MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

December 6, 2016

TO: File for Ethylbenzene (CAS No. 100-41-4)

FROM: Mike Depa, Air Quality Division, Toxics Unit

SUBJECT: Derivation of Screening Levels

The Initial Risk Screening Level (IRSL) for ethylbenzene is 0.4 µg/m³ with annual averaging time. The Secondary Risk Screening Level (SRSL) for ethylbenzene is 4 µg/m³ with annual averaging time. The Inhalation Unit Risk (IUR) for ethylbenzene is 2.5E-6 per µg/m³. The IRSL and SRSL are calculated pursuant to Rule 231(1):

IRSL = 1E-6/IUR
IRSL = 1E-6/2.5E-6 per µg/m³
IRSL = 0.4 µg/m³

SRSL = 1E-5/IUR
SRSL = 1E-5/2.5E-6 per µg/m³
SRSL = 4 µg/m

AQD established an ITSL of 1,000 ug/m^3 on December 20, 1990, based on the U.S. Environmental Protection Agency (EPA, 1991a) inhalation Reference Concentration (RfC) for ethylbenzene of 1,000 µg/m³. AQD assigned a 24-hr averaging time (AT) at that time, because the critical effect is developmental toxicity. The current review finds that a 24 hr AT is appropriate for the ITSL.

EPA (1991a) Inhalation RfC Summary	Exposures*	UF	MF	RfC
<u>Critical Effect:</u> Developmental toxicity Rat and Rabbit Developmental Inhalation Studies (Andrew et al., 1981; Hardin et al., 1981)	NOAEL: 434 mg/m^3 (100 ppm) NOAEL(ADJ): 434 mg/m^3 NOAEL(HEC): 434 mg/m^3 LOAEL: 4340 mg/m^3 (1000 ppm) LOAEL(ADJ): 4340 mg/m^3 LOAEL(HEC): 4340 mg/m^3	300	1	1E+0 mg/m^3

*Conversion Factors: MW = 106.18g. Assuming 25℃ an d 760 mmHg, NOAEL(mg/m^3) = 100 ppm x MW/24.45 = 434 mg/m^3. For developmental effects, this concentration is not adjusted; therefore, NOAEL(ADJ) = NOAEL. The NOAEL(HEC) was calculated for a gas:extrarespiratory effect, assuming periodicity was attained. Since b:a lambda values are unknown for the experimental animal species (a) and humans (h), a default value of 1.0 was used for this ratio. NOAEL(HEC) = NOAEL(ADJ) x (b:a lambda(a)/lambda(h)) = 434 mg/m^3.

Discussion

The EPA (2014) publishes screening values for long-term (chronic) inhalation and oral exposures (see: Dose-Response Assessment for Assessing Health Risks Associated With Exposure to Hazardous Air Pollutants; https://www.epa.gov/fera/dose-response-assessment-assessing-health-risks-associated-exposure-hazardous-air-pollutants). EPA's Office of Air Quality Planning and Standards (OAQPS) uses these peer-reviewed values for risk assessments of hazardous air pollutants. EPA adopted the IUR for ethylbenzene of 2.5E-6 per µg/m³. The IUR was adopted from California Office of Health Hazard and Assessment (OEHHA).

The IUR for ethylbenzene was derived from a 2-year inhalation study performed by the National Toxicology Program (NTP, 1999) in male and female rats and mice (OEHHA, 2011). In NTP (1999), the incidences of renal tumors (adenoma and carcinoma in males; adenoma only in females) were significantly increased among rats of both sexes in the high-dose group (males: 3/50, 5/50, 8/50, 21/50; females: 0/50, 0/50, 1/50, 8/49 in control, 75 ppm, 250 ppm and 750 ppm groups, respectively. NTP concluded that there was clear evidence of carcinogenicity in male rats and some evidence in female rats, based on the renal tumorigenicity findings.

An internal dose metric representing the amount of ethylbenzene metabolized per kg body weight per day (metabolized dose) was used in the dose response analysis with published physiologically-based pharmacokinetic (PBPK) modeling parameters (see Table 1). OEHHA (2011) stated:

> The metabolized dose metric is considered the most appropriate metric for assessment of carcinogenic risks when the parent compound undergoes systemic metabolism to a variety of oxidative metabolites which may participate in one or more mechanisms of carcinogenic action, and the parent compound is considered unlikely to be active. In this case the dose response relation is likely to be more closely related to the internal dose of metabolites than of the parent compound.

