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Efficacy of an Advanced Sewage Treatment Plant in Southeast Queensland, Australia, to Remove Estrogenic Chemicals

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The estrogenicity profile of domestic sewage during treatment at a medium-sized (3800 EP) advanced biological nutrient removal plant in Queensland, Australia, was characterized using a sheep estrogen receptor binding assay (ERBA) and the MCF-7 breast cancer cell proliferation assay (E-Screen). The raw influent was highly estrogenic (20−54 ng/L EEq), and primary treatment resulted in a slight increase in estrogenicity that was detected in one of the assays (6−80 ng/L). Concurrent chemical analysis suggested that most of the estrogenicity in the influent was due to natural hormones (>48%). Secondary activated sludge treatment followed by nitrification/denitrification effectively removed >95% of the estrogenic activity (to <0.75−2.6 ng/L), and estrogenicity of the final tertiary-treated effluent was below the detection limit of both assays (<0.75 ng/L).

Introduction

Fish exposed to treated sewage have been shown to exhibit reproductive abnormalities consistent with estrogenic endocrine disruption (*1*). Several studies have identified the natural steroids 17β-estradiol (E_2), estrone (E_1), and the synthetic estrogen ethynylestradiol (EE_2) as the most potent estrogenic compounds in treated municipal sewage (*2*, *3*). Laboratory exposures have confirmed these chemicals to be estrogenic to fish in vivo at very low (ppt) concentrations, with effects similar to those observed in the field (*4*−*7*). The main source of these chemicals in domestic sewage is from human waste (*8*, *9*). As hormones are naturally excreted, the focus of a strategy to manage the potential environmental impact of sewage must shift to identifying and implementing effective treatment technologies to remove these chemicals effectively before discharge into the environment.

Studies have shown that secondary treatment of sewage, and in particular activated sludge treatment, is very effective at removing estrogens (*10*−*14*). A German municipal sewage treatment plant (STP) removed over 98% of the natural estrogens (E_1 and E_2) and more than 90% of EE_2, mostly during activated sludge treatment (*15*). In experiments with activated sludge from STPs, E_2 was quickly converted into E_1, which was then slowly degraded (*16*, *17*). In STPs in the United States, Layton et al. (*18*) showed that 70−80% of E_2 was degraded within 24 h through mineralization and 10−20% was sorbed to biosolids, for a total removal of E_2 from the aqueous phase of over 90%. However, in aerobic batch experiments with activated sludge, EE_2 was not significantly degraded after 48 h (*16*). Only a small fraction (20%) was degraded by activated sludge, but removal from the aqueous phase was still about 80% because of high sorption to the biosludge (*18*). Joss et al. (*19*) reported significant and rapid degradation of E_2 and E_1 in both anaerobic and aerobic conditions (albeit at a faster rate in the latter), while EE_2 was only significantly degraded in aerobic conditions.

Because of the broad socioeconomic differences between Australia and Europe or North America, data generated abroad must be confirmed locally. In 2004, for example, agriculture was a more important part of the economic landscape in Australia (3.5% of the GDP) than in the United Kingdom (0.9%), Germany (1%), the United States (1.4%), or even France (2.7%) (*20*). Social factors can also have an impact on the presence of hormones in the environment, with much higher use of the pill as a form of contraceptive by women of reproductive age in Western Europe (48.2%) than in North America (15.5%) or Australia (24.0%) (*21*). There are also climatic differences, for example, the monthly average temperature in Brisbane in the four seasons prior to sampling was similar to that of Houston, Texas, and much warmer than other selected European and North American cities (Table 1). Layton et al. (*18*) showed that temperature can significantly affect the rate of degradation of E_2 and EE_2 in activated sludge, and these differences could significantly affect the presence and degradation of hormones in sewage in Queensland, Australia. To the authors' knowledge, there are to date no other studies on the estrogenicity profile of sewage during treatment in Australia. The present study used chemical extraction methods followed by two different bioassays to examine the estrogenic profile of sewage along the treatment train at an advanced STP with an activated sludge system in subtropical Australia. The main purpose was to determine the efficacy of each step of the treatment train at removing estrogenic compounds.

