestrogens and anthropogenic estrogenic compounds. Environ. Tox. Chem. **17**: 119–126.

Shore, L.S., Correll, D.L., and Chakraborty, P.K. 1995. Relationship of fertilization with chicken manure and concentrations of estrogens in

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small streams. *In* Animal waste and the land-water interface. *Edited by* K.F. Steele. Lewis Publishers, Boca Raton, Fla. pp. 155–162.

Sonnenschein, C., and Soto, A.M. 1998. An updated review of environmental estrogen and androgen mimics and antagonists. J. Steroid Biochem. Molec. Biol. **65**: 143–150.

Ternes, T.A., Stumpf, M., Mueller, J., Haberer, K., Wilken, R.D., and Servos, M. 1999. Behavior and occurrence of estrogens in municipal sewage treatment plants — I. Investigations in Germany, Canada and Brazil. Sci. Total Environ. **225**: 81–90.

Topp, E., and Starratt, A. 2000. Rapid mineralization of the endocrine-disrupting chemical 4-nonylphenol in soil. Environ. Toxicol. Chem. **19**: 313–318.

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