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The Styrene Information & Research Center

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Dr. Michael D. Shelby
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RE: Expert Panel Report on Styrene, Request for Public Comment Per 70 *Federal Register* 42,064 (July 21, 2005)

Dear Dr. Shelby:

The Styrene Information and Research Center (SIRC)[1] appreciates the opportunity to provide comment on the National Toxicology Program's (NTP's) Center for the Evaluation of Risks to Human Reproduction (CERHR) final *Expert Panel Report on Styrene* ("Report").

SIRC provided comments on the Expert Panel draft Report, and attended and commented at the June 1-3, 2005 public meeting of the Panel. We complement the NTP CERHR for a comprehensive documentation and assessment of the potential for styrene to affect human reproduction and development. SIRC wishes to continue to assist the CERHR in ensuring the accuracy and proper context of the information reviewed and summarized in the Panel's final Report. Accordingly, SIRC respectfully submits the following comments on the Report on styrene.

The principal point we wish to convey in these comments is that we do not concur with the inclusion of the following statement in the Panel's summary conclusion:

> "There is suggestive evidence that exposure to styrene in occupational settings is associated with increased serum prolactin and depletion of peripheral blood dopamine metabolizing enzyme activities relative to unexposed individuals. The interpretation of the clinical relevance of these

[1] The Styrene Information and Research Center's (SIRC's) mission is to evaluate existing data on potential health effects of styrene, and develop additional data where it is needed. SIRC has gained recognition as a reliable source of information on styrene and helping ensure that regulatory decisions are based on sound science. For more information, visit http://www.styrene.org.

effects is uncertain because the average elevation was not outside the normal range and because menstrual function and other reproductive endpoints were not evaluated in these studies."

In light of the lack of clinical effects, questionable increases of prolactin within the normal range, and lack of clear mode of action data, we believe the Expert Panel placed unwarranted emphasis on the prolactin findings. Accordingly, SIRC urges that CERHR's own Brief on styrene, to be included in the final Monograph, should not include reference to styrene affecting prolactin, for the reasons outlined below.

Prolactin is secreted by the pituitary and it has well described influences on the gonads, so that when it is excessively secreted in females it produces abnormal or absent menses, infertility, thinning of the bones due to estrogen deficiency, and non-pregnancy related lactation. In males it produces low testosterone levels and may cause thinning of the bones. These abnormalities occur with prolactin levels above the upper range of normal (e.g. greater that 20 ng/ml in women and 15 ng/ml in men), and often require levels greater than 100 ng/ml.

Four papers report higher levels of prolactin in styrene exposed workers than in the controls used in those studies (references #108, 109, 110 and 29 in Report). Although there were different first authors, three of these (#108, 109, 110 in Report) came from Dr. Mutti's laboratory.

Reference #108. Mutti *et al* (1984) reported levels of 622+/-372 pM (14.6+/-8.6 ng/ml) prolactin in 30 styrene-exposed women (average exposure 130 ppm) compared to 313 +/-175 pM (7.2+/-4.0 ng/ml) for 30 controls. The values for the styrene-exposed women are within the normal range and the paper does not describe whether there were any clinical abnormalities that could be related to increased prolactin. Further, although blood samples were collected from all women between 8 and 9 a.m., there is no indication that dietary factors that can affect prolactin levels were controlled for, or even ascertained. Given the lack of abnormal prolactin values, this is not evidence of a styrene-induced hyperprolactiniemia.

Reference #109. In Arfini *et al* (1987), the group's basal prolactin data are not easily seen in Figure 1. However it is stated the basal prolactin levels of the styrene group were minimally increased over the controls, but not into the abnormal range. The CERHR Report estimates those as 20 ng/ml for the styrene-exposed women and 12 ng/ml for the controls. The investigators then administered a supraphysiologic dose of thyrotropin-releasing hormone (TRH) and measured prolactin 10 minutes later. They reported a greater increase in prolactin in the exposed workers than the controls. Such stimulation does not occur in the workplace and this has no relevance to workplace styrene exposures. Further, the editors of the journal provided a commentary on this article which stated: "First, the baseline levels of prolactin were normal in the exposed group as well as the unexposed group. Second, there has been no clear demonstration of a functional role for TRH stimulation of prolactin in the human. Third, the dose of TRH stimulation used is clearly a supraphysiologic dose. Fourth, it is difficult to assess the importance of TRH stimulated prolactin as an indicator of intrinsic dopaminergic tone as opposed to basal prolactin levels." The authors reported that

one woman had amenorrhea for more than one year during exposure, had high response to TRH stimulation, and a normal cycle returned within 40 days of stopping exposure. It should be noted that this woman had a normal baseline prolactin level (18.7 ng/ml). Hyperprolactinemia was not the cause of her amenorrhea. Thus the basal prolactin levels in this study are normal and the role of TRH stimulation is questionable.

Reference 110. Bergamaschi *et al* (1997) reported prolactin levels of 8.9+/-1.9 ng/ml for 33 exposed males and 12.6+/-1.6 ng/ml for exposed females, compared to 6.0+/-1.6 ng/ml and 9.3+/-1.6 ng/ml for unexposed males and females, respectively. The authors said that more styrene exposed workers had prolactin values outside the range of normal but didn't provide the data for evaluation. The paper states that blood samples were collected from the exposed workers between 8 a.m. and 9 a.m., but does not state when they were collected from the controls. Prolactin levels are higher during the night and early morning, and if the controls were obtained later in the day there would be a strong bias for prolactin to be higher in the exposed group, all of whom were studied between 8 a.m. and 9 a.m..

Platelet monoamine oxidase activity and plasma dopamine-beta-hydroxylase (DBH) activity were assessed as surrogate markers of CNS dopamine metabolism. The authors found a negative relationship between plasma DBH and urinary MAPGA, metabolites of styrene. We do not agree with the authors' rationale of using platelet rich plasma for determining monoamine oxidase activity and plasma beta-hydroxylase activity, which they have performed in these studies. They are using changes in these levels as surrogate markers for what is happening with hypothalamic dopamine metabolism. We are unaware of any data which support using peripheral levels to draw conclusions concerning the central control of dopamine / prolactin regulation.

Reference 29. Luderer *et al* (2004) assert that for every 10-fold increase in styrene concentration there is a two-fold increase in prolactin, based on assays in 259 men and 43 women exposed to styrene in reinforced plastics manufacture. The mean values were reported as 10.4 (0.4 SEM) ng/ml for males and 12.7(1.0) ng/ml for exposed females; there were no controls in this study. The authors do not state how many, if any, workers were above the upper limits of normal.

The authors stated that blood for styrene and prolactin was collected within 10 minutes of the end of the shift. The specimens were handled appropriately and sent to reputable laboratories for measurement. Some error may have been added to the data by several design issues. Prolactin has a diurnal variation with much greater levels occurring at night. Since many of the workers were involved in shift work, the samples drawn from those working the evening or graveyard shift have an important bearing on the results. Thus, workers from the later shifts would be expected to have higher values than workers from the day shift. Additionally, it appears food ingestion was not controlled for. Prolactin can be acutely stimulated by the protein content of food (i.e. certain amino acids). Additionally, certain individuals may have had a prolactin disorder at baseline unrelated to styrene. Also a few may have had macroprolactinemia; a term in the medical literature that refers to an elevated serum prolactin level that is measured by the standard assays, but is about 6 times greater in size and is biologically less active. Its presence is of little clinical significance.

One study (#127 in Report) reported decreased brain dopamine in rabbits exposed to styrene as an explanation for increased prolactin in human studies, while another one in rats (#125, 126 in Report) reported no effect on brain dopamine or serum prolactin in rats.

Mutti *et al* (1984) reported that decreased tuberoinfundibular dopamine levels were observed in rabbits exposed by inhalation to 1500 ppm for 12 hours/day for 3 or 7 days (#127 in Report). Decreased hypothalamic dopamine levels were also observed in rats treated orally with styrene at 500 mg/kg bw/day for 13 weeks (#128 in Report). Neither of these studies reported serum prolactin levels. Conversely, Jarry *et al* (#125 & 126 in Report) reports that styrene exposure at 150, 500 and 1500 ppm in Wistar rats for 6 hours/day for 5 consecutive days did not produce any significant effect on hypothalamic dopamine levels at any dose, and did not significantly alter peripheral blood prolactin levels.

In the Jarry *et al* paper they point out the fact that serum prolactin was not measured by Mutti *et al,* which raises the question if stress might have occurred in the handling of the animals, contributing to the changes in dopaminergic activity reported. The Jarry *et al* paper raises a question about the underlying postulated mechanism of styrene's effect on serum prolactin levels proposed by Mutti *et al*.

