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Bioassay analysis (T47DKBluc cell line) for Estrogenic Activity Results:

Reference Test Articles for ERTA	CAS No	Approximate IC50 (M)	Results
17 β -estradiol (E2)	50-28-2	-11.5	Strong Agonist
ICI-182,780 (ICI)	129453-61-8	-9.0	Strong Antagonist
Bisphenol A	80-05-7	-6.5	Agonist
Methoxychlor	72-43-5	-5.0	Mild Agonist
Client Sample Submitted for Testing	CAS No	Approximate IC50 (M)	Results
PLA Containers	NA	NA	No measurable activity

Notes on Sample Submitted for Estrogen Testing: Chemicals that mimic or antagonize the actions of naturally occurring estrogens are defined as having estrogenic activity (EA), which is the most common form of endocrine disruptor activity. There are currently several methods being used to assess EA activity but no standard method is currently approved for testing plastics for EA. Recently several stable cell lines have become available that specifically express the estrogen receptor (ER). Chemicals having EA typically interact with one or more of the classical nuclear ER subtypes: ER α or ER β . The T47D-KBluc cell line developed by the U.S. EPA is a commercially available cell line that expresses both ER α and ER β subtypes. It has been well characterized for use with endocrine disrupting chemicals and it was selected for use in this study.

Plastic test article was cut into approximately 5mm squares and incubated with cellular media for 120 and 240 hours at 40^oC. Media without plastic were incubated concurrently for comparison. After incubation was complete the media were separated from the plastic and tested for EA in the presence of the T47D-KBluc cells. Several controls were also tested to show the cells responded appropriately to ER agonists or antagonists that may be present in the media. The plastic test article extracts showed no EA after incubation compared to the non-plastic incubation controls or reference controls.

There was no measurable EA in the T47D-KBluc cell model under the experimental conditions outlined in the report. (Please see Report for further details)



Study No. 9342-101118 Addendum to Report

Respectfully Submitted for Cyprotex, US, LLC.

By David H. Blakeman 18 Jul 2014

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