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HYPOTHESIS-DRIVEN WEIGHT OF EVIDENCE EVALUATION INDICATES ETHYLBENZENE LACKS ENDOCRINE DISRUPTION POTENTIAL BY EATS PATHWAYS

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Table of Contents

Supplemental Material B
OSRI Evaluation for Ethylbenzene

Summaries of Studies

1. Estrogen Agonist Hypothesis

1.1 Rank 2: Repeat Dose Toxicity – Epididymis histopathology

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (*n* = 5/ dose/sex) and 13 weeks (*n* = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histologic changes in the epididymides of ethylbenzene-exposed mice or rats.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

1.2 Rank 2: Repeat Dose Toxicity – Epididymis weight

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. Additionally, high doses were used. It is unclear if the endpoint responses are secondary to general toxicity.

Results included in WOE: The tabular data indicate there was a significant decrease in the epididymal weight in mice exposed to ethylbenzene in the 1000-ppm group. The authors note that this was not considered biologically significant since spermatid counts, sperm motility, and caudal weight were normal. The narrative portion of the report states that this significant difference was found in the epididymal weight of rats, not mice – a likely error (p. 17). The tabular data show that there was no difference in the epididymal weight of rats at any ethylbenzene exposure level.

1.3 Rank 2: Repeat Dose Toxicity – Ovary histopathology

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the ovaries of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

1.4 Rank 2: Repeat Dose Toxicity – Testis histopathology (atrophy)

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no effects observed on sperm or testicular morphology in rats exposed to ethylbenzene.

1.5 Rank 2: Repeat Dose Toxicity – Testis weight

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Weights of testes in mice and rats were not affected by ethylbenzene.

1.6 Rank 2: Repeat Dose Toxicity – Uterus histopathology

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to

ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the uterus of mice or rats compared with controls.

[10] ***Cragg and colleagues (1989)*** Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The uterus of high-exposure and controls animals of all species was subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in this organ.

1.7 Rank 2: Repeat Dose Toxicity – Vaginal histopathology

[1] NTP (National Toxicology Program). (1999) Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results used for WOE: The histological examination of vaginal tissue did not reveal significant differences between the chamber controls and any of the exposure groups in rats or mice.

[3] Mellert and colleagues (2007) Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

1.8 Rank 2: Developmental Toxicity – Corpora lutea

[5] ***Saillenfait and colleagues (2003)*** The developmental toxicity of ethylbenzene was studied in

Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean number of corpora lutea per dam did not differ between dams in any of the treatment groups and control dams.

[13] ***Andrew et al., (1981); Hardin et al. (1981)*** Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: The number of Corpora Lutea was unchanged by exposure to 100 and 1,000 ppm ethylbenzene relative to controls.

1.9 Rank 2: Developmental Toxicity – Post-implantation loss

[4] ***Saillenfait and colleagues (2007)*** The combined effects of EB and butyl acetate (BA) were investigated. Groups of 18 bred rats (15– 18 pregnant) were exposed to vapors of EB or BA, separately or in combination, 6 h day−1, on days 6–20 of gestation. There were nine experimental groups: Control; 250 or 1000 ppm EB; 500 or 1500 ppm BA or mixtures of 250 ppm EB + 500 ppm BA, 250 ppm EB + 1500 ppm BA, 1000 ppm EB + 500 ppm BA, or 1000 ppm EB + 1500 ppm BA

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no effect of treatment on the mean number of implantations and of live fetuses, and on the incidence of non-live implants and resorptions.

[5] ***Saillenfait and colleagues (2003)*** The developmental toxicity of ethylbenzene was studied in Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: Clinical signs of toxicity (ataxia, decreased motor activity) were seen at 2000 ppm. Maternal weight was significantly reduced on GD 21 at 1000 ppm and on GD 13 and 21 at 2000 ppm. Dams exposed to 1000 or 2000 ppm showed significant decreases in maternal weight gain and food consumption throughout exposure, and in corrected weight gain

Results included in WOE: The number of implantations was comparable among groups. Although the difference was not statistically significant, the incidence of non-live implants and resorptions was higher at 2000 ppm than in the control group. This was likely due to the 100% postimplantation loss seen in three of the 21 pregnant females exposed to 2000 ppm (0 in other

groups).

[6] ***Saillenfait and colleagues (2006)*** Pregnant Sprague–Dawley rats were exposed to ethylbenzene (EB; 0, 250, or 1000 ppm) and methylethylketone (MEK; 0, 1000, or 3000 ppm), alone and in combination, by inhalation, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: EB and MEK, alone or in combination, have no effect on the average number of implantations and live fetuses, and in the incidence of non-live implants and resorptions.

[11] ***Ungváry, G. & Tátrai, E. (1985)*** Groups of CFY rats were exposed to inhalation of ethylbenzene at 0, 138, 276 or 553 ppm for 24 h/day from day 7 to day 15 of pregnancy. Fetuses were evaluated on pregnancy day 21. CFLP mice were exposed to inhalation of ethylbenzene at 0, 115 or 230 ppm for 24 h/day (no data provided for these groups) or for 3-4 hours/day intermittently from day 6 to 15 of pregnancy. The fetuses were evaluated on pregnancy days 18. NZ rabbits were exposed to 0, 115, or 230 ppm ethylbenzene for 24 h/day from day 7 to day 20 gestation. Fetuses were examined on pregnancy day 30. The three rabbit does in the 230-ppm dose group aborted.

Limitations: The data for mice was only provided for the animals in the 115-ppm exposure group and maternal toxicity information was lacking. The authors mention that the maternal toxic effects of ethylbenzene in rats were "moderate and dose-dependent" but fail to describe or quantify these effects. The contribution of general toxicity effects to all study findings should be considered.

Results included in WOE: The percentage of dead or resorbed fetuses was significantly increased in all ethylbenzene-exposed groups in rats (138, 276 and 553 ppm). There was no significant difference in the percentage of dead or resorbed fetuses in ethylbenzene exposed mice or rabbits compared with controls.

[13] ***Andrew et al., (1981); Hardin et al. (1981)*** Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: The number of implantations was comparable among groups. Post-implantation loss was inferred from the number of live fetuses, which was slightly reduced in rabbits, but not in rats, exposed to ethylbenzene at 1,000 ppm, a concentration that produced some indications of maternal systemic effects. This finding is therefore unlikely to have been produced by and endocrine mode of action.

1.10 Rank 2: Developmental Toxicity – Pre-implantation loss

[13] ***Andrew et al., (1981); Hardin et al. (1981)*** Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for

3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: Pre-implantation loss was inferred from the number of implantations per corpora lutea, which was comparable between control groups and groups exposed to 100 and 1,000 ppm ethylbenzene in both rats and rabbits.

1.11 Rank 2: Reproductive Toxicity – Estrous cyclicity

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean estrous cycle length (4.0 ± 0.3 days) was significantly reduced for the F_0, 500 ppm group when compared to the F_0 control group value (4.4 ± 0.8 days). However, the authors felt this difference was not biologically important because all females in this group were cycling normally and this strain of rat normally exhibits 4- to 5-day estrous cycles. Mean estrous cycle length did not differ between control and experimental F_1 offspring.

1.12 Rank 2: Reproductive Toxicity – Fertility

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F_0 or F_1 animals.

1.13 Rank 2: Developmental Toxicity – Gestational length

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: No effects from ethylbenzene exposure of F_0 or F_1 animals were observed on reproductive performance parameters (mating and fertility indices, gestation lengths, former implantation sites and unaccounted sites).

1.14 Rank 2: Reproductive Toxicity – Implantations

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-hr inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: No effects from ethylbenzene exposure of F_0 or F_1 rats were observed on reproductive performance parameters (mating and fertility indices, gestation lengths, former implantation sites and unaccounted sites).

1.15 Rank 2: Reproductive Toxicity – Litter size

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean number of F_1 and F_2 pups born, live litter size, percentage of males per litter at birth, and postnatal survival were unaffected by ethylbenzene exposure.

1.16 Rank 2: Reproductive Toxicity – Mating index

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The male and female mating indices (%) were not different between any of the treatment animals and controls.

1.17 Rank 2: Reproductive Toxicity – Ovarian follicle count in offspring

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70

consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: In the F_1 females, the mean number of primordial follicles in the 500-ppm dose group was no significantly different from controls.

1.18 Rank 2: Reproductive Toxicity – Sperm count

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean sperm number (millions/g tissue) in the left cauda epididymis for F_0 and F_1 males were not different between any treatment group and controls.

1.19 Rank 2: Reproductive Toxicity – Time to mating

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22.

Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-hr inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F_0 or F_1 animals.

1.20 Rank 2: Reproductive Toxicity – Time to vaginal patency

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean age of acquisition of vaginal patency for all exposed groups (25, 100 and 500 ppm ethylbenzene) was statistically significantly lower than the mean for the concurrent control group value in F_1 female offspring; similar differences were not observed in the F_2 female pups. The authors felt these differences were not biologically important because the mean values were comparable to the historical control mean value.

1.21 Rank 3: Repeat Dose Toxicity – Gross pathology

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Gross pathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

2. Estrogen Antagonist Hypothesis

2.1 Rank 2: Repeat Dose Toxicity – Epididymis histopathology

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the epididymides of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

2.2 Rank 2: Repeat Dose Toxicity – Ovary histopathology

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the ovaries of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

2.3 Rank 2: Repeat Dose Toxicity – Prostate histopathology

[1] NTP ***(National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the prostates of mice or rats compared with controls.

2.4 Rank 2: Repeat Dose Toxicity – Seminal vesicle histopathology

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 hper day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the seminal vesicles of mice or rats compared with controls.

2.5 Rank 2: Repeat dose toxicity – Testis histopathology (atrophy)

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in testes of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

2.6 Rank 2: Repeat Dose Toxicity – Testis weight

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Weights of testes in mice and rats were not affected by ethylbenzene.

2.7 Rank 2: Developmental Toxicity – Corpora lutea

[5] ***Saillenfait and colleagues (2003)*** The developmental toxicity of ethylbenzene was studied in Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean number of corpora lutea per dam did not differ between dams in any of the treatment groups and control dams.

[13] ***Andrew et al., (1981); Hardin et al. (1981)*** Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: The number of Corpora Lutea was unchanged by exposure to 100 and 1,000 ppm ethylbenzene relative to controls.

2.8 Rank 2: Reproductive Toxicity – Estrous cyclicity

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean estrous cycle length (4.0 ± 0.3 days) was significantly reduced for the F_0, 500ppm group when compared to the F_0 control group value (4.4 ± 0.8 days). However, the authors felt this difference was not biologically important because all females in this group were cycling normally and this strain of rat normally exhibits 4-5 day estrous cycles. Mean estrous cycle length did not differ between control and experimental F_1 offspring.

2.9 Rank 2: Reproductive Toxicity – Fertility

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F_0 or F_1 animals.

2.10 Rank 2: Reproductive Toxicity – Litter size

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean number of F_1 and F_2 pups born, live litter size, percentage of males per litter at birth, and postnatal survival were unaffected by ethylbenzene exposure.

2.11 Rank 2: Reproductive Toxicity – Sperm count

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of

the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean sperm number (millions/g tissue) in the left cauda epididymis for F_0 and F_1 males were not different between any treatment group and controls.

2.12 Rank 2: Reproductive Toxicity – Time to mating

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F_0 or F_1 animals.

2.13 Rank 2: Reproductive Toxicity – Time to vaginal patency in offspring

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean age of acquisition of vaginal patency for all exposed groups (25, 100 and 500 ppm ethylbenzene) was statistically significantly lower than the mean for the concurrent control group value in F_1 female offspring; similar differences were not observed in the F_2 female pups. The authors felt these differences were not biologically important because the mean values were comparable to the historical control mean value.

2.14 Rank 3: Repeat Dose Toxicity – Gross Pathology

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (*n* = 5/ dose/sex) and 13 weeks (*n* = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Gross pathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

3. Androgen Agonist Hypothesis

3.1 Rank 2: Repeat Dose Toxicity – Ovary histopathology

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (*n* = 5/ dose/sex) and 13 weeks (*n* = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the ovaries of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

3.2 Rank 2: Repeat Dose Toxicity – Sperm count

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Sperm counts (sperm count/gram testis) were not significantly different between control and ethylbenzene-exposed mice or rats.

3.3 Rank 2: Repeat dose toxicity – Testis histopathology (atrophy)

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (*n* = 5/ dose/sex) and 13 weeks (*n* = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in testes of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

3.4 Rank 2: Repeat Dose Toxicity – Testis weight

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action.

Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Weights of testes in mice and rats were not affected by ethylbenzene.

3.5 Rank 2: Developmental Toxicity – Implantations

[4] ***Saillenfait and colleagues (2007)*** The combined effects of EB and BA were investigated. Groups of 18 bred rats (15– 18 pregnant) were exposed to vapors of EB or BA, separately or in combination, 6 h day−1, on days 6–20 of gestation. There were nine experimental groups: Control; 250 or 1000 ppm EB; 500 or 1500 ppm BA or mixtures of 250 ppm EB + 500 ppm BA, 250 ppm EB + 1500 ppm BA, 1000 ppm EB + 500 ppm BA, or 1000 ppm EB + 1500 ppm BA

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no effect of treatment on the mean number of implantations and of live fetuses, and on the incidence of non-live implants and resorptions.

[5] ***Saillenfait and colleagues (2003)*** The developmental toxicity of ethylbenzene was studied in Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean number of implantation sites per litter did not differ between any of the treatment groups and controls.

[6] ***Saillenfait and colleagues (2006)*** Pregnant Sprague–Dawley rats were exposed to ethylbenzene (EB; 0, 250, or 1000 ppm) and methylethylketone (MEK; 0, 1000, or 3000 ppm), alone and in combination, by inhalation, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: EB and MEK, alone or in combination, have no effect on the average number of implantations and live fetuses, and in the incidence of non-live implants and resorptions.

[13] ***Andrew et al., (1981); Hardin et al. (1981)*** Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: In both rats and rabbits, the number of implantations per doe and per corpora lutea was unaffected by exposure to 100 or to 1,000 ppm ethylbenzene relative to unexposed controls.

3.6 Rank 2: Developmental Toxicity – Litter size

[4] ***Saillenfait and colleagues (2007)*** The combined effects of EB and BA were investigated. Groups of 18 bred rats (15– 18 pregnant) were exposed to vapors of EB or BA, separately or in combination, 6 h day−1, on days 6–20 of gestation. There were nine experimental groups: Control; 250 or 1000 ppm EB; 500 or 1500 ppm BA or mixtures of 250 ppm EB + 500 ppm BA, 250 ppm EB + 1500 ppm BA, 1000 ppm EB + 500 ppm BA, or 1000 ppm EB + 1500 ppm BA

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no effect of treatment on the mean number of implantations and of live fetuses, and on the incidence of non-live implants and resorptions.

[5] ***Saillenfait and colleagues (2003)*** The developmental toxicity of ethylbenzene was studied in Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: Clinical signs of toxicity (ataxia, decreased motor activity) were seen at 2000 ppm. Maternal weight was significantly reduced on GD 21 at 1000 ppm and on GD 13 and 21 at 2000 ppm. Dams exposed to 1000 or 2000 ppm showed significant decreases in maternal weight gain and food consumption throughout exposure, and in corrected weight gain

Results included in WOE: The number of implantations was comparable among groups. Although the difference was not statistically significant, the incidence of non-live implants and resorptions was higher at 2000 ppm than in the control group. This was likely due to the 100% postimplantation loss seen in three of the 21 pregnant females exposed to 2000 ppm (0 in other groups).

[6] ***Saillenfait and colleagues (2006)*** Pregnant Sprague–Dawley rats were exposed to ethylbenzene (EB; 0, 250, or 1000 ppm) and methylethylketone (MEK; 0, 1000, or 3000 ppm), alone and in combination, by inhalation, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: EB and MEK, alone or in combination, have no effect on the average number of implantations and live fetuses, and in the incidence of non-live implants and resorptions.

[13] ***Andrew et al., (1981); Hardin et al. (1981)*** Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: Litter size, inferred from the number of live fetuses per litter, was slightly reduced in rabbits, but not in rats, exposed to ethylbenzene at 1,000 ppm, a concentration that produced some indications of maternal systemic effects. This finding is therefore unlikely to have been produced by and endocrine mode of action.

3.7 Rank 2: Developmental Toxicity – Sex ratio

[5] ***Saillenfait and colleagues (2003)*** The developmental toxicity of ethylbenzene was studied in Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The percentage of males per litter did not differ between any of the treatments groups and controls.

[13] ***Andrew et al., (1981); Hardin et al. (1981)*** Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: Sex ratio in rats and rabbits was unaffected by exposure to 100 or to 1,000 ppm ethylbenzene relative to unexposed controls.

3.8 Rank 2: Reproductive Toxicity – Estrous cyclicity

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart).

Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean estrous cycle length (4.0 ± 0.3days) was significantly reduced for the F_0 500ppm group when compared to the F_0 control group value (4.4 ± 0.8 days). However, the authors felt this difference was not biologically important because all females in this group were cycling normally and this strain of rat normally exhibits 4-5 day estrous cycles. Mean estrous cycle length did not differ between control and experimental F_1 offspring.

3.9 Rank 2: Reproductive Toxicity – Fertility

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F_0 or F_1 animals.

3.10 Rank 2: Reproductive Toxicity – Implantations

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: No effects from ethylbenzene exposure of F_0 or F_1 rats were observed on reproductive performance parameters (mating and fertility indices, gestation lengths, former implantation sites and unaccounted sites).

3.11 Rank 2: Reproductive Toxicity – Litter size

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean number of F_1 and F_2 pups born, live litter size, percentage of males per litter at birth, and postnatal survival were unaffected by ethylbenzene exposure.

3.12 Rank 2: Reproductive Toxicity – Mating index

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The male and female mating indices (%) were not different between the any of the treatment animals and controls.

3.13 Rank 2: Reproductive Toxicity – Prostate weight

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500 ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-hr inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were statistically significant decreases in absolute prostate weights in the F_0 male 500 ppm group but not when these organ weights were expressed as relative to body weight. There was no significant difference in absolute or relative prostate weights in F_1 males.

3.14 Rank 2: Reproductive Toxicity – Sex ratio

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The sex distribution, measured by the % males/litter, was not different in either F_1 or F_2 litters compared with control litters.

3.15 Rank 2: Reproductive Toxicity – Sperm count

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout

mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean sperm number (millions/g tissue) in the left cauda epididymis for F_0 and F_1 males were not different between any treatment group and controls.

3.16 Rank 2: Reproductive Toxicity – Time to balano-preputial separation

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500 ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean age at acquisition of balanopreputial separation was significantly greater in the F_1 offspring in the 500-ppm treatment group compared with controls (PND 44.7± 2.0 vs. PND 43.5 ± 2.2). The mean value for the 500 ppm F_1 male group (PND 44.7) was similar to the value obtained in the F_2 generation control group (PND 45.3) and essentially equivalent to the mean historical control value (44.8 days) for the laboratory and as such, the authors stated that the significant finding was not considered biologically important. F_2 data for this measure were not published. However, the data is available in the full study report.

3.17 Rank 2: Reproductive Toxicity – Time to mating

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND)

21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F_0 or F_1 offspring.

3.18 Rank 2: Reproductive Toxicity – Time to vaginal patency

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean age of acquisition of vaginal patency for all exposed groups (25, 100 and 500ppm ethylbenzene) was statistically significantly lower than the mean for the concurrent control group value in F_1 female offspring; similar differences were not observed in the F_2 female pups. The authors felt these differences were not biologically important because the mean values were comparable to the historical control mean value.

3.19 Rank 3: Repeat Dose Toxicity – Gross pathology

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/ kg bodyweight/day (mg/kg bw/day), administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Gross pathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

4. Androgen Antagonist Hypothesis

4.1 Rank 2: Repeat Dose Toxicity – Epididymal weight

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. Additionally, high doses were used. It is unclear if the endpoint responses are secondary to general toxicity.

Results included in WOE: The tabular data indicate there was a significant decrease in the epididymal weight in mice exposed to ethylbenzene in the 1000-ppm group. The authors note that this was not considered biologically significant since spermatid counts, sperm motility, and caudal weight were normal. The narrative portion of the report states that this significant difference was found in the epididymal weight of rats, not mice – a likely error (p. 17). The tabular data show that there was no difference in the epididymal weight of rats at any ethylbenzene exposure level.

4.2 Rank 2: Repeat Dose Toxicity – Epididymis histopathology

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was

studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the epididymides of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

4.3 Rank 2: Repeat Dose Toxicity – Ovary histopathology

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was

studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the ovaries of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

4.4 Rank 2: Repeat Dose Toxicity – Prostate histopathology

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the prostates of mice or rats compared with controls.

4.5 Rank 2: Repeat Dose Toxicity – Seminal vesicle histopathology

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the seminal vesicle of mice or rats compared with controls.

4.6 Rank 2: Repeat Dose – Testis histopathology (atrophy)

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in testes of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

4.7 Rank 2: Repeat Dose Toxicity – Testis weight

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Weights of testes in mice and rats were not affected by ethylbenzene.

4.8 Rank 2: Repeat Dose Toxicity – Uterus histopathology

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the uteri of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The uterus of high-exposure and controls animals of all species was subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in this organ.

4.9 Rank 2: Reproductive Toxicity – Estrous cyclicity

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean estrous cycle length (4.0 ± 0.3days) was significantly reduced for the F_0, 500ppm group when compared to the F_0 control group value (4.4 ± 0.8 days). However, the authors felt this difference was not biologically important because all females in this group were cycling normally and this strain of rat normally exhibits 4- to 5 day estrous cycles. Mean estrous cycle length did not differ between control and experimental F_1 offspring.

4.10 Rank 2: Reproductive Toxicity – Fertility

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three

equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F_0 or F_1 animals.

4.11 Rank 2: Reproductive Toxicity – Gross pathology

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-hr inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: No adverse exposure-related macroscopic pathology was noted at any level.

4.12 Rank 2: Reproductive Toxicity – Litter size

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean number of F_1 and F_2 pups born, live litter size, percentage of males per litter at birth, and postnatal survival were unaffected by ethylbenzene exposure.

4.13 Rank 2: Reproductive Toxicity – Prostate weight

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were statistically significant decreases in absolute prostate weights in the F_0 male 500ppm group but not when these organ weights were expressed relative to body weight. There was no significant difference in absolute or relative prostate weights in F_1 males.

4.14 Rank 2: Reproductive Toxicity – Sperm count

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean sperm number (millions/g tissue) in the left cauda epididymis for F_0 and F_1 males were not significantly different between any treatment group and controls.

4.15 Rank 2: Reproductive Toxicity – Sperm motility

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean percentage of motile sperm did not differ significantly between any of the treatment group animals compared with controls.

4.16 Rank 2: Reproductive Toxicity – Time to balano-preputial separation

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 hr/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 hr apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 hr after a 6-hr inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean age at acquisition of balanopreputial separation was significantly greater in the F_1 offspring in the 500ppm treatment group compared with controls (PND 44.7± 2.0 vs. PND 43.5 ± 2.2). The mean value for the 500ppm F_1 male group (PND 44.7) was similar to the value obtained in the F_2 generation control group (PND 45.3) and essentially equivalent to the mean historical control value (44.8 days) for the laboratory and as such, the authors stated that the significant finding was not considered biologically important. F_2 data for this measure were not published. However, the data is available in the full study report.

4.17 Rank 2: Reproductive Toxicity – Time to mating

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F_0 or F_1 animals.

4.18 Rank 3: Repeat Dose Toxicity – Gross pathology

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Gross pathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

5. Thyroid Inhibition Hypothesis

5.1 Rank 2: Repeat Dose Toxicity – Thyroid follicular cell histopathology

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Positive trends in the incidences of thyroid follicular cell hyperplasia occurred in mice in both males (control: 21:50; 75 ppm: 21:50; 250 ppm: 29:50; 750 ppm: 32:50) and females (18:50, 23:50, 25:50, 35:50) with significant increases in incidences relative to chamber

controls in 750 ppm males and females. There were no significant differences between control and exposed rat thyroids upon histopathological examination.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (*n* = 5/ dose/sex) and 13 weeks (*n* = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic changes seen in the thyroid glands of mice or rats.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

5.2 Rank 2: Developmental Toxicity – Fetal survival

[4] ***Saillenfait and colleagues (2007)*** The combined effects of EB and BA were investigated. Groups of 18 bred rats (15– 18 pregnant) were exposed to vapors of EB or BA, separately or in combination, 6 h day−1, on days 6–20 of gestation. There were nine experimental groups: Control; 250 or 1000 ppm EB; 500 or 1500 ppm BA or mixtures of 250 ppm EB + 500 ppm BA, 250 ppm EB + 1500 ppm BA, 1000 ppm EB + 500 ppm BA, or 1000 ppm EB + 1500 ppm BA

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no effect of treatment on the mean number of implantations and of live fetuses, and on the incidence of non-live implants and resorptions.

[5] ***Saillenfait and colleagues (2003)*** The developmental toxicity of ethylbenzene was studied in Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The percent dead fetuses per litter was not significantly different between ethylbenzene treated and control dams.

[6] ***Saillenfait and colleagues (2006)*** Pregnant Sprague–Dawley rats were exposed to ethylbenzene (EB; 0, 250, or 1000 ppm) and methylethylketone (MEK; 0, 1000, or 3000 ppm), alone and in combination, by inhalation, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no increase in embryolethality for fetuses whose mothers were exposed to ethylbenzene alone or in combination with methylethylketone.

[11] ***Ungváry, G. & Tátrai, E. (1985)*** Groups of CFY rats were exposed to inhalation of ethylbenzene at 0, 138, 276 or 553 ppm for 24 h/day from day 7 to day 15 of pregnancy. Fetuses were evaluated on pregnancy day 21. CFLP mice were exposed to inhalation of ethylbenzene at 0, 115 or 230 ppm for 24 h/day (no data provided for these groups) or for 3-4 h/day intermittently from day 6 to 15 of pregnancy. The fetuses were evaluated on pregnancy days 18. NZ rabbits were exposed to 0, 115, or 230 ppm ethylbenzene for 24 h/day from day 7 to day 20 gestation. Fetuses were examined on pregnancy day 30. The three rabbit does in the 230-ppm dose group aborted.

Limitations: The data for mice was only provided for the animals in the 115-ppm exposure group and maternal toxicity information was lacking. The authors mention that the maternal toxic effects of ethylbenzene in rats were “moderate and dose-dependent” but fail to describe or quantify these effects. The contribution of general toxicity effects to all study findings should be considered.

Results included in WOE: All rabbit dams (3/3) in the 230 ppm dose group aborted resulting in the loss of all fetuses.

[13] ***Andrew et al., (1981); Hardin et al. (1981)*** Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: The number of implantations was comparable among groups. The number of live fetuses was slightly reduced in rabbits, but not in rats, exposed to ethylbenzene at 1,000 ppm, a concentration that produced some indications of maternal systemic effects. This finding is therefore unlikely to have been produced by and endocrine mode of action.

5.3 Rank 2: Developmental Toxicity – Fetal weight

[4] ***Saillenfait and colleagues (2007)*** The combined effects of EB and BA were investigated. Groups of 18 bred rats (15– 18 pregnant) were exposed to vapors of EB or BA, separately or in combination, 6 h day−1, on days 6–20 of gestation. There were nine experimental groups: Control; 250 or 1000 ppm EB; 500 or 1500 ppm BA or mixtures of 250 ppm EB + 500 ppm BA, 250 ppm EB + 1500 ppm BA, 1000 ppm EB + 500 ppm BA, or 1000 ppm EB + 1500 ppm BA

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. Additionally, high doses were used. It is unclear if the endpoint responses are secondary to general toxicity.

Results included in WOE: Fetal body weight was significantly decreased after exposure to 1000 ppm EB alone.

[5] ***Saillenfait and colleagues (2003)*** The developmental toxicity of ethylbenzene was studied in Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: Clinical signs of toxicity (ataxia, decreased motor activity) were seen at 2000 ppm. Maternal weight was significantly reduced on GD 21 at 1000 ppm and on GD 13 and 21 at 2000 ppm. Dams exposed to 1000 or 2000 ppm showed significant decreases in maternal weight gain and food consumption throughout exposure, and in corrected weight gain

Results included in WOE: Ethylbenzene produced a concentration-related reduction in fetal weights that achieved statistical significance at 1000 ppm. These decreases amounted to 7 and 18% of the control values at 1000 and 2000 ppm, respectively.

[6] ***Saillenfait and colleagues (2006)*** Pregnant Sprague–Dawley rats were exposed to ethylbenzene (EB; 0, 250, or 1000 ppm) and methylethylketone (MEK; 0, 1000, or 3000 ppm), alone and in combination, by inhalation, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. Additionally, high doses were used. It is unclear if the endpoint responses are secondary to general toxicity.

Results included in WOE: The body weight of the fetuses (all, males, females) was significantly lower than control after exposure to the high concentration of EB, 1000 ppm.

[11] ***Ungváry, G. & Tátrai, E. (1985)*** Groups of CFY rats were exposed to inhalation of ethylbenzene at 0, 138, 276 or 553 ppm for 24 h/day from day 7 to day 15 of pregnancy. Fetuses were evaluated on pregnancy day 21. CFLP mice were exposed to inhalation of ethylbenzene at 0, 115 or 230 ppm for 24 h/day (no data provided for these groups) or for 3-4 h/day intermittently from day 6 to 15 of pregnancy. The fetuses were evaluated on pregnancy days 18. NZ rabbits were exposed to 0, 115, or 230 ppm ethylbenzene for 24 h/day from day 7 to day 20 gestation. Fetuses were examined on pregnancy day 30. The three rabbit does in the 230-ppm dose group aborted.

Limitations: The data for mice was only provided for the animals in the 115-ppm exposure group and maternal toxicity information was lacking. The authors mention that the maternal toxic effects of ethylbenzene in rats were "moderate and dose-dependent" but fail to describe or quantify these effects. The contribution of general toxicity effects to all study findings should be considered.

Results included in WOE: The percentage of weight-retarded fetuses was significantly greater in the group of rats exposed to ethylbenzene at a concentration of 553 ppm and in female rabbit fetuses at 115 ppm compared with controls. There was not a significant difference in mean fetal weights in mice exposed to ethylbenzene 3-4 hours/day intermittently at 115 ppm.

[13] ***Andrew et al., (1981); Hardin et al. (1981)*** Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: Fetal weights were unchanged relative to controls in rats or rabbit exposed to 100 or to 1,000 ppm ethylbenzene.

5.4 Rank 2: Reproductive Toxicity – Pup growth

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean body weight gain of males and females in the F_1 and F_2 offspring, between postnatal days 1-4, did not differ significantly from the control animals.

5.5 Rank 2: Reproductive Toxicity – Pup survival

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-hinhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The percentage of F_1 and F_2 pups surviving from birth to PND 4 and from PND 4-21 did not differ between treatment animals and controls.

5.6 Rank 2: Reproductive Toxicity – Thyroid weight

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. The authors note that because this finding was only found in the F_1 males and not in the F_1 females or the F_2 treatment animals that the finding was considered to be the result of normal biological variation and not related to ethylbenzene exposure. However, we could not ascertain that a histopathologic examination of the thyroid tissue was carried out to rule out pathologic changes.

Results included in WOE: Increases (approximately 18–20% and statistically significant) in absolute and relative thyroid weights in the F_0 males in the 100 and 500 ppm groups were not replicated in the F_1 male group nor were they observed in the female groups exposed to these concentrations of ethylbenzene.

5.7 Rank 3: Repeat Dose Toxicity – Liver weight

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. However, the histological examination of liver tissue demonstrated only centrilobular hypertrophy of hepatocytes suggesting an adaptive response.

Results included in WOE: Liver weight was increased in a dose-related fashion in both male and female rats in the mid and high dose exposure groups.

[6] ***Saillenfait and colleagues (2006)*** Pregnant Sprague–Dawley rats were exposed to ethylbenzene (EB; 0, 250, or 1000 ppm) and methylethylketone (MEK; 0, 1000, or 3000 ppm), alone and in combination, by inhalation, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. High doses were used, and it is unclear if the endpoint responses are secondary to general toxicity. Additionally, histological evaluation of the liver revealed no pathological effects attributable to solvent exposures therefore we agree with the authors that the positive liver weight changes are likely an adaptive response.

Results included in WOE: Compared with control, both absolute and relative liver weight were significantly elevated in animals treated with 250 and 1000 ppm ethylbenzene.

[7] Li and colleagues (2010) In the neurotoxicity study, ethylbenzene was administered orally via gavage twice daily to Sprague-Dawley male and female rats at 0, 25, 125, or 250 mg/kg per dose (total daily dosages of 0, 50, 250, or 500 mg/kg bw/day) for 13 weeks and the functional observational battery (FOB), automated tests for motor activity and neuropathological examination were conducted.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. In addition, there were no treatment-related microscopic lesions observed in the liver suggesting the increased relative liver weight finding represents an adaptive response rather than a pathological change.

Results included in WOE: The weights of the liver relative to terminal body weights were significantly increased ($p \leq 0.05$) in male rats at 250 and 500 mg/kg bw/day and in female rats at 500 mg/kg bw/day.

[8] ***Stott and colleagues (1999)*** Male and female Fischer 344 rats and B6C3F1 mice were exposed to 0 or 750 ppm ethylbenzene vapor 6 h/day for one or four weeks. Livers from 6 (one-week study) or 8 (four-week study) mice/sex/dose were examined and weighed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis,

a positive result could be due to several mechanisms. In addition, increased liver weights were not accompanied by histological changes suggesting an adaptive rather than a pathologic response.

Results included in WOE: The relative liver weight of male and female mice exposed to 750 ppm EB for one week and female mice exposed to 750 ppm for four weeks were significantly higher than those of control animals. There were no significant differences in liver weight between male mice and male controls in the four-week study.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. Additionally, high doses were used. It is unclear if the endpoint responses are secondary to general toxicity. There were no treatment-related microscopic lesions associated with the increased liver weights, therefore this change is considered to be an adaptive response rather than a pathological finding.

Results included in WOE: Significant increases in liver weights were seen in male rats in the 250-, 500-, 750- and 1000 ppm exposure groups and in female rats in the 500-, 750- and 1000 ppm exposure groups; significant increases in liver weights were seen in male and female mice in the 750- and 1000-ppm exposure groups.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were statistically significant increases in the liver/body weight ratios of male rats in the 782 ppm dose group and female rats in the 382 ppm and 782 ppm dose groups. Absolute liver weight was increased for female rats at 782 ppm and for male rats at 382 and 782 ppm. The absolute liver weight for female mice was also significantly increased at 782 ppm. We agree with the authors note that the absence of accompanying liver histopathology or abnormal clinical chemistry indicates that the increases were due to an adaptive induction of hepatic function rather than toxicity. Liver weights were unchanged in rabbits exposed to EB at any concentrations up to 1610 ppm.

5.8 Rank 3: Reproductive Toxicity – Liver weight

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of

the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms. Additionally, histological evaluation of the liver revealed no pathological effects attributable to solvent exposures therefore we agree with the authors that the positive liver weight changes are likely an adaptive response.

Results included in WOE: Absolute and relative liver weights were slightly increased (3–7%) in the 500ppm groups compared to the control group. The increases in relative liver weight were statistically significant in the F_0 and F_1 females. These increases in the liver weights were considered related to ethylbenzene exposure but not a pathological finding.

5.9 Rank 3: Developmental Neurotoxicity – Auditory startle

[12] ***Faber and colleagues (2007)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500 ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed. Neurobehavioral development of one F2-generation treatment derived offspring/sex/litter was assessed in a functional observational battery (FOB; PND 4, 11, 22, 45, and 60), motor activity sessions (PND 13, 17, 21, and 61), acoustic startle testing (PND 20 and 60), a Biel water maze learning and memory task (initiated on PND 26 or 62), and in evaluations of whole-brain measurements and brain morphometric and histologic assessments (PND 21 and 72).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no statistically significant differences in parameter of the acoustic startle test for the F_2 treatment derived offspring of either gender. On PND 20, the maximum startle amplitudes in the control group were much lower than the mean historical control values for this assessment age. Also on PND 60, a statistically significant main effect of treatment was obtained for maximum startle amplitude in the F_2 males, however there was an outlier response of three control males which inflated the mean. When these responses were removed, the within group distributions were not markedly different and closely matched historical control values. Therefore, the differences noted in males at this age were attributed to unusual control mean values and were not considered to be related to parental ethylbenzene exposure.

5.10 Rank 3: Developmental Neurotoxicity – Brain morphometry

[12] ***Faber and colleagues (2007)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500 ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed. Neurobehavioral development of one F2-generation treatment derived offspring/sex/litter was assessed in a functional observational battery (FOB; PND 4, 11, 22, 45, and 60), motor activity sessions (PND 13, 17, 21, and 61), acoustic startle testing (PND 20 and 60), a Biel water maze learning and memory task (initiated on PND 26 or 62), and in evaluations of whole-brain measurements and brain morphometric and histologic assessments (PND 21 and 72).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: No brain morphometric changes were noted at either age (PNDs 21, 72) in the measurements taken in the height of the hemisphere and vertical thickness of the cortex, the radial thickness of the cortex, vertical heights between hippocampal pyramidal neuron layers, vertical height of the dentate hilus, the length of the ventral limb of the dentate hilus or the vertical thickness of the brainstem and base of cerebellar lobule 9, in animals of either gender.

5.11 Rank 3: Developmental Neurotoxicity – Learning and memory

[12] ***Faber and colleagues (2007)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500 ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed. Neurobehavioral development of one F2-generation treatment derived offspring/sex/litter was assessed in a functional observational battery (FOB; PND 4, 11, 22, 45, and 60), motor activity sessions (PND 13, 17, 21, and 61), acoustic startle testing (PND 20 and 60), a Biel water maze learning and memory task (initiated on PND 26 or 62), and in evaluations of whole-brain measurements and brain morphometric and histologic assessments (PND 21 and 72).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Assessments of straight-alley escape times and aspects of learning and memory in the Biel water maze task were initiated on PND 26 and PND 62. There were no biologically meaningful differences noted in animals of either gender at either testing age.

5.12 Rank 3: Developmental Neurotoxicity – Motor activity

[12] ***Faber and colleagues (2007)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500 ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed. Neurobehavioral development of one F2-generation treatment derived offspring/sex/litter was assessed in a functional observational battery (FOB; PND 4, 11, 22, 45, and 60), motor activity sessions (PND 13, 17, 21, and 61), acoustic startle testing (PND 20 and 60), a Biel water maze learning and memory task (initiated on PND 26 or 62), and in evaluations of whole-brain measurements and brain morphometric and histologic assessments (PND 21 and 72).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no statistically significant differences among the groups in cumulative session activity counts during the pre-weaning period (PND13, 17, 21). There was a significant main effect of treatment found in the repeated measure of analysis of variance for total activity counts for females on PND 61, but due to the relatively slight change in this behavior and the lack of any suggested dose-response relationship in either gender, this difference was not considered to be related to parental ethylbenzene exposure.

6. Interaction with Steroidogenesis Enzymes Hypothesis

6.1 Rank 2: Repeat Dose Toxicity – Ovary histopathology

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (*n* = 5/ dose/sex) and 13 weeks (*n* = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***(National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the ovaries of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

6.2 Rank 2: Repeat Dose toxicity – Testis histopathology

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in

females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the testes of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The gonads (ovaries or testes with epididymides) and thyroids of high-exposure and controls animals of all species were subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in any of these tissues.

6.3 Rank 2: Repeat Dose Toxicity – Uterus histopathology

[1] ***NTP (National Toxicology Program). (1999)*** Groups of 50 male and 50 female Fischer rats and B6C3F1 mice were exposed to ethylbenzene by inhalation at 0, 75, 250, and 750 ppm 6 h per day, 5 days per week, for 104 weeks (rats) and for 103 week (mice).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no significant histological differences between the chamber

control animals and treatment group animals, in either specie, for uterine, vaginal or ovarian tissues in females or for testes, prostate, epididymides or seminal vesicles for males.

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (n = 5/ dose/sex) and 13 weeks (n = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/kg bw/day, administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Histopathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

[9] ***NTP (National Toxicology Program). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies) (1992)*** Inhalation toxicity of ethylbenzene was studied by exposing groups of seven-week-old F344/N rats and B6C3Fi mice of each sex to ethylbenzene vapor at chamber concentrations of 0, 100, 250, 500, 750, or 1000 ppm, 6 h per day, 5 days per week for 13 weeks.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There were no histopathologic lesions and no chemically related histopathologic changes identified in the uteruses of mice or rats compared with controls.

[10] Cragg and colleagues (1989) Mice, rats and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapors for 6 h/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382 or 782 ppm EB while rabbits received 0, 382, 782 or 1610 ppm.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The uterus of high-exposure and controls animals of all species was subjected to histopathological examination. There were no gross or microscopic changes attributable to ethylbenzene in this organ.

6.4 Rank 2: Developmental Toxicity – Sex ratio

[5] ***Saillenfait and colleagues (2003)*** The developmental toxicity of ethylbenzene was studied in Sprague–Dawley rats after inhalation exposure. Animals were exposed to ethylbenzene at 100, 500, 1000 or 2000 ppm, for 6 h/day, during days 6–20 of gestation.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The percentage of males per litter did not differ between any of the treatments groups and controls.

[13] ***Andrew et al., (1981); Hardin et al. (1981)*** Groups of 29-33 Female Wistar rats and New Zealand White rabbits were exposed to 0, 100, or 1000 ppm ethylbenzene for 7 h/day, 5 days/week for 3 weeks, then mated with unexposed males. Pregnant females were further exposed to 0, 100, or 1000 ppm 7 h/day through Gestational Day 19 (rats) and 24 (rabbits).

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in the WOE: Sex ratio in rats and rabbits was unaffected by exposure to 100 or to 1,000 ppm ethylbenzene relative to unexposed controls.

6.5 Rank 2: Reproductive Toxicity – Estrous cyclicity

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean estrous cycle length (4.0 ± 0.3days) was significantly reduced for the F_0, 500ppm group when compared to the F_0 control group value (4.4 ± 0.8 days). However, the authors felt this difference was not biologically important because all females in this group were cycling normally and this strain of rat normally exhibits 4-5 day estrous cycles. Mean estrous cycle length did not differ between control and experimental F_1 offspring.

6.6 Rank 2: Reproductive Toxicity – Fertility

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 hr after the last

gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: There was no impairment of fertility or increased time to mating in the F_0 or F_1 offspring.

6.7 Rank 2: Reproductive Toxicity – Live births

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean number of F_1 and F_2 pups born, live litter size, percentage of males per litter at birth, and postnatal survival were unaffected by ethylbenzene exposure.

6.8 Rank 2: Reproductive Toxicity – Mating index

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The male and female mating indices (%) were not different between the any of the treatment animals and controls.

6.9 Rank 2: Reproductive Toxicity – Sex ratio

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 hr after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The sex distribution, measured by the % males/litter, was not different in either F_1 or F_2 litters compared with control litters.

6.10 Rank 2: Reproductive Toxicity –Sperm count

[2] ***Faber and colleagues (2006)*** Four groups of Crl:CD(SD)IGS BR rats (30/sex/group for F_0 and 25/sex/group for F_1) were exposed to 0, 25, 100, and 500ppm EB for 6 h/day for at least 70 consecutive days before mating. Inhalation exposure for the F_0 and F_1 females continued throughout mating, gestation through gestation day (GD) 20, and lactation days (LD) 5–21. On LD 1–4, females received EB in corn oil via oral gavage at dose levels of 26, 90, and 342 mg/kg/day (divided into three equal doses, approximately 2 h apart). Pups were weaned on postnatal day (PND) 21 and exposure of the F_1 generation started on PND 22. Estimates of internal exposure were determined by measuring EB concentrations in blood collected from F_1 dams (4/group) and their culled pups 1 h after the last gavage dose on PND 4. On PND 22, blood was collected from these same F_1 dams and their weanlings for EB analysis 1 h after a 6-h inhalation exposure. The remainder of the F_2 generation was not directly exposed.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: The mean sperm number (millions/g tissue) in the left cauda epididymis for F_0 and F_1 males were not different between any treatment group and controls.

6.11 Rank 3: Repeat Dose Toxicity – Gross Pathology

[3] ***Mellert and colleagues (2007)*** Ethylbenzene was administered to groups of male and female Wistar rats by gavage for 4 (*n* = 5/ dose/sex) and 13 weeks (*n* = 10/dose/sex) (OECD 408) at doses of 0 (vehicle control), 75, 250, and 750 mg/ kg bodyweight/day (mg/kg bw/day), administered am/pm as half doses.

Limitations: This study was not specifically designed to investigate an endocrine mode of action. Although a negative result supports the conclusion that the response is not indicative of the hypothesis, a positive result could be due to several mechanisms.

Results included in WOE: Gross pathological evaluation of the epididymis, mammary glands, ovaries, prostate, seminal vesicles, testes, thyroid, uterus, and vagina did not identify any adverse effects.

Supplemental Material C
Rationale for Excluding Studies

Gong et al., 2023.

Gong, X., Huang, Y., Duong, J., Leng, S., Zhan, F.B., Guo, Y., Lin, Y., Luo, L. 2023. Industrial air pollution and low birth weight in New Mexico, USA. Journal of Environmental Management, 348, part. no. 119236, DOI: 10.1016/j.jenvman.2023.119236.

This study evaluated the relationship between exposure to air pollution and Low Birth Weight (LBW) among 22,375 LBW cases and 233,340 controls in New Mexico, where the incidence of LBW has exceeded the national average in recent decades. Exposure focused on 14 common chemicals listed in the Toxic Release Inventory (TRI) and monitoring datasets, which have abundant monitoring samples. The Emission Weighted Proximity Model (EWPM) was used to calculate maternal air pollution exposure intensity. Adjusted odds ratios (adjORs) were calculated using binary logistic regressions to examine the association between maternal residential air pollution exposure and LBW, while controlling for potential confounders, such as the maternal age, race/ethnicity, gestational age, prenatal care, education level, consumption of alcohol during pregnancy, public health regions, child's sex, and the year of birth. Multiple comparison correction was applied using the False Discovery Rate approach. Maternal residential exposure to 1,2,4-trimethylbenzene, benzene, chlorine, ethylbenzene, and styrene was associated with LBW in offspring, with adjusted odds ratios ranging from 1.10 to 1.13. These five chemicals remained as significant risk factors after dividing the estimated exposure intensities into four categories. In addition, significant linear trends were found between LBW and maternal exposure to each of the five identified chemicals. Furthermore, 1,2,4-trimethylbenzene was identified as a risk factor to LBW for the first time.

Although statistically significant, the adjusted odds ratios reported in this study are of such low magnitude that their biological relevance is uncertain. Despite reporting significant linear trends for all five chemicals individually, the adjusted odds ratios are not quantifiable for ethylbenzene exposure alone, as the exposure was to air pollution generally rather than to specific chemicals. Exposures were estimated based on residential locations, excluded potential workplace exposures, and did not consider several potentially-important confounders for LBW, such as genetic factors, exposure to tobacco smoke, and exposure to other air pollutants, including those on the Criteria Air Pollutants (CAPs) list. As such, the results are not interpretable for use in the WoE evaluation.

Gong et al., 2018.

Gong, X., Lin, Y., Bell, M. L., & Zhan, F. B. (2018). Associations between maternal residential proximity to air emissions from industrial facilities and low birth weight in Texas, USA. Environ Int, 120, 181–198. https://doi.org/10.1016/j.envint.2018.07.045

Gong et al., (2018) is a forerunner to Gong et al., 2023 that investigated associations between maternal residential exposure to industrial air pollutants during pregnancy and low birth weight (LBW) in offspring using a case-control design that included 94,106 term LBW cases and 376,424 controls. The analysis covered 78 air pollutants common to both the Toxic Release Inventory and ground air quality monitoring databases in Texas during 1996 – 2008. The authors report an adjusted odds ratios for ethylbenzene of 1.05 (95% CI: 1.03 – 1.06). Although statistically significant, the biological significance of such a low odds ratio is questionable. Confounding exposures and inadequate control of other factors that could influence birth weight render the results uninterpretable for a WoE analysis of potential endocrine activity for ethylbenzene.

Supplemental Material C
Rationale for Excluding Studies

Harrath et al., 2022.

Harrath AH, Alrezaki A, Jalouli M, Aldawood N, Aldahmash W, Mansour L, & Alwasel S. (2022). Ethylbenzene exposure disrupts ovarian function in Wistar rats via altering folliculogenesis and steroidogenesis-related markers and activating autophagy and apoptosis. Ecotoxicol Environ Saf, 229, 113081. doi:10.1016/j.ecoenv.2021.113081.

In this repeat dose toxicity study, Harrath et al., (2022) exposed rats to EB for 30 minutes per day for 30 consecutive days to 2,000 ppm, 4,000 ppm, and 8,000 ppm EB. Ovary weight was slightly reduced at 2,000 ppm, but not at 4,000 or 8,000 ppm. Abnormal follicles were observed at 2,000 and 4,000 ppm but the effect was barely significant at 8,000 ppm. Circulating estradiol levels were increased at 4,000 but not at 2,000 or 8,000 ppm. Circulating testosterone was increased at 2,000 and 8,000 ppm, but not at 4,000 ppm and estrogen receptor numbers were also altered.

The lack of clear dose-response relationships for these various effects makes the results difficult to interpret, but this is the least of the problems with this study. All exposure levels produced significant apoptosis in the affected organs, which confounds the interpretation of an endocrine MoA as each endpoint was likely affected secondary to induction of apoptosis. The authors assert that apoptosis is the primary mechanism underlying various other effects observed in the study. Moreover, the reported levels of exposure strain credulity. The reported exposure concentrations are equivalent to 2.5X, 5X, and 10X the IDLH value* for human occupational exposures, and near within 1/4, 1/2, and 1X the explosive limit of the chemical. The lowest exposure level used in this study equals or exceeds the highest level used in other studies, and is tenfold above the KMD for EB (Burgoon et al., 2023). Even though the exposure durations were short (30 minutes), the degree of kinetic overload these would produce and the obvious confounding by apoptosis and other unknown mechanisms renders the effects reported by Harrath et al. (2022) unreliable and uninterpretable for the purposes of an endocrine WoE analysis.

Lei, T., Qian, H., Yang, J., Hu, Y.

Lei, T., Qian, H., Yang, J., & Hu, Y. (2023). The association analysis between exposure to volatile organic chemicals and obesity in the general USA population: A cross-sectional study from NHANES program. Chemosphere, 315, 137738. https://doi.org/10.1016/j.chemosphere.2023.137738

This study attempted to evaluate whether recent reports of an association between exposure to volatile organic chemical (VOC) pollutants, measured as urinary metabolites, and obesity are general, or associated specifically with abdominal obesity. Data from the 6 survey cycles (2005–2006, 2011–2018, 2017–2020) of the NHANES program were analyzed by 4 separate models in a cross-sectional study among a total of 17,524 participants (4965 obesity, 7317 abdominal obesity). Participants in the obesity or abdominal obesity groups showed higher VOCs in urine than were present in the control group. OR for obesity in the Q2 to Q4 of model 3 was 1.169 (Q2, $p < 0.05$), 1.306 (Q3, $p < 0.001$) and 1.217 (Q4, $p < 0.01$) respectively. The OR for abdominal obesity in the Q2 to Q4 of model 3 was 1.222 (Q2, $p < 0.01$), 1.448 (Q3, $p < 0.001$) and 1.208 (Q4, $p < 0.05$) respectively. A significantly positive association between urine levels of VOCs (Acrolein, Acrylamide, Acrylonitrile, 1,3-Butadiene, Crotonaldehyde, Cyanide, N,N-Dimethylformamide, Ethylbenzene, Styrene, Propylene oxide, Toluene and Xylene) and BMI and waist circumference was reported.

None of the analyses were specific to ethylbenzene, and the models employed were incapable of determining whether the direction of the associations, i.e., whether exposure begat obesity or obesity enhanced absorption of VOCs. The endpoints measured were unusable in the WoE evaluation due to confounding by multiple chemical exposures.

Supplemental Material C
Rationale for Excluding Studies

Nakhjirgan et al. 2019.
Nakhjirgan P, Kashani H, Naddafi K, Nabizadeh R, Amini H, & Yunesian M. (2019). Maternal exposure to air pollutants and birth weight in Tehran, Iran. J Environ Health Sci Eng, 17(2), 711-717. doi:10.1007/s40201-019-00386-7

Although the authors mention EB in the context of air pollutants in urban Tehran, Iran, the study evaluated potential associations between air pollutants broadly and health outcomes in pregnant women. Since exposures to EB were unspecified and uncertain and the results indicative only of potential associations with urban air generally, the results of the study are not informative regarding potential endocrine MoAs for EB.

Rouget et al., 2021.
Rouget F, Bihannic A, Cordier S, Multigner L, Meyer-Monath M, Mercier F, Pladys P, Garlantezec R. (2021). Petroleum and Chlorinated Solvents in Meconium and the Risk of Hypospadias: A Pilot Study. Front Pediatr, 9, 640064. doi:10.3389/fped.2021.640064

Rouget et al. (2021) conducted a pilot case-control study in the maternity unit of the University Hospital in Rennes, France to evaluate possible associations between the occurrence of hypospadias and fetal exposure to petroleum and chlorinated solvents measured in meconium. Since exposures to EB were unspecified and uncertain and the results indicative only of potential associations with petroleum and chlorinated solvents generally, the results of the study are not useful for an endocrine WoE evaluation.

Werder et al. 2019.
Werder EJ, Engel LS, Blair A, Kwok RK, McGrath JA, & Sandler DP. (2019). Blood BTEX levels and neurologic symptoms in Gulf states residents. Environ Res, 175, 100-107. doi:10.1016/j.envres.2019.05.004

Werder et al. (2019) evaluated potential associations between blood levels of BTEX chemicals (benzene, toluene, EB, and xylene) in Gulf coast residents of the United States who were transiently exposed to BTEX during the Deepwater Horizon oil spill and/or the response to it. Although the publication mentions endocrine disruptive effects of BTEX, the authors generated no data relevant to specific endocrine effects of EB.

Werder et al. 2020.
Werder EJ, Beier JI, Sandler DP, Falkner KC, Gripshover T, Wahlang B, . . . Cave MC. (2020). Blood BTEXS and heavy metal levels are associated with liver injury and systemic inflammation in Gulf states residents. Food Chem Toxicol, 139, 111242. doi:10.1016/j.fct.2020.111242.

Werder et al., 2020 conducted a clinical cross-sectional analysis to evaluate possible associations of biomarkers with serum livery injury and adipocytokine biomarkers in a sample of 214 men. No data relevant to specific endocrine effects of EB were reported. However, with respect to endocrine disruptive effects as speculated by the authors, their results suggest that rather than and endocrine mechanism, liver toxicity would be the likely mechanism that secondarily affects endocrine parameters.

Supplementary Table 1. Estrogen Agonist Hypothesis; Guideline Toxicity Studies					
Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
1	FSTRA	Vitellogenin	↑♂		
	Uterotrophic	Uterus weight (blotted or wet)	↑		
2	Uterotrophic	Conversion to estrus	↑		
	ERTA	Reporter gene activation	↑		
	FSTRA	Behavioral (sexual, mating)	Δ♂		
		Gonad histopathology	Δ♂		
		Tubercle score	↓♂		
	Female Pubertal	Age and body weight at vaginal opening	↓		
		Age at first estrus	↓		
		Ovary histopathology	Δ		
		Ovary weight	↓		
	Male Pubertal	Testis histopathology (atrophy)	Δ		
		Testis weight	↓		
	Repeat Dose Toxicity	Epididymis histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Epididymis weight	↓	[9s,m]	[9s,r]
		Mammary histopathology	Δ		
		Ovary histopathology	Δ		[1c,m,r][3s,r] [9s,m,r] [10s,m,r,rb]
		Ovary weight	↓		
		Prostate weight	↓		
		Seminal vesicle weight	↓		
		Testis histopathology (atrophy)	↑		[1c,m,r] [9s,m,r]
		Testis weight	↓		[9s,m,r]
		Uterus histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Uterus weight	↑		
		Vaginal histopathology	Δ		[1c,m,r] [3s,r]
	Developmental Toxicity	Corpora lutea	↓		[5r][13r,rb]
		Post-implantation loss	↑	[11r] [13rb]	[4r] [5r] [6r] [11m,rb] [13r]

Supplementary Table 1. Estrogen Agonist Hypothesis; Guideline Toxicity Studies					
Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
2	Developmental Toxicity	Pre-implantation loss	↑		[13r,rb]
		Time to vaginal patency	↓		
	Reproductive Toxicity	Anogenital distance	Δ ♂, ♀		
		Corpora lutea	↓		
		Epididymis histopathology (atrophy)	Δ		
		Epididymis weight	↓		
		Estrous cyclicity	Δ	[2r,F_0]	[2r,F_1]
		Fertility	↓ ♂, ♀		[2r,F_0,F_1]
		Gestational length	↓♀		[2r,F_0,F_1]
		Implantations	↓		[2r,F_0,F_1]
		Litter size	↓		[2r,F_0,F_1]
		Mammary histopathology	Δ ♀		
		Mating index	↓ ♂, ♀		[2r,F_0,F_1]
		Ovarian follicle count in offspring	Δ		[2r,F_1]
		Ovary histopathology	Δ		
		Ovary weight in offspring	↓		
		Prostate histopathology (atrophy)	Δ		
		Prostate weight	↓		
		Seminal vesicle weight	↓		
		Sperm count	↓		[2r,F_0,F_1]
		Testis histopathology (atrophy)	Δ		
		Testis weight (absolute)	↓		
		Time to mating	↑ ♂, ♀		[2r,F_0,F_1]
		Time to preputial separation	↑ ♂		
		Time to vaginal patency	↓	[2r,F_1]	[2r,F_2]
		Uterus histopathology	Δ		
		Uterus weight in offspring	↑		
		Vaginal histopathology	Δ		
3	ERBA	Displacement of Estradiol	↑		
	FSTRA	Behavior	Δ		
		Estradiol level	↓♀		
		Fecundity	↓		

Supplementary Table 1. Estrogen Agonist Hypothesis; Guideline Toxicity Studies					
Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
3	FSTRA	Fertilization success	↓♀		
		Follicular atresia	↑		
		Gonad somatic index	↓♂, ↑♀		
		Testosterone level	↓♂		
	Female Pubertal	Estrous cyclicity	↑		
		Growth	↑		
	Male Pubertal	Epididymis histopathology	Δ		
		Growth	Δ		
		Ventral prostate weight	Δ		
	Steroidogenesis	Estradiol level	↑		
	Repeat Dose Toxicity	Gross pathology	Δ♂, ♀		[3s,r]

♂= males; ♀= females; ↑ = increase relative to controls; ↓ = decrease relative to controls; Δ = altered; ? = altered but not as expected. Numbers correspond to numbered studies in Appendix C; r = rat; m = mouse; rb = rabbit; s = subchronic; c = chronic; n = non-guideline; F_0 = F_0 generation; F_1 = F_1 generation; F_2 = F_2 generation.

Supplementary Table 2. Estrogen Antagonist Hypothesis; Guideline Toxicity Studies					
Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
1	Uterotrophic	Uterus weight increase w E2	↓		
2	ERBA	Displacement of estradiol	↑		
	FSTRA	Gonad histopathology	Δ ♀		
		Vitellogenin	↓ ♀		
	Female Pubertal	Age and body weight at vaginal opening	↑		
		Age at first estrus	↑		
	Repeat Dose Toxicity	Epididymis histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Ovary histopathology	Δ		[1c,mr] [3s,r] [9s,m,r] [10s,m,r,rb]
		Prostate histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r]
		Seminal vesicle histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r]
		Testis histopathology (atrophy)	↑		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Testis weight	↓		[9s,m,r]
	Developmental Toxicity	Corpora lutea	↓		[5r] [13r,rb]
		Time to vaginal patency	↓		
	Reproductive Toxicity	Corpora lutea	↓		
		Epididymis histopathology (atrophy)	Δ		
		Estrous cyclicity	Δ	[2r,F_0]	[2r,F_1]
		Fertility	↓ ♂, ♀		[2r,F_0,F_1]
		Litter size	↓		[2r,F_0,F_1]
		Ovary histopathology	Δ		
		Prostate histopathology (atrophy)	Δ		
		Seminal vesicle histopathology	Δ		

Supplementary Table 2. Estrogen Antagonist Hypothesis; Guideline Toxicity Studies

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
2	Reproductive Toxicity	Sperm count	↓		[2r,F_0,F_1]
		Testis histopathology (atrophy)	Δ		
		Testis weight (absolute)	↓		
		Time to mating	↑ ♂ , ♀		[2r,F_0,F_1]
		Time to vaginal patency	↑	[2r,F_1]	[2r,F_2]
3	Aromatase Inhibition	Aromatase inhibition	↓		
	FSTRA	Behavior	Δ ♀		
		Estradiol level	↓ ♀		
		Fecundity	↓		
		Fertilization success	↓ ♀		
		Gonad somatic index	↓ ♀ , ♂		
		Testosterone level	↓ ♂		
	Female Pubertal	Estrous cyclicity	Δ		
		Ovary histopathology (atrophy)	Δ		
		Ovary weight (with atrophy)	↓		
	Steroidogenesis	Estradiol level	↓		
	Repeat Dose Toxicity	Gross pathology	Δ ♂ , ♀		[3s,r]

♂= males; ♀= females; ↑ = increase relative to controls; ↓ = = decrease relative to controls; Δ = altered; ? = altered but not as expected. Numbers correspond to numbered studies in Appendix C; r = rat; m = mouse; rb = rabbit; s = subchronic; c = chronic; n = non-guideline; F_0 = F_0 generation; F_1 = F_1 generation; F_2 = F_2 generation.

Supplementary Table 3. Androgen Agonist Hypothesis; Guideline Toxicity Studies					
Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
1	Hershberger	Concordance of 5 endpoints	↑		
	FSTRA	Secondary sexual characteristics: tubercles in females	↑		
2	ARBA	Displacement of testosterone	↑		
	FSTRA	Gonad histopathology	Δ		
		Vitellogenin	↓♀		
	Male Pubertal	Age & weight at preputial Separation: if accelerated	↓		
		Dorsolateral prostate weight	↑		
		Epididymis histopathology	Δ		
		Epididymis weight	↑		
		LABC weight	↑		
		Seminal vesicle + coagulating gland weight	↑		
		Testis histopathology (atrophy)	Δ		
		Testis weight	↑		
		Ventral prostate weight	↑		
	Hershberger	Concordance of 2 to 4 endpoints	↑		
	Repeat Dose Toxicity	Ovary histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Ovary weight	↑		
		Prostate weight	↓		
		Seminal vesicle weight	↓		
		Sperm count	↓		[9s,m,r]
		Testis histopathology (atrophy)	↑		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Testis weight	↓		[9s,m,r]
		Uterus weight	↑		
	Developmental Toxicity	Implantations	↓		[4r] [5r] [6r] [13r,rb]
		Litter size	↓	[13rb]	[4r] [5r] [6r] [13r]
		Masculinization of female offspring	↑		
		Sex ratio	Δ♂, ♀		[5r] [13r,rb]
		Time to balano-preputial separation	↓♂		
		Time to vaginal patency	↑		

Supplementary Table 3. Androgen Agonist Hypothesis; Guideline Toxicity Studies					
Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
2	Reproductive Toxicity	Anogenital distance	↑♀		
		Estrous cyclicity	↓	[2r,F_0]	[2r,F_1]
		Fertility	↓♂,♀		[2r,F_0,F_1]
		Implantations	↓		[2r,F_0,F_1]
		Litter size	↓		[2r,F_0,F_1]
		Masculinization of female offspring	↑♀		
		Mating index	↓♂,♀		[2r,F_0,F_1]
		Nipple retention	↑♂		
		Ovarian follicle count	↓		
		Ovary histopathology	Δ		
		Ovary weight in offspring	↑		
		Prostate weight	↓		[2r,F_0,F_1]
		Seminal vesicle weight	↓		
		Sex ratio	Δ♂,♀		[2r,F_1,F_2]
		Sperm count	↓		[2r,F_0,F_1]
		Testis histopathology (atrophy)	Δ		
		Testis weight	↓		
		Time to balano-preputial separation	↓♂	[2r,F_1]	[2r,F_2]
		Time to mating	↑♀		[2r,F_0,F_1]
		Time to vaginal patency	↑	[2r,F_1]	[2r,F_2]
3	Aromatase	Aromatase activity	↑		
	FSTRA	Behavior	Δ		
		Estradiol level	Δ		
		Fecundity	Δ		
		Fertilization success	Δ		
		Gonad somatic index	Δ		
		Testosterone level	Δ		
	Female Pubertal	Adrenals weight	↓		
		Age & weight at vaginal opening	↑		
		Growth	↑		
		Ovary histopathology	Δ		
		Ovary weight	↓		
		Uterus histopathology	Δ		
		Uterus weight	↑		
	Male Pubertal	Growth	↑		
		Testosterone level	↓		
	Steroidogenesis	Testosterone level	Δ		
	Hershberger	Concordance of 1 endpoint	↑		
	Repeat Dose Toxicity	Gross pathology	Δ♂,♀		[3s,r]

♂= males; ♀= females; ↑ = increase relative to controls; ↓ = decrease relative to controls; Δ = altered; ? = altered but not as expected. Numbers correspond to numbered studies in Appendix C; r = rat; m = mouse; rb = rabbit; s = subchronic; c = chronic; n = non-guideline; F_0 = F_0 generation; F_1 = F_1 generation; F_2 = F_2 generation.

Supplementary Table 4. Androgen Antagonist Hypothesis; Guideline Toxicity Studies					
Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
1	Hershberger	Concordance of 5 endpoints	↓		
2	ARBA	Displacement of testosterone	↑		
	FSTRA	Gonad histopathology	Δ ♂		
		Secondary sexual characteristics	↓ ♂		
		Vitellogenin	↑ ♀		
	Male Pubertal	Age & weight at preputial separation: if delayed	↑		
		Dorsolateral prostate weight	↓		
		Epididymis histopathology	Δ		
		Epididymis weight	↓		
		LABC weights	↓		
		Seminal vesicle + coagulating gland weight	↓		
		Testis histopathology (atrophy)	Δ		
		Testis weight	↓		
		Ventral prostate weight	↓		
	Hershberger	Concordance of 2 to 4 endpoints	↓		
	Repeat Dose Toxicity	Epididymal weight	↓	[9s,m]	[9s,r]
		Epididymis histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Ovary histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Prostate histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r]
		Prostate weight	↓		
		Seminal vesicle histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r]
		Seminal vesicle weight	↓		
		Testis histopathology (atrophy)	↑		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Testis weight	Δ		[9s,m,r]
		Uterus histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]

Supplementary Table 4. Androgen Antagonist Hypothesis; Guideline Toxicity Studies					
Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
2	Developmental Toxicity	Time to balano-preputial separation	↑♂		
	Reproductive Toxicity	Anogenital distance	↓♂		
		Epididymis weight	↓		
		Epididymis histopathology	Δ		
		Estrous cyclicity	↓	[2r,F_0]	[2r,F_1]
		Fertility	↓♂,♀		[2r,F_0,F_1]
		Gross pathology	Δ♂,♀		[2r,F_0,F_1]
		Litter size	↓		[2r,F_0,F_1]
		Nipple retention	↑♂		
		Ovary histopathology	Δ		
		Prostate histopathology	Δ		
		Prostate weight	↓		[2r,F_0,F_1]
		Seminal vesicle histopathology	Δ		
		Seminal vesicle weight	↓		
		Sperm count	↓		[2r,F_0,F_1]
		Sperm motility	↓		[2r,F_0,F_1]
		Testis histopathology (atrophy)	Δ		
		Testis weight	↓		
		Time to balano-preputial separation	↑♂	[2r,F_1]	[2r,F_2]
		Time to mating	↑♀		[2r,F_0,F_1]
		Uterus histopathology	Δ		
3	FSTRA	Behavior	Δ		
		Estradiol level	Δ		
		Fecundity	↓		
		Fertilization success	↓		
		Gonad-somatic index	↓		
		Testosterone level	↑♂		
	Male Pubertal	Testosterone level	↑		
	Steroidogenesis	Testosterone level	Δ		
	Hershberger	Concordance of 1 endpoint	↓		
	Repeat Dose Toxicity	Gross pathology	Δ♂,♀		[3s,r]

♂= males; ♀= females; ↑ = increase relative to controls; ↓ = decrease relative to controls; Δ = altered; ? = altered but not as expected. Numbers correspond to numbered studies in Appendix C; r = rat; m = mouse; rb = rabbit; s = subchronic; c = chronic; n = non-guideline; F_0 = F_0 generation; F_1 = F_1 generation; F_2 = F_2 generation.

Supplementary Table 5. Thyroid Inhibition Hypothesis; Guideline Toxicity Studies

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
1	Male Pubertal	Thyroid histopathology	Δ		
		Thyroid weight	↑		
	Female Pubertal	Thyroid (colloid area & follicular cell height)	↑		
		Thyroid weight	↑		
	AMA	Asynchronous development	↑		
		Thyroid histopathology	Δ		
2	Female Pubertal	Age & weight at vaginal opening	↑		
		T4 level	↑		
		TSH level	↑		
	Male Pubertal	Liver weight	Δ		
		T4 level	↑		
		TSH level	↑		
	Repeat Dose Toxicity	Thyroid follicular cell histopathology	Δ ♂ , ♀	[1c,m]	[1c,r][3s,r] [9s,m,r] [10s,m,r,rb]
		Thyroid hormones	Δ ♂ , ♀		
		Thyroid weight	↑ ♂ , ♀		
	Developmental Toxicity	Fetal survival	↓ ♂ , ♀	[11rb] [13rb]	[4r] [5r] [6r] [11r] [13r]
		Fetal weight	↓ ♂ , ♀	[4r] [5r] [6r] [11r,rb]	[11m] [13r,rb]
		Thyroid follicular cell histopathology	Δ ♂ , ♀		
		Thyroid hormones	Δ ♂ , ♀		
		Thyroid weight	↑ ♂ , ♀		
	Reproductive Toxicity	Fetal weight	↓ ♂ , ♀		
		Pup growth	↓ ♂ , ♀		[2r,F_1,F_2]
		Pup survival	↓ ♂ , ♀		[2r,F_1,F_2]
		Pup weight	↓ ♂ , ♀		
		Thyroid follicular cell histopathology	Δ ♂ , ♀		
		Thyroid hormones	Δ ♂ , ♀		
		Thyroid weight	↑ ♂ , ♀	[2r,F_0♂]	[2r,F_0♀,F_1]
3	Male Pubertal	Age & weight at preputial separation	↑		
		Growth	↓		
		Pituitary weight	↓		

Supplementary Table 5. Thyroid Inhibition Hypothesis; Guideline Toxicity Studies

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
3	Female Pubertal	Estrous cyclicity (diestrus)	Δ		
		Ovary histopathology	Δ		
		Ovary weight	↓		
	AMA	Delayed development	↑		
		Hind limb length	↓		
		Snout-vent length	Δ		
		Wet weight	↑		
	Repeat Dose Toxicity	Liver weight	↑ ♂ , ♀	[3s,r] [6r] [7s,r] [8s,m] [9s,m,r] [10s,m,r]	[10s,rb]
	Reproductive Toxicity	Liver weight	↑ ♂ , ♀	[2r,F_0,F_1]	
	Developmental Neurotoxicity	Auditory Startle	↓ ♂ , ♀		[12,F_2]
		Behavioral Ontogeny	↓ ♂ , ♀		
		Brain Morphometry	Δ ♂ , ♀		[12F_2]
		Learning and Memory	↓ ♂ , ♀		[12F_2]
		Liver weight	↑ ♂ , ♀		
		Motor Activity	Δ ♂, ♀		[12F_2]
		Myelination	Δ ♂ , ♀		
		Pup growth	↓ ♂ , ♀		
		Pup survival	↓ ♂ , ♀		

♂= males; ♀= females; ↑ = increase relative to controls; ↓ = decrease relative to controls; Δ = altered; ? = altered but not as expected. Numbers correspond to numbered studies in Appendix C; r = rat; m = mouse; rb = rabbit; s = subchronic; c = chronic; n = non-guideline; F_0 = F_0 generation; F_1 = F_1 generation; F_2 = F_2 generation.

Supplementary Table 6. Interaction with Steroidogenesis Enzymes Hypothesis; Guideline Toxicity Studies

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
1	Female Pubertal	Uterus weight	↓		
2	Female Pubertal	Ovary weight	↓		
	FSTRA	Gonad histopathology: males	Δ		
		Vitellogenin	↓ ♀		
	Steroidogenesis	Estradiol level	↓		
		Testosterone level	↓		
	Repeat Dose Toxicity	Ovary histopathology	Δ		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Ovary weight	↓		
		Testis histopathology (atrophy)	↑		[1c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Uterus histopathology	Δ		[1,c,m,r] [3s,r] [9s,m,r] [10s,m,r,rb]
		Uterus weight	↓		
	Developmental Toxicity	Sex ratio	Δ ♂ , ♀		[5r] [13r,rb]
	Reproductive Toxicity	Estrous cyclicity	Δ	[2r,F_0]	[2r,F_1]
		Fertility	↓ ♂ , ♀		[2r,F_0,F_1]
		Live births	↓ ♀		[2rF_0,F_1]
		Mating index	↓ ♂		[2r,F_0,F_1]
		Ovary histopathology	Δ		
		Parturition	↓		
		Post-implantation loss	↑		
		Resorptions	↑		
		Sex ratio	Δ ♂ , ♀		[2r,F_0,F_1]
		Sexual behavior	Δ ♂		
		Sperm count	↓		[2r,F_0,F_1]
		Testicular histopathology (atrophy)	Δ		
		Uterus histopathology	Δ		
		Uterus weight	↓		

Supplementary Table 6. Interaction with Steroidogenesis Enzymes Hypothesis; Guideline Toxicity Studies

Rank	Assay	Endpoint(s)	Expected Response	Response to Ethylbenzene	No Response to Ethylbenzene
3	Aromatase	Aromatase activity	↓		
	Female Pubertal	Age & weight at vaginal opening	↑		
		Age at first estrus	↑		
	Male Pubertal	Testosterone level	Δ		
	FSTRA	Behavior	Δ		
		Estradiol level	Δ		
		Fecundity	Δ		
		Fertilization success	Δ		
		Gonad-somatic index	Δ		
		Testosterone level	Δ		
	Repeat Dose Toxicity	Gross pathology	Δ ♂ , ♀		[3s,r]

♂= males; ♀= females; ↑ = increase relative to controls; ↓ = decrease relative to controls; Δ = altered; ? = altered but not as expected. Numbers correspond to numbered studies in Appendix C; r = rat; m = mouse; rb = rabbit; s = subchronic; c = chronic; n = non-guideline; F_0 = F_0 generation; F_1 = F_1 generation; F_2 = F_2 generation.

Supplementary Table 7. Summary of Endpoints from all Tables

MoA	Fraction of Rank 1 Endpoints Tested	# of Rank 1 Endpoints Responding to Ethylbenzene	# of Rank 1 Endpoints Showing No Response to Ethylbenzene	Fraction of Rank 2 Endpoints Tested	# of Rank 2 Endpoints Responding to Ethylbenzene	# of Rank 2 Endpoints Showing No Response to Ethylbenzene	Fraction of Rank 3 Endpoints Tested	# of Rank 3 Endpoints Responding to Ethylbenzene	# of Rank 3 Endpoints Showing No Response to Ethylbenzene
Estrogen Agonist - Table 1	0 (2)	0	0	20 (53)	4	20	1 (15)	0	1
Estrogen Antagonist - Table 2	0 (1)	0	0	13 (26)	2	13	1 (12)	0	1
Androgen Agonist - Table 3	0 (2)	0	0	18 (47)	4	18	1 (19)	0	1
Androgen Antagonist - Table 4	0 (1)	0	0	17 (45)	3	17	1 (10)	0	1
Thyroid Inhibition - Table 5	0 (6)	0	0	6 (21)	4	6	6 (21)	2	5
Steroidogenesis - Table 6	0 (1)	0	0	10 (25)	1	10	1 (11)	0	1
Hershberger endpoints counted as one concordance response									