Balancing the budget of environmental estrogen exposure: the contribution of recycled water
Frederic D. L. Leusch, Michael R. Moore and Heather F. Chapman

ABSTRACT

Estrogenic endocrine disrupting compounds (e-EDCs) are present in treated sewage and there is concern about estrogenicity of potable recycled water. However e-EDCs are also present in other environmental media and intake from water needs to be considered in relation to these other sources. The concentrations of 13 e-EDCs in foodstuffs and drinking water are reviewed, their predicted concentrations in recycled water are estimated, and the daily estrogenic intake as 17β-estradiol equivalent (EEq) based on both in vitro and in vivo potencies is calculated as 1.39 and 9.65 μg EEq/d, respectively. Dietary intake accounts for more than 99.8% of that total, and more than 84.2% is due to phytosterols. Drinking 2 L of recycled water per day is expected to add 0.001 to 0.016 μg EEq/d based on in vitro and in vivo potencies, respectively. Exposure to e-EDCs in recycled water is therefore likely to be insignificant compared to current dietary intakes.

Key words | daily exposure, diet, e-EDCs, estrogenic compounds, recycled water, urban water

INTRODUCTION

Estrogenic endocrine disrupting compounds (e-EDCs) are chemicals that can mimic and/or interfere with the action of natural estrogen hormones in organisms. Estrogen hormones are involved in a variety of biological functions such as development, puberty, behavior, gametogenesis and integrated sexual function. Some e-EDCs such as industrial compounds, plant sterols, natural hormones, pharmaceuticals, pesticides and metals are present at low concentrations in treated sewage (NRMCC/EPHC/NHMRC 2008), which raises concerns about potential exposure to e-EDCs from water reclaimed from treated sewage. It should however be recognized that environmental pollutants are ubiquitous not only in water but also in air, soil and food. Drinking water is only one source of exposure, and other pathways such as diet, cosmetics or medical applications may result in significant exposures to xeno-estrogens.

In this paper, we review concentrations of 13 known e-EDCs in a variety of foodstuffs and water and compare the estimated daily intake from dietary sources with intake from consumption of 2 L of recycled water (assuming a 3-log removal from the highest reported concentration in treated sewage).

Compounds considered

Thirteen chemicals were considered for this study:

- 4 industrial compounds: 4-nonylphenol (4-NP), 4-t-octylphenol (4tOP), bisphenol A (BPA) and di-n-butylphthalate (DnBP),
- 2 phytosterols: genistein and daidzein,
- 3 natural hormones: 17β-estradiol (E2), estrone (E1) and estriol (E3),
- 1 pharmaceutical: 17α-ethynylestradiol (EE2),
- 2 pesticides: p,p'-dichlorodiphenyltrichloroethane (DDT) and endosulfan, and
- 1 metal: cadmium (Cd).

All have been shown to possess estrogenic activity in vitro and/or in vivo (Table 1).

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Industrial compounds

Industrial xeno-estrogens are generally not very potent endocrine disruptors (Table 1) but being produced in very large volumes they can be found as pollutants at high concentration in water and other sources. 4-NP is a degradation product of a widely used group of nonionic surfactants, nonylphenol polyethoxylates (NPEOs). 4tOP is also a by-product of alkylphenol polyethoxylate nonionic surfactants used in industrial processes. BPA is one of the highest volume chemicals produced worldwide, and is used in the production of polycarbonate plastics, epoxy resins used to line metal cans, and many plastic consumer products. Finally phthalates are used in the production of various plastics and are among the most common industrial chemicals. Several million tons of these compounds, including DnBP, have been used as plasticizers for more than 40 years worldwide.

Phytosteros

Phytoestrogens are compounds produced naturally in plants that are estrogenic (Kuiper et al. 1998; Jefferson et al. 2002; Diel et al. 2004) and can cause estrogen-like effects in the animals that consume them, in the more severe cases leading to infertility (e.g. “clover disease” in sheep; Adams 1998). Phytoestrogens are relatively potent estrogen mimics (Table 1) and high amounts can be ingested through diet. This paper focuses on two potent isoflavones, daidzein and genistein.

