The Detection of Endocrine Disrupting Chemicals Leaching from Reusable Plastic Water Bottles

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Introduction

Endocrine disruption is a particular form of toxicity, where natural and/or endogenous compounds, known as “Endocrine Disrupting Chemicals”, cause adverse health effects by resulting in disruption to the endogenous hormone system in mammals, including humans, particularly in foetal and infant stages [1].

It is generally agreed that much of this exposure to humans is through the consumption of food and beverages that are stored in plastic packaging. Bisphenol A (BPA) has been identified as an endocrine disruptor that is widely used in the production of polycarbonate plastic. Numerous scientific studies have established that BPA has the potential to leach from plastic products which are in direct contact with food or beverages and as such the main route of exposure of humans to BPA is through ingestion. BPA has been shown to interfere with the normal functioning of the endocrine system causing disruption and as a result having a direct effect on reproductive and thyroid function and blocking testosterone production. The leaching of chemicals, such as BPA, increases when these plastics are exposed to common, every-day stresses. Concerns over the safety of BPA and the legal restrictions which have been implemented on BPA containing plastics and their uses, have given rise to many manufacturers seeking substitutes for BPA resulting in an alternative polycarbonate backbone which can be labelled as “BPA-Free” and as such are perceived to be safer [2]. Currently, there is little information available as to whether these alternative polycarbonate plastics are free from possessing oestrogenic activity or if they have the potential to release compounds that may cause endocrine disruption.

Aims & Objectives

The aim of this research is to assess the water in reusable plastic bottles for endocrine disrupting chemicals and to test the hypothesis that bioactive plasticisers e.g. BPA, BPS & BPF, are released from these bottles following exposure to conditions which are representative of normal consumer usage including typical sunlight exposure and short-term exposure to hot and cold temperatures.

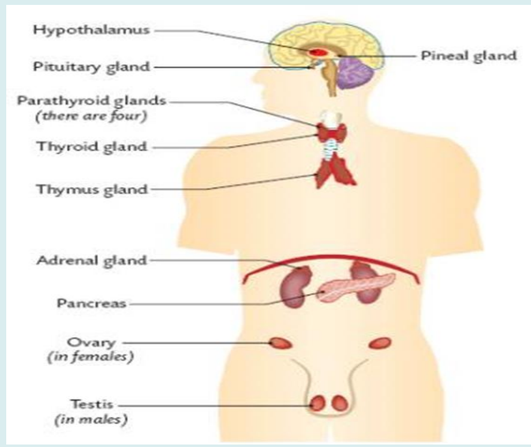

Figure 1: Human endocrine system

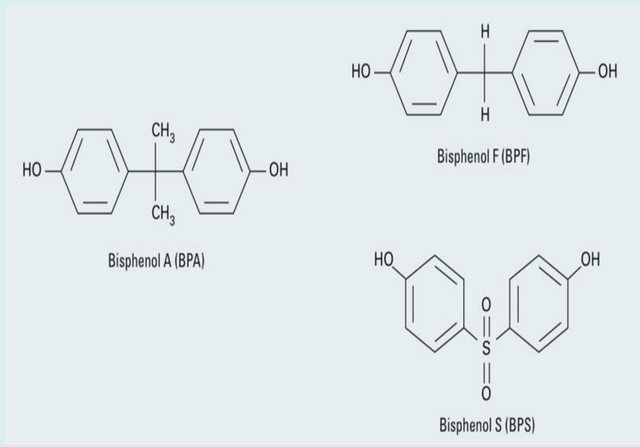

Figure 2: Chemical structures of Bisphenol A, Bisphenol F & Bisphenol S

Results

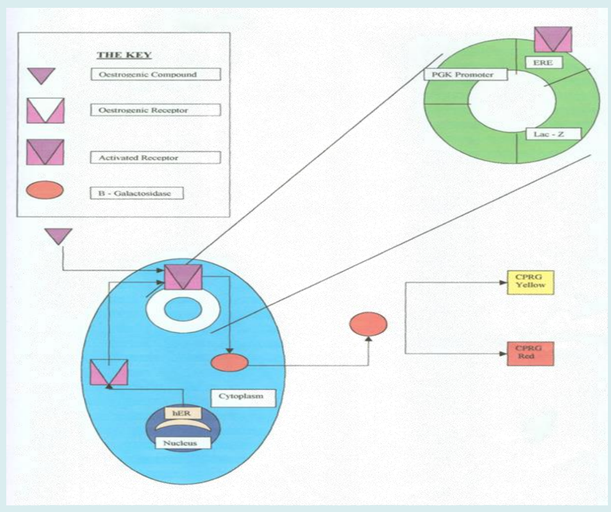

Figure 4: Schematic diagram of the oestrogen-inducible expression system in yeast obtained from Glaxo

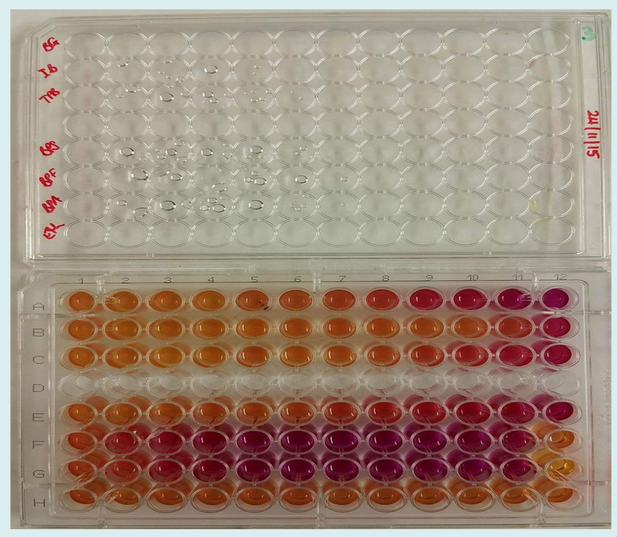

Figure 5: Oestrogenicity of water bottle extractions and standards in the YES Assay

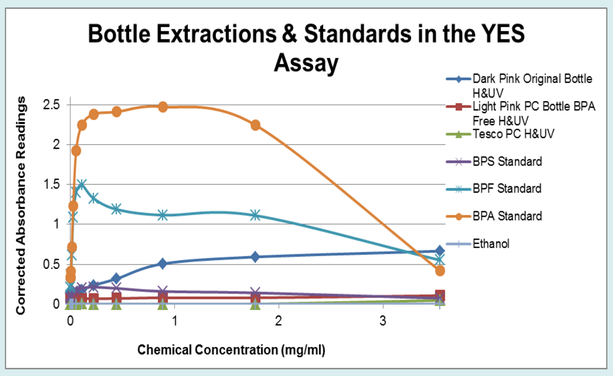

Figure 6: Oestrogenicity of BPA, BPS , BPF and water bottle extractions in the YES Assay

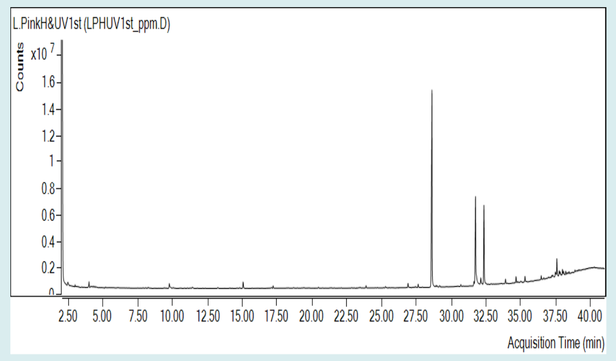

Figure 7: Chromatogram of Light Pink Polycarbonate Bottle labelled as BPA-Free

Methodology

Preparation of samples for solvent extraction

Plastic drinking bottles were treated to conditions representative of normal consumer usage. Liquid-liquid solvent extractions were performed and all extracted samples were dried under a steady stream of nitrogen gas and reconstituted in sterile molecular grade ethanol as needed.

Yeast Oestrogen Screen (YES) Assay

The yeast oestrogen screen, previously described by Routledge and Sumpter 1996 [3], was used to assess the oestrogenic potency of various bisphenol chemicals and extractions from reusable water bottles. The YES Assay is a colorimetric assay which is comprised of a yeast strain that is stably transfected with the human estrogen receptor (hERα) gene and an expression plasmid which contains oestrogen response elements (ERE) that control the β-galactosidase-encoding reporter lac-Z.

Gas Chromatography/Mass Spectrometry

Samples were analysed using a Agilent 7890B with MSD attached. A HP5 non-polar column was used. All injections were carried out using an auto sampler with an injection volume of 0.1µl for each solution.

Discussion & Conclusion

To date 28 reusable water bottles have been exposed to various conditions including heat, cold and UV light. Extractions from these bottles were analysed through the YES Assay for oestrogenicity. In total 29% of extractions produced a positive oestrogenic response and 79% produced a negative response in the YES Assay. Analysis using GC/MS was carried out to identify the compounds present in the water bottle extractions. A retention time of 28.6mins was obtained for a standard solution of BPA analysed using GC/MS. In total 25% of extracted water samples examined using GC/MS were deemed to contain BPA, of which 5 of these bottles were labelled as BPA Free. Three of the bottles analysed produced negative responses for oestrogenicity in the YES Assay, therefore indicating that there may be a limit of detection associated with the YES Assay which must be considered. The safety concerns that surround BPA and the substitute bisphenols are increasingly controversial. BPA and phthalic acid esters are known to possess oestrogenic activity albeit weakly, they therefore have the potential for low level endocrine disruption. Exposure to these detrimental chemicals occurs numerous times throughout the day through the ingestion of food and beverages which are contained within plastic packaging. Ongoing studies are underway on a range of available drinking water bottles to ensure “BPA-Free” means BPA-Free and that the plasticisers being substituted for BPA are equally not as oestrogenic, mutagenic or carcinogenic Further studies are needed in order to obtain a better understanding of the release of BPA and other bisphenols from polycarbonate bottles which are exposed to real use conditions in order to more accurately identify and quantify leachates from the plastic matrix.

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