March 20, 2012

Via email

Ms. Cynthia Oshita
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Re: OEHHA's Notice Requesting Information Relevant to the Assessment of the Evidence of Developmental and Male and Female Reproductive Toxicity for Xylene (CAS RN 1330-20-7), January 2012

Dear Ms. Cynthia Oshita:

The American Chemistry Council's Toluene and Xylene Panel (Panel) appreciates the opportunity to provide the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA) with these comments on your January 20, 2012 notice requesting information relevant to the assessment of the evidence of developmental and male and female reproductive toxicity for xylene (CAS RN 1330-20-7). According to the notice, OEHHA has selected xylene for the Developmental and Reproductive Toxicant Identification Committee's (DARTIC) review for possible listing under Proposition 65 as a developmental and reproductive toxicant.

The following highlights key evidence in the Saillenfait et al 2003, Hass et al 1995 and1997, Ungváry and Tátrai 1985, and Taskinen et al 1994 studies identified by OEHHA in their preliminary toxicological evaluation of evidence on the development and reproductive toxicity of xylene (preliminary toxicology evaluation of xylene), as well as, the Xylene Teratology Study in Rats (API,1978). The information provided herein is critical to the assessment of the reproductive and developmental endpoint for xylene; thus, it should be included in OEHHA's draft hazard identification materials for xylene to ensure that the materials for review are complete and scientifically sound.

A. Animal Developmental and Reproductive Toxicity Studies

i. API 1978

The study the American Petroleum Institute (API 1978) performed at Litton Bionetics was not included in OEHHA's preliminary toxicology evaluation of xylene. This study concluded that

no maternal or developmental effects were observed following exposure of pregnant CD rats to 0, 100, or 400 ppm mixed xylenes (52% *m*-, 11% *o*-, 0.31% *p*-xylene, and 36% ethylbenzene), 6 hours/day, during GD6-15. The NOAEL for maternal and developmental effects was 400 ppm (highest concentration tested). In the teratogenicity portion of the 1-generation reproduction study, one-half of the control and 500 ppm high dose pregnant females were euthanized on GD21 for developmental toxicity evaluation. Animals had been exposed to mixed xylenes for 151 days prior to and during mating, and GD1-20. No effects were observed on maternal body weight, food consumption or utilization, or postmortem examination. No statistically significant differences were reported for mean number of corpora lutea, implantations, live fetuses, live fetus/implantation, or fetal sex ratios. A slight increase in mean number of resorption sites (1.6 vs. 1.2 in controls) was not statistically significant. Fetuses from exposed dams had slightly higher incidence of unossified sternebrae and incompletely ossified cervical vertebral processes, reported by fetal incidence. Mean body weight of female fetuses on GD21 was marginally but statistically decreased (93% of control values), but male fetuses were comparable to controls. This marginal decrease occurring in only one sex was difficult to assess due to small sample size (12 litters compared to 20 litters in controls). The maternal toxicity NOAEL was 500 ppm and the developmental LOAEL was 500 ppm (decreased female fetal weight) with a NOAEL of 250 ppm. In the animals allowed to deliver litters, mean pup weights were statistically significantly decreased in treated litters on lactation day (LD) 4 probably as a consequence of elevated mean pup weights in the control group where the mean litter size was smaller than all treated litters in any group (9.6 live pups/litter in controls vs. 10.8-12.5 pups/litter in treated groups). Marginally decreased mean pup weight in 500 ppm (both sexes treated) litters was observed on LD21 and at 49 days of age (92% males, 93% females of control values), but no decrease in pup weights was observed in offspring of litters in which only the dam or the male parent was exposed to 500 ppm, suggesting these marginal weight decreases were not an effect of xylenes exposure. Offspring NOAEL was 500 ppm. The Panel considers these findings consistent with those reported by Saillenfait et al 2003, and indicate that mixed xylenes are not hazardous to development.

ii. Saillenfait et al 2003 and 2002

Saillenfait et al 2003 and 2002 are listed in OEHHA's preliminary toxicology evaluation of xylene. Saillenfait et al 2003 is a peer reviewed article and Saillenfait et al 2002 is only available in abstract. The Panel contacted Sailenfait who advised that the 2002 reference doesn't exist and it appears that the study referenced in the second reference is the same as the one described in the first reference. We suggest that OEHHA remove the second reference (Reprod. Toxicol. 2002;16(4):417) for future review or make it clear that this is the same study.

