Styrene Information and Research Center (SIRC)

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THIS DOCUMENT SUBMITTED ELECTRONICALLY

RE: Comments on Nomination of Ethylbenzene for Review for the Report on Carcinogens

Dear Dr. Lunn:

The Styrene Information & Research Center[1] (SIRC) appreciates the opportunity to provide comments to the National Toxicology Program (NTP), in response to its *Federal Register* notice requesting public comment on its nomination of substances for review under the Report on Carcinogens (RoC) program. ***77 Fed. Reg. No.12, 2728 (January 19, 2012)***

SIRC recently has assumed oversight for ethylbenzene scientific and regulatory issues, and thus wishes to offer comment on NTP's nomination of ethylbenzene for review for potential listing in the RoC.

Summary Assessment of EB RoC Nomination

The stated purpose of the RoC is to identify substances that are "known human carcinogens" or "reasonably anticipated to be human carcinogens." Ethylbenzene should not be considered further for potential inclusion in the RoC, because it is not carcinogenic in humans nor is it reasonably anticipated to be so. There are no reliable studies of carcinogenicity in human populations exposed to ethylbenzene. Animal studies indicate increased tumors at very high

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

inhalation exposures by modes of action that are non-genotoxic and are not relevant to human cancer.

Genotoxicity studies of ethylbenzene are negative. Inhalation of 750 ppm ethylbenzene for 2 years produced increased kidney renal tubule tumors in male and female rats as a result of exacerbation of chronic progressive nephropathy (CPN). CPN does not occur in humans and these rat kidney tumors are not an indication of possible human cancer from ethylbenzene. Inhalation of 750 ppm ethylbenzene for 2 years produced increased lung tumors in male mice as a result of cytotoxicity caused by CYP2F2 metabolism of ethylbenzene. Human CYP2F1 does not produce sufficient metabolites to cause cytotoxicity; therefore, the mouse lung tumors are not an indication of possible human cancer from ethylbenzene (this factor also is pertinent to NTP's nomination of cumene for possible RoC review). Inhalation of 750 ppm for 2 years produced increased liver tumors in female mice via a Phenobarbital type mode of action of induction of CYP2B. This mode of action does not occur in humans; therefore, the mouse liver tumors are not an indication of possible human cancer from ethylbenzene. More detail on the above is provided in the attached summary assessment.

NTP Should Rely on US EPA's Existing Assessment of EB Cancer Data

A comprehensive review of ethylbenzene's carcinogenic potential already has been documented under the U.S. Environmental Protection Agency's Voluntary Children's Chemical Evaluation Program (VCCEP). Copies of all the related VCCEP ethylbenzene documents may be found on the EPA's VCCEP web site at http://www.epa.gov/oppt/vccep/pubs/chem8c.html, although copies of pertinent documents are being submitted with SIRC's comments for NTP's reference.

The VCCEP Revised Ethylbenzene Sponsor Document characterizes the ethylbenzene carcinogenicity data as follows:

> In summary, ethylbenzene has been evaluated in rats and mice for chronic and carcinogenicity effects. The strongest evidence of cancer was kidney tumors found in male rats that inhaled 750 ppm ethylbenzene, a concentration that also significantly reduced the male rats' survival. There was some evidence of kidney tumors in female rats at this concentration that was detected only after extended evaluation. Exacerbation by ethylbenzene of chronic progressive nephropathy, a pathway that is considered to have no relevance for extrapolation to humans, is postulated as the mode of action underlying the development of the rat renal neoplasia. Male rats that inhaled 750 ppm ethylbenzene also appeared to have an exacerbation in testicular tumors, a type of tumor that occurs in nearly all aged rats of this strain. There was some evidence at 750 ppm ethylbenzene of liver and lung tumors in mice. The incidences of lung tumors in male mice and liver tumors in female mice were greater than those in concurrent control but were within the NTP historical control ranges. Increases in regenerative cell proliferation are postulated to play

> a key role in the mouse tumor findings. Chronic nonneoplastic toxicity by ethylbenzene principally targeted the kidneys in rats and the liver and lungs of mice at concentrations of 75 ppm and above. Changes of uncertain relevance to ethylbenzene were also apparent in the rat prostate and the mouse thyroid and pituitary glands. No further chronic or carcinogenicity testing of ethylbenzene is recommended.

As noted above, research subsequent to the VCCEP assessment on the mouse lung tumor mode of action has shown increased lung tumors in male mice are the result of cytotoxicity caused by CYP2F2 metabolism of ethylbenzene, that human CYP2F1 does not produce sufficient metabolites to cause cytotoxicity, and thus the mouse lung tumors are not relevant to a human carcinogenicity concern.

Conclusion

SIRC strongly believes that a thorough, balanced and transparent weight-of-the-evidence assessment of the full ethylbenzene data base does not provide evidence that ethylbenzene can be characterized as either "reasonably anticipated" or "known" to be a human carcinogen. Available assessments (i.e. – EPA VCCEP document) and data in the published literature support this conclusion. Accordingly, SIRC urges NTP to remove ethylbenzene from its list of substances proposed for assessment under the Report on Carcinogens program.

SIRC would be pleased to provide any references cited herein. We sincerely hope that NTP will carefully consider the evidence summarized in these comments, and will avail itself of the existing VCCEP reports in accurately characterizing the carcinogenic potential of ethylbenzene.

Very truly yours,

[Redacted]

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Attachment:
SIRC Comments on Nomination of Ethylbenzene for Report on Carcinogens Review

SIRC COMMENTS ON NOMINATION OF ETHYLBENZENE FOR REPORT ON CARCINOGENS REVIEW

February 28, 2012

Summary Statement

The stated purpose of the Report on Carcinogens (RoC) is to identify substances that are "known human carcinogens" or "reasonably anticipated to be human carcinogens." Ethylbenzene should not be included in the RoC, because it is not carcinogenic in humans nor reasonably anticipated to be so. There are no reliable studies of carcinogenicity in human populations exposed to ethylbenzene. Animal studies indicate increased tumors at very high inhalation exposures by modes of action that are non-genotoxic and are not relevant to human cancer.

Genotoxicity studies of ethylbenzene are negative. Inhalation of 750 ppm ethylbenzene for 2 years produced increased kidney renal tubule tumors in male and female rats as a result of exacerbation of chronic progressive nephropathy (CPN). CPN does not occur in humans and these rat kidney tumors are not an indication of possible human cancer from ethylbenzene. Inhalation of 750 ppm ethylbenzene for 2 years produced increased lung tumors in male mice as a result of cytotoxicity caused by CYP2F2 metabolism of ethylbenzene. Human CYP2F1 does not produce sufficient metabolites to cause cytotoxicity; therefore, the mouse lung tumors are not an indication of possible human cancer from ethylbenzene. Inhalation of 750 ppm for 2 years produced increased liver tumors in female mice via a Phenobarbital type mode of action of induction of CYP2B. This mode of action does not occur in humans; therefore, the mouse liver tumors are not an indication of possible human cancer from ethylbenzene.

Human Data

There are no reliable human epidemiology studies that have evaluated ethylbenzene exposure and cancer. No reliable epidemiology studies of workers involved in ethylbenzene manufacture have been found nor are there reliable epidemiology studies that examined cancer rates from solvent or gasoline exposure in relation to ethylbenzene concentrations.

A medical monitoring study of some 200 (exact number not stated) Czechoslovakian ethylbenzene production workers (Bardoděj, and Círek, 1988) contained statements on cancer findings. The workers were exposed between 1964 and 1985 and their mean age was 36.6 years and their mean length of employment was 12.2 years. The authors stated that the cancer incidence among chemical workers in the industrial complex (of comparable age and length of employment) not engaged in ethylbenzene production was about three times the national average, whereas in the group of ethylbenzene production workers, no tumors were reported over the previous years. In IARC's review (2000) of this study, they noted that no precise data

was provided to substantiate the author's assertions, there was co-exposure to benzene, and the age of the workers and length of the follow-up were not sufficient for a proper evaluation of cancer risk in relation to exposure to ethylbenzene.

