Assessment of Endocrine Disrupting Activity of Commercially Available Sanitary Products – A Pilot Study

Report for Organic Initiative

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Purpose of Report

Many sanitary products on the market are fully or partially made from synthetic fibres, with little known about their health impact.

Organic Initiative is a New Zealand owned company which sells organic cotton sanitary products. Organic Initiative sought to understand the quality and health implications of widely available sanitary products in New Zealand and globally.

In 2022, Organic Initiative contracted Zestt Wellness[1] to assess the prevalence of endocrine-disrupting chemicals (EDCs), with xenoestrogenic potential, in feminine hygiene products. Zestt worked with Insitugen to undertake the laboratory work and analysis for this report[2].

Scope of work

The scope of the work was to determine if there were endocrine-disrupting chemicals (EDCs) present in a range of commercially available sanitary products.

Background

The global feminine hygiene products market size was valued at US$39.66 billion in 2022[3]. and is expected to continue to grow. Vaginal hygiene products include washes, wipes, moisturisers and creams, and other products. Whilst little is known of their endocrine-disrupting potential, these products have been reported to exhibit adverse effects on the vaginal microbiome (Wissel et al., 2020[4]),

One study in Canada (1,435 women) found that 95% of participants reported using at least one vaginal hygiene product. Participants who used any vaginal product had nearly three times higher odds of an adverse health condition, such as reported history of BV, yeast infection, urinary tract infection, or a sexually transmitted disease (Cran et al., 2018[5]).

In addition to concerns regards feminine hygiene products, there is growing concern about the materials used in sanitary products especially as they have changed from mostly cotton-based products to synthetic fibre products (Nicole 2014[6]). There is some concern about the health impact of the synthetic fibres, given the sensitivity of the vaginal microbiome and the permeability of the vagina to chemical compounds.

Mucous membranes in the vagina and vulva rapidly absorb unmetabolised chemicals[7] due to the high water permeability of the vaginal epithelium. Vaginally delivered pharmaceuticals have been shown to enter the blood stream faster and more efficaciously than orally delivered products. The application of estradiol, resulted in blood serum levels 10 times

[1] www.zesttwellness.com
[2] https://www.insitugen.com/
[3] https://www.fortunebusinessinsights.com/feminine-hygiene-products-market-103530
[4] A Narrative Review on Factors Shaping the Vaginal Microbiome: Role of Health Behaviors, Clinical Treatments, and Social Factors (authorea.com)
[5] Vaginal health and hygiene practices and product use in Canada: a national cross-sectional survey - PMC (nih.gov)
[6] https://ehp.niehs.nih.gov/doi/full/10.1289/ehp.122-A70
[7] https://ehp.niehs.nih.gov/doi/full/10.1289/ehp.122-A70

higher than oral dosing (Tourgeman et al, 1999[8]). Thus it is likely toxic compounds, which might have negative health effects, will also be easily absorbed via the vaginal tract.

Health risk of endocrine-disrupting compounds

Endocrine-disrupting chemicals (EDCs) are a potential health threat to humans (Toppari et al., 2005[9], Da Chen et al., 2016[10]; Davis et al., 1996[11], T Hadibarata et al., 2020[12]) and have been recognised as such for over 50 years (Johnson et al., 1964[13]).

Xenoestrogens, a class of EDC that mimics estrogen in the body by binding to estrogen receptors, are of particular importance to women due to their disruption of normal physiologic functioning. Prolonged xenoestrogen exposure can lead to increased risks of many types of endocrine-related syndromes and diseases (Kaaks et al., 2005[14]; Cutolo and Straub, 2020[15]) including, but not limited to:

- Endometriosis;
- Polycystic ovarian syndrome;
- Hypogonadism;
- Cardiovascular complications;
- Metabolic and autoimmune diseases;
- Neurodegenerative and psychiatric diseases;
- Hormone-dependent cancers.

Sanitary products as vehicles for endocrine-disrupting chemicals

Tampons, menstrual pads, and other menstrual products present a potential risk to women through the introduction of endocrine-disrupting hormones, given the direct contact with the permeable vaginal tract (Aguirre 2022[16]).

