

## Report for 2004AZ51B: Measurement of estrogenic activity in sludges and biosolids

- Conference Proceedings:
  - Arnold, R. Quanrud, D., Ela, W., Conroy, O., Zhang, J., and Leung, C. 2004. Total estrogenic activity and nonylphenol concentration at the Roger Road Wastewater Treatment Plantfates during effluent polishing and implications for water reuse. In: Proceedings, Seventeenth Annual Symposium of the Arizona Hydrological Society. Tucson, AZ. September 15-18, 2004.
  - Quanrud, D., Zhang, J., Teske, S., Dong, H., Orosz-Coghlan, P., Littlehat, P., Conroy, O., Arnold, R., Ela, W., and Lansey, K. 2004. The fate of nonylphenol and total estrogenic activity during wastewater treatment and sludge digestion: a mass balance analysis. In: Proceedings, 4th International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water. Minneapolis, MN. October 13-15, 2004.
  - Teske S., J. Zhang, H. Dong, P. Orosz-Coghlan, D. Quanrud, R. Arnold, W. Ela. 2004. Estrogenic Compounds in Wastewater Treatment. Arizona Water Pollution Control Association 77th Annual Conference, Mesa, Arizona, May 5-7, 2004.
  - Zhang, J., Dong, H., Arnold, R. Ela, W., Quanrud, D., and Lansey, K., 2004. The fate of nonylphenol and total estrogenic activity during sludge treatment processes. In: Proceedings, Seventeenth Annual Symposium of the Arizona Hydrological Society. Tucson, AZ. September 15-18, 2004.
- Dissertations:
  - Teske, S. 2004. Mass flux of total estrogenic activity and nonylphenol for a wastewater treatment plant. Unpublished M.S. Thesis. Chemical and Environmental Engineering. The University of Arizona.

Report Follows

## A. Problem and Research Objectives

There is widespread speculation that exposure to endocrine disrupting compounds (EDCs) in the environment is responsible for recently observed increases in several types of human cancers and worldwide declining sperm levels in men. In fact, the effect of exposure to EDCs on human health is not known with certainty, and relevant epidemiological data is not likely to arise in the near future. A number of organic compounds that are responsible for estrogenic activity in municipal wastewater readily survive conventional wastewater treatment processes (Huang and Sedlak, 2001). They are either discharged to surface waters that serve as effluent receiving waters or they are separated from the aqueous phase onto solid materials that are captured as primary or waste activated sludge. Nonylphenol and several other compounds thought to be responsible for estrogenic activity in wastewater effluent are moderately hydrophobic. Consequently, these compounds should partition, in some measure at least, with sludges derived from wastewater treatment. The extent to which these compounds survive sludge stabilization and dewatering processes is not known. Their fate in biosolids that are used as soil amendments (as in Arizona) is of environmental relevance.

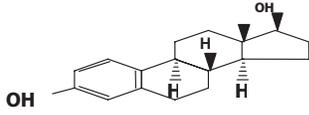
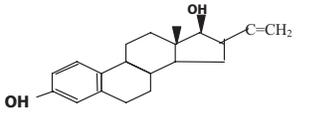
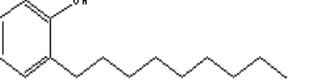
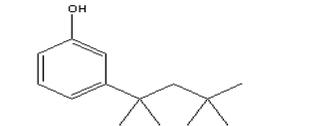
The hydrophobic nature of compounds reputed to contribute to total estrogenic activity in wastewater and wastewater effluent (Table 1) suggests that estrogenic activity is strongly associated with sludges produced during wastewater treatment. That is, the through-plant reduction in total estrogenic activity that typically accompanies the treatment of municipal wastewater (Ternes *et al.*, 1999a,b; Holbrook *et al.*, 2002) is due to not only biochemical destruction of responsible organic compounds but also transfer of the same chemicals to sludges and biosolids. Anaerobic digestion is a widely used sludge treatment process in municipal wastewater treatment plants because of advantages such as low energy consumption, possible production of energy, and reduced sludge volume. However, due to the persistence of estrogenic compounds under anaerobic condition (Ying *et al.*, 2003, 2004; Lee and Liu, 2002), the fate of estrogenic activity during anaerobic sludge digestion is of environmental interest.

Several relatively recent investigations suggest that levels of estrogenic contaminants and estrogenic activity decrease significantly during conventional wastewater treatment. Due to the hydrophobic nature of these compounds, however, it seems likely that most of the difference in estrogenic activity between wastewater treatment plant influent and effluent can be accounted for in biosolids produced via wastewater treatment. Until recently, however, meaningful investigation of mechanisms for estrogen removal during wastewater treatment was impeded by a lack of reliable methods for extracting hydrophobic organics from sludges and biosolids. That is, it was not possible to extract estrogens from biosolids with confidence, making it difficult to assign mechanisms (biodegradation versus phase transfer, etc.) for treatment-related improvements in water quality.

The two goals of this project were to develop methods to extract estrogenic compounds from sewage sludges/biosolids and to then perform a preliminary assessment of the fate of estrogenic activity and nonylphenol content in sludges/biosolids during sludge digestion processes at a few selected full-scale wastewater treatment plants. Nonylphenol

was chosen for analysis because it is an important estrogen mimic that is always present in municipal wastewater.

**Table 1.** Structures and properties of wastewater compounds with estrogenic behavior.

Chemical	Structure	Molecular Weight	Log $K_{ow}$	Relative Estrogenic Activity (YES bioassay)
17 $\beta$ -estradiol (E <sub>2</sub> )		272	3.94	1.0
17 $\alpha$ -ethinyl estradiol (EE <sub>2</sub> )		296	4.15	1.4
Nonylphenol		220	4.48	10 <sup>-4</sup> – 2 × 10 <sup>-4</sup>
Octylphenol		206	4.12	5 × 10 <sup>-4</sup>

A specific objective of this work was to measure total estrogenic activity and nonylphenol mass fluxes during wastewater treatment and solids handling operations at operational wastewater treatment plants. Those results would then be used to support analysis of removal mechanisms for estrogenic compounds during wastewater treatment operations.

The investigation at the wastewater treatment plants focused on the fate of nonylphenol and total estrogenic activity during anaerobic sludge digestion and subsequent dewatering processes. Sludge composting at one plant was also investigated. Total estrogenic activity and nonylphenol mass fluxes across each operation unit at treatment plants were determined.

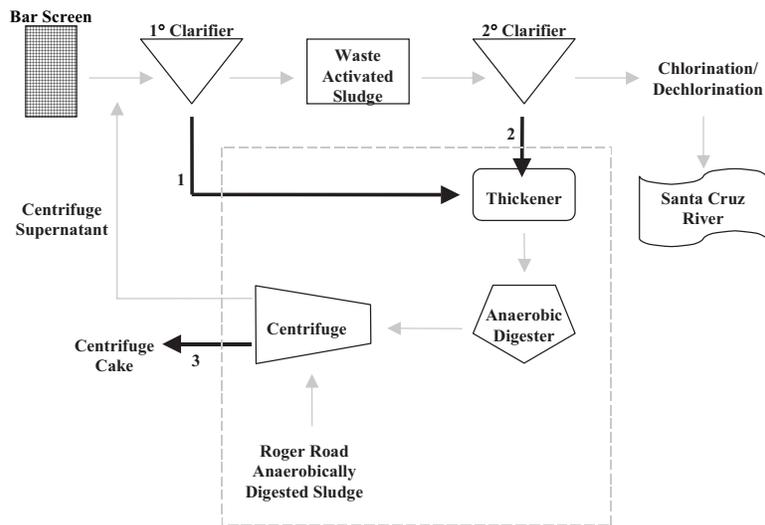
There have been only a few successful efforts to extract estrogenic compounds from soil, sediment, sludge, or biosolids. See, for example, Furbacker *et al.*, (1999); Korner *et al.*, (2000); Matsui *et al.*, (2000); Ternes *et al.*, (2002); and Holbrook *et al.*, (2002). Holbrook *et al.* (2002) extracted raw and digested sludge in pentane, leading to measurement of total estrogenic activities using a reporter-gene assay. Removal of estrogenic activity from raw wastewater via secondary treatment was 55-70 percent. Relatively little of the estrogenic activity lost during secondary treatment, however, was

recoverable from waste activated sludge, suggesting that most of the observed loss of activity was due to biodegradation. This result is at apparent odds with theoretical considerations that suggest hydrophobic estrogenic compounds (Table 1) should partition with organic-rich solids. A more likely explanation is that the extraction procedure used did not produce complete desorption of estrogenic compounds from sludge particles, leading to low recoveries. Both anaerobic and aerobic digestion processes increased the mass of extractable estrogenic compounds detected on residual biosolids. The survival of specific estrogenic contaminants during anaerobic treatment of sludge and sediments has been reported by others (Fauser *et al.*, 2003).

## B. Methodology

### Sampling Sites

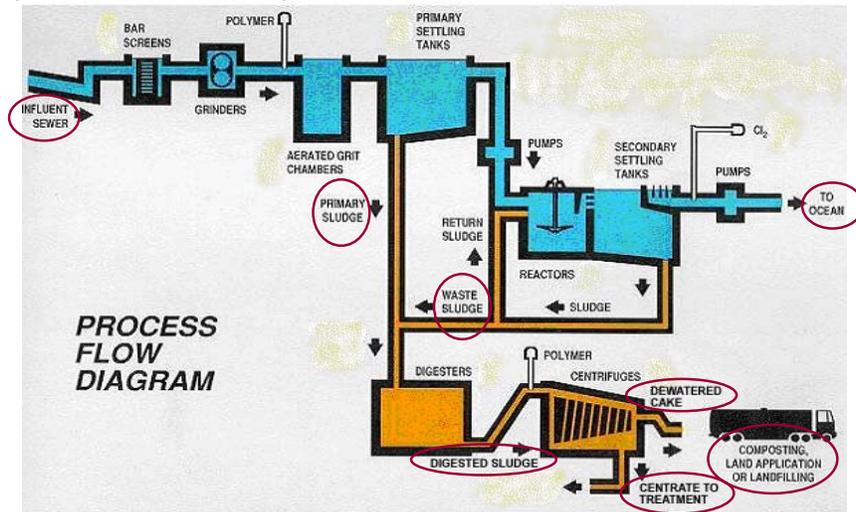
The fate of estrogenic compounds that are separated with sludge was determined by subjecting solids to the same extraction/bioassay procedures before and after sludge digestion, dewatering and composting. This work was carried out using sludges and biosolids produced at the Ina Road Wastewater Pollution Control Facility (IRWPCF) (Pima County, Arizona), Joint Water Pollution Control Plant (Carson, CA), and Hyperion Wastewater Treatment Plant (Los Angeles, CA). Additional data was taken from the Ina Road and JWPCP plants (raw wastewater, primary effluent, secondary effluent) to support a crude through-plant balance on estrogenic activity. Sampling points (numbered as 1, 2, 3) at the Ina Rd WWTP are shown in Figure 1.



**Figure 1.** Simplified schematic of the Ina Road Wastewater Treatment Plant (Tucson, AZ) showing sampling points (numbered as 1, 2, 3).

The JWPCP is a 350 million gallon per day (MGD) municipal wastewater treatment plant owned and operated by the County Sanitation Districts of Los Angeles County (CSDLAC), which serves a heavily industrialized section of Los Angeles County, including a population of about 3.5 million. The Hyperion Treatment Plant receives sewage from a 515 square mile area covering most of the greater Los Angeles area, with a capacity of approximately 450 MGD and a current inflow of about 360 MGD. The sludge digestion processes used at the JWPCP and Hyperion Plants are classified as

mesophilic and thermophilic respectively. Wastewater, solids, etc., samples were taken at the JWPCP points indicated (Figure 2) by CSDLAC personnel and shipped overnight to University of Arizona for analysis.



**Figure 2.** Sampling locations at JWPCP. The circle at lower right represents composted biosolids.

At the Hyperion WWTP, samples of primary and waste activated sludges, a digested blend of primary and waste-activated sludges, and dewatered sludge (four samples total) were collected by plant personnel and shipped overnight to the University of Arizona for analysis. At each plant, one set of grab samples were obtained for analysis within this study. Thus, this project provides only a “snapshot” of sludge digestion performance at each plant.

#### *Laboratory Procedures*

In general, samples were stored at 40° - 60°C for 48 hours to produce a dry residual for extraction and analysis. Water content was determined using subsamples that were dried for 12 hours at 103°C. Influent and effluent liquid samples were passed through a 0.80 µm membrane filter, and filtrate was stored for subsequent analyses of total nonylphenol and total estrogenic activity.

**Sample Extraction.** Organic extracts from sludges/biosolids were obtained using a microwave assisted extraction (MAE) procedure in a CEM MDS-2100 Microwave Digestion System. In general, 0.1 g of the dry solid was extracted in 20 mL of reagent grade methanol using the following program. Pressure was ramped from 0 to 20 psig over five minutes by heating the closed extraction vessel and held constant for 30 minutes. Reactor contents were then allowed to cool for 45 minutes. Liquid-phase subsamples were subsequently taken for further processing leading to analyses of nonylphenol and total estrogenic activity.

Post-extraction sample clean-up steps were designed to separate estrogenic compounds from other organic material that might compromise measurements of total estrogenic activity. Methanol-based extracts were diluted to ~1% methanol in Nanopure water.

Hydrophobic organics in the dilute mixture were then adsorbed on C-18 SPE cartridges. Adsorbed organics were separated via differential elution in a methanol/water gradient that initially varied in volume fraction methanol from 0.2 to 1.0 by increments of 0.2. Only the 0.60 and 0.80 v/v methanol fractions proved to be estrogen. Consequently, a standard protocol was adopted in which 5 mL of 0.2 v/v methanol/water was passed through the C-18 cartridge and discarded. Estrogenic compounds were then eluted in 10 mL of 0.8 v/v methanol and saved. The extracts so obtained were directly analyzed for total extractable nonylphenol via HPLC with fluorescence detector.

The process blank was derived using a blank microwave extraction step (methanol only), dilution of the methanol “extract” in Nanopure water, adsorption on a C-18 cartridge and elution, per above.

The organic separation process for samples that were predominantly liquid (raw wastewater, secondary effluent, centrate from sludge dewatering, etc.) was different. At times, the entire sample in its original form was dried and resuspended in methanol for MAE, etc. Occasionally, samples were filtered, and then applied directly to the C-18 disks without a solids extraction step.

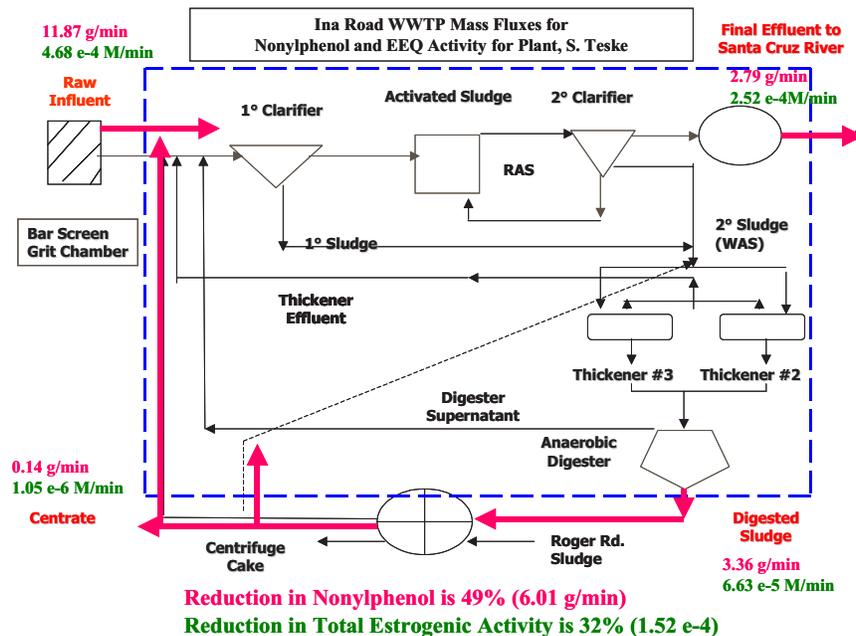
*Nonylphenol Measurement.* Total nonylphenol was determined via high performance liquid chromatography (HPLC) following sample preparation/concentration. Samples in which dry solids comprised a significant fraction of the total mass were dried for 12 hours at 103°C and resuspended in methanol. The ratio of dry sample mass to methanol volume was a function of the expected contaminant mass in the original sample (0.1 g dry weight to 20 ml reagent for most sludge samples). The Hewlett-Packard HPLC-FLD system used for nonylphenol measurement consisted of an autosampler, solvent delivery system, reverse-phase C18 column and a fluorescence detector (1046A). The mobile phase was an acetonitrile (ACN) gradient in ultrapure water and a flow rate of 1mL/min. The ACN gradient program was 0.30 ACN/0.70 water from 0 - 5 min; 0.40 ACN/0.60 water from 5 - 10 min; 0.60 ACN/0.40 water from 10 - 20 min; 0.80 ACN/0.20 water from 20 -25 min; and an isocratic purge from 25 - 30 min after which the eluent composition was returned to 0.30 ACN/0.7 water. The injection volume was 25µL and the excitation and emission wavelengths were 230nm and 305nm.

*Estrogenic Activity Measurement.* Total estrogenic activity in extracts was measured by using a trans-activation reporter gene assay. A portion of each extracted sample was dried and re-dissolved in Nanopure water for measurement of total extractable estrogenic activity using the yeast estrogen screening (YES) protocol of Routledge and Sumpter (1996). The yeast estrogen screen is an *in vitro* transactivation bioassay based on estrogen-dependent synthesis of  $\beta$ -galactosidase by a recombinant strain of *Saccharomyces cerevisiae*. When applied to chemically complex samples, results can be converted to equivalent concentrations of 17 $\alpha$ -ethinyl estradiol (EE<sub>2</sub>) via reference to a suitable EE<sub>2</sub> standard response curve. Positive (EE<sub>2</sub>) and negative controls were run with each sample set. The process blank was derived using a blank microwave extraction step (methanol only), dilution of the methanol ‘extract’ in Nanopure water, adsorption on a C-18 disk and elution, per above.

For the YES protocol, sample organics were ultimately redissolved in the yeast growth medium. For liquid samples, the overall procedure consisting of adsorption, elution, drying and redissolution resulted in nominal concentration factors of 200-500 based on the ratio of initial to final sample volumes. When applied to chemically complex samples such as those encountered here, results can be converted to equivalent concentrations EE<sub>2</sub> via reference to a suitable EE<sub>2</sub> standard response curve. Positive (EE<sub>2</sub>) and negative controls were run with each sample set. The process blank was derived using a blank microwave extraction step (methanol only), dilution of the methanol “extract” in Nanopure water, adsorption on a C-18 cartridge and elution, per above.

### C. Principal Findings and Significance

*Ina Road WWTP (Tucson, AZ).* At the Ina Road WWTP, analyses of samples collected from raw influent, final effluent, digested sludge, and centrate indicated overall reductions of estrogenic activity and nonylphenol of 49% and 32%, respectively (Figure 3).



**Figure 3.** Mass fluxes for nonylphenol and total estrogenic activity at the Ina Rd WWTP, Tucson, AZ.

*JWPCP (Los Angeles County, CA).* Nonylphenol measurements were combined with mass (solids) or volume (liquids) fluxes corresponding to various points at JWPCP as shown (Table 2) to yield daily mass fluxes of nonylphenol at those positions. From the results of the analysis (Figure 4), it is evident that secondary treatment is capable of lowering the flux of nonylphenol from influent to effluent by more than 90 percent (here, 93%). Of the 93 percent through-plant loss, however, more than two-thirds (72%) was accounted for as extractable nonylphenol in the dewatered sludge. Considering both the dewatered cake and effluent as sinks for nonylphenol at JWPCP, all but a fourth of the influent nonylphenol is accounted for, and (net) biotransformation removed at most 26%

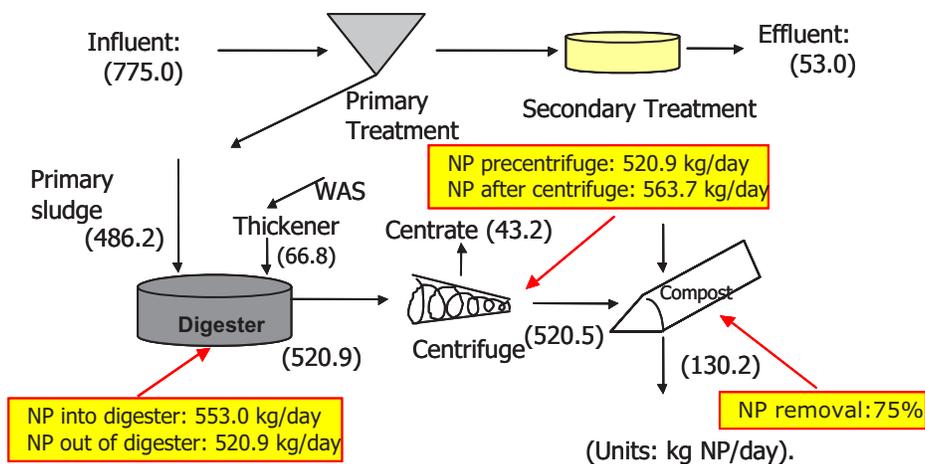
of the influent nonylphenol. There were more circumscribed balances around the anaerobic digester and JWPCP sludge dewatering operations. These show that mesophilic digestion and physical dewatering have a very limited effect on nonylphenol mass. That is, total extractable nonylphenol was essentially unchanged by digestion and centrifugation. A comparison of nonylphenol in primary and waste activated sludges shows that primary sludge accounts for almost 90 percent of the nonylphenol that enters the anaerobic digester. There was little or no loss of extractable nonylphenol during mesophilic anaerobic digestion. As expected, dewatering had little effect on nonylphenol levels or fluxes. A balance around the composting operation, which precedes sale of composted sludge and fertilizer/soil conditioner, suggests that perhaps 75% of the nonylphenol that enters the composting process associated with the dewatered cake is lost, perhaps due to aerobic biochemical activity. This encouraging result should be further examined and verified in future research studies to establish the efficacy of using aerobic decomposition processes, such as composting, for nonylphenol destruction.

**Table 2.** Measurements and calculations leading to mass balance analyses of nonylphenol fate at JWPCP.

Sample Description	Flow Rate or Mass Flux	Water Content (Mass Fraction)	Nonylphenol Concentration	Nonylphenol Flux (kg/day)
Influent	350 MGD	1.0	0.59 mg/L	775
Effluent	350 MGD	1.0	0.04 mg/L	53
Primary sludge	3.5 MGD	0.968	1150 µg/g	486.2
Thickened waste (activated sludge)	1.1 MGD	0.944	286.6 µg/g (dry wt.)	66.8
Digested sludge (pre centrifugation)	4.6 MGD	0.975	1190 µg/g	520.9
Dewatered cake (post centrifugation)	1650 wet tons/day	0.737	1320 µg/g (dry wt.)	520.5
Centrate	4.16 MGD	1.0	2.74 mg/L	43.2
Composted biosolids	550 wet tons/day	0.169	314.0 µg/g (dry wt.)	130.2

Overall, the balance on nonylphenol fluxes at the JWPCP indicates that two-thirds of the nonylphenol that enters the treatment plant leaves with the dewatered cake. Aerobic biodegradation during secondary treatment may remove as much as 25 percent of the influent nonylphenol.

By comparing nonylphenol levels in filtered versus unfiltered influent and effluent samples, about 80 percent of the nonylphenol in the JWPCP influent was associated with particles larger than 0.8 µm. Thus, relatively low nonylphenol levels in the plant effluent (40 µg/L, equivalent to the highest level recorded in the USGS nationwide survey, Kolpin *et al.*, 2002) are more a product of suspended solids removal than of biochemical treatment of nonylphenol.



**Figure 4.** Nonylphenol fluxes throughout JWPCP. Fluxes were calculated based on mass flux or volume rate of flow, water content and NP concentration at the points shown.

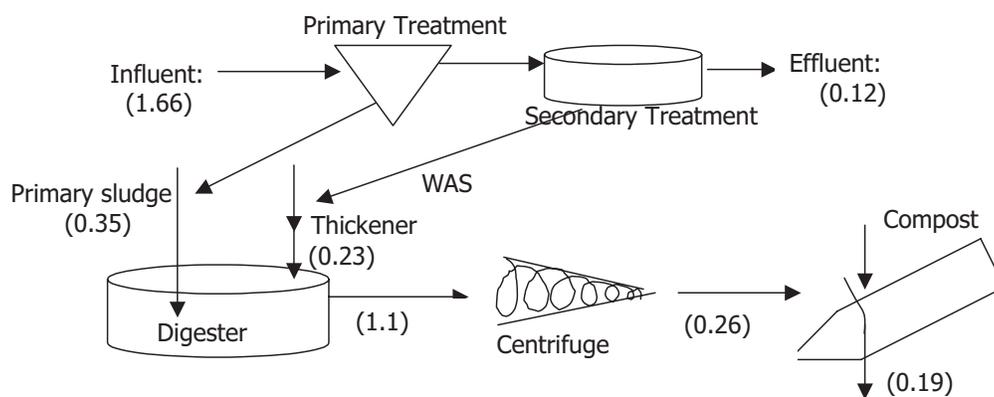
A similar overall picture emerges from total estrogenic activity data at JWPCP (Table 3, Figure 5). That is, the  $EE_2$  equivalent mass fluxes indicated that 93 percent of the total estrogenic activity in the plant influent was missing from JWPCP effluent. The  $EE_2$  equivalent concentration of estrogenic activity in JWPCP effluent was  $8.9 \times 10^{-11}$  M (26.3 ng/L), still much higher than minimum levels known to disrupt estrogen physiology in exposed animals. Again, more than 90 percent of the influent estrogenic activity was associated with particles removed on a 0.8  $\mu$ m filter. In this case, >50 percent of the estrogenic activity lost (based on comparison of JWPCP influent and effluent concentrations) was accounted for in an extract derived from the dewatered sludge. There is some evidence of experimental error in the total extractable estrogenic activity in sludge samples. Estrogenic activity in the dewatered cake was low compared to that of digested sludge before centrifugation. Nevertheless, it is probable that the fraction of total estrogenic activity lost to biochemical processes ( $\leq 45$  percent based on Figure 5 data) was larger than the fraction of nonylphenol biodegraded ( $\leq 26$  percent). The balances on total estrogenic activity around anaerobic digestion, dewatering and composting were suspect, probably due to error that is essentially unavoidable in the YES assay. The flux of estrogenic activity out of the digester seems particularly high. As a consequence, it was not possible in this limited study to estimate the efficiencies of individual unit operations (anaerobic digestion, dewatering and composting) for removal of total estrogenic activity.

It may be significant that the total estrogenic activity in sludges entering the digester was just half the activity measured in the digested solids and three-fourths of the activity in the dewatered cake. While this at first seems incongruous and perhaps a consequence of error in sampling, extraction or application of the YES bioassay, the increase in estrogenic activity through the digester may also result from destruction of anti-estrogenic compounds during anaerobic digestion. That is, a primary source of anti-estrogenic activity in the YES bioassay consists of compounds that bind to hER $\alpha$  (human

estrogen receptor) without stimulating synthesis of  $\beta$ -galactosidase. A number of natural and synthetic organics have this property. If anti-estrogens are removed or transformed to some extent during anaerobic digestion, those reactions would produce an apparent increase in estrogenic activity, as competition for hER $\alpha$  by anti-estrogen decreased. Other explanations are possible, however.

**Table 3.** Measurements and calculations for mass balance analysis of total estrogenic activity at JWPCP.

Sample Description	Total estrogenic activity (equivalent EE <sub>2</sub> concentration)	Flux of estrogenic activity (mol EE <sub>2</sub> /day)
Influent	1.25 nM	1.66
Effluent	0.089 nM	0.12
Primary sludge	0.8 nMol/g dry wt.	0.34
Thickened WAS	1.0 nMol/g dry wt.	0.23
Digested sludge (precentrifugation)	2.5 nMol EE <sub>2</sub> /g dry wt.	1.1
Dewatered cake (post centrifugation)	2.0 nMol EE <sub>2</sub> /g dry wt.	0.79
Centrate	no data	—
Composted biosolids	0.15 nMol EE <sub>2</sub> /g dry wt.	0.062



(Units: mols of EE<sub>2</sub>/day)

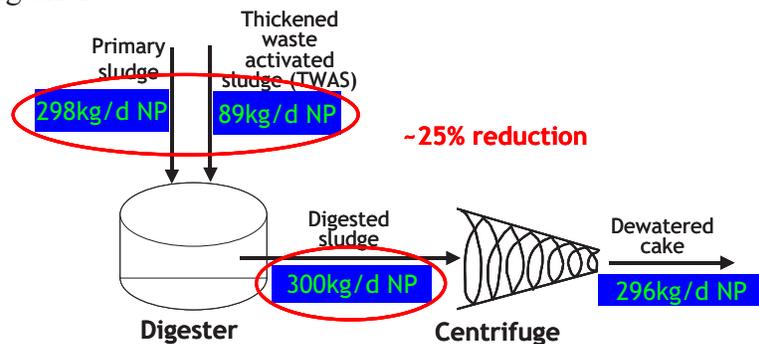
**Figure 5.** Total estrogenic activity flux at different stages of treatment at JWPCP. Total estrogenic activity fluxes were calculated based on flow rate, water content and estrogenic activity as mol EE<sub>2</sub> equivalents/day at each position shown.

