SIRC Styrene Information & Research Center

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May 31, 2018

Submitted electronically via science@acgih.org

American Conference of Governmental Industrial Hygienists
ACGIH® Science Group at science@acgih.org
1330 Kemper Meadow Drive
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Re: Comments on Notice of Intended Change to TLV™ for Styrene

Dear ACGIH® Science Group,

ACGIH® issued a Notice of Intended Change (NIC) for styrene that proposes a reduced TLV-TWA value and notations for ototoxicity and carcinogenicity A3. The Styrene Information & Research Center (SIRC) appreciates the opportunity to provide comments on the styrene NIC. In North America, SIRC serves as a resource for industry, federal and state governments, and international agencies on issues related to the potential impact of exposure to styrene on human health and the environment. Headquartered in Washington, D.C., SIRC was formed in 1987 as the principal focal point for the public information and research on styrene. SIRC is a non-profit organization comprising voting member companies involved in the manufacturing or processing of styrene, and associate member companies that fabricate styrene-based products. Collectively, SIRC's membership represents approximately 95% of the North American styrene industry. SIRC's charter also addresses the interests of ethylbenzene producers.

SIRC agrees the proposed ototoxicant notation is warranted for styrene, however, we find that the TLV-TWA value of 2 ppm proposed for styrene is not supported by ACGIH's draft documentation or available evidence. Styrene is ototoxic in experimental animals and there is human evidence of hearing deficits associated with styrene occupational exposures, although the exposure response information is not convincing. From our understanding of these workplaces, it is likely that past higher exposures and peak exposures contributed to hearing effects, but these concentrations are not accounted for in current studies. Protection from hearing effects is an appropriate basis for a TLV-TWA value, but evidence from reliable studies with exposure response information demonstrates that the existing TLV-TWA is protective against adverse effects on hearing. The reasons for proposing a lower value (2 ppm rather than 20 ppm) are not transparent.

Since ACGIH's previous review and continuing until recently, animal cancer studies demonstrate that styrene produces tumors in the lungs of mice but not rats. Mechanistic studies, however, indicate these tumors are not relevant to humans.

Regarding the overall draft TLV documentation, in general the content is outdated in its review of styrene's health effects.

SIRC urges ACGIH to reconsider the TLV-TWA value and update the documentation for styrene. Specifically, SIRC asks ACGIH® to:

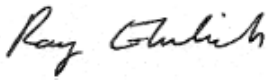
1. Reconsider the human and animal evidence for styrene hearing effects and establish a TLV-TWA value that is transparent and justified for the available information.
2. Reconsider the animal carcinogenicity and mechanistic data assignment for A3 cancer notation.
3. Improve the TLV documentation to include new and missing physico-chemical, exposure, and health effects information.

Because the proposed TLV-TWA value of 2 ppm is not supported by existing documentation, ACGIH should defer consideration of styrene until 2019 to provide adequate time for reconsideration.

SIRC appreciates the requested page limit for comments but, due to the limitations of the existing documentation, the applicable science requires more extensive summarization.

We would be pleased to answer questions or provide additional details to support these comments.

Sincerely,



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Enclosure: Comments of the Styrene Information & Research Center

Comments of the

STYRENE INFORMATION & RESEARCH CENTER (SIRC)

on the

AMERICAN CONFERENCE OF GOVERNMENTAL INDUSTRIAL HYGIENISTS (ACGIH®)

Notice of Intended Change to Establish a

THRESHOLD LIMIT VALUE (TLV®) FOR STYRENE

May 31, 2018

1. Executive Summary

ACGIH® issued a Notice of Intended Change (NIC) for styrene that proposes a reduced TLV-TWA value and notations for ototoxicity and carcinogenicity A3. The ototoxicant notation is warranted for styrene. Styrene is demonstrated ototoxic in experimental animals and there is human evidence of hearing deficits associated with styrene occupational exposures although the exposure response information does not provide a clear picture. Past higher exposures and peak exposures likely contributed to hearing effects, but these concentrations are not accounted for in current studies. There is evidence that styrene may produce other neurological effects including symptom reports, neurobehavioral changes and changes in color discrimination, however, these effects are reported at higher concentrations than those that are reported for hearing effects. Therefore, protection from hearing effects is the appropriate basis for the proposed TLV-TWA value but the available evidence from reliable studies with exposure response information support a value in the range of the current TLV-TWA as protective against hearing effects. The reasons for ACGIH proposing a TLV-TWA value lower than the present value are not transparent and the proposed value is not supported by the available evidence. Since ACGIH's previous review, new animal cancer studies demonstrate styrene produces tumors in the lungs of mice but not rats and hence is a confirmed animal carcinogen. However, mechanistic studies demonstrate these tumors are not relevant to humans. In general, the TLV documentation is outdated in its review of styrene's health effects. SIRC urges ACGIH to reconsider the TLV-TWA value and documentation for styrene.

2. List of Recommendations/Actions

SIRC urges ACGIH® to:

1. Reconsider the human and animal evidence for styrene hearing effects and establish a TLV-TWA value that is transparent and justified for the available information.
2. Reconsider the animal carcinogenicity and mechanistic data assignment for A3 cancer notation.
3. Improve the TLV documentation to include new and missing relevant physico-chemical, exposure and health effects information.

Because the proposed TLV-TWA of 2 ppm is not supported by existing documentation, ACGIH should defer consideration of styrene until 2019 to provide adequate time for reconsideration.

3. Rationale

1) Reconsider the human and animal evidence for styrene hearing effects and establish a TLV-TWA value that is transparent and justified for the available information.

Styrene is ototoxic in experimental animals and has been linked to hearing loss in humans in the workplace, particularly in conjunction with exposure to noise. Therefore, protection from hearing effects is the appropriate basis for the styrene TLV-TWA value. The reasons for ACGIH proposing a TLV-TWA value lower than the present TLV-TWA value are not transparent and the proposed value is not supported by the available evidence. The document does not adequately justify the basis for lowering the TLV-TWA to the value proposed. The document would benefit from and be more transparent by explicitly identifying the specific styrene exposure level that is considered a concern from the database and the rationale for selecting the proposed value. Why was 2 ppm selected, versus 1 ppm, 5 ppm or 10 ppm or 15 ppm? The human reports of color vision effects and neurobehavioral effects are at exposures of 50 ppm and greater, hence the present TLV-TWA value is more than protective for these effects. Only for ototoxicity are there reports of effects at exposures in the present TLV-TWA range and lower, but these studies are not convincing given a variety of limitations. The most reliable study is Triebig (2009) and its conclusions indicate that exposure levels in the range of the present TLV-TWA of 20 ppm should protect against hearing damage.

The evidence for styrene effects to hearing is supported by a number of high-quality animal studies and a threshold for ototoxicity is evident in animals exposed via both oral (gavage) and inhalation routes. There is human evidence of hearing loss in workers, but the exposure response information is less clear given the uncertainties inherent in exposure estimation in epidemiological studies and the confounding influences of other chemical exposures, alcohol use, and noise in many workplaces. Moreover, hearing loss in workers has been reported to occur in some studies only after years of occupational exposure (especially prior to institution of modern exposure controls), suggesting that uncharacterized historical peak exposures may have been responsible for the effect.

The draft documentation review of the human hearing effects literature should provide a thorough review and assessment of this information, in particular as these findings are among those reported at the lowest styrene exposure concentrations. The present draft documentation, however, reports limited information on these studies (mainly just the findings) and does not provide a critical assessment of the strengths and limitations of the studies.

Comments are offered only on the more recent studies that indicate effects as SIRC agrees the earlier 1980s to 1990s studies provide insufficient information upon which to reach a conclusion.

The study by Morata et al. (2002) is moderately sized in its number of workers from fiberglass product factories and included a number of exposure measurements and the interaction of noise. Of note the reported styrene air concentrations were correlated with mandelic acid (MA)

concentrations in urine and were found to be significantly correlated although there was great variability. Noise exposures were determined as 8-hour dB(A) L_{eq} values for 185 of the workers; the remaining 128 subjects were assigned the mean exposure of other workers doing the same tasks. Each worker's previous noise exposure was estimated from questionnaire responses or database information. Using the measured or assigned exposure for current employment, and the approximate values for previous employment, lifetime noise exposure was estimated as an 8-hour L_{eq} over each working day. For the noise only group, the current exposures ranged from 75 to 116 dB(A) L_{eqbh}. An unstated number of this group did not receive "excessive" noise, above 85 dB(A) L_{eqbh} and therefore could have been placed in the control group. The styrene only subjects had a range of current styrene exposures from 0.05 ppm to 23 ppm, while for the styrene and noise group, styrene ranged from 0.007 ppm to 12 ppm. Such wide ranges of noise and styrene measurements suggest that the exposure groups were not rigorously stratified. Each subject gave an audiogram which was evaluated for hearing loss at 1, 2, 3, 4, 6 and 8 kHz. An audiogram was considered "normal" if no threshold exceeded 25 dB Hearing Level at any frequency. A "high-frequency hearing loss" was noted if the thresholds were poorest in the frequency range 3 to 6 kHz. Over the 4 exposure groups, prevalence of high-frequency hearing loss ranged from 33% to 48%; the differences between groups were not statistically significant. The binary hearing variable, normal versus high-frequency loss, was also employed in a further analysis. The medical, lifestyle and occupational data (including current and past exposure to noise and to styrene) were subjected to a multiple logistic regression analysis to estimate the odds of subjects developing a high-frequency hearing loss. Only age, current noise exposure and MA in the urine were significant. Neither current nor lifetime styrene exposure achieved statistical significance. The authors concluded that exposure to styrene, at concentrations below 20 ppm, produced high-frequency hearing losses. However, considering the unclear information on styrene exposure of the subjects and the lack of exposure-response relationships, this conclusion is not substantiated by the available evidence.

The Sliwinska-Kowalska et al. (2003) study examined a moderate number of workers employed in boat-building or plastics factories. Of note, more than 80% of the workers in the 'styrene-only' cohort were exposed to other solvents (including toluene, dichloromethane and acetone) at levels that exceeded the occupational exposure levels (OELs) in Poland (100 mg/m^3, 50 mg/m^3, and 200 mg/m^3 respectively). Peak levels (unclear if these were 8-hour TWA values) were 225 mg/m^3 for toluene, 307 mg/m^3 for acetone, and 145 mg/m^3 for dichloromethane. This study shows some evidence of styrene-induced hearing loss and an additive or synergistic effect with other ototoxic agents such as toluene or noise. However, since in the 'styrene only' group, workers were also exposed to relatively high levels of noise (around 80 dB(A)), it is difficult to interpret whether the effect observed was due to styrene exposure only or to noise or both. Furthermore, interpretation is hampered by co-exposure to other substances (dichloromethane and acetone).

The Mascagni et al. (2007) study is a very small study. This study is expected to suffer from the same limitations as the larger studies but was not acquired and reviewed due to it being a non-English publication.

The findings of the Morata et al. (2011) study are important as the study included a large cohort of workers from different countries. Styrene exposure and noise were again examined, and this study purported finding hearing deficits in workers with exposure to normal noise levels. A weakness of this study is that styrene exposure measurements were contemporary mean TWA concentrations. No information was provided on short-term peak exposures or factory historical exposures which were likely higher than current measurements and especially important for workers with long tenure employment (some workers had decades of exposure up to 40 – 50 years which indicates exposures occurring during the 1970s when workplace concentrations were known (documented) to be significantly higher). The authors' themselves acknowledge that:

> "It is important to remember however that participants could have been exposed to higher styrene concentrations in the past, and that exposure to peaks of high styrene levels could explain the observed effects. Moreover, in both study locations in Poland, styrene exposure occurred in combination with other ototoxicants in a few instances, and information about those exposures was not complete nor entered in the analysis. Because of the potential co-exposure to other ototoxicants in the fiberglass products manufacturing industry, caution is suggested when extrapolating the findings of this study to other industries in which styrene exposures occur."

The authors also state that "Although our data indicate that low exposures to noise and styrene are associated with an increased probability of hearing loss, they do not allow us any attempt on dose-response calculations or to speculate about a safe styrene exposure concentration to prevent hearing loss. At the moment, the best information for the risk assessment of styrene comes from studies with experimental animals (Lataye et al, 2005)". Therefore, caution is needed when considering this study's finding for occupational exposure level setting.

The Sisto et al. (2013) study also found hearing deficits in a small group of workers with occupational exposures at or below the present TLV-TWA limit of 20 ppm. As with earlier reported studies, possible higher exposures in the past causing irreversible damage were not accounted for in their study design. The authors acknowledge that their study analyzed a small sample of subjects and hence a larger study is needed to confirm their findings. In their subsequent study (Tognola et al., 2015), the authors further acknowledged caution in interpreting this study's results as age, sex distribution, and size of subjects in the control group

were similar but not perfectly matched to those of the exposed group (the control group, for example, had a higher number of females and was slightly younger than the exposed group).

The most recently reported study by Tognola et al. (2015) is an extension of the Sisto et al. (2013) study that considered the same workers but for this study they "perfectly" matched the control group by age and sex (and size) to exposed workers. Otherwise, the examinations and analyses were reported to be the same. As with their previous study, subclinical but statistically significant differences were found between the transient evoked otoacoustic emissions (TEOAEs) of exposed and control subjects. This study, as with the other earlier studies, does not account for the possibility of past higher exposures of styrene or noise that could have impacted their exposed subjects.

As commented, all these studies suffer from the possibility that styrene exposures prior to those evaluated under contemporary workplace conditions or peaks of higher concentrations could have impacted hearing loss, and, being irreversible, these already could be present in the workers that were studied with lower concentrations during the course of the study. Therefore, the contribution of chronic past exposure to higher concentrations or peak exposures cannot be ruled out. Overall, although there is evidence that styrene is ototoxic in humans, the exposure-response for effects remains unclear and is confounded by earlier exposures, noise, and exposures to other ototoxic agents.

In view of the state of knowledge on human ototoxicity, the study reported by Triebig et al. (2009) was performed as cross-sectional evaluation with repeated effects measurements with 3 critical questions in mind: (1) Are there measurable hearing losses in styrene-exposed workers using standard audiometric tests? (2) Can dose–response relationships and measurable thresholds of effects be identified? (3) Are measured effects altered when exposure is not occurring? Subgroups of workers were objectively identified as having current low (n = 99), medium (n = 118), and high (n = 31) styrene exposures as defined by biomonitoring data (urinary mandelic acid (MA) + phenylglyoxylic acid (PGA) concentrations and blood styrene concentrations), thereby deriving individual exposure measurements without confounding by personal protection measures. The urinary metabolite data were converted to airborne exposure concentrations of 1.7–3.4 ppm for the low group, 7.6–15.2 ppm for the medium group, and 38.8–48.5 ppm for the high group. Historical air monitoring data in the lamination department of this facility indicated median styrene concentrations of 100 ppm (range 10 – 200 ppm) in the 1980s and 80 ppm (range 5 – 150 ppm) from 1990 to 1995. Using the historical data, a "high-long" exposure group (n = 17, mean job tenure 14.6 years) and a "low-short" exposure group (n = 34, mean job tenure 6.4 years) were defined. Workers exposed to noise levels of 85 decibels

(dB) and above were excluded in order to focus on the effects of styrene alone.[1] Hearing in eligible participants without hearing disorders was evaluated for specific frequencies by both pure tone audiometry, a behavioral test based on the patient's response, and recording of TEOAE, an objective method able to detect subtle disturbances of cochlear function. The measurements of TEOAE did not exhibit significant exposure-related changes. A strong relationship between age and pure tone hearing threshold was observed, but no significant relationship with current styrene exposure levels was observed. However, the subgroup exposed to high styrene levels (30 – 50 ppm average, and concentrations above 50 ppm in the past) for a longer period of time (more than 10 years) showed elevated thresholds at frequencies up to 1.5 kHz and 8 – 12.5 kHz. Grouping subjects into cases and non-cases according to the criterion of more than 25 dB hearing loss, a statistically significantly elevated odds ratio (7.46) was obtained between the low-short and high-long groups. No dose-response relationship could be determined using biomonitoring data or cumulative exposure measures. The repeated audiometric measurements indicated improvements in hearing function during holidays among all participants, perhaps due to a "training" effect. Interestingly, the high-long subgroup demonstrated greater "improvement" than the low-short subgroup, suggesting that the observed hearing deficit in this group may not be permanent. Therefore, in this study no hearing deficits were found for exposures of 12.5 ppm and 50 ppm at the time of investigation going back about 10 years. However, there was an indication for styrene-induced hearing losses in a subgroup of "high-long" exposed workers assumed to be exposed at 25-33 ppm over about 15 years (range up to 26 years). Higher exposures of 80-100 ppm existed for a time more than 10 years before this study was initiated. When the irreversibility of styrene induced hearing deficits is considered, these effects were likely due to the former high exposures. In summary, from the findings of this key study, 20 ppm is suggested as an exposure level of low concern for human hearing loss.

In addition to the review articles cited in the draft documentation, there are additional and more recent reviews reported by Vyskocil et al. (2012) that applied a weight of evidence approach to the assessment of ototoxic chemicals including styrene and the most recent review by Pleban et al. (2017). The Vyskocil et al. (2012) review of animal and human epidemiological evidence for ototoxicity of styrene concluded that, although the human data were less clear than animal data in terms of establishing a dose-response relationship, the overall evidence favors the conclusion that styrene is ototoxic. Similarly, while recognizing the complications imposed by multiple confounding factors (including exposure to noise and/or other chemicals as

[1] While this study used 85dB, we note that the lower action level in the EU is 80 dB under DIRECTIVE 2003/10/EC on the minimum health and safety requirements regarding the exposure of workers to the risks arising from physical agents (noise).

well as age and sex) the 2017 systematic review by Pleban et al. (2017) recommended that chronic styrene-exposed workers be routinely evaluated with a comprehensive audiological test battery to identify early signs of hearing impairment.

Overall there is considerable uncertainty in the majority of studies that have evaluated styrene and occupational hearing effects. A qualitative conclusion that styrene is ototoxic in humans is supported by the existing epidemiological literature and the new ototoxicant notation is warranted and should serve as a useful alert for workers. However, the present available information does not provide a robust basis for identification of a threshold styrene exposure level for such effects. In particular, it is well known that high exposures with inconsistent use of personal protection were historically common in reinforced fiberglass plastics industries, especially for laminators (the workforce most often studied for effects on hearing). Therefore, suggested thresholds from studies lacking reliable exposure data for former high exposures and defaulting to a current or recently measured time-weighted average concentrations would inevitably underestimate the true threshold. The most useful study on exposure response information is reported by Triebig (2009) which found no hearing deficits for exposures of 12.5 ppm and 50 ppm at the time of investigation going back to about 10 years. There was however an indication for styrene induced hearing losses in a subgroup of "high-long" exposed workers assumed to be exposed at 25-33 ppm over about 15 years (range up to 26 years). Higher exposures of 80-100 ppm existed for a time more than 10 years before this study was initiated. Taking into account the irreversibility of styrene induced hearing deficits, these effects are considered to have resulted from the former high styrene exposures. Taken together, exposures of 25 ppm and below are indicated to be a low concern for human hearing loss. Therefore, a TLV value for styrene in the range of the present TLV-TWA should protect against hearing loss in workers.

2) Reconsider the animal carcinogenicity and mechanistic data assignment for A3 cancer notation.