Potential endocrine-disrupting chemicals have been found in in feminine hygiene products (Gao and Kannan 2020[17]), although the estrogen binding potential of the compounds were not measured. Compounds found included:

- Pthalates which are plasticisers used to increase flexibility, transparency and durability of sanitary products;
- Bisphenols used in the making of plastics, and now ubiquitous in the environment;

[8] https://www.sciencedirect.com/science/article/abs/pii/S0002937899700426
[9] Male reproductive health and environmental xenoestrogens. | Environmental Health Perspectives | Vol. 104, No. suppl 4 (nih.gov)
[10] https://pubs.acs.org/doi/10.1021/acs.est.5b05387
[11] Medical hypothesis: xenoestrogens as preventable causes of breast cancer. | Environmental Health Perspectives | Vol. 101, No. 5 (nih.gov)
[12] https://iwaponline.com/jwh/article/18/1/38/71709/Occurrence-of-endocrine-disrupting-chemicals-EDCs
[13] https://doi.org/10.1016/B978-012507751-4/50032-X
[14] https://pubmed.ncbi.nlm.nih.gov/16322344/
[15] https://www.nature.com/articles/s41584-020-0503-4
[16] https://repository.usfca.edu/capstone/1399
[17] https://www.sciencedirect.com/science/article/pii/S0160412019333859?via%3Dihub

- Parabens which are chemical preservatives used extensively in pharmaceutical, cosmetic, lotions, and creams and menstrual products;
- Triclocarban, an antibacterial chemical, which is banned in over-the-counter hand and body washes in the United States but is still present in a proportion of tampons and pads around the world (Lacopetta et al., 2021[18]).

Project Aim

We set out to determine if commercially available tampon products (including Organic Initiative brand) cause endocrine-disrupting activity.

Results

Measurable estrogen activity was analysed in the different tampon brands as follows:

There was no measurable estrogen activity in the Organic Initiative tampons (green triangle) or two of the other commercially available tampon brands (A-black diamond, B-black circle). There was measurable estrogen activity in one commercially available tampon (C-red square) (Figure 1). When purified estradiol was spiked into tampons prior to extraction estradiol was detected in all brands, as expected (Figure 1 and Appendix 2).

Data for non-spiked tampons is shown more clearly in Figure 2 (different scaled axes) showing the estrogen activity of brand C.

[18] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8126057/

Figure 1. Estrogen activity measured in four commercially available tampon brands, spiked and unspiked with estradiol. Sample testing SOP ISG/002, in triplicate. Lumineniscence output is measured in (RLU) Relative Luminescence Units.

