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Via email

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Re: OEHHA's Draft Hazard Identification Document for Xylene

Dear Ms. Cynthia Oshita:

The American Chemistry Council's Toluene and Xylene Panel[1] ("Panel") appreciates the opportunity to provide these comments on the Office of Environmental Health Hazard Assessment ("OEHHA") Draft Hazard Identification Document[2] ("Draft HID") for xylene to the Proposition 65 Developmental and Reproductive Toxicity Identification Committee ("DARTIC"). The following comments on the Draft HID show that xylene does not meet the Proposition 65 criteria for listing: specifically, xylene has not "been clearly shown through scientifically valid testing according to generally accepted principles to cause … reproductive toxicity."

Based on the current xylene dataset it is important to recognize that no authoritative body has formally identified xylene as causing reproductive toxicity. Thus, xylene has been placed before the DARTIC to address the question of whether xylene has "been clearly shown through scientifically valid testing according to generally accepted principles to cause … reproductive toxicity."

I. Summary

The Draft HID[3] identifies a large number of epidemiological and animal studies that have evaluated the potential developmental toxicity, male reproductive toxicity and female reproductive toxicity of xylene. Many of these studies have significant limitations and shortcomings that undermine their usefulness for purposes of Proposition 65; this concern is also reflected in the Draft HID as the document notes that the quality and reliability of these studies varies. In order for the DARTIC to recommend listing xylene pursuant to Proposition 65, it must be "clearly shown through scientifically valid testing according to generally accepted principles to cause" reproductive toxicity.

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[1] The members of the ACC Toluene and Xylene Panel are CITGO, Chevron Phillips, Exxon Mobil Chemical Company, Flint Hills Resources, and Total.
[2] OEHHA (2012) Draft Hazard Identification Document, Evidence on the Developmental and Reproductive Toxicity of Xylene, September, 2012.
[3] OEHHA (2012) Draft Hazard Identification Document, Evidence on the Developmental and Reproductive Toxicity of Xylene, September, 2012.

The overall scientific evidence in humans and animals is insufficient to demonstrate that xylene has been clearly shown to cause developmental or reproductive toxicity.

Human Evidence of Developmental or Reproductive Toxicity: The epidemiological studies do not provide convincing evidence to support a causal relationship between exposure to xylene and any endpoint of reproductive toxicity, including developmental toxicity, male reproductive toxicity, and female reproductive toxicity. The epidemiological studies of xylene are of limited value in assessing the potential for xylene to cause reproductive toxicity since there are no epidemiological studies of exposure to xylene alone. All of the epidemiological studies of xylene involve populations with concomitant exposures to other solvents. In fact, humans exposed to xylene are frequently exposed to other substances such as benzene and toluene, two chemicals on the Proposition 65 list of substances "known to the state to cause" reproductive toxicity. The Proposition 65 listing criteria require, among other factors, "the proper control of confounding factors." Exposures to other chemicals represent obvious confounding factors in all of the epidemiology studies of xylene. It is virtually impossible to control for these confounding exposures.

Animal Evidence of Developmental Toxicity: The results of the animal studies do not support a conclusion that xylene is clearly shown to cause developmental toxicity. In animal studies conducted under current state-of-the-art testing methodology, there is little evidence of developmental toxicity, even at high, maternally toxic dose levels of xylene. Other studies identified in the HID do not constitute "scientifically valid testing according to generally accepted principles." These studies, which are described herein, have been critically reviewed by U.S. Environmental Protection Agency (U.S. EPA, 2003) and the Agency for Toxic Substances and Disease Registry (ATSDR, 2007). Examples of the limitations of certain studies include: unknown composition of the test material, inadequate group size, insufficient number of doses to evaluate a dose-response relationship, lack of detail with regard to both methods and results, and improper statistical analysis.

It is a generally accepted principle that the proper statistical unit for developmental toxicity studies is the litter, not the fetus; the statistical analyses in many of the developmental toxicity studies of xylene identified in the HID are inappropriate because the fetus (not the litter) was incorrectly used as the statistical unit. In certain studies, the test material contained ethylbenzene, in addition to xylene. When developmental effects were reported, these effects occurred at doses or concentrations above those known to cause adult systemic toxicity, and in some cases, occurred only at maternally lethal doses. The evidence does not meet the statutory standard that must be met before xylene can be listed.

Animal Evidence of Male Reproductive Toxicity: No evidence of male reproductive toxicity was observed in a one-generation reproductive toxicity study of male and female rats exposed by inhalation to 0, 60, 250, and 500 ppm of xylene daily for 6 hours per day for 131 days premating and during a 20-day mating period (Biodynamics, 1983). In a 90-day oral study conducted by the National Toxicology Program (NTP), no adverse effect on the histopathology of the testes or prostate was observed among male rats and mice administered mixed xylenes for 5 days per week at doses as high as 1000 and 2000 mg/kg/day, respectively (NTP, 1986). NTP also conducted chronic toxicity studies of mixed xylenes in both rats and mice. No evidence of an effect on the histopathology of the male reproductive organs was observed in rats or mice at doses as high as 500 and 1000 mg/kg/day administered 5 days per week for 103 weeks (NTP, 1986). The only evidence of male reproductive toxicity in animals comes from

unreliable studies that do not represent "scientifically valid testing according to generally accepted principles." These studies are reviewed herein.

Animal Evidence of Female Reproductive Toxicity: No evidence of female reproductive toxicity was observed in a one-generation reproductive toxicity study of male and female rats exposed by inhalation to 0, 60, 250, and 500 ppm of xylene daily for 6 hours per day for 131 days premating and during a 20-day mating period (Biodynamics, 1983). Similarly, xylene had no significant effect on the female reproductive organs of rats or mice administered the test substance in a 90-day study at doses as high as 1000 and 2000 mg/kg/day, respectively (NTP, 1986) or in a 2-year study in rats or mice at doses as high as 500 and 1000 mg/kg/day, respectively (NTP, 1986). The HID notes that out of 13 animal studies providing information relevant to evaluating female reproductive toxicity, only three showed any evidence of xylene-induced female reproductive toxicity.[4] Those three studies had serious flaws and limitations. More importantly, the evidence of female reproductive toxicity reported in those three studies was limited to exposure concentrations that caused significant maternal toxicity, and in two of three cases, maternal death.

II. Statutory Standard and the Guidance Criteria

The statutory standard for Proposition 65 established the criteria that the DARTIC must apply criteria in determining whether to list a chemical as a reproductive toxicant under Proposition 65. The statute provides:

> "A chemical is known to the state to cause cancer or reproductive toxicity . . . if in the opinion of the state's qualified experts it has been ***clearly shown*** through ***scientifically valid testing*** according to ***generally accepted principles*** to ***cause*** cancer or reproductive toxicity . . . ." [emphasis added]

Anything less does not permit listing under this statute. The question before the DARTIC is restricted to reproductive toxicity. In reviewing the Draft HID and the data cited therein, the DARTIC must determine whether the standard for Proposition 65 listing has been met for xylene.

Specifically, it is the DARTIC's duty is to:

> "Render an opinion . . . as to whether specific chemicals have been ***clearly shown, through scientifically valid testing according to generally accepted principles***, to cause reproductive toxicity."[5]

Although this statutory standard is stringent, it is not very specific, so the DARTIC has developed Guidance Criteria (DART IC, 1993) that provide general principles and specific factors for the Committee to weigh in assessing whether the scientific data on a particular chemical satisfy the "clearly shown" standard. The general principles include the following:

> "In evaluating the sufficiency of data, a ***weight of evidence approach*** shall be used to evaluate the body of information available for a given chemical."[6]

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[4] OEHHA (2012) HID. p., 45.
[5] Cal. Code Regs., tit. 27, § 25305(b)(1) (emphasis added).
[6] DARTIC Guidance Criteria at 1.D. (emphasis added).

> "In determining whether a chemical is to be . . . listed as known . . . to cause reproductive toxicity, the ***biological plausibility*** of the association between the adverse reproductive effects observed and the chemical in question should be considered. Confidence is increased when . . . a sound scientific basis exists for the observed adverse effects and the known characteristics of the particular chemical. Conversely, confidence is decreased if the observed adverse effects are contradictory to the known characteristics of the particular chemical."[7]

> "Developmental, and female and male reproductive effects ***shall meet at least one of the following criteria*** for recommendation as known to the State to cause reproductive toxicity.
>
> (a) ***Sufficient evidence*** in humans. . . .
>
> (b) Limited evidence or suggestive evidence in humans, supported by sufficient experimental animal (mammalian) data . . . .
>
> (c) ***Sufficient evidence*** in experimental animals (mammals), such that extrapolation to humans is appropriate."[8]

The Guidance Criteria identify specific factors for the Committee to consider in evaluating whether the available data on a particular compound qualify as "sufficient evidence in humans" or "sufficient evidence in experimental animals."

> "Sufficient evidence in humans," in the case of epidemiology studies, means studies that provide:
>
> > "***convincing*** evidence to support ***a causal relationship*** between exposure to the chemical and the . . . effect in question. This requires ***accurate exposure and toxicity endpoint classification*** and ***proper control of confounding factors***, bias, and effect modifiers."[9]

As discussed below, there is no epidemiological evidence that would support listing xylene since exposures to xylene are confounded by concomitant exposures to other solvents. Therefore, any decision to list xylene as a reproductive toxicant would need to be supported on the basis of "sufficient evidence in experimental animals." Whether animal studies are "sufficient evidence" to support extrapolation to humans, in most cases, is based on the following:

> "(1) The ***experimental design,*** including overall protocol and numbers of animals and ***presence of appropriate controls***.
>
> (2) The exposure, in terms of ***route of administration***, is ***relevant to expected human exposures*** . . . .
>
> (3) Number of dose levels, so that the presence of a ***dose-response relationship*** can be evaluated . . . .

[7] Guidance Criteria at 4.B. (emphasis added).
[8] Guidance Criteria at 3.A.-C. (emphasis added).
[9] Guidance Criteria at 3.A.(1) (emphasis added).

(4) ***Consideration of maternal and systemic toxicity***."[10]

Finally, with respect to developmental toxicity in particular, Proposition 65 regulates developmental effects caused only by pre-natal exposures, and not by post-natal exposures.[11]

III. Human Studies Do Not Support a Finding that Xylene Is Clearly Shown to Cause Reproductive Toxicity

The epidemiological studies do not provide convincing evidence to support a causal relationship between exposure to xylene and any endpoint of reproductive toxicity, including male reproductive toxicity, female reproductive toxicity and developmental toxicity. The epidemiological studies of xylene are of limited value in assessing the potential for xylene to cause reproductive toxicity since there are no epidemiological studies of exposure to xylene alone. All of the epidemiological studies of xylene involve populations with concomitant exposures to other solvents. In fact, humans exposed to xylene are also frequently exposed to substances such as benzene and toluene, two chemicals on the Proposition 65 list of substances "known to the state to cause" reproductive toxicity.

As noted earlier, the listing criteria require, among other factors, "the proper control of confounding factors." Exposures to other chemicals represent obvious confounding factors in all of the epidemiology studies of xylene. It is virtually impossible to control for these confounding exposures to other solvents in the epidemiological studies of xylene.

The Draft HID repeatedly notes the problem of confounding exposures in the epidemiological studies, including the following examples:

> "For each type of male reproductive outcome, the epidemiologic evidence for effects of xylene is sparse. The two studies did not clearly separate effects of xylene from those of other exposures, and had few subjects exposed (Sallmen et al., 1998) or were not reported in detail (Xiao et al., 1999, 2001)."[12]
>
> "The only epidemiologic study that examined effects of occupational exposure to xylene on menstrual cycles found that low-level exposure to xylene was associated with oligomenorrhea in women working in petroleum and chemical processing plants in China (Cho et al., 2001), though the women were exposed to multiple other industrial chemicals that could have contributed to the observed association."[13]
>
> "As with other endpoints, most studies of developmental endpoints reported results for xylene but could not separate potential effects of xylene from those of other organic solvents."[14]

[10] Guidance Criteria at 3.C.(1)-(4) (emphasis added).
[11] OEHHA General Counsel William Soo Hoo, 1996.
[12] OEHHA, HID (2012), p. 30.
[13] Id., p. 46.
[14] Id., p. 47.

In summary, confounding factors were not controlled as required and the epidemiological evidence is insufficient to clearly show that xylene causes developmental or reproductive toxicity.

IV. Animal Studies Do Not Support a Finding that Xylene Is Clearly Shown to Cause Reproductive Toxicity

The Draft HID identifies a large number of studies in animals of xylene and various endpoints of developmental and reproductive toxicity. The quality and reliability of these studies varies. This section includes a description of the strengths and weaknesses of the key animal studies. Some of the studies identified in the Draft HID do not constitute "scientifically valid testing according to generally accepted principles." Examples of the limitations of certain studies include: unknown composition of the test material, inadequate group size, insufficient number of doses to evaluate a dose-response relationship, lack of detail with regard to both methods and results, and improper statistical analysis (not on a per litter basis). These studies should not be afforded any weight in determining whether xylene has been "clearly shown through scientifically valid testing according to generally accepted principles to cause reproductive toxicity."

The results of the animal studies do not support a conclusion that xylene is clearly shown to cause developmental toxicity, male reproductive toxicity, or female reproductive toxicity. Each of these is discussed separately in the sections below.

A. Developmental Toxicity

The Draft HID identifies 13 animal developmental toxicity studies of inhaled xylene and 2 animal developmental toxicity studies of orally-administered xylene in Tables 7a and 7b, respectively. The developmental toxicity studies in animals do not "clearly show, through scientifically valid testing according to generally accepted principles" that xylene causes reproductive toxicity. Several studies do not demonstrate any evidence of developmental toxicity. Other studies report developmental effects, but there is no consistency in findings. In all cases where developmental effects were reported, these effects occurred at doses or concentrations above those known to cause adult systemic toxicity, including neurotoxicity

i. <u>Neurodevelopmental Toxicity</u>

Some evidence of neurodevelopmental toxicity was reported in a series of studies of rats exposed prenatally to xylene conducted by Dr. Hass and colleagues at Denmark's National Institute of Occupational Health, but the evidence is relatively weak. The reported changes in neurodevelopment are limited to (1) impaired cognitive (but not motor) performance in the Morris water maze test in female but not male offspring of rats exposed to 500 ppm xylene for 6 hours per day on GD 7-20 (Hass et al., 1995, 1997) and (2) decreased rotarod performance in offspring of rats exposed to 200 ppm xylene for 6 hours per day on GD 6-20 (Hass and Jakobsen, 1993).

The effect in the water maze test observed in female rat offspring was not observed in male rat offspring exposed to xylene. In addition, no effect in the water maze test was seen in the same study in another group of female rat offspring with the same exposure to xylene but raised in "enriched" housing with

various toys. Thus, the effect of xylene observed in female rat offspring was not confirmed by the same investigators in either male rat offspring or in female rat offspring housed under different conditions.

The decreased rotarod performance observed by Hass and Jakobsen (1993) was not confirmed in a subsequent study by the same group of investigators. The effect on rotarod performance in the earlier study was questioned by Hass et al. (1995) because it was not conducted by experimenters who were blind to the exposure status of the rats. Furthermore, no effect on neurodevelopment was observed among the offspring of rats exposed to 800 or 1600 ppm of p-xylene on GD 7-16 in an acoustic startle response test on PND 13, 17, 21, and 63 and a figure eight maze activity test on PND 22 and 65 (Rosen et al., 1986).

In summary, there is no convincing evidence that xylene causes neurodevelopmental toxicity.

ii. <u>Malformations and Variations</u>

Over 30 years ago, investigators at the State Institute Occupational Health in Budapest, Hungary reported statistically significant increases in the incidences of fetal abnormalities among the offspring of pregnant rats exposed to xylene. However, these studies are seriously flawed. For example, no litter specific data was provided; the fetus, not the litter, was considered the statistical unit. It is a generally accepted principle of developmental toxicity studies that the litter, not the fetus, is the proper statistical unit.

Hudak and Ungvary (1978) reported statistically significant increases in the incidences of fused sternebrae and extra ribs among the offspring of pregnant rats exposed to 230 ppm of xylene for 24 hours per day on GD 9-14. However, the statistical analysis was scientifically inappropriate since it considered the fetus, not the litter, to be the statistical unit. This study suffered from many other limitations. The purity of the test material was only 80%; the authors reported that 20% of the test material was ethylbenzene, not xylene. The Methods section was inadequate, consisting of a total of only six sentences. Only one dose level was studied, precluding any assessment of a dose-response relationship. And maternal body weight was measured only twice and reported as “percent of starting weight”; however, neither the starting point nor the ending point was stated. There are other deficiencies in this study, but they are too numerous to list.