<u>Reports on Human menstrual cycles</u>

The CERHR Report concludes Lemasters (#78 in Report) found no effect of styrene exposure on menstrual cycle in women exposed for several years at up to 85 ppm styrene, and that Härkönen and Holmberg (#114 in Report) observed no effect of styrene exposure on irregular menstruation or changes in menstrual patterns in reinforced plastics workers. In contrast, Cho *et al* (#107 in Report) reported that exposure to organic solvents, including styrene, is associated with menstrual periods longer than 35 days, which is their definition of oligomenorrhea. In the no-exposure-to-solvent group there was an 8.5 percent frequency of oligomenorrhea, and in women exposed to styrene they found a 14.5 percent frequency of oligomenorrhea. More work years of exposure were associated with more oligomenorrhea.

One major concern with this study has to do with their method for determining whether oligomenorrhea was present. They used a 12-month recall instrument in this cross-sectional study, but do not present any validation of the accuracy of this method for retrospectively determining menstrual history. For example, studies which have used food frequency questionnaires to determine 12-month intake of general food groups have correlated poorly with 7-day recall instruments. These women might easily remember amenorrhea (absent menses for more than 90 days) over the previous 12 months, but whether they would remember oligomenorrhea (an average cycle length greater than 35 days) is problematic, unless it was consistent during the year. Often it can be intermittent or the person may never have had regular 28 day cycles. Secondly, there was no indication what their menstrual status was before they were exposed to organic solvents. Thirdly, only 3 of 276 women exposed to styrene were exposed <u>only</u> to styrene. Furthermore, their styrene exposure levels were quite low (<1 ppm) compared to exposure levels in Lemasters or Härkönen and Holmberg.

Clinical Significance

It is important to recognize that the extant literature on prolactin physiology does not support a role for minimal prolactin increases on any untoward physiology in humans. Therefore it is not possible to make a convincing argument that the increase in prolactin levels harmed these individuals. Individuals can have elevated prolactin for years, much higher than those sustained by these workers, without any observable or measurable health effects.

Is there a biological basis for styrene influencing prolactin levels? It is proposed by Mutti *et al* that styrene exposure increases dopamine catabolism in the hypothalamus, leading to less inhibition of prolactin release by endogenous dopamine. Because dopamine is the primary regulator of prolactin release, there is reason to believe that if styrene causes a decrease in brain dopamine, prolactin levels could increase in humans. Mutti *et al* reported decreased levels of dopamine in rabbits exposed to styrene, but no changes in hypothalamic dopamine were found in rats by another investigator (Jarry *et al*). Thus, the underlying mode of action is questionable.

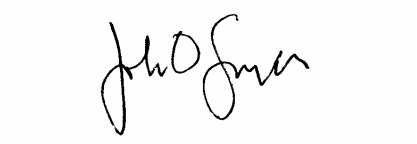
Conclusions about the effect of styrene on serum prolactin levels in humans.

- Styrene when inhaled in the workplace may produce a mild increase in serum prolactin levels, but the serum prolactin levels associated with styrene inhalation are rarely outside the range of normal.
- There is no evidence that long term exposure to styrene results in any of the clinical features of increased prolactin levels in woman. This includes galactorrhea (breast milk and lactation not associated with pregnancy) and/or amenorrhea (absent menses for at least 90 days). Clinical findings would not be expected with such minimal increases in prolactin reported in these studies.
- Most of the papers suggest that styrene does not influence menstrual regularity, which is a common side effect of increased serum prolactin levels. The one paper (Cho *et al*) that suggested increased menstrual cycle length has serious design flaws.
- The few subjects noted in these reports with prolactin levels outside the range of normal have not been examined for a wide variety of etiologic considerations which would have to be considered before styrene could be implicated in either the elevation of prolactin and/or the clinical manifestations.
- Many of the studies have design issues, such as sampling times and relationship of the blood sample to previous meals (food intake increases serum prolactin levels), which could bias the results.

As noted previously, in light of the lack of clinical effects, questionable increases of prolactin within the normal range, and lack of clear mode of action data, we believe that the Panel should not have suggested this as a concern in the summary conclusions of the final Panel Report. Accordingly, we urge CERHR not to include this reference to styrene affecting prolactin in its Brief on styrene reproductive and developmental toxicity.

We hope that our comments will be helpful to the CERHR in crafting its Brief document on the Styrene Expert Panel Report, and appreciate your consideration of them. SIRC would be pleased to provide additional information or discuss any questions that these comments might raise.

Sincerely,



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