

May 29, 2007

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Texas Commission on Environmental Quality

Delivered Electronically: mhoneycu@tceq.state.tx.us

RE: Scientific and Policy Considerations Concerning Effects Screening Levels for Styrene

Dear Dr. Honeycutt:

The American Composites Manufacturers Association (ACMA) appreciates the opportunity to submit these comments to the Texas Commission on Environmental Quality (TCEQ) concerning the effects screening level (ESL) for styrene.¹ The TCEQ proposed to replace the current permit by rule (PBR) for thermoset resin facilities with a new air quality standard permit. The proposed standard permit conditions were written to ensure that ground level concentrations near the plant do not exceed the ESL for styrene (and alpha methyl styrene and methyl methacrylate).² Air dispersion models prepared by the TCEQ suggest that maximum 1-hour ambient air concentrations of styrene under the proposed permit would not exceed the current ESL, but the resultant emission, and by extension raw material usage limits have, and continue to impose significant constraints on the operation and growth of the Texas composites manufacturing industry, particularly when compared to competing facilities in surrounding states.

ESLs are based on data concerning health effects, potential nuisance odor, and vegetative effects, but are not ambient air standards. As the TCEQ notes, “if ambient air concentrations exceed the ESL, it does not necessarily indicate a problem, but triggers a more in-depth review.”

Summary

As detailed in these comments and the enclosed materials ACMA submits that:

- A. It is inappropriate to base the styrene ESL on odor, if consideration is taken of the policy thrust of the federal Clean Air Act, the recommendations of the TCEQ-sponsored peer review process, and TCEQ’s own record for developing ESLs.
 - a. TCEQ should consider the fact that the Federal provision governing toxic air pollutants does not authorize EPA to regulate odor, but only health and ecological effects. Where EPA is arguably authorized to regulate odor (in the standards for ozone, sulfur oxides, and other “ambient” pollutants), it has rarely done so. This provides some helpful

¹ The American Composites Manufacturers Association is the national trade group for the composites industry. Our members include small and medium-sized companies that use combinations of thermoset plastic resin, glass fiber and other materials to make underground gasoline storage tanks and pollution control equipment, wind turbine blades, modular tub/shower units and bathroom vanities, ballistic panels and armor for military vehicles, fiberglass boats, automotive, truck and motorhome components, window lineal and ladder rail, bridge decks and concrete reinforcing bars, playground equipment, components for commercial and military aircraft, signs and building fascia, and thousands of other composites products, as well as the suppliers of raw material to this industry.

The more than 150 composites manufacturing companies in Texas directly employ over 7,500 workers and have combined annual sales revenue of more than \$950 million.

² http://www.tceq.state.tx.us/assets/public/permitting/air/Announcements/draft_thermosetresin4_07.pdf.

guidance about the importance of odor vs. health in the overall framework for regulating air toxics.

- b. Although TCEQ contracted with the Toxicology Excellence for Risk Assessment (TERA) to conduct a peer review of its methodology for developing ESLs, TCEQ has apparently chosen to disregard the advice it received regarding setting ESLs for odor. This undermines the scientific validity of the resulting ESLs.
 - c. Our review of TCEQ's past practices has revealed that in less than a quarter of the cases has TCEQ followed its own policies in developing odor ESLs, and in the case of five well-known chemicals, TCEQ appears to have chosen to establish the ESL on the basis of a higher health level and not on the lower odor level for these chemicals. Similarly, TCEQ needs to tailor its development of a styrene ESL to the underlying science and not follow a rigid formula for development of an ESL that results in an unacceptable number.
- B. In calculating the proposed odor ESL for styrene, TCEQ did not follow the opinions of authoritative bodies and the best scientific literature.
- a. Both USEPA and the Agency for Toxic Substance and Disease Registry (ATSDR) concluded that the odor threshold for styrene is 1,360 $\mu\text{g}/\text{m}^3$ and both agencies rely on the same study, J.E. Amooore and E. Hautala (1983). Amooore and Hautala (1983) derived the odor threshold from an average value because none of the studies available at the time, including Stalker (1963) on which TCEQ relies, met all of the applicable good-methodology and study design criteria.
 - b. TCEQ's reliance on Stalker (1963) is flawed because that study did not employ the forced-choice method associated with best practice methodology. The very low odor threshold reported by Stalker (1963) is highly likely to be an artifact or outlier stemming from this methodological weakness. In addition, TCEQ made no adjustment for the difference between the potential for clinical perception and the concentration of styrene that will be detected in ambient air by the public, which would increase the level by a factor of 4 or 5. It is precisely because of the limitations in the study and variations in odor detection methodology generally, that USEP and ATSDR turned to the averaging approach reflected in Amooore and Hautala (1983).
 - c. The only odor detection level study for styrene that meets all of the good methodology criteria is Dalton et al. (2003). The methodology used in this study meets all applicable criteria, including the forced choice procedure. The main strengths of this work are the accuracy of the analytical measurements and the large size of the sample (67 subjects). Thus, the odor detection threshold of 1,905 $\mu\text{g}/\text{m}^3$ in Dalton (2003) is a very reasonable data point. TCEQ does not present a compelling basis for rejecting the only fully acceptable study, particularly when the odor detection level in Stalker (1963) is both methodologically compromised and one or two orders of magnitude less than other studies of comparable quality.
 - d. TCEQ ignored the scientific literature that stresses the substantial difference between the concentration of an odorant that *can* be detected in clinic studies and the concentration that *will* be detected under normal conditions. When TCEQ calculates an odor ESL, the clinical odor detection threshold should be multiplied by a factor of 4 or 5 to determine the odor awareness threshold. The odor awareness threshold would be a conservative criteria when contrasted with an actual nuisance odor level.

- C. ACMA's review of odor is not intended to endorse the use of an odor ESL for styrene, but demonstrates that best approach is to base the styrene ESL on health effects. Based on a review of the toxicology data for styrene, which is summarized in an enclosure to these comments, the physiological functions affected by the lowest exposures to styrene are neurological; e.g., neuromuscular reaction times and color discrimination, reported in studies of workers in the composites industry. An appropriate annual ESL based on a best science review of potential health effects would be the same as the US Environmental Protection Agency's (USEPA) reference concentration for styrene, which is 1 mg/m³ (1,000 µg/m³) averaged over a lifetime. A reasonable derivation of an hourly value results in values comparable to those developed by other states which are detailed in Enclosure 1. ACMA looks forward to exploring this topic further with TCEQ.

ACMA has endeavored to keep its comments concise. They are supported by enclosures that present a styrene toxicology summary, a survey of styrene air regulation in more than 20 states and a review of styrene odor threshold values. Naturally, ACMA would be happy to elaborate on the points raised in these comments if it would assist TCEQ.

I. The styrene ESL should not be based on odor

1. TCEQ should take guidance from the federal Clean Air Act in determining the weight it should give to odor concerns

TCEQ should inform the exercise of its regulatory discretion by considering the longstanding policies and practices of USEPA under the Clean Air Act with regard to the regulation of odor and protection of welfare generally. Under the section of the Clean Air Act that most closely parallels the Texas ESL authority, Section 112(f) [toxic air pollutants], USEPA is not authorized to consider odor in establishing standards. Only public health and significant and widespread ecological effects can be considered. With regard to ambient air quality standards for carbon monoxide, lead, nitrogen dioxide, particulate matter, ozone, and sulfur oxides the Administrator of EPA may set standards for the protection of both health (primary standards) and welfare (secondary standards). Welfare is defined in section 302(h) of the CAA to include personal comfort and well being, which presumably includes odor, although odor is not explicitly mentioned.³ USEPA has given strong preference to setting health (primary) standards and, in most instances, when USEPA has set secondary standards they are the same as the primary standards and odor has not been considered.

The comparison of federal and state systems does not mean that Texas can not consider odor. Rather, it means that odor should not be given great weight. The absence of any direct reference to odor under the Texas statute that TCEQ is implementing simply reinforces the secondary nature of odor in the regulatory framework.

³ Section 302(h) states: "All language referring to effects on welfare includes, but is not limited to, effects on soils, water, crops, vegetation, man-made materials, animals, wildlife, weather, visibility, and climate, damage to and deterioration of property, and hazards to transportation, as well as effects on economic values and on personal comfort and well-being, whether caused by transformation, conversion, or combination with other air pollutants."

2. TCEQ's Treatment of Odor is Inconsistent with the Advice Provided by the Peer Review Toxicology Excellence for Risk Assessment (TERA) Panel

TCEQ contracted with Toxicology Excellence for Risk Assessment (TERA) to conduct a peer review of its procedures for developing ESLs. It appears that in the case of odor, TCEQ has not followed the guidance of the TERA peer review panel. Throughout its report, the TERA Panel consistently questioned the approach that TCEQ had laid out for the establishment of odor ESL's and recommended that TCEQ use sensory irritation rather than odor as the basis of their ESL's:

The panel suggested that the choice of a 50% odor threshold for setting the odor ESL should be better explained in the document because the ability to perceive odor does not necessarily correlate with concentrations associated with toxicity, and odor detection also involves cognitive issues not related to chemical concentration. The panel suggested that the potential for sensory irritation, as measured by the concentration that results in a 50% reduction in respiratory rate in rodents (the RD50), would be a better basis for an ESL than odor.⁴

TERA also asked an odor expert to review the TCEQ document with regard to odor and his written comments are contained in Appendix D of the TERA Report. In his analysis, Dr. William Cain, Professor of Surgery (Otolaryngology), in the Chemosensory Perception Laboratory at the University of California, San Diego, stated that:

These data lead me to believe that the EPA/AIHA set is [sic] systematically overestimates threshold concentrations and has many values that are incorrect by substantial factors. In light of these new results, I certainly question the use of the EPA/AIHA values at all and believe that new data gathering is probably the only way to fix the problem.⁵

The response of a TCEQ representative to the TERA panel serves to highlight a critical issue in controlling odors:

In addition, panel members note that cognitive issues are involved with odor perception, TCEQ replied that odor perception is an important factor to consider when working with the public, since the public tends to believe that if they can smell a chemical, it must be harmful.⁶

It is ACMA's understanding that odor detection is not regarded as a toxicologically relevant endpoint -- annoyance does not represent a sensory or psychological effect, but rather a psychological discomfort from the presence and increasing concentration of an odor.⁷ The detection of foul odors is a defense mechanism which occurs through both olfactory and trigeminal stimulation. The olfactory stimulation relays messages to the brain using the first cranial nerve for odor perception while trigeminal stimulation is responsible for sensing the ocular and nasal irritation of a chemical using the fifth cranial nerve.⁸

⁴ Report of the Peer Review Meeting on Development and of Effects Screening Levels, Reference Values, and Unit Risk Factors for the Texas Commission on Environmental Quality (TCEQ), organized by the Toxicology Excellence for Risk Assessment (TERA), Executive Summary at 5 (Oct. 12, 2005).

⁵ *Id.* at D-10.

⁶ *Id.* at 30.

⁷ Arts et al. (2006b); McGinley (1999).

⁸ Paustenbach and Gaffney (2005); McGinley (1999).

Studies have shown that even a pure odorous substance, lacking any trigeminal stimulation, can elicit reports of sensory irritation.⁹ For the majority of chemicals, odor has a zero correlation with actual exposure risk, but odor may have a substantial correlation with perceived exposure risk. However, odor detection may tap into a person's aversions to unpleasant odors.¹⁰ Because the vast majority of volatile chemicals stimulate the olfactory system at concentrations well below that at which they will elicit trigeminal activation, the evaluation of irritation from volatiles is often confounded by the perception of odor.¹¹

Styrene is not an irritant at its odor threshold; but in the fence-line context, it is possible for the public to perceive the substance and its odor as harmful. Such perception may influence the reporting of irritation where only odor exists. As a result, measurements of sensory irritation can strongly be biased by subjective feelings and interpretations, in many instances caused by the odor of the compound.

The TCEQ representative's reply to the peer review panel that odor is important because the public (incorrectly) tends to believe that if they can smell a chemical, it must be harmful suggests the need to educate the public that the odor of certain chemicals (such as styrene) does not indicate risk. The alternative of regulating the detection of unharmed odors has the undesirable effect of confirming the public's misconceptions about odor.

3. The TCEQ Odor ESLs Have Not Been Applied Consistently

The ACMA identified several instances in which the ESL determination process was applied inconsistently. These inconsistencies raise significant concerns about the fundamental fairness of the styrene ESL development process. We reviewed the 38 odor ESLs that are listed as acceptable in the AIHA study and discovered that only 9 of these followed the ESL determination process. The number of "acceptable" ESLs would increase to 14 were the TCEQ to utilize recognition threshold instead of detection threshold. But at this juncture, only 24% of these 38 odor ESLs comply with the ESL Guidelines.

In addition, a random review of the TCEQ ESLs revealed that, for at least five well-known substances, the selected health-based ESLs are significantly higher than the AIHA-approved odor thresholds:

Substance	Health-Based ESL	Lowest Odor Threshold
MIBK	2,050	352
Toluene	1,880	603
Acetic acid	250	91
Propyl alcohol	4900	76
Butyl alcohol	610	364

As in the case of these five substances, the ACMA respectfully submits that odor ESLs are fundamentally unsuitable as a basis for setting a short-term ESL for styrene and that health represents a much sounder scientific basis for regulating this chemical.

⁹ van Thriel (2006).

¹⁰ Paustenbach and Gaffney (2005).

¹¹ Arts et al. (2006b).

4. TCEQ erred in basing a styrene odor ESL on Stalker (1963)

a. *Methodological Flaws*

When we consider Stalker (1963), upon which TCEQ relies, the critical feature of this study concerns the presentation procedure. Indeed, the subject had to indicate the initial perception of the odorant by raising his hand. Contrary to the forced choice method which is the procedure of choice in such studies today, this procedure induces anticipation effects and increases the bias associated with a subject's criterion for responding. Given this choice of presentation methodology, it is not surprising that Stalker (1963), which reported an odor detection threshold of $73.6 \mu\text{g}/\text{m}^3$, is at the very bottom end of the range of 11 studies that might be considered (73.6 to $8530.1 \mu\text{g}/\text{m}^3$) and where the average of those studies is $1,736.46 \mu\text{g}/\text{m}^3$, as reflected in the enclosed review of styrene odor threshold values in air. This out-of-date outlier study should not serve as the basis of any odor ESL.

b. *Odor Detection and Odor Awareness*

Although awareness of odor can occur at concentrations where the odor is just detectable, other information such as odor quality, identifiability and unpleasantness typically requires exposure to higher concentrations (Sucker et al, 2001). For this reason, evaluating the relationship between the detectability of environmental odorants and the concentration at which they become unpleasant and elicit annoyance or concern is a critical step in their regulation and management.

Overall, as for most chemical compounds, reported odor thresholds for styrene span a considerable range. Indeed, it is not uncommon for reported odor thresholds values to range over four orders of magnitude for the same chemical. For example, 29 values were reported for n-butyl alcohol, ranging from 1.45×10^{-7} to 1.88×10^{-4} g/L (Amoore and Hautala, 1983). The lack of standardization of method used to determine odor thresholds as well as the large variability of human sensitivity are responsible for the wide range of thresholds concentrations usually found in the literature. The enclosure to these comments entitled "Review of Styrene Odor Threshold Values in Air," reviews the methodology for determining odor threshold, including methods of delivery, methods of stimulus presentation, and methods of odor detection threshold measurement.

It is important for regulatory officials to consider the factors affecting odor detectability in real-world environments. There is a substantial difference between the concentration of an odorant that *can* be detected in a clinical setting and the concentration that *will* be detected in an ambient setting.

In a study evaluating the influence of various degrees of distraction on responsiveness to warning odors the researchers found significant differences in the chemical concentration needed for subjects to accurately detect odor in directed vs. undirected tests (Amoore and Hautala, 1983a). When subjects were not given instructions to detect an odor, they showed a four-fold decrease in detectability when compared with a more typical laboratory experimental condition in which subjects were specifically instructed to attend to and detect the target odor. It should also be noted that laboratory odor detection thresholds are typically obtained in an environment where the air is purified and filtered and hence the background for the target signal is much lower. Signal detection theory has shown that as background noise increases, signal detectability decreases (Swets *et al.*, 1961). In a non-laboratory environment, the ambient background typically contains many other odorous compounds which have the potential to significantly increase the signal-noise ratio. This would serve to reduce the detection threshold for a chemical when compared with the concentration necessary for detection obtained in a laboratory setting.

Based on awareness of the above modifying factors, for example, van Dorn and colleagues (van Dorn *et al.*, 2002) applied a four-fold correction (increase) to the odor detection threshold as the initial step in the process for determining community awareness of industrial odor.

To assist in the interpretation of such field data, a number of studies have been conducted to establish the relationship between odor annoyance and odor detection threshold (Adams et al, 1968; Hellman & Small, 1974; NCASI, 1971; Steinheider & Winneke, 1993; Sucker et al, 2001; Winneke & Kastka, 1977). Results from these studies suggest that, as a general rule of thumb, the ratio of perceived annoyance to odor detection levels for unpleasant odorants is approximately 5-1, although this obviously could vary across compounds having different hedonic qualities.

Thus, taking into consideration factors such as the relationship of perceived odor nuisance or annoyance to odor detection levels, as well as the likelihood of other background odors rendering any odor signal less detectable, various states have set fence-line emission limits at dilution to threshold ratios that range from as much as 7-1 to as little as 4-1. In combination with regulations on the number of exceedances of this limit (for both frequency and duration) this approach seems reasonable, given the numerous factors which can affect odor detectability and nuisance impact at distances beyond the fence-line.

Given the frequent disparity in reported odor thresholds found in the published literature, one additional question to be addressed is which value to use in setting regulations. Without question, studies need to be evaluated with regard to adherence to the criteria for testing and analytical integrity set forth earlier. Once having passed this initial review, however, one can still find differences in odor thresholds spanning an order of magnitude. While it may be tempting to assume the lowest value in a data set is the most protective, there are compelling reasons to seek a 'weight of evidence' approach with regard to convergence of threshold data values across laboratories and methods. Indeed, depending on the test panel composition (gender and number) a very low average threshold may occur as a function of having selected a small number of extremely sensitive and well-trained individuals for participation. When we consider studies where a larger number of individuals are tested, the average thresholds are typically higher and may be more representative of the general, naïve population.

Thus the use of the odor detection threshold reported in Stalker is doubly flawed. First, there is a methodological flaw that plainly resulted in an exceptionally low threshold calculation. Given the extremely high variability in the studies on styrene, choosing the study with the lowest level is not scientifically sound, giving regulatory validity to an outlier study. Further, TCEQ did not make any adjustment for the difference between the potential for clinical perception and the concentration of styrene that will be detected in ambient air by the public. Thus, even if TCEQ were to revisit Stalker, the appropriate odor ESL would be four or five times greater than the reported threshold plus an additional increasing adjustment factor based on the failure to use the forced-choice method.

The state of the science relating to odor detection and perception coupled with the flaws in the study on which TCEQ seeks to base a styrene odor ESL, simply reinforce the ultimate conclusion: the styrene ESL should be based on health effects and not odor.

II. Deriving a styrene ESL based on health effects

The enclosed summary of styrene toxicology is based on much more extensive work by the Styrene Information and Research Center, Inc. (SIRC), which serves as a liaison between industry, federal and state governments, and international agencies on health-related issues involving styrene. Through great expense, SIRC and its members have developed sound medical, scientific and technical information for

the use by industry and federal/state regulators on health and environmental issues associated with styrene.

Based on a review of human, animal and other data, the physiological functions affected by the lowest exposures to styrene are neurological; e.g., neuromuscular reaction times and color discrimination, reported in studies of workers in the reinforced plastics industry. The US EPA Reference Concentration (RfC) for styrene is 1 mg/m^3 , derived from the study of Mutti et al. (1984), one of four studies examining a total of 193 composites workers with reported exposures of three to 280 ppm reporting slower reaction times than the controls. The American Council of Governmental Industrial Hygienists (ACGIH) adopted a Threshold Limit Value (TLV) of 85 mg/m^3 (20 ppm) for workplace exposures of eight hours per day, five days per week, for a working lifetime, based on effects reported in humans on the peripheral and central nervous systems. Such effects are not specifically characteristic of exposures to styrene, but rather are characteristic of exposures to a very large class of aromatic and nonaromatic hydrocarbons. Our scientists believe that the US EPA RfC provides a conservative estimate for the Environmental Screening Level (ESL): $1,000 \text{ } \mu\text{g/m}^3$ (1 mg/m^3) averaged over a lifetime. ACMA sees this as a departure point for discussing the derivation of an hourly effects analysis used by TCEQ for health effects ESLs.

In this regard, it is important to note that a health-based ESL for styrene would bring Texas in line with neighboring states. One enclosure consists of a table showing the ambient air toxics regulatory level for styrene in more than 20 states, including all EPA Region 6 states, Georgia and Tennessee. The 1-hour equivalent values adopted by these states range from 1,704 to $21,000 \text{ } \mu\text{g/m}^3$. These states have many composite operations that compete with those in Texas. Those operations benefit significantly from less-restrictive styrene screening limits at the expense of Texas businesses.

III. Conclusion

Based on the foregoing, the ACMA submits that a health-based ESL is the best method for regulating styrene. As demonstrated by the enclosed summaries of styrene toxicology and neighboring states' limits, an appropriate health effects ESL for styrene can be derived by studying the USEPA RfC of $1,000 \text{ } \mu\text{g/m}^3$ and the regulatory approach taken in many other states. An odor-based ESL for styrene is inappropriate for policy, fairness, and practical considerations. Clearly, the odor threshold approach proposed by TCEQ for styrene is undermined by serious scientific flaws and oversights.

Thank you very much for the opportunity to provide these brief comments on the scientific and policy considerations affecting the development of short-term ESLs for styrene. We hope that this discussion will also assist the TCEQ in developing appropriate ambient air limitations for the proposed standard air permit. Please contact me if you have questions or would like to discuss any aspect of these comments.

Respectfully submitted,



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Enclosures:

1. Comparison of State Ambient Limits for Styrene
2. Summary of Styrene Toxicology
3. Summary of Odor Science

Comparison of Ambient Air Toxic Impact Limits for Styrene Vapor

last revised on 5/29/07

State	Limit name	Value (µg/m ³)	Averaging Period	Equivalent 1-hr value (µg/m ³)	Basis	Equation or Source	Citation
AL	n/a	2,130	1-hour	2,130	Health	<u>ACGIH '06 TLV</u> 40	ADEM Modeling Guidelines - April 2006
		203	annual			<u>ACGIH '06 TLV</u> 420	
AR	PAIL	852	24-hour	2,130	Health	<u>ACGIH '06 TLV</u> 100	Nuisance odor is treated case-by-case as a local issue Non-Criteria Pollutant Control Strategy – 1996 Screening Modeling Protocol
AZ	AAAQC	3,500	1-hour	3,500	Health	Appendix B AAAQC list	Air Modeling Guidelines for AZ Air Quality Permits July 1992
		1,700	24-hour				
CA	AIL	21,000	1-hour	21,000	Health	n/a	Consolidated Table of OEEHA/ARB Approved Risk Assessment Health Values
CT	HLV	21,500	30-min	21,500	Health	Table 29-3 April 4, 2006	RCSA 22a-174-29
		4,300	8-hr				
FL	none	n/a	n/a	n/a	NAAQS	n/a	FL DEP only requires NAAQS/PSD modeling FL repealed air toxic modeling under FAC 62-210.500
GA	AAC	1,000	annual	12,500	Health	IRIS RfC	Guideline for Ambient Impact Assessment of Toxic Air Pollutants – June 1998
IA	none	n/a	n/a	n/a	NAAQS	n/a	IA DNR only requires NAAQS/PSD modeling
IL	none	n/a	n/a	n/a	NAAQS	n/a	IL EPA only requires NAAQS/PSD modeling
IN	none	n/a	n/a	n/a	NAAQS	n/a	IN DEM only requires NAAQS/PSD modeling
KY	none	n/a	n/a	n/a	NAAQS	n/a	Odor is regulated by direct measurement Appendix A - 401 KAR 53:010
LA	AAS	5,070	8-hour	7,243	Health	<u>ACGIH '93 TLV</u> 42	LAC 33.III Chapter 51 Table 51.1
MD	TAP screening limits	1,704	1-hour	1,704	Health	<u>ACGIH '06 TLV STEL</u> 100	COMAR 26.11.16.07
		852	8-hour			<u>ACGIH '06 TLV</u> 42	
MI	ITSL	1,000	24-hour	2,500	Health	Table 1	MAC R 336.1225
MN	acute HRV	21,000	1-hour	21,000	Health	n/a	MN DoH Health Risk Values for Air - March 2002
	chronic HRV	1,000	annual			IRIS RfC	
MO	RAL	2,240	1-hour	2,240	Health	non-public - must call DNR	List maintained by state toxicologist
		333	annual				
NC	AAL	10,600	1-hour	10,600	Health	<u>OSHA PEL</u> 40	15A NCAC 2D.1104
NH	AAL	1,000	24-hour	2,500	Health	Table 1450-1	NH ARAR Env A 1400
		1,000	annual				

Comparison of Ambient Air Toxic Impact Limits for Styrene Vapor

last revised on 5/29/07

State	Limit name	Value (µg/m ³)	Averaging Period	Equivalent 1-hr value (µg/m ³)	Basis	Equation or Source	Citation
NJ	RfC _{ST}	21,000	1-hour	21,000	Health	CARB OEEHA May 00	"Technical Manual 1003" - NJAC 7:27-17
NM	none	n/a	n/a	n/a	MACT	n/a	20.2.72.502 NMAC Modeling Guidelines – Section 2.8
NV	none	n/a	n/a	n/a	NAAQS	n/a	NV DEP only requires NAAQS/PSD modeling
NY	SGC	17,000	1-hour	17,000	Health	SGC/AGC Tables	6NYCRR part 212 Air Guide 1 - November 1997
	AGC	1,000	annual			IRIS RfC	
OH	MAGLC	2,028	1-hour	2,028	Health	<u>ACGIH '06 TLV</u> 42	OAC 3745-114-01
OK	MAAC	4,260	24-hour	10,650	Health	<u>ACGIH '86 TLV</u> 50	OAC 252:100-41-40
OR	ABC	none	n/a	n/a	Health	styrene not listed or modeled as air toxic	OAR 340-246-0010
RI	AAL	20,000	1-hour	20,000	Health	Table 1	Reg 22 RI Air Toxics Guidelines - June 2005
		1,000	24-hour				
		100	annual				
SC	MAC	5,325	1-hour	5,325	Health	n/a listed in table	SC R.62.5 Standard 8 (D.) Facilities subject to MACT are exempt from modeling
TN	none	n/a	n/a	n/a	MACT	n/a	No modeling or nuisance odor citations appear in TN regulations or permitting policies
VA	SAAC	4,259	1-hour	4,259	Health	<u>ACGIH '06 TLV STEL</u> 40	9 VAC 5-60-230
		170	annual			<u>ACGIH '06 TLV</u> 500	
VT	HAAS	512	annual	1,280	Health	<u>OSHA PEL</u> 420	VA PER 5-261 Appendix B Cat II chronic / Appendic C acute Cat III styrene is not listed or modeled as acute air toxic
WA	ASIL	1,000	24-hour	2,500	Health	Class B Table	WAC Chapter 173-460-160
WI	SAAC	2,045	1-hour	2,045	Health	Table A	WAC NR 445.07
		1,000	annual			IRIS RfC	
WV	none	n/a	n/a	n/a	MACT	styrene is <u>not</u> not listed as state TAP	45 CSR 27 Toxic Air Pollutants
WY	none	n/a	n/a	n/a	NAAQS	n/a	Odor is regulated by direct measurement WAQS&R Chapter 2 Section 11(a)(i)
TX	ESL	110	1-hour	110	Odor	TARA mean of two lowest odor AIHA studies	MERA (RG-324 - Oct 2001) 2003 ESL Tables

Styrene Toxicology Summary

The following summary of styrene toxicology is based on much more extensive work and review by the Styrene Information and Research Center (SIRC), which serves as a liaison between industry, federal and state governments, and international agencies on health-related issues involving styrene. Through great expense, SIRC has developed sound medical, scientific and technical information for the guidance of industry and federal/state regulators on health and environmental issues associated with styrene.

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I. Introduction¹²

Styrene (ethenylbenzene) is a commercially important chemical used in the production of polymers, copolymers, and reinforced plastics. Exposure mainly occurs in industries and operations using styrene, and industrial sources are the most likely cause of general population exposure. Other potential sources of general population exposure include motor vehicle exhaust, tobacco smoke, and other combustion/pyrolysis processes. Low-level exposure of the general population can occur through the ingestion of food products packaged in polystyrene containers.

General population exposure levels are usually orders of magnitude lower than occupational exposure levels - though the latter vary considerably depending on the operations concerned. While some exposure occurs in styrene and polystyrene manufacturing plants, the highest levels of exposure are found in the industries and operations concerned with the fabrication and application of plastics.

II. Exposure Level Presenting No Significant Risk of Harm¹³

The physiological functions affected by the lowest exposures to styrene are neurological; e.g., neuromuscular reaction times and color discrimination, reported in studies of workers in the reinforced plastics industry.¹⁴ The US EPA Reference Concentration (RfC) for styrene is 1 mg/m³, derived from the study of Mutti et al. (1984), one of four studies examining a total of 193 reinforced plastics workers with reported exposures of three to 280 ppm reporting slower reaction times than the controls. The American Council of Governmental Industrial Hygienists (ACGIH) adopted a Threshold Limit Value (TLV) of 85 mg/m³ (20 ppm) for workplace exposures of eight hours per day, five days per week, for a working lifetime, based on effects reported in humans on the peripheral and central nervous systems. Such effects are not specifically characteristic of exposures to styrene, but rather are characteristic of exposures to a very large class of aromatic and nonaromatic hydrocarbons. The US EPA RfC provides a conservative estimate for the Environmental Screening Level (ESL): 1 mg/m³.

III. Summary of Information from Human Exposures

The most consistently reported effects of styrene in humans are a slowing of reaction time and reduction in color discrimination. One study examined the relationship of styrene concentration and reaction time and concluded that the no observed adverse effect level (NOAEL) was 25 parts per million (ppm).

Two studies have examined dose-response in color discrimination by dividing the exposed workers in to high and low-exposed groups. In both studies, the highly exposed workers had decreased color discrimination. In one group the lowest-exposed cohort with an exposure of less than 50 ppm were not affected and in the other lowest-exposed cohort those with exposure less than 30 ppm were not affected. Most commonly, symptoms of central nervous system (CNS) depression (light-headedness, dizziness, incoordination, etc.) occur after exposures of greater than 100 ppm styrene for several hours.

¹² Abbreviations: NOEL (no observed effect level); NOAEL (no observed adverse effect level); LOEL (lowest observed effect level); LOAEL (lowest observed adverse effect level); SO (styrene oxide); R-SO (the R optical isomer of styrene oxide); S-SO (the S optical isomer of styrene oxide).

¹³ To convert ppm to mg/m³ the following formula was used: mg/m³ = ppm x MW/24.45. For styrene, the ratio MW/24.45 corresponds to 4.2536.

¹⁴ The literature does not refer to the slowing of reaction time as neuromuscular reaction time. It is true that this effect involve neurological processes and muscular actions, but it is usually referred to as "reaction time")

Exposures of workers at levels above 25 ppm have been inconsistently reported to result in a variety of reversible neurological effects. Exposure levels vary considerably and establishing a true effect level and no observed effect level (NOEL) has been very difficult due to the subtle nature of the effects. The levels of exposure that have been studied were very high compared to ambient levels of styrene or levels from plant effluents.

IV. Summary of Information from Test Animal Exposures

From animal studies of controlled exposure to styrene, the following toxicity endpoints have been reported:

a. General Toxicity (*decreased body weight or survival*)

NOAELs reported were:

500 mg/kg bw/day after 78 weeks of exposure by F344 rats;

600 ppm after 78 weeks (males) or 87 weeks (females) by Sprague-Dawley rats; 200 ppm after 104 weeks by Sprague-Dawley rats.

b. Nasal Olfactory Toxicity

LOAEL: 50 ppm after 2 years of exposure by rats;

LOAEL: 20 ppm after 2 years of exposure by mice.

This effect is highly dependent on the metabolism of styrene in the specific species studied. Metabolism of styrene in human nasal tissue had not been detected. Olfactory function was not affected in reinforced plastics workers exposed to styrene for at least 5 years at exposure levels greater than 30 ppm. This endpoint does not appear relevant for human risk assessment.

c. Lower Respiratory Toxicity

This effect is dependent on metabolism specific to a particular type of cell most prominent in the lower respiratory system of mice, Clara cells. Toxicity to Clara cells in the terminal bronchioles of mice (LOAEL, 20 ppm), but not rats, has been reported in several studies. In human upper respiratory and lung tissue, the metabolism of styrene is considerably less than in mice and rats, and not detectable in most samples. This endpoint does not appear relevant for human risk assessment.¹⁵

¹⁵ Clara cells are found in medium sized bronchioles and terminal bronchioles (leading into alveoli), not in the upper respiratory tract which is generally understood to consist of the nose, trachea and bronchus.

d. *Superficial Neurological Effects*

Cognition and behavior may be affected after exposure to high concentrations of styrene and other hydrocarbons. However, major effects only occur at very high exposures, on the order of several hundred ppm, and exposure durations of several hours. A typical NOAEL for a neurological effect, ototoxicity, was reported after exposures of rats to 200 ppm styrene by inhalation six hours per day five days per week for 13 weeks.

e. *Liver Toxicity*

Short inhalation exposures by several strains of mice for up to 3 days of 160 ppm styrene or greater resulted in liver toxicity. Liver toxicity was not reported for rats after exposures as high as 1,500 ppm.

V. Studies of Humans Exposed to Styrene

A. Short-term Human Exposures

Humans acutely exposed by inhalation to 800 ppm (3,400 mg/m³) for 3 hours experienced immediate eye and throat irritation, increased nasal mucous secretion, metallic taste, drowsiness, and vertigo. After test termination, slight muscular weakness, accompanied by inertia and depression were noted.¹⁶

Nine human volunteers were exposed to styrene vapor at concentrations of 50, 100, 216, and 376 ppm for varying periods up to 7 hours. None of the volunteers exposed at 50 ppm for 1 hour experienced any subjective symptoms or abnormal objective clinical findings. Vapor exposure at 100 ppm, however, produced mild, untoward, but transient subjective responses in half of those exposed. At 376 ppm, the majority of test subjects experienced unpleasant subjective symptoms and definite signs of neurological impairment.¹⁷

Human volunteers were exposed to styrene vapor for periods of 1-7 hours. At doses of 52 and 117 ppm subjects reported smelling a moderately strong, but not objectionable, odor of styrene upon entering the chamber. The odor diminished during the one or two hours of exposure. No symptoms or objective signs of illness were reported. One of nine subjects reported nasal irritation following 20 minutes of exposure to 216 ppm styrene. There were no neurological symptoms or physical findings. At 376 ppm styrene, two subjects reported mild ocular irritation within three minutes and two more within 15 minutes, and all reported nasal irritation. One subject reported a burning sensation of the skin of the face. The odor decreased but remained perceptible during the exposure period. Neurological impairments, measured by performance on a modified Romberg test, a dexterity test, and a coordination test, were noted during this exposure. One subject reported nausea, two reported a feeling of slight inebriation, and two reported headaches. Clinical lab studies, including complete blood count, erythrocyte sedimentation rate, reticulocyte count, serum glutamic pyruvic transaminase (SGPT), lactic dehydrogenase (LDH), alkaline

¹⁶ Clayton, G.D., F.E. Clayton (eds.) Patty's Industrial Hygiene and Toxicology. Volumes 2A, 2B, 2C, 2D, 2E, 2F: Toxicology. 4th ed. New York, NY: John Wiley & Sons Inc., 1993-1994., p. 1353.