Ungvary et al. (1980) reported statistically significant increases in the incidences of extra ribs among the offspring of rats exposed to 692 ppm of p-xylene or m-xylene (but not o-xylene) for 24 hours per day on GD 7-14. Once again, no litter specific information was provided. The statistical analysis was improper since it was based on the designation of the fetus (not the litter) as the statistical unit. Of note, the effects on skeletal development reported in the earlier study (Hudak and Ungvary, 1978) were not observed in this study among the offspring of rats exposed to a higher concentration (346 ppm of xylene) for 24 hours per day for a longer duration (GD 7-14).

More recently, Saillenfait et al. (2003) at the National Institute of Research and Safety in France conducted a well-designed standard developmental toxicity study. This study was chosen as a key study by the U.S. EPA for its VCCEP Tier 1 submission (U.S. EPA, 2005). It was also considered “the most reliable developmental toxicity assay” in ATSDR’s Toxicological Profile for Xylene (ATSDR, 2007). Importantly, this study correctly considered the litter to be the proper statistical unit. Each of the

individual isomers of xylene tested had ≥99.5% purity. In comparison, the technical (mixed) xylene tested contained 21.3% ethylbenzene.

Saillenfait et al. (2003) exposed pregnant rats to 0, 100, 500, 1000, and 2000 ppm of p-, o-, or m- or technical xylene for 6 hours per day on GD 6-20. They concluded that none of these substances was teratogenic at concentrations up to 2000 ppm (the highest level tested). Although significant increases in the mean percent of fetuses with skeletal variations of all types were seen at 2000 ppm of p- and o-xylene (but not with m-xylene or technical xylene), no single skeletal variation occurred at significantly increased incidence compared to controls.

In pregnant mice given large oral doses of "mixed xylene," an increased incidence of malformations, primarily cleft palate, was reported among the offspring. Marks et al. (1982) at Research Triangle Institute administered 0, 0.6, 1.2, 2.4, 3.0, 3.6 and 4.8 ml/kg/day of "mixed xylene" by gavage in three divided daily doses. The Draft HID incorrectly reports these doses as 0, 0.52, 1.03, 2.06, 2.58, 3.10 and 4.13 mg/kg/day. However, when expressed on a mg/kg/day basis, the actual doses are 0, 520, 1030, 2060, 2580, 3100 and 4130 mg/kg/day of "mixed xylene." The error in the Draft HID occurred because there is a typo in the Methods section of the Marks et al. (1982); "mg/kg/day" should have been "g/kg/day." U.S. EPA and others have recognized this typo in the paper and agree that actual doses are as stated in this paragraph.[15]

The test material used by Marks et al. (1982) contained 17.0% ethylbenzene, as well as 13.6% p-xylene, 60.2% m-xylene, 9.1% o-xylene, and <0.3% other volatile impurities. As such, it is not possible to distinguish between the effects of xylene and ethylbenzene in this study.

The highest dose (4130 mg/kg/day) was lethal to all of the pregnant mice. The next dose (3100 mg/kg/day) was lethal to about 32% of the pregnant mice. An increased incidence of malformations, primarily cleft palate, was observed at 2016, 2580 and 3100 mg/kg/day. These investigators correctly considered the litter to be the statistical unit.

It is well recognized that mice are extremely susceptible to cleft palate, which can be induced by a variety of sources of stress. It is likely that the increased incidence of cleft palate in this study can be explained by the degree of maternal toxicity associated with these maternally lethal and near lethal doses.

In its evaluation of the Marks et al. (1982) study, ATSDR (2007) concluded:

> "It is unclear whether the observation of cleft palate in this study is associated with maternal toxicity or a predisposition of mice under stress to give birth to offspring with this birth defect. In a teratology screening study, 2000 mg/kg/day of m-xylene produced no evidence of fetal toxicity in mice (Seidenberg et al. 1986). Given the limited amount of animal data, no conclusion can be

[15] U.S. EPA (2003) Toxicological Review of Xylenes. p. 32. Footnote 3 on page 32 states: "Marks et al. (1982) noted that xylene dissolved in cottonseed oil at concentrations (v/v) of 0 (vehicle control), 2, 3, 8, 10, 12, and 16% were administered by gavage in individual doses three times a day and that the daily doses were 0, 0.6, 1.2, 2.4, 3.0, 3.6, and 4.8 ml/kg-day. It was noted that a density value of 0.86 g/ml was used to convert these to units of mg/kg-day, but the actural numbers in the report should be in units of g/kg-day. This was an apparent typographical error. The mg/kg-day doses cited herein appar to be the correct administered doses."

