

April 8, 2014

Environment Canada
Chemicals Management Division
Gatineau Quebec K1A 0H3

RE: SIRC Comments on Environment Canada/Health Canada Draft Screening Assessment Report – Ethylbenzene, February 2014

Dear Sir or Madam:

The Styrene Information and Research Center (SIRC)¹ appreciates the opportunity to provide comments on the Draft Screening Level Assessment Report (DSAR) published by Environment Canada/Health Canada regarding potential environmental and human health concerns associated with ethylbenzene, pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA, 1999).

SIRC supports the overall conclusions of the DSAR and endorses the key DSAR conclusions that ethylbenzene is not genotoxic and that extrapolation of the relevance of chronic animal toxicity and tumorigenicity findings to human health should assume existence of toxicological thresholds for such findings. As a conservative-based approach appropriate to a screening level assessment the DSAR assumes human relevance of ethylbenzene-induced tumors in mouse lung and liver and rat kidney and testes. However, it appropriately acknowledges that mode of action investigations have questioned the qualitative and/or quantitative relevance of these toxicity and tumor endpoints to human cancer risks.

SIRC endorses the DSAR conclusion that ototoxicity reported in several animal studies represents the most appropriate and likely human relevant endpoint on which to evaluate potential risks resulting from short-term consumer exposures to ethylbenzene.

The detailed and informative exposure data presented for both environmental species and humans, when coupled with toxicological and other health data, fully support the conservatively constructed DSAR screening-level conclusions that ethylbenzene does not constitute a danger to the environment or to humans with the possible limited exceptions of human consumer use of certain products in which ethylbenzene is present as a non-additive

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 on styrene and helping ensure that regulatory decisions are based on sound science. For more information, visit <http://www.styrene.org>.

co-product in mixed xylene solvents (concrete sealants, lacquers, stains, and varnishes). However, as further detailed in specific comments below, a more detailed comparison of reasonable quality toxicokinetic data available for ethylbenzene in rats and humans suggests that short-term risk of ototoxicity associated with the consumer product scenarios of concern may not present unacceptable margins of exposure as defined under CEPA. In addition, as acknowledged in the DSAR (p.2, paragraph 2), ethylbenzene is not directly added as a novel solvent component to mixed xylenes, but rather is a variable concentration co-product in mixed xylene solvents used in the consumer products of concern identified in the DSAR (concrete sealants, lacquers, stains, varnishes). Environment Canada/Health Canada has noted that the commercial mixed xylenes obtained from petroleum or coal-tar sources contain up to 15 percent ethylbenzene, and importantly has determined that such mixed xylenes “are not entering the environment in quantities or under conditions that may be harmful to the environment in quantities or under conditions that may be harmful to the environment, or that constitute a danger to the environment on which human life depends, or to human life or health.” (Environment Canada/Health Canada, 1993). Thus, the conclusion of the DSAR that ethylbenzene in mixed xylene solvents may present a health risk is inconsistent with the primary conclusion presented in the Priority Substances List Assessment Report for Xylenes in that ethylbenzene is an inextricable co-product in mixed xylene solvents, which do not present a health risk.

As noted in the associated Environment Canada/Health Canada Risk Management Scope document for ethylbenzene, provision of other information clarifying concentrations, labeling, use and other information also will further inform decision-making on these consumer product applications.

Overall, the DSAR was clearly constructed and sufficiently detailed to support the primary conclusions presented in the draft or point to areas of refinement that may improve the DSAR conclusions. The detailed comments offered below are intended to affirm key conclusions as well as provide suggestions to further clarify and/or strengthen the information and conclusions presented in the DSAR.

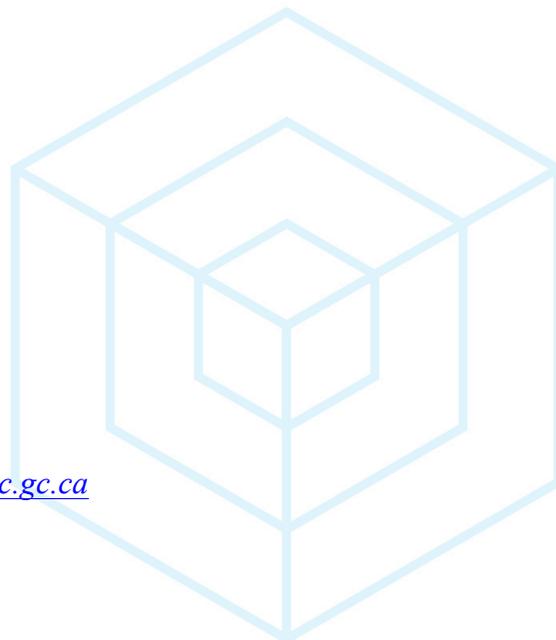
SIRC very much appreciates the Chemicals Management Division’s consideration of these comments, and would be pleased to provide clarification or additional details if needed.

Very truly yours,



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Styrene Information & Research Center Detailed Comments on DSAR

p.25, bottom: SIRC agrees with conclusion that the extensive toxicity data available from mammalian (rat, mouse) laboratory toxicity studies provides a reasonable screening-level data surrogate to assess potential impacts of ethylbenzene to terrestrial wildlife, and that selection of a Low Observed Effect Concentration (LOEC) of 75 ppm based on increased nephropathy in female rats exposed to ethylbenzene for 104 weeks is appropriate. Likewise, the DSAR selection of 4.40 $\mu\text{g}/\text{m}^3$ ethylbenzene as a representative “worst case” predicted environmental concentration, based on 95th percentile determinations of ambient air reported at a Montreal, Quebec, sampling site, is appropriate.

p.30: The conclusion that environmental species exposure to ethylbenzene in air, surface water, and sediment and soil “probably do not exceed concentrations associated with effects” is appropriate given the large Risk Quotient (RQ) values obtained for most exposure scenarios and associated large and clearly defined uncertainties used in derivation of these values.

p.34 and 38: SIRC agrees with selection of a cross-Canada survey of ethylbenzene indoor air concentrations ranging from 1.8 to 15.3 $\mu\text{g}/\text{m}^3$ with 95th percentile concentrations of 5.0 to 54.3 $\mu\text{g}/\text{m}^3$ as the most appropriate dataset to use in population screening-level human health assessments.

p.41: Given the lack of data available for ethylbenzene concentrations in drinking water, it is appropriate to use the conservatively established Canadian Drinking Water Guideline of 2.4 $\mu\text{g}/\text{L}$ as an upper bound estimate of daily ethylbenzene intake from drinking water.

p.53, top: It should be noted that the positive mouse lymphoma assay finding was specifically not replicated. A detailed description of the replication study is presented in the peer-reviewed Voluntary Children’s Chemical Evaluation Program (Siedel et al, 2006, cited subsequently at bottom of p.53 of the DSAR); VCCEP; 2007). The summary of the genotoxicity information presented here should also cite the review of Henderson et al. (2007) in which it was similarly concluded that “the available data from the standard battery of genotoxicity assays do not support a genotoxic mechanism for ethylbenzene”.

p.53, Mode of action for Carcinogenicity, 1st para.: Because the study of Midorikawa et al. (2004) reporting oxidative DNA damage has been cited by others (ATSDR, 2010; OEHHA,2007) as key evidence supporting for ethylbenzene genotoxicity, the DSAR should consider briefly noting the significant weaknesses of this study, i.e., use of high *in vitro* test concentrations of putative ethylbenzene metabolites ethylhydroquinone and ethylcatechol; use of phenobarbital-induced microsomes; addition of copper catalyst promoting oxidative stress; and use of (unphysiologic) calf thymus DNA. SIRC agrees, however, with the DSAR overall conclusion that, despite the report of Midorikawa et al. (2004), the overall weight of evidence indicates ethylbenzene is not genotoxic.

p.53, bottom: SIRC commends the DSAR citation and review of the comprehensive VCCEP (2007) evaluation addressing ethylbenzene exposures, animal and human toxicity

assessments, and associated Hill-criteria-based mode of action reviews of mouse lung and liver and rat kidney and testes tumor responses. However, it would be further informative to note that the VCCEP (2007) review was subjected to a peer-review (TERA, 2007) which endorsed the VCCEP conclusions that mode of action assessments indicated a lack of human relevance for both mouse liver and rat kidney tumors, while mouse lung tumors might be quantitatively relevant to humans but operating through a threshold-based (non-genotoxic) mode of action.

p.53, bottom: The meaning of the sentence “Furthermore, there was no temporal concordance in the *in vivo* studies” is unclear. This sentence appears to be addressing genotoxicity, but temporality of this endpoint is generally not a consideration in mode of action assessments.

p.54, top: The phrase “however, it is not clear whether reactive metabolites formed in the liver could also distribute through blood to lung [Huff et al., 2010]” should be deleted in that this concern was robustly rebutted in a detailed response published to the Huff et al. commentary (Saghir et al., 2010a). The mode of action data for both ethylbenzene and its close structural analog styrene (also a mouse lung carcinogen) strongly support the conclusion that mouse lung tumors are mediated through local mouse lung specific microsomal CYP2F2-mediated metabolism to cytotoxic metabolites, e.g., ethylhydroquinone and ethylcatechol, and not through systemic distribution of toxic metabolites generated in liver to lung (Saghir et al., 2009; Saghir et al., 2010; Cruzan et al., 2009).

p.54, bottom of top paragraph: The discussion regarding the potential lack of human relevance of the CPN-mediated mode of action for rat kidney tumors should be further clarified to note that ethylbenzene has been specifically identified with this mode of action (Hard et al., 2002; Lock and Hard, 2004). Using highly refined criteria to differentiate the CPN mode of action from other identified modes of action of rat kidney toxicity, Lock and Hard (2004) concluded that “the criteria for implicating exacerbated CPN as the mechanism underlying the increased renal tumor incidence with ethylbenzene were therefore fulfilled.”

p.54, second paragraph: SIRC strongly endorses the conclusion presented in this paragraph that the overall data support the conclusion that, even if conservatively assuming human relevance of the animal tumor responses, such responses should be evaluated assuming a threshold for toxicity expression. However, given the emphases of the immediately preceding text of the DSAR, this paragraph should also note that mode of action evaluations have strongly indicated a potential lack of qualitative and/or quantitative relevance of animal tumor findings to humans. In addition, the mode of action findings also include kidney, which is not specifically noted in the parenthetical “(lung, Leydig cell, liver)” in the draft.

p.55, top paragraph: The DSAR should also cite Mellert et al.(2007) in which no evidence of ethylbenzene-induced toxicity to male or female reproductive organs was reported in rats orally dosed at 750 mg/kg/day for 90 days.

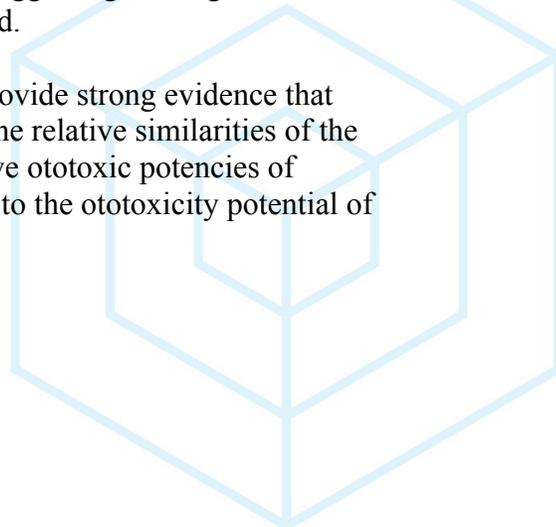
p.56, top: Since ototoxicity is identified as the key endpoint for critical elements of the DSAR screening level risk assessment, it should note that the endpoints identifying the NOAEC of 300 ppm in Cappaert et al. (2000) were based on effects to both auditory thresholds and loss of outer hair cells (OTC) observed at 400 ppm, while the LOAEC of 200 ppm reported in Gagnaire et al. (2007) was conservatively based on a loss in OTC only (auditory thresholds were altered at 400 ppm).

The likely human relevance of ethylbenzene-induced ototoxicity as a key endpoint for human risk assessment could be further amplified by comparison of ethylbenzene findings to those of its close structural analog, styrene. Styrene ototoxicity has not only been characterized in animals using test protocols similar to those for ethylbenzene, but also has been examined in workers with relatively specific exposures to styrene, thus providing direct evidence of human ototoxicity that can be directly correlated to animal findings.

Loquet et.al (1999) evaluated the impact of styrene exposure (500, 650, 850, 1000, and 1500 ppm, 6 hr/day, 5 day/week, 4 weeks) in Long-Evans rats on both audiometric and outer hair cell (OHC) loss. Other than using a different strain of rat, this study protocol was similar to the inhalation ethylbenzene ototoxicity study conducted in Sprague-Dawley rats reported by Gagnaire et.al. (2007), which exposed animals to 200, 400, 600 and 800 ppm, 6 hr/day, 5 days/week, up to 13 weeks. After a 4-week exposure to styrene, Loquet et al. identified an audiometric LOEL of 570 ppm (520-620 95%C.L.) and a 60-65% loss in OHC₃ in the 650 ppm exposure group. For ethylbenzene, Gagnaire and co-workers reported an audiometric LOEL of 400 ppm and a 67% loss in OHC₃ at 400 ppm. A comparison these toxicity responses suggests that ethylbenzene has an approximate 40% greater ototoxicity potency relative to styrene in rats, exclusive of any potential rat strain toxicity differences and differences in lengths of exposure.

Ototoxicity of styrene, assessed by changes in audiometric thresholds, also has been reported in a group of fiberglass boat fabricators who had relatively specific exposures to styrene (Triebig et.al, 2009). Styrene exposure was characterized by cumulative urine concentrations of mandelic acid and phenylglyoxylic acid. The worker population was divided into 3 exposure groups based on the urinary biomarkers and projected past history of styrene exposures. The findings of this study indicated that only workers exposed to styrene in the 30-50 ppm range over a period of 15 years, and who also experienced exposures greater than 50 ppm in past, were at risk for increased hearing thresholds. These investigators also concluded that previous studies suggesting hearing loss in workers at styrene exposures below 20 ppm could not be confirmed.

The animal and human styrene studies described above provide strong evidence that ethylbenzene is a likely human ototoxicant. In addition, the relative similarities of the animal test protocols provide useful insight into the relative ototoxic potencies of ethylbenzene to that of styrene that can then be compared to the ototoxicity potential of styrene identified in fiberglass composite workers.



p. 56, paragraph beginning “Other nervous system effects...”: Should also cite the lack of developmental neurotoxicity in rats at exposures up to 500 ppm (Faber et al., 2007) and adult rats orally treated up to 500 mg/kg/day (Li et al., 2010).

p.57, top paragraph: As noted in an early comment, this paragraph might be further clarified if the study of Lock and Hard (2004) was also cited in that it demonstrated a specific association of ethylbenzene with CPN using highly defined pathological criteria that differentiated the CPN mode of action from that of other modes of action of kidney toxicants.

p.58, top: The cited immunotoxicity references also should include Li et al., 2010.

p.58, bottom, citation of Faber et al., 2006 blood concentrations: Since the primary exposure scenario of concern identified in the DSAR involves inhalation exposure (consumer exposure to cement sealants, varnishes, lacquers), the DSAR also should cite data describing blood concentrations following inhalation exposure to ethylbenzene in both rats and humans. Both rat and human blood data following inhalation exposure to ethylbenzene have been described in Tardif et al. (1997) as a means to validate a proposed PBPK model. Although numerical data were not specifically provided in this study, blood concentrations can be visually estimated from graphical data presentations describing rat blood concentrations following 4 hour exposures to either 100 or 200 ppm ethylbenzene and humans exposed for up to 7 hours to 33 ppm ethylbenzene. The data indicate peak blood concentrations of approximately 2 and 8 µg/ml for 100 and 200 ppm rat exposures, respectively, and 0.5 µg/ml for human exposures to 33 ppm ethylbenzene. The approximate 4-fold increase in peak rat blood concentration resulting from a 2-fold increase in exposure suggests saturation of metabolic clearance at exposures between 100 and 200 ppm. In addition, the toxicokinetic data reported in Tardif et al. (1997) also indicate that blood concentrations are near or at plateau after 4 hours of exposure in both rats and humans, suggesting that potential extended exposures to ethylbenzene as might be encountered in home consumer use scenarios are unlikely to significantly impact the reported equilibrium blood concentrations seen in shorter-term exposures.

The above comparative cross-species toxicokinetic data presented in Tardif et al. (1997) provide an opportunity to further examine potential screening level ototoxicity health concerns identified in the DSAR as associated with certain consumer product exposures (concrete sealants, varnishes, lacquers). As described in Table 18 of the DSAR (p.63), a conservatively modeled maximum mean air concentration of 48 mg/m³ (11 ppm) ethylbenzene was estimated for potential human exposures associated with consumer use of concrete sealants. Using peak blood concentrations of 0.5 µg/ml determined in volunteers exposed to 33 ppm ethylbenzene for 7 hours (Tardif et al., 1997), and assuming linear toxicokinetics at exposures less than 100 ppm, a peak human blood concentration of 0.17 µg/ml is predicted from exposure to 48 mg/m³ (11 ppm) ethylbenzene, the maximum exposure associated with concrete sealant consumer use. As noted in comments above, the DSAR has appropriately selected the ototoxicity NOAEC of 300 ppm identified in rats exposed for 5 days to ethylbenzene (Cappaert et al., 2000) as the key toxicity endpoint for establishing margins of exposure associated with short-term consumer use scenarios. The

