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February 25, 2013

Via Federal Express

Document Processing Center (Mail Code 7407M)
Room 6428
Attention: 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
1201 Constitution Ave., NW
Washington, DC 20004

8EHQ-12-18717

Dear 8(e) Coordinator:

8EHQ-12-18717A
Generic Name: Substituted Nitrogen Containing Heterocycle

This letter is to inform you of the results of the human adrenocortical carcinoma (H295R) cell Steroidogenic Assay with the above referenced R&D test substance. The test substance is an R&D substance and to the best of our knowledge not on the public inventory.

The human adrenocortical carcinoma (H295R) cell Steroidogenic Assay was utilized to determine the potential for the test substance to interact with the steroidogenic pathway. H295R cells, cultured in 24-well plates, were incubated with the test substance at concentrations of 100, 10, 1, 0.1, 0.01, 0.001, or 0.0001 μM in triplicate for 48 hours. The stock solution vehicle was dimethyl sulfoxide (DMSO). Positive controls, a known inducer (forskolin) and inhibitor (prochloraz) of testosterone and 17ß-estradiol production, respectively, were also conducted to confirm the test performance. After 48 hours in culture, media was collected for analyses of testosterone and 17ß-estradiol concentrations using HPLC/MS/MS. As expected, forskolin showed effects consistent with testosterone and 17β estradiol synthesis induction, and prochloraz showed effects consistent with testosterone and 17β estradiol synthesis inhibition. The test substance caused statistically significant reductions in testosterone or 17β-estradiol synthesis relative to the vehicle control in 3 independent experimental runs only at the highest treatment dose of 100μM.

Sincerely,

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Company Sanitized