> made regarding the relationship between oral exposure of xylene and adverse developmental effects."[16]

No significant increase in fetal malformations or variations was observed in two large, conventional studies conducted at contract laboratories and submitted to regulatory agencies (Litton Bionetics, 1978; Biodynamics, 1983). In the Litton Bionetics study, pregnant rats were exposed to levels up to 400 ppm of xylene for 6 hours per day on GD 6-15. However, the test material evaluated in the Litton Bionetics study contained a high percentage (36%) of ethylbenzene. In the Biodynamics study, pregnant rats were exposed to levels of up to 500 ppm of xylene for 6 hours per day throughout pregnancy.

In summary, there is no clear evidence that xylene causes malformations or skeletal variations.

iii. <u>Fetal Body Weight</u>

Decreased fetal body weight was reported in several studies. But, many of these studies were seriously flawed. And the reported effects on fetal body weight occurred at concentrations above those at which neurobehavioral effects were reported in adult animals.[17]

Decreased fetal body weight was reported in certain developmental toxicity studies conducted by Dr. Ungvary and colleagues at the State Institute of Occupational Health in Budapest, Hungary (Ungvary et al, 1980; Ungvary and Tatrai, 1985). In contrast, no decrease in fetal body weight was observed in a similar study by the same investigators (Hudak and Ungvary, 1978). As noted earlier, these studies exhibited a number of serious limitations. Fetal body weights were not expressed as the mean of the litter means. The statistical analysis of the fetal body weight data was inappropriate since the fetus, not the litter, was incorrectly considered to be the statistical unit in all of these studies. Other limitations of these studies included the high concentration of ethylbenzene in the test material, poor reporting of methods and results, and inconsistencies in or lack of dose-response relationships.

Mirkova et al. (1983) at the Research Institute of Hygiene and Occupational Diseases in Bulgaria reported a significant decrease in fetal body weight in a study of offspring of pregnant rats exposed to 0, 3, 12, and 110 ppm of xylene for 6 hours per day, 5 days per week, during GD 1-21. The decrease in fetal body weight was reported at 12 and 110 ppm, but no dose-response relationship was apparent since the magnitude of the decrease was virtually the same at both exposure concentrations. The data from this study are limited by numerous factors, including lack of information on the purity and composition of the test material, incomplete description of methods, inadequate litter size, lack of proper statistical analysis (the fetus was considered the statistical unit), and incomplete reporting of results.[18] Further, the overall health of the animals used for this study has been called into question by the U.S. EPA because of the poor conception rate of the control dams and the high incidence of hemorrhages in the control fetuses.[19]

In a well-designed developmental toxicity study of individual isomers of xylene, as well as mixed xylenes, Saillenfait et al. (2003) observed decreased fetal body weights in the offspring of pregnant rats exposed to ≥1000 ppm of m- or p-xylene for 6 hours per day. Statistically significant maternal effects,

[16] ATSDR (2007) Toxicological Profile for Xylene. p. 108.
[17] U.S. EPA (2003) Toxicological Review of Xylenes. p. 58.
[18] U.S. EPA (2003) Toxicological Review of Xylenes. p. 41.
[19] U.S. EPA (2003) Toxicological Review of Xylenes, p. 79.

including reduced corrected body weight gain (body weight gain exclusive of gravid uterine weight), indicating that the decreased fetal body weight is likely secondary to maternal toxicity. In comparison, o-xylene and mixed xylenes (but not m-xylene or p-xylene) produced decreased fetal body weight at 500 ppm; reduced corrected maternal body weight gain was observed with these test substances at ≥1000 ppm in this study. Although statistically significant, the decrease in fetal body weight observed at 500 ppm was slight (i.e., a 4.3% and 4.6% decreases compared to controls for mixed xylenes and o-xylene, respectively). A 5% decrease in fetal body weight is at the limit for statistics to be able to show a significant difference in fetal body weight in a standard size developmental toxicity study in rats. In comparison, the mean fetal body weight of offspring of pregnant rats exposed to 500 ppm of m-xylene or p-xylene was 100.5% and 98.9%, respectively, of their control values. It is important to note that 500 ppm of xylene is well above the exposure concentration known to produce neurotoxicity in adult rats in other studies. Saillenfait et al. (2003) concluded:

> "All chemicals [mixed xylenes, m-, o-, p-xylene, and ethylbenzene] produced fetal toxicity at 1000 and 2000 ppm, in the presence of maternal toxic effects. There was also a slight indication of fetal growth retardation at 500 ppm of o-xylene and mixed xylenes, as evidenced by the lower fetal body weight. None of these agents was teratogenic up to 2000 ppm."[20]

Marks et al. (1982) reported decreased fetal body weights among the offspring of pregnant mice administered 2060, 2580, or 3100 mg/kg/day of "mixed xylene" by gavage in three divided daily doses. As noted earlier, significant maternal lethality (32%) was observed at 3100 mg/kg/day. Thus, the decreases in fetal body weight in this study occurred at lethal or near-lethal doses. This study did not adequately assess the effect of xylene on maternal body weight or body weight gain. The test material used for this study contained 17.0% ethylbenzene, as well as 13.6% p-xylene, 60.2% m-xylene, 9.1% o-xylene, and <0.3% other volatile impurities. As such, it is not possible to distinguish between the effects of xylene and ethylbenzene in this study.

No significant effect on fetal body weight was observed in other developmental toxicity studies of xylene (Litton Bionetics, 1978; Biodynamics, 1983; Rosen et al., 1986). Most of the studies where an effect on fetal body weight was reported suffer from multiple flaws and limitations that eliminate them for consideration because they are not "scientifically valid testing according to generally accepted principles." The sole exception appears to the Saillenfait et al. (2003) study where a decrease in fetal body weight generally appears to be secondary to maternal toxicity. In the case of two xylene isomers, a slight decrease (less than 5%) in fetal body weight was observed at a concentration (500 ppm) at which the authors did not observe maternal toxicity, but others have reported to cause adult neurotoxicity. Thus, the scientific evidence does not clearly show that xylene causes developmental toxicity in animals.

B. Male Reproductive Toxicity

No effect on mating or fertility was observed in a one-generation reproductive toxicity study of male and female rats exposed by inhalation to 0, 60, 250, and 500 ppm of xylene daily for 6 hours per day for 131

[20] Saillenfait et al. (2003), p. 428.

days premating and during a 20-day mating period (Biodynamics, 1983). Testicular weights were unaffected by exposure to xylene.

In a 90-day oral study conducted by the National Toxicology Program (NTP), no adverse effect on the histopathology of the testes or prostate was observed among male rats and mice administered mixed xylenes for 5 days per week at doses as high as 1000 and 2000 mg/kg/day, respectively (NTP, 1986). NTP also conducted chronic toxicity studies of mixed xylenes in both rats and mice. No evidence of an effect on the histopathology of the male reproductive organs was observed in rats or mice at doses as high as 500 and 1000 mg/kg/day administered 5 days per week for 103 weeks (NTP, 1986).

The only evidence of male reproductive toxicity in animals comes from two unreliable studies that do not represent "scientifically valid testing according to generally accepted principles." In an unconventional study, Washington et al. (1983) reported an increase in abnormal sperm in rats five weeks after being given intraperitoneal injections of o-xylene for two days. According to the study authors, the increase in abnormal sperm was observed at 0.5 ml/kg/day, but not at 1.5 ml/kg/day. In addition, this effect occurred only at a room temperature of 24-30°C. No effect was observed at a room temperature of 20-24°C. Importantly, the study had a number of major limitations. The group size was not reported. The methods and results are poorly reported. The purity and composition of the test material are unknown. And, the choice of the intraperitoneal route of exposure is not representative of the route of human exposure.

The only other study to report an effect of xylene on male reproduction was a highly unconventional study by Yamada (1993). This study reported reductions in the weights of the male reproductive organs and decreased numbers of epididymal sperm in rats exposed by inhalation. However, the exposure concentrations of xylene were not measured. Instead, rats were exposed to xylene until they lost the righting reflex twice per day for seven days. This exposure regimen also produced decreased body weights, and the role of systemic toxicity on the observed effects is unclear. There is no opportunity to evaluate a possible dose-response relationship from the results of this study since only one exposure concentration was tested, and the concentration is unknown. The group size was small (n=5).

In summary, the animal evidence does not support a conclusion that xylene has been clearly shown to cause male reproductive toxicity.