Natural estrogens

E2 is excreted as glucuronide or sulfate conjugate in urine, but is deconjugated (re-activated) by microbial activity in sewer and sewage treatment plants. E2 can thus be present in sewage, and potentially in source waters. Natural hormones including E2, E1 and E3 are also present in animal-derived foods such as meat or milk (Table 2).

Pharmaceuticals

Pre-menopausal women may also be exposed to very high concentrations of synthetic estrogens from birth control pills, which contain 20–50 μg of the very potent estrogen EE2 per pill, depending on the formulation. Natural estrogens (E2 and E1) are also used at high concentrations (mg/pill) in hormone replacement therapy.

Pesticides

Pesticides are used worldwide and provide significant benefits in agriculture. Pesticide residues in food do however pose risks to human populations, and they are

<table>
<thead>
<tr>
<th>Table 1</th>
<th>in vitro and in vivo estrogenic potency* of selected e-EDCs relative to 17β-estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro potency</strong> (E-SCREEN assay)</td>
<td><strong>In vivo potency</strong> (vitellogenin induction in fish)</td>
</tr>
<tr>
<td>4-Nonylphenol (4NP)</td>
<td>0.000078†</td>
</tr>
<tr>
<td>4-t-Octylphenol (4tOP)</td>
<td>0.000065†</td>
</tr>
<tr>
<td>Bisphenol A (BPA)</td>
<td>0.00003†</td>
</tr>
<tr>
<td>di-n-Butylphthalate (DnBP)</td>
<td>0.00000034pp,0.000001††,‡‡</td>
</tr>
<tr>
<td>Genistein</td>
<td>0.0005§§</td>
</tr>
<tr>
<td>Daidzein</td>
<td>0.0005§§</td>
</tr>
<tr>
<td>17β-Estradiol (E2)</td>
<td>1</td>
</tr>
<tr>
<td>Estrone (E1)</td>
<td>0.012†</td>
</tr>
<tr>
<td>Estriol (E3)</td>
<td>0.071‡‡‡</td>
</tr>
<tr>
<td>Ethynylestradiol (EE2)</td>
<td>1.25§§§</td>
</tr>
<tr>
<td>p,p'-DDT</td>
<td>0.000004×××</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>0.000001*****</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>0.0097††††</td>
</tr>
</tbody>
</table>

*Potency is a measure of biological activity. Chemicals with a high potency (such as E2 or EE2 in the table above) evoke a response at a lower concentration than those with a lower potency (such as DnBP). NA = not available.
†Leusch et al. (2006).
‡3-week waterborne exposure with adult female zebrafish.
†Van den Belt et al. (2004).
§2-week waterborne exposure with adult male fathead minnow.
§Brian et al. (2005).
××Körner et al. (2001).
††3-week waterborne exposure with juvenile rainbow trout.
‡‡Van den Belt et al. (2003).
kkMatsumura et al. (2005).
kkk3-week waterborne exposure with juvenile fathead minnow.
kkBrian et al. (2002).
××××intraperitoneal injection over 10 d in juvenile male Siberian sturgeon.
×××××-d waterborne exposure with juvenile zebrafish.
×××××Volbesch et al. (2006).
××××Yang et al. (2003).
×××××Andersen et al. (2002).
××××Choe et al. (2003).
×××××7-week waterborne exposure with adult male Japanese medaka.
××××××××Fitzon et al. (2003).
<table>
<thead>
<tr>
<th>Foodstuffs (in µg/kg unless otherwise indicated)</th>
<th>Meat, poultry, fish, eggs</th>
<th>Vegetables and legumes</th>
<th>Fruits</th>
<th>Cereals</th>
<th>Dairy</th>
<th>Miscellaneous</th>
<th>Drinking water</th>
<th>Recycled water estimate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Nonylphenol</td>
<td>16.1–236†</td>
<td>7.5–31.0†</td>
<td>22.0–27.4†</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Processed foods: 0.1–19.4‡</td>
<td>0.003–2.7§</td>
</tr>
<tr>
<td>4-t-Octylphenol</td>
<td>0.4–44.9**, NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Up to 0.005§</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>13.3–213**, NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Canned foods: up to 95.3‡‡</td>
<td>0.002–0.025§</td>
<td></td>
</tr>
<tr>
<td>DnBP</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Total diet: 10–190§§,</td>
<td></td>
</tr>
<tr>
<td>Genistein</td>
<td>Up to 4,400***</td>
<td>Up to 841,000†††</td>
<td>NA</td>
<td>Up to 17,100***</td>
<td>Soy milk: 137,000</td>
<td></td>
<td></td>
<td>Beer: 0.05–23 µg/L</td>
</tr>
<tr>
<td>Daidzein</td>
<td>Up to 2,300***</td>
<td>Up to 560,000†††</td>
<td>NA</td>
<td>Up to 5,000***</td>
<td>Soy milk: 114,000</td>
<td></td>
<td></td>
<td>Beer: 0.02–13 µg/L</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>Up to 0.73****,††††</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Up to 0.06 µg/L</td>
<td>§††††</td>
<td>Therapy: 2,000 µg/pill</td>
<td></td>
</tr>
<tr>
<td>Estrone</td>
<td>Up to 0.51††††</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Up to 0.12 µg/L</td>
<td>§††††</td>
<td>NA</td>
<td>Up to 0.0006§</td>
</tr>
<tr>
<td>Estriol</td>
<td>Up to 0.60††††</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Up to 0.12 µg/L</td>
<td>§§§§</td>
<td>NA</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Ethynylestradiol</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Birth control: 20–50 µg/pill</td>
<td>Up to 0.0005§</td>
<td>0.00027</td>
</tr>
<tr>
<td>p,p'-DDT</td>
<td>Up to 28****</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>&lt; 0.005****</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>NA</td>
<td>Up to 82****</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>&lt; 0.005****</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Up to 500*</td>
<td>Up to 60††††</td>
<td>Up to 80††††</td>
<td>Up to 230††††</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Up to 0.2††††</td>
</tr>
</tbody>
</table>

closely monitored by food safety agencies (FSANZ 2003). Several pesticides such as DDT, endosulfan and dieldrin have been shown to possess estrogen-like activity both in vitro and in vivo (Bitman et al. 1968; Soto et al. 1994; Andersen et al. 2002).

Metals

Finally, some metals have also been shown to have estrogenic properties both in vitro and in vivo, particularly cadmium (Choe et al. 2003; Johnson et al. 2003). Cadmium is used in several industrial applications, including the production of NiCd batteries.

DATA SOURCES AND CALCULATIONS

Reported concentrations in foodstuffs and current drinking water

Concentrations in foodstuffs and current drinking water were sourced from the scientific literature and national regulatory agency reports (Table 2).

Estimated recycled water concentrations

Concentrations in recycled water were estimated by applying a 3-log (i.e. 99.9%) removal to the highest concentration in treated sewage reported in the Australian Guidelines for Water Recycling (NRMMC/EPHC/NHMRC 2008). This assumption is a conservative estimate based on the combined removal efficacy of advanced water reclamation and drinking water treatment technologies prior to consumption (Snyder et al. 2003; Drewes et al. 2005; Kim et al. 2007).

Estimated daily intake (EDI)

Dietary EDI was sourced from the scientific literature and national regulatory agency reports (Table 3). EDI from drinking and recycling water was based on an average consumption of 2 L/day used in Australian regulatory guidelines (NRMMC/EPHC/NHMRC 2008) and the concentrations reported in Table 2.

Estimated daily estrogenic intake

The estimated daily estrogenic intake as 17β-estradiol equivalents was calculated as the sum of the product of EDI and relative potency for each individual chemical, based on in vitro and in vivo potencies. When a range was available for EDI, the median was used. When a concentration or relative potency was stated as “less than” (<), that value was used to assume a “worst-case scenario”. When only the maximum EDI was available then that value was used.

For phytosterols, there can be a large difference in dietary intake between Western-style and soy-rich Asian-style diets (Clarke & Lloyd 2004). The EDI from a Western-style diet was used in this study. The contribution of dietary phytosterols to total estrogenic intake would be approximately 10-fold higher from a soy-rich Asian-style diet.

With EE2, the primary source of exposure is for medical use in women. Because of the sexual dichotomy, the contribution of these pharmaceuticals from medical applications (e.g. contraceptive, cycle regulation) was not integrated into the overall daily estrogenic intake as men would not be exposed to these drugs from this particular type of application. Their contribution from other sources that would affect the whole population (e.g. residues in drinking water) was however of course included.

DISCUSSION

The concentrations of the 13 chemicals in foodstuffs and current drinking water worldwide as well as the predicted “worst” concentration in Australian recycled water are reported in Table 2, EDIs are reported in Table 3.

Concentration of individual compounds and estimated daily intakes

In general, EDI from food is several orders of magnitudes higher than that from drinking water (Table 3). 4-NP is ubiquitous and can be found at high concentrations in foodstuffs (Table 2). 4tOP is also produced in large amounts, and is found at high concentrations in food (much like 4-NP). BPA can be found at high concentrations
in processed foodstuffs due in part to leaching from consumer plastics and epoxy resin linings (Vandenberg et al. 2007). The concentration of BPA in the liquid phase in canned vegetables can be high, containing as much as 450 μg/L in canned peas (Brotons et al. 1995). The vegetables themselves can contain BPA at concentrations as high as 95.3 μg/kg in canned corn (Yoshida et al. 2001). There are also less conventional sources of exposure to BPA. For example, BPA is used in dental sealants (as high as 670 μg/mg; Olea et al. 1996), and leaching can result in high concentrations of BPA in saliva (with up to 30 μg/mL of saliva 1 h after application; Olea et al. 1996). Human exposure to phthalates such as DnBP occurs during production, distribution and final use of products made of PVC and other polymers because phthalates are easily released from the matrix (e.g. plastic food wrap or food packaging) by evaporation and abrasion (Fromme et al. 2007). Food and consumer products (e.g. cosmetics) are the main source of phthalates in humans (Wormuth et al. 2006).

Genistein and daidzein, two potent isoflavone phytoestrogens, can be found at high concentrations in leguminous plants, with concentrations as high as 841,000 and 560,000 μg/kg in soybean (Table 1; Mazur & Adlercreutz 1998). Plant-derived beverages such as beer and bourbon also contain high amounts of genistein and daidzein (Table 1; Lapcik et al. 1998), as well as other phytoestrogens such as β-sitosterol (Rosenblum et al. 1993), biochanin A (up to 33 μg/L; Clarke & Lloyd 2004) and the very potent 8-prenylnaringenin (up to 138 μg/L; Clarke et al. 2004). Wine also contains high concentrations of resveratrol (red wine in particular, up to 3,000 μg/L; Klinge et al. 2003).

