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Sent: Mon, Mar 3, 2014 11:00 am

Subject: Submission of a genomics evaluation of styrene

On behalf of Jack Snyder, Executive Director of the Styrene Information and Research Center (SIRC), I am submitting the following communication to the NRC Panel on Styrene regarding a just-completed genomics evaluation of styrene.

**The Styrene Information and Research Center (SIRC) has just completed a genomics evaluation of styrene at The Hamner Institute (formerly CIIT). While we plan to submit this for publication, we have attached the full study report here which we just received because we know that your Panel is working against a tight deadline.**

**SIRC has submitted to the Panel a number of publications on the mode of action (MOA) for the formation of lung tumors in mice exposed to styrene. Based on the available evidence, SIRC has hypothesized that styrene is metabolized in mouse lung club (formerly known as Clara) cells by CYP2F2 to ring-oxidized metabolites, which are lethal to the club cells. The terminal bronchiolar stem cells respond by producing more club cells to replace the dead cells. This repeated cycle of cell death and replication leads to hyperplasia and eventually tumors. SIRC hypothesized that styrene-induced mutations were not a part of this MOA because a tumor-initiation assay in strain A mice and chromosomal aberration study in the lungs of B6C3F1 mice were negative.**

**SIRC previously submitted to the Panel 2012 and 2013 publications demonstrating that the increased cell replication (as measured by BrdU incorporation) from styrene and styrene-7,8-oxide was completely eliminated in CYP2F2 knockout (KO) mice.**

**In the just-completed Hamner Institute's genomics study, C57BL/6(WT), CYP2F2 KO and CYP2F1 TG (2F2KO 2F1, 2A13, 2B6 transgenic) mice were exposed to 0, 40 or 120 ppm styrene by inhalation for 6 hrs/day for 1 or 4 weeks (5 days/week). BrdU incorporation was determined in wild-type (WT) mice exposed to 0 or 120 ppm for 5 days. WT mice exposed to 120 ppm had a 7 fold increase in BrdU labeling in the terminal bronchioles. This response is equivalent to previous studies where styrene was administered at 200 mg/kg/day by oral gavage or ip injection for 5 days.**

**After 5 days of exposure to 40 ppm styrene, about 500 genes in WT mice had significantly different expression levels from the WT controls. In WT mice exposed for 5 days to 120 ppm, about 700 genes were different. Gene ontology enrichment of the 1 week WT genes showed a strong dominance of cell cycle regulatory pathways. Pathways for cholesterol synthesis were also enriched in WT mice exposed to 40 (but not 120 ) ppm for 5 days. Fewer genes were differentially expressed after 4 weeks. There was no indication of differential expression of genes related to DNA damage or repair, as would be expected in a mutational MOA.**

**There were no gene changes in kKO mice exposed to 40 or 120 ppm styrene. One gene related to circadian rhythm was differentially expressed in transgenic mice.**

**This study has provided valuable support for the hypothesized CYP2F2 MOA:**

- 1. Increased BrdU labeling by inhalation at concentrations that caused tumors;**
- 2. Demonstration of gene enhancements regulating cell cycle in WT mice;**
- 3. Lack of gene responses indicative of a mutagenic MOA; and**
- 4. Lack of gene enhancements in the absence of CYP2F2 metabolism.**

**If you or members of the panel have any questions, please do not hesitate to contact us.**

Chuck Elkins, Consultant to SIRC