Styrene is an animal carcinogen producing increases in mouse lung tumors. There is a large body of metabolism and mechanistic evidence available demonstrating that these mouse lung tumors are not relevant to human cancer risk. Two recent publications provide an overview of the available body of mode of action (MOA) evidence that has been supported by a new 2-year inhalation exposure study of styrene using genetically-modified mouse strains and evaluations for lung changes including changes on gene expression (Cruzan et al., 2018; Andersen et al., 2018). Briefly, the MOA for mouse lung tumors consists of styrene metabolism to ring-oxidized compounds in mouse lung club cells. When produced in sufficient quantity, these metabolites cause tissue responses in mouse lungs with enhanced gene expression. These data sets support a molecular initiating event for styrene of direct mitogenicity from mouse-specific CYP2F2-mediated metabolites that appear to activate *Nr4a* signaling. Longer-term modifying factors

include down regulation of *Nr4a* genes and shifts in both circadian clock transcription factors (TFs) and other TFs, linking circadian clock to cellular metabolism. There were no gene expression changes indicative of cytotoxicity or activation of p53-mediated DNA-damage pathways indicated at any time of exposure. In the absence of CYP2F2 metabolism, or CYP2F1 metabolism in transgene (TG; humanized) mice, neither styrene nor styrene oxide produce cytotoxic or proliferative changes in lung (styrene includes 104-week exposure to 120 ppm styrene. The lack of styrene oxide toxicity in both the knock-out (KO) and TG mouse strains supports a conclusion that styrene oxide is not the proximate metabolite accounting for the lung toxicity and tumorigenicity of styrene in mice. Importantly, the MOA evidence also supports a conclusion that styrene oxide formed in humans primarily by CYP2E1 is not subsequently metabolized by CYP2F1 to ring-oxidized metabolite(s) in amounts sufficient to present a lung toxicity or tumorigenicity risk. The role of CYP2F2 metabolism as an essential MOA gateway event responsible for mouse lung toxicity is consistent with toxicity and MOA data in rats. Rats have less CYP2F (2F4) than mice and therefore produce less ring-oxidized metabolites. No cytotoxic, proliferative or tumorigenic changes are found in the lungs of rats exposed to styrene, even up to 1000 ppm for 2 years. Overall, the animal MOA data unequivocally demonstrate that styrene-induced lung tumors are unique to the mouse due to mouse-specific metabolism of styrene by CYP2F2. Thus, the MOA demonstrates that these styrene-induced mouse lung tumors are qualitatively, or possibly quantitatively, not relevant to humans.

SIRC urges ACGIH to review this new key information and reconsider the carcinogenicity notation proposed for styrene.

3) Improve the TLV documentation to include new and missing relevant physico-chemical, exposure and health effects information.

   The draft documentation includes many pages of study information that for a large amount of the content contains outdated or incomplete information and therefore citable materials and detailed comments on this information are offered that would improve this content. Given the volume of information in the draft documentation, the comment volume is also necessarily large and hence is offered separately for ACGIH's consideration. We hope ACGIH will consider our offered comments.

Citable Material and Specific Comments on Draft Documentation

Our identification of citable materials and detailed comments on the draft documentation are organized per the ACGIH identified section headers. Only the citable references not identified in the ACGIH draft documentation are listed.

Chemical and Physical Properties

The chemical and physical properties of styrene are reasonably well described in the draft documentation. However, a couple of the listed parameters are noted to not have information available, whereas there is information reported for these parameters.

For autoignition temperature, there is a reported value of 490 °C at atmospheric pressure of 1013 hPa (Fire Protection Guide to Hazardous Materials, 1997)

For octanol/water partition coefficient, there is a reported value of 2.96 log Pow at 25 °C (ECHA, 1987).

Fire Protection Guide to Hazardous Materials. (1997). 12th Ed. Quincy MA: National Fire Protection Association. (cited in HSDB).

European Chemical Agency (ECHA). (1987). Registration Dossier for Styrene. Unnamed study report. Entry: Partition coefficient, Key Experimental Result (date). Online at: https://echa.europa.eu/registration-dossier/-/registered-dossier/15565/4/8/?documentUUID=3b56748b-bff0-4c26-9ae4-55c7fa15f830

Major Sources of Occupational Exposure

In addition to the styrene manufacturing method of catalytic dehydrogenation of ethylbenzene, styrene is also manufactured by a second method which involves oxidation of ethylbenzene to ethylbenzene hydroperoxide, that is reacted with propylene to yield propylene oxide. The co-product of this process, methyl phenyl carbinol, is dehydrated to form styrene (IARC, 2002).

There is more current data available on U.S. annual styrene production than reported in the draft documentation, although the volumes produced today are similar to the amounts reported in the draft documentation for the 1990s. The latest U.S. styrene production is reported in the U.S. EPA 2012 Chemical Data Reporting (CDR) information and is 10,245,086,182 lbs./year (https://chemview.epa.gov/oppt_chemical_search/) which is approximately equivalent to 5 million tons/year.

The potential for styrene exposure is not limited to industrial sources. Styrene may be present as a combustion product of cigarette smoke and automobile exhaust (ATSDR, 2010) and in certain foods due to its natural presence or from limited migration from food packaging materials (ATSDR, 2010).

The reported worker exposure statistics in the draft documentation (NIOSH, 1983) is of historical interest but this data is now obsolete. In 2011, the Styrene Information & Research Center (SIRC) estimated that 90,000 American workers, employed by approximately 5,000 plants across the U.S., participated in the manufacture of styrene products (SIRC, 2011). This study did not include composites manufacturers; the number of workers and plants cited would be as much as twofold larger if the composites industry had been included.

It is apparent that a higher proportion of workers in plastics industries are female: over 26% of workers manufacturing plastics products, one-third of the workforce producing rubber products, and 30% of the workers in the resin, and synthetic rubber, and fiber industry are women U.S. Bureau of Labor Statistics, 2014), and the plastics industry in Canada has a higher proportion of female workers than any other industry in the manufacturing sector (Dematteo et al., 2013).

Regarding industrial exposures, styrene and styrene-based polymers or copolymers are generally manufactured using closed processes that limit potential exposure to the monomer (Miller et al., 1994). During processing of thermoplastics, heating of the plastics can release small quantities of styrene (and other hydrocarbons), with amounts emitted dependent upon the types of polymer, processing, and ventilation equipment, as well as process temperatures and residence time (Miller et al., 1994). Exposures to styrene are also reported to be relatively low during the processing of polymers or copolymers, such as styrene-butadiene rubber (IARC 2002, NTP CERHR, 2006).

Worker exposures have decreased over the past several decades. Miller et al. (1994) estimated average worker exposure for all segments of the industry except fiberglass-reinforced plastics (RFP) to be 5 ppm or less in 1994. Ten exposure studies conducted between the 1960s through 1990s in styrene monomer, polymer, and copolymer manufacturing plants (many in the U.S.) reported mean air levels of styrene of ≤ 35 ppm, with most values below 10 ppm, and higher values generally associated with older studies (IARC 2002, Cohen et al., 2002). Occasional peaks of up to 50 ppm occurred during filling of drums, occasional bursts, or equipment leaks (IARC 2002, Cohen et al., 2002). Sampling data from 5 U.S. styrene-butadiene rubber plants in the period 1978 – 1983 (n = 3,649) reported an average styrene concentration of 3.53 ppm (ca. 16,000 $\mu g/m^3$), with a standard deviation of 14.3 ppm (Matanoski et al., 1993). A study of the same facilities by Macaluso et al. used industrial hygiene data and air dispersion models to estimate changes in time-weighted average (TWA) styrene concentrations associated with typical tasks and job groups since the 1940s (Macaluso et al., 2004). Mean TWA styrene exposures decreased 15% to 97% by task, and 64% to 98% by job (Macaluso et al., 2004).

The highest occupational styrene exposures occur during the production of fiberglass-reinforced plastics, especially for large items such as boats that involve manual application of materials (Miller et al., 1994, Cohen et al., 2002, NTP CERHR, 2006, Tranfo et al., 2012). Trend analyses of styrene exposure data from 60 reports providing data on 24,145 TWA personal air samples from open-mold workers in the European

fiberglass-reinforced plastics industry showed that the average styrene concentration in the breathing zone decreased on average by 5.3% per year during the period 1966 –1990, but only 0.4% in the period from 1990 to 2002 (Van Rooij et al., 2008). Mean TWA styrene concentrations in 2003 for all regions ranged from 9.2 – 32.5 ppm (Van Rooij et al., 2008). Urinary biomonitoring data showed a steeper decline in styrene exposure (8.9%), perhaps because urine samples were collected in companies that showed a greater decrease of styrene exposure in air (Van Rooij et al., 2008). In a recent study of workers in 4 fiberglass reinforced plastics plants in Italy, the median styrene exposures ranged from 5.5 to 21.6 ppm, and molders had the highest exposures (maximum 36.2 ppm) (Bonanni et al., 2015).

Summarizing data from 4 U.S. plants, Lees et al. (2003) reported TWA styrene exposures ranging from 9.2 to 55 ppm without adjusting for respiratory protection (9.2 to 24 ppm including adjustment). Personal TWA styrene concentrations collected from 1996 to 1999 for workers at 17 U.S. fiberglass reinforced plastics factories ranged from non-detect to 142 ppm, and means ranged from 9.2 to 27.3 ppm (Luderer et al., 2004). A further evaluation of these data showed that the type of product and job title were important predictors of styrene exposure. The job titles “laminator” and “grinder/sander” were associated with higher exposures, as was production of recreational vehicles (Serdar et al., 2006).

The American Composites Manufacturers Association (ACMA) and the National Marine Manufacturers Association (NMMA) sponsored 2 industrial hygiene surveys in 6 open-mold composite plants located in the U.S. Midwest and California typifying the range of open molding processes in order to characterize relatively highly exposed workers’ time-weighted average styrene exposures in 2001 and 2003 (Lipiro et al., 2004). No estimation of exposure with respirator use was presented. Styrene concentrations in 62 8-hour TWA samples ranged from 4 to 122 ppm, with a mean of 43.5 ppm and 95% UCL of 48 ppm calculated using EPA’s ProUCL software (U.S. EPA, 2016). These data are summarized in Table 1.

Table 1. Summary of 8-hour TWA styrene concentrations in 6 U.S. open-mold plants

Plant no.	n	Average 8-hour TWA styrene (ppm)	Range
1	8	33.3	11.8-49.5
2	5	22.3	20.1-23.5
3	10	35.6	20-52.7
4	15	53.7	19-88
5	9	38.8	4-83
6	15	53.9	20-122
	Mean	**43.5**	
	95% UCL	**48.0**	
	Percentile		
	10	20.0	
	25	25.8	

Plant no.	n	Average 8-hour TWA styrene (ppm)	Range
	50	44.0	
	75	54.5	
	90	64.8	
	95	81.6	

Source: Lipiro *et al.* (2004)

ACMA recently provided a summary of employees' breathing-zone styrene concentrations at 11 reinforced plastic/composite manufacturing facilities, of which 5 use open-mold processes (1-5), 3 are compression molding facilities (6-8), and 3 others use the pultrusion operation (Lipiro et al., 2004). Both compression molding and pultrusion are closed processes with a lower potential for worker exposures, and generally do not require respiratory protection. As noted previously, processes in the open molding facilities associated with higher styrene exposures are gelcoat and lamination. In these operations, styrene-containing gelcoats and resins are applied to an open mold by mechanical atomized (gelcoat spray), mechanical nonatomized resin, or manual (bucket and brush) application. Because some workers in open processes use respiratory protection, breathing zone samples do not represent actual inhalation exposures. Therefore, the OSHA-recommended ten-fold Assigned Protection Factor for a half-mask organic vapor cartridge was applied to estimate the actual inhalation exposures for workers wearing respirators in plants 1 to 5 (Lipiro et al., 2004) (Table 2). Figure 1 is a box and whisker plot displaying range, mean, median, and quartile 8-hour TWA styrene concentrations for these conditions. Average 8-hour TWA styrene concentrations in the 3 pultrusion plants (the only data given in the ACMA report) ranged from 7.8 ppm to 29.5 ppm (Lipiro et al., 2004).

Conservative estimates of central tendency (95% UCL) and upper bound (95th percentile) 8-hour TWA styrene exposures in the U.S. fiberglass-reinforced plastic industry were developed for closed-mold and open-mold plants with and without respiratory protection based on the most recent data provided by ACMA (2018) (Table 3). The averages provided for the 3 pultrusion plants were added to the individual measurements for plants 6 to 8 for the closed process exposure estimates. Of note, the OSHA respirator protection factors should not be confused with actual worker exposures. The Assigned Protection Factors are provided by OSHA as a convenience to allow employers to use respiratory protection avoid satisfy regulatory requirements (PELs). The only way to determine actual exposures to workers using respirators is through blood or urine tests.

Table 2. Summary of 8-hour TWA styrene concentrations in 8 U.S. plants using open vs. closed molding processes

Plant no.	Plant type	n	Average 8-hour TWA styrene (ppm)	Range	Adjusted average 8-hour TWA styrene (ppm)*	Range
1	Open molding processes - very large parts molded in large room with high volume filtered exhaust and makeup air	5	48.7	0.6-67	5.0	0.6-6.7
2	Open molding processes conducted in filtered exhaust ventilation booths	7	21.3	6-41	4.1	1.4-9
3	Open molding processes conducted in filtered exhaust ventilation booths	8	11.4	3-21	1.1	0.3-2.1
4	Open molding processes conducted in large booths with filtered exhaust and makeup air	11	23.0	1-52	4.3	1-10
5	Open molding, filament winding, and resin infusion processes conducted in large room with high volume filtered exhaust and makeup air	5	28.4	12-51	2.8	1.2-5.1
6	Compression molding, SMC compounding and covered mixing processes	8	10.4	0.7-18		
7	Compression molding and covered mixing processes	6	10.2	3.6-18		
8	Compression molding, SMC compounding and covered mixing processes	14	6.1	0.3-13.8		

Source: ACMA (2018)
* OSHA Assigned Protection Factor for half-mask organic vapor cartridge (10)

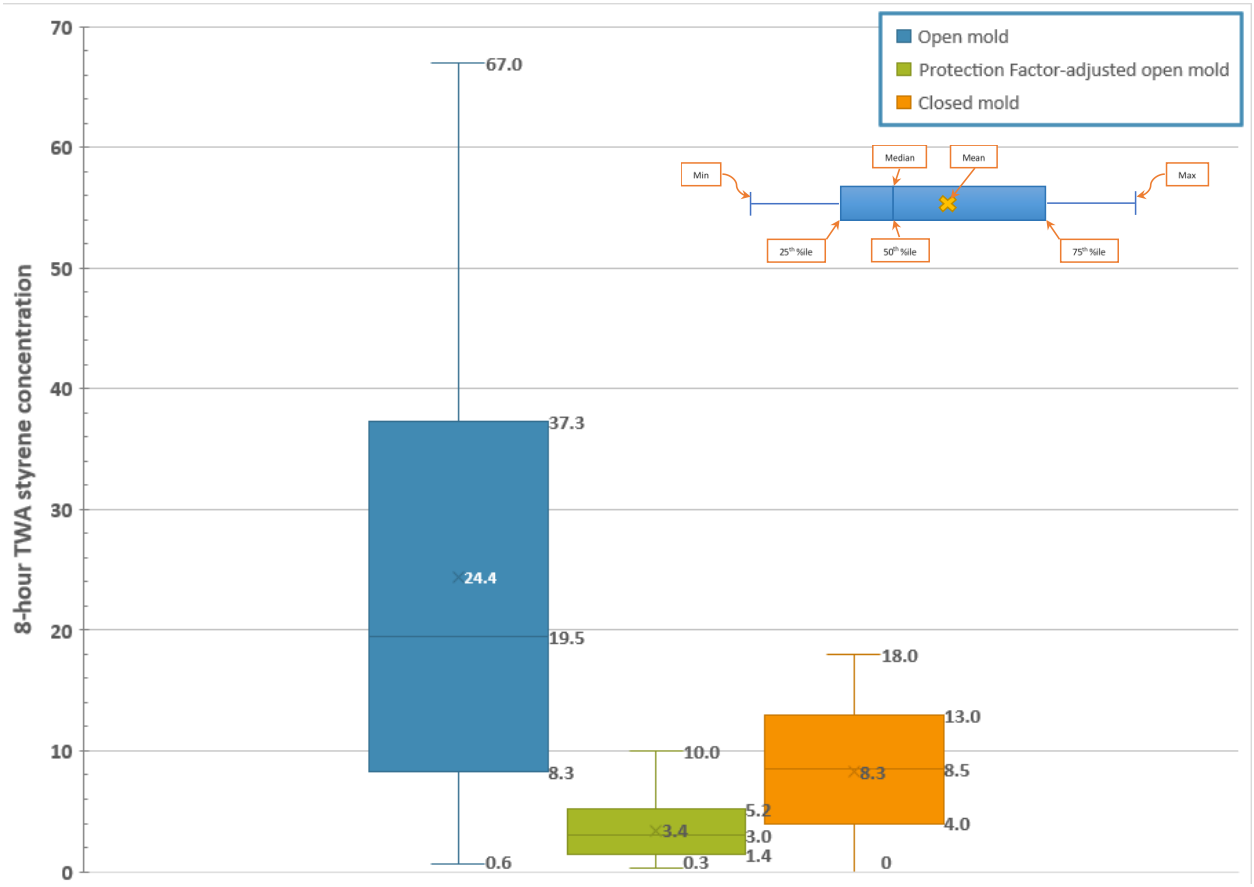

Figure 1. Distribution of 8-hour TWA styrene concentrations in eight U.S. plants using closed vs. open molding processes (with and without OSHA Assigned Protection Factor adjustment) (data from ACMA [2018])

Table 3. Central tendency and upper-bound 8-hour TWA styrene exposure concentrations for workers in plants using open (with and without respiratory protection) and closed molding processes

Plant type	n	8-hour TWA styrene concentration (ppm/µg/m³)[a]	
		Central tendency (95% UCL)	Upper bound (95th percentile)
Open molding	36	29.9/129,000	61.5/266,000
Open molding with Assigned Protection Factor[b]	36	4.3/19,000	7.3/32,000
Closed mold[c]	31	11.2/48,000	18.0/78,000

Source: ACMA (2018)

[a] Rounded

[b] OSHA Assigned Protection Factor for half-mask organic vapor cartridge (10)

[c] Includes averages from three pultrusion plants

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Animal Studies

Acute

The reference assigned to the rat acute inhalation study is incorrect. The correct reference for this study is Shugaev (1969). In addition to acute toxicity studies via inhalation and oral routes, there is available a recently conducted acute dermal toxicity studies reported for rats (ECHA, 2005). The study was compliant with OECD Guideline 402 and tested 5 Sprague-Dawley rats with skin exposures to 2000 mg/kg bw styrene applied under semi-occlusive dressing for 24 hours. This study found no deaths or gross necropsy abnormalities but clinical signs indicative of CNS depression (e.g., decreased spontaneous locomotion) were observed. The LD50 for this study was > 2000 mg/kg bw.

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Subchronic

The focus of the subchronic toxicity section is on studies that reported auditory system neurological effects and, while these effects are important, a number of other repeated exposure studies are available that examined styrene for general systemic toxicity, other target organ effects, or other neurotoxicities that were not addressed in the draft documentation. Including some of this other repeated exposure information would provide a more thorough perspective of this endpoint for styrene. Only some of the available subchronic inhalation studies that were not included in the draft documentation are described below.

Thirteen-week subchronic inhalation toxicity studies conducted in rats and mice are reported for styrene (Cruzan et al., 1997). In the 13-week rat study, 10 male and 10 female Sprague-Dawley rats were exposed to 0, 200, 500, 1000 or 1500 ppm styrene for 6 hours/day for 5 days/week and comprehensively evaluated for effects on body weight, food and water consumption, clinical chemistry and haematology (at 4 and 13 weeks), organ weights, and macroscopic and microscopic pathology in a wide range of organs and tissues were assessed at 13 weeks. The study also included a satellite group of

15 males that was exposed using the same regimen for 2, 5 or 13 weeks, at which time lung and liver cell proliferation was determined by 5-bromo-deoxyuridine (BrdU) labelling. The study found no treatment-related deaths and clinical signs were limited to evidence of local irritation as evidenced by closing of the eyes at 200 ppm and greater concentrations during exposure. Body weight gain was reduced to 81% of control values in males at 1500 ppm and food consumption was also reduced in this group. The only other exposure-related effects were histopathological changes in the olfactory epithelium: focal disorganisation or hyperplasia of basal cells, single cell necrosis or cell loss were recorded. These changes were observed at 500 -1500 ppm, the incidence and severity of the microscopic effects being dose-related. BrdU labelling showed no evidence of increased cell proliferation in the rat liver cells or in the cells of the bronchiolar or alveolar regions of the rat lung at any of the time points.

Additional rat inhalation studies have also reported upper respiratory irritation effects but little evidence of significant systemic toxicity. In a study reported only as an abstract, Roycroft et al. (1992) examined groups of F344 rats (number per group not specified) exposed to 0 or 125 to 1500 ppm styrene for 90 days (exposure regimen not detailed). No treatment-related deaths were reported, and no clinical signs of toxicity were observed. Body weight gain was reportedly reduced (not quantified) in rats at 1500 ppm. Relative liver weights were apparently increased (not quantified) in rats at the two exposures. Hematological and biochemical analysis apparently revealed no treatment-related variations in rats of either sex in any of the exposure groups. Histopathology revealed degeneration and necrosis of the nasal olfactory epithelium and goblet cell hypertrophy of the nasopharyngeal duct, apparently observed in animals of all styrene-exposed groups, although no information on the incidence of severity of these findings was reported. Ohashi et al. (1985) investigated the respiratory toxicity of styrene (cited in the review by Bond (1989). In the study, groups of rats (strain and number not specified) were exposed to up to 800 ppm styrene for 4 hours/day for 8 weeks. Animals were sacrificed and examined histopathologically 3 weeks after the last exposure. The authors reported that changes in the nasal mucosa occurred at 50 ppm and above, including vacuolation of epithelial cells, nuclear pyknosis, and 'fall-off' of epithelial cells. The incidence and severity of the findings is not given. The damage was reportedly more severe in the upper respiratory tract compared with the lower respiratory tract. In a follow-up study, Ohashi et al. (1986) (also cited in the review by Bond (1989), rats (strain and number not specified) were exposed to 150 or 1000 ppm styrene for 4 hours/day, 5 days/week for 3 weeks. Animals were sacrificed and examined histopathologically at up to 12 weeks after the last exposure. At day 1 after the end of the exposure period, it is reported that there was a dose-dependent decrease in tracheal and nasal ciliary activity, and that ciliastasis was observed in animals exposed to 1000 ppm. At week 12 post-exposure, in animals exposed to 150 ppm, ciliary activity and nasal tracheal mucosal morphology were comparable with controls. In animals exposed to 1000 ppm, ciliary activity was only 50 to 75% of that of control values, and nasal mucosal morphology remained abnormal, whereas tracheal mucosal morphology was comparable with controls.

In a study specifically designed to investigate the effects of styrene exposure on the lung, groups of male and female CD rats (5/sex/group) were exposed to styrene vapor at 0 or 500 ppm, for 6 hours/day, 5 days/week for up to 2 weeks (Green, 1999). Groups of 5 animals were sacrificed at 17 hours after the 1st, 5th, 6th and 10th exposure, and the lungs removed for histopathological examination and quantitation of cell division rates (3 days prior to sacrifice each animal was fitted with a BrdU mini-pump). No treatment-related macroscopic or microscopic findings were observed in the lungs of any of the exposed animals. No increases in the labelling indices or in cell division were observed in any region of the lungs of treated animals at any time point.

Gamer et al. (2004) conducted a mechanistic study investigating lung effects of styrene in rats. Female CD rats (10/group) were exposed to 0, 688 or 2150 mg/m^3 (0, 160, 500 ppm) styrene for 6 hours/day for 1 or 5 exposures and sacrificed immediately at the end of the exposure period. Lung lavage was performed on 5 animals per group for each exposure duration. Cytological and humoral analyses were conducted together with the measurement of levels of proteins and CC16 (club cell [formerly known as Clara cell] specific protein), lactate dehydrogenase, alkaline phosphatase, γ-glutamyl transferase, N-acetyl-β-D-glucosaminidase and catalase activities. The blood of the remaining animals was sampled and the lungs, together with the trachea and larynx were removed for histopathological examination. Statistically significant reduction in body weights was observed in the 160 and 500 ppm group. The CC16 level and the measured enzyme activities were unaffected by styrene exposure. No exposure-related histopathological findings were seen in the respiratory tract and lungs of any of the exposed animals at the end of the exposure periods. Overall, this study showed that inhalation exposure up to 500 ppm styrene did not induce any club (Clara) cell toxicity in rats.

Mutti et al. (1999) studied styrene for potential kidney toxicity in a group of 10 female rats exposed to styrene at 300 ppm, for 6 hours/day, 5 days/week for 12 weeks. Additional groups included animals administered the known nephrotoxicant adriamycin. The styrene-exposed animals exhibited some changes in urinary indicators of kidney function that were described as slight but statistically significantly elevated. Kidney weights in styrene-exposed animals were comparable with controls. At histopathological examination the scores for kidney interstitial fibrosis, cystic dilatations and hyaline were all statistically significantly elevated in styrene-exposed animals compared with controls. Scores for cellular infiltrates in styrene-exposed animals were comparable with controls. This study suggests that repeated exposure of rats to styrene produced kidney changes, however, these effects have not been observed in longer studies or studies that tested higher concentrations. Viau et al. (1987) also examined styrene for kidney effects in 10 male and 10 female Sprague Dawley rats exposed to 0 or 135 ppm styrene, for 7 hours/day, 5 days/week for 13 weeks. Only the kidney (weight, function (glomerular filtration rate, urinary concentration ability) and histopathology) was investigated. No adverse effects on the kidney were observed in this study.

Overall from the variety of rat studies, the two identified target organ sites that have been identified are the nasal epithelium and the auditory system.

Species differences in target organ effects associated with styrene repeated exposure emerge from the studies in mice. The mouse is peculiarly sensitive to toxicity arising from repeated inhalation exposure to styrene, although there is some variation between different strains. The three well-characterized target sites that have been identified for styrene in the mouse are: the lung, the nasal epithelium and the liver.

The Cruzan et al. (1997) 13-week mouse study examined groups of 10 male and 10 female CD-1 mice that were exposed to 0, 50, 100, 150 or 200 ppm styrene for 6 hours/day for 5 days/week. A satellite group of 30 males was exposed by the same regimen for 2, 5 or 13 weeks, at which time lung and liver cell proliferation was determined by BrdU labelling. Animals were assessed during the study for deaths, body weight changes and clinical signs of toxicity. Extensive analyses of hematological and clinical chemistry parameters were conducted. Extensive gross pathological and histopathological examination was performed. Additional satellite groups of 5 mice/sex/group were examined at week 1 with respect to serum sorbitol dehydrogenase (SDH) and alanine transferase (ALT), bile and acid levels and liver histopathology. Two female mice exposed to 200 ppm died during the first week of the study. Evidence of centrolobular necrosis and congestion was found in the livers of the decedents and was reported as the cause of death. Abnormal histopathology of the nasal passages (atrophy of the olfactory epithelium) was also observed in the decedents. In relation to the surviving animals, (females at 200 ppm were cold to touch, lethargic and had slow respiration during the first week of exposure. No other clinical signs of toxicity were observed. In males at 200 ppm body weights and food consumption were reduced (no quantitative data reported). Organ weights, hematological and clinical chemistry parameters were comparable with controls in all animals investigated at each time point. Abnormalities in the nasal passages of animals in all exposure groups were observed. The primary lesion reported was atrophy of the olfactory epithelium and of the olfactory nerve fibres with or without focal respiratory metaplasia, and dilatation, hypertrophy and hyperplasia of the Bowman's gland. Abnormalities of the lungs were observed in the lungs of the majority of animals at 100, 150 and 200 ppm. These findings included decreased eosinophilia of the bronchial epithelium with focal crowding of non-ciliated cells in the bronchioles. No abnormalities of the lungs were observed in animals at 50 ppm. No abnormalities were observed in the livers of animals at 50 or 100 ppm. Abnormal liver histopathology (inflammation, fibrosis and hepatocyte loss) was observed after 13 weeks in 2 males at 200 ppm and in 5 females at 150 ppm and all surviving females at 200 ppm. The severity of these liver effects was more marked in females than in males. BrdU labelling showed no increase in cell proliferation in the liver cells at any exposure level. An increase in the labelling index of the respiratory epithelium club (Clara) cells was observed at weeks 2 and 5 in some animals at 150 and 200 ppm. No increase was observed at week 13. No such effects were observed at 100 ppm. Cell proliferation in nasal tissue was not investigated.

There are also many additional repeated exposure inhalation toxicity studies reported for styrene in mice with primary focus on liver and lung effects. Morgan et al. (1993a) studied groups of B6C3F1 mice that received exposures up to 500 ppm styrene for 14 days. Deaths were observed in significant numbers of the animals that inhaled 250 and 500 ppm in the first couple of days and necropsy of these animals found severe liver changes including hepatocyte necrosis. Surviving animals also showed liver lesions that included pigmentation, focal necrosis and inflammation. Morgan et al. (1993b) further studied hepatotoxicity in different strains of mice ((DBA/2, B6C3F_1, C57BL/6 and Swiss strains) administered styrene at up to 500 ppm for 4 days. The results demonstrated that the DBA/2 strain of mouse was much less susceptible to hepatotoxicity caused by styrene than the other strains tested. The author's continued investigations in mice strains (Morgan et al., 1995) found that B6C3F1 mice mice were more susceptible to styrene-induced hepatoxicity than Swiss mice, and that female mice of this strain are more sensitive than the males. The authors speculated that the possible reason for these differences (intra-species and between sexes) is probably a result of different rates of metabolism of styrene to styrene oxide, as indicated by differences in GSH depletion rates.

The mouse respiratory tract toxicity related to styrene exposure has been thoroughly studied to support understanding the lung tumor findings in the chronic mouse study. An overview of the many mice respiratory tract studies is presented in Cruzan et al. (2002). Several of the key studies are detailed below.

Foster (1999) investigated the effects of styrene exposure on the nasal tissue of groups of 10 male mice that were administered concentrations of 0, 40 or 160 ppm for 6 hours/day for 3 days (Foster, 1999). Additional groups of males (10/group) were administered orally 200 mg/kg of the cytochrome P450 2F2 inhibitor 5-phenyl-1-pentyne (5-P-1-P) prior to styrene exposure. The study found no effects on body weights or macroscopic necropsy findings. There were no treatment related microscopic findings observed in any of the control animals (including those administered 5-P-1-P but not styrene) or any of those exposed to 40 ppm styrene (including those administered 5-P-1-P) or in animals administered 5-P-1-P and exposed to 160 ppm styrene. In animals exposed to 160 ppm styrene alone, localized atrophy of the olfactory epithelium in the dorsal regions of the nasal passages and focally decreased numbers of Bowman's glands in these regions was observed. No changes in other parts of the nasal passages were apparent. This study demonstrates a necessity for cytochrome P450-catalysed metabolism of styrene in the mouse respiratory tract before toxicity is expressed.

Green (2001) studied the effects of styrene exposure on the lungs of groups of male and female CD1 mice (5/sex/group) exposed to styrene concentrations of 0, 40 or 160 ppm, for 6 hours/day, 5 days/week for 2 weeks. No treatment related macroscopic findings were observed in any of the exposed mice. Following repeated exposure to styrene (both concentrations) treatment related focal loss of apical cytoplasm of on ciliated cells, principally in the terminal bronchioles, was observed. The magnitude of this finding varied between individual animals, with females appearing to be slightly more

sensitive than males. Crowding of non-ciliated cells throughout the bronchiolar tree was observed. There was no obvious dose related dependent increase in the cell labelling indices of the terminal bronchioles of animals, with the largest increase (3-4 fold) being reported in animals exposed to 160 ppm. This author extended his work in a study of groups of male CD1 mice (5/sex/group) exposed to styrene concentrations of 0, 40 or 160 ppm for 6 hours/day for 4 days (Green, 2001). In a second part to this study, as with that of Foster et al. (1999), additional groups of 10 animals/sex were administered orally 0 or 200 mg/kg of the cytochrome P450 inhibitor 5-P-1-P. On microscopic analysis, evidence of necrosis and loss of cells from the large bronchioles (thought to be club (Clara) cells by the authors) was observed in animals examined after only one exposure to 40 ppm. At later time points (after 2 and 4 exposures), changes in the appearance of the club (Clara) cells and of their organisation within the epithelium remained prominent. No information was given on histopathological findings with 160 ppm. BrdU studies demonstrated a statistically significant increase in pulmonary cell replication in the epithelium of the terminal and large bronchioles after 3 and 4 days of exposure to both 40 and 160 ppm styrene. There was no evidence of increased cell replication in the alveolar region. Pre-treatment with 5-P-1-P prevented the styrene induced increases in bronchiolar epithelium cell replication, indicating the importance of P450-catalysed metabolism of styrene to styrene oxide in the process by which styrene affects the respiratory epithelium in mice.

In a study specifically designed to investigate the effects of styrene exposure on the lungs, groups of female CD-1 mice (35/group) were exposed whole body to styrene concentrations of 0, 40 or 160 ppm for 6 hours/day, for 1 to 5 days or for 4 weeks (5 days/week) (Gamer et al., 2004). No consistent concentration- and time- related changes were observed in enzyme activity (catalase, superoxide dismutase, GSH reductase and GSH peroxidase) in the lung homogenates. The concentration of 8-OHdG was lowered in all the exposed groups except for the 160 ppm animals after 20 exposures. Statistically significant increases in lung lavage alkaline phosphatase (AP) were noted in the 160 ppm group at all exposure durations and in lung lavage LDH levels after 1 and 5 exposures. In animals at 160 ppm only, increases in malondialdehyde levels and in γ-glutamyl transferase levels were also reported and the glutathione concentration was significantly decreased in the homogenates. The serum levels of CC16 were significantly decreased in animals at 40 and 160 ppm; the effect was most pronounced after 1 exposure. Similarly, a marked decrease in CC16 protein in lavage fluid was observed at the same concentrations following 1 and 5 exposures. Histopathological examination of the lungs showed epithelial desquamation, ballooning and vacuolation in the terminal bronchioles, large and medium airways in animals at 160 ppm after 1 exposure. Cellular crowding, expressed as an irregular epithelial lining (which is indicative of very early hyperplasia), and a reduction of apical blebs and secretory granules in club (Clara) cells were seen at the same concentration (i.e., 160 ppm) after 5 and 20 exposures. Electron microscopy of lung tissue further revealed degenerative lesions (vacuolar cell degeneration and cell necrosis) or slightly different structures (absence of electron dense oval granules) in the Clara cells of the 160 ppm animals. The results of this study suggest that club (Clara) cell destruction may be the underlying mechanism for the pneumotoxic effects seen in mice exposed to

styrene. Even though, slight glutathione depletion was observed at 160 ppm after 20 exposures, the other parameters did not indicate evidence of oxidative stress. Malondialdehyde (an indicator of lipid peroxidation) was only slightly elevated after 1 exposure at 160 ppm but decreased after 20 exposures at both 40 and 160 ppm. This lack of consistency in the observations for the oxidative stress parameters did not support previous conclusions about the role of oxidative stress in styrene-induced lung damage in mice. However, club (Clara) cell toxicity was indicated by marked decreases in CC16 protein concentrations in both lavage fluid and blood serum, and by the microscopic observation of degenerative lesions. The irregular epithelial lining observed after 5 or 20 exposures was considered to represent regenerative hyperplasia. Club (Clara) cell toxicity and (regenerative) cell proliferation respectively were also indicated by increased γ-glutamyl transferase and AP activities noted at 40 ppm after 5 or 20 exposures and at 160 ppm after 1, 5 or 20 exposures. Overall, the findings of this study suggest that early biochemical changes in club (Clara) cells (observed from 40 ppm), sustained cell damage and regenerative cell proliferation may be the MOA for the carcinogenic effect of styrene in mice.

Neurotoxicity, other than ototoxicity, has been studied for styrene in a number of animal studies. In a study of neurotoxicity parameters only, groups of 8 male Wister-derived rats were exposed to 0, 350, 700 or 1400 ppm styrene for 16 hours/day, 5 days/week for 18 weeks (Kulig, 1989) followed by a 6-week post exposure period. Automated assessment of coordinated hindlimb movement and tests for grip strength, spontaneous activity, peripheral nerve conduction time and visual discrimination performance learning test were carried out at 3-week intervals during exposure and the recovery period (animals were trained on the apparatus for each test prior to exposure). Hearing was not investigated. The results of the study indicate that at exposure concentrations of up to 1400 ppm for 18 weeks, styrene did not express significant neurotoxicity; there was a finding of delayed visual learning observed in animals exposed to 1400 ppm for the first week of exposure only (at later times, the 1400 ppm group was comparable with controls) that may be indicative of a transient reversible CNS depressive effect.

The study by Albee et al. (1992) was included in the draft documentation but only for ototoxicity findings. This 13-week rat study examined groups of 14 male Fischer 344 rats that were exposed to 0, 50, 200 or 800 ppm styrene for 6 hours/day, 5 days/week also in a functional observational battery (including observations of salivation, abnormal movements or behavior) and hind limb grip strength performance test were conducted monthly. Evoked potential tests (12 rats per group) were included after 13 weeks and neuropathology at terminal necropsy was performed on 8 rats per group after electrophysiological tests were carried out. This study found no exposure-related effects on mortality, bodyweight, functional observations, or grip strength and no treatment-related alterations in histopathological lesions in nerve tissues or limb muscles, other than in the auditory system.

Yamamoto et al. (1997) (also reported in Teramoto et al. 1993) studied the effects of styrene on nerve conduction velocity in rats. Groups of 8 Wistar rats were exposed whole-body to airborne

concentrations of 0, 200 or 2000 ppm styrene for 8 hours/day, 5 days/week for 32 weeks. Nerve-conduction velocity of the tail nerve (using 3 sampling points between the base and end of tail) was assessed every 2 weeks from age 8 weeks (i.e. from the start of the experiment for older rats and after 4 weeks of exposure for young rats) until 16 weeks after the end of exposure. The following measurements were made at each of these times: motor nerve conduction velocity (MCV), distal latency (DL) and sensory nerve conduction velocity (SCV); the latter was determined for distal, proximal and whole tail areas. No differences between treated and controls groups were observed in either MCV or DL at any time. Slight differences in distal, proximal and whole-body SCV between the 200 ppm group and controls were occasionally observed (i.e., in 1 or 2 weeks out of the study) but these were not consistent and in some cases the values were slightly lower and in others, slightly higher. In contrast, although not seen at all weeks, in general the SCV of all 3 types were lower at 2000 ppm compared to controls over most of the exposure period, and in some cases after, in both ages of animals. The data were presented graphically but it would appear that the reductions in SCV seen at 2000 ppm were by around 5-10% compared to controls. Overall, in this study no effects were seen on tail nerve conduction velocity after exposure of rats to 200 ppm styrene with slight but consistent reductions in SCV at 2000 ppm.

In a similar study, exposure of rats to 300 ppm styrene for 6 hours/day, 5 days/week for 11 weeks resulted in an increase in tail motor nerve conduction velocity after 6 weeks, but no difference between control and exposed groups at 8 and 11 weeks (Seppalainen et al., 1978). This result is in contrast to the findings of the study summarized above. No other parameters were measured, and the experiment was only reported in brief.

Fumiko (1988) investigated the effects of styrene on operant behavior in groups of 6-7 (13 for controls) male JCL:SD rats that were exposed whole-body to 0, 50, 150, 500 or 1000 ppm for 4 hours/day, 5 days/week for 3 weeks. The Fixed Ratio Food Reinforcement schedule 30 (FR30) was essentially unaffected compared to control at 50 or 150 ppm (a small non-statistically significant reduction in the number of lever presses was observed). The number of lever presses was reduced compared to controls at 500 (by 20%) and 1000 ppm (by ~35-50%). However, there was recovery during each 2-week exposure-free period and after the end of the exposures, suggesting that the effects may reflect an acute response immediately following exposure. For Low Response Ratio Differential Reinforcement Schedule 20 (DRL20), data were only presented for 50 and 1000 ppm. No difference in the number of lever presses, food acquired for the ratio of the two was observed between the 50 ppm and control groups. At 1000 ppm the number of lever presses was increased, and food acquisition and ratio were reduced compared to controls. These differences were observed during the first half-week and the end of the exposure period but thereafter showed a trend to recovery and from the second week returned to pre-exposure levels. This study demonstrated that exposure to styrene at 500 ppm and above affected the operant behavior of rats, but no significant effects were seen at 50 or 150 ppm.

Several studies have examined the effects of styrene inhalation on brain biochemistry in rats. Mutti et al. (1984) studied groups of 8 male rats that were exposed to a control atmosphere or styrene for 12 hours/day. The control group was exposed for 7 days and styrene-exposed groups inhaled 750 or 1500 ppm for 3 days or 1500 ppm for 7 days. Additional animals (20/group) were included for the turnover experiment. Eight hours after the end of exposure, animals were sacrificed, and brains were removed immediately and dissected to collect tissue from the hypothalamus, median eminence (tubero-infundibular), striatum, cerebellum, hippocampus, and cortex. A variety of nervous system substances were assessed including catecholamine, homovanillic acid, dopamine and norepinephrine. Relative to controls, there was a marked dose-dependent decrease in dopamine in the striatum of animals exposed to 750 and 1500 ppm styrene and in tubero-infundibular areas at 1500 ppm, accompanied by an increase in homovanillic acid in the same regions. Norepinephrine levels were not altered. The dopamine turnover rates were similar in control and exposed groups, suggesting that no increase in the activity of monoamine oxidase (MAO) could be detected in this study.

The effects of styrene inhalation on brain MAO-A and B activities was investigated by Coccini et al. (1999). In this study groups of 7-8 male Sprague Dawley rats were exposed to either 50 ppm styrene for 6 hours/day, 5 days/week for 13 weeks or 300 ppm for 6 hours/day, 5 days/week for 4 weeks; controls were sham-exposed under similar conditions. Further groups of 4 or 5 rats received daily i.p. injections of 100 or 400 mg/kg bw/day styrene for 14 days, controls receiving corn oil. The rats were killed immediately after the last exposure, the brain dissected into the cerebral cortex, cerebellum striatum, hippocampus, brainstem and remaining brain. MAO-A and B activities were then determined in these brain regions. Inhalation exposure to both 50 and 300 ppm was stated to induce signs at lethargy during exposure but no changes in body weight, brain weight or brain protein content were observed. MAO-A activity was unaffected by treatment with styrene. MAO-B activity was reduced across all brain regions by exposure to both 50 ppm (38-50% across all regions studied) and 300 ppm (20-34% reductions across the regions). Following i.p. dosing, MAO-A activity was unchanged and MAO-B activity was only reduced statistically significantly in the brainstem (by about 45% for both doses). No effects were seen on either MAO-A or B activities in the studies performed in vitro. Overall, changes were seen in MAO-B activities in this study, but their toxicological significance is unknown.

Another inhalation study was designed to assess potential effects on behavior and changes to the brain following exposure of rats to styrene (Savolainen et al., 1980). Groups of 26 male Wistar rats were exposed to 0 or 300 ppm styrene for 6 hours/day, 5 days/week for 4-17 weeks. Cerebral samples taken were used to isolate glial cells. The glial glutathione (GSH) concentration and activity of acid proteinase and NADPH-diaphorase were determined. Brain samples were taken from the animals that had been withdrawn from exposure at 8 weeks and RNA content and activity of acid proteinase and NADPH-diaphorase analysed. Behavior was analysed in an open field situation after 4, 9 and 13 weeks of exposure. Body weight was unchanged relative to the control group. Generally, the analyses indicated no consistent pattern of differences in styrene-exposed animals when compared with the control group.

There were also no significant changes in behavioral parameters in styrene-exposed animals. These authors earlier studied the effects of styrene on brain proteins and brain and serum enzyme activities in rats exposed by inhalation (Savolainen and Pfaffli, 1977). Groups of 40 adult male Wistar rats were exposed to either 0 or 300 ppm styrene by whole body-exposure for 6 hours/day, 5 days/week for between 2 and 11 weeks. Brain, spinal cord, blood and perirenal fat were taken. The right hemisphere of the brain and perirenal fat were assessed for the styrene content. The left hemisphere of the brain was assessed for protein and RNA content and for acid proteinase (AP), acetyl cholinesterase (ACh) and creatine kinase (CK) activities. A water-soluble brain fraction and spinal cord axonal fraction were analysed for protein profile by polyacrylamide gel electrophoresis (PAGEP). Serum non-specific cholinesterase and CK activities were also measured. Animals were stated to be "somnolent" during exposure in the early stages of the study with this effect "levelling out" (it is unclear whether this meant signs were less prominent or were seen to be at the same degree) towards the end of the experiment. No other clinical signs of altered neurological function were observed. Styrene levels in brain and perirenal fat increased during the first four weeks of exposure but subsequently dropped to between one-half (fat) and one-fifth (brain) of these levels by the 11th week of exposure. Occasional small (5-10%) statistically significant differences between exposed and control groups were seen in brain protein and RNA content, and AP, ACh and CK activities. These were seen generally towards the end of the exposure period when brain styrene levels were lower, were inconsistent (i.e. would be different from concurrent controls at one time point but not the next) and generally fell within the range of control values seen throughout the whole experiment. Serum non-specific cholinesterase activity was lower by 25-30% after 2-4 weeks of exposure to styrene but was subsequently similar in both exposed and control groups. No changes in protein profile were observed in PAGEP analysis of the water-soluble brain fraction. The expression of one protein near the cathode in the axonal spinal cord preparation was reduced from week 9 of exposure but major protein bands remained unchanged. Overall, no consistent effects were seen in the brain biochemistry endpoints measured in this study in rats exposed to 300 ppm styrene for up to 11 weeks. An early reduction in serum non-specific cholinesterase activity was seen but subsequent adaptation appears to have accommodated for this reduction.

The draft documentation did include the study performed by Rosengren and Haglid, (1989) in which brain protein was examined in rats exposed continuously to styrene for 90 days. Groups of 8 (exposed) or 16 (control) Sprague Dawley rats inhaled 0, 90, 320 ppm. Animals were then kept for 4 months free of exposure, sacrificed, the brains removed, and various regions of the brain isolated by dissection. Tissue samples were homogenised and the astrological markers S-100 and glial fibrillary acidic proteins (GFAP) measured. Relative to the controls, GFAP concentrations in the 320 ppm group were significantly increased in the sensory motor cortex and in the hippocampus but the toxicological significance of this change is unknown.

The draft documentation provides a limited review of the available animal auditory system studies. As effects to this system are among the most important findings in animals as well as in humans, a more thorough review of the available information is warranted in the ACGIH documentation.

The early auditory system study by Albee et al. (1992) is included in the documentation, although it would be useful to note what changes were identified, specifically lesions were seen in the organ of Corti as indicated by the loss of two outer hair cells per cross section from the upper basal turn, and the occasional absence of an outer hair cell from the lower middle turn and elevations in auditory brainstem response (ABR) thresholds by approximately 40 dB at 16, 25 and 30 kHz. In addition to the Albee study, several other early investigations on styrene ototoxicity with similar findings were reported by Pryor et al. (1987), Yano et al. (1992), Rebert et al. (1993), Makitie (1997), Loquet et al. (1999).

In addition to the Lataye et al. (2000) study reported in the draft documentation, this investigator performed several additional studies that provide additional useful perspectives on animal ototoxicity. A further rat and guinea pig study is reported by Lataye et al. (2003). The rat study examined groups of 5-6 male Long-Evans rats exposed to 0 or 1000 ppm styrene 6 hours/day for 5 days. At regular intervals during the exposure period urine samples were taken for measurement of urinary metabolites of styrene. Cochlear function was tested before, 20 minutes after the end of the 5 days of exposure, and 2 and 4 weeks post-exposure using cubic distortion product otoacoustic emissions (DPOAE) recorded from the external ear canal in anaesthetized animals. A satellite group of animals was anesthetized immediately after the last styrene exposure, killed and subjected to blood sampling for analysis of styrene levels. An additional group of 5-6 male styrene-exposed rats was sacrificed 4 weeks after the last exposure for removal and analysis of the aural bones and tissues. In styrene-exposed rats, DPOAE amplitudes were statistically-significantly decreased at 2 and 4 weeks post-exposure, although the magnitude of the effect was no greater at 4 weeks than at 2. Histologically, hair cell loss was observed. Blood styrene levels on completion of 5 days of styrene exposure were 22.8 µg/g. Urinary hippuric acid levels at 3 and 4 days post-exposure were around 4 g/g creatinine. The companion guinea pig study examined groups of 6 males exposed also to 0 or 1000 ppm styrene for 6 hours/day for 5 days and using a similar study design. This study found no significant changes in the cochlear function between styrene-exposed animals and the control animals. Blood styrene levels on completion of 5 days of styrene exposure were 4.9 µg/g. Urinary hippuric acid levels at 3 and 4 days post-exposure were around 8 g/g creatinine. The differences in ototoxicity in guinea pigs receiving the same concentration as rats may be due to marked differences in OHC lateral membrane morphology, tissue glutathione content, and styrene uptake, pharmacokinetics, and metabolism between these two rodent species (Cappaert et al., 2002, Lataye et al., 2003). Humans resemble rats more than guinea pigs in terms of both the pharmacokinetics and metabolism of styrene, and therefore should be considered similar in sensitivity (Morata and Campo, 2002; Campo and Maguin, 2007). The authors subsequently evaluated rats for age differences in ototoxicity response to styrene (Lataye et al., 2004) and found that styrene-induced hearing loss was greater in 3-month old Long-Evans rats compared to 4-month old rats exposed to 700

ppm styrene 6 hours/day, 5 days/week for 4 weeks. These data suggest that younger adult rats may be more susceptible to styrene ototoxicity compared to older adult animals.

Lataye et al. (2005) continued their investigations with examination of rat activity levels and ototoxicity. The authors compared styrene-induced ototoxicity in active rats and sedentary/ordinary rats and investigated the combined effects of noise and styrene on hearing. Groups of 8 male Long-Evans rats were exposed under sedentary/ordinary conditions to 0, 500, 650, 850 or 1000 ppm styrene for 6 hours/day, 5 days/week for 4 weeks. Additional groups of 4 male Long-Evans rats, forced to run (for 2 minutes every 3 minutes) in a special wheel during the exposure, were exposed whole body to 0, 300, 400, 500 or 600 ppm styrene for the same exposure duration. Furthermore, groups of 6 active male Long-Evans rats were exposed to 0, 400 ppm styrene alone, noise alone (an octave band noise centered at 8 kHz) or styrene (400 ppm) and noise combined. Audiometric testing was performed prior to styrene exposure and at six weeks after the exposure period. Following audiometry testing histopathological and scanning electron microscopic (SEM) examination was used to assess damage to the inner and outer hair cells (IHC and OHC) of the organ of Corti. In the sedentary animals, hearing loss and OHC cell damage were observed at 650 ppm and above, but not at 500 ppm. In the active rats, functional and histological damage was observed at 400 ppm and above, but not at 300 ppm. These results suggest that the ototoxic potency of styrene exposure depends on the physical activity of the animals as this is related to the ventilation rate and, in turn, to the uptake of the chemical via the lungs. Overall, based on these findings, NOAEC values of 500 and 300 ppm can be identified in sedentary/ordinary rats and active rats, respectively. In the experiment investigating the combined effects of noise and styrene, noise alone or styrene alone were found without effect; however, both hearing loss and OHC cell damage were observed in the animals exposed to noise and styrene combined. These results suggest that styrene-induced ototoxicity can be potentiated by exposure to noise.

The other identified ototoxicity studies in the draft documentation are reasonably well reported. Some additional perspectives of the Chen and Henderson (2009) results are that this study shows that styrene leads to ototoxicity not only after inhalation but also after oral dosing. In addition, a styrene/noise synergism was demonstrated even at exposures that lead to no or only marginal effects on the parameters investigated by either noise or styrene alone.

The draft documentation would also benefit from an overall summary discussion of the animal ototoxicity data. This information provides clear evidence of ototoxicity (both functional and histological in sedentary/ordinary rats repeatedly exposed to styrene by inhalation at concentrations of 600 ppm and above. In 3 different studies, no such effects were seen at 200 ppm (13 weeks), 300 ppm or 500 ppm, although in the latter 2 cases the studies were only of 4 weeks' duration and a small hair cell loss in row 3 of the cochlea was still observed at 500 ppm. One study in active rats exposed to styrene for 4 weeks showed that styrene-induced ototoxicity tends to occur at lower exposure concentrations than those at which ototoxicity is observed if sedentary/ordinary rats are employed. This is considered to be

due to the increased styrene uptake, which is the consequence of the increased ventilation rate and, in turn, of the increased physical activity. In this study ototoxic effects were seen at 400 ppm and above, but not at 300 ppm apart from a small hair cell loss in row 3 of the cochlea. Comparative studies using rats and guinea pigs exposed to 1000 ppm for 5 days indicate an obvious species-difference, as similar findings were not observed in guinea pigs (Lataye et al., 2003). Ototoxicity has been seen at similar exposure levels with other aromatic organic solvents, such as toluene and xylene. The underlying toxicological mechanism has not been clearly elucidated. This effect should be regarded as of potential relevance to human health. The histological damage consists in the destruction of the outer hair cells (OHC; especially of row 3) of the cochlea. These changes are accompanied by an elevation of the hearing thresholds in the mid-frequency range (10-20 kHz). The destruction of the hair cells is irreversible and occurs at slightly lower exposure concentrations than those producing the audiometric hearing threshold shifts. Mechanistic investigations indicate that styrene reaches the sensory hair cells of the cochlea via the blood stream and that styrene itself and/or its metabolites cause a serious disturbance of the membranous organization of these target cells. A number of studies suggest that styrene-induced ototoxicity can be potentiated by exposure to noise. The available evidence also shows that ototoxicity appears after relatively short exposures (1 week) and that continued treatment (4 weeks up to 19 months) does not enhance the intensity of the ototoxic response. Overall, the available inhalation repeated dose toxicity studies have identified ototoxicity as the most sensitive and relevant effect of styrene repeated inhalation exposure with NOAEC values of 500 ppm (2165 mg/m^3) and 300 ppm (1300 mg/m^3) for 4 weeks in sedentary/ordinary and active rats respectively.

The temporal aspect of the auditory effects is also an important discussion point. The damage once present is permanent but there is strong evidence that the maximum of hearing impairment is already reached after one to a few weeks of exposure and that ototoxicity does not increase with prolongation of the exposure period. In the Campo et al. (2001) study, the exposed rats to 1000 ppm styrene (6 hours/day, 5 days/week) for 1, 2, 3, or 4 consecutive weeks exhibited permanent hearing loss using electrophysiological examinations 6 weeks after the end of each exposure period. An exposure duration of 1 week was enough to obtain the maximal hearing deficit without any further increase by prolongation of exposure. This was confirmed by histopathology of the cochlea carried out 6 weeks post-exposure: hair cell loss was virtually the same after an exposure duration of 1 or 3 weeks. The findings of Albee et al. (1992) and Mäkitie et al. (2003) provide indirect evidence that maximal hearing impairment is already reached after a few weeks of exposure. Albee et al. (1992) exposed male Fisher-344 rats (6 hours/day, 5 days/week) to 0, 50, 200 and 800 ppm styrene over 13 weeks and examined the auditory system by electrophysiology and histopathology of the cochlea a few days after the end of exposure. Clear effects were noted at an exposure of 800 ppm and the NOAEC was 200 ppm. This compares well to the NOAEC of 300 ppm determined in male Wistar rats by Mäkitie et al. (2003) after an exposure to 0, 100, 300 and 600 ppm (12 hours/day, 5 days/week) over only 4 weeks. Therefore, an exacerbation of hearing deficits is not to be expected by prolongation of exposure beyond a few weeks to life time.

Not reported in the draft documentation is the recent mechanistic study that provides further definition of cochlear responses to styrene treatment. Fetoni et al. (2016) evaluated the efficacy of the water-soluble coenzyme Q_{10} analog Q_{ter}® in protecting against ototoxicity (evaluated by auditory brainstem response [ABR] threshold and amplitude of DPOAEs) and combating oxidative stress (as evidenced by production of superoxide anion and lipid peroxidation) in both OHCs and Deiters' cells from rats administered 400 mg styrene/kg via gavage in olive oil for one, two, or three weeks. As reported by others, styrene at this dose impacted hearing most strongly at mid-frequencies and caused preferential damage to Deiters' cells and OHCs in the cochlear middle turn, with a gradient of increasingly severe injury from the third to first OHC rows. There was a much smaller impact on spiral ganglion neurons (SGNs) in the middle turn. Styrene increased superoxide levels in the organ of Corti, SGNs, and the *stria vascularis*, and increased 8-isoprostane expression (biomarker of lipid peroxidation) primarily in the *stria vascularis* and SGNs, with greater involvement of Deiters' cells than OHCs. Q_{ter}® ameliorated the styrene-induced decrease in DPOAE amplitude (especially at the later time points) and ABR threshold shift. Loss of OHCs and SGNs was reduced by Q_{ter}®, but the treatment notably did not save Deiters' cells. Different patterns were also evident in the two indicators of oxidative stress. Q_{ter}® treatment significantly decreased superoxide production, and also stimulated superoxide dismutase expression in OHCs but not in Deiters' cells. Whereas Q_{ter}® was able to ameliorate lipid peroxidation in OHCs, Deiters' cells were not protected. These results provide support for a free radical-mediated mode of toxicity for styrene in the inner ear but suggest a different mechanism of styrene toxicity and/or greater sensitivity of the apparent critical target, Deiters' cells.

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Chronic/Carcinogenicity

The chronic/carcinogenicity section would benefit from an update to include a new mouse 2-year inhalation study and MOA information that has been developed to understand the styrene induced mouse lung tumors. There are also some inaccuracies in the data reported in the draft documentation section.

The cited rat study by Jersey et al. (1978) should note correctly the high exposure level used in this study. Specifically, 1200 ppm was the high exposure level at study start but after 2 months this level was reduced to 1000 ppm (4.26 mg/L) for the duration of the study. Therefore, the tested high exposure concentration should be expressed as 1200/1000 ppm.

The cited studies by Ponomarkov and Tomatis (1978) are reported in the draft documentation as daily dosages, however, these were weekly dosages.

The draft documentation includes chronic/carcinogenicity information on styrene oxide, however, this data has questionable value to this endpoint assessment for styrene. The routes of exposure used in the styrene oxide studies (topical, oral) do not correlate with how styrene oxide is produced following

styrene exposure and hence the findings of these studies do not appear to be relevant to the overall styrene carcinogenicity assessment. Styrene oxide is also not discussed consistently throughout the endpoint sections. Therefore, this styrene oxide content could be deleted from the documentation.

In addition to identifying the existing animal carcinogenicity studies, there is a large body of information on mechanistic studies that have been performed that inform on the mouse lung tumors associated with styrene exposure. One of the many published studies was cited in the ADME section of the draft ACGIH documentation but there are several recent publications regarding a new chronic study and related genomic analyses that are significant to this endpoint and hence warrant inclusion in the documentation (Cruzan et al., 2017, Andersen et al., 2017, 2018).

The Cruzan et al. (2017) publication reports on the new 2-year inhalation study that studied CD-1, C57BL/6 (WT), CYP2F2(-/-) (KO), and CYP2F2(-/-) (CYP2F1, 2B6, 2A13-transgene) (TG; humanized) mice following exposure for up to 104 weeks to 0- or 120-ppm styrene vapor. Groups of mice were sacrificed at 1, 26, 52, and 78 weeks and 104 weeks of exposure. The study found cytotoxicity present in the terminal bronchioles of some CD-1 and WT exposed mice but not in KO or TG mice. Hyperplasia in the terminal bronchioles was present in CD-1 and WT exposed mice but not in KO or TG mice. Increased cell proliferation, measured by KI-67 staining, occurred in CD-1 and WT exposed mice that inhaled styrene for 1 week, but not after 26, 52, or 78 weeks, nor in KO or TG mice. Styrene was found to increase the incidence of bronchioloalveolar adenomas and carcinomas in CD-1 mice, whereas no increase in lung tumors was found in WT despite clear evidence of lung toxicity, or, KO or TG mice. The absence of preneoplastic lesions and tumorigenicity in KO and TG mice in this study indicates that mouse-specific CYP2F2 metabolism is responsible for both the short-term and chronic toxicity and tumorigenicity of styrene, and activation of styrene by CYP2F2 is a rodent MOA that is neither quantitatively or qualitatively relevant to humans.

In parallel with the new 2-year inhalation mouse study, Andersen and colleagues (2017, 2018) studied gene expression in the lungs of the various mouse strains over different exposure periods. The first Andersen study (2017) examined changes up through 26 weeks of exposure. This study found that after 1-day of exposures at 1, 5, 10, 20, 40 and 120 ppm there were significant increases in differentially expressed genes (DEGs) only in parental strain lungs where there was already an increase in DEGs at 5 ppm and then many thousands of DEGs by 120 ppm. The enrichment findings for 1-day and 1-week exposures included cell cycle, mitotic M-M/G1 phases, DNA-synthesis and metabolism of lipids and lipoproteins pathways. Over time, the numbers of DEGs decreased steadily with no DEGs meeting both statistical significance and foldchange criteria at 26 weeks. The results at 4 and 26 weeks indicated that some key transcription factors (TFs) - Nr1d1, Nr1d2, Dbp, Tef, Hlf, Per3, Per2 and Bhlhe40 - were upregulated (|FC| > 1.5), while others - Npas, Arntl, Nfil3, Nr4a1, Nr4a2, and Nr4a3 - were down-regulated but at all times, consistent changes in gene expression only occurred in the parental strain. These results support as the molecular initiating events (MIE) for styrene of direct mitogenicity from

mouse-specific CYP2F2-mediated metabolites activating Nr4a signaling. Longer-term modulating factors (MFs) include down-regulation of Nr4a genes and shifts in both circadian clock TFs and other TFs, linking circadian clock to cellular metabolism. This study found no gene expression changes indicative of cytotoxicity or activation of p53-mediated DNA-damage pathways.

Andersen continued to study the gene expression during the full course of the new 2-year inhalation mouse study and prepared an overall review of their findings (Andersen et al., 2018). This study expanded the findings to include gene expression data at 52, 78 and 104 weeks. Their study found that very few exposure-related responses occurred at any time in KO or TG mice. For short term exposures, male mice of the strains expressing Cyp2f2 had altered expression of thousands of genes and the pathways enriched were similar (including cell cycle, mitosis, DNA replication/repair, lipid/cholesterol metabolism, and immune response) but with very different time courses. The WT mice responded within a single day while the CD-1 mice response required several days of exposure. Benchmark doses for pathway activation were found to be lower by a factor of two in CD-1 mice. At all later times/ages, the changes in gene expression were greatly reduced. With the longer times, exposure-related differentially expressed genes were enriched for the broad category of metabolism – biological oxidations in WT and metabolism of lipids and lipoproteins in CD-1 mice. Changes in circadian cycle transcription factor (TF) gene expression over time were also more persistent for the CD-1 mice. These gene expression results indicate a non-genotoxic, rodent and rodent strain specific MOA related to activation of nuclear receptor signaling with attendant cell proliferation, changes in cellular metabolism and activation of immune response pathways followed by diminished response and adaptation with continued exposure. The differences in lung tumor susceptibility between the C57BL/6 mice and the CD-1, a more tumor-prone strain, appear to relate to the presence of the Pas1 loci, differential basal and styrene-dependent Cyp expression and both greater immune system/inflammatory pathway activation and more persistent changes in key circadian TFs in CD-1 mice.

The above new mouse 2-year inhalation study and gene expression findings are significantly important in understanding how these animal tumors develop in the mouse and their human health relevance. Taken together this information does not warrant ACGIH's cancer classification as A3 – Confirmed Animal Carcinogen with Unknown Relevance to Humans.

In addition, a thorough overview and discussion on the MOA of mouse lung tumors and evaluation of human relevance has been published (Cruzan et al., 2018). This review found that key events in the mouse lung tumor development are: metabolism of styrene by CYP2F2 in mouse lung club cells to ring-oxidized metabolites; changes in gene expression for metabolism of lipids and lipoproteins, cell cycle and mitotic M-M/G1 phases; cytotoxicity and mitogenesis in club cells; and progression to preneoplastic/neoplastic lesions in lung. It also discusses the lack of a role for styrene oxide that although it is a common genotoxic styrene metabolite in in vitro studies, the data clearly demonstrate that styrene oxide is not the proximate toxicant and that styrene does not induce a genotoxic MOA.

Based on complete attenuation of styrene short-term and chronic toxicity in CYP2F2 knockout mice and similar attenuation in CYP2F1 (humanized) transgenic mice, limited metabolism of styrene in human lung by CYP2F1, 2+ orders of magnitude lower styrene oxide levels in human lung compared to mouse lung, and lack of styrene-related increase in lung cancer in humans, there is no evidence to suggest that styrene presents a risk of cancer to humans.

The IARC 2002 Monograph on styrene is the latest published monograph. IARC reviewed styrene again in March 2018, and, according to a summary published in *Lancet Oncology*, found sufficient evidence in experimental animals (IARC Monographs Vol 121 Group, 2018). The reported findings would characterize the mouse lung tumor findings described in 2002 as "limited evidence" but now are considered "sufficient evidence" due in part to changes in the IARC preamble criteria for animal data assessment.

The McConell and Swenberg review of styrene is outdated for the present body of information on styrene carcinogenicity and hence could be removed from the documentation.

Andersen ME, Cruzan G, Black MB, Pendse SN, Dodd D, Bus JS, Sarang S, Banton MI, Waites R and McMullen PD. (2017). Assessing molecular initiating events (MIEs), Key events (KEs) and modulating factors (MFs) for styrene responses in mouse lungs using whole genome gene expression profiling following 1-day and multi-week exposures. Toxicol Appl Pharmacol. 335:28-40.

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IARC Monographs Vol 121 Group. (2018). Carcinogenicity of quinoline, styrene, and styrene-7,8-oxide. Lancet Oncol. Published Online April 18, 2018 http://dx.doi.org/10.1016/S1470-2045(18)30316-4

Reproductive/Developmental

Reproductive and developmental toxicity is a focus of concern for solvents generally and hence styrene has been well studied for these effects. The draft documentation does not detail all of the available studies, but as many have limitations, the studies that are included appear to be the key studies. In particular the Cruzan et al. (2005 a,b) studies that examined two generations of rats for fertility, developmental and developmental neurotoxicity were included, and these studies support a low concern for these endpoints.

The Luderer (2005) review's conclusion regarding prolactin and dopamine findings was further examined in a review performed to evaluate styrene and endocrine disruptor activity (Gelbke et al., 2015). This review broadly evaluated endocrine system endpoints and concluded that in vitro and in vivo screening studies, as well as non-guideline and guideline investigations in experimental animals indicate that styrene is not associated with (anti)estrogenic, (anti)androgenic, or thyroid-modulating activity or with an endocrine activity that may be relevant for the environment. Regarding the studies in exposed workers that suggested elevated prolactin levels, these have been further examined in a series of human and animal investigations and, while there is only one definitively known physiological function of prolactin, namely stimulation of milk production, there are many normal stress situations that may lead to elevations without any chemical exposure. The available animal studies on various aspects of dopamine, the prolactin-regulating neurotransmitter, in the central nervous system did not give mechanistic explanations on how styrene may affect prolactin levels. The authors concluded that overall a neuroendocrine disruption of prolactin regulation cannot be deduced from a large experimental database. The effects in workers were not consistently reproduced in experimental animals and the findings in humans represented acute reversible effects clearly below clinical and pathological levels. The authors determined that unspecific acute workplace-related stress is proposed as an alternative MOA for elevated prolactin levels in workers. ACGIH is encouraged to consider the Gelbke et al. (2015) review in the prolactin discussion in the draft documentation.

To support the statements comparing the Beliles et al. (1985) rat oral drinking water reproductive toxicity study to the Srivastava et al. (1989a) oral presumably gavage rat study, it would be useful to include the estimated daily oral dosages for the Beliles et al. (1985) drinking water concentrations. Specifically, the high drinking water concentration of 250 ppm was estimated by Luderer et al. (2005) to be approximately 18 mg/kg bw/day for males and 23 mg/kg bw/day for females. This dosage is significantly lower than the very high oral daily dosages of up to 400 mg/kg bw/day administered in the Srivastava et al. (1989a) study.

Genotoxicity

Although, the overall conclusions on the genotoxicity potential of styrene reflected in the draft documentation have not significantly changed since the data cited were published, there are more recently conducted studies and reviews published that would provide a useful update to the documentation. In addition to the cited review by Henderson and Speit (2005), reviews of styrene genotoxicity have been reported by: Scott and Preston (1994 a,b), Cohen et al. (2002), IARC (2002), Nestman et al. (2005), NTP CERHR (2006) and ATSDR (2010). When considering the extensive literature on the genotoxicity of styrene, it is important to remember the timeframe when studies were conducted. Study methods have evolved over time as the field of genetic toxicology has developed hence older studies now may be considered to have significant technical deficiencies that make these data difficult to interpret or uninterpretable.

Besides the prokaryotic organism studies cited in the draft documentation, styrene has also been evaluated in vitro in mammalian cell systems including Chinese hamster ovary and lymphocyte cells and rat and human blood or lymphocytes for changes in chromosomal aberrations, micronuclei or sister chromatid exchanges finding variable results (DeRaat et al., 1978; Linnainmaa et al., 1978, Matsuoka et al., 1979; Norppa et al., 1980, 1983; Norppa and Tursi, 1984; Pohlova et al., 1985; Jantunen et al., 1986; Chakrabarti et al., 1993; Lee and Norppa, 1995). A comet assay using freshly isolated murine hepatocytes (and in human lymphocytes) is also reported for styrene that found positive results at non-cytotoxic concentration (Fontaine et al., 2004). Co-incubation with the cytochrome P-450 inhibitor SKF-525A strongly attenuated the effect in the hepatocyte cultures. Strafella et al. (2009) investigated the effects of styrene on cell viability, cell proliferation, DNA damage and its repair and on colony formation in soft-agar (as an indication for carcinogenicity) in mesothelial and epithelial alveolar cell in vitro. Styrene inhibited cell proliferation and induced low cytotoxicity in mesothelial cells, but induced cell proliferation in alveolar cells. DNA damage was not induced in both cell types, but DNA repair was inhibited in mesothelial cells. No effect on colony formation was reported. There are also several in vitro studies that examined the possible impact of polymorphism in humans on genotoxicity. Bernardini et al. (2002) studied the induction of sister chromatid exchanges (SCE) by styrene in human lymphocyte cultures from donors with polymorphism of glutathione S-transferase (GSTM1 and GSTT1). High concentrations of styrene (1.5 mm) induced SCE in cultures of all donors and SCE induction was significantly higher in subjects lacking both GSTM1 and GSTT1. If only one of these enzymes was missing, the SCE induction was intermediate between those subjects having either both or none of these genes. The authors suggest that the concurrent lack of GSTM1 and GSTT1 may increase the genotoxic effect of styrene on human cells. Laffon et al. (2003) incubated styrene itself with mononuclear leukocytes of 30 human donors. An influence of polymorphism on genotoxicity as measured by the alkaline comet assay was suggested for the activating enzymes CYP1A1 and CYP2E1 and for the detoxifying GSTP1. Together, these in vitro data suggest that polymorphism of toxifying or detoxifying enzymes may modulate the genotoxicity of styrene.

As with the in vitro genotoxicity information, there are additional reported in vivo clastogenicity studies available for styrene beyond the few older studies cited in the draft documentation. These data add to the overall weight of evidence that there is no convincing evidence of styrene clastogenicity in experimental animals when the quality of the studies and the plausibility of the test results are considered. Equivocal results were obtained after exposure of rodents to high doses causing lethality (Simula and Priestly, 1992; Norppa, 1981). However, overall, negative results were obtained from in vivo chromosome aberration and micronucleus studies in the rat (Sinha et al., 1983; Simula and Priestly, 1992; Kligerman et al., 1993; Preston and Abernethy, 1993), hamster (Norppa et al., 1980) and mouse (Loprieno et al., 1978; Sbrana et al., 1983; Sharief et al., 1986; Kligerman et al., 1992; 1993; Engelhardt et al., 2003) following single or repeated exposures to styrene up to concentrations and/or doses causing systemic toxicity, via the inhalation (Norppa et al., 1980a; Sinha et al., 1983 Kligerman et al., 1992; 1993; Preston and Abernethy, 1993; Engelhardt et al., 2003), oral (Loprieno et al., 1978; Sbrana et

al., 1983) and intraperitoneal routes (Sharief et al., 1986; Simula and Priestly, 1992) in the tissues examined (bone marrow, peripheral lymphocytes, splenocytes and whole blood). Of note, the new OECD Test Guidelines for in vivo genetic toxicology studies consider the intraperitoneal route to be an irrelevant route of exposure. A micronucleus test in bone marrow cells of mice conforming to the current OECD guideline was clearly negative (Engelhardt et al., 2003). The general pattern of SCE results in wide range of tissues examined (lymphocytes, splenocytes, bone marrow, alveolar macrophages, regenerating liver cells) from both the rat (Simula and Priestly, 1992; Kligerman et al., 1993; Preston and Abernethy, 1993) and the mouse (Conner et al., 1979; 1980a; Sharief et al., 1986; Simula and Priestly, 1992; Kligerman et al., 1992; 1993) following inhalation (Conner et al., 1979; 1980a; Preston and Abernethy, 1993; Kligerman et al., 1992; 1993) or i.p. exposure (Sharief et al., 1986; Simula and Priestly, 1992) to styrene has been positive (Conner et al., 1979; 1980; Simula and Priestly, 1992; Kligerman et al., 1992; 1993). However, it is important to note that in most cases concomitant chromosome aberration and/or micronucleus assays involving the same animals and, in some cases, the same tissues were carried out and that negative results were obtained for these indicators of chromosome damage. Therefore, this clearly reduces the significance of the SCE findings in relation to mutagenicity. When rats were exposed to styrene by inhalation DNA strand breaks were not observed in the comet assay (Kligerman et al., 1993). Again, the significance of these findings is unclear, given the repeated failure of styrene to demonstrate mutagenic activity in standard clastogenicity assays. In contrast to the weakly positive findings in indicator tests detecting SCEs, DNA strand breaks and DNA adducts, the in vivo UDS test (is cited in the draft documentation) performed in accordance with international guidelines did not reveal a genotoxic effect of styrene in mouse liver (Clay, 2004). The most recent reported styrene clastogenicity study exposed rats to styrene (75, 300, 1000 ppm) or styrene-7,8-oxide (styrene oxide) by inhalation (25, 50, 75 ppm) 6 hours/day, 5 days/week over 4 weeks (Gate et al., 2012). Micronuclei in circulating reticulocytes using the very sensitive flow cytometry procedure and DNA strand breaks in leukocytes by the Comet assay were studied at the end of the 3rdand 20thdays of exposure. Blood was collected from the carotid artery to measure styrene oxide directly coming from the lung avoiding first-pass hepatic metabolism. After styrene oxide exposure the styrene oxide blood concentrations increased nearly linearly and 1000 ppm styrene led to blood styrene oxide concentrations between those after 25 and 50 ppm styrene oxide. Reticulocytes in blood were significantly decreased after exposure to styrene and styrene oxide and this effect was more pronounced after 3 days of exposure that after 20 days. Thus, the two compounds or their metabolites had reached the bone marrow. Neither styrene nor styrene oxide induced a significant increase of micronuclei frequency in reticulocytes or DNA strand breaks in white blood cells. However, in the presence of formamidopyridine DNA glycosylase (Fpg), an enzyme able to recognize and excise oxidized DNA bases, a significant increase of DNA damage was observed at the end of the 3rdday (but not after the 20thday) of treatment with styrene but not with styrene oxide. But no dose response relationship was observed at styrene exposures between 75 to 1000 ppm. According to the authors the results obtained with the Fpg-modified Comet assay may suggest oxidative stress in white blood cells after styrene exposure. In

summary, inhalation exposure to styrene and styrene oxide over up to 20 days did not lead to an increase in micronuclei or DNA strand breaks in blood reticulocytes of rats. At the dose levels used the bone marrow was reached. Positive results in the Comet assay using Fpg are difficult to interpret as these were obtained only with styrene on day 3 (but not on day 20 or with styrene oxide) and did not show a dose response relationship. Overall, considering those studies tested using standardized guidelines, there is no convincing evidence that styrene possesses significant mutagenic/clastogenic potential in vivo from the available data in experimental animals.

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Absorption, Distribution, Metabolism, and Excretion

The absorption, distribution, and excretion of styrene are reasonably well described in the draft documentation. The available information supports this substance to be well absorbed across the respiratory tract, widely distributed through the body with some partitioning into fat and eliminated relatively rapidly and primarily in the urine as metabolites. For dermal uptake, a guideline skin in vitro study is now available that supports the low skin penetration of styrene. This OECD- and GLP-compliant study examined skin membranes prepared from human samples and found for styrene liquid uptake to be approximately 2% of the applied dose (ECHA, 2003).

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The content of the draft documentation on styrene metabolism is significantly outdated. Numerous studies have been conducted in the last several decades to improve our understanding of styrene metabolism.

There is now a large body of information that importantly highlight the significant differences in the metabolism of styrene between species and between tissues. The side chain oxidation reaction to styrene oxide is quantitatively important but there is evidence that oxidation of the aromatic ring may be toxicologically important and species differences are notable with this pathway much more used in mice than in rats and in humans having only a very small role. A general observation from studies of human tissues is that, apart from the liver, very little styrene oxide is produced and these tissues have a greater capacity to hydrolyze styrene oxide with epoxide hydrolase (EH) than rodents. This difference is most pronounced in human nasal and lung tissues where production of styrene oxide is minimal or undetectable and is also associated with a greater capacity to hydrolyze styrene oxide by EH. The mouse lung and nasal tissues produce the greatest amount of styrene oxide among the species tested, and, in general, have less EH activity, suggesting that significantly high local concentrations of styrene oxide will be present in these tissues. It is also evident that other toxic metabolites, particularly 4-vinylphenol (4-VP) and its reactive downstream products, are produced to a far higher extent in mouse lung than in rat (14-79% of the mouse concentrations) or human lung (1.5-5% of the mouse concentrations).

A useful overview of styrene metabolism is provided in Cruzan et al. (2002) publication and the EU REACH dossier (ECHA). In recent years, there have been a number of studies on the influence of genetic polymorphisms of the metabolic enzymes on styrene metabolism.

Some of the many metabolism studies that have been performed on styrene in recent years are listed below:

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Wang Y. (1998). Effects of styrene exposure on middle latency auditory evoked potentials and glial cells in rat. Hokkaido J. Med. Sci. 73(2):157-170.

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Zhang F, Lowe ER, Rick DL, Qiu X, Leibold E, Cruzan G and Bartels MJ. (2011). In vitro metabolism, glutathione conjugation, and CYP isoform specificity of epoxidation of 4-vinylphenol. Xenobiotica, 41(1): 6-23.

The last study entry in this section, Cruzan et al. (2013) describes research that has been conducted on mouse lung tumor MOA. These studies have a metabolism link as metabolism plays a role in these tumors but the mouse response information may be better suited for the carcinogenicity section of the draft documentation. There is also more recent information on this line of research described above that warrants inclusion in the carcinogenicity section.

Human Studies

Genetic Effects

As with the animal and in vitro genetic effects information, there is an extensive literature on human genetic studies for styrene.

The draft documentation provides a limited review of the available styrene human genotoxicity information, although a number of the more recent studies have been included. In addition to the reports on chromosomal abnormalities, there are a few human studies that evaluated changes in gene mutations and, although these studies have limitations as do the clastogenicity studies, information on these studies should be included to more completely present the available information. Two mutation assays have been used in humans to study styrene, the Hprt gene mutation test and the Glycophorin A (GPS) assay. The studies that evaluated occupationally exposed subjects for increases in the frequency of Hprt mutants in relation to control individuals were reported by Vodicka et al. (1995, 1999, 2001) and Lambert et al. (1995). Two studies are reported that used the GPA gene to assess mutation induction (Compton-Quintana et al., 1993; Bigbee et al., 1996). None of these studies provides a clear conclusion as to whether styrene can induce mutation in human lymphocytes. In general, the experimental designs suffered from too few study subjects, workers and controls who were not well matched, insufficient exposure assessment, wide ranges in the exposure levels for workers, confounding exposures (particularly smoking) and low cloning efficiencies (for the Hprt assay).

In addition to the select human studies on chromosomal aberrations associated with styrene exposure reported in the draft documentation, there are many more studies that have been published. ATSDR (2010) provides a compilation of the studies by endpoint through the time period of their review. Summaries of the more recent human studies were included in the draft documentation; however, a

few studies were overlooked, including Jin et al. (2009), Hanova et al. (2010, 2011) and Helal and Elshafi (2012). Although there are a relatively large number of chromosomal aberration studies for occupationally exposed humans, as with the mutation studies, the study designs of these studies lack key elements important for understanding the results. Many studies were lacking in external or internal exposure information (some lacked both). When exposure was assessed there were only a few studies that assessed exposure in both the occupationally exposed workers and the controls. Often the control and worker populations did not appear to be well matched and an overall exposure assessment, to actually evaluate confounding exposures (beyond whether individuals were or were not active smokers) was not presented. Furthermore, most studies had a very small number of subjects. Overall, the weight of evidence indicates that there is insufficient information to conclusively determine whether styrene induces chromosome aberrations in humans.

Recently a review and meta-analysis focused on the induction of micronuclei was published by Costa et al. (2016). From their meta-analysis, the authors conclude that overall there was an increase in micronuclei among styrene-exposed workers and that that the findings of an increase were consistent across studies even though they reported significant heterogeneity in the meta-analysis of all studies. However, the authors also indicate that study quality is problematic in the reported literature and they identify a number of issues, such as poorly matched controls and exposed individuals, small number of study subjects, inadequate exposure assessment, confounding lifestyle exposures (i.e. combustible cigarette use) and poor epidemiological design. Of note, in the presence of substantial heterogeneity across studies, it is difficult to propose an overall meta-mean difference of micronuclei increase that represent the results of all studies. One study had a decrease in the meta-mean differences (Van Hummelen et al., 1994), five studies were consistent with neither an increase or a decrease (Anwar & Shamy, 1995; Holz et al., 1995; Mäki-Paakkanen, 1987; Tomanin et al., 1992; Vodicka et al., 2004), and five studies showed an increase (Godderis et al., 2004; Hanova et al., 2010; Laffon et al., 2002; Migliore et al., 2006; Teixeira et al., 2004). Overall, these studies taken together do not show consistent findings. Exposure-response is an important causal guideline. The Yager et al. (1993) longitudinal study of 48 workers, 26 males and 22 females, is an important study for exposure considerations and is particularly useful for assessing exposure-response relationship over time. In this one-year longitudinal study, there were multiple individual measurements made for both external and internal exposure. Personal breathing zone air monitors were worn for an entire work-shift and samples taken at approximately 6-week intervals for up to 7 randomly chosen days during the year. Exhaled air was evaluated for styrene 3 times in a work day for 7 monitoring days. 38% of the individuals were exposed to a mean 31 ppm styrene level and the remainder exposed to 3-6 ppm). Blood was taken for micronucleus analysis on up to 4 occasions. A minimum of 1000 binucleated cells were scored per sample and the scoring was done by a single individual. The data for micronucleus frequency are presented by individual. While there was an association between micronucleus frequency and both age and gender, there was no association between the level of styrene exposure and the frequency of micronuclei.

Overall, the increase in micronuclei frequencies among styrene workers occurred in the studies with lowest quality. Given the lack of consistency across studies and the equivocal finding on exposure response, there does not currently appear to be sufficient data to determine if an increase in micronucleus frequencies may be due to high styrene exposure.

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Mäki-Paakkanen J. (1987). Chromosome aberrations, micronuclei and sister-chromatid exchanges in blood lymphocytes after occupational exposure to low levels of styrene. Mutat Res. 189(4):399-406.

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Cancer

The human cancer information provided in the draft documentation is generally up to date for studies reported from occupational cohort studies conducted on monomer/polystyrene production and styrene butadiene rubber (SBR) workers but is outdated for the reinforced plastic worker (RFP) cohorts as there have been a number of updates to these cohort studies reported in 2017 and 2018. In addition, there has recently been published a systematic review and meta-analysis of the styrene epidemiology

literature (Collins and Delzell, 2018) which evaluated studies published through mid-2017 that may be useful to include in the documentation.

For the human cancer studies, the discussion content would benefit with inclusion of comments on the strengths and limitations of the studies to provide context in considering the results. For the studies presented in this section, brief comments are provided below on the study's strengths and limitations.

In addition, the human cancer information discussion in the draft documentation is presently organized by study publication dates. However, it may be more useful to organize the cancer information by study type as this impacts the importance of the studies. The occupational cohort studies are the most informative studies and there are studies available from 3 industry sectors: the manufacture of reinforced plastics (RFP), the production of styrene and polystyrene, and the manufacture of synthetic rubber (styrene-butadiene rubber, SBR). Of these studies, the studies of RFP industry workers are particularly important. These workers have had relatively high levels of styrene monomer (10s to 100s parts per million (ppm) with peak exposures more than 200 ppm) (NIOSH 1982; IARC 2002; Collins et al. 2013), while in the styrene/polystyrene production and SBR industries exposure to styrene has typically been below 10 ppm (Frentzel-Beyme et al., 1978; Ott et al., 1980; Meinhardt et al., 1978; Macaluso et al,. 2004). In addition, the RFP industry workers have not been widely exposed to known or suspected carcinogens (NIOSH 1982; Collins et al. 2013), whereas workers at styrene/polystyrene production facilities potentially have been exposed to several established or suspected carcinogens (e.g., benzene, dyestuffs, ethylene oxide, 1,3-butadiene, formaldehyde, acrylonitrile, mineral oil, carbon black, cadmium, arsenicals, vinyl chloride, asbestos and others (Bond et al., 1992; Nicholson et al.,, 1978; Hodgson and Jones 1985. RFP industry studies also have included over 100,000 workers exposed to styrene, whereas studies of the other two industry sectors were considerably smaller (styrene/polystyrene manufacturing, about 6,000, and synthetic rubber industry, about 15,000 workers exposed to styrene). Finally, the RFP industry is the only one of the sectors for which a large, comprehensive study of cancer incidence, rather than cancer mortality, has been conducted (Christensen et al., 2016). Besides the occupational cohort studies, there are other studies that report about styrene and cancer including cohort and nested case-control studies of workers control studies of workers in various industries in which styrene was not a main exposure of interest (Marsh et al., 2001; Guénel et al., 2002; Radican et al., 2008; Budroni et al., 2010); population- and hospital-based case-control studies that evaluated the relation between occupational exposure to styrene and other agents and various cancers (Flodin et al., 1986; Siemiatycki et al., 1991; Cantor et al., 1995; Gérin et al., 1998; Dumas et al., 2000; Scélo et al., 2004; Miligi et al., 2006; Seidler et al., 2007; Costantini et al., 2008; Cocco et al, 2010; Karami et al., 2011); a prospective cohort study of environmental exposure to styrene and other hazardous outdoor air pollutants and the risk of invasive breast cancer (Garcia et al., 2015); and ecologic studies that investigated environmental exposure to styrene and cancer (Coyle et al., 2005; Bulka et al,. 2016). These study types are less informative than the occupational cohort studies given

generally lower number of subjects and imprecise exposure information. The draft documentation appropriately limited its review to the more important occupational cohort studies.

Nicholson et al. (1978) – it would be useful to identify that the cohort studied was comprised of workers from a plant that manufactured styrene and polystyrene. The study's limitations were its very small size, lack of quantitative estimates of exposure to styrene, short follow-up, lack of information on lifestyle factors and lack of clarity regarding methods.

Okun et al. (1985) – This study was of a cohort of workers at 2 RFP boatbuilding plants. This cohort study was subsequently updated by Ruder et al. (2004) and then again by Ruder et al. (2016, 2017) and Bertke et al. (2018), hence it may be useful to mention this study in the draft documentation discussion of these subsequent cohort updates. Of note, this cohort is the smaller of the 2 US reinforced plastic worker studies, the other being the Collins et al (2013) and predecessor study by Wong (1990, 1994).

Bond et al. (1992) – it would be useful to identify the cohort studied was comprised of workers exposed to styrene monomer. Study strengths included long follow-up and a detailed job classification based on individual subjects' work histories. Study Limitations were lack of quantitative estimates of cumulative exposure to styrene; exposure to multiple agents other than styrene, including carcinogens such as butadiene, formaldehyde and benzene; and lack of information on lifestyle factors.

Hodgsen and Jones (1985) – It would be useful to identify that the study cohort consisted of workers at a styrene production, polymerization and processing facility. Study strengths included the analysis of cancer registrations (lymphohematopoietic cancer (LHC) was the only specific form of cancer included in the analysis of cancer registrations) in addition to cancer mortality; study limitations were the small numbers of styrene-exposed subjects, lack of quantitative styrene exposure estimates and lack of information on other workplace exposures and lifestyle factors.

Meinhardt et al. (1978) – As a study of SBR plants and with the potential for co-exposures, the cohort in this study may be of lower importance than the RFP workers and styrene and polystyrene worker cohorts, hence SIRC recommends separately discussing these studies. This study also is further limited as results were not presented for workers and styrene exposure. Of note, the most recent study of the SBR industry (Sathiakumar et al., 2015) included work forces at the plants investigated by Meinhardt and colleagues (Meinhardt et al., 1978: Meinhardt et al., 1982; Lemen et al., 1990).

Matanoski et al. (1990) – It would be useful to identify this study as a cohort study of SBR workers and, as with Meinhardt, the results were not presented for workers and styrene exposures, results are only presented for the combined exposures. Of note, the most recent study of the SBR industry (Sathiakumar et al., 2015) included work forces at 7 of the 8 plants investigated in the earlier studies by Matanoski and colleagues (Matanoski and Schwartz, 1987; Matanoski et al.,1993, 1997; Santos-Burgoa et al., 1992).

Collins et al. (2013) – This is the larger of the 2 available cohort studies of U.S. RFP workers and covers workers at 30 plants. The mean length of follow-up in the Collins updated study was 35 years. This study used data from industrial hygiene surveys conducted at each plant when the study began, as well as historical industrial hygiene and process records linked to subjects' detailed work histories, in order to develop quantitative estimates of workers' exposure to styrene. Workers had styrene TWAs of about 35 ppm in 1967 and about 25 ppm in 1977. Over the entire study period, the average styrene TWA was 28 ppm. The lung cancer findings observed in the Collins et al. (2013) study and other studies that have found increases may be confounded by smoking. In the Collins et al. (2013) study excesses of non-malignant respiratory disease, which is associated with smoking, paralleled lung cancer excesses. An earlier study of the same cohort investigated by Collins et al. (2013) included a nested case-control study of respiratory cancer in which data on smoking was obtained (Wong 1990). The latter study, which included 40 cases who had died of respiratory cancer and 102 deceased controls, reported that respiratory cancer was associated positively with smoking but not with direct exposure to styrene, duration of direct exposure to styrene or type of styrene process. Exposure to asbestos in the reinforced plastics plants studies has also been suggested as another potential confounder. The strengths of this study were its industry-wide design, the relatively high quality of its styrene exposure estimation, the relatively high styrene exposure of the workers, the long follow-up period and the thorough exposure-response analyses performed for certain cancers. The study's limitations were its reliance on mortality data to determine cancer outcomes and lack of information on lifestyle factors that were potential confounders for some of the cancers analyzed and, because of its moderate size and reliance on cancer mortality, the study's results for some cancers (for example, rare cancers and cancers associated with good survival) were imprecise.

Kogevinas et al. (1993, 1994) – The Kogevinas study is a large international cohort study of European RFP workers that included several hundred facilities in the RFP industry in Denmark, Finland, Norway, Sweden, Italy and the United Kingdom. This study included workers reported in Danish studies (Christensen et al. 2016; Kolstad et al. 1993, 1994, 1995), and workers included in studies in the United Kingdom by Coggon (1987) and Coggon et al. (2015). Workers entered the international cohort of Kogevinas in 1945 through 1970 and were traced to determine their mortality experience through a calendar year that varied by country and ranged from 1987 to 1991. Styrene exposure estimation was based on detailed job histories available for some facilities, on industrial hygiene and production data from several countries or on biological monitoring data. The workers that were laminators had the highest average styrene exposures, estimated to have been about 200 ppm in the late 1950s, 100 ppm in the late 1960s and falling to about 20 ppm in the late 1980s. Styrene exposure estimation for individuals was based on workers' longest-held job (e.g. laminators - 43 ppm, unspecified jobs – 29 ppm, other exposed jobs – 15 ppm, unexposed job, unknown). Due to lack of detailed work histories, all Danish and Finnish workers and many Swedish workers were classified as having unspecified jobs and were assigned an average of 29 ppm. Strengths of this study were the inclusion of comprehensive analyses of several indices of styrene exposure (job type, duration of employment, ppm-years) for many

forms of cancer, as well as analyses by time since first exposure. Limitations of the study included lack of detailed job information for a high proportion of cohort members, lack of information on lifestyle factors, a high proportion (40% overall) of subjects employed in the industry for less than one year and the use of cancer mortality, rather than cancer incidence, as the outcome of interest.

This international cohort study has been the subject of a recent further update that is reported in Loomis et al. (2018). The original study on RFP industry workers included 40,668 workers with 539,479 person years. The countries in this study include Denmark, Finland, Italy, Norway, Sweden, and the United Kingdom. The Loomis et al. (2018) study updates a portion of this study population and has 37,021 workers with 506,459 person-years. The Loomis study did not include data from Denmark, United Kingdom or Norway in their update. The Loomis et al. study concentrated on cancers of the lymphatic and hematopoietic tissues and used the most recent classifications of what is considered a non-Hodgkin lymphoma (NHL). Cancers of secondary interest were lung esophagus, kidney and pancreas. The exposure assessment in the study appears to use the same approach as the original study. The Looms et al. study examines cumulative exposure, mean exposure, duration of exposure and jobs with likely high exposure to styrene (i.e., laminators). Poisson regression is used to calculate rate ratios with follow-up time treated as the time axis. Other factors considered were age, calendar date, sex, country, length of follow-up and time since first exposure. The "best model" was determined by the set of variables which produced the best fit and the largest change in the relative risk. Latency effects were examined by lagging exposures 0, 5, 10 and 20 years. The findings for cancers of the lymphatic and hematopoietic tissues including were NHL were similar to the original study. With the exception of NHL, cancers in this category were not statistically associated with styrene exposure. However, NHL was related to mean styrene exposure but not cumulative exposure. There was no association with styrene exposure for lung cancer and kidney cancer. However, there were statistically significant associations for esophageal cancers for both cumulative and mean exposure (sometimes referred to as average exposure). Pancreatic cancers were associated with mean styrene exposure but not for cumulative exposure. The authors conclude the findings for lung cancer and NHL are unchanged from the original study, but the new findings for esophageal and pancreatic cancer merit further investigation. This study was well analyzed in keeping with the current accepted methodology for occupational cohort studies. The exposure characterization appears well done and misclassification of exposures are examined. Further, this study update does a good job in allowing consideration of the findings of this study in the context of recent updates of the Danish and United Kingdom studies of reinforced plastic workers. There are some limitations to this study. First, the unexposed group of workers should have been discussed in more detail. The authors mention some of these workers were "clerical" and thus may be very different from exposed workers in their education, lifestyles and habits. It is not clear if clerical workers were included in the exposed groups. Second, there were many models examined but only a few models presented in the results. The authors mention that models presented in the paper were selected by fit and with the variables which "appreciably changed" the relative risk. For NHL the variables in the model were age, calendar time, and sex, but for esophageal and pancreatic cancer only age was included. To

help the reader fully assess the finding, the results for the full model with all variables for each cancer evaluated should also have been presented. It is not clear how many models were examined and how many of these did not have statistically significant findings. Finally, most of the findings of effects in this study were observed when mean (average) styrene exposure is used in the model. Mean exposure is the arithmetic mean of past exposures and has been used in occupational studies to examine partially reversible effects such as pulmonary function. When examining cancers, however, cumulative exposure, the product of intensity and duration is generally considered more appropriate for examining irreversible effects. The authors should have discussed the lack of associations when cumulative exposure models were employed as in the case of pancreatic cancer and in most of the models of esophageal cancer.

The studies of the Danish RFP industry workers are missing from the draft documentation, although there is overlap in reporting of these studies in the international cohort study discussed above. (Kolstad, 1993, 1994, 1995). This cohort was recently updated by Christensen et al. (2017), Christensen et al. (2018) and Nissen et al. (2018). The earlier Kolstad studies included workers employed between 1964 and 1988 at 386 companies. Christensen et al. (2017) expanded the cohort to include workers employed between 1964 and 2007 at 443 companies and extended follow-up to identify incident cancer cases diagnosed from 1968 through the end of 2012, adding more than 20 years of follow-up to the earlier studies. In addition, Christensen et al. (2017) used data from a worker survey to estimate the probability of exposure to styrene at each company and to obtain data on workers' tobacco smoking patterns; analyzed cancer incidence in relation to three surrogate measures of styrene exposure; and assessed potential confounding by smoking. The main conclusion of Christensen et al. was that "Occupational exposure may be associated with Hodgkin lymphoma, myeloid leukemia, and cancer of nasal cavities and sinuses. Further studies are needed to evaluate if the observed associations are likely to be causal." Also of importance are the findings that an increased incidence of lung/bronchus/trachea cancer seen in some analyses could have been due to confounding by smoking; that an observed 2.5-fold increase in mesothelium cancer (mesothelioma) was likely attributable to shipyard employment, rather than to exposure to styrene; and that NHL, lymphoid leukemia, multiple myeloma and a number of other cancers of a priori interest were not associated positively with styrene exposure. The study has several notable strengths, including large size, long follow-up and use of cancer incidence rather than cancer mortality as the endpoint of interest. Major limitations are lack of information on specific jobs held by workers and the consequent lack of quantitative estimates of cumulative exposure to styrene; lack of analyses of cancer incidence by exposure categories specified on the basis of both probability of exposure and duration of employment/potential exposure; lack of internal analyses of styrene exposure proxies; and the possibility of residual confounding by tobacco smoking, other non-occupational factors and occupational exposures other than styrene. The statistical associations reported by Christensen et al. (2017) for styrene and Hodgkin lymphoma, myeloid leukemia and cancer of the nasal cavities and sinuses lack external support and are not interpreted as causal associations, and the study's results for myeloid leukemia were not statistically significant. The study provides evidence that previously reported

associations between styrene and cancers of the esophagus, pancreas, lung, kidney and bladder may be non-causal, and it found no positive association between styrene and NHL, Hodgkin lymphoma, lymphoid leukemia or multiple myeloma.

Christensen et al. (2018) reports on a study of LHC incidence among workers exposed to styrene in the Danish reinforced plastics industry. This study adds to a Christensen et al. (2017) study of the same cohort of workers (with minor changes). Christensen et al. (2018) enhanced the methodologic approach of the earlier paper in several major ways, including improved exposure estimation, analysis of subtypes of LHC not examined in the 2017 study (the 2018 study analyzed a total of 21 types of LHC), use of an internal referent group and inclusion of analyses of LHC incidence in relation to multiple time-dependent exposure indices and induction time/latency. The follow-up period of the new study was 1968 through 2011. The 2017 study by Christensen et al. includes findings of positive statistical associations between surrogate styrene exposure variables and overall myeloid leukemia and Hodgkin lymphoma but the authors acknowledged limitations and concluded that "Further studies are needed to evaluate if the observed associations are likely to be causal." The 2018 study of Christensen et al. is a further investigation that contributes to the knowledge base for styrene and LHC. It analyzed subtypes of myeloid leukemia, lymphoid leukemia and non-Hodgkin lymphoma, included new analyses of multiple myeloma and Hodgkin lymphoma and analyzed several types of LHC not included in the earlier paper by Christensen et al. (2017). Findings of the new study highlighted by Christensen et al. (2018) included a positive association between cumulative exposure to styrene and acute myeloid leukemia in a subgroup of their cohort and positive but statistically less impressive associations with Hodgkin lymphoma and T-cell lymphoma. Other important findings included null results for styrene and overall NHL, the most numerically important subtypes of NHL (including multiple myeloma), other subtypes of leukemia, myelodysplastic syndrome and polycythemia vera. The evidence for a causal association between styrene and acute myeloid leukemia remains inconclusive for several reasons. Limitations of the Christensen et al. (2018) study are the uncertain styrene exposure estimates and consequently a high likelihood of exposure misclassification, lack of control of confounding by cigarette smoking, lack of information on other occupations held by short-term workers and, despite the large size of the overall study, the relatively small numbers of outcomes available for analysis (acute myeloid leukemia, 50; Hodgkin lymphoma, 57; T-cell lymphoma, number not reported) and the resulting statistical imprecision of the results. The results of Christensen et al. (2018) for Hodgkin lymphoma and T-cell lymphoma are not persuasive in terms of causality. The null results for other forms of NHL are based on large numbers and are an important contribution to the relevant literature.

In a case-control study nested within the Danish reinforced plastics industry cohort, Nissen et al. (2018) analyzed the association between styrene exposure and sinonasal cancer incidence among the 73,092 workers included in the Christensen et al. (2017) study. The original study reported 40 sinonasal cancers, whereas Nissen et al. (2018) reports on 37 sinonasal cancer from the original study including 9 adenocarcinomas, 15 squamous cell carcinomas, and 13 other histologic types. There was no

explanation for the differences in the numbers between the two versions of study. The observed 9 cases of sinonasal adenocarcinomas corresponded with a fivefold increased age, sex and wood industry-adjusted odds ratio (OR) for high versus low cumulative styrene exposure (OR 5.11, 95% CI 0.58-45.1). The incidence increase was confined to exposure received during the recent 15 years. No association was found for the other histological subtypes. Given the potential for confounding exposure to wood dust in this industry, the authors should have committed more time to understanding the exact nature and levels of potential wood dust exposures. The categories used in this study to classify employment in the wood industry (never, ever, unknown) are far too crude to examine such a powerful potential risk factor as wood dust. A strength of this study was the specific histological information, but major limitations were the small numbers of cancers included and the potential confounding with wood dust exposure in this study which make it difficult to assess the impact of styrene exposure on the risk of sinonasal cancer. This study does not support a causal interpretation of the reported association between styrene and sinonasal adenocarcinoma because chance and residual confounding by exposure to wood dust cannot be reasonably ruled out.

Coggon et al. (2015) – The earlier study by this investigator of these United Kingdom RFP workers (Coggon, 1987) is included in the study by Kogevinas (1993, 1994). The study's strengths are the inclusion of a substantial proportion of cohort members with high exposure, long follow-up, inclusion of nested case-control analyses of LHC deaths and registrations and analyses of time-dependent exposure variables. Limitations of the study are a lack of quantitative estimates of cumulative exposure to styrene, lack of data on lifestyle factors, lack of analyses of cancer incidence for forms of cancer other than LHC and a relatively high proportion of subjects lost to follow-up.

Ruder et al. (2004, 2016) – As noted previously, these studies are updates to the cohort first reported by Okun et al. (1985) and are the smaller of the 2 US RFP worker studies. The mean length of follow-up in the last update was 35 years. The original study included a survey of styrene exposure levels in specific departments at the facilities. Although quantitative historical exposure estimation for the cohort members was performed, Ruder et al. (2016) did not report numerical data on the results of cumulative exposure analyses but rather reported the detailed analyses of specific forms of cancer were limited to exposure proxies including duration of employment and employment in high-exposure jobs (fibrous glass or lamination) versus other jobs with lower exposure. Other limitations of this study included imprecision due to small study size, lack of information on lifestyle factors and failure to analyze high versus low styrene exposure in a time-dependent manner.

Two additional updates to this cohort have been published since 2016, including Ruder and Bertke (2017) and Bertke et al. (2018). Ruder and Bertke (2017) examined the cancer incidence from 1991-2007 among 3704 workers of the Washington cohort living in Washington state with no restriction on duration of employment and using data from the Washington state cancer registry and applying statistical methods that were used by Ruder et al. (2016). The results found standardized incidence

ratios (SIR) of lung cancer of 1.11 (95% CI 0.89-1.37) and 1.42 (95% CI 1.00-1.95) for the total population of workers and for workers with potential high exposure to styrene, respectively. Corresponding SIR values for these groups were 1.03 (95% CI 0.77-1.35) and 0.99 (95% CI 0.59-1.57) for LHC cancers, 1.00 (95% CI 0.75-1.32) and 1.17 (95% CI 0.70-1.82) for urinary tract cancers, and 0.81 (95% CI 0.50-1.23) and 0.88 (95% CI 0.49-1.45) for breast cancers. The main strengths of this study are its inclusion of workers with relatively high exposure to styrene and its use of cancer incidence, rather than cancer mortality, as the outcome of interest. The study has important limitations, including statistical imprecision, lack of analyses that considered time since first exposure to styrene and duration of exposure/employment and inability to control for potential confounding by lifestyle risk factors or occupational exposures in work before and after employment at the study facilities. Positive associations reported by Ruder and Bertke were due in large part to unexplained deficits of cases in the low styrene exposure subcohort compared to the general SEER population, not to any excess in the high exposure subcohort. Furthermore, the elevated SIRs and SRRs for specific forms of cancer seen in this study were statistically imprecise. Thus, non-causal explanations of the results are plausible. The cancer incidence study adds little to the previous mortality study (Ruder et al., 2016) of workers at the same facilities.

Bertke et al. (2018) reported on cancer mortality from 1959-2016 for 5201 workers in the Washington state cohort. Exposure levels reported between 1978 and 1979 for full shift average for workers ranged from 42.5 to 71.7 ppm styrene. In this update, for workers with more than 1 year of employment, many cancer sites showed increased SMRs when compared to Washington state expected values including lung cancer (SMR 1.20, 95% CI 0.95-1.51) whereas increases were not observed for cancer of buccal cavity and pharynx, breast, NHL or leukemia. In internal analyses, leukemia showed increasing mortality with duration of employment in a high exposed category with duration (RR 0.9, 95% CI 0.7-1.) while this estimate was 1.1 (95% CI 0.7-1.3). Study strengths were the high styrene exposure levels, long follow up and few competing risk factors. Limitations of this study were important, including, the small size of the study, the limited exposure characterization and the lack of information on smoking status and other lifestyle factors. In addition, as mentioned by the authors, their observed association between styrene exposure and leukemia does not have consistent external support from studies of other cohorts occupationally exposed to styrene. Consequently, firm conclusions of cancer risks, or lack thereof, due to styrene exposure are not warranted. As the authors noted, the study results should be interpreted cautiously given these limitations.

IARC has recently completed review of the styrene human cancer epidemiology information and concluded that there is limited evidence in humans for carcinogenicity of styrene (IARC Monographs Vol 121 Group, 2018). This is the same conclusion IARC made in their previous 2002 review. In their present review, for lymphohematopoietic malignancies, IARC noted increased incidence or mortality of subtypes of leukemia and lymphomas in several studies, with greater consistency for leukemia, and in particular myeloid leukemia. They found the incidence of sinonasal adenocarcinoma, a rare cancer, was increased in one large cohort of reinforced plastics workers, but cases were few and chance and confounding

could not be discounted. The evidence for solid tumors, including lung cancer, was found to be sparse or inconsistent. Overall, IARC concluded that the human cancer information provides credible evidence that exposure to styrene causes lymphohematopoietic malignancies, but confounding, bias, or chance cannot be ruled out.

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Reproductive Effects

The human reproductive/developmental toxicity information provided in the draft documentation is generally up to date for the limited reports of these human effects associated with styrene exposure. The studies cited appear to be accurately described as well as their limitations. This endpoint was thoroughly reviewed by Brown et al. (2000) and a NTP CERHR expert committee (Luderer et al., 2005). Brown et al. (2000) found that overall there is little indication that styrene can exert any specific developmental or reproductive or endocrine toxicity and that putative effects on female reproduction and neurobehavior development could have a CNS site of action, which is compatible with the known neurotoxic actions of styrene at high exposure levels. The CERHR experts concluded that based on the styrene experimental animal data, there is negligible concern for reproductive or developmental toxicity in humans; although noting that the human epidemiological evidence is insufficient and an outstanding question of the clinical relevance of prolactin findings observed with occupational exposures (Luderer et al., 2005). The Brown et al. (2000) and Luderer et al. (2005) publications are included in the draft documentation reference list but are not cited or described in the reproductive effects section, hence inclusion of a statement on this committee review in this section would be useful. Regarding, the CERHR committee's comment on a prolactin finding, this was subsequently reviewed by Gelbke et al. (2015), which is included in the draft documentation, and the finding was suggested to be related to acute workplace stress.

Ototoxicity

SIRC's detailed comments on the human ototoxicity information were offered in SIRC's main comments and hence are not repeated here. Below are citations that were referenced in SIRC's main comments that are not presently listed in the TLV documentation.

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Neurological Effects

As neurological effects are among the basis for the proposed TLV change, the draft documentation review for this information should be thorough and current. However, the draft documentation on

human neurological effects consists mostly of pre-1990 reports, noting only one recent report by Sato et al. (2009). The United Kingdom performed a thorough review and assessment of styrene human neurological effects in their 2008 European Union Risk Assessment which provides another 20 years of human study information.

Since 1990, studies that evaluated neurophysiological measurements associated with styrene exposure were reported by Matikainen et al. (1993) for EEG, Murata et al. (1991) and Stetkarova et al. (1993) for somatosensory evoked potentials, and Murata et al. (1991) and Yuasa et al. (1993) for peripheral nerve conduction parameters. A strength of these studies is that they provide measures of nervous system function that are independent of the level of collaboration of the subject and, with the possible exception of EEG recordings, effects seen are not likely to represent simply acute effects that may be reversible within hours. These studies, however, have important limitations including methodological validation, confounders, statistical chance findings, and toxicological significance of the changes. The results of these and the previously reported studies of neurophysiological measures are mixed for styrene. The evaluation of EEG by Matikainen et al. (1993) in a group of 100 workers, selection not specified, found no excess of abnormal EEG on visual assessment but, using an automated analysis, found that the group with the highest cumulative exposure had an excess of recordings judged abnormal and an increase in total EEG power in the α and β bands, particularly in the frontal and temporal regions of the brain. Generally, for EEG studies, a major problem is the lack of a clear, validated and generally accepted interpretational framework for assessing the significance of the results. Because EEG can vary with arousal, it is important to control for the state of arousal of the subject and it is not clear that this was done in studies that evaluated EEGs. Additionally, substances that cause CNS depressions have the potential to produce diffuse, non-specific changes in EEG patterns. Therefore, the overall findings do not provide robust evidence for the absence or presence of styrene-induced EEG changes in exposed workers. The studies measuring somatosensory evoked potential also evaluate the functioning of the cortical region. The results reported by Murata et al. (1991) found no change in somatosensory evoked potentials between the exposed and comparison groups. The results from Stetkarova et al. (1993) are unclear but there appears to be slowing in central conduction time and prolonged latency of cortical evoked potentials following styrene exposure. In this study, peripheral conduction velocity was slower in the styrene-exposed workers compared to the comparison group on stimulation of both the median and tibial nerves. Murata et al. (1991) found a greater (and significant) slowing on the sensory rather than motor conduction in the median nerve. Yuasa et al. (1993) reported a greater (and significant) slowing of motor than sensory conduction in the ulnar nerve, with significant slowing also in the peroneal (motor) nerves but not in the sural (sensory) nerve. Murata et al. (1991) also reported a reduced R-R coefficient of variation in the exposed workers, which they interpret as a significant change in the autonomic nervous system in the styrene-exposed workers. Considering these and earlier reported nerve conduction studies, the results are inconsistent for different groups of workers exposed to similar levels of styrene. Some studies have indicated a correlation between styrene exposure and small (<10%) decreases in nerve conduction velocity, compared with unexposed controls, whereas others have shown

similar nerve conduction velocities in styrene-exposed and unexposed workers. Therefore, it is not clear if styrene exposure can produce a small decrease in nerve conduction velocity. A difficulty with these studies is the number of measures taken, which might be expected to lead to some positive results from chance variation. Additionally, the clinical significance of this effect is questionable, as all subjects appeared to be healthy workers.

Studies since 1990 that performed neurobehavioral testing have been reported by Letz et al. (1990), Jegaden et al. (1993), Edling et al. (1993), Mergler et al. (1996), Viaene et al. (1998, 2001), Tsai and Chen (1990) and Yokoyama et al. (1992). The first 3 studies considered cross shift (acute) effects. Letz et al. (1990) found no relation between exposure during the shift and scores on the continuous performance test (of complex reaction time) or on a test of hand-eye performance. Scores on the symbol digit test at end of shift were related to styrene in air corrected for respirator use (by a method not specified) and to urinary MA. Although the data are not presented, it appears that those exposed to >50 ppm had the lowest symbol digit scores. Jegaden et al. (1993) conducted a similar study in a French naval shipyard. The analysis across shift was not reported adequately but there appears to have been no significant difference between changes across the shift in the exposed and comparison groups. Significant pre (and post) shift differences were observed which the authors interpret as chronic effects but could equally reflect either inadequate adjustment for confounding or incomplete clearance of styrene and its metabolites from the previous day's exposure. Edling et al. (1993) found no change in test score over shift between the exposed and comparison groups but within the exposed group there was a relationship between complex reaction time and various measures of exposure during the shift including 8-hour time weighted average. No effect was seen on the symbol digit test, the only test found by Letz et al. (1990) to have an exposure-related cross shift change. Letz explicitly says that most of the effects are found in those exposed to more than 50 ppm on the day of testing (this data is not presented). Jegaden et al. (1993) measured exposure on the day of testing and the highest level of concentration was 55 ppm with a mean of 23 ppm; no notable cross shift effects were found. The exposures in the Edling study were low (8.6 ppm on average and nobody exposed to more than 15.4 ppm). He was however able to measure excursions greater than 50 ppm and found that greater number and duration of excursions above 50 ppm were related to poor scores on the complex reaction time test. Taken together these three surveys do suggest that at the exposures below 50 ppm, and without excursions above this level, cross shift change in test score may not be detectable using current techniques. Two studies reported the effects of reducing or removing exposure on behavioral test scores. These were Mergler et al. (1996) and Viaene et al. (1998; 2001). In the study reported by Mergler et al. (1996) 3 workplaces were assessed and baseline measurements made of scores of performance on neurobehavioral tests. Two years later the exposure and neurobehavioral tests were repeated, following interventions at one of the three companies to improve working conditions. The overall exposures were low and did not change essentially but it appears that those whose MA concentrations increased over time did worse on the second occasions, and importantly, those whose MA concentrations were reduced did better on at least some tests. The study reported by Viaene and colleagues (1988 and 2001) has such

a weak design that it is not possible to draw any conclusions about the reversibility of effects. The authors would appear to deduce that the better test scores seen for people laid off three years earlier would reflect such a reversal, but no baseline data are available. Neither study is sufficiently reliable to support conclusions. Tsai and Chen (1990) report on a study from Taiwan that presented simply cross sectional data comparing behavioral tests in an exposed group and a non-exposed comparison group. This study used an internal comparison group of workers from within the same six manufacturing plants in Taiwan and information given on potential confounders suggesting they were in fact very well matched. This rather thorough study, in plants where the mean concentration was 22 ppm, found worse performance on the continuous performance test (of complex reaction time test) and on vibration perception where the threshold was high in both the hand and foot for the exposed. The duration of employment was 8.3 years and it is not clear whether high levels of exposure had pertained in the past. Yokoyama et al. (1992) reported on neurobehavioral testing of 12 styrene-exposed workers and 11 non-exposed steel workers. The exposed workers had a mean duration of exposure of 5 years and current exposure of around 26 ppm with a maximum 8 hour TWA of 77 ppm. It is not known whether there had been higher exposures in the past. In comparison to the comparison group the exposed workers did less well on the picture completion test but there was no difference in scores on the digit symbol test. It does not appear that the comparison group was particularly well matched, but statistical adjustment for confounders was attempted. In the Jegaden study (1993), the exposed group did significantly worse than the comparison group on a test of simple reaction time, complex reaction time and digit span. The authors suggest this was likely a result of chronic exposure. In the preceding year, mean exposures had been approximately 30 ppm with perhaps 20% of exposures more than 50 ppm. There are, however, a number of difficulties in interpretation of this study. Viaene et al. (2001) compared scores of the currently exposed and the previously exposed with a comparison group of never exposed. The scores on the symbol digit substitution and digit span forward test appeared to be worse for never-exposed workers than those who had been exposed to styrene.

Overall, although a number of studies are reported, the information is insufficient to base any conclusions about the potential long-term effects of styrene exposure on neurobehavioral parameters. There are few studies in which the potential for acute effects can be entirely excluded. One study suggests a possible deficit in a single neurobehavioral parameter (manual dexterity); another provides no evidence for a functional deficit in reaction time; and the studies by Viaene et al. (1998; 2001) failed to demonstrate that the effects seen were due to styrene exposure because of inadequate adjustment/matching for educational level.

Benignus et al. (2005) conducted a meta-analysis of several of the studies that have reported on the relationship between styrene exposure and effects on reaction time (simple and choice) and color vision (discussed separately below). Four studies of choice reaction time (CRT) and 3 studies of simple reaction time (SRT) were selected. No clear inclusion/exclusion criteria for the selected studies were provided. Airborne styrene concentrations were estimated from urinary styrene or metabolite data by fitting

regression equations. No standard extrapolations were used. Cumulative exposures were then calculated as the product of concentration and time by assuming that the current exposure concentration was a representative measure of the workers' exposure history. In the interest of homogeneity, study group means for both concentration and duration of exposure rather than individual data were computed. Outcome data were transformed to a common metric of effect magnitude (percentage of baseline) and linear equations were fitted to the pooled data to produce dose-response relationships. In the majority of the reports, it is unclear whether or not the different tests were administered in a blind manner. Statistically significant relationships were found between cumulative styrene exposure and increased choice reaction time. Eight years of exposure to 20 ppm styrene was estimated to produce a 6.5% increase in CRT and to increase the CCI as much as 1.7 additional years of age in men. No significant effects were observed on SRT. It is considered that a number of methodological weaknesses in the analysis question the validity and reliability of these findings. Firstly, it is noted that the meta-analysis presented failed to reflect the available literature, as several additional studies could have been included in the model calculations but were not. Secondly, given that study group means for both concentration and duration of exposure rather than individual data were used and that there was no information on past exposure, the dose-response and time-response evaluations presented are likely to be unreliable. Thirdly, by using percentages of baseline response, the variability of the control groups was unaccounted for. Fourthly, by imposing a linear mathematical model to the dose-response relationships, the study failed to reflect the available data and the threshold nature of these effects. Also, it is difficult to understand the plausibility as to why CRT is affected but not SRT; such a specific effect is of doubtful toxicological significance.

In addition to the United Kingdom HSE review, there are other comprehensive reviews of the literature conducted in the 1990s that reached differing conclusions regarding these effects. Pahwa and Kaira (1993) considered the evidence for a variety of effects (including prenarcotic effects, electroencephalographic abnormalities, slowing of motor, sensory and distribution nerve conduction velocities that reveal the possibility of polyneuropathy, dysfunction of the autonomic nervous system, slowing of reaction times, and centrally-controlled otoneurotoxicity) sufficiently compelling to justify reduction of occupational exposure limits. In contrast, Rebert and Hall (1994), surveying much of the same literature, found no indications of persisting damage to the nervous system and concluded that reported effects were "false positive outcomes" due to type I statistical error, the effects of factors other than styrene, and misinterpretation of data. A critical review of 20 studies published between 1990 and 2003 conducted by Bagirzadeh et al. (2006) observed that they generally avoided the more severe pitfalls of the earlier studies, with conclusions that were "…usually appropriately modest and point to the relatively slight effects that can be attributed to styrene in the populations studied."

In view of the conflicting results among studies and lack of clear dose-response relationship, Seeber et al. (2009a) undertook a cross-sectional study of workers with repeated measurements in a German boat-building plant with 3 critical questions in mind: (1) Are the published findings for neurobehavioral

impairment reproducible? (2) If such effects exist, are they related to current exposure or chronic exposures? (3) If effects exist, are they reduced during an exposure-free period? The same workplace setting was also used to investigate effects on color vision (Seeber et al. 2009b) and ototoxicity (Triebig et al. 2009), providing a unique opportunity for comparison of these different effects in the same exposed population. Air monitoring data for laminators (a relatively highly exposed job) available since 1982 indicate decreasing average styrene exposure levels in the intervals 1982-1990, 1990-1995, and post-1995 of 100, 80, and 30 ppm. Current exposure subgroups were objectively defined using creatinine-normalized urinary MA + PGA concentrations (mg/g creatinine) as low (n = 83, 53 mg/g creatinine), medium (n = 101, 230 mg/g creatinine), and high (n = 29, 928 mg/g creatinine), thereby deriving individual exposure measurements without confounding by personal protection measures. Job tenure was about 6 years. In addition, subgroups chronically exposed to low-short (n = 30, lifetime weighted average exposure mean 184 mg/g creatinine for 6 years) and high-long (n = 16, 693 mg/g creatinine for 15 years). Thus, the high-long group was employed before technological innovations allowed significant reduction of styrene levels in the plant. The urinary metabolite data were converted to airborne exposure concentrations of 10 ppm for the medium group, and 40 ppm for the high group. The authors estimated that the high-long group experienced a lifetime weighted average exposure of approximately 27 ppm over 15 years. Examinations consisted of a symptom questionnaire, tests of Benton visual retention, symbol digit substitution and digit span for cognitive functions, and choice reaction, aiming, peg board, tapping, and steadiness for psychomotor functions. Tests of vocabulary and personality traits were included for evaluation of intelligence and affect as potential confounders. Testing was conducted during normal working days and during company holidays. Self-reported symptoms were not related to styrene exposure, but did correlate with the personality trait Negative Affectivity. Among tests for cognitive function, only Benton visual retention test (visual perception and memory of figures, quality of immediate reproductions) results showed significant correlation with parameters of chronic exposure when evaluated by linear regression (but not analysis of variance), which did not resolve during the exposure-free holiday period. Given the possibility of false positives, the authors argue that the absence of exposure-related cognitive effects in the multivariate analysis evidences a lack of styrene-related effects. Among tests for psychomotor function, only the peg board test (placing sticks in holes) results showed significant correlations with chronic exposure. This effect disappeared during holidays. The fact that the position of laminator was significantly associated with this and all other psychomotor variables suggests that the daily work activities of laminators might themselves affect psychomotor control. The authors' overall conclusion was that their data supports "no convincing evidence" for clearly styrene-related neurobehavioral effects, emphasizing that the modest effects noted for the Benton and peg board tests were associated with high long-term exposure but not current exposure.

Overall, the literature concerning neurobehavioral effects of styrene is conflicting in its findings, its interpretation hampered by confounded or questionable results. As a result, available neurobehavioral endpoints do not provide a sound basis for development of regulatory criteria for the protection of occupational or public health.

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Visual Disturbances

In addition to the studies on visual disturbances, including color vision (usually of the tritan type affecting blue-yellow color discrimination) and/or visual contrast sensitivity, that are discussed in the draft ACGIH documentation, a number of critical reviews on this subject are available (Sheedy, 1997; Gobba, 2000; Gobba and Cavalleri, 2000; Kishi et al., 2000; Gobba and Cavalleri, 2003; Lomax et al.; 2004; Paramei et al., 2004; Benignus et al;. 2005; Good and Nichols, 2006; UK HSE, 2008; Betancur-Sánchez et al., 2017; Choi et al., 2017) that provide further insights into the reported findings. Overall, the available evidence indicates visual disturbance effects may occur at high styrene concentrations (≥ 50 ppm, 8h TWA), but the magnitude of these effects is small, their dose-response and reversibility are unclear, and their implications for instrumental activities for daily living (IADL) are uncertain (Benignus et al., 2005; Good and Nichols, 2006; Choi et al., 2017). In particular, a threshold is difficult to identify in available epidemiological studies due to a number of shortcomings in the available data base, including limited sample sizes, control groups inadequately matched for potential confounding variables, lack of accurate measures of current and historical exposures, lack of baseline measures prior to exposure, small effect sizes, and effect heterogeneity. The impact of such effects on IADL have not been determined, even for highly exposed workers.

The visual disturbance studies identified in the draft documentation include the majority of the historical and recent reports for styrene, however the discussion on these studies does not assess the study information which is critical for consideration of the validity and significance of these effects. Additional comments on the study design and appropriateness of the conclusions would better inform on the study findings and significance and whether these should form a basis of the proposed TLV-TWA change. For several of the discussed studies, issues are identified below that should be considered in the assessment of the study findings.

The Gobba et al. (1991) study provides no discussion of the sampling methods used to identify styrene-exposed subjects within the 7 fiberglass plants, nor the control group. It is important to know the total number of styrene-exposed workers in all plants, and a random sample could have been taken from this subject pool eliminating any potential selection bias. With the currently described method, it is possible that certain low-exposure subjects may have been excluded due to the tendency to select those with more severe disease or exposures. There is no mention of masking of the examiners performing the test procedures regarding the exposure status of the subjects. Another issue regarding the subjects is that females were included in the styrene-exposed group. This may pose a problem as up to 15% of women are heterozygous for color vision deficiencies and will show errors on the dD-15 panel. This could spuriously inflate the CCI values associated with the styrene-exposed group leading to significant differences from the control group. The styrene-exposed sample should have included only male subjects free of congenital color vision deficiencies. Also, alcohol use should have been controlled for in the analyses even when weekly consumption values were less than 250 g/week. This also could have

increased the CCI values; however, alcohol consumption would have affected the CCI values in both the styrene-exposed and control groups. Another concern in this study was the apparent lack of testing data for normality, as inspection of the data shows a highly skewed range of data. This could have accounted for the significant differences found between age-matched groups. The authors did not perform the appropriate analysis to derive their conclusion regarding a dose-response effect. Given that three groups were present in the subgroup analysis, the appropriate statistical analysis would have been analysis of variance (ANOVA) with post-hoc testing of groups as necessary. The ANOVA technique would have allowed the authors to control for the effects of age as well. It is unclear why excreted MA did not predict CCI values. The authors did not provide power calculations for their observed findings in this case as it appears there is much variability in the styrene-exposed group in this outcome (i.e., the standard deviation is larger than the associated mean for MA concentration). Also, the authors did not present data and analyses regarding the effect of short-term elimination of styrene exposure (i.e., the 4 week vacation) on color discrimination.

The study reported by Fallas et al. (1992) presents a conclusion that styrene leads to impairment in color vision; however, this conclusion is not well-supported by the study results and this study was significantly criticized in a letter to the editor by Muttray (1993). There was no statistical difference in error scores when comparing the styrene-exposed workers and the control group. The authors did not state whether one orientation of defect class was more prevalent than the other nor how they determined whether a defect was predominantly red-green or blue-yellow. The analyses presented are of poor quality, and there is no mention of controlling for confounders (i.e., age). It is likely that the analysis of error scores was probably underpowered to find a difference between these two groups given the variability presented. Although age-based subgroups are proposed in the methods, there is no indication that statistical analysis of these groups was carried out in the results. Further, it is unclear why there was no attempt to evaluate any association between urinary MA concentration, PGA, and color vision test results (i.e., after controlling for age in the styrene-exposed workers, does MA or PGA predict the error score). There is lack of sufficient details regarding the sampling methods used in order to ascertain any potential selection bias associated with subject recruitment. There is no mention of masking of the examiners who performed the testing regarding subject exposure status. Also, the styrene exposure sampling method was for the workshop rather than through personal monitors and there is no indication of duration or frequency of exposure to the peak styrene levels (469 ppm).Of note, the actual error scores presented are much higher than what might be expected, both in the styrene-exposed workers and the control group. The authors stated that they used “a specially devised automated procedure for analysis of the results” but do not sufficiently describe the method. Moreover, the actual testing methods used for color vision testing are not described sufficiently. The lighting used was not optimal, and there is no mention of whether testing and scoring was carried out monocularly or binocularly. Muttray’s criticized the Fallas et al. study for improper illumination, lack of control over subject’s glasses and contact lens status, failure to exclude subjects with congenital color vision defects, failure to screen vision or for ophthalmic conditions, skepticism of error scores results given their high means compared.

Fallas did respond to Muttray's criticisms but the responses were less than reassuring which suggests Fallas and his colleagues were unfamiliar with many aspects of color vision and testing methods.

The Gobba and Cavalleri (1993) study presents data published in Gobba et al. (1991) plus some additional data. Due to the problems identified for the previous study group, specifically no matching was conducted, their second study assembled age, gender, alcohol, and tobacco matched controls. Although this strengthens the study's validity, there is still lack of details regarding sampling methods used to assemble the styrene-exposed workers and controls. With the new sample, the conclusion that styrene exposure can impair color vision seems valid. However, the conclusion that age and styrene exposure have a synergistic effect on impairment of color vision is not supported. It appears that no interaction terms were examined in the multiple regression analyses, therefore, it is not appropriate to suggest this relation between these two predictor variables on color vision impairment. The data as presented suggest that there is a dose-response effect of styrene on color vision when comparing high and low level styrene exposure groups. However, as in their previous work (Gobba et al. 1991), it appears as if the low exposure CCI mean (1.11) probably does not statistically differ from the control group mean CCI (1.053). This finding in a second sample of patients may suggest that low exposures of styrene (< 50 ppm) do not impair color vision. In fact, the low exposure group CCI mean (1.11) is actually very similar to the previous control group CCI mean (1.151). The conclusion regarding the short-term eliminations of styrene exposure does not seem to be valid, as only a very small sample of patients underwent this test protocol. The authors conclude that most color vision discrimination alterations are blue-yellow in origin, although no data are presented to that end. Finally, there is no discussion of masking of the examiners who performed testing in terms of exposure status of the subjects.

The Castillo et al. (2001) study was a follow-up study to the initial reports regarding RFP plant workers of Campagna et al. (1992, 1995, 1996) and Mergler et al. (1996). The authors found a significant increase in the CCS score between the first two assessment periods, but not between the earlier assessments. It is confusing why the authors chose to analyze color vision in terms of the CCS, rather than the traditional CCI, which they used in their previous reports. The CCS differs from the CCI in that it assigns a score equivalent to the percentage increase in distance traveled around the color circle based upon the specific color cap replacement. Thus, it makes it difficult to compare across studies, and it is unclear why the authors chose to analyze data in this manner. Another concern is the analysis of longitudinal data in this study. The statistical models may not be appropriate, as repeated measures ANOVA was not used. Repeated measures ANOVA is generally a more powerful design, as it is based on within-subjects variance, providing a more precise estimate of experimental error. If repeated measures ANOVA was used here, it may have shown a significant difference between the 3 color vision assessment periods in terms of the CCS. It would have also allowed the investigators to control for age, although age adjustments appear to have been made. Additionally, when using vision testing at nearpoint within a longitudinal study, it is extremely important to control refractive status. The normal reduction in uncorrected near vision seen with age can greatly influence visual acuity and contrast sensitivity testing.

A possible limitation of these studies is the lack of an unexposed control. It is difficult to draw reliable conclusions from the first study because of inconsistencies between the different statistical analyses of the results, and uncertainties as to the possible influence of direct eye irritation. It should also be noted that the majority of CCI scores for the workers in this study are likely to have been within the normal age-related range of reference values. The follow-up investigation at 2 years showed no changes in the mean CCI scores over this time period. In some workers within the study population there was a correlation between increases in urinary MA concentration and a decline in CCI scores. Among the small subgroup of workers re-evaluated at 9 years, no changes in mean CCI were detected, although styrene exposure had decreased. At best these data are only suggestive of a possible effect of styrene on color discrimination, but it is difficult to ascertain the magnitude of any deficiency. Overall, these 3 Canadian studies appear to contribute little to understanding styrene's potential effects on color vision.

The preceding studies by Campagna et al. (1992, 1995, 1996) of Canadian RFP workers are also difficult to interpret. One statistical analysis suggested a positive relationship between CCI and urinary MA, but this was not confirmed by the analysis of those with and without color vision deficiency. From the mean urinary MA concentration of the workers in this study and using the ACGIH conversion, it is estimated that the mean amount of styrene inhaled by workers is equivalent to an airborne concentration of 30 ppm (8-hourTWA). Although there was no control group, it is noted that the mean CCI score from all the exposed workers (1.14) is between the 50^{th} and 90^{th} percentile of normal reference values (Table b), given the mean worker age (29 years). The reports of lacrimation and blurred vision also raise doubts over the interpretation of this study. Overall, there was no consistent evidence for a clear-cut effect of styrene exposure on color discrimination, and it does not seem possible to draw any firm conclusions from these studies.

Kishi et al. (2001), the group of investigators that reported the Eguchi et al. (1995) study of Japanese workers, conducted a second study in workers in 7 Japanese fiberglass RFP factories. Issues with this study include its stated purpose - to evaluate a dose-response effect of styrene on color vision with a 'larger sample of patients'; yet only 105 styrene-exposed workers were included. It is unclear how this sample size was determined to be large enough for the analyses required. The authors suggests that color discrimination is altered in styrene-exposed individuals compared with age-matched controls, although no supportive data are presented. Regarding dose-response evaluations, it appears that the analyses were underpowered to detect a difference between low, moderate, and high exposure groups. However, an additional analysis comparing these groups to age-matched controls shows that the CCI is significantly higher in the moderate and high-exposure groups than the control group. Thus, there was some evidence of a dose-response affect. It is unclear why no data were presented regarding the quantitative analysis of color vision (number of blue-yellow and red-green defects in the sample).

Two meta-analyses found high variability in effect size across the studies (related to both exposure-related parameters and differences in subject characteristics, tests, and testing conditions), but reached

different conclusions (Paramei et al., 2004; Benignus et al., 2005). Paramei et al. (2004) conducted a meta-analysis based on effect size in 5 studies that used the Lanthony D-15d test, had quantitative exposure estimates, and included a matched control group. Mean CCIs for styrene-exposed workers ranged from 1.21 to 1.29 vs. 1.05 to 1.17 for referents, and effect sizes ranged from 0.19 (Kishi et al., 2000) to 2.39 (Gobba and Cavalleri, 1993). Effect sizes varied to a greater extent than expected from within-study sampling error, and this non-homogeneity could not be explained by lighting conditions, levels or duration of exposure, or differences in age between subjects and referents. Although the average effect size for styrene was positive, it was not statistically significant. Indeed, the authors stated that "[t]he result for styrene scarcely failed a significant negative effect" (Paramei et al., 2004). Discussing this seemingly paradoxical finding, the authors noted that the insensitivity and inaccuracy of exposure measurements may play a dominant role, with testing protocol vagaries and confounding factors both known and unknown also contributing. In particular, they noted exposure characterization using current mean exposure levels as a source of error, given "accumulating evidence that the impact of cumulative exposure and of high past exposure levels is also of importance for explaining effects of solvents on colour vision…" (Castillo et al., 2001; Viaene et al., 2001; Gong et al., 2002).

Benignus et al. (2005) presented CCI results obtained from 6 studies where exposure was quantified as the product of inhaled-air styrene concentrations (estimated by converting urinary styrene or styrene metabolite concentrations to air concentrations) and exposure duration (ppm-years). In contrast with the approach taken by Paramei et al. (2004), these authors calculated a normalized effect value (percentage of baseline, defined as control or low-dose individuals according to study data) so that "…magnitude estimates were not conflated with variances…" (Benignus et al. 2005). The relationships between both mean and individual CCI results and exposure were significant but highly variable. A nomogram for predicting the magnitude of effect at different styrene concentrations and exposure durations of 2, 4, 6, and 8 years indicated a 2.23% increase in CCI after 8 years of exposure to the TLV-TWA (20 ppm), approximately equivalent to the change a person would be expected to experience with 1.7 additional years of age over that timeframe (Benignus et al. 2005). There are a number of methodological weaknesses in the analysis that question the validity and reliability of these findings. Firstly, it is noted that the meta-analysis presented failed to reflect the available literature, as several additional studies could have been included in the model calculations, but were not. Secondly, given that study group means for both concentration and duration of exposure rather than individual data were used and that there was no information on past exposure, the dose-response and time-response evaluations presented are likely to be unreliable. Thirdly, by using percentages of baseline response, the variability of the control groups was unaccounted for. Finally, by imposing a linear mathematical model to the dose-response relationships, the study failed to reflect the available data and the threshold nature of these effects.

In view of the conflicting results among studies, Seeber et al. (2009a) undertook a cross-sectional study of workers with repeated measurements in a German boat-building plant with three critical questions in

mind: (1) Are the published findings for color vision deficiencies and impaired contrast sensitivity reproducible? (2) If effects exist, are they related to current or chronic exposures? (3) If effects exist, do they diminish or disappear during an exposure-free period (during company holidays)? As mentioned previously, the same work setting was used to investigate effects on neurobehavioral effects (Seeber et al. 2009a) and ototoxicity (Triebig et al. 2009). Current exposure subgroups were objectively defined as low (n = 97), medium (n = 115), and high (n = 30) styrene exposures as defined by biomonitoring data (urinary MA + PGA concentrations and blood styrene concentrations), thereby deriving individual exposure measurements without confounding by personal protection measures. Job tenure was about 6 years. The urinary metabolite data were converted to airborne exposure concentrations of 1.7–3.4 ppm for the low group, 7.6–15.3 ppm for the medium group, and 39.1–48.9 ppm for the high group. Historical air monitoring data in the lamination department of this facility indicated median styrene concentrations of 100 ppm (range 10 – 200 ppm) in the 1980s and 80 ppm (range 5 – 150 ppm) from 1990 to 1995. Using the historical data, a "high-long" exposure group (n = 17, mean job tenure 14.6 years) and a "low-short" exposure group (n = 34, mean job tenure 6.4 years) were defined. CCI was assessed with the Lanthony D-15d test (1,000 lux, daylight fluorescent lamp) and contrast sensitivity with the Vistech chart VCTS 6500 during normal working schedule and company holidays. There were no significant associations between any styrene exposure parameters and CCI, which as expected was strongly associated with age, and not as expected, with native language, with native German speakers having significantly lower CCI than non-German speakers. CCI performance improved significantly during holidays, but not in relation to styrene exposure. Overall, the analyses for contrast sensitivity showed a strong relationship with age but not with styrene exposure. The authors concluded that neither acute styrene exposure levels of 40 ppm (range of standard deviation up to 54 ppm) nor long-term exposures to 27 ppm (range of standard deviation up to 44 ppm) for 15 ± 7 years with past exposures of 50 to 100 ppm were associated with elevated risks for the parameters of color vision and contrast sensitivity (Seeber et al. 2009b). They also pointed out that their sample size was sufficient to ensure detection of "non-tolerable" functional changes in visual test performance.

The systematic review and meta-analysis recently performed by Choi et al. (2017) concluded that available evidence supports the hypothesis that chronic occupational exposure to styrene at "levels below most regulatory agency-prescribed exposure limits" can induce subclinical deficits in color vision. However, the analysis was not designed to yield a threshold concentration or NOAEL, and the authors reported no consensus regarding a threshold exposure concentration that causes acquired dyschromatopsia. Findings regarding the reversibility of dyschromatopsia were described as sparse and mixed. Of the 15 studies evaluated qualitatively by Choi et al. (2017), only the Seeber et al. (2009a) study did not support the authors' hypothesis. Of possible relevance in this regard is Seeber et al. (2009a) excluded participants on the basis of demonstrated red-green deficiency (as well as "congenital color-blindness") in order to avoid potential impacts of this predominantly inherited deficiency on the color discrimination test that could have "inflated" styrene impacts in other studies. Taking a contrary view, Choi et al. (2017) suggested that elimination of such individuals could have "inadvertently excluded

advanced cases of acquired dyschromatopsia, with more clinically significant symptoms." Given that congenital color-blindness is generally of the red-green variety (Deeb, 2004), it is not clear to what extent this criterion actually eliminated participants not identified as "congenitally" color-blind. Acknowledged limitations of the Choi et al. (2017) analysis include (1) the previously mentioned propensity for false positives in the D-15 d protocol, (2) the lack of blinding in 10 of the 15 studies, and (3) perhaps most importantly, the lack of knowledge regarding the magnitude of past exposures.

Applying a variance-stabilizing, log-scale transformation of mean CCI, Choi et al. (2017) reported low- to-moderate heterogeneity in CCI among 8 studies of styrene-exposed workers selected for meta-analysis (which did not include Seeber et al. 2009a). For this evaluation, studies with significant co-exposure to chemicals other than styrene were not considered, nor were studies that did not contain a direct measurement of styrene exposure, either through airborne measurements or urinary biomarkers. In total, 352 exposed and 355 control workers were included. Pooled analysis using a random effects model yielded an overall standardized mean difference (SMD) of 0.56 (95% confidence interval [CI]: 0.37, 0.76; $P < 0.0001$), and a fixed effects model yielded a SMD of 0.53 (95%CI: 0.37, 0.68; $P < 0.0001$), stated to indicate a "medium-size effect," which was not further defined in terms of functional significance. A "mild" publication bias against smaller studies with negative results was suggested, but the implications were not discussed.

Considering the sum of available evidence in humans and animal models, critical questions remain regarding the MOA, dose-response, functional significance, and reversibility of occupational styrene-induced color vision deficiencies. As such, the data base is considered insufficient for purposes of developing toxicological criteria based on this endpoint.

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