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January 13, 2011

TSCA Confidential Business Information Center (7407M)
EPA East - Room 6428 Attn: FYI
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-0001



Subject: TSCA 8(e) Notice – “For Your Information” (FYI) submission on In vitro Endocrine Assays

Dear Section 8(e) Coordinator:

In accordance with EPA’s March 16, 1978, Policy Statement on Section 8(e) reporting under the Toxic Substances Control Act (TSCA), and the EPA’s June, 1991 TSCA Section 8(e) Reporting Guide, Intertek Corporation wishes to bring to the attention of the Environmental Protection Agency the results of endocrine screening assays conducted for a number of chemicals. While we have not determined that the information supports a conclusion that the chemicals pose a substantial risk of injury to human health or the environment, we feel it is necessary to submit these test results in view of the Agency’s current concern about chemical-induced endocrine disruption.

This study was performed to screen a diverse set of substances for estrogenic and androgenic activity. The assays followed the procedures by Routledge and Sumpter (1996). The test also incorporated an assessment of anti-estrogenic and anti-androgenic activity. The assays were performed using a 96-well microtitre plate serial dilution procedure. Dilutions of the test substance in the range 100 mg/L – 50 µg/L were incubated in the presence of the recombinant yeast strains, *Saccharomyces cerevisiae* hER, containing the human estrogen receptor gene and *Saccharomyces cerevisiae* hAR, containing the human androgen receptor gene. Two positive reference substances, 17-β-estradiol and dihydrotestosterone, were tested in this study. High levels of estrogenic and androgenic activity were demonstrated by these positive reference substances, consistent with the responses expected for these substances. Detection of estrogenic and androgenic activity (binding of the test substance to the human receptor genes) was indicated by the activation of a reporter gene (*lac-Z*, encoding the enzyme β-galactosidase) and was measured by photometric measurement of the conversion of the chromogenic substrate, CPRG, from yellow to red. Detection of anti-estrogenic or anti-androgenic activity was performed by incubating the dilutions of the test substance in the presence of a set concentration of either 17-β-estradiol or dihydrotestosterone and observing any reduction of the photometric measurement. Results were depicted graphically by plotting corrected absorbance values at 540 nm against test

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concentration for each test substance and reference substances. A summary of the findings is presented in Table 1 and a list of the list chemicals is shown in Table 2.

In a first series of tests, two test substances, 4,4-Biphenol and Bisphenol A, demonstrated significant estrogenic activity as has been reported by other researchers. One test substance mixture, 2-methyl-1,3-propanediol – 4-hydroxyphenylacetic acid (HPAA) reaction products demonstrated significant androgenic activity. This test substance mixture also demonstrated a clear anti-estrogen effect.

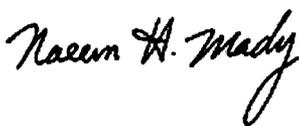
Seven test substances in this series showed a combination of anti-estrogenic and/or anti-androgenic activity ranging from slight effects to marked reductions in receptor binding at high test substance concentrations. These were HPAA, Isosorbide-HPAA reaction products, Tricyclodecanedimethanol (TCDM)-HPAA reaction products, 1,3-phenylene-bis(2-oxazoline), Cardanol, Euganol and 1,4-Cyclohexanedimethanol (CHDM)-HPAA reaction products.

In a second series of tests, three test substance mixtures demonstrated estrogenic activity. 2,2,4,4-Tetramethyl-1,3-cyclobutanediol – 3-Hydroxybenzoic acid (3-HBA) showed a significant estrogenic response at 3 days of incubation. TCDM-3-HBA and 2,2,4,4-Tetramethyl-1,3-cyclo-butanediol-HPAA showed weaker estrogenic responses that only developed during the extended incubation up to 7 days. In the assessment for anti-androgenic activity, each of these test substance mixtures showed some reduction of the anticipated androgen response in the presence of the dihydrotestosterone reference substance. These test substance mixtures did not show anti-estrogenic activity.