C. Female Reproductive Toxicity

No effect on mating or fertility was observed in a one-generation reproductive toxicity study of male and female rats exposed by inhalation to 0, 60, 250, and 500 ppm of xylene daily for 6 hours per day for 131 days premating and during a 20-day mating period (Biodynamics, 1983).

In a 90-day oral study conducted by the National Toxicology Program (NTP), no adverse effect on the histopathology of the female reproductive organs was observed among female rats and mice administered mixed xylenes for 5 days per week at doses as high as 1000 and 2000 mg/kg/day, respectively (NTP, 1986). NTP also conducted chronic toxicity studies of mixed xylenes in both rats and mice. No evidence of an effect on the histopathology of the female reproductive organs were observed in rats or mice at doses as high as 500 and 1000 mg/kg/day administered 5 days per week for 103 weeks (NTP, 1986).

The Draft HID notes that out of 13 animal studies providing information relevant to evaluating female reproductive toxicity, only three showed any evidence of xylene-induced female reproductive toxicity.[21] The evidence of female reproductive toxicity reported in these three studies is unconvincing. The only evidence of female reproductive toxicity in animals comes from three unreliable studies that do not represent "scientifically valid testing according to generally accepted principles."

In the first study purported to show an effect, Ungvary and Tatrai (1985) reported no live fetuses in rabbits at inhalation concentrations of xylene that were associated with maternal deaths. According to U.S. EPA, "These findings are indicative of severe maternal toxicity." When severe maternal toxicity is taken into account, the inability to bear live offspring under such extreme conditions is not a generally to demonstrate a substance causes developmental toxicity even in a well-designed and well implemented study. The Ungvary and Tatrai (1985) had major limitations, including unknown purity of the test material, no information on the inhalation chamber, no analytical results of the chamber air, small group size, inadequate description of the methods and results, and inappropriate statistical methods.

The second study that was alleged to show some evidence of female reproductive toxicity was an earlier study by Unvgary and colleagues. Ungvary et al. (1980) reported statistically significant decreases in mean litter size among pregnant rats exposed to 692 ppm of p-xylene (but not m- or 0-xylene) for 24 hours per day on GD 7-14. The study authors reported decreased maternal weight gain (data not provided) at 692 ppm of p-xylene (but not o- or m-xylene), but the difference in maternal weight gain was not apparent by the end of the study (GD 21). The Ungvary et al. (1980) study had many limitations, including unknown purity of the test materials, inadequate description of methods and results, no data on litters, and inappropriate statistical analysis. This inadequate study provides no consistent evidence of female reproductive toxicity.

The third study that was purported to provide some evidence of female reproductive toxicity is the Marks et al. (1982) developmental toxicity study. This study reported an increased incidence of fetal resorptions in the offspring of pregnant mice given daily oral doses of 3100 mg/kg/day of xylene, a dose associated with 32% maternal mortality. An increase in resorptions at a dose that produces death in about a third of the pregnant mice is very weak evidence of female reproductive toxicity. As noted earlier, the xylene used by Marks et al. (1982) contained 17.0% ethylbenzene. Even if female reproductive toxicity could be clearly attributed to the test material in this study (which it can't), it would not be possible to attribute this effect to xylene since the test material contained 17% ethylbenzene.

In contrast to the three studies described above, ten animal studies of xylene showed no evidence of female reproductive toxicity according to the Draft HID. These ten studies included better designed and more reliable studies than the three flawed studies which allegedly showed some evidence of female reproductive toxicity. In summary, xylene has not been clearly shown to cause female reproductive toxicity in animal studies.

V. Conclusion

The Draft HID identifies a large number of epidemiological and animal studies that have evaluated the potential developmental toxicity, male reproductive toxicity and female reproductive toxicity of xylene.

[21] OEHHA (2012) HID. p., 45.

However, the Draft HID notes the quality and reliability of these studies varies. Many of these studies have significant limitations and shortcomings that render them not "scientifically valid testing according to generally accepted principles." The overall scientific evidence does not support a conclusion that xylene has been clearly shown to cause developmental toxicity, male reproductive toxicity or female reproductive toxicity.

Again, thank you for the opportunity to provide these comments. If you have any questions, please do not hesitate to contact me at 202-249-6717or David_Fischer@americanchemistry.com.

Sincerely,
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