Furthermore, Saillenfait et al. 2003 is a well-documented and robust study. It was selected as a key study for the US EPA's VCCEP tier 1 xylene assessment (2005) and xylene REACH registration dossier submitted to ECHA (2010). It was also considered as "the most reliable

developmental toxicity assay" in ATSDR's toxicological profile for xylene (2007). Saillenfait et al. 2003 concluded that none of the substances tested (technical xylene, 3 xylene isomers, and ethylbenzene) were teratogenic up to 2000 ppm (highest concentration tested). Although a significant increase in mean percent fetuses with skeletal variations of all types and litter was seen at 2000 ppm concentrations of o- and p-xylene, and ethylbenzene, no single skeletal variation occurred at an incidence significantly higher than that in the control animals. Fetal body weight reductions occurred at 1000 and 2000 ppm which also caused significant maternal toxicity (reduced body weight gain and food consumption).

iii. Hass et al. 1995, 1997

Hass et al. (1995, 1997), while it considered only a single dose level of 500 ppm, was also a well-conducted evaluation of the postnatal development and behavior of rats exposed prenatally to mixed xylenes. There were some slight effects on learning and memory performance based on the Morris water maze in female offspring that were not seen significantly in males or in females with various toys in their cages. US EPA concluded in its xylenes IRIS assessment that the LOAEL for developmental neurobehavioral effects is 500 ppm, based primarily on the Hass 1995 study, and that the developing organism is not more sensitive than the adult to xylenes exposure (EPA 2003).

The currently available developmental neurotoxicity studies are limited by the absence of dose response data and a definitive NOAEL level. However, they provide very comprehensive evaluation of the critical endpoint of concern during a period of in utero development when the brain is rapidly developing. Hass et al. (Hass and Jakobsen, 1993, Hass 1995, Hass 1997) has done the majority of this research, conducting two separate developmental neurotoxicity analyses of mixed xylenes in rats. The first study conducted at 200 ppm (Haas and Jakobsen, 1993) was not considered by the authors to be as reliable as the second study (Haas et al., 1995) which was conducted at 500 ppm. Some animals from the second study were maintained for up to a year for additional follow–up examination (Haas et al., 1997).

The only developmental neurotoxicity evaluation from the Hass and Jakobsen (1993) study was a decrease in rotarod performance in rats exposed in utero to 200 ppm mixed xylenes. In a later publication, these researchers noted methodology concerns with the rotarod evaluation of the 1993 study. These effects were not considered treatment related because there were no effects at 500 ppm mixed xylenes when experimental conditions to reduce bias were better controlled (Hass 1995).

There were no statistically significant effects on rotarod performance at 500 ppm indicating that the investigators were unable to repeat effects on rotarod performance at 200 ppm in their earlier study (Haas and Jacobson, 1993). The mean time on the rotarod was shorter and a higher percentage of animals in the exposed group failed to stay on the rod for 30 seconds compared to

controls, with females more affected than males. The air-righting reflex was delayed by one day in exposed litters due to the ability of only 4 pups from 4 litters to right themselves. All animals were able to right themselves on postnatal day 17. No differences were observed in open field activity. Offspring from xylene-exposed rats raised in cages with various toys showed no difference in the Morris water maze compared to controls. Offspring raised in standard housing showed impaired performance at 12 weeks when a non-significant trend for increased latency in finding the platform in the beginning of the learning test was evident. The effect was attributed to a slight increase in latency during the first 2 trials of a 5-trial learning phase with no difference in learning during the last 3 trials. At 16 weeks, females also used significantly more time to find a hidden platform in the center of the pool and showed an increase in swimming length, while speed was not affected. Although these effects are statistically significant, the latency for treated females was less than 6-13 seconds which is comparable to male control values and also similar to the latency during the 5th day of the initial learning phase for all control offspring. Male offspring of exposed dams performed comparably to controls. The overall effects on developmental neurotoxicity from this study, especially noting the lack of effects in a separate group of treated females that had various toys in their cages, appear to be relatively slight effects.