A mortality study was conducted among 560 styrene production and polymerization workers employed for at least 5 years on May 1, 1960 at a US plant (Nicholson *et al*., 1978). Exposures present at the plant were ethylbenzene, benzene, toluene, and styrene. There were 83 deaths observed in the cohort versus 106.4 expected deaths, including 17 cancer deaths (versus 21 expected). Among the deaths, one was from leukemia (0.79 expected) and one was from lymphoma (1.25 expected). A further review of additional death certificates from recent years revealed additional cases of leukemia and lymphoma. IARC (2000) concluded that this study was not useful for evaluation of cancer risk because of deficiencies in the reporting and analysis of the mortality data.

A meta-analysis of 22 cohorts and 13 case-control studies examined the risk of NHL among persons employed in the downstream petroleum industry. No positive associations of NHL and exposures were found with the exception of one study. The pooled risk estimate from a random-effects meta-analysis was 1.02 (95% confidence interval (CI) 0.94-1.12). Although there were no estimates available, exposure is likely to have varied by occupation, location and time period; there was no evidence however that risk estimates varied by any of these factors. NHL is a heterogeneous disease, yet no data were reported for NHL subtypes. In summary, there is no suggestion across an extensive literature that exposure to gasoline at the levels workers' experience in an occupational setting increases the risk of NHL (Kane and Newton, 2010).

Animal Cancer Data

Ethylbenzene is carcinogenic in animals following lifetime exposures to high vapor concentrations. Tissue sites observed with increased tumor incidence following exposure to EB include the kidney (male and female rats), lung (male mice), and liver (female mice).

Inhalation chronic toxicity and carcinogenicity studies have been conducted for ethylbenzene in rats and mice by the U.S. National Toxicology Program (NTP, 1999). Groups of 50 male and 50 female Fischer 344/N rats and 50 male and female B6C3F1 mice, beginning at 6 weeks of age, were exposed to ethylbenzene by inhalation in whole-body exposure chambers at concentrations of 0, 75, 250 or 750 ppm (0, 325, 1085, or 3255 mg/m^3) for 6 hours/day, 5 days/week for 104 and 103 weeks, respectively.

In the rat study, survival was similar among the female groups but was significantly decreased in the high-dose males compared to the control males (number of males surviving to study termination: 15/50, 14/50, 13/50 and 2/50 at 0, 75, 250 and 750 ppm, respectively). The mean terminal body weights of exposed males and females were 5 to 10% lower than those of the

control animals. For chronic (nonneoplastic) effects, the kidney was the major target organ of toxicity in the rat, with renal tubular hyperplasia noted in both males and females at the 750 ppm level only (17/50 vs.10/50 in controls for males and 8/49 vs. 1/50 in controls for females). The severity of nephropathy was significantly increased relative to the chamber controls in 750 ppm male (3.5 vs. 2.3 in controls) and all exposed female rats (2.3, 1.7, 1.6 and 1.3 for 750, 250, 75 ppm and control groups). The enhanced nephropathy was more severe in males than in females.

Ethylbenzene-related tumor findings in the rats were present in the kidney and testis at the highest exposure concentration only. Ethylbenzene administered at 750 ppm was associated with an increase in renal tubule tumors in males after standard evaluation of a single section of each rat's kidney, and in both males and females after evaluation of step-sectioned kidneys. The standard histopathological evaluation found a significantly greater incidence in the 750 ppm male rats of renal tubule adenoma (4/50 vs. 0/50 in controls) and adenoma or carcinoma (combined)(7/50 vs. 0/50) than found in the chamber controls. The findings from an extended evaluation (step-section) of the kidney showed a significant increase in the incidences of renal tubule adenoma (17/50 vs. 3/50 in controls for males and 7/49 vs. 0/50 in controls for females); the incidence of renal tubule adenoma or carcinoma (combined) was significantly increased in 750 ppm males (18/50 vs. 3/50 in controls).

In the testis, the incidence of interstitial cell adenoma in 750 ppm males was significantly greater than in the chamber control group (44/50 vs. 36/50 in controls). The incidence of bilateral testicular adenoma was also significantly increased at 750 ppm (40/50 vs. 27/50 in controls). Although testicular adenomas develop in nearly all aged Fischer rats, in this study ethylbenzene appeared to enhance its development since 92% (22 of 24 rats) of the 750 ppm rats that died between day 400 and day 600 had testicular adenoma, whereas only 33% (3 of 9 rats) of the control that died early had testicular adenoma. The study NOAEL for tumors was 250 ppm in both male and female rats. NTP's cancer conclusion for the study was there was clear evidence of carcinogenicity in male rats due to increased incidences of kidney (renal tubule neoplasms) and testes tumors (testicular adenoma) and some evidence of carcinogenicity in female rats that also showed kidney tumors (renal tubule adenomas), but in a lower incidence and only detected after extended evaluation by step sections.

In the mouse study (NTP, 1999) survival was unaffected by exposure to ethylbenzene and no consistent exposure-related effects were observed on body weights. The mean body weights of 750 ppm females were generally lower than the controls from week 24 through week 68 but were similar to the controls from week 72 until the end of the study. For chronic (nonneoplastic) effects, the lung and liver were the principal target organs of toxicity with lesions also present in the thyroid and pituitary glands. In the lung, the incidence of alveolar epithelial metaplasia was significantly elevated in 750 ppm males compared to controls. Nonneoplastic liver lesions in females consisted of eosinophilic foci that were increased in incidence at 750 ppm. Male mice exhibited increases in the incidences of hepatocyte syncytial alteration at 250 and 750 ppm and increases in the incidences of hypertrophy and necrosis at 750 ppm. Pathology in the thyroid gland consisted of positive trends in the incidence of thyroid

follicular cell hyperplasia in males and females with significant increases in incidences relative to controls in both sexes at 750 ppm. The pituitary gland of 250 and 750 ppm females had significantly increased incidences of hyperplasia of the pars distalis. The NOAEL for chronic toxicity was 75 ppm in females and males based on pituitary and liver pathology, respectively. Ethylbenzene-related tumor findings in the mice were present in the lung and liver of the high exposure concentration group only. Ethylbenzene administered at 750 ppm was associated with an increase in lung tumors in males only and liver tumors in females only. The 750 ppm male mice exhibited a significantly greater incidence of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma (16/50 vs. 5/50 in controls) or carcinoma (combined)(19/50 vs. 7/50 in controls) although these incidences were within the NTP historical control range. The 750 ppm female mice compared to chamber controls had a significantly greater incidence of hepatocellular adenoma (16/50 vs. 6/50) and hepatocellular adenoma or carcinoma (combined) (25/50 vs. 13/50) but were within the NTP historical control range. The increase in eosinophilic foci, a lesion that is judged to be a precursor of hepatocellular adenomas, was a supporting finding in the 750 ppm females. The study NOAEL for tumors was 250 ppm in both male and female mice. NTP's cancer conclusion for the study was there some evidence of carcinogenicity in both sexes; for male mice due to lung tumors (alveolar/bronchiolar neoplasms) and for female mice due to liver tumors (hepatocellular neoplasms).