Table 3 | Estimated daily intake of selected e-EDCs from dietary sources, current drinking water and recycled water (all in μg/d)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dietary Intake (70-kg adult)</th>
<th>Drinking Water (2L/d)</th>
<th>Recycled Water (2L/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Nonylphenol (4-NP)</td>
<td>3.0–35.3†††‡‡‡</td>
<td>0.006–5.4</td>
<td>0.006</td>
</tr>
<tr>
<td>4-t-Octylphenol (4tOP)</td>
<td>Up to 0.05‡</td>
<td>Up to 0.01</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>Bisphenol A (BPA)</td>
<td>100‡</td>
<td>0.004–0.05</td>
<td>0.024</td>
</tr>
<tr>
<td>di-n-Butylphthalate (DnBP)</td>
<td>8.4–114**</td>
<td>Up to 0.064</td>
<td>0.0018</td>
</tr>
<tr>
<td>Genistein†††</td>
<td>Western-style diet: 2,250‡‡‡</td>
<td>&lt;0.004</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>Estrone (E1)</td>
<td>0.10</td>
<td>Up to 0.012</td>
<td>0.0002</td>
</tr>
<tr>
<td>Estradiol (E2)</td>
<td>0.045–0.155†,‡</td>
<td>Up to 0.004</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>Ethynylestradiole (EE2)</td>
<td>0.01–0.018§,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P,p'-DDT</td>
<td>0.035–1.8†††, §§</td>
<td>&lt;0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>0.16–1.5†††, §§</td>
<td>&lt;0.01</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>4.9–20.3††</td>
<td>Up to 0.4</td>
<td>0.008</td>
</tr>
</tbody>
</table>

20–50 μg/day for 21 d followed by 7 d without. As previously stated, the intake from medical applications (e.g. contraceptive) is not included in the calculated EDI as it does not affect the entire population. It would be unlikely to find residues of EE2 in foodstuffs, and the dietary EDI is therefore nil. Residues of EE2 have been reported in drinking water up to 0.0005 mg/L (Kuch & Ballschmiter 2000).

Pesticides are mostly found in agricultural products, however some residues can be found in source waters due to run-off from agricultural practices (Table 2). The EDI of DDT from consumption of 2 L of recycled water (0.04 μg/d) can be equivalent to the lowest EDI of DDT from dietary sources (0.035–1.8 μg/d) (Table 3). Note that this is based on a relatively high estimate of 0.02 μg/L of DTT in recycled water, and the actual figure is likely to be negligible.

Finally metals such as cadmium are more likely ingested from food rather than air or water (Table 3), although tobacco smoking will significantly add to the body burden (FSANZ 2003).

**Total estrogenicity**

The estrogenic activity of many of the above compounds has been established both *in vitro* and *in vivo* (Table 1). It is thus possible to roughly estimate the daily estrogenic intake (as estradiol equivalent, EEq) from both dietary sources and consumption of 2 L of recycled water (Table 3). Based on the data presented in the tables above, the total estimated daily estrogenic intake in adults from dietary sources is 1.39 μg EEq/d based on *in vitro* potencies and 9.65 μg EEq/d based on *in vivo* potencies, with 84.5% and 97.6% of the estrogenicity from dietary phytoestrogens (*in vitro* and *in vivo*, respectively; Figure 1).

These figures are in agreement with previously published literature, which clearly highlights the significant intake of estrogenic compounds from dietary phytoestrogens and contraceptives (Safe 1995; Pugh & Moore 1998).

In comparison, the estimated daily estrogenic intake from consumption of 2 L of recycled water results in approximately 0.001 and 0.016 μg EEq/d based on *in vitro* and *in vivo* potencies, respectively. This is much less than the dietary exposure, and in fact is only 0.07 and 0.16% of the total daily *in vitro* and *in vivo* estrogenic intake, respectively. It is interesting to note that the intake from recycled water would be less than that for current drinking water (0.010 and 0.035 μg EEq/d based on *in vitro* and *in vivo* potencies, respectively). It should be noted that women on the contraceptive pill can take the equivalent of up to 62.5 μg EEq/d (*in vitro*) or 1,400 μg EEq/d (*in vivo*), dwarfing the total intake from all other sources combined.