In a follow-up study to assess persistence of the decreased water maze performance, these same female offspring raised in standard housing were maintained and evaluated at 28 and 55 weeks. (Hass et al, 1997). At 28 and 55 weeks, an increased latency in finding the platform in the center was not repeated. There was an effect in finding the platform when placed in a new position not earlier used on the rim (original starting point) and animals were placed in a new starting point. Increased latency again corresponded to increased swimming length as measured on the first trial but resolved itself by the second and third of three trials. No other significant differences were observed for other testing situations in the water maze and no statistically significant differences of any kind were observed at 55 weeks. Xylene exposure during development may cause subtle deficits in some learning and memory tasks of offspring, which eventually resolve in the absence of additional exposure as the animal matures. These data were limited because no dose-response can be determined and no clear effect in other neurological tests was observed (EPA, 2003). The learning and memory test involved 10 component phases each with four to five trials. While this extended evaluation may increase the possibility of detecting very subtle effects, there is also increased opportunity for statistically significant effects that may not be treatment related.

In summary, developmental neurotoxicity data have been developed on mixed xylenes, by Hass et al. (1993, 1995, 1997), in which exposure of pregnant rats at 500 ppm during most of gestation resulted in some learning and memory effects in offspring tested from 2 days to 16 weeks postnatal. These investigators questioned the significance of their own results at 200 ppm conducted in the earlier study (Hass 1993) because of the lack of control of the experimental bias and because these decreases in rotarod performance could not be replicated at the higher exposure levels of 500 ppm with improved methods (Hass 1995). A different group of

investigators from EPA Health Effects Research Laboratory demonstrated that p-xylene did not affect neurobehavior in offspring of dams exposed to 800 and 1600 ppm prenatally (Rosen 1986). Taken together, these studies provide comprehensive sophisticated evaluation of many neurobehavioral endpoints at multiple time points following exposure during the vulnerable period of rapid brain development. These slight effects were measured primarily in female offspring and were not measured in males or in females with various toys in their cages. These studies support a LOAEL of 500 ppm for developmental neurobehavioral effects and support EPA's conclusion that the developing organism is not more sensitive than the adult to xylene exposure (EPA 2003).

The Panel believes it is important to include ATSDR's assessment of Hass 1995 in the HID found in the 2007 Toxicological Profile for Xylene. ATSDR (2007) noted many limitations of this study and concluded that it is difficult to determine from this study whether xylene is selectively toxic to the fetus or the observed developmental toxicity was secondary to maternal toxicity.

iv. Ungváry and Tátrai 1985

Ungváry and Tátrai (1985) exposed pregnant CFY rats (19-23/group) to a wider range of doses - 0, 60, 440, and 780 ppm (0, 250, 1900, and 3400 mg/m3) xylenes again for 24hours/day, GD 7-15; animals were euthanized on GD 21. Pregnant CFLP mice (17-18/group) and pregnant New Zealand white rabbits (10) were exposed to 0, 115, or 230 ppm (0, 500 or 1000 mg/m3) mixed xylene 3 times for 4 hours/day intermittently or to 115 ppm o-, p-, or m-xylene on GD6-15 or 7-20, respectively; animals were sacrificed on GD 18 or 30, respectively. Although an increase in percentage of skeletal retardation (no specifics given) was reported in mouse and rat fetuses, the increases were not concentration-related, the occurrence/litter was not reported, and incidences were not statistically significant compared to controls. Rats exposed to 780 ppm had an increased percentage of dead or resorbed fetuses (13% vs. 5% in controls). Maternal toxicities in rats were reported as moderate and concentration-related. Rats exposed at all doses had approximately 30% incidence of fetal skeletal abnormalities and an average 13% weight reduction at 780 ppm. Mice had a slight increase in skeletal abnormalities and fetal weight reduction at 115 ppm for each isomer and for mixed xylenes at 230 ppm but not at 115 ppm. In rabbits, exposure to 230 ppm resulted in 3 maternal deaths, increased relative liver weight in dams, and an increased number of abortions. Exposure of pregnant rabbits to 115 ppm o-, m-, p-xylene or mixed xylenes, produced only slight decrements in fetal weight of female offspring. ATSDR (2007) noted many limitations of this study and concluded that it is difficult to determine from this study whether xylene is selectively toxic to the fetus or the observed developmental toxicity was secondary to material toxicity. The Panel places lower confidence on the value of this study in hazard assessment because the method description was inadequate to assess the validity of reported exposures, no information on the source and purity of test materials was included, non-standard mouse and rat strains were used, and reported fetal

mortality findings are inconsistent with results described by multiple sources. The Hazard Identification Document should include a description of these limitations.