One test substance mixture, CHDM/3-HBA, showed reduction of the anticipated estrogen and androgen responses in the presence of the respective reference substances. However, these observed anti-estrogenic and anti-androgenic responses may be attributable to toxicity of the test substance mixture towards the yeast assay organisms, evident from reduction of the absorbance curves below the ethanol solvent blank at high test substance concentrations.

The *Saccharomyces cerevisiae* hER – hAR system is a useful, cost-effective screening tool which we believe the Agency should include among the recommended *in vitro* assays for endocrine disrupter screening. If you have any questions, please contact me at 201-952-8110, or email at: naeem.mady@intertek.com

Very truly yours,



Naeem Mady
Vice President, Regulatory Services

Table 1. Results of Yeast Screening Assays for Endocrine Response

Test substance	Endocrine response ¹			
	Estrogenic	Anti-estrogen	Androgenic	Anti-androgen
4-hydroxyphenylacetic acid (HPAA)	-	(+)	-	(+)
2-Methyl-1,3-propanediol-HPAA Reaction Products	-	(+)	+	(+)
4,4-Biphenol	+	-	-	-
Bisphenol A	+	-	-	-
Eugenol	-	(+)	-	(+)
Tricyclodecanedimethanol (TCDM)- HPAA Reaction Products	-	(+)	-	(+)*
Isosorbide- HPAA Reaction Products	-	(+)	-	-
1,4-Cyclohexanedimethanol (CHDM) / HPAA Reaction Products	-	(+)	-	(+)
Cardanol	-	(+)*	-	(+)
1,3 phenylene bis (2-oxazoline)	-	(+)	-	(+)*
CHDM / 3-Hydroxybenzoic acid (3-HBA) Reaction Products	-	(+)*	-	(+)*
TCDM /3-HBA	+ ¹	-	-	(+)
2,2,4,4-Tetramethyl-,3-cyclobutanediol / 3-HBA Reaction Products	+	-	-	(+)
2,2,4,4-Tetramethyl-1,3-cyclobutanediol / HPAA Reaction Products	+ ¹	-	-	(+)

+ Positive response

¹ Weak positive response that developed between 3 to 7 days incubation

- Negative response

(+) Evidence of anti-oestrogenic/anti-androgenic activity

* Test substance exhibited toxicity towards the yeast assay organism (this can contribute/appear as an anti-'endocrine' response).

¹ The testing was conducted for Intertek by Huntingdon Life Sciences, Cambridgeshire, UK.

Table 2. Test Substances

Chemical Name	CAS No.
4-Hydroxyphenylacetic acid (HPAA)	99-96-7
2-Methyl-1,3-propanediol	2163-42-0
4,4-Biphenol	92-88-6
Bisphenol A	80-05-7
Eugenol	97-53-0
Tricyclodecanedimethanol (TCDM)	26160-83-8
Isosorbide	652-67-5
1,4-Cyclohexanedimethanol (CHDM)	105-08-8
Cardanol	37330-39-5
1,3-Phenylenebis(2-oxazoline)	34052-90-9
3-Hydroxybenzoic acid (3-HBA)	99-06-9
2,2,4,4-Tetramethyl-1,3-cyclobutanediol	3010-96-6

References

ROUTLEDGE, E. J. & SUMPTER, J. P. 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Environmental Toxicology and Chemistry*, 15 (3), 241-248.

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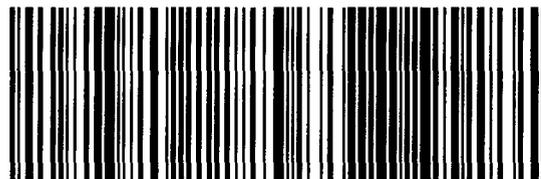
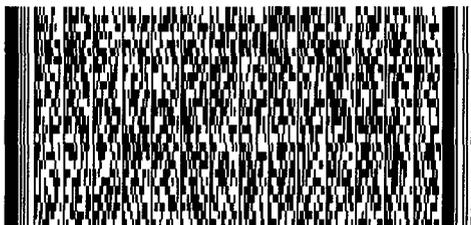
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