¹⁷ American Conference of Governmental Industrial Hygienists, Inc. Documentation of the Threshold Limit Values and Biological Exposure Indices. 6th ed. Volumes I, II, III. Cincinnati, OH: ACGIH, 1991., p. 1441.

phosphatase, blood urea nitrogen, creatinine, glucose, and urinalysis, were normal and unchanged from pre-exposure values.¹⁸

To simulate a work day the same human volunteers were exposed to 99 ppm styrene for an initial 3.5 hours, followed by 30 minutes in an uncontaminated area, then 3.5 hours of additional exposure. Subjectively, three subjects reported mild eye or throat irritation within 20 minutes. At the end of the exposure period, the odor of **styrene** was barely perceptible and none of the subjects reported nausea, headache, or irritation of the eyes, nose, or throat. All reported no objection to working in this concentration for an indefinite period of time. There were no objective signs of impairment of balance or coordination. Results of clinical lab studies were again normal and unchanged from pre-exposure values.

B. Summary of Epidemiological Data

A number of epidemiological studies of workers exposed to styrene involving the production of polystyrene (PS), styrene-butadiene rubber (SBR), and reinforced plastics and composites (RPC) have been published. Of these, studies in the RPC industry provide the most definitive assessment of cancer risk in light of higher exposure to styrene and less confounding from other chemical exposures. In RPC studies, there is no clear evidence that exposure to styrene increases the risk of cancer. In the SBR and PS manufacturing industries, exposures to styrene are much lower, and are confounded by a variety of other chemicals, a number of which are known or suspected carcinogens. While several studies of workers in these two industries have shown increases in certain types of cancer, most notably leukemia, the data either strongly implicate other chemicals present, or show no pattern consistent with the presence of styrene. Overall, the combined weight of the evidence from studies of all industry segments supports the conclusion that styrene presents no demonstrable risk of cancer in humans.

The data on hematological evaluations of workers exposed to styrene do not present evidence of significant hematological alterations in humans. No effects on red blood cells were reported in seven studies, which included a total of 873 styrene-exposed workers at exposure levels generally exceeding 50 ppm but as high as hundreds of ppm styrene. A minority of studies reported slight alterations in hematologic parameters, but the effects reported were different in each study (not supported by any other study), were very slight (not of clinical significance), and other studies reported no such effects at similar or higher exposure concentrations.

Checkoway and Williams (1982) reported a slight decrease in red blood cell count among tank farm workers, a subset of 154 workers in a styrene-butadiene rubber manufacturing plant (average styrene level 14 ppm, butadiene 20 ppm). Little effect from styrene was assigned using multiple regression analysis.

VI. Studies of Test Animals Exposed to Styrene

1. General Toxicology (non-target organ) Toxicology of Styrene

Many of the animal studies of styrene produced general toxicity at the dose or exposure levels used. This toxicity was usually manifested as dose-related growth (body weight) decrements relative to the controls. Other aspects of this response included decreased feed consumption and non-specific clinical signs. Mortality was also affected in some of these studies. The best data for assessing general toxicity are available from several long-term bioassays of styrene (discussed below).

¹⁸ Rom, W.N. (ed.). Environmental and Occupational Medicine. 2nd ed. Boston, MA: Little, Brown and Company, 1992., p. 1000.

B. Toxicity after Acute Exposures¹⁹

Non-Human Toxicity Values

LC50, Mouse inhalation: 4,940 ppm (21,013 mg/m³)²⁰ for an exposure of 2 hours.

LD50, Mouse oral: 316 mg/kg bw.

LC50, Rat inhalation: 2,770 ppm (11,783 mg/m³) for an exposure of 4 hours.

LC50, Rat inhalation: 5,642 ppm (24,000 mg/m³) for an exposure of 4 hours.

LD50, Rat oral, male and female: 5,000 mg/kg bw.

LD50, Rat oral: 1,000 mg/kg bw.

C. Short-Term or Long-Term Repeated Exposures

a. *Nasal and Olfactory Toxicity*

Nasal olfactory lesions in rats and mice are the result of local metabolism of styrene. Differences in toxicity are explained by metabolic differences. Olfactory lesions are not expected in humans exposed to styrene because styrene metabolism has not been detected in human nasal tissue. Decreased olfactory function was not found in a study of reinforced plastics workers exposed to styrene for at least 5 years. A second study, of nasal histopathology, found no abnormalities in reinforced plastics workers.

b. *Lung Toxicity*

Because exposure to ambient styrene occurs necessarily through the lungs, this route of exposure is important. Because obvious lung effects due to styrene exposures have been reported only for mice, a full description of the type of reasoning that has evolved from the studies in this area is provided below. Available reports of studies of styrene using other species indicate that, except for the mouse, styrene is not a pulmonary toxicant. Species that have been studied include Rhesus monkeys, guinea pigs, rabbits, and pigs. Pulmonary toxicity by styrene has not been observed after oral exposures. There are no reports of direct toxicity to the lung in humans from styrene inhalation.

The lung has consistently been reported to be a target organ in mice exposed by the inhalation of styrene, but lung effects have been reported only rarely for rats and have not been reported for other species. The mouse lung effects are characterized by minimal acute effects in the cells of the terminal bronchioles after as little as one exposure. However, upon repeated exposures, there are subtle cellular structural changes (decreased staining of cells and cell crowding) involving the smallest divisions of the airway. With longer duration of exposure, hyperplasia of the terminal bronchioles, sometimes extending into the alveolar ducts but not the alveoli themselves, is reported. With long-term (lifetime) exposure, lung tumors, primarily benign, are also reported for mice. Lung histopathological effects have been reported for mice exposed to levels as low as 20 ppm styrene for two years. Lung effects are not commonly reported for rat studies, even for exposure levels as high as 1000 ppm for two years. The primary

¹⁹ The summarized information in this section was taken directly from the Hazardous Substances Data Bank at <http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?/.temp/~VfaWVp:1>. Minor revisions were made to make the format consistent with this report. Citations are in the form given in the Data Bank.

²⁰ To convert ppm to mg/m³ the following formula was used: mg/m³ = ppm x MW/24.45. For styrene, the ratio MW/24.45 corresponds to 4.2536; for this specific example, 4,940 x 4.2536 = 21,013.

cell type in the terminal bronchioles is a non-ciliated columnar epithelial cell called the Clara cell. Marked species differences of morphology, numbers, and physiologic capabilities of this specific cell type have been reported.

Morphological changes or cell proliferation were not seen in the alveoli in any of the mouse studies. Toxic effects in Clara cells have been reported following oral and inhalation exposure to styrene by mice. These findings indicate that the Clara cell is the target cell for the toxic action of styrene in the mouse respiratory system. In contrast to mice, lung toxicity in rats from styrene exposure is either not existent, or covert. There are no reports of lung toxicity in humans or other species.

a) Effects in Mice After Inhalation Exposure

Several studies of repeated inhalation exposure to styrene ranging from 2 weeks to 2 years duration have demonstrated morphologic effects in the smallest airways (terminal bronchioles) in mice. In addition to conventional toxicology studies, several studies have been conducted to more fully characterize the lung effects in mice and compare the responses reported in other species. The conclusion of the studies was that hyperplasia and carcinogenic activity occur exclusively in mice. This toxicological activity is attributed to a specific type of cell, called the Clara cell, in the upper respiratory system of mice. Similar effects in lung cells most similar to those of other test species and humans have not been reported.

Several studies have reported lung lesions of a specific nature in mice.²¹ However, the most definitive characterization of the lesions were performed in a two-year chronic toxicity/oncogenicity study in which groups of 70 CD-1 mice/sex were exposed to 0, 20, 40, 80 or 160 ppm styrene, 6 hours/day, 5 days/week for up to 2 years (SIRC, 1998; Cruzan et al., 2001). Subgroups of 10 mice/sex/exposure level were necropsied after 52 weeks and 78 weeks with the remaining 50 mice/sex/exposure level necropsied either after spontaneous death, euthanized when in moribund condition, or terminated after 98 weeks (females, due to high mortality in the controls) or 104 weeks (males). The morphologic pattern of effects upon the terminal bronchiolar epithelium first noted in the 13-week study was further refined in this study. There was a general pattern of the progression of the effects from:

Decreased eosinophilia of the epithelium of the terminal bronchioles, to

Hyperplasia of the terminal bronchiolar epithelium, finally to

Hyperplasia of the terminal bronchiolar epithelium extending into alveolar ducts.

The above histopathological effects were all unique to styrene-exposed mice. Additionally, foci or areas of hyperplasia of distal lung parenchyma, not associated with the terminal bronchioles (at least in the plane of the histological section) were found in mice from the terminal necropsy. These foci, which were termed bronchioloalveolar hyperplasia, were also present in control mice although the incidence was greater in exposed mice. The effect upon the terminal bronchioles progressed over the two years of this study. as reflected in the severity grades, incidence and effect levels.

²¹ Cruzan et al. (1997); SIRC, 1996; Cruzan et al., 1997; Roycroft et al. (1992).

More definitive characterization of the cells affected in the terminal bronchioles of mice by immunohistochemical and ultrastructural examination of additional lung specimens from the terminal necropsy of the two-year chronic toxicity/oncogenicity study has been conducted (Cruzan et al., 2001). The cells comprising the areas of hyperplasia in the terminal bronchioles generally stained well for CC10, a protein found in Clara cells, while immunostaining for alveolar surfactant, a protein more characteristic of alveolar Type II cells, was only rarely present. Electron microscopy of epithelial hyperplasia of the terminal bronchioles identified the predominant cell type as the Clara cell. Decreased numbers of intracellular organelles, likely the secretory granules, were apparent in some of the Clara cells and may correlate with the decreased eosinophilia noted in the terminal bronchiolar cells.

Proliferation of lung cells was examined in subgroups of 10 male mice/exposure level sacrificed after 2, 5 and 13 weeks of exposure (Cruzan et al., 1997). Cell proliferation was quantified by microscopic identification and counting of immunohistochemically-stained nuclei incorporating the nucleotide analogue bromo-deoxyuridine (BrdU), delivered by an osmotic minipump implanted subcutaneously one week prior to necropsy (thus, the cell proliferation was only for the second, fifth and thirteenth weeks of exposure, respectively). The results of this study (Table 6) indicate that there was an early increase in Clara cell proliferation, which returned to normal levels by week 13. Type II alveolar cells did not have increased rates of proliferation at any time and hepatocytes also did not have increased proliferation at any time.

Further work by Green et al. (2001b) confirmed the results summarized above. This study examined the early time course of cell proliferation in mouse lung cells. Groups of five CD-1 mice/sex were exposed to 0, 40 or 160 ppm styrene for 1, 5, 6 or 10 exposures. The exposure regimen was 6 hours/day, 5 days/week; thus the sixth exposure followed two week-end days of non-exposure. Osmotic minipumps containing BrdU were implanted three days prior to necropsy. At necropsy, which was conducted the morning following the proscribed exposure period, the lungs were collected for histopathological examination and quantitation of cell proliferation. Histopathological examination disclosed individual cell necrosis, exfoliated or desquamated cells or intrabronchiolar debris in the terminal bronchioles in low numbers of mice after one exposure. These were of minimal severity and were present in individual male and female mice at each exposure level. Most of the female mice also had decreased cytoplasm of the non-ciliated cells of the terminal bronchioles. Mice from the groups necropsied after 5, 6 or 10 exposures had focal crowding of non-ciliated cells in the terminal bronchioles. After 5 or 6 exposures, this was noted for about one-half of the mice of either sex exposed to either exposure level while after 10 exposures this finding was noted for 3 males and all 5 females exposed to 160 ppm but only one female exposed to 40 ppm styrene. Cell proliferation indices showed increased cell proliferation in the terminal bronchioles of both sexes and both exposure levels after 5 exposures. There was increased cell proliferation, of lesser degree, in the large bronchioles in both sexes after either 5 or 10 exposures to 160 ppm styrene (also females exposed to 40 ppm for 5 exposures). There were no differences in proliferation rates of alveolar cells.

b) Effects in Rats After Inhalation Exposure

Several inhalation studies of styrene conducted using rats have confirmed that lung lesions have not usually been associated with styrene exposure. Rats tolerate much higher levels of styrene than mice, which experience fatal hepatic necrosis at exposure levels greater than 200 ppm. In a recent subchronic toxicity study, there were no effects in the lungs related to styrene exposure (Cruzan et al., 1997; SIRC, 1992b). Although the available data are limited, Roycroft et al. (1992) reported similar findings; i.e., for Fischer 344 rats exposed to styrene for 90 days at

dose levels up to 1500 ppm no effect was seen in lung. Three long term chronic toxicity/oncogenicity inhalation studies of styrene have been conducted in rats without identifying the lung as a target organ. These include Jersey et al. (1978), Conti et al. (1988) and Cruzan et al. (1998) (SIRC, 1996).

Jersey et al. (1978) exposed groups of 96 Sprague-Dawley rats to levels of 0, 600 or 1000 ppm (1200 ppm for the first two months) styrene, 6 hours/day, 5 days/week for 18.3 (males) or 20.7 (females) months with survivors held until 2 years for necropsy. Subgroups of 5 or 6 rats/sex/exposure level were necropsied after 6 and 12 months.

Conti et al. (1988) exposed Sprague-Dawley rats, 30/sex (except controls where 60/sex were used) to levels of styrene of 0, 25, 50, 100, 200 or 300 ppm, 4 hours/day, 5 days/week for one year. No effects were reported for the lung.

Cruzan et al. (1998) and SIRC (1996) exposed groups of 70 males and 70 female Sprague-Dawley rats to 0, 50, 200, 500 or 1000 ppm styrene for two years. After one year, 10/sex/exposure level were necropsied. The only pulmonary effect related to styrene was slightly increased incidence of foci of foamy alveolar macrophages in females exposed to the highest exposure level for two years (1 of 60 control rats vs. 8 of 60 exposed to 1000 ppm styrene).

Cell proliferation studies of the cells of the terminal bronchioles and type II alveolar cells have also been conducted using Sprague-Dawley rats exposed to levels up to 1500 ppm styrene for 2, 5 or 13 weeks (Cruzan et al., 1997; SIRC, 1992b) or 500 ppm for 1, 5, 6 or 10 exposures (Green, 2001b). There were no effects present on labeling indices in either bronchiolar cells or type II of rats at any exposure level or time point.

The only studies that report lung lesions in rats exposed to styrene by inhalation are by Coccini et al. (1997; 1998). In these studies, rats were exposed to 300 ppm styrene, 6 hours/day, 5 days/week, for two weeks. At necropsy, immediately after exposure, small samples of lung and trachea were collected for light and electron microscopic examination with remaining lung and liver frozen for biochemical analyses (nonprotein sulfhydryls, protein content and lipid peroxidation). These authors reported patchy effects in the trachea and lung. The pulmonary changes were characterized by thickened interalveolar septae at the light microscopic level. By electron microscopy, a few alveolar type II cells or bronchiolar cells were noted to have dilated endoplasmic reticulum. Increased thickness of the alveolar septae was reported to be due to the presence of collagen fibrils. The ultrastructural effects were reported to be almost completely reversed three weeks after cessation of exposure with only mild alterations in the cytoplasm of some bronchiolar or type II cells. The tracheal effects were characterized as surface blebs of ciliated cells by light microscopy and alterations of the cilia along with vacuolation, dilated endoplasmic reticulum and mitochondrial changes by electron microscopy. Effects were also noted ultrastructurally for the tracheal goblet cells (decreased secretory granules) and the intermediate and basal cells (dense bodies and fibrillary inclusions). The tracheal changes were almost completely recovered after a 3-week post-exposure period. Additional studies by these authors (Coccini et al., 1998) repeated these findings after the same inhalation exposure regimen but reported that ingestion of high levels of ethanol did not affect the severity of the lesions. The disparity of these findings with those previously presented is unexplained. The exposure level and time interval used by Coccini et al. is lower than those used by several other investigators that report a lack of lung effects.

Ohashi et al. (1988) also reported effects upon the tracheal mucosa of male Sprague-Dawley rats exposed to styrene by inhalation to levels of 0, 171 or 1108 ppm styrene, 4 hours/day, and 5 days/week for 3 weeks. Samples of nasal respiratory mucosa from the nasal septum and the trachea were collected from 5 rats/exposure level after exposure; five additional rats/exposure level were held for an additional 12 weeks. Portions of the tracheal samples were evaluated for ciliary activity (beats/minutes) with other portions used for scanning and transmission electron microscopy. Ciliary activity (beats/minute) was decreased in an exposure-related manner the day following exposure. By 12 weeks post-exposure, ciliary activity was normal for those rats previously exposed to 171 ppm and was only slightly decreased for the group previously exposed to 1108 ppm. There were minor ultrastructural changes (dense bodies, small vacuoles and small compound cilia in some of the epithelial cells) in rats exposed to 171 ppm immediately after exposure. Dense bodies were found only sporadically in tracheal epithelial cells after 12 weeks post-exposure for this group. For the 1108 ppm group, cytoplasmic protuberances, vacuolation, dense bodies and decreased numbers of ciliated cells were found after exposure. Only mild vacuolation and low amount of dense bodies were present after 12 weeks post-exposure for this exposure group.

c. *Developmental and Reproductive Effects*

In animals exposed to styrene by inhalation or gavage, styrene did not produce developmental toxicity at non-maternally toxic exposure levels. There is some evidence of slight embryo-fetal toxicity at exposure levels that resulted in maternal toxicity (e.g. decreased body weight gain). There are very limited data to suggest transient postnatal developmental retardation induced by 300 ppm styrene 6 hours/day throughout gestation.

A study designed to conform to EPA guidelines for developmental neurotoxicity study and employing whole body exposures to styrene at concentrations up to 500 ppm throughout gestation, did not cause developmental neurotoxicity. Because the parents were the F2 generation of a multi-generation reproduction study, some growth retardation was seen in the parents and offspring with a NOAEL of 50 ppm. This weight reduction resulted in very slight developmental delays (less than 1 day) in a number of parameters.

d. *Neurological Effects*

Styrene is typical of hydrocarbons that induce narcosis at high exposure levels. Acute exposure to excessive levels of this class of chemicals results in depression of the central nervous system, leading to drowsiness, dizziness, light-headedness, tiredness, headache, and disturbed balance. These acute pre-narcotic effects have been reported in reinforced plastics workers exposed to styrene at concentrations above 100 ppm, but are not consistently reported at lower concentrations.

In addition to pre-narcotic effects, deficits in neurobehavioral tests, alterations in nerve conduction or electroencephalogram, color discrimination, and hearing response have been reported. Results of neurobehavioral tests offer many challenges in their interpretations including the subjective nature of some endpoints, the lack of clearly established norms, and, in studies using styrene-exposed workers, the need to establish an appropriate control comparison group matched for potential confounding factors. The difficulty in assessing the hazard represented by these reports is compounded by inconsistent results, inappropriate statistical analyses or study designs, controversial clinical significance of marginal findings, relationship of the findings to styrene exposure vs. other factors (e.g., coexposures), and uncertainty of the styrene

concentration that causes adverse effects. These problems make it difficult to establish a NOAEL with a large degree of certainty and consensus. Collectively for all endpoints, NOAELs in reinforced plastics workers appear to be between 25 and 75 ppm.

e. *Effects on the Immune System*

Increased susceptibility to virus induced encephalomyocarditis and malaria of mice after exposure to styrene was reported in limited experiments. Some cell-mediated and humoral immunological responses were also reported to be altered after exposure to styrene. These observations have not been confirmed. There is limited evidence from human and animal studies that exposure to styrene may affect the distribution of T and B lymphocyte subsets and natural killer (NK) cells; in general, the reported changes are small and cells were found to be immunocompetent. There is one study, which found decreases in host resistance in mice and rats exposed to styrene orally. Additional studies are needed to characterize the immunotoxicity potential of styrene.

f. *Sensitization*

There is insufficient evidence to consider styrene to be a dermal or respiratory sensitizer. Styrene was not a sensitizer in one report of a guinea pig skin maximization test (Sjoberg et al., 1982) while it caused sensitization in 11% of the animals tested in another report (Senma et al., 1978). Both studies are reported only briefly and do not report the use of negative or irritation control groups. There is one case report of a positive patch test in a single person exposed to styrene in a unsaturated polyester plastic repair kit (Sjoberg et al., 1982, 1984). There are also case reports of three people who responded to bronchial challenge to styrene (Hayes et al., 1991; Moscato et al., 1990). These cases are briefly reported. The lack of reports in the literature despite the routine dermal and inhalation exposure in the workplace supports the conclusion that styrene is not a sensitizer.

g. *Hematology: Effects on Red Blood Cells*

No effects on red blood cells have been reported in rats (NCI, 1979a, b; Jersey, et al., 1978; Cruzan et al., 1997; 1998) exposed by inhalation for up to 2 years at levels up to 1000 ppm or by gavage for up to 2 years at levels 1000 mg/kg bw/day or 2000 mg/kg bw/day for 1 year. No hematologic effects have been reported in mice (Cruzan et al., 1997; SIRC, 1998), although acute liver toxicity to mice precluded exposure to levels as high as those used for rats.

Slightly lower red blood cell counts, hemoglobin, and hematocrit were seen in male beagle dogs exposed orally to 600 mg/kg bw/day styrene for 316 days, followed by 154 days without styrene and an additional 90 days of styrene administration (Quast et al., 1979); these values were occasionally significantly different from control. During the recovery period, these parameters were not significantly different from control and did not become significantly different after 90 days of re-exposure. No effect was seen in females.

Increased incidence of percent of red blood cells with Heinz bodies present was seen in males and females when first measured on Day 243; Heinz bodies were not seen until after exposure was stopped and reoccurred when exposure was restarted. Administration of 400 mg/kg bw/day continuously for 561 days resulted in a slight increase in red blood cells with Heinz bodies present; no consistent effect on other RED BLOOD CELL parameters was seen. At 200 ppm an occasional Heinz body was seen in female dogs. Heinz bodies are thought to be oxidative denaturation of hemoglobin (Fertman and Fertman, 1955; Jandl, 1963). No effect on bone

marrow, erythrocyte fragility, or serum erythropoietin was seen. The formation of Heinz bodies in the red blood cells of dogs exposed to 400 or 600 mg/kg bw/day styrene appear to be unique to the dog and are not seen in humans exposed to styrene at levels of at least 50 ppm.

VII. Genetic Toxicity of Styrene

The genotoxicity data on styrene are mixed. Most studies of styrene do not show genetic toxicity. Chromosomal aberrations and micronuclei are not seen in most animal studies. Several studies indicate a weak induction of sister chromatid exchange. The frequency of alkali labile sites/DNA strand breaks is increased in some studies. A minority of reinforced plastics worker studies indicate increased chromosomal aberrations, micronuclei, or sister chromatid exchange.

Styrene is metabolized, at least in part, to styrene oxide, which is mutagenic in several *in vitro* systems. The R-enantiomer of styrene oxide has been shown to be slightly more reactive than the S-enantiomer in the Ames assay, although not in all reports. Chromosome aberrations were induced in mouse bone-marrow cells in one study by the S-enantiomer of styrene oxide, but not with the R-enantiomer.

VIII. Carcinogenicity

Styrene has been confirmed to induce lung tumors in mice following lifetime inhalation exposures but not in chronically exposed rats. Studies of the metabolism of styrene indicate a secondary mechanism of tumorigenesis induced by local toxicity and loss of control of tissue repair processes.

Available studies indicate that styrene causes increased lung tumors in mice following repeated and prolonged inhalation exposures. Although four studies by gavage administration conducted in the 1970s had inconsistent results and all had significant design deficiencies, an increase in lung tumors was suggested in two of the studies. A subsequent GLP-compliant inhalation study found that exposure of CD-1 mice to 20 to 160 ppm styrene for up to two years resulted in increased lung tumors in males (at 40, 80 and 160 ppm) and females (at 20, 40 and 160 ppm) at two years. Toxicity to lung Clara cells and nasal olfactory cells was also reported. Chronic exposures of mice to styrene result in toxicity specific to Clara cells leading to lung tumors, nasal and olfactory lesions, both of which appear to be point of contact site-specific effects.

Inhalation or oral exposure to styrene has been reported to produce lung toxicity in mice, but not in rats. Effects in mice have been seen consistently in the terminal bronchioles, but no effects are reported in alveolar cells.

No morphologic or cell proliferation effects were seen in the alveolar region in any of the mouse studies. Toxic effects in Clara cells have been reported following oral and inhalation exposure to styrene. Overall, these findings indicate that the Clara cell is the target cell for the toxic action of styrene in mouse lungs.

In contrast to the effects observed in mice, there were no styrene-related effects in the lungs of Sprague-Dawley rats (Cruzan et al., 1997) or F344 rats (Roycroft et al., 1992) at concentrations up to 1500 ppm for 3 months.

Proposed Mode of Action of Carcinogenicity in Mice and its Relevance to Humans

In mouse lung Clara cells and nasal olfactory epithelium, styrene is metabolized primarily by the enzyme CYP2F2 to produce R-SO and ring-oxidized metabolites. These tissues are also highly sensitive to cytotoxicity following styrene exposure. Inhibition of CYP2F2 eliminates the styrene-induced cytotoxicity. In mouse lung, a regenerative hyperplasia results from the cytotoxicity, and increased incidences of lung tumors were found only after more than 18 months of repeated exposures.

In rats CYP2F4 is much less prevalent in lung tissue; rat lung produces primarily S-SO and limited amounts of ring-oxidized metabolites, and cytotoxicity is not seen. No increases in lung tumors have been reported in any of the eight chronic rat studies of styrene. In rat nasal tissue, CYP2F4 is present to a large extent and a high level of R-SO is produced. However, epoxide hydrolase in rat tissue is more active than in mouse nasal tissue, resulting in a more efficient removal of SO and less nasal toxicity in rats than in mice.

In humans very limited metabolism of styrene occurs in the lung or nasal tissue. Both lung and nasal tissue possess epoxide hydrolase activity and, thus, should be able to rapidly remove any SO formed either in the tissue or which might have migrated from the blood.

Lung tumors in mice most likely result from a non-genotoxic mode of action as a result of cytotoxicity leading to hyperplasia. No lung tumors are seen in rats exposed to styrene, where there is no evidence of cytotoxicity. No styrene-induced lung tumors would be expected in humans, which possess even less ability to metabolize styrene in lungs than rats. Indeed, no styrene-related increase in lung tumors has been reported in human cohort mortality studies.

Nasal olfactory lesions in rats and mice are the result of local metabolism of styrene. Differences in toxicity among species are explained by metabolic differences. Olfactory lesions are not expected in humans exposed to styrene because no styrene metabolism was detected in human nasal tissue. Loss of olfactory function was not found in a study of workers exposed to styrene for at least 5 years.

In conclusion, styrene respiratory tract toxicity in mice and rats, including mouse lung tumors, appears to be mediated by CYP2F-generated metabolites. The PBPK model predicts that humans do not generate sufficient levels of these metabolites in the terminal bronchioles to reach a toxic level. Therefore, the postulated mode of action for these effects indicates that respiratory tract effects in rodents are not relevant for human risk assessment.

IX. Absorption, Distribution and Excretion of Styrene

The metabolic and biokinetic data obtained from test animals and humans lead to the following conclusions:

- There are significant species differences in the activities of styrene and SO metabolizing enzymes. In those tissues with high levels of CYP2F (mouse lung Clara cells, rat and mouse nasal olfactory epithelium), R-SO is the main first metabolite from styrene and tissue damage is found.
- The toxicity of styrene in lung and nasal tissues is caused by one or more metabolites derived from oxidation of styrene by CYP2F which include R-SO and 4-vinylphenol (4-VP) or derivatives from 4-VP.

- The concentration of SO in lung and nasal tissue in mice is mainly due to the local metabolism of styrene. Exposure due to the presence of SO in the blood is of little importance.
- Differences in nasal toxicity from styrene in rats and mice are consistent with a greater ability of rat nasal tissue to remove SO, especially R-SO, by epoxide hydrolase.
- Little if any SO is formed in human lung or nasal tissue. However, epoxide hydrolase is very active in both human lung and nasal tissue and could remove small amounts of formed or that migrates into the cell from the blood. Human lung is incapable of producing sufficient SO to achieve the bronchiolar concentration of 0.1 ppm produced in mice. Thus no lung or nasal toxicity is expected in humans from styrene exposure.

Styrene is absorbed by humans and animals by all routes of exposure (oral, dermal, and inhalation). The most substantial human exposures occur via pulmonary uptake of styrene vapors in occupational settings. Pulmonary absorption has been evaluated in several human studies that show uptake values ranging from 61 to 77% of the inspired air concentration.

The rate of absorption of liquid styrene through the skin is apparently quite low, on the order of 0.06 mg/cm²/hr (60 μg/cm²/hr) for humans. Older reports seriously overestimated the uptake of styrene through the skin. Dermal absorption of styrene vapors in humans is negligible, only about 2% of that observed from the respiratory system. In rats, dermal absorption was estimated to be 9.4% of the total amount absorbed when vapor is inhaled. The absorption rate of liquid styrene across excised rat skin was determined to be 30 μg/cm²/hr.

There have been no human studies in which absorption of styrene from the gastrointestinal tract has been systematically evaluated. However, studies in rats indicate that absorption from the gastrointestinal tract is rapid and virtually complete.

Styrene and/or its metabolites can cross the placenta to the fetus in pregnant animals after inhalation or intravenous exposure. Styrene concentrations in the placenta were reported to be slightly lower than that in maternal blood, while those in amniotic fluid and fetal tissue were on the order of 5-times lower.

Human studies have shown that 90-97% of absorbed styrene is eliminated as urinary metabolites, primarily mandelic and phenylglyoxylic acids. Some styrene is eliminated unchanged in urine or expired air but the amounts detected have been very small.

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REVIEW OF STYRENE ODOR THRESHOLD VALUES IN AIR

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Executive Summary

The aim of this paper was to conduct a comprehensive literature search of published odor thresholds for styrene and to evaluate those studies on the basis of accepted criteria in olfactometry, for their utility in determining a reasonable odor threshold and odor nuisance level for styrene vapor. Some of the reviewed thresholds are derived from original research and some are from literature reviews of many compounds where the original research study is not presented and the details of the methodology have to be inferred.

Although odor thresholds have been measured and reported for more than a hundred years, even now no single methodology has been uniformly adopted for measuring olfactory sensitivity in humans. It is not uncommon to find reported odor thresholds in the literature for a single compound to vary by more than an order of magnitude and in this regard, styrene is no exception. The variation in thresholds among studies can be traced to differences in odor presentation method, panel composition and size, and/or procedures for analyzing the stimulus concentration and calculating the threshold. By way of providing the background for evaluating odor threshold studies, this review describes criteria for collecting odor thresholds, including the selection of test subjects, methods of stimulus delivery, and the various procedures that have been used to measure olfactory sensitivity in humans.

Of the styrene odor threshold studies that were reviewed, a number were rejected for not meeting sufficient criteria for the data reported to be considered reliable. Among the studies that were not rejected, however, we still noted a number of issues in sensory or analytical methodology that raised questions about the reliability of the thresholds reported. This was particularly true for studies where extreme (low and high) threshold values were reported, as some of the methods used for measuring sensitivity were not objective and could have led to anticipation and bias on the part of the panelists. In order to assist in the use of these data, a critique of each reviewed study is included in the appendix, highlighting the concerns we noted and their implications for the results.

In this review, we also review factors present in the real-world which serve to increase the concentration needed for an individual to become aware of or annoyed by an odor and summarize the research establishing a relationship between odor detection thresholds and annoyance. Studies evaluating the relationship between odor concentration and annoyance have generally found that a five-fold increase above the odor detection threshold is the point at which odors become annoying. State regulations employing field olfactometry to determine odor nuisance levels have adopted this reasoning to set the fence line emissions levels at multiples of the odor threshold. In combination with regulations on the number of exceedances of this limit (for both frequency and duration) this approach seems reasonable, given the numerous factors which can affect odor detectability and nuisance impact at distances beyond the fence line.

REVIEW OF STYRENE ODOR THRESHOLD VALUES IN AIR

1. Introduction

The human sense of smell, although not as sensitive as that of other species, allows the detection of many odorants at extremely low concentrations. However, although awareness of odor can occur at concentrations where the odor is just detectable, other information such as odor quality, identifiability and unpleasantness typically requires exposure to higher concentrations (Sucker et al, 2001). For this reason, evaluating the relationship between the detectability of environmental odorants and the concentration at which they become unpleasant and elicit annoyance or concern is a critical step in their regulation and management.

The aim of this paper was to conduct a comprehensive literature search of published odor thresholds for styrene. Styrene is a clear colorless liquid with a highly characteristic pungent odor. The primary use of styrene is in the production of polymers and copolymers, including polystyrene, styrene-butadiene-rubber, styrene-butadiene latex, and a variety of different resins. Styrene monomer is combined with polyester resins and serves as a cross-linking agent in the manufacture of numerous fiber-reinforced products. The production of styrene has steadily increased each year since 1990. Production data indicate that 11.9 billion pounds of styrene were produced in the US 1999 (Chemical and Engineering News, 2000).

Some of the reported thresholds are derived from original research and some are from literature reviews of many compounds where the original research study is not presented. Overall, as for most chemical compounds, reported odor thresholds for styrene span a considerable range. Indeed, it is not uncommon for reported odor threshold values to range over four orders of magnitude for the same chemical. For example, 29 values were reported for n-butyl alcohol, ranging from 1.45×10^{-7} to 1.88×10^{-4} g/L (Amoore and Hautala, 1983). The lack of standardization of method used to determine odor thresholds as well as the large variability of human sensitivity are responsible for the wide range of threshold concentrations usually found in the literature.

2. Odor Perception

A brief review of odor characteristics and methods used to evaluate the perceptual characteristics of odors in both indoor and outdoor environments is presented to facilitate understanding of odor thresholds values.

2.1. Odor characteristics

For describing the features of olfactory perception, it is important to make a distinction between odorants and odor. Odorants (or volatiles) are molecules. They are properties of the external world and can be described in physicochemical terms. On the other hand, odors are the sensations resulting from the reception of a stimulus (the odorant) by the olfactory sensory system.

The process of odor perception begins with the binding of volatile chemical molecules to specialized receptors in the nasal cavity. The process ends when information from the many different receptors is organized into patterns that can be recognized by the brain as distinct odors, with different characteristics. The sensory perception of odors is characterized by four major attributes: detectability, intensity, hedonic tone, and odor quality. Numerous methods are available for the investigation of olfactory function in humans, yet no one is optimal for measuring odor impact in all situations. Techniques for measuring odor characteristics include those that evaluate concentrations at perithreshold and suprathreshold levels. At perithreshold level, the odorant is at a concentration where its presence can be first differentiated from ambient air, but little other information about the odorant is available. This level corresponds to the detectability (or odor threshold) which refers to the lowest concentration of odorant stimulus necessary for detection in some specified percentage of the test population or panel. Olfactory thresholds are known to be extremely variable across subjects and are dependent on a number of factors, including the individual's sensitivity to that particular compound or mixture. At higher concentrations (suprathreshold), the detectability of the odor stimulus is assumed, and interest is focused on characteristics such as intensity (refers to perceived strength or magnitude of the odor sensation, which increases as a function of concentration), odor quality (categorization of odorant sensations into categories such as "pungent", "putrid" or "floral"), and hedonics (on dimensions such as "pleasant-unpleasant" or "acceptable-unacceptable").

This review will focus on styrene odor detectability and through a review of the existing literature will seek to determine the mean concentration at which this volatile becomes detectable.

Table 1. Overview of Relevant Odor Characteristics and Assessment Methods

Levels of Exposure	Odor Characteristics	Index	Method
Perithreshold	Detectability	Odor detection Threshold	Static Olfactometry
			Dynamic Olfactometry
Suprathreshold	Intensity	Intensity Rating	Scaling Methods
	Hedonics	Acceptability and Annoyance Ratings	Qualitative Descriptive Analysis
	Quality	Descriptive Labels	

2.2. Irritation potential

In addition to odor sensations, most odorants have the propensity to stimulate free endings of the trigeminal nerve and induce sensations such as irritation, tickling, burning, warming, cooling, and stinging (Doty *et al.*, 1978; Silver, 1991). The capacity for a chemical to induce these irritant sensations largely depends on the concentration (Cometto-Muniz and Cain, 1990). For example, acetic acid at low concentrations will elicit a smell of vinegar; however, at higher concentrations the smell will be accompanied by a pungent sensation in the nose. The difference between the concentration at which a chemical is detectable by odor and the concentration at which it begins to elicit irritation can be considered as a measure of its irritant potency (Cometto-Muniz and Cain, 1995). Similarly to odor sensitivity, nasal irritant sensitivity varies considerably across different odorant chemicals and exhibits significant inter-individual variation (Shusterman, 2002).

Table 2. Relationship between Odor Detectability, Recognition and Annoyance.

Threshold Type	Description	Concentration Needed
Odor Detection	Lowest concentration that can be reliably discriminated from clean air 50% of the time	
Odor Recognition	Lowest concentration that can be reliably recognized or identified 50% of the time	3x Detection Threshold
Odor Annoyance	Lowest concentration that is judged unacceptable	5x Detection Threshold

3. Methodology for Determining Odor Detection Threshold

3.1. Concept of odor threshold

Generally the odor detection threshold is considered as the minimum amount of stimulus concentration necessary to discriminate an odor from a blank sample of ambient air (Dalton, 2002). It is largely recognized that an individual's threshold for any odorant is an estimate of sensitivity obtained at one point in time and consequently is subject to variations. Thus in studies investigating odor detection threshold it appears important that any obtained threshold for an individual or a group could be considered as representing a range of sensitivity.

3.2. Criteria for acceptability of odor threshold measurement techniques

As the olfactory modality is very sensitive, the following issues should receive careful attention in reviewing studies that report olfactory thresholds values. Methods for stimulus delivery and measurement are important factors contributing to the variability of published odor threshold values. These issues have been considered as criteria elements in our review of studies reporting styrene olfactory threshold values.

3.2.1. Research environment

The environment in which tests of olfactory thresholds are conducted should be odor-free, maintained at constant temperature, and equipped with a ventilation system for the expedient turnover air.

3.2.2. Selection of research subjects or panelists

3.2.2.1. Panel size

The size of the subjects sample or panel is important to approximate the distribution of olfactory sensitivity in the population. Although a large number of subjects is recommended to represent the general population, most studies rely on a smaller group of reliable panelists who meet the selection criteria below. At a minimum, the panel should be comprised of 10 individuals.

3.2.2.2. Panel selection

Research subjects or panelists should be selected carefully insofar as olfactory thresholds values may vary widely among individuals (Stevens *et al.*, 1988). Prospective panelists should be first

evaluated for their general olfactory sensitivity in order to exclude panelists with extreme performance, either sensitive (hyperosmics) or insensitive (anosmics).

Indeed, olfactory sensitivity can be impacted by many factors. Subjects should be screened for such factors, which include: gender; age; the presence of respiratory disease that may affect olfactory sensitivity (i.e, asthma, seasonal allergies, or active colds); medication use; smoking habits; occupational history; and chemical sensitivities.

3.2.3. Methods of stimulus delivery

3.2.3.1. Vapor modality

In odor detection threshold assessment, the measured odor could either be in the form of a gas-air mixture or vapor over an aqueous or other solution. The choice of vapor modality is determined by the test purpose of the study. The diluent, which could be liquid or gaseous should be consistent with the chemical compounds tested. When the diluent is liquid, it is generally odorless mineral oil or polyethylene glycol or, less frequently, water.

In earlier studies odor-detection thresholds were also measured in water instead of air. In the water-dilution procedure the odorant is prepared as a series of aqueous dilutions in closed, partially filled vessels from which the headspace vapors can be sniffed.

Overall, the majority of the reported thresholds are gas-air measurements.

3.2.3.2. Methods of delivery

At the present time, three general methods are widely used to deliver odorants to the nasal cavity for detection tests: static olfactometry, dynamic olfactometry and chamber tests. In static olfactometry the odorant to be smelled is presented as a fixed concentration of the liquid chemical in a closed container, and the stimulus is the odorized air or headspace over the liquid (Prah *et al.*, 1995). A series of plastic squeeze or glass bottles or flasks into the nose is inserted is typically used. Each bottle contains a different concentration of the odorant dissolved in a diluent according to a predetermined dilution sequence (e.g., binary, tertiary, or logarithmic series).

In contrast, dynamic olfactometry involves delivering a continuous, well-regulated gas flow that contains odorized air mixed in varying proportions with a carrier gas, typically odorless air or nitrogen (Prah *et al.*, 1995). The more common delivery systems were nose ports held under the nostrils, vents into which the whole head is inserted or syringes that impinge vapor into the nose.

Less frequently, studies used whole rooms into which the odorant was injected. Each method has its advantages and disadvantages. Static olfactometry typically requires little in the way of instrumentation and can be field-portable. Dynamic olfactometry although requiring greater technical proficiency to set up and administer is most useful when the odor of interest is coming from a mixture with unknown components and the sample can be diluted to the point where odor detection just occurs. Chamber exposures are typically more labor-intensive; while they represent more realistic exposure conditions, they also limit the number of concentrations which can be presented within a given session.

3.2.4. Methods of stimulus presentation

3.2.4.1. Concentration presentation

In odor detection threshold assessment, it is recommended to present odor stimuli in a sequence going from weakest to strongest (ascending series) rather than from strongest to weakest (descending series). The reason is that exposure to high concentrations of the odorant early on may cause olfactory adaptation in the panelists. More recent advances have used adaptive procedures to obtain thresholds, in which the concentrations presented are determined by the subject's response (correct or incorrect) to a prior stimulus presentation. This method allows the determination of threshold using a fewer number of trials and omits the possibility that exposure to higher concentrations will elicit olfactory adaptation and an artificially high threshold.

3.2.4.2. Presentation procedure

Regardless of the odorant delivery system (bottles in static olfactometry or sniffing ports/vents or syringes in dynamic olfactometry) panelists are usually presented two kinds of samples in succession – the odorant sample and one or more samples of clean air – and are instructed to identify in which sample they smelled an odor. This “forced choice” procedure is generally preferred over the yes-no procedure, in which subjects are just asked whether they smelled an odor or not. Indeed, using the forced choice procedure the bias associated with a subject's criterion for responding is eliminated (Dalton, 2001). In olfactory research, unless measures of criterion are specifically being evaluated, the forced-choice procedure has become the method of choice, however many older studies relied on yes-no procedures.

3.2.4.3. *Number of trials*

In the ascending stimulus concentration procedure, a fixed number of trials should be presented at each concentration (typically three to five), from weakest to strongest, until the subject is able to correctly identify the odor stimulus on all trials. Then the procedure may be repeated for reliability (Table 3).

Table 3. Ascending Procedure with three trials at each concentration

High	Concentrations	Trial 1	Trial 2	Trial 3
	...			
	6	+	+	+
	7	+	+	+
	8	+	+	-
	9	+	-	-
	10	-	-	-
Low	...	-	-	-

- = no detection

+ = detection

Concentration 7: odorant detected on the three trials (potential odor threshold)

Concentration 6: repetition of the procedure to test odor threshold reliability

In the adaptive procedure, the number of trials at each concentration is determined by the respondents' accuracy, but the number of reversals (the concentration step at which an incorrect judgment becomes correct or vice versa) is typically set at 5-7 for reliability (Table 4, example with five reversals). With this procedure an incorrect detection of the bottle that contains the odorant on any trial results in the presentation of the next higher concentration. However, a correct detection of the bottle containing the odorant results in a second presentation at that concentration. Presentation of increasing or decreasing concentrations continues until the subject achieves five or seven reversals. A reversal is defined as two correct decisions followed by one incorrect or one incorrect decision followed by two correct one at a given concentration step.

Table 4. Adaptive procedure with five reversals

High	↓	Concentrations																		
		...			1			3			5									
		8			+	+		+	+		+	+								
		9			-			-			-									
		10	-				2			4										
	Low	...																		

- = no detection
 + = detection
 ○ reversal

In both methods the interval between subsequent odor presentations is critical, as short interstimulus intervals can produce adaptation and higher thresholds (Dalton, 2001).

At the present time, the adaptive procedure is preferred to the ascending method. Indeed, detection threshold values based upon a single ascending presentation series have been shown to be much less reliable than those based upon a staircase procedure (Doty *et al.*, 1995). This may be due to the test length, as the adaptive procedure results on a threshold determination with fewer trials than ascending method

3.2.5. Methods of odor detection threshold measurement

3.2.5.1. Calculation of the odor threshold

Odor thresholds can be calculated in two different ways. From a psychophysical tradition, the odor detection threshold is an absolute threshold, i.e., a concentration of the odorant that an individual can detect on 50% of the trials (McNicol, 1972). Thus, the concentration that lies between the step at which the subject could not identify the odorant sample and the step where the subject could always identify the odorant sample is considered to be the odor threshold for that subject. The individual thresholds can be averaged across the group of subjects who are tested.

An alternative method of estimating odor thresholds for a population is to determine the concentration step at which 50% of the panelists can reliably detect the odor 100% of the time (Frechen, 1994; Miedema and Ham, 1988). Different values of odor thresholds will result from either method. Since these thresholds cannot be use interchangeably, one should always pay attention to how the odor detection threshold was determined in a particular instance.

In the present review, some of the studies (e.g., Nagata, 2003, Deadman & Prigg, 1958) calculated thresholds by taking the geometric mean of the individuals' thresholds; in other studies, (e.g., Nagy, 1991), the reported threshold was the concentration at which 50 % of the panelists could detect the odorant, and in other studies, the method used to calculate threshold is not described. The potential for differences in threshold calculation provide yet another source of variation in reported values.

3.2.5.2. Analytic measurement

The concentration of odorant as it reaches the panelist should be measured accurately. Indeed, in static olfactometry, vapor-phase concentration in the headspace of the bottles/flasks can not be simply predicted from the amount of odorant in the solution as it is dependent on many factors. At the present time, it is most reliably assessed using gas chromatography (Dalton, 2002) to express the concentration in parts per million (ppm) or parts per billion (ppb) of air. This method of concentration assessment has occurred only recently and a major problem with early threshold studies is the absence of such analytic methodology.

4. Factors Modifying Odor Detectability in Real-World Environments

It is important to realize that there is a substantial difference between the concentration of an odorant that *can* be detected and the concentration that *will* be detected. In a study evaluating the influence of various degrees of distraction on responsiveness to warning odors the researchers found significant differences in the chemical concentration needed for subjects to accurately detect odor in directed vs. undirected tests (Amoore and Hautala, 1983a). When subjects were not given instructions to detect an odor, they showed a four-fold decrease in detectability when compared with a more typical laboratory experimental condition in which subjects were specifically instructed to attend to and detect the target odor. It should also be noted that laboratory odor detection thresholds are typically obtained in an environment where the air is

purified and filtered and hence the background for the target signal is much lower. Signal detection theory has shown that as background noise increases, signal detectability decreases (Swets *et al.*, 1961). In a non-laboratory environment, the ambient background typically contains many other odorous compounds which have the potential to significantly increase the signal-noise ratio. This would serve to increase the concentration required to detect a chemical when compared with the concentration necessary for detection obtained in a laboratory setting.

Based on awareness of the above modifying factors, for example, van Dorn and colleagues (van Dorn *et al.*, 2002) applied a four-fold correction (increase) to the odor detection threshold as the initial step in the process for determining community awareness of industrial odor.

5. Use of Odor Detection Thresholds for Establishing Odor Regulations

At the present time there is no single standard procedure for establishing a fence line emission level that will result in acceptable air quality to the surrounding community. Some states rely on the frequency of odor complaints at the emissions site, which can result in an over-estimation of the odor nuisance impact, as perception and complaints can be modulated by a number of non-sensory factors (Smeets and Dalton, 2005). In recent years, field olfactometry devices such as the “Scentometer™” or its successor, the “Nasal Ranger™” (St. Croix Sensory, Lake Elmo, MN¹) have been used successfully to determine the number of volumes of clean air needed to dilute a single volume of odorous air such that the resulting dilution is odorless. This value is known as the ‘dilution to threshold’ ratio. These devices can be utilized at varying distances from the facility property line in real-time to assess, in a standardized manner, the degree to which an ambient odor exceeds the odor threshold.

To assist in the interpretation of such field data, a number of studies have been conducted to establish the relationship between odor annoyance and odor detection threshold (Adams *et al.*, 1968; Hellman & Small, 1974; NCASI, 1971; Steinheider & Winneke, 1993; Sucker *et al.*, 2001; Winneke & Kastka, 1977). Results from these studies suggest that, as a general rule of thumb, the ratio of perceived annoyance to odor detection levels for unpleasant odorants is approximately 5-fold, although this obviously could vary across compounds having different hedonic qualities.

¹ Full references and device description can be found at www.fivesenses.com

Thus, taking into consideration factors such as the relationship of perceived odor nuisance or annoyance to odor detection levels, as well as the likelihood of other background odors rendering any odor signal less detectable, various states have set fenceline emission limits at dilution to threshold ratios which range between 4 to 1 and 7 to 1², effectively setting the odor nuisance level at 5 or 8-fold higher than the detection limit. In combination with regulations on the number of exceedances of this limit (for both frequency and duration) this approach seems reasonable, given the numerous factors which can affect odor detectability and nuisance impact at distances beyond the fenceline.

Given the frequent disparity in reported odor thresholds found in the published literature, one additional question to be addressed is which value to use in setting regulations. Without question, studies need to be evaluated with regard to adherence to the criteria for testing and analytical integrity set forth earlier. Once having passed this initial review, however, one can still find differences in odor thresholds spanning an order of magnitude. While it may be tempting to assume the lowest value in a data set is the most protective, there are compelling reasons to seek a ‘weight of evidence’ approach with regard to convergence of threshold data values across laboratories and methods. Indeed, depending on the test panel composition (gender and number) a very low average threshold may occur as a function of having selected a small number of extremely sensitive and well-trained individuals for participation. When we consider studies where a larger number of individuals are tested, the average thresholds are typically higher and may be more representative of the general, naïve population.

6. Literature Search and Review

Literature threshold values have been grouped in water and air dilution thresholds, placed in chronological order, and all converted (Table 2), as necessary, to concentrations expressed $\mu\text{g}/\text{m}^3$ of styrene gas in air. Odor thresholds originally measured in water dilution were converted to the equivalent air dilution using the formula shown below.

² A dilution to threshold ratio of 7-1 means that no odor will be detected when 1 volume of odorous air is mixed with 7 volumes of clean air (an actual dilution of 1 part odor in 8 parts air). As an example, assume that for compound X, the threshold for odor detection is just above 1 ppm (meaning at 1 ppm the odor cannot be smelled) and odor nuisance regulations allow up to a 7-1 dilution to threshold off the property line. As long as the (undiluted) ambient concentration is not greater than 8 ppm, then the limit is not exceeded.

Table 5. Characteristics and Properties of Styrene

Common Synonyms	Vinyl benzene Phenylethene Ethenyl benzene Cinnamene
CAS (Chemical Abstracts Services) number	100-42-5
Molecular Formula	C ₈ H ₈
Molecular Weight (g/mole)	104.14
H ^(*) (atm-m ³ /mole)	2.75x10 ⁻³
Air/water partition coefficient^(**)	0.128
Water/air distribution ratio^(***)	7.8
Conversion Factors	1 ppm = (mg/m ³ x 24.4)/ molecular weight = 4.33 mg/m ³ 1 mg/m ³ = (ppm x molecular weight)/24.4 =0.23 ppm (Verschueren, 1983)
Equivalent of odor threshold in air calculated from value in water	<u>odor threshold in water x 24400</u> 104.14 x 7.8

(*) H = Henry's Law Constant

(**) Parameter that describes the concentration ratio of a substance in equilibrium between air and water

(***) Reciprocal of the air-water partition coefficient

6.1. Rejected papers (Table 6)

Some papers were rejected on the basis of the following criteria:

- Not located papers
- Insufficient methodology (odor delivery/procedure/analysis)
- Extreme value

Table 6. Rejected Papers

References	Odor Detection Thresholds		Major Reason of Rejection
	Original data	$\mu\text{g}/\text{m}^3$ *	
(Wolf <i>et al.</i> , 1956)	10 - 60 ppm	43300 - 259800	- Insufficient Methodology (Odor delivery and Procedure) - Extreme value
(Shen, 1961)	0.02 mg/m ³ (from unpublished data)	20	Not Located
(Baker, 1963)	Thresholds in water, 60°C Range = 0.02-2.6 ppm Average = 0.73 ppm = 21.9 ppm in air **	94827	Extreme value

(*) 1 ppm = 4.33 mg/m³

(**) odor threshold in air (ppm) = $\frac{\text{odor threshold in water (ppm)} \times 24400}{104.14 \times 7.8}$

6.2. Reviewed papers (Table 7)

Table 7. Reviewed Papers

References	Methods	Vapor Modality	Odor Detection Thresholds	
			Original Data	$\mu\text{g}/\text{m}^3$ ^c
(Rosen et al., 1963)	<i>Water-dilution</i>	Water	37mg/1000L (= 0.037 ppm in water ^a = 1.11 ppm in air ^b)	4806.3
(Zoeteman et al., 1971)	<i>Water-dilution</i>	Water (15°C)	0.05 ppm (= 1.5 ppm in water ^b)	6495
(Alexander et al., 1982)	<i>Static olfactometry</i> (modification of the ASTM method D-1292)	Water (60°C)	0.004 mg/L (= 0.004 ppm ^a) = 0.12 ppm in air ^b)	519.6
Summary of Styrene Odor Detection Thresholds in WATER			Average = 3940.3 $\mu\text{g}/\text{m}^3$ Range = 519.6 – 6495 $\mu\text{g}/\text{m}^3$	
(Deadman and Prigg, 1958)	<i>Dynamic olfactometry</i> (odorimeter tests)	Air	140 mill.cu.ft/lb ^d (= 0.026 ppm in air)	112.58
(Stalker, 1963)	<i>Dynamic olfactometry</i>	Air	0.017 ppm	73.61
(Hellman and Small, 1973; Hellman and Small, 1974)	<i>Odor fountain</i> (air dilution method)	Air	0.05-0.148 ppm (average = 0.1ppm)	216.5 – 640.84 (average = 433)
(Dravnieks, 1974)	<i>Dynamic olfactometry</i>	Air	4900 ^e (= 1.97 ppm in air)	8530.1
(Amoore and Hautala, 1983b)	Average of literature data, non cited references	Air	0.32 ppm	1385.6
(Nagy, 1991)	<i>Dynamic olfactometry</i> (binary port odor panel)	Air	1300 $\mu\text{g}/\text{m}^3$	1300
(Nagata, 2003)	<i>Triangular odor bag method</i> (air dilution method)	Air	0.035 ppm	151.55
(Dalton et al., 2003)	<i>Static Olfactometry</i>	Air	0.44 ppm ^f	1905.2
Summary of Styrene Odor Detection Thresholds in AIR			Average = 1736.46 $\mu\text{g}/\text{m}^3$ Range = 73.6 – 8530.1 $\mu\text{g}/\text{m}^3$	

(a) 1 mg/L = 1 ppm

(b) odor threshold in air (ppm) = $\frac{\text{odor threshold in water (ppm)} \times 24400}{104.14 \times 7.8}$

(c) 1 ppm = 4.33 mg/m³

(d) Value of odorosity. Odorosity of a gas is defined as the volume to which unit volume of the gas must be diluted to obtain the threshold of smell. For liquids, the odorosity is expressed as the volume in mill.cu.ft to which 1 lb of the vaporized material has to be diluted to reach the threshold (Deadman and Prigg, 1958).

(e) Odors thresholds are given in terms of E.D.₅₀ dilution factors. It signifies the total number of volumes to which one volume of air saturated with the odorant vapor at 25°C must be diluted (with non odorous air) to reach detection level (Dravnieks, 1974).

(f) Values in µg/m³ were calculated from the data published as dilution steps in the styrene series.

APPENDIX

Critique of reviewed papers

Overall the number of papers dealing with styrene odor detection thresholds is quite limited. Some of the thresholds are derived from compilations, published (Amoore and Hautala, 1983c; Verschueren, 1983) or unpublished, of numerous chemicals. In this case, the reported thresholds could be average data of different studies references whose references are not always cited. The majority of styrene odor thresholds are reported from experimental studies conducted a long time ago, sometimes more than 50 years ago. Thus, one has to be aware that most of the reported odor thresholds were not obtained under the same conditions of methodological precision that are taken for granted today. Moreover, some values are reported from many interdisciplinary sources in which the main goal was not threshold measurement per se (Rosen *et al.*, 1963; Zoeteman *et al.*, 1971). As for many other chemicals compounds, styrene odor thresholds found in the literature are distributed over the concentration of large range depending on the method used but also on the vapor modality (i.e., air vs water).

WATER DILUTION THRESHOLDS

Rosen *et al.*, 1963; Zoetman *et al.*, 1971 and Alexander *et al.*, 1982

Overall, our opinion is that water thresholds should not be given the same consideration as air dilution thresholds. The major reason is that the rapid evaporative loss during the experiments may be a potential source of error in such measurements (i.e., changes in solution concentration). Moreover, the data reported in the right column of the table were converted from measurements made with water solutions, which are likely to be in error due to the rather high water/air distribution ratio.

AIR DILUTION THRESHOLDS

Hellman and Small, 1973; 1974

Except for the odor delivery system ('odor fountain') which is maybe not as stringent as the devices used today, these studies are in conformity with acceptability criteria required for

modern threshold determination. The only limitation of these works is that the number of panelists is not mentioned.

Stalker, 1963

The critical point of this study concerns the presentation procedure. Indeed, the subject has to indicate the initial perception of the odorant by raising the hand. Contrary to the forced choice method, this procedure induces anticipation effects and increases the bias associated with a subject's criterion for responding. In particular, since there is no indication in the report that clean air (blanks) are ever introduced to ensure that subjects are accurately responding in the presence (or absence) of odorant, it is likely that individuals may have reported 'detecting' odor even on trials when it was below their detection threshold. It is also noteworthy that the median threshold (i.e. the threshold that would be calculated if they used the 50% panel detection at 100% probability) is nearly an order of magnitude above the individual geometric threshold suggesting that a few very low thresholds may have skewed the mean value.

Deadman and Prigg, 1958 and Dravnieks, 1974

Contrary to other studies, odor thresholds are not reported in term of concentration but in term of odorosity (Deadman and Prigg, 1958) and E.D.₅₀ dilution factors (Dravnieks, 1974). These values have been converted in concentration values (i.e., ppm in air) by authors using an unspecified method. For this reason, we can not put as much credibility into these data as in those reported by other studies.

Nagy, 1991

The methodology used in this study meets all the criteria mentioned earlier in this paper except for the presentation procedure, i.e. "yes-no procedure". Moreover, as odor thresholds are already expressed in $\mu\text{g}/\text{m}^3$, we consider that this study yields an acceptable odor threshold value.

Nagata, 2003

Together with Stalker's study, the odor threshold reported here is one of the lowest values found in the literature. The method used, i.e. triangular odor bag method, appears to be an accurate means to measure odor thresholds, as it uses a forced-choice methodology. Indeed, it is reported

that at least for n-butanol the threshold measured by this method is almost the same as measured with classical dynamic olfactometry (Ishikawa and Nishida, 2000). Despite that, one major limitation of this study is the small size of the sample (only 6 panelists).

Dalton *et al*, 2003

The methodology used in this study is in accordance with all the criteria previously cited, including the forced choice procedure. The main strength of this work is the procedure to eliminate subject bias and the accuracy of the analytic measurement. Although the published manuscript expressed threshold concentration in terms of the dilution step, headspace measurements of each concentration were collected at each test point using gas chromatography and calibration curves developed; thus, styrene thresholds could be accurately calculated in $\mu\text{g}/\text{m}^3$ for this purpose. Thus, we consider that it is a very reasonable data point, especially given the large size of the sample (67 subjects).

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