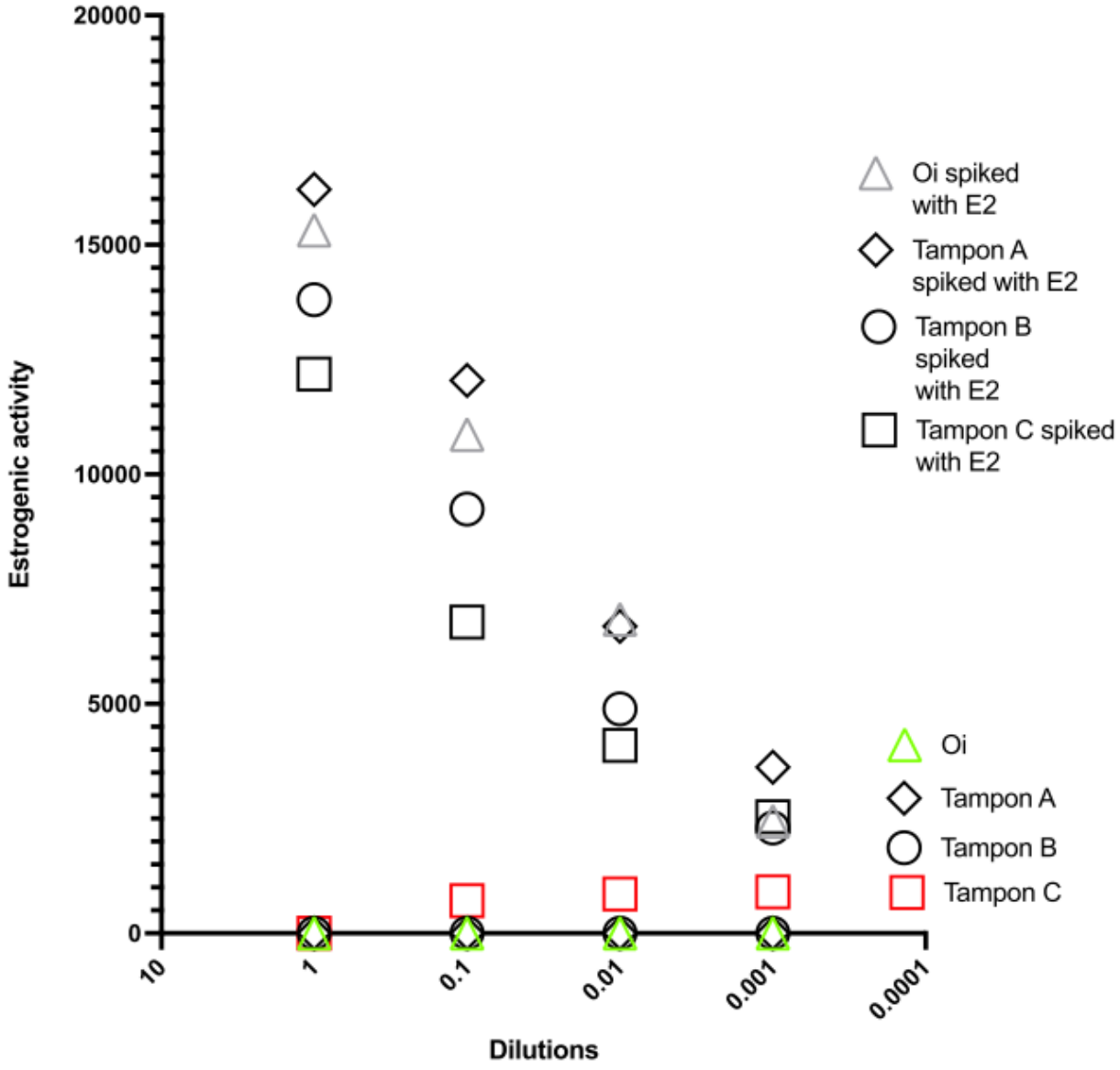
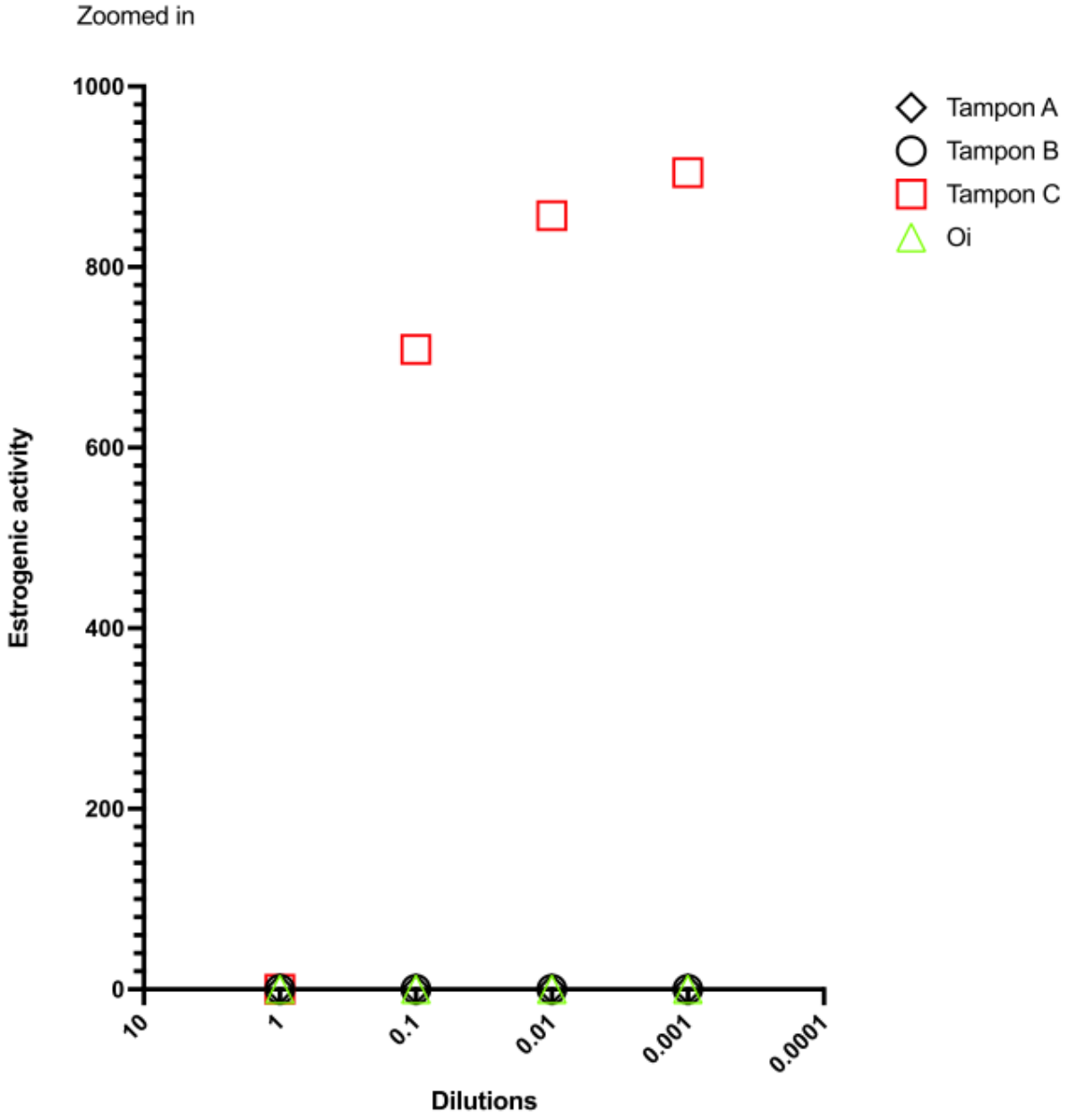