There are also other sources of exposure to e-EDCs that have not been included in these estimates. Air breathing may also contribute to the total estrogenic intake. For example air (Klein et al. 2006) and cigarette smoke extracts (Takamura-Enya et al. 2005) have been shown to be estrogenic *in vitro*. In a recent study, Kennedy et al. (accepted for publication) found an average of 54 pg EEq/m³ in indoor air using the E-SCREEN bioassay. Volume of air breathed depends heavily on activity type (CEPA 1994), but using a conservative average daily breathing volume of 10 m³/d translates into a daily *in vitro* estrogenic equivalent intake of 0.0005 μg/d, slightly less than the estimated intake from recycled water and much less than that from dietary sources. Furthermore, some cosmetics can contain high levels of xeno-estrogens, and dermal exposure can result in absorption of these chemicals through the skin. Cosmetics such as deodorant, perfumes, aftershaves, shampoos and skin care products can contain very high concentrations of phthalates (as high as 10,000 μg/g DnBP in aftershave or nail care product for example; Wormuth et al. 2006). In the case of diethylphthalate, up to 80% of the estimated daily intake is caused by dermal application or incidental ingestion of personal care products (Wormuth et al. 2006). The alkyl esters of p-hydroxybenzoic acid (parabens) are also added in concentrations of up to 0.8% as preservatives to thousands of cosmetic products (Darbre 2006). Parabens can be absorbed rapidly through the skin (Darbre 2006) and they have weak estrogenic activity *in vitro* (from 0.0000002 for short chain parabens such as methylparaben to 0.00002 for longer chain parabens such as n-butylparaben; Byford et al. 2002; Darbre 2006). Assuming a daily application of 0.3 mL of perfume containing 0.8% of a mixture of parabens (with an average relative potency of 0.000002 compared to 17β-estradiol) and 50% absorption, up to 0.0024 μg *in vitro* EEq/d could be attributable to cutaneous perfume application, or more than twice
the total intake from drinking recycled water (but still dwarfed by the dietary intake).

These numbers however must also be kept in perspective with endogenous natural estrogens. Estrogens are produced daily by human endocrine systems, as high as 140 μg/d in men, 630 μg/d in pre-menopausal women, and 54–100 μg/d in pre-pubertal children (17β-estradiol and estrone combined; Kushinsky 1983 cited in Hartmann et al. 1998). These numbers are 5–65-fold higher than the total estimated estrogenic intake from all sources combined (based on in vivo potencies). In estrogen replacement therapy, post-menopausal women are administered 17β-estradiol orally at a dose of approximately 1,000–2,000 μg/day, which results in a 10-fold increase in plasma estrogen concentrations (Geisler et al. 1999). It is therefore unlikely that a combined oral estrogenic intake of 9.65 μg EEq/d (in vivo) would significantly affect estrogen homeostasis in humans.

The predicted concentrations (and estimated daily intake) of e-EDCs in recycled water presented in this paper are based on an assumed 3-log removal from the worst concentration in treated sewage. The authors did not have access to recycled water samples to confirm these predictions at this time, but once it becomes available the

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**Figure 1** | Estimated daily estrogenic intake (expressed in 17β-estradiol equivalents) from dietary sources and consumption of 2 L of recycled water. (A) Based on in vitro relative potencies. (B) Based on in vivo relative potencies.
real concentrations of these 13 chemicals should be used to fine-tune the assessment presented here.

CONCLUSIONS

When considering possible intake of xeno-estrogen compounds from recycled water, other sources need to be considered. The predicted intake from consumption of 2 L of recycled water (conservatively estimated as 3-log removal from the highest reported concentration in treated sewage) is significantly lower than intake from dietary sources (1,400 and 620-fold lower for in vitro and in vivo estimates, respectively). Dietary phytoestrogens alone constitute the large majority of the dietary estrogenic intake (84.2 and 97.7% based on in vitro and in vivo potencies, respectively). This suggests that ingestion of e-EDCs from recycled water is unlikely to contribute significantly to the total estrogenic intake.

ACKNOWLEDGEMENTS

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