Experimental Section

Site Description. The Landsborough STP operated by CalAqua (Caloundra City Council) treats approximately 0.98 ML of raw sewage per day (3800 equivalent people) from the town of Landsborough in southeast Queensland, Australia, >95% of which is from domestic sources. It is an advanced tertiary treatment facility originally designed for biological and nutrient removal (BNR) and has been the focus of a recent study where a suite of suspected EDCs (such as pesticides, herbicides, PCBs, lead, mercury, cadmium, E_2, E_1, and EE_2) were measured by chemical analysis (*23*). Primary treatment consists of a screen and a grit and grease chamber (Figure 1). Secondary treatment includes anaerobic selectors, a large sequencing batch reactor (bioreactor) followed by nitrification/denitrification, and a secondary clarifier. The

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TABLE 1. Mean Monthly Air Temperature (°C) in the Four Seasons Prior to Sampling[a]

city	June 1999[b]	Oct 1999	Jan 2000	April 2000
Brisbane, Australia	15.2	20.5	23.6	21.0
Brussels, Belgium	19.7	10.7	4.0	10.5
Berlin, Germany	21.4	10.1	1.6	12.1
Nice, France	24.3	16.9	7.3	13.3
Manchester, U.K.	17.9	11.1	5.2	8.3
San Francisco, CA	16.7	17	11.5	14.5
Houston, TX	28.4	22.6	14.9	21.7
Toronto, Canada	24.3	9.4	−5.8	6.7

[a] Data from GHCN (Global Historical Climatology Network) v2 (*22*). [b] Seasons are reversed in the southern hemisphere, so that June corresponds to the peak of winter and Jan to summer.

sludge is returned from the secondary clarifier to the anaerobic selectors, while the aqueous phase flows to tertiary treatment. Average sludge retention time at the time of sampling was 25.8 days. Tertiary treatment includes sand filters, ozone contact tanks, bioactivated carbon filters, and UV disinfection banks.

Sample Collection and Extraction. Samples were collected at seven different stages of the sewage treatment train (Figure 1, labeled A−G) on August 21, 2000 at 0900 h. In the 48 h prior to sampling, there was no rain in the sampling area and the minimum and maximum air temperature were 9−25 °C (data courtesy of the Commonwealth Bureau of Meteorology). The water temperature at time of sampling was 19 °C. Duplicates of each sample were collected in methanol-rinsed 1-L glass Schott bottles and were kept on ice until extraction within 24 h of collection. The solid-phase extraction (SPE) protocol was based on the method of Lee and Peart (*24*). Before extraction, each sample was vacuum filtered through a Whatmann filter with a pore size of 1.2 μm. Polypropylene cartridges with 1 g of end-capped octadecyl reversed-phase sorbent and 6 mL reservoir (International Sorbent Technology no. 221-0100-C) were used to extract organic contaminants from the aqueous phase. The cartridges were loaded on an SPE manifold and preconditioned with 5 mL acetone, 5 mL methanol, and 10 mL double-distilled water. The filtered sample (1 L) was applied to the SPE cartridge under −65 kPa vacuum, at approximately 10 mL/min. When extraction was complete, the cartridges were dried on the manifold for 5 min, rinsed with 2 × 5 mL acetone/water (1:4, v/v), wrapped in aluminum foil, and stored at −20 °C. Recovery efficiency for E_2, determined by spiking 1 L of water with 12 ng of E_2, was 75%. Blanks were made from distilled water and put through the same manipulation as the samples.

Estrogen Receptor Binding Assay (ERBA). For analysis in the ERBA, each SPE cartridge was eluted with 2 × 2.5 mL acetone (polar fraction), 2 × 2.5 mL diethyl ether (midpolar fraction), and 2 × 2.5 mL *n*-hexane (nonpolar fraction), under low vacuum. The solvent in each fraction was evaporated under gentle nitrogen stream, and the samples were reconstituted in 100 μL dimethyl sulfoxide (DMSO).

The ERBA protocol has been described previously (*25*). Briefly, uteri were excised from adult ewes, trimmed of fat, and homogenized in TEDG buffer (10 mM Tris, 2 mM EDTA, 10% glycerol, pH to 7.2 at 4 °C; 1 mM dithiothreitol immediately before use). The homogenate was centrifuged at 1000*g* for 10 min at 4 °C. The pellet was discarded and the supernatant was centrifuged at 105 000*g* for 50 min at 4 °C. The final supernatant, containing the unoccupied cytosolic receptors, was aliquoted into 2-mL polypropelene eppendorf tubes and snap-frozen in liquid nitrogen. The aliquots were stored at −80 °C. Typical results for sheep ER exhibited kinetics linked to limited capacity (B_{max} = 570 ± 150 fmol/mg) and high affinity (K_d = 0.17 ± 0.01 nM). For the ERBA, serial dilutions of the samples were incubated with a known concentration of radiolabeled 17β-estradiol ([2,4,6,7-^{3}H]E_2; $^3HE_2$; Amersham Pharmacia Biotech NZ, Auckland, New Zealand) and a fixed amount of receptors (standardized between preparations by the number of binding sites B_{max}) for 18 h at 4 °C. At the end of the incubation period, free $^3HE_2$ was stripped from the incubation medium by addition of dextran-coated charcoal (DCC; 0.5% w/v charcoal, 0.05% w/v dextan T70, in TEDG buffer) and the remaining bound $^3HE_2$ was measured by liquid scintillation. The EC_{50} (or in the case of a water sample the EV_{50}, the equivalent volume of sample needed to displace $^3HE_2$ from half the receptor binding sites) was determined by least-squares regression of a Verhulst curve (y = bottom + (top − bottom)/(1 + 10∧((log EC_{50} − log X)slope))) with a VBA6 module for Excel9 written by F. Leusch.

The estrogenicity of the samples was expressed as estradiol equivalents (EEq), or the equivalent concentration of E_2 that would have to be present in the sample to achieve a response of the same amplitude. EEq is calculated as the ratio between the amount of E_2 in the incubation tube at EC_{50} in the standard curve and the equivalent volume at EC_{50} (EV_{50}). With 1 L of sample, the method detection limit (MDL) was 0.75 ng/L.

MCF-7 Breast Cancer Cell Proliferation Assay (E-Screen). For the E-Screen, organics were eluted from the cartridges with 2 × 2.5 mL acetone. For samples A and D, a second elution was performed with 2 × 2.5 mL ethyl acetate and was analyzed separately in the E-Screen assay to check for completeness of elution. For sample A, 5.5% of the estrogenic activity of the first eluate (expressed in EEq) was found in the second eluate, while no estrogenicity was detectable in the second eluate of sample D. Fifty microliters of DMSO was added to each extract, and the solvent evaporated completely under a gentle stream of nitrogen. Stock solutions of the extracts were prepared with steroid-free experimental medium [phenol red-free Dulbecco's modification of Eagle's medium (DMEM) supplemented with 5% charcoal-dextran treated fetal calf serum (CD-FCS), 10 mM HEPES buffer, 2 mM L-glutamine, 1% of a solution of nonessential amino acids (NEAA), and 1% of a penicillin/streptomycin/amphotericin solution]. The preparation of the medium is described in detail elsewhere (*26*). Experimental medium (4.95 mL) was added to each sample and was homogenized for 1 min, and the clear solution was filtered sterile through a 0.22-μm Millex-GS filter (Millipore, France). These stock solutions containing 1% (v/v) DMSO were stored in sterile 5-mL glass flasks at 4 °C. For cell culture testing, aliquots of the stocks were diluted 10- to 2000 fold (0.05−10 L final volume assuming that the whole extract is diluted) with steroid-free experimental medium using sterile 15-mL polypropylene vials (Sarstedt, Germany). The maximum solvent concentration in the culture medium did not exceed 0.1%, a concentration that did not affect cell proliferation.

The assay was based on Körner et al. (*27*) with minor modifications. Estrogen receptor-positive human MCF-7 breast cancer cells were cultivated in 25 cm^2 flasks (Sarstedt) in DMEM with 15 mg/L phenol red and 2 mM *N*-acetyl-L-alanyl-L-glutamine (Biochrom, Germany) at 37 °C in a water-saturated atmosphere of 5% CO_2/95% air. The culture medium was supplemented with 5% FCS, 1% of NEAA, and 1% of penicillin/streptomycin/amphotericin. Subconfluent MCF-7 cells were trypsinized, washed with culture medium, and resuspendend in steroid-free experimental medium.

Cells were seeded into 96-well plates (Sarstedt) in 75 μL of experimental medium at a density of 1500 cells per well. After 24 h, 75 μL of experimental medium was added to each well, containing a series of dilutions of the stock solutions of sewage extracts. Each dilution was tested in eight replicates per assay. Eight wells per assay without hormones acted as negative control. E_2 in five final concentrations between 10^{-12}

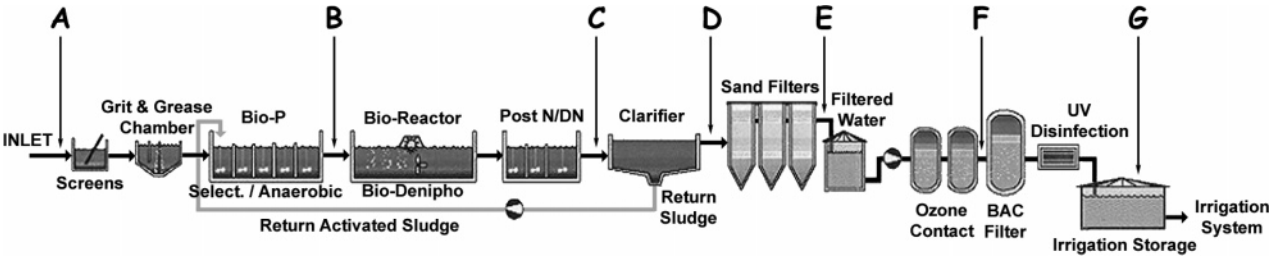

FIGURE 1. Treatment train at the Landsborough sewage treatment plant, including sample collection points: (A) raw; (B) post anaerobic selectors; (C) post nitrification/denitrification; (D) final secondary treated; (E) post sand filtration; (F) post ozonation; (G) final tertiary treated.

M and 10^{-9} M was the internal positive control in each assay. Previous experiments have shown that higher E2 concentrations do not induce a higher proliferative response (*26*). Five days later (i.e., day 6), the assay was terminated during the late exponential phase of proliferation, and the cell number in each well was determined by measuring total protein content using the sulforhodamine B (SRB) assay (*28*). In brief, cells were fixed with cold 10% (w/v) trichloroacetic acid, stained with 0.4% (w/v) solution of sulforhodamine B in 1% acetic acid, washed, and dried. The dye was dissolved in 100 μL cold 10 mM Tris buffer (pH 10.5) per well and the extinction at 550 nm (ref 630 nm) was measured in a microplate reader (Dynex MR 1200). The extinction of SRB at 550 nm is directly proportional to the cell number within a wide range (*28*, *29*).

The end point of the E-Screen assay is the cell number relative to the hormone free control. The proliferative effect (PE) of a sample is the ratio of the highest cell number achieved with the sample to the cell number of the negative control. The estrogenic activities of sewage samples were evaluated quantitatively by determining the relative proliferative effect (RPE) and the estradiol equivalent concentration (EEq). RPE is a measure of relative estrogenic efficacy and compares the maximum proliferation induced by a sample with that induced by the positive control E_2 at 1 nM. This enables the distinction of full agonistic activity (RPE = 80−100%) from partial agonistic activity (RPE < 80%) (*30*). The EEq is the total amount of estrogenic active compounds in a sample normalized to E_2 and is computed as described above. EEq values for sewage samples were calculated irrespective of whether a full response was obtained or not. PE, RPE, EC_{50}, and EEQ values of sewage samples were calculated for each individual experiment. The log-probit regression analysis and calculation of EC_{50} values were done with a VBA module for Excel5 written by Josef Greve (Fraunhofer Institute of Environmental Chemistry and Ecotoxicology, Schmallenberg, Germany).

Chemical Analysis (GC/MS). GC/MS analyses were carried out by the Australian Government Analytical Laboratory (AGAL; Pymble, NSW, Australia). Analytical methods to measure the levels of E_2, E_1, and EE_2 were developed by AGAL on the basis of the methods of Lee and Peart (*24*). Briefly, samples were extracted by SPE as described above and derivatized with PFPA followed by GC/MS−SIM analysis. The limit of reporting was 5 ng/L for all three chemicals.

Results

Simple Fractionation. In the ERBA, none of the midpolar and nonpolar eluates (eluted with diethyl ether and *n*-hexane, respectively) significantly displaced E_2 from the ER binding site (data not shown). All the estrogenic activity of the samples was contained in the polar fraction (eluted with acetone), and the EEq was calculated from those polar eluates. Likewise with the E-Screen, almost all estrogenic activity (94−100%) of the sample was eluted with acetone.

Estrogenicity Profile Along the Treatment Train. Estrogenicity of the samples (expressed as EEq) during the treatment train is shown in Figure 2. The two bioassays

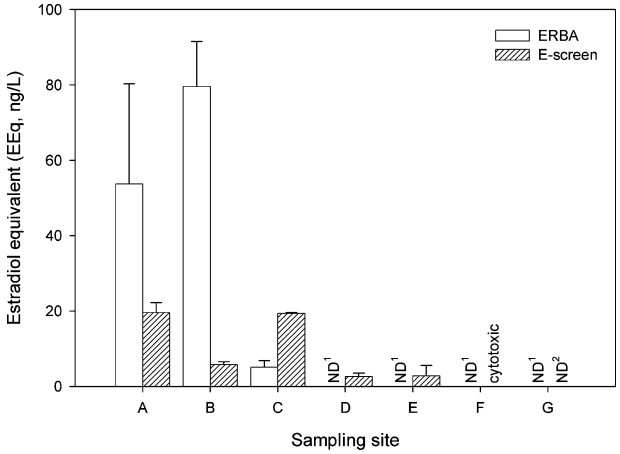

FIGURE 2. Estradiol equivalents (EEq ± SE) of the samples determined by ERBA (open bars) and E-Screen (hatched bars) at each of the sampling points defined in Figure 1. Method detection limit for the ERBA (ND^1) was 0.75 ng/L. Method detection limit for the E-Screen (ND^2) was 0.03 ng/L.

TABLE 2. Estrogenic Activity Relative to 17β-Estradiol (Relative Proliferative Effect, RPE) of the Samples in the E-Screen. Each Value Represents Mean ± SD of Two Independent Assays

sample	RPE (%)	interpretation
A	86 ± 14	full agonism
B	73 ± 10	partial agonism
C	35 ± 9.3	partial agonism
D	28 ± 0.3	partial agonism
E	46 ± 5.8	partial agonism
F	N.A.[a]	N.A.
G	N. A.	N. A.

[a] N. A. = not active.

yielded somewhat different results, although both showed a significant decrease of EEq during treatment and could not detect estrogenic chemicals in the final treated effluent (Figure 2).

With the ERBA (Figure 2, open bars), EEq in the influent was 54 ± 27 ng/L. This level increased to 80 ± 12 ng/L after primary settling and anaerobic selection (Figure 2, sample B) but decreased to 5.1 ± 1.7 ng/L after aerobic sludge treatment and nitrification/denitrification (Figure 2, sample C). After secondary settling, the level dropped below detection limit (<0.75 ng/L) (Figure 2, samples D−G).

With the E-Screen (Figure 2, hatched bars), EEq in the influent was 20 ± 2.7 ng/L (Figure 2, sample A) of a fully agonistic E_2 mimic (RPE > 80%, Table 2, sample A). Following primary settling and anaerobic selection, EEq decreased by 72% to 5.8 ± 0.8 ng/L (Figure 2, sample B) but later increased to 19 ± 0.2 ng/L after aerobic sludge treatment and nitrification/denitrification (Figure 2, sample C). The estrogenic chemicals were, however, only weakly agonistic (RPE

TABLE 3. Octanol−Water Partition Coefficient (K_{OW}) and Relative Potency of Selected Estrogens and Estrogen Mimics Compared to 17β-Estradiol in the Sheep ERBA and the E-Screen[a]

compound	CAS-RN[b]	log K_{OW}	ERBA RBA	E-Screen RP
17β-estradiol (E_2)	50-28-2	3.94[c]	1.0	1.0[f]
estrone (E_1)	53-16-7	3.43[c]	0.15	0.096[f]
ethynylestradiol (EE_2)	57-63-6	4.15[c]	1.9	0.91[f]
p-nonylphenol	84852-15-3	4.48[d]	0.000 074	0.000 076[f]
4-*t*-octylphenol	140-66-9	4.12[d]	0.000 34	0.000 076[f]
bisphenol A	80-05-7	3.32[e]	0.0023	0.000 053[f]

[a] For the ERBA, relative binding affinity (RBA) is calculated as $[E2]_{EC50}/[compound]_{EC50}$. For the E-Screen, relative potency (RP) is calculated as $[E2]_{EC50}/[compound]_{EC50}$. [b] CAS−RN = Chemical Abstracts Service Registry Number. [c] Lai et al. (*35*). [d] Ahel and Giger (*36*). [e] Ying et al. (*37*). [f] Körner et al. (*27*).

TABLE 4. Concentration of 17β-Estradiol (E_2) and Estrone (E_1) in Selected Samples Determined by GC/MS and Predicted EEq Evaluated from the ERBA and E-Screen

sample	E2 (ng/L)	E1 (ng/L)	predicted ERBA EEq (ng/L) [% actual]	predicted E-Screen EEq (ng/L) [% actual]
A	19	45	25.8 [31−95%]	23.3 [102−134%]
D	<LOR[a]	<LOR[a]		
G	<LOR[a]	<LOR[a]		

[a] LOR = limit of reporting, 5 ng/L.

< 50%, Table 2, samples C−E). After secondary settling, EEq level dropped markedly to 2.6 ± 0.9 ng/L (Figure 2, sample D) and remained unchanged following sand filtration (Figure 2, sample E). The effluent from the ozone contact tanks was cytotoxic to MCF-7 cells up to a dilution volume of 0.5 L, revealing cell counts significantly lower than the hormone-free negative control (Figure 2, sample F), and thus EEq could not be determined for that sample. The level of EEq in UV-treated effluent was below the limit of detection of 0.03 ng/L (Figure 2, sample G).

The relative potencies of E_2, E_1, and EE_2 relative to E_2 in the sheep ERBA and the E-screen are presented in Table 3.

EE_2 was not detectable by GC/MS in any of the three selected samples for chemical analysis (data not shown, limit of reporting of 5 ng/L). E_2 and E_1 in the influent were 19 and 45 ng/L, respectively (Table 4, sample A), and were below the limit of reporting (LOR) after secondary settling (<5 ng/L, Table 4, samples D−G).

Discussion

Raw Sewage. The concentrations of estrogenic compounds present in raw sewage at the Landsborough STP were comparable to those reported by previous studies for municipal sewage treatment plants abroad (*2*, *8*). As in previous studies, most of the activity was associated with the most polar fraction, where compounds such as E_2, E_1, and EE_2 are found (*2*, *3*). In municipal sewage, these compounds originate from human excretion and are therefore present wherever humans are. With the E-Screen, the predicted EEq from the natural hormones E_2 and E_1 alone was 102−134% of the actual EEq of raw sewage (Table 4). With the ERBA, they accounted for 31−95% of the activity (Table 4; the wide spread is due to the large amount of variability in estrogenicity of sample A in the ERBA). The difference between the two assays suggests the presence of other chemicals that bind to the ER but are not potent inducers of estrogenic effects (such as nonylphenol, bisphenol A, and octylphenol; Table 3) which could account for the remainder of the activity measured in the bioassay.

At the highest concentration tested, chemicals in the raw sewage interfered with the ER binding assay. It is postulated that the high lipid content in raw sewage may have artificially increased nonspecific binding at the highest concentration, an effect that disappeared after dilution of the sample. This interference with the assay resulted in more variability for the EEq levels in the influent than for other samples (Figure 2, sample A).

Primary and Secondary Treatment. The slight increase in EEq levels measured with the ERBA and the concomitant decrease in EEq in the E-screen after primary treatment and anaerobic sludge treatment (Figure 2, sample B) suggest either the formation of a less potent chemical with a higher affinity for the ER or the presence of large quantities of a much less potent chemical with less affinity for the ER. A combination of reactivation of steroid estrogens by cleavage of the glucuronide conjugates during primary treatment (*16*) with the degradation of E_2 into E_1 early in activated sludge treatment (*31*) could be one of the explanations for this phenomenon.

Aerobic sludge treatment and post nitrification/denitrification decreased EEq levels measured by the ERBA by 96% (Figure 2, open bars, sample C). At the same time, EEq levels in the E-screen increased appreciably, but only with partial agonistic effects (RPE = 35%; Table 2). The combination of low binding to the ER with low RPE suggests the presence of weakly estrogenic substances (e.g., phenols and derivatives, phthalates, pesticides) and of estrogen antagonists.

The combination of primary and secondary treatments removed 87 and 98% of the initial estrogenic activity from the aqueous phase (E-screen and ERBA, respectively; Figure 2, sample D). These figures are similar to those reported for municipal STPs in other parts of the world (*8*) and clearly indicate that activated sludge treatment is very effective at removing estrogenic activity from sewage water. On the basis of GCMS results, at least 74% of all E_2 and 89% of all E_1 was removed by secondary treatment (Table 4). However, quantification of the removal efficacy for individual compounds with GC/MS analysis was not very useful because of the relatively high limit of reporting of this method (5 ng/L).

In a study on the fate of estrogens during sewage treatment, Andersen et al. (*15*) showed that most estrogens were either eliminated or bound to sludge during activated sludge treatment. Estrogen concentrations in the sludge were not measured in this study, but on the basis of their relatively high K_{OW} values (Table 3), a similar scenario would be expected. Further studies at this plant will investigate estrogen concentrations in the sludge.

Tertiary Treatment. The cytotoxicity of the effluent extract after ozonation to MCF-7 breast cancer cells (sample F) could be the result of the formation of some toxic ozonation byproducts. The sewage samples before and after sand filtration (samples D and E) were very similar in their estrogenicity, indicating this treatment step may not be efficient in the removal of estrogenic substances. A comparable result with slow sand filtration as tertiary treatment step was found in a municipal sewage treatment plant in Bavaria, southern Germany (Körner, unpublished results).

Overall Efficacy of the Treatment Plant. Full treatment removed in excess of 95−98% of all estrogenic activity, depending on the bioassay (Figure 2, E-Screen and ERBA, respectively), and estrogenicity was below detection limit of the bioassays in the final effluent (Figure 2 and Table 4, sample G) clearly indicating that treatments successfully removed or sequestered most of those compounds.

In laboratory exposures, median effective concentrations for a significant induction of the egg-yolk precursor vitellogenin in juvenile rainbow trout after 2 weeks of exposure were estimated to be 10−20 ng/L for E_2, 25−60 ng/L for E_1, and 1 ng/L for EE_2 (*32*, *33*). Although the analytical detection limit for EE_2 was higher than this level (5 ng/L), the fact that the overall estrogenic potency of the effluent samples was

below detection limit of the bioassays (0.75 ng/L in the ERBA, 0.03 ng/L in the E-Screen) suggests that the potential for the effluent to induce estrogenic effects in exposed wildlife is very small, at least in the short term. Further studies need to be undertaken to examine the long-term effects of treated sewage containing trace concentrations (ppt) of estrogenic chemicals on exposed wildlife as well as to determine if the unique Australian wildlife is more susceptible to estrogenic chemicals.

Future Direction. The two bioassays (ERBA and E-Screen) yielded slightly different results, although the major trends were similar (Figure 2). This is attributable to the difference in biological complexity between the two assays. The E-Screen is a cellular end point and integrates the full response of cells to estrogenic compounds. This includes crossing of the cell membrane, binding to the ER, activation of the ER, as well as the whole genetic machinery involved in transduction, transcription, and translation of the signal. The ERBA, on the other hand, is a molecular end point, and estrogenicity is measured solely by the ability of chemicals to bind to the ER. The ERBA can thus be thought of as a bioassay for potential estrogenic disruption via the ER, while the E-Screen is more representative of the full cellular response. These in vitro bioassays still do not account for the complex feedback mechanisms that occur in the endocrine system, and the results must be interpreted with caution. For instance, when comparing E_2 equivalency factors (EEF, a measure of relative potency) determined from an ER binding assay with those determined in vivo with transgenic zebrafish, Legler et al. (*34*) showed that some synthetic chemicals were more potent in vivo than when evaluated with the ERBA. We are currently working on an in vivo bioassay for mosquitofish, a species of fish relevant to subtropical Australia, which could be used to examine in situ responses to the effluents.

The samples in this study were taken on 1 day, and further sampling is required to provide a more complete understanding of the day-to-day variation in estrogenicity at that plant. Also, the high level of tertiary treatment in place at the Landsborough STP is not representative of municipal STPs in Australia, which are often limited to secondary treatment followed by disinfection. A survey of estrogenic compounds in sewage at several STPs in Queensland and New Zealand will be published shortly.

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