The contribution of nonylphenol to total estrogenic activity is also of some interest. YES bioassays with pure 17 $\alpha$ -ethinyl estradiol and a mixture of nonylphenol isomers indicated that EE<sub>2</sub> is 5000 – 10,000 times more estrogenic than nonylphenol (data not shown). The difference in potency is compensated, at least in part, by the relatively large expected concentration of nonylphenol in wastewater and wastewater effluent. Thus, by expressing total estrogenic activity in terms of an equivalent EE<sub>2</sub> concentration, it is

possible to speculate on the contribution of nonylphenol to the YES bioassay response. Here we applied a factor of 1/7500 to convert nonylphenol measurements to EE<sub>2</sub>-equivalent concentrations. In the JWPCP influent, for example, the nonylphenol concentration was  $5.90 \times 10^5$  ng/L, for an EE<sub>2</sub>-equivalent concentration of 79 ng/L. Total estrogenic activity in the same sample, expressed as an EE<sub>2</sub>-equivalent concentration, was 370 ng/L, so that the measured nonylphenol concentration accounted for just over 20 percent of the total estrogenic activity. In the plant effluent, nonylphenol accounted for just 2 percent of the total estrogenic activity. Results suggest that nonylphenol is removed with greater efficiency than other components of estrogenic activity during conventional wastewater treatment, perhaps because the affinity of nonylphenol for organic-rich solids is greater than those of most other estrogens and estrogen mimics. The analysis ignores the possibility of synergy or antagonism among compounds contributing to total estrogen activity and, consequently, should be considered cautiously.

The nonylphenol concentration was estimated at 1300 µg/g in the dried dewatered cake, and the total extractable estrogenic activity was 600 ng EE<sub>2</sub>/g in the same sample. Consequently, nonylphenol accounted for perhaps 30 percent of the extractable estrogenic activity in the dewatered cake. Evidently, nonylphenol is an important component of estrogenic activity in the JWPCP wastewater and solid products derived from its treatment.

*Hyperion WWTP (Los Angeles, CA).* At the Hyperion Treatment Plant in Los Angeles, the one-time sampling effort indicated that there is little loss of estrogenic activity during thermophilic sludge digestion and subsequent dewatering operations (Figure 6; Table 4). The daily mass flux values obtained for total nonylphenol and estrogenic activity suggest that thermophilic sludge digestion probably offers little advantage in terms of estrogen and particularly nonylphenol destruction. Nonylphenol concentrations were probably not affected by the digestion process. The through-digestion increase in total nonylphenol could have arisen from the nature of the experimental design (one-time grab samples), or from conversion of ethoxylated nonylphenol forms to nonylphenol during digestion. Error introduced by sample preparation steps is also a possibility, although no such error was evident in the JWPCP samples reported above. A modest reduction in total estrogenic activity is apparent in the data, and this could be real. Additional data collection is warranted to confirm this result before it is accepted on the basis of a one-time monitoring effort.



**Figure 6.** Total daily flux of nonylphenol (NP) during sludge digestion processes at the Hyperion wastewater treatment plant, City of Los Angeles, CA.

**Table 4.** Measurements and calculations leading to mass balance analyses of nonylphenol and total estrogenic activity at Hyperion WWTP.

Sample Description	Flow Rate or Mass Flux	Water Content (Mass Fraction)	Nonylphenol Concentration ( $\mu\text{g/g}$ dry sludge)	Nonylphenol Flux (kg/day)
<i>Hyperion Treatment Plant</i>				
Primary sludge	2.17MGD	0.961	915	298.4
Thickened waste activated sludge	0.93MGD	0.965	715	88.8
Digested sludge	3.1MGD	0.980	1289	299.5
Dewatered cake	800 tons/day	0.683	1286	296.1
		Total estrogenic activity (ng EE2 Equivalent per g dry sludge)		Flux of estrogenic activity (g/day)
Primary sludge		223		72.6
Thickened waste activated sludge		465		57.8
Digested sludge		620		144
Dewatered cake		544		125

## Publication Information

### *Dissertations*

Teske, S. 2004. Mass flux of total estrogenic activity and nonylphenol for a wastewater treatment plant. *Unpublished M.S. Thesis*. Chemical and Environmental Engineering. The University of Arizona.

### *Conference Proceedings*

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