Two chronic oral studies in rats of limited design and detail were performed for ethylbenzene by Maltoni and co-workers (1985, 1997). The first study was comprised of groups of 50 male and 50 female Sprague-Dawley rats that received gavage doses of 0 or 800 mg/kg bwt ethylbenzene (purity, 99.57%) in olive oil solution daily on 4 days/week for 104 weeks. In the second study, groups of 40 male and 40 female Sprague-Dawley rats received 500 mg/kg bwt/day ethylbenzene according to the weekly schedule above while 50 male and 50 female Sprague-Dawley rats were used as control group and received only olive oil. The rats in this study were permitted to live out their natural life span, up to 145 weeks. In these studies, animal survival was affected by treatment with ethylbenzene as indicated as an "intermediate reduction" in animal numbers in both males and females. Reportedly, at 800 mg/kg bwt/day, there was an increase in the incidence of tumors of the nasal cavity (type unspecified)(2% incidence in females versus 0% in controls) and neuroesthesioepitheliomas (6% in males versus 0% in controls) and a borderline increase in tumors of the oral cavity (6% in females versus 2% in controls). The reporting of this study is deficient in details such as numbers of animals with specific tumors, adjustments for survival, historical control data, and statistical analysis; hence an assessment of the study and its results is not possible.

Mode of Action

Information regarding the cancer mode of action for each tissue site is discussed below. A direct mutagenic mode of action for ethylbenzene is not supported by available information for any of the observed tumor types. Instead, ethylbenzene appears to exert its carcinogenic effects via a nongenotoxic mode of action. Exacerbation by ethylbenzene of chronic progressive nephropathy, a pathway that is considered to have no relevance for extrapolation

to humans, is postulated as the mode of action underlying the development of the rat renal cancer. Increases in regenerative cell proliferation, as a result of the formation of one or more reactive metabolites (catechols, quinones), are postulated to play a key role in the mouse lung tumor findings. Liver tumors in mice may be related to either a phenobarbital-like induction, which is not considered to be a relevant mode of action for humans, or as a result of the formation of one or more reactive metabolites (catechols, quinones).

Mutagenicity

Ethylbenzene has been extensively tested for toxicity to genetic material using nearly every available type of genetic toxicity test. Ethylbenzene is negative for genotoxicity in all *in vivo* studies that have been conducted and predominately negative for genotoxicity in *in vitro* studies. Overall, these study results do not indicate that ethylbenzene is a concern for genotoxicity (Table 1).

Rat Kidney Tumors

A further evaluation of the rat kidneys from the NTP study was conducted by Hard (2002) to define the mode of action underlying the tumor development. The reevaluation confirmed the increases in renal tubule incidence in the 750 ppm groups and the increases in the precursor lesion, atypical tubule hyperplasia. The vast majority of the proliferative lesions were of basophilic type and, apart from three carcinomas in the 750 ppm males, either small adenomas or foci of atypical tubule hyperplasia. Also found was a marked exacerbation of chronic progressive nephropathy, an age-related spontaneous disease involving both degenerative and regenerative components, in the 750 ppm males (68% vs. 12% in control males) and a modest exacerbation in 750 females (8% vs. 0% in control females). Almost all of the basophilic tumors occurred in the rats with advanced, usually end-stage, chronic progressive nephropathy, and they were located in areas of parenchyma involved in the chronic progressive nephropathy process. Statistical analysis of the proliferative lesion and the chronic progressive nephropathy data indicated a highly significant correlation between atypical tubule hyperplasia/renal tumor incidence and end-stage chronic progressive nephropathy, and adjusting for end-stage chronic progressive nephropathy removed any statistically significant difference in renal tubule incidence between the ethylbenzene-treated groups and controls. Further, the microscopic examination of renal tubules revealed no evidence of renal tubule injury or increased mitotic activity that would indicate sustained cytotoxicity/cell regeneration as a mode of action of tumor development. Also there was an absence of granular casts and linear papillary mineralization that discounted the possibility of α-2u-globulin nephropathy as the primary underlying basis in male rats, even though subchronic studies had shown a modest accumulation of hyaline droplets in proximal tubules (Stott *et al.*, 2003). Hard's overall conclusion, based on the close association of atypical tubule hyperplasia and renal tumors with chronic progressive nephropathy, was that ethylbenzene–induced exacerbation of chronic progressive nephropathy was the mode of action underlying the development of renal

neoplasia, a pathway that is considered to have no relevance for extrapolation to humans. A recent publication found a positive association between severe CPN (grades 6-8 on scale of 1-8) and renal tubule tumors in male and female B6C3F1 control rats from 28 NTP studies (Hard et al., 2012), providing further evidence that exacerbated CPN, not genetic damage or a-2u-globulin, is responsible for the increased tumors from ethylbenzene exposure in the rats.

A short-term exposure study was conducted in rats by Stott *et al.* (2003) to further explore the mode of rat kidney tumorigenesis. Male and female Fischer 344 rats were administered 750 or 75 ppm (3255 or 325 mg/m^3) ethylbenzene vapor 6 hours/day, 5 days/week for 1 or 4 weeks. Kidneys were evaluated for changes in organ weights, mixed function oxygenases, glucuronosyl transferase activities, S-phase DNA synthesis, apoptosis, α-2u-globulin deposition, and histopathology. In male rats, exposure to the tumorigenic level of 750 ppm ethylbenzene vapor resulted in an increase in kidney weight and an initial increase in hyaline droplets and α-2u-globulin in the proximal tubule epithelial cells of the cortex accompanied by an increase in regenerative cell proliferation during the first week of exposure. Early changes were followed by a diminution in α-2u-globulin deposition but continued elevation of S-phase DNA synthesis and histopathologic changes, suggesting chronic progressive nephropathy and a more chronic regenerative cell proliferation, findings consistent with the evaluation by Hard (2002). Both α-2u-globulin nephropathy and chronic progressive nephropathy associated increases in cell turnover are recognized kidney tumor risk factors. The male rats exhibited modest induction of mixed function oxygenase and glucuronosyl transferase activities, primarily following 1-week exposure suggestive of an adaptive response to the metabolic load of ethylbenzene and its metabolites. In contrast to males, female rat kidneys did not display significant histopathological changes nor increased S-phase DNA synthesis and instead a nearly 50% decrease in S-phase synthesis was noted following 1 week exposure with no discernable changes in apoptotic rates. Minimal decreases in all mixed function oxygenase activities were found following 4 weeks of exposure that, combined with S-phase synthesis findings, suggested an alteration or loss of the mixed function oxygenase competent cells in female kidney with increasing exposure period. This change was suggested to possibly serve to accelerate development of chronic progressive nephropathy at a level that does not elicit significant morphological changes or measurable elevations in S-phase DNA synthesis over the time periods examined. Exposure to the nontumorigenic level of 75 ppm for one week caused few changes to the rat kidney.

Rat Leydig Cell Tumors

The incidence of testes Leydig (interstitial) cell tumors in male rats in the NTP study appear to be increased by exposure to 750 ppm ethylbenzene. However, Leydig cell tumors are one of the most frequently occurring endocrine tumors in rodents in chronic toxicity/carcinogenicity studies (Capen, 2001). The incidence of Leydig cell tumors in old rats varies considerably depending upon the strain, with the highest incidence found in Fischer rats (the strain used for the ethylbenzene chronic/ carcinogenicity study) with the incidence at 2 years of age often approaching 100% (Capen, 2001; Haseman and Elwell, 1996). With such a high background

incidence, any additional contribution from chemical exposure is difficult to discern. Also, in contrast to rats, Leydig cell tumors in humans are rare and are different in cellular origin (Haseman and Arnold, 1990; Capen, 2001; Clegg *et al*., 1997). Hormonal imbalances and a number of clinical substances that cause increases in Leydig cell tumors in rats have not resulted in an increased incidence of Leydig cell neoplasia in man (Capen, 2001). Therefore, Leydig cell tumors, a frequent tumor type in male rats, are not considered an appropriate model for assessing the potential risk to human males of developing this rare testicular tumor (Capen, 2001).

Mouse Lung Tumors

Brown (2000) conducted a further evaluation of the mouse lungs from the NTP study. This re-evaluation confirmed the increases in lung tumor incidences in male mice at 750 ppm. The re-evaluation also revealed decreased eosinophilia of the terminal bronchiolar epithelium of male and female mice of the 750 ppm group. Also, a dose-related increased incidence in multifocal hyperplasia of the bronchiolar epithelium with extension to the peribronchiolar alveolar epithelium (reported as "alveolar epithelial metaplasia" in NTP report) was observed in all male treated groups and 250 and 750 ppm females.

A short-term exposure study was conducted in mice by Stott *et al.* (2003) to further explore the mode of lung and liver tumorigenesis. Male and female B6C3F1 mice were administered 750 or 75 ppm (3255 or 325 mg/m^3) ethylbenzene vapor 6 hours/day, 5 days/week for 1 or 4 weeks. Lungs and livers were evaluated for changes in organ weights, mixed function oxygenases, glucuronosyl transferase activities, S-phase DNA synthesis, apoptosis, and histopathology. In the mouse lung at 750 ppm, increases in S-phase biosynthesis and loss/renewal of metabolic capacity in bronchiolar epithelium indicated a shift in cell populations, likely in Clara cells. The changes in lungs and liver are suggestive of the formation of a toxic metabolite and regenerative cell proliferation. The nontumorigenic exposure level of 75 ppm resulted in few changes in the lungs and livers of mice.

It is likely that metabolism by CYP2F2, expressed primarily in mouse lung, generates the metabolites that are toxic to mouse lung. Similar patterns of increased lung tumors in mice, but not rats have been found for a number of similar aromatic compounds, such as styrene and cumene[2]. A common mode of action has been hypothesized (Cruzan et al., 2009) that involves ring oxidation to 4-ethylphenol and 2-ethylphenol. These metabolites are further metabolized to catechols and hydroquinones, which then undergo additional auto-oxidation to reactive, cytotoxic quinone metabolites capable of binding to cellular macromolecules, causing cytotoxicity including cell death. Metabolism by CYP2E1 has been demonstrated to not play a role in this lung toxicity because there was no diminution of lung toxicity from styrene in CYP2E1-knockout mice. The essential role of CYP2F2 in lung toxicity was demonstrated by the complete elimination of cytotoxicity from styrene, naphthalene, and 3-methylindole in CYP2F2-knockout mice (Cruzan et al., 2012a, Li et al., 2011, Zhou et al., 2011). Because rats have less

[2] This mode of action also is pertinent to NTP's assessment of cumene for potential assessment under the RoC

CYP2F4, less of these toxic metabolites are produced and neither cytotoxicity nor lung tumors happen from exposure to these chemicals. Human lungs contain even less CYP2F1, so neither cytotoxicity nor tumors would be expected from exposure to ethylbenzene. In a preliminary study, CYP2F2KO, 2F1-transgenic (i.e., no mouse 2F2, but human 2F1) did not develop cytotoxicity from exposure to styrene (Cruzan et al., 2012b).

Mouse Liver Tumors

Brown (2000) conducted a further evaluation of the mouse livers from the NTP study. This re-evaluation confirmed the increases in liver tumor incidences in female mice at 750 ppm. The author noted that the necrotic hepatocytes in the 750 ppm males were usually that of a coagulation-type necrosis of single or small groups of cells, usually the enlarged, hypertrophied centrilobular hepatocytes. The morphology of this necrosis was histomorphologically different from "apoptosis." Also, the syncytial cells were not the predominant cell type with necrosis.

As noted in the MOA for mouse lung tumors, a short-term exposure study was conducted in mice by Stott *et al.* (2003) to further explore the mode of lung and liver tumorigenesis. Male and female B6C3F1 mice were administered 750 or 75 ppm (3255 or 325 mg/m^3) ethylbenzene vapor 6 hours/day, 5 days/week for 1 or 4 weeks. Exposure of mice to 750 ppm ethylbenzene vapor caused sustained increases in the levels of cell proliferation in liver as evidenced by increases in mitotic figures and S-phase biosynthesis. A greater incidence of mitotic figures was observed in females than males, consistent with the occurrence of liver tumors in this sex. Levels of S-phase synthesis were also higher in females than males at every location and exposure period. A regiospecificity of increases in S-phase synthesis in both sexes was apparent with the greatest response in centrilobular hepatocytes.
A primary metabolite of ethylbenzene in the liver is 1-phenylethanol (Engstrom, 1984; Saghir *et al.*, 2006). The primary cytochrome P450 isozyme responsible for this reaction is the CYP2E1 (Imaoka and Funae, 1991; Sequeira *et al.*, 1992, Yuan *et al.*, 1997a,b; Sams *et al.*, 2004). In rats, metabolic saturation and induction of CYP2E1 is found, as well as induction of CYP2B1 and 2B2 (Imaoka and Funae, 1991; Sequeira *et al.*, 1992, Yuan *et al.*, 1997a,b). These observations are indicative of a phenobarbital-type liver response (CYP2B1-specific enzyme induction and hepatocellular proliferation, eosinophilic foci) (Bus, 2006). Chronic induction of P450 isozymes has been associated with liver tumors in rodents (Grasso and Hinton, 1991). Eosinophilic foci are considered to be a precursor to liver tumors (NTP, 1999; Chan *et al.*, 1998). Increased liver weights and liver tumors associated with eosinophilic foci are characteristic of a phenobarbital-type liver response (Dalton *et al.*, 2003). In addition, the Phenobarbital-type liver tumor MOA only applies to nongenotoxic compounds, entirely consistent with the negative genotoxic profile of ethylbenzene. Therefore, data are available to support this MOA. The incidence of thyroid gland follicular cell hyperplasia was significantly increased in male and female mice exposed to 750 ppm ethylbenzene (NTP, 1999), an effect that is also consistent with a phenobarbital-like alternation in thyroid hormone clearance (Meek *et al.*, 2003). The critical role of the liver physiologic and metabolic adaptive responses to high-dose ethylbenzene exposure in mediating the female mouse liver tumor response is further supported by findings

from chronic oral studies of the ethylbenzene metabolite, 1-phenylethanol, in mice (NTP, 1990). Treatment of both sexes of B6C3F1 mice with doses up to 750 mg/kg-day, 5 days/week for two years produced no evidence of liver tumors or changes in hepatocellular histopathology. Since direct administration of 1-phenylethanol does not produce phenobarbital-like metabolic and physiologic adaptive responses, the absence of liver tumors observed in mice exposed to 1-phenylethanol is consistent with the MOA proposed for ethylbenzene. Taken together, these findings provide strong support that the MOA of ethylbenzene-induced female mouse liver tumors are secondary to a phenobarbital-like enzyme induction and cell proliferation.

Summary

Ethylbenzene has been evaluated in rats and mice for chronic and carcinogenicity effects. The strongest evidence of cancer was kidney tumors found in male rats that inhaled 750 ppm ethylbenzene, a concentration that also significantly reduced the male rats' survival. There was some evidence of kidney tumors in female rats at this concentration that was detected only after extended evaluation. Exacerbation by ethylbenzene of chronic progressive nephropathy, a pathway that is considered to have no relevance for extrapolation to humans, is postulated as the mode of action underlying the development of the rat renal neoplasia. Male rats that inhaled 750 ppm ethylbenzene also appeared to have an exacerbation in testicular tumors, a type of tumor that occurs in nearly all aged rats of this strain. There was some evidence at 750 ppm ethylbenzene of liver and lung tumors in mice. The incidences of lung tumors in male mice and liver tumors in female mice were greater than those in concurrent control but were within the NTP historical control ranges. Increases in regenerative cell proliferation are postulated to play a key role in the mouse tumor findings. Chronic nonneoplastic toxicity by ethylbenzene principally targeted the kidneys in rats and the liver and lungs of mice at concentrations of 75 ppm and above. Changes of uncertain relevance to ethylbenzene were also apparent in the rat prostate and the mouse thyroid and pituitary glands. No further chronic or carcinogenicity testing of ethylbenzene is recommended.

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