v. Rosen et al., 1986

An additional developmental neurotoxicity study was conducted, by a separate laboratory, on p-xylene in rats (Rosen et al., 1986). In this study, the Chernoff/ Kavlock screen was used on exposed pregnant Sprague Dawley rats (25/group) to 0, 800 or 1600 ppm para-xylene (99% pure) on GD7-16. No adverse effects were observed on litter size or pup weight at birth or on postnatal day 3. No effects were seen on central nervous system (CNS) development measured by acoustic startle response on postnatal day 13, 17, 21 and 63, or the figure 8 maze activity on days 22 and 65; or on pup growth rate. The only effect of exposure was a significant decrease in maternal body weight in 1600 ppm dams (74% of control values). The maternal LOAEL was 1600 ppm; the developmental neurotoxicity NOAEL was 1600 ppm.

B. Epidemiologic Development and Reproductive Toxicity Studies

OEHHA identified a number of epidemiological studies of xylene and reproductive toxicity in its May 2011 summary of the literature. These studies have various strengths and weaknesses, which should be described in detail in the Hazard Identification Document (HID).

For example, Taskinen et al. (1994) describes the methods and results of a case-control study designed to examine the relationship between (1) working in a laboratory and (2) exposure to specific chemical substances commonly encountered in laboratories and spontaneous abortion among Finish women and congenital malformations and birth weight among their offspring. It is important that the HID include the strengths and weakness of this epi study including the following areas.

Numerous methodological limitations present in this study compromise the ability to draw conclusions, regarding the reported associations between specific chemicals and classes of chemicals encountered in laboratory-based work and spontaneous abortion, with conclusions regarding xylene particularly weak. Primary areas of concern include: (1) poor characterization of the study population and lack of eligibility criteria for participation (e.g. non-smokers; geographic location); (2) lack of any information on the distribution of potentially important covariates/confounders (e.g. smoking, alcohol consumption, chronic illness, coffee consumption, medication use) the pattern of which could be explanatory in the observed results; (3) lack of explicit information on employment and how it was included in the statistical analysis (e.g. current v. formerly employed, duration of employment); and most importantly (4) exposure assessment is crude: (a) data are self-reported or based on professional opinion, creating a high probability of exposure misclassification; (b) exposure to mixtures and the inability to separate xylene (only 2 cases and 2 controls were exposed to xylene alone) invalidates statements made

about the independent effects (ie. OR) of this chemical on spontaneous abortion or any of the other outcomes; (c) there is no clear exposure referent (who are the unexposed and how was this determined?); and (d) there is a very, very long (potentially decades) time period between when a women had a spontaneous abortion (e.g. 1973) and when the survey was mailed (time not reported). Such a lag in time period further complicates the reporting of exposure when recall is known to decay as time passes. In addition, the woman may have had subsequent pregnancies potentially further dimming the memory of a prior pregnancy.

This study was not designed to answer the question of whether xylene is associated with increased odds of adverse pregnancy outcomes, and as such, it contributes little if any information on the subject. Appropriately, the authors invoke caution in interpreting the results with respect to individual chemicals examined in this study. OEHHA should take particular note that the authors themselves noted: "Because of the multiexposure situation, the results concerning individual chemicals have to be interpreted cautiously."

C. Conclusion

In summary, it is imperative that OEHHA's hazard identification materials for xylene include the evidence discussed in this document to ensure that the materials for review are complete and scientifically sound. Again, thank you for the opportunity to provide these comments. If you have any questions, please do not hesitate to contact me at (202) 249.6716 or at Leslie_Berry@americanchemistry.com.

Sincerely,

Leslie Berry

Leslie Berry
Toluene and Xylene Panel Manager,
American Chemistry Council

Reference List

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