Figure 2. Estrogen activity measured in four commercially available tampon brands unspiked with estradiol (showing only unspiked data but enlarged scale from Figure 1). Sample testing SOP ISG/002 in triplicate. Lumineniscence output is measured in (RLU) Relative Luminescence Units.



Discussion and conclusions

A range of materials, both synthetic and naturally derived, are used to produce sanitary products globally. There has been concern that some of these materials, especially those which are synthetically derived, contain chemicals which may have negative health effects.

The presence of these chemicals was confirmed by Gao and Kannan in (2020)[19], but they did not determine if these compounds had endocrine-disrupting activity.

The aim of this pilot study was to determine whether there was endocrine-disruption potential in four commercially available tampon brands, including Organic Initiative.

We found no endocrine-disrupting activity in three of the four brands tested (including Organic Initiative). However, we did find that in one of the commercially available brands, there was significant endocrine-disrupting activity. Highlighting the potential of this product to disrupt normal endocrine functioning (Figure 1 and 2). In this study, we only evaluated estrogen activity, but the presence of these chemicals may also disrupt other endocrine hormones.

Humans are exposed an increasing number of endocrine-disrupting chemicals and environmental toxicants every day. It has been proposed that this escalating, yet constant exposure, may partially explain a concurrent decline in human fertility that has occurred over the last 50 years (reviewed in Green et al, 2001[20]). A recent study showed that the decline in human fertility may be in part due to the impact of endocrine-disrupting chemicals on the gut microbiome, however the researchers did not assess the vaginal microbiome as part of this study (Fabozzi et al., 2022[21]). Impacts of endocrine-disrupting chemicals have also been postulated for female cancers (Rachoń, 2016[22], Morgan et al 2017[23], Davis et al., 1993[24]) and disease susceptibility (Schug et al., 2011[25]).

Overall, the results from this study show that further investigation of a wider range of commercially available sanitary products is warranted. In addition, it would be of value to determine which endocrine-disrupting chemical/s are present in the brand/s that display estrogenic activity.

Considering the global extensivity of synthetic sanitary products and their impact on human health and fertility, further investigation is time-critical.

Next Steps

1. Survey a wider range of sanitary products, including pads, panty liners (note these had the highest endocrine-disrupting chemicals in the study by Gao and Kannan (2020[26]) and period and incontinence underwear.
2. In those samples that displayed estrogen activity, determine which xenoestrogens are present.
3. Determine the level of activity and likely health impacts.

Note: these steps would make a good PhD programme potentially (funded commercially).

Appendix 1.