rat toxicokinetic data presented in Tardif et al. (1997) can be used to estimate a 4 hour peak blood concentration of 12 µg/ml after a 300 ppm ethylbenzene exposure, assuming linear toxicokinetic behavior above 200 ppm (ethylbenzene blood concentrations were approximately 8 µg/ml at 200 ppm). The 12 µg/ml peak estimate at 300 ppm is likely conservative given evidence of toxicokinetic saturation at ethylbenzene exposures between 100 and 200 ppm (see above), and is consistent with peak rat blood concentrations of 23.2 µg/ml measured immediately after an 8 hour exposure to 500 ppm ethylbenzene (Cappaert et al., 2002). These rat vs. human comparative peak blood concentrations translate to an internal systemic dose margin of exposure of approximately 71 ($12 \mu\text{g/ml} \div 0.17 \mu\text{g/ml} = 71$). Importantly, since this margin of exposure calculation is based on internal systemic dose comparisons that have intrinsically compensated for toxicokinetic differences within species, the traditional approximate 3-fold factors applied to correct for pharmacokinetic variations within both rat and humans (total of 10X) need not be applied. Thus, viewed in context of these comparative rat and human toxicokinetic data, the total systemic dose margin of exposure of 71 indicates that consumer exposures to concrete sealants may not present an unacceptable screening level health risk as is currently proposed in the DSAR.

p.59, first full paragraph: Although Charest-Tardif et al. (2006) concluded that toxicokinetic saturation was likely present in mice at exposure concentrations above 500 ppm, the blood C_{max} and AUC data presented in this study indicate that saturation might have been present at exposures between 75 and 200 ppm (i.e., both C_{max} and AUC increased non-linearly between these two test concentrations). Regardless, these data nonetheless suggest that ethylbenzene tumor data observed in mice only at 750 ppm in the NTP bioassay have limited if any relevance to human risk given the large margins of exposure to human general population and consumer exposures identified in the DSAR (Carmichael et al, 2006; Slikker et al., 2004).

p.63, Table 18: As noted above, margins of exposure calculated for the concrete sealant exposure scenario may indicate an acceptable screening level of risk if such margins are estimated on a data-driven analysis of comparative systemic doses (blood concentrations) between rats and human exposed by inhalation to ethylbenzene.

p.65, second paragraph: The analysis presented above addressing margins of exposure as calculated from comparison of systemic inhalation doses between rats and humans suggests the current draft screening level proposal that ethylbenzene presents an inadequate margin of exposure for certain consumer product uses and thus enters the environment at concentrations constituting a danger to human health should be reconsidered.

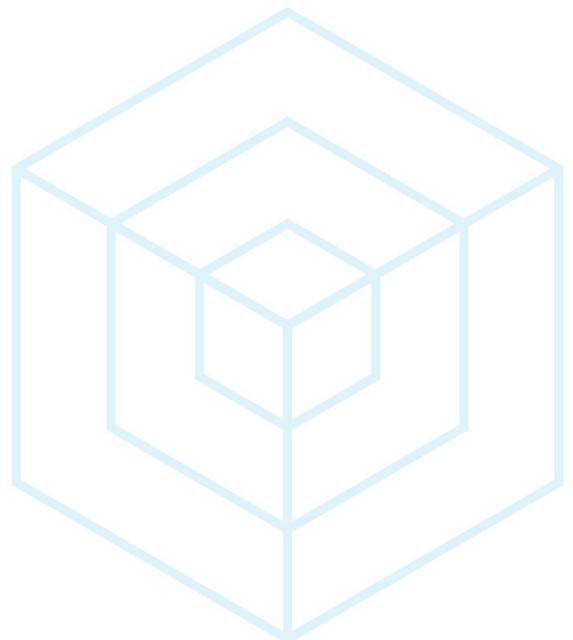
p.65, Uncertainties, paragraph 2: The DSAR has correctly noted the substantial uncertainty created in the screening level assessment by assuming existing toxicokinetic and/or PBPK modeling data are insufficient to impact risk decisions. The earlier described analyses indicate such uncertainty is likely to be substantially reduced if existing pharmacokinetic differences between rats and human are considered.

p.65, second paragraph from bottom: SIRC commends the DSAR conclusion that the human relevance of ethylbenzene-induced tumors from both rat and mouse chronic is

uncertain, and believes that existing mode of action and toxicokinetic data establish a low qualitative and/or quantitative relevance to humans.

p.127, Genotoxicity: Should note here, as was done in main body of text, that the mouse lymphoma finding was not replicated in an OECD guideline study (Seidel et al, 2006).

p.128, Developmental toxicity: Should note that the increased incidence of extra ribs in rats exposed only during the gestation period was not apparent in rats exposed continuously during both the pregestational and gestational periods (see statement at end of this section that extra ribs were not observed in “rats exposed to a low concentration of ethylbenzene during both pregestational and gestational periods”), calling into question the toxicological significance of the 100 ppm LOEC value.



REFERENCES (new additions beyond those cited in DSAR)

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