Table 1. Details on the Dose Metrics Used by OEHHA (2011)*(excerpt)*

Table 2. Incidence of renal tubule adenoma or carcinoma in male rats exposed to ethylbenzene via inhalation and relevant dose metrics (from NTP, 1999).

Chamber concentration (ppm)	Average concentration[a] (mg/m^3)	LTWA dose[b] (mg/kg-day)	PBPK metabolized dose[c] (mg/kg-d)	Tumor incidence[d]		Statistical significance[e]	
				Quantal Response	%	Fisher Exact Test	TrendTest
0	0	0	0	3/42	7.1		
75	60.7	35.6	19.09	5/42	11.9	$p = 0.356$	$p < 0.001$
250	202	119	58.78	8/42	19.0	$p = 0.0972$	
750	607	356	124.26	21/36	58.3	$p < 0.001$	

a. Average concentration during exposure period calculated by multiplying chamber concentration by 6.25 hours/24 hours, 5 days/7 days, and 4.35 mg/m^3/ppm.
b. Lifetime weighted average doses determined by multiplying the lifetime average concentrations during the dosing period by the male rat breathing rate (0.264 m^3/day) divided by the male rat body weight (0.450 kg). The duration of exposure was 104 weeks, so no correction for less than lifetime exposure was required.
c. Rodent PBPK models were used to estimate internal doses under bioassay conditions; methods are described in detail below.
d. Effective rate. Animals that died before the first occurrence of tumor (day 572) were removed from the denominator. Total number of tumors/number of survivors.
e. The *p*-value listed next to each dose group is the result of pair wise comparison with controls using the Fisher exact test. The *p*-value listed for the trend test is the result obtained by the National Toxicology Program (NTP, 1999) using the life table, logistic regression and Cochran-Armitage methods, with all methods producing the same result.

Linearized multistage (LMS) methodology was used with dose and tumor incidence data in order to construct a straight line for best fit to data for various tumor types. Organ specific dose metrics were derived from previous studies examining the fraction of cardiac output of blood delivered to the tissue for each tumor type. Physiologically Based Pharmacokinetic (PBPK) modeling was used to estimate internal dose.

> An internal dose metric representing the amount of ethylbenzene metabolized per kg body weight per day (metabolized dose) was used in the dose response analysis with published PBPK modeling parameters.
>
> *From OEHHA, 2011, page B287*

> The PBPK models were comprised of compartments for liver, fat, vessel poor tissues (e.g., muscle), vessel rich tissues, and lung.
>
> *From OEHHA, 2011, page B295*

> The rat PBPK model was based on Dennison et al. (2003). Simulations were conducted using Berkeley Madonna (v.8.3.9) software (e.g., 6.25 hr exposure/day x 5 days/wk for one week simulations of bioassay exposure levels, see sample model equations in Appendix A). The chemical partition coefficients used in the Haddad et al. model were: blood:air, 28.0; fat:blood, 55.57; liver:blood, 2.99; muscle:blood, 0.93; and vessel rich:blood, 2.15 (Haddad et al., 2001). For the Dennison et al. rat model the chemical partition coefficients were: blood:air, 42.7; fat:blood, 36.4; liver:blood, 1.96; muscle:blood, 0.609; and vessel rich:blood, 1.96. The metabolic parameters from Haddad et al. (2001) were: VmaxC = 6.39 mg/hr/kg body weight scaled to the ¾ power of body weight; Km = 1.04 mg/L. For the rat model the metabolic parameters were: VmaxC = 7.60 mg/kg-d scaled to the 0.74 power of body weight and Km = 0.1 mg/L. A second set of human metabolic parameters from Sams et al. (2004) was also used. In this case constants for low and high affinity saturable pathways were incorporated into the models: high affinity Vmax = 689 pmol/min/mg microsomal protein, Km = 8.0 µM; low affinity Vmax = 3039 pmol/min/mg protein, Km = 391 µM. A value of 28 mg/mL liver for microsomal protein concentration was assumed. Published values we reviewed ranged from 11 to 35 mg/g tissue. The value we used was similar to that of Kohn and Melnick (2000) (30 mg/g liver) and Medinsky et al. (1994) (35 mg/g liver). All model units were converted to moles, liters, or hours for simulation.
>
> *From OEHHA, 2011, pages B295 – B296*

Doses were calculated as lifetime weighted average (LTWA) doses, described as:

> Male and female rats (NTP, 1999) were exposed to ethylbenzene for 6.25 hours/day, five days/week for 104 weeks. Male and female mice (NTP, 1999) were exposed to ethylbenzene for 6.25 hours/day, five days/week for 103 weeks. Average concentrations, expressed in mg/m^3, during the exposure period were calculated by multiplying the reported chamber concentrations by 6.25 hours/24 hours, five days/seven days and 4.35 mg/m^3/ppm.
> The average body weights of male and female rats were calculated to be 0.450 kg and 0.282 kg, respectively, based on data for controls reported by NTP (1999). The average body weights of male and female mice were estimated to be approximately 0.0429 kg and 0.0389 kg, respectively, based on data for controls reported by NTP (1999).
> Inhalation rates (I) in m^3/day for rats and mice were calculated based on Anderson et al. (1983):

$I_{rats} = 0.105 \times (bw_{rats}/0.113)^{2/3}$
$I_{mice} = 0.0345 \times (bw_{mice}/0.025)^{2/3}$

Breathing rates were calculated to be 0.264 m^3/day for male rats, 0.193 m^3/day for female rats, 0.0494 m^3/day for male mice, and 0.0463 m^3/day for female mice. Lifetime weighted average (LTWA) doses were determined by multiplying the average concentrations during the dosing period by the appropriate animal breathing rate divided by the corresponding animal body weight. For mice, the exposure period (103 weeks) was less than the standard rodent lifespan (104 weeks), so an additional factor of 103 weeks/104 weeks was applied to determine lifetime average doses.

From OEHHA, 2011, pages B294 – B295

Table 2 shows the various IURs considered by OEHHA (2011) for quantitative assessment of human cancer risk from ethylbenzene exposure.

Table 2. Tumor Types Modeled by OEHHA (2011) based on Data from NTP (1999)

Sex, Species	**Site, Tumor Type**	**Human Inhalation Unit Risk (IUR) Value** $(mg/m^3)^{-1}$	**Potential IRSL*** IRSL = 1E-6/IUR (µg/m³)
Male, rats	Renal tubule carcinoma or adenoma	0.0025	0.40
Male, rats	Testicular interstitial cell adenoma	0.0066	0.15
Female, rats	Renal tubule adenoma	0.00063	1.6
Male, mice	Lung alveolar/ bronchiolar carcinoma or adenoma	0.0015	0.67
Female, mice	Liver hepatocellular carcinoma or adenoma	0.0017	0.59

* IRSL = 1E-6/IUR

It should be noted that the relevance of the results from NTP (1999) to human carcinogenic risk has been questioned by Texas Commission on Environmental Quality (TCEQ, 2015):

> … Gaylor (2005) reviewed 156 NTP chronic bioassays and found that 62% (97/156) of the chemicals tested were identified by the NTP as showing some or clear evidence of carcinogenicity. The lifetime exposure studies were typically conducted in rats and mice (50 per sex per group), incorporated the maximum tolerated dose (MTD), and included many non-genotoxic chemicals. Results of the investigation estimated the probability that almost all chemicals (e.g., 92%) would produce a statistical incidence with larger sample sizes (e.g., from 50 to 200 rats or mice per sex per group). The analysis suggested that exposure to the MTD can result in cytotoxicity, which can lead to increased carcinogenicity due to increased opportunities for mutagenic activity during the regenerative cell proliferation process. It also suggested that a chemical's carcinogenic activity may be related to one or more nearly universal MOAs (e.g., regenerative cell proliferation at the MTD) rather than to some unique carcinogenic

> property for the chemical. Considering that the NTP (1999) study was conducted with 50 per sex per group and that 750 ppm = MTD, the observed carcinogenic responses may be unrelated to inherent ethylbenzene carcinogenicity, but rather to some universal MOA operative at the MTD.
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> *From TCEQ (2015), page 15*

Michigan Department of Environmental Quality (MDEQ) disagrees with the conclusions of TCEQ (2015) with regard to ethylbenzene carcinogenicity. MDEQ finds that there is sufficient evidence that ethylbenzene is carcinogenic to animals and that the results are relevant to humans. OEHHA (2011) describes their reasoning, in which MDEQ concurs:

> A proposed MOA for ethylbenzene-induced tumors, especially those in the mouse lung, involves the generation of quinone metabolites. This is analogous to the actions of styrene and naphthalene, which are also carcinogenic. OEHHA recognizes the plausibility of quinone metabolites participating in a potential MOA for ethylbenzene induced lung cancer in mice (see Genotoxicity above). However, a suggestion that the role of these metabolites is confined to cytotoxicity (resulting in promotion of spontaneous tumors) is not convincing. The observation of oxidative DNA damage in vitro (Midorikawa et al., 2004) supports a role for quinone metabolites in carcinogenic initiation, following the analogy with benzene (a well-known genotoxic carcinogen targeting multiple sites in various species including humans). The observation of chromosomal damage in peripheral blood lymphocytes of workers exposed to ethylbenzene and benzene (Sram et al., 2004) may be indicative of quinone metabolite induced DNA damage. Thus, the involvement of quinone metabolites is plausible and supported by at least some data. Although this does not of itself establish the quantitative nature of the dose-response relationship, a mechanism involving oxidative DNA damage might display low-dose linearity. Since ring oxidation may produce a genotoxic epoxide metabolite it is possible that more than one metabolic process which generates genotoxic intermediates may be operating. In our view the genotoxicity of ethylbenzene, particularly with respect to oxidative DNA effects, merits further investigation.
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> OEHHA therefore concludes that the limited data do not conclusively establish any particular MOA for ethylbenzene carcinogenesis. However, one or more genotoxic processes appear at least plausible and may well contribute to the overall process of tumor induction. Because of this, the default linear approach has been used for extrapolating the dose-response curve to low doses.
>
> *From OEHHA, 2011, page B-286*

Animal tumor findings are generally judged to be relevant to humans (EPA, 2005; page 1-10). However, some chemicals induce accumulation of alpha 2u-globulin, a low molecular weight protein, in the male rat kidney. A statistically significant positive association of renal tubule tumors with an advanced stage of chronic progressive nephropathy (CPN) has been shown for carcinogenicity studies of ethylbenzene (Hard 2002). In Hard (2002), the kidneys of all rats in the NTP (1999) bioassay were histopathologically reevaluated with the purpose of attempting to define a mode of action underlying the development of the renal tumors. Hard (2002) concluded that based on the close association of atypical tubule hyperplasia and renal tumors with CPN, that chemically induced exacerbation of CPN was the mode of action underlying

the development of renal neoplasia, a pathway that Hard (2002) considered to have no relevance for extrapolation to humans.

Under certain circumstances, chemicals exhibiting CPN and the accumulation of alpha 2µ-globulin, the use of kidney lesions are not appropriate for extrapolation to humans and should not be used in the risk assessment process. EPA (1991b) describes this phenomenon:

> The alpha 2µ-globulin accumulation initiates a sequence of events that appears to lead to renal tubule tumor formation. Female rats and other laboratory mammals administered the same chemicals do not accumulate low molecular weight protein in the kidney, and they do not develop renal tubule tumors. Because humans appear to be more like other laboratory animals than like the male rat, in this special situation, the male rat is not a good model for assessing human risk.

EPA stresses the need for full scrutiny of a substantial set of data to determine when it is reasonable to presume that renal tumors in male rats are linked to a process involving alpha 2u-globulin accumulation (EPA, 1991b). In the case of ethylbenzene, alpha 2µ-globulin accumulation is not present in the male rat kidney. NTP (1999) stated:

> No clear evidence of hyaline droplets was seen in the kidneys of male F344/N rats exposed to ethylbenzene for 13-weeks (NTP, 1992) or 2 years, and, thus, this proposed mechanism did not appear to contribute to the proliferative renal tubule lesions in male or female F344/N rats in the studies reported here.

Additionally, kidney tumors were observed in female rats in the NTP (1999) study, which indicates that kidney tumors resulting from ethylbenzene do not meet the conditions described in EPA (1991b) necessary for exclusion from quantitative cancer risk assessment. Hard (2002) concluded that:

> Careful examination of renal tubules revealed no evidence of renal tubule injury or increased mitotic activity that would support sustained cytotoxicity/cell regeneration as a mode of action for tumor development. An absence of granular casts and linear papillary mineralization discounted the possibility of alpha-2u-globulin nephropathy as the primary underlying basis in male rats

Hard's (2002) observation that cytotoxicity and cell regeneration is not occurring in the kidneys of male rats in in the NTP (1999) study does not support TCEQ's conclusion that kidney tumors should not be used for extrapolation to quantitate human cancer risk. Hard (2002) also concurs that alpha-2µ-globulin nephropathy is not observed in male rat kidney tumors. Given EPA's (1991b) guidance concerning kidney tumors and alpha-2u-globulin nephropathy, as well as the absence of cytotoxicity and cell regeneration, the extrapolation of kidney dose-response data of kidney lesions (both cancer and non-cancer endpoints) in the male rat are appropriate to use to estimate human health risks from exposure to ethylbenzene.

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