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[19] https://www.sciencedirect.com/science/article/pii/S0160412019333859?via%3Dihub
[20] https://www.sciencedirect.com/science/article/abs/pii/S0013935120315930
[21] https://www.mdpi.com/2073-4409/11/21/3335
[22] https://link.springer.com/article/10.1007/s11154-016-9332-9
[23] https://www.sciencedirect.com/science/article/abs/pii/S0303720716304117
[24] Medical hypothesis: xenoestrogens as preventable causes of breast cancer. | Environmental Health Perspectives | Vol. 101, No. 5 (nih.gov)
[25] https://www.sciencedirect.com/science/article/abs/pii/S096007601100166X
[26] https://www.sciencedirect.com/science/article/pii/S0160412019333859?via%3Dihub

Methodology

Standards and reagents

Estradiol - Sigma Aldrich β-Estradiol, ≥98%, isopropanol, Optima™ LC/MS Grade - Fisher Chemical, methanol (Methanol, For HPLC analysis) - J.T. Baker, and water (HPLC grade) - J.T. Baker, Acetonitrile (Acetonitrile HPLC Plus, ≥99.9%) - Sigma Aldrich

Sample collection and analysis

In November of 2022, tampon samples were provided by Organic Initiative. Both their product and three other competitive products were used in the analysis. The tampons were four global brands of varying prices.

To avoid possible contamination and cross-contamination samples were disposed of one week after opening, they were prepared progressively from first to last sample and no two samples were open at the same time.

The chromotragraphic separation was carried out using a Shimadzu Nexera LC-40D HPLC with a Shimadzu UV-VIS SPD-40 UV detector. Shim-Pack C18 100mm x 3mm column. The injection volume was 10 µL. The mobile phase comprised of acetonitrile 50% and water 50%. Flowrate 1.5ml/min at 40ºC

To confirm accuracy of methodology for detection, we were able to show through HPLC analysis that we were only able to detect estrogen activity when estradiol was spiked into all four tampon samples prior to extraction, otherwise no estrogen activity was detected. Samples were run in triplicate.

Methodology & Sample Preparation

HPLC

1. Estradiol Controls
   (i) Soak 1g of product in 1mL ethanol/water (50% v/v) and spike with 5µL of 2µM estradiol.
   (ii) Cover lightly, and leave to dry overnight in fume hood.
   (iii) Tube/Blank control: 10mL of ethanol/water (50% v/v).
2. Soak 1g of product (including spiked controls) in 10mL ethanol/water (50% v/v).
3. One hour agitation.
   (i) 10min sonication
   (ii) 40min shaker (200-210 rpm)
   (iii) 10min sonication
   (iv) Vortex: 2min
4. Injection of 10 µL. The mobile phase - acetonitrile 50% and water 50%. Flowrate 1.5ml/min at 40ºC through C18 column.

Hormone Testing

1. Estradiol controls:
   (i) Soak 1g of product in 1mL ethanol: milliQ (50% v/v) and spike with 5µL of 2µM estradiol.
   (ii) Cover lightly, and leave to dry overnight in fume hood.
   (iii) Tube/Blank control: 10mL of ethanol: milliQ (50% v/v).
2. Soak 1g of product (including spiked controls) in 10mL ethanol: milliQ (50% v/v).
3. 1 hour agitation.
   (v) 10min sonication
   (vi) 40min shaker (200-210 rpm)
   (vii) 10min sonication
   (viii) Vortex: 2min
4. Centrifuge: 15min 4000g
5. Wash 5mL through SPL column:

(i) Pre-condition a C18 column (2g Bond Elut, Agilent Technologies) with 3 mL methanol, followed by 3mL sodium acetate (pH 4.8).

(ii) Load the sample into the column.

(iii) Wash the column sequentially with 2mL water, 1.5 mL sodium carbonate (10% w/v), 2mL water, 2mL water/methanol (1:1 v/v).

(iv) Air dry the column.

(v) Elute sample with 4 mL acetonitrile.

6. Transfer supernatant into glass tube.
7. Concentrate elute by evaporation:
   (i) Dispense elute in 200µL volumes into a 96-well plate. Leave in fume hood overnight, covered lightly.
8. Resuspend in 200µL 100% ethanol in total.
9. Transfer to glass tube.
10. Perform cell assay in accordance to InsituGen SOP ISG/002.