THE SIRC REVIEW

RESEARCH • TECHNOLOGY • PUBLIC POLICY

Vol. 5, No. 1

Styrene Exposure and Color Vision

Update on the Research Program of the Styrene Information and Research Center

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ONE OBSERVER'S VIEWS

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POTENTIAL APPLICATION TO STYRENE

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THE SIRC REVIEW

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Introduction

The SIRC Review, as noted in the subtitle, focuses on information from research, technology, and public policy that is relevant to the safe use of styrene and protection of the public health and the environment. The articles that have been included in this issue encompass the breadth of this intent.

This issue of *The SIRC Review* begins with a technical article on color vision. Several recent reports in the scientific literature have presented subtle, subclinical (i.e. not serious enough to be noticed) effects upon color discrimination in styrene-exposed workers. Dr. James E. Sheedy, University of California, Berkeley, reviews the principles of color vision and methods of testing color vision. He then reviews the published studies on the potential effects of styrene on color vision. This report was commisioned by the Styrene Information and Research Center (SIRC) and has been presented to the HOC (Hydrogen, Oxygen, Carbon) Subcommittee of the American Conference of Governmental Industrial Hygienists (ACGIH) at a presentation by Dr. Sheedy and SIRC scientists regarding this and other health endpoints in studies of humans. This ACGIH subcommittee is involved in recommending a suggested Threshold Limit Value for styrene levels in the workplace.

The remaining articles in this issue of *The SIRC Review* examine the research program of SIRC and explore recent developments in the regulatory environment with regard to styrene. Dr. George Cruzan, Science Consultant to SIRC and former Chairman of SIRC's Science and Technology Task Group, provides a review of the numerous research projects that SIRC has sponsored. Since its inception in 1987, SIRC has consistently promoted state-of-the-art scientific studies of a diverse nature on potential health effects of styrene. Since then, SIRC has invested over $10 million on this effort. The SIRC research program was previewed previously (*The SIRC Review*, Vol 2, No. 1, 1991). Since that time, many of the proposed studies have been completed with other studies nearing completion. As these, and other, studies have identified or refined effects due to styrene exposure, SIRC's Science & Technology Task Group has investigated or proposed new areas of research.

Geoff Granville, Shell Canada Limited, and Dr. Steve Williams, BP Chemicals Limited, describe recent review processes by the Organization for Economic Cooperation and Development (OECD) and the European Union (EU), respectively. These reviews of styrene were based upon a draft document prepared by the Health and Safety Executive (HSE) of the United Kingdom for these forums. Several of the research projects sponsored by SIRC were prominent in this review. The HSE evaluation, although very thorough and lengthy, was a draft document for discussion by the appropriate international regulatory authorities; the necessity of completing several studies, notably the SIRC mouse cancer study, and addressing several other issues were recognized before the evaluation could be finalized. We have summarized the health risk assessment section of this document to provide perspective on the regulatory status of styrene in these two organizations.

Finally, the evolving changes in the way the United States Environmental Protection Agency assesses the carcinogenic risk of a chemical is reviewed in an article by Drs. Colin Park and Keith Johnson of the Dow Chemical Company. The EPA has proposed a new risk assessment procedure. As the carcinogenic potential of styrene has not been formally classified by the EPA, it is likely that these new guidelines will influence this process.

Keith A. Johnson, DVM, PhD
Editor

Styrene Exposure and Color Vision

By James E. Sheedy, OD, PhD

ABSTRACT

Color vision deficiencies (dyschromatopsia) have been reported in association with occupational exposure to styrene. This report investigates the published literature on this topic. Although some of the studies in this area are inconclusive because of methodology issues, those of Gobba et al. (1991), Gobba and Cavalleri (1993), Eguchi et al. (1995), and Campagna, Mergler et al. (1995) provide substantial evidence that styrene exposure is associated with color vision deficiencies. Each used the Desaturated D-15 panel, a sensitive color vision test, for assessing color vision in a styrene-exposed group of workers compared to either a matched control group or a lower exposed group. They all showed statistically significant poorer color discrimination in the higher exposed group.

Styrene exposure at higher concentrations, but not lower concentrations, is associated with color vision deficiency. Gobba et al. (1991) and Eguchi et al. (1995) split their exposed population according to exposure level and determined a significant difference between the high exposure group and a control group, and both also showed a non-significant difference between the low exposure group and a control group. High exposure levels are associated with color vision deficiency, whereas low exposure levels are not. The Gobba et al. study used 50 ppm styrene as the division between high and low exposure groups, the Eguchi et al. study used 30 ppm equivalent (based upon mandelic acid) for the division. The mean exposure levels of the high and low groups were not reported by Gobba et al., but were 93 ppm and 8 ppm respectively in the Eguchi et al. study. Based upon these studies, the minimum exposure level (or duration) at which significant color vision discrimination deficiency occurs cannot be precisely determined, but is between 93 ppm and 8 ppm based upon the Eguchi data. Campagna, et al. (1996) performed a reanalysis of 118 subjects from 2 previous studies. They calculated that a dose-response relationship between styrene exposure and color confusion index (CCI), a measure of discrmination of shaded discs, began at 4 ppm, with an upper limit of confidence interval (5%) of 26 ppm.

Styrene-induced color vision deficiencies statistically improve when exposure is decreased, the time course for improvements is between one month and two years.

The effects of styrene, as an independent solvent, upon color vision have been studied much more than those of other solvents.

Research shows that the degree of styrene-induced color vision deficiency is likely not subjectively noticeable. One way of characterizing the styrene-induced color deficiency is as approximately 1.5 additional transpositions on the Desat D-15 test, or approximately 0.35 transpositions on the less sensitive D-15 test which is more commonly used for occupational color vision screening.

Color vision deficiencies (dyschromatopsia) have been reported in association with occupational exposure to styrene. High exposure levels are associated with color vision deficiency, whereas low exposure levels are not. Styrene-induced color vision deficiencies statistically improve when exposure is decreased. In this article Dr. James Sheedy reviews the literature on these reported effects, noting in his conclusion that research shows that the degree of styrene-induced color vision deficiency is likely not subjectively noticeable.

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INTRODUCTION

Styrene is a clear, colorless liquid which evaporates easily and has a sweet smell. Styrene has a wide range of uses in plastics, synthetic rubbers, polyesters and latex coatings that are produced by the polymerization of styrene or copolymerization with other polymers. Since styrene evaporates very readily, styrene can be released into the air by industries that use it in their products. The primary human exposure to styrene is usually through respiration. However, it is also possible for styrene to enter orally or through the skin.

The most common health problems involve the nervous system. The problems include concentration problems, muscle weakness, tiredness, depression, and nausea at high styrene concentrations. There have been no reports of death as a result of styrene exposure. (US Public Health Service, 1992). The Environmental Protection Agency (EPA) has determined that 0.1 ppm is the maximum amount of styrene that may be present in drinking water. This was determined to be a safe lifetime exposure level against the non-cancer effects of styrene. The Occupational Safety and Health Administration has set limits for 40 hr/week, 8 hour/day employees of 100 ppm for a time weighted average with a short term exposure limit of 200 ppm.

Color vision disorders (dyschromatopsia) have been reported in association with occupational exposure to styrene. This report investigates the published literature on this topic.

COLOR VISION TESTING

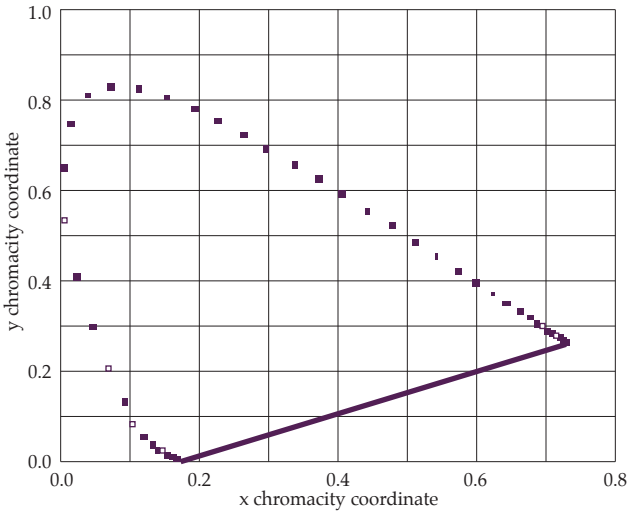
General

The sense of color is entirely subjective, although it is such a strong sensation that we perceptually treat it as a fixed physical attribute of the viewed object. Our sensation of color is based upon our sensory assessment of the relative amounts of the various visible wavelengths of light that enter our eye from each particular object. With modern instruments, we are able to accurately measure the exact amount of energy at each wavelength in the visible spectrum (from 400 nanometers [nm] to 700 nm) coming from an object — the result is a graph of the relative spectral energy. However, the eye does not determine the amount of energy at each wavelength. Instead, there are three different types of cone receptors in the retina — each of which is optimally sensitive to different regions of the visible light spectrum. Therefore, when we look at an object with a particular relative spectral energy distribution, it results in a particular ratio of stimulation of the three cone receptor types which, after considerable neural interpretation, is subjectively appreciated as a particular color. Most objects that we view result in different ratios of cone receptor stimulation and we therefore interpret them as being different colors.

Human color vision is surprisingly consistent across individuals thereby enabling the development of a color space which represents human color vision. Mathematical transformations of the wavelength content of any color stimulus can locate it on a color space diagram. The Commission Internationale de l'Eclairage (CIE) is the recognized agency which has standardized these functions and the associated color space(s). Details of the color spaces are available in textbooks (Wyszecki and Stiles, 1967). A sample of such a color space is shown in Figure 1.

FIGURE 1.

The CIE XYZ color space.



The two-dimensional color space which represents human color vision is derived from color matches made by human observers. White is in the middle of the space (0.333, 0.333) and the edges of the space contain the saturated colors arranged in their spectral (rainbow) order. Part of the edge of the color space contains the line of purples (the straight line in Figure 1) — these are not spectral colors, but represent the purples that are, in

effect, caused by simultaneous stimulation of red and blue. Desaturated colors are located between the middle and the edge of the space, e.g. a line between white (the center) and a particular red on the edge of the diagram would represent a smooth flow of color from white to red and contain the range of pinks that have the same hue as the particular red on the edge of the diagram.

Color vision tests are typically designed to test whether a person's color vision is normal or whether they have a color vision deficiency. A color vision deficiency manifests itself as an inability to discriminate between some stimuli that a person with normal color vision would be able to perceive as being different in color. Color vision deficiencies can be acquired or inherited as a sex linked recessive trait. About 8% of males and about 0.5% of females have a congenital color vision deficiency.

Most color vision tests, including those used in the assessment of the effects of styrene upon color discrimination, are designed around the characteristics of the color space. Human color discrimination within the color space has been quantified, i.e. the distance that is necessary to travel across the color space for a "just noticeable difference" (JND) in color. The JND for each location on the color space can be represented by a small circle or ellipse (Committee on Colorimetry of the Optical Society of America, 1963).

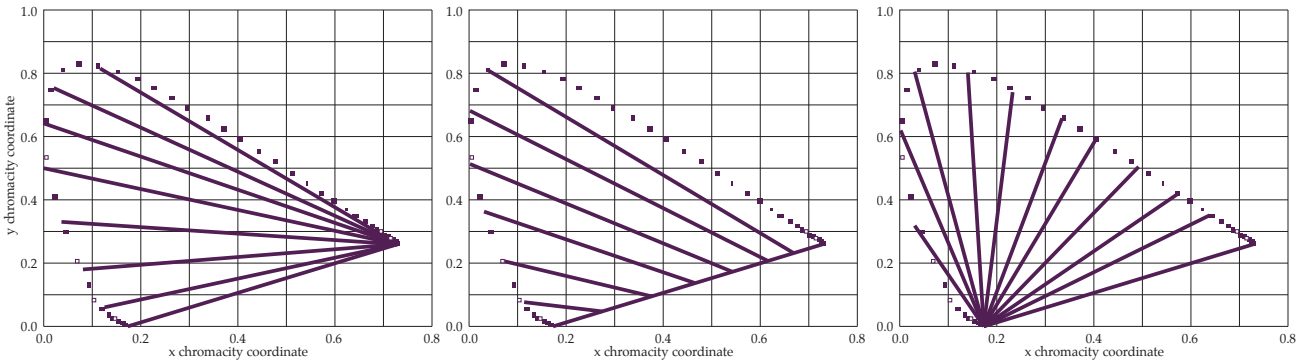
An important characteristic of the color space is that congenital and some acquired dyschromatopsias cause a pattern of decreased discrimination on the diagram, as shown in Figure 2. The pattern is that there are "lines of confusion" — discrimination is reduced along the lines of confusion relative to normal. The lines of confusion in Figure 2 represent the loci of colors which are perceived by normal individuals but for which discrimination is reduced for the person with the color loss. Dichromatism (missing one receptor system and therefore having only two) results in complete loss of discrimination along the lines of confusion, anomalous trichromatism results in partial loss of discrimination along the lines of confusion.

Furthermore, the lines of confusion for congenital dyschromatopsia intersect at a common point (the copunctal point) on the edge or just outside of the diagram (Figure 2). There are three copunctal points on the diagrams — one associated with each of the three cone mechanisms that subserve color vision. Theoretically, the copunctal point represents the color that would be perceived with pure stimulation of that particular cone system — a person with a "weakness" in that mechanism has difficulty discriminating colors along lines that radiate from that point.

The color vision tests described below can be used to identify the particular receptor system which is "weak" or missing. Anomalies of the long wavelength (red) system are termed "protan," anomalies of the middle wavelength (green) are termed "deutan," and anomalies of the short wavelength (blue) are termed "tritan." The color vision tests do not utilize saturated colors on the edge of the color space — instead they utilize desaturated colors which are quite near the center of the space. In this region, each congenital dyschromatopsia has an orientation of the lines of confusion which is unique to the affected receptor The tests are designed to identify the

FIGURE 2.

CIE chromaticity diagrams showing lines of confusion for the three fundamental types of congenital color deficiencies.



axis of the confusion. The protan and deutan axes of confusion are similar — therefore they are sometimes jointly referred to as the "red/green" axis. The tritan axis is often referred to as the "blue/yellow" axis.

The primary tests which have been used to assess styrene-induced dyschromatopsia are the Farnsworth Munsell 100 Hue Test (FM 100), the Panel D-15 (D-15), and the Lanthony or Desaturated D-15 test (Desat D-15). All of these tests use color chips which are embedded into the top of a cap or button. The caps are randomly displayed on a table surface and the subject is requested to arrange them in their proper color order, i.e. so that they represent a smooth flow of color along the line of arrangement. The examiner scores the performance by looking at the underside of the color buttons and recording the numerical order in which they have been arranged.

D-15 and Desaturated D-15 Tests

The D-15 test is quick and easy to perform and one for which there is substantial literature. The test involves the presentation of 15 color chips which the subject arranges in a smooth flow of color starting from one of the chips. The color chips form a circle around white in the color space. A person with a congenital dyschromatopsia will have reduced discrimination such that they mis-arrange the buttons by crossing the color circle rather than arranging the color around the circle — and the axis of the crossing can identify the particular disorder. The test is designed so that people with normal, or with low to moderate color vision deficiencies will pass. Because of this sensitivity, it is often used as a criterion for occupational standards such as for police or firefighters. The Desat D-15 test is identical except that the colors are desaturated compared those of the D-15. Therefore the colors form a tighter circle around white in the color space and the test is more sensitive — persons with milder forms of dyschromatopsia will make arrangement errors.

Bowman (1982) introduced a scoring technique for the D-15 test in which the total differences of the colors as they were arranged are summed to create a total color difference score (TCDS). In effect, this is a calculation of the travel in color space as the subject ordered the color chips. The TCDS score would be at its lowest if the chips were arranged in the intended order. A perfect arrangement results in a TCDS score of 117.0. If the chips are arranged in an incorrect order, it results in an increased travel through color space. If the increased color space travel was 30, then the total TCDS would be 147.0. A derived value is the Color Confusion Index (CCI), which is the TCDS for the particular arrangement divided by the TCDS for a perfect arrangement. In the above example, the CCI would be 1.256. The same scoring system is applied to the Desat D-15, but a perfect arrangement results in a TCDS of 56.

In a subsequent study, Bowman et al. (1984) determined normative values on 120 subjects with normal color vision, 20 from each decade between 10 and 70. The age effect is not very large. They determined norms for the D-15 test and the desaturated D-15. The normative values appear in Table 1.

TABLE 1.

Normative TCDS and CCI values for the D-15 and Desaturated D-15 tests as determined by Bowman et al. (1984).

Age Range	D-15 TCDS	D-15 CCI	Desat D-15 TCDS	Desat D-15 CCI
10-20	118.0	1.009	61.1	1.090
20-30	117.2	1.002	58.8	1.050
30-40	120.4	1.029	62.0	1.107
40-50	122.8	1.050	64.8	1.157
50-60	122.3	1.045	70.7	1.263
60-70	125.1	1.069	73.5	1.313

Perfect arrangements of the D-15 and Desat D-15 result in color space travel of 117.0 and 56 respectively. The Desat D-15 forms a tighter circle in color space and therefore has a shorter travel. Since there are 16 inter-chip distances in each test, the average color space distance between chips in those tests is 7.3 and 3.5 respectively. The ratio of these 2 numbers (7.3/3.5 = 2.09) can be taken as a measure of the relative sensitivities of the two tests. More subtle color vision disorders will be measured with the Desat D-15 than with the D-15.

On the average, color space confusion must be 2.09 times more severe to be detected with the D-15 test than on the Desat D-15 test. This means that more subtle forms of color discrimination deficiency will be detected by the Desat D-15 test and will be undetected by the D-15 test. It also means that for any color deficiency which can be detected by both tests, it would be expected that the increased color space travel will be greater for the Desat

TABLE 2.

Mean TCDS values as a function of age for the D-15 and Desaturated D-15 tests as determined by Bowman et al. (1984). The final column gives a measure of the increased color space travel for the Desat D-15 compared to the D-15 test.

Age Range	D-15 TCDS	D-15 TCDS-117	Desat D-15 TCDS	Desat D-15 TCDS-56	Desat TCDS-56/D15 TCDS - 117
10-20	118.0	1.0	61.1	5.1	5.1
20-30	117.2	0.2	58.8	2.8	14.0
30-40	120.4	3.4	62.0	6.0	1.8
40-50	122.8	5.8	64.8	8.8	1.5
50-60	122.3	5.3	70.7	14.7	2.8
60-70	125.1	8.1	73.5	17.5	2.2

D-15 test than for the D-15 test. This is because there are more (a factor of 2.09 on the average) chips per color space distance in the Desat D-15 test therefore resulting in greater opportunity for misarrangement. This also predicts that the Desat D-15 CCI will be higher for a given color vision defect.

These predictions are verified by analysis of the Bowman et al. (1984) age normative data as presented in Table 2.

When the perfect arrangement performances of 117 and 56 are subtracted from the mean age performances of the D-15 and Desat D-15 respectively it can be seen that in the younger age groups, for whom there is little measurable error, there is little or no increased color space travel for the D-15 test (column #3) whereas there is considerable increase in color space travel for the Desat D-15 (column #5). This is because of the increased sensitivity of the Desat D-15 test. For the older groups, there is increased color space travel for both tests, but considerably more for the Desat D-15 as predicted. The last column gives a measure of the increased color space travel for the Desat D-15 compared to the D-15 test. For the ages of 30-70, the mean factor is 2.075 — nearly identical with the 2.09 factor calculated above.

A single transposition on either test results in an increased color space travel which is approximately twice the distance between chips. Therefore, the approximate average increased color space travel caused by a single transposition would be 14.6 (range of 10-28) and 7.0 (range of 4.3-10.7) for the D-15 and Desat D-15 respectively, resulting in TCDS values of 131.6 (range of 127-145) and 63.0 (range of 60.3-66.7). Each mean results in the same CCI value of 1.125.

Of course, these values of 14.6 and 7.0 for the D-15 and Desat D-15 are only approximate averages, as can be seen from the ranges above, the location of a transposition is important in terms of the additional color space travel required. It must also be pointed out that the ratios of color space travel between the same numbered chips in the D-15 and Desat D-15 tests are not equal, i.e. the relative spacings of the color chips are different between the two tests.

Farnsworth Munsell 100 Hue Test (FM 100)

The Farnsworth Munsell 100 Hue Test (FM 100) (Farnsworth, 1943) is, similar to the D-15 test, a series of color chips which form a circle around white in the color space. There were initially 100 chips in the series, but 15 were so close in color to their neighbors that they were eliminated — subsequently the test consists of 85 color chips. Only one fourth of the chips, representing one fourth of the color circle, are presented to the subject at a time. The end color chips of that portion of the color circle are fixed and identified to the subject — the subject's task is to arrange the intervening chips in a smooth flow of color in between the fixed chips. The four quarters of the color circle are tested sequentially. The FM 100 test is a more critical analysis of the ability to discriminate fine color differences than is the D-15. Persons with "normal" color vision usually make mistakes on the test.

The color chips in the FM 100 test are numbered 1-85. The test is scored by calculating a value for each chip which is the sum of the differences in chip number between that chip and the ones on either side of it as

TABLE 3.

Normative values for the Farnsworth-Munsell 100 hue test from Verriest et al. (1982).

Age range	Monocular	Mono sqr root	Binocular	Bino sqr root
10-14	92.4	9.61	83.4	9.13
15-19	54.0	7.35	43.9	6.63
20-29	41.1	6.41	32.4	5.69
30-39	55.8	7.47	45.0	6.71
40-49	80.5	8.97	67.7	8.23
50-59	105.9	10.29	75.3	8.68
60-69	121.4	11.02	91.6	9.57
70-80	178.8	13.37	131.3	11.46

arranged by the subject. Therefore, the lowest score given to any chip would be two. The greater the mis-arrangement, the greater will be the calculated value for that chip. Normative age-related data were determined by Verriest et al. (1982) on 232 subjects screened for normal color vision. The mean monocular and binocular error scores are presented in Table 3. The error score is the total score in excess of a perfect performance (i.e., minus 170). The square root values are also reported, since their distribution is satisfactorily Gaussian. When interpreting the values, it is useful to recall that a single transposition in order results in an error score of 4. This can be demonstrated with the following example of a single transposition of numbers — the number in parentheses is the score for that number in the sample arrangement: ...20, 21(2), 22(3), 24(3), 23(3), 25(3), 26(2), 27...

Since only one fourth of the color circle is tested at a time in the FM 100 test, there is not an opportunity for a color deficient observer to cross the color circle as is possible in the D-15 test. However, the color deficient observer will have particular difficulty in the areas of the color circle where the axis of a line of confusion is tangent to the color circle. Therefore, the type of dyschromatopsia can be identified by determining the areas of the circle where the greatest mis-arrangement has occurred — or where the scores are the highest. There are several mathematical methods for doing this (Kitahara, 1984; Smith et al., 1985; Knoblauch, 1987).

STYRENE AND COLOR VISION STUDIES

This section contains a summary and assessment of each styrene color vision study.

Aliyeva et al. (1985)

Aliyeva et al. (1985) studied 705 workers from an air conditioner factory. The workers were placed into three groups as follows: 153 workers from the plastics shop who were exposed to styrene, 374 workers in the compressor shop exposed to tetrachloroethylene, and a control group of 178 office and maintenance workers. The exposure level to styrene was reported as; "Planned analyses of air samples in the plastic shop showed periodic excess of styrol vapors over the maximum permissible concentrations (mpc) by an average factor of three (15 mg/m^3)." It is difficult to determine the actual exposure level from this information — it is unlikely that the actual exposure level was 15 mg/m^3 — or only 3.5 ppm.

Color vision test plates were used to eliminate those with congenital color vision disorders. The remaining subjects had their color vision assessed with an AN-5-9 anomaloscope — an instrument which is not available in western countries nor could descriptions of it be found in western literature. Descriptions in the study indicate that it is an instrument that allows the measurement of a threshold amount of red, green, and blue. The exact threshold that is measured is not determinable from the study report. It appears that the AN-5-9 anomaloscope is not a standardized instrument since they had to first establish "the average threshold of color perception for the given apparatus" on a separate test group of 30 subjects.

Aliyeva et al. (1985) report that the "color perception threshold was increased in the styrene-exposed group 2.2 times more often than in the control group ($p<0.05$). The decreased sensitivity was most often to red."

Assessment

Assessment of this study is hampered by a lack of reported details about the styrene exposure levels of the population and about the color vision measurements. Assessment is further limited by unfamiliarity with the instrument used for color vision testing. The study lacks controls for other substances such as alcohol or tobacco. The actual results are also not presented (i.e., neither the actual measurements nor even the numbers of subjects who failed the color vision test).

Conclusions about the effects of styrene upon color vision cannot be determined from this study.

Gobba et al. (1991)

Gobba et al. (1991) studied 73 styrene exposed workers from seven fiberglass reinforced plastics factories in Italy compared to 57 workers employed in the production of rock wool. Exposure times for the test group ranged from one to 324 months, with a mean of 84 months or seven years. Environmental and biological measurements were made on a Thursday. There was a wide range in the measurements across the test group, mean styrene was 69.02 mg/m^3 (equivalent to 16 ppm), end shift urinary styrene was 49.5 μg/L and end-shift urinary mandelic acid was 342.9 mg/L. No historical exposure levels were reported.

All subjects were medically evaluated and screened to exclude poor vision, congenital color vision disorders, or systemic disease, drug or alcohol use which could affect color vision. The Lanthony desaturated D-15 test was used to evaluate color vision. It was administered under a daylight fluorescent lamp with a color temperature of 5000 K and providing an illumination of 1200 lux. The color confusion index (CCI) was the primary indicator used in analysis. Since Lanthony performance is affected by age, the data were analyzed by age category and are shown in Table 4.

TABLE 4.

Lanthony desaturated D-15 results from Gobba et al., (1991).

Age group		Control group	Test group	Significance
<29	N	17	42	
	Median CCI	1.043	1.078	NS
30-39	N	16	10	
	Median CCI	1.108	1.172	NS
>40	N	24	21	
	Median CCI	1.203	1.301	<0.05

Although the CCI values were higher for the exposed group in each age category, they were statistically significant only for the older category.

In order to test whether the differences between the exposed and control groups were significant independent of age, the two groups were reduced to 41 subjects each who were matched according to age (+/- 2 years). The method of selecting the sub-populations is not described. The results are shown in Table 5.

TABLE 5.

Lanthony desaturated D-15 results for 41 age-matched subjects from Gobba et al. (1991).

	Controls	Exposed	Significance
Age – Mean	36.2	36.1	NS
CCI Mean	1.151 (0.141)	1.265 (0.223)	<0.01
CCI median	1.110	1.265	

They also determined a dose-related effect of styrene exposure upon CCI. When the entire group of exposed workers was divided by the TLV-TWA exposure limit of 215 mg/m^3 (50 ppm), the higher exposed group had a significantly poorer CCI compared to control, but there was no significant difference between the lower exposed group and control. The mean exposure levels of the low and high exposure groups were not reported. The results are presented in Table 6.

TABLE 6.

Lanthony desaturated D-15 results for high and low exposure subjects compared to control, from Gobba et al. (1991).

	N	Median CCI	Comparison to Control
Control group	57	1.108	—
Low exposure (<50 ppm)	55	1.078	NS
High Exposure (>50 ppm)	15	1.297	p<0.05

From these results it appears that the CCI differences between the test and control groups are entirely due to the color vision measurements on those with levels of styrene exposure which exceed the TLV-TWA exposure limit of 215 mg/m^3 (50 ppm).

On a sub-group of 20 subjects, they also measured color vision after one month of interruption of exposure and determined that there was no improvement. This indi-

cates that the color vision deficiencies were persistent for at least this period of time. They also reported that most of the color vision deficiencies were in the blue-yellow region and not the red/green region of the color circle.

Muttray et al. (1992), in a letter to the editor were critical that Gobba et al. did not specify the means of selecting those with congenital dyschromatopsia for exclusion, that the authors didn't state whether tinted glasses or contact lenses were excluded, and that ethanol should still be considered as a confounder even if weekly consumption is below 250 g. The author's response (Galassi et al. 1992) stated that congenital color vision disorders were determined by case history, that their subjects did not wear tinted lenses, and that their subjects alcohol intake was considerably less than 250 g weekly and therefore was not a factor.

Assessment

The responses to the comments of Muttray et al. were largely satisfactory — however, the case history method of eliminating congenital color vision disorders is less than optimal and could have resulted in false positives and/or negatives in group selection. Although this may have affected the results, it is unlikely that it had a serious influence.

The retrospective selection of two sub-populations which were age matched is also of some concern. There are numerous methods by which the populations could be reduced to obtain age-matched groups — and it would be difficult to do it in a manner that was unbiased. This may affect the statistical test showing a significant difference between the entire exposed group compared to the entire control group.

The primary findings of the study were that 1) the older exposed group had a significantly poorer Desat D-15 result than the control group of the same age, and 2) the high exposure group had a significantly poorer color discrimination than control but not the low exposure group. The authors did not perform a multi-variate analysis, so the relative effects of age and exposure level are not reported. However, since CCI scores were poorer for all age groups (but only significant for the older group) and since the low exposure group actually had a better CCI than the control group, this is an indication that the exposure effect was greater than the age effect.

There is considerable overlap in the CCI values of the exposed and control groups, evidenced by the fact that the CCI difference between the two groups was 0.114 and the standard deviations for the exposed and control groups were higher (0.141 and 0.223 respectively) than this difference. The standard deviations for the high and low exposure groups were not reported, but a graph of the CCI values shows extensive overlap of the CCI values for the two groups. Although statistically significant differences are measured between groups, there is considerable overlap of the CCI values. The most significant result shown by this study is that the CCI for the Desat D-15 test was reduced relative to control for the high exposure group (levels greater than 50 ppm) and not for the low exposure group. However, the mean exposure levels of the high and low exposure groups were not reported.

Gobba and Cavalleri (1993)

This publication reports upon two test and control groups. The first groups are the same as reported previously by Gobba et al. (1991). In the Gobba and Cavalleri (1993) report, however, there is an additional group of 36 styrene-exposed workers who were tested along with 36 control workers matched for age, alcohol consumption and tobacco smoking. Data collection methods appear the same as for the earlier study. The mean exposure level of the test group was 68.2 mg/m^3 (15 ppm) and the mean urinary styrene level was 41.4 $\mu g/L$.

The mean CCI value (mean [SD]) for the exposed group was 1.206 (0.2) and for the control group it was 1.053 (0.07). The exposed group had significantly poorer ($p<0.001$) color discrimination compared to the control group.

Assessment

The differences between the two populations are highly statistically significant — and show that a group with a relatively low mean exposure of 15 ppm has significantly poorer color discrimination on the Lanthony compared to a matched control group.

They did not perform an analysis of high and low exposure groups, as in the previous study, nor was the relationship between exposure level and color deficiency analyzed in another manner.

Mergler et al. (1992)

Mergler et al. (1992) studied 128 workers who were divided into high and low mandelic acid groups as measured by post-shift urinary testing. The value of 0.60 mmole/mmole creatinine was used to separate the 2 groups. This value is reported by the authors to be the permissible level proposed by ACGIH (1991-2). Truchon et al. (1992) report that 0.67 mmole/mmole creatinine is the expected metabolite level in urine drawn at the end of

TABLE 7.

Color vision test results from Campagna et al. (1994).

	Styrene < 50 ppm	Styrene > 50 ppm	Mandelic acid <0.6 mmol/mmol cr	Mandelic acid >0.6 mmol/mmol cr
	n=60	n=28	n=67	n=21
acquired dyschromatopsia	20(33%)	10(36%)	18(27%)	12(57%)

*Chi square: $p<0.05$

a work shift in individuals exposed for 8 hours to inhalation of 213 mg/m^3 (50 ppm) of styrene.

Mergler et al. (1992) report that 52% in the high exposure group and 26% in the low exposure group showed color vision deficiency (Chi-square: $p<0.05$). However, the reporting of color vision testing and analysis is very limited (perhaps a limitation of the translation from French). Therefore, strong conclusions cannot be made from this report. However, better reports of analyses on this same study population appear below (Campagna et al., 1994; Mergler et al., 1995).

Campagna et al. (1994 and 1995)

These 2 publications are further analyses on the same population as Mergler et al. (1992). Analysis was performed on 88 of the initial 128 workers, exclusion criteria were: less than six months of exposure, poor near visual acuity, eye injury, congenital color blindness, cataracts, diabetes, use of potentially ophthalmotoxic medication, non work-related solvent exposure and incomplete exposure data. Mean styrene exposure was 205.78 (SD=262.35) mg/m^3. Mean mandelic acid was 0.36 (SD=0.52) mmol/mmol creatinine. The Desat D-15 test was used as a measure of color vision. Subjects were considered to have acquired dyschromatopsia if the caps were misplaced by at least 2, a single transposition was considered passing. Their results are shown in Table 7.

The results above show that the exposure level per se was not related to acquired dyschromatopsia, however, when the subjects were segregated by urinary mandelic acid there was a significant relationship with acquired dyschromatopsia.

Assessment

These studies provide convincing evidence that workers with levels of mandelic acid exceeding 0.6 mmol/mmol creatinine are at greater risk for acquired dyschromatopsia than those with lower levels of mandelic acid. The evidence indicates that the mandelic acid level is related to the color vision deficiency, and not the exposure level as measured with passive dosimeters.

Fallas et al. (1992)

Fallas et al. (1992) studied the color vision of a group of styrene-exposed workers compared to a control group. Their results were subsequently challenged (Muttray et al., 1993) and then defended (Fallas, 1993) in an exchange of letters to the editor of the journal.

Fallas et al. (1992) studied 60 men employed in a shipyard who had been exposed to styrene for a mean of 6.5 years. Workers were divided into four groups based upon duration of exposure (< 1 year, 1-5 years, 5-10 years, > 10 years). A psychometric test battery was measured on test and control subjects at the end of a shift. Color vision was tested during working hours "in daylight" with the Farnsworth 100 hue test. A control group of 60 age-matched male workers from the same employer was established.

During the three month study period, the mean styrene concentration was measured at 24.3 ppm, peak exposures were as high as 469 ppm. Some tasks were performed in restricted areas where concentrations may have been higher than the ambient air measurements that were made. Urinary concentrations of mandelic acid (MA) and phenylglyoxylic acid (PGA) were measured at the end of the shift on the day of the psychometric tests. The mean values were MA 230 mg/g creatinine and PGA 57.4 mg/g creatinine. Very low concentrations of polyvinyl alcohol and isophtalic resins were also reported.

The authors stated that they used a "specially devised automated procedure" for analysis of the Farnsworth 100

results — but don't specify or provide a reference for the procedure. The test results are shown in Table 8.

TABLE 8.

Farnsworth-Munsell 100 hue test results, Fallas et al. (1992).

FM 100 Hue test	Exposed workers	Controls	p value
Error score [mean(SD)]	259.9 (136.9)	262.7 (114.0)	NS
Blue/yellow and/or red/green ranges (Number)	32	20	<0.05

Assessment

It is difficult to place confidence in the data due to the many procedural problems. Also, the data that are reported do not support a difference between the exposed and control groups.

The mean error scores are significantly higher than those which are expected (Verriest et al., 1982). However, it is likely that they have reported the total scores which include the base value of "2" for each chip — subtraction of 170 (2 x 85 chips) from their reported numbers makes them consistent with reported normal values.

Their results show no difference in the error score between the test and control groups. However, they show that more workers in the exposed group than the control group had abnormal "discrimination between blue and yellow and between red and green." The authors do not further elaborate upon a description of what this is or means. They use these results to state that there was an "abnormally high frequency of dyschromatopsia in the exposed subjects." They also stated that the anomalies found in the study were "infra clinical and therefore not perceived by those concerned." They do not state how they determined that they were infra clinical nor how they determined that the subjects did not perceive them.

Muttray et al. (1993) were critical of the Fallas et al. study on the following bases:

1) The lighting used for the Farnsworth 100 testing.
2) Subjects may have been wearing colored glasses.
3) They didn't distinguish between congenital and acquired color deficiencies.
4) They didn't define the term "range."
5) No other eye measures (such as visual acuity) were taken to screen for eye disorders.
6) The mean of the error scores was significantly higher than those reported in previous studies, possibly indicating a large number of congenital disorders or possibly measurement procedure problems.
7) Testing during the shift did not allow for differentiation between acute and chronic effects.

They conclude by stating that the Fallas et al. (1992) study provides no evidence of a color vision impairment caused by styrene.

Fallas et al. (1993) responded accordingly by number:

1) "The Farnsworth Munsell procedural guidelines indicate that "sunlight" is irrelevant and that "daylight" together with fluorescent lighting is more appropriate. We have therefore applied the Farnsworth Munsell procedure."
2) No subjects wore colored glasses or contact lenses.
3) They find it difficult to understand that there would be a difference in the numbers of congenital color vision disorders between the 2 groups.
4) "Range" refers to circumferential errors.
5) All workers had annual examinations by an occupational physician and subjects were not used if they had any disorders that may have influenced the testing.
6) Not addressed.
7) They never claimed to distinguish between acute and chronic effects.

The criticisms by Muttray et al. are valid and the responses by Fallas et al. are only partially re-assuring. Colored glasses were probably not a problem (#2) and it is likely that most of the workers did not have eye disorders that would have otherwise influenced the color vision (#5). Also, any positive results can be annotated by stating that they could have been chronic or short term (#7).

The methods section of the original study and the later response by Fallas et al. do not enable a good determination of the lighting conditions during testing (#1) — nor whether they were even consistent across the study population. The high error scores (#6 — a criticism to which Fallas et al. did not respond) are likely the result of including the value of "2" for each chip. It is clear that no screening for congenital vision disorders (#3) was performed and that Fallas et al. allowed it to chance that the numbers of congenital vision disorders and their severity were equal in the two groups. The relative paucity of details about the color vision results and the short response to the criticism about the "range" do not indicate that the authors have a good understanding of the test measures.

It is also disappointing that they do not state the analysis method nor do they provide a reference for it.

Even if the procedural issues are set aside, the author's conclusions are not supported by their data. The most important outcome measure is the total error score — which was essentially the same between the two groups. Given that the total error score was the same between the two groups, it is intriguing that they found more R/G and B/Y defects in one group compared to the other. The "R/G and B/Y ranges" which Fallas et al. report are an indication of the percentage or extent of the errors that occur within the color ranges normally associated with R/G and B/Y disorders. (The authors, however, do not state how they mathematically made this determination.) If the total error score is the same in both groups, and the "R/G and B/Y range" scores are greater in the exposed group compared to the control group, then this would necessarily indicate that the exposed group performed better than the control group in the non-range areas of the color circle.

Chia et al. (1994)

Chia et al. (1994) studied the color vision of 21 styrene-exposed workers in comparison to 21 controls from the same factory who had not been exposed to styrene or other toxicants. The groups were age-matched and screened for low alcohol consumption. Medical assessments were performed on all subjects to rule out significant health conditions. No screening for congenital dyschromatopsia is reported.

The mean styrene exposure duration for the test group was 18.8 years. Urinary analysis was performed on all subjects on the day of testing. MA and PGA/creatinine values for the test group were 84.0 and 66.0 mg/g respectively, and for the control group they were 3.3 and 0.7 respectively. Projecting from the results of a previous study in the same factory, the authors calculated that the exposed workers were exposed to a mean environmental concentration of about 6.0 ppm styrene. Previous investigations in the same factory had measured daily means of 11.0 ppm in 1991 and 9.7 ppm in the 80's. The authors conclude that the workers were generally exposed to less than 10 ppm styrene.

Color vision was assessed on Monday mornings with the Lanthony D-15 Desaturated Panel. It was administered under a daylight fluorescent lamp providing 1000 lux of illumination. The total color difference score (TCDS) was used to score the test performances.

The TCDS values [mean (SE)] for the exposed and control groups were 164.0 (0.04) and 131.8 (0.04). The mean TCDS score for the exposed group was significantly higher ($p<0.0006$) than the control group, indicating poorer color discrimination. There was a linear (r of 0.33) but non-significant correlation between urinary MA and TCDS. The authors state the urinary MA is the best correlate of environmental styrene exposure.

Assessment

The authors didn't state anything about screening out those with congenital dyschromatopsia. In the relatively small subject population, this could result in significant errors.

More importantly, the reported TCDS numbers are not understandable. As population means, they are completely out-of-scale compared to normative values for the Desat D-15 test established by Bowman et al. (1984). The reported numbers are also considerably above normative ranges established for the D-15 test — thereby discouraging an assumption that the authors either mis-reported the test they used or that they used D-15 scoring of Desat D-15 test results.

The extremely low standard error values are also highly unlikely. A standard error of 0.04 on a population of 21 means that the standard deviation was only 0.18. The scatter that is normally encountered in administering the Desat D-15 or D-15 tests is magnitudes greater than this.

Conclusions cannot be drawn from this study without an understanding of the data.

Eguchi et al. (1995)

These authors assess color discrimination in 64 styrene-exposed male workers in comparison to 69 age matched male controls. Subjects were excluded on the basis of congenital dyschromatopsia (method not specified), hypertension, poor visual acuity, alcohol consumption over 250 g/week, or drug usage. Mean atmospheric styrene concentration (by area, not subject) was 18.5 ppm (6.6-35.4). Urinary mandelic acid concentration was measured on the day of color vision testing.

Color discrimination was tested with the Desat D-15 test. A perfect arrangement or a single transposition error was graded as normal color vision — all others failed. The TCDS and CCI were calculated for each subject. Non-parametric statistical testing was appropriately used for analysis of TCDS and CCI data.

Effects of age upon the TCDS values were noted in both the test and control groups and were consistent with normative data of Bowman (1982). The linear correlation for the test group was: TCDS = 54.14 + 0.46 X age, for the control group it was: TCDS = 52.53 + 0.34 X age.

TABLE 9.

Data [mean (SD)] for the high and low exposure groups compared to control (Eguchi et al., 1995)

	Low exposure		High exposure	
	test	control	test	control
n	40	40	17	17
mandelic acid (g/l)	0.20 (0.11)	—	1.06 (0.93)	—
equiv atm styrene (ppm)	8	—	93	—
CCI	1.173(0.191)	1.118(1.30)	1.332(0.293)	1.125(0.126)

The test group had a higher intercept value and a greater slope. The greater slope points to a greater age effect in the test group.

The test and control populations were then paired for age — resulting in a reduction to 57 in each group. The mean (SD) CCI for the exposed group was 1.220 (0.235) and for the control group 1.120 (0.128), the difference was significant ($p<0.01$).

To test dose effects, the control population was divided into those workers with mandelic acid concentration greater or less than 0.42 g/l — an amount stated to be equivalent to an atmospheric styrene concentration level of about 30 ppm. The results are presented in Table 9.

The difference in CCI scores between test and age-matched controls in the low exposure group was not statistically significant, whereas it was for the high exposure group ($p<0.01$). The high exposure test group also had a significantly higher CCI than the low exposure test group ($p<0.05$).

Assessment

The test and analysis methods in this study are appropriate. The results show that high styrene-exposure results in color discrimination deficiency relative to an age matched control group and also relative to a low exposure group. The exposure differences between the high and low exposure groups were large (equivalent atmospheric styrene levels of 8 and 93 ppm). Although there was a higher CCI in the low exposure group compared to age matched control, it was not statistically significant ($p<0.12$) on the paired groups of 40 subjects. As the authors state, their population size is not large enough to support further segmentation based upon exposure level, thus the data cannot be analyzed to determine the minimum threshold level at which there is a significant CCI difference.

OTHER SOLVENTS AND COLOR VISION

Nakatsuka et al. (1992) studied color vision deficiency in 261 workers exposed to toluene and tetrachloroethylene in comparison to 120 controls. The Lanthony test (Desaturated D-15) and the Ishihara test failed to identify any color vision defects other than congenital ones, therefore they concluded that no color vision deficiencies were detected in their sample of exposed workers. However, the negative results must be viewed with caution for two reasons. First, the exposure levels of the exposed groups were low (46 ppm for toluene group, 13 ppm for tetrachloroethylene group, and 13 ppm of tetrachloroethylene and 7 ppm trichloroethylene for a mixed group). Secondly, the Lanthony test was not administered in its usual fashion. Instead, subjects were asked to select Lanthony chips from among other "monochromatic chips." No basis is given for this non-standard method of using the Desat D-15 test. In fact, "monochromatic chips" indicates highly color saturated chips (although it is not technically possible that the chips were actually monochromatic). Therefore, the task of identifying Lanthony chips (which are very desaturated) from among very saturated chips becomes a very easy task and therefore not sensitive at identifying color defectives. Ishihara testing was performed on only those subjects who failed this unusual Lanthony testing. The negative results in this study would be expected on the basis of the unusual testing procedures.

Mergler and Blain (1987) studied color vision in a group of 23 solvent-exposed workers in a paint manufacturing plant. Exposure was to a mixture of solvents including acetone, methyl-ethyl ketone, toluene, xylene,

TABLE 10.

Perchloroethylene exposure levels and CCI from Cavalleri et al. (1994)

	# subjects	mean ppm	Mean CCI workers	Mean CCI controls	Sig level
all workers	35	6.23	1.143	1.083	0.025
dry cleaners	22	7.27	1.192	1.089	0.007
ironers	13	4.80	1.061	1.061	ns

styrene, MIBK, 2-ethanoxy ethanol and 2-ethanoxy ethanol acetate. The high exposure group (n=10) had significantly more color vision deficients than the moderate exposure group (n=13) as measured with both the Desat D-15 test and the FM100 test.

Cavalleri et al. (1994) studied color vision deficiency in 35 workers exposed to perchloroethylene in comparison to an age, sex, alcohol, and nicotine matched control group. The mean exposure level of the 35 workers was 6.23 ppm segregated into dry-cleaners (n=22, 7.27 ppm) and ironers (n=13, 4.80 ppm). They used the Lanthony desaturated D-15 test for color vision assessment. The results of testing are shown in Table 10.

The data show that a relatively low level (7.27 ppm) of perchloroethylene exposure was significantly related to color vision deficiency on the Desaturated D-15 test. The difference from control (CCI of 1.192 vs. 1.089) was somewhat less than the difference measured by Gobba et al. (1991), Gobba and Cavalleri (1993), and Eguchi et al. (1995) for somewhat higher levels of styrene (see summary in Table 12).

Broadwell et al. (1995) studied 25 workers who had been exposed to unmeasured levels of several solvents including 2-propanol, toluene, 1,1,1 trichloroethane, methylene chloride, chlorophene, acetone, trichloroethylene, freon TF, methanol and xylene (ordered on basis of quantity of solvents used in plant) in comparison to an age, gender, education and ethnicity matched control group. There was no significant difference in the CCI (Desaturated D-15 test) between the exposed and control groups, although there were significant differences in general symptoms, mood, finger tapping, simple reaction time and symbol-digit substitution. It is difficult to attach much significance to the lack of measured color vision deficiency since no information on exposure level is reported.

Baird et al. (1994) studied the color vision of 82 print shop workers who were exposed to acetone, isopropyl alcohol, toluene, xylene and 2-ethoxy ethanol. No significant differences in color vision testing (Desat D-15) were measured between the different exposure groups. However, the exposure levels to the solvents were generally about 1% or less of the ACGIH TLVs. Therefore, it can only be concluded that very low levels of exposure to the various solvents did not create color vision differences between the exposure groups.

The effects of styrene, as an independent solvent, upon color vision have been studied much more than those of other solvents. Perchloroethylene exposure has been shown to have effects upon color vision. Conclusions about the effects of other solvents upon color vision cannot be made.

OTHER SUBSTANCES AND COLOR VISION DEFICIENCY

Exposure to heavy metals has been shown to be associated with color vision deficiency. Cavalleri et al. (1995) studied 33 workers exposed to mercury vapor in comparison to an age, sex, alcohol, and nicotine matched control group. Lanthony desaturated D-15 test was used to assess color vision. Those with urinary mercury levels per g creatinine less than 50 μg/g had no difference between the exposed and control group. However, those with urinary mercury levels per g creatinine greater than 50 μg/g (n#) had significantly poorer CCI (1.285) compared to control (1.101). These decreases in color vision are somewhat greater than those measured by Gobba et al. (1991), Gobba and Cavalleri (1993), and Eguchi et al. (1995) for somewhat higher levels of styrene. (See summary in Table 12.)

Mergler et al. (1988) studied the effects of weekly alcohol intake upon performance on the Desat D-15 test. They divided their study group into light (<250 g/week), moderate (251-750 g/week) and heavy (>750 g/week) alcohol

consumption. The mean CCI performance based upon age and alcoholic consumption level are shown in Table 11. Multiple regression analysis showed that color vision deficiency was significantly related ($p<0.001$) to both age and alcohol intake.

The relative effects of age and alcohol upon the color vision deficiency cannot be determined from the study presentation. Also, in the study of alcohol intake, nutritional factors such as vitamin A intake can be confounders (Birch, 1993).

TABLE 11.

Mean CCI performance (Desat D-15) based upon age and alcoholic consumption level. (Mergler et al., 1988)

age range	light	moderate	heavy
20-29	1.00	1.03	1.17
30-39	1.09	1.13	1.14
40-49	1.14	1.14	1.48

Exposure to carbon disulphide has been shown to be associated with color vision deficiency (De Rouck et al., 1986; Ruijten et al., 1990). Color vision deficiencies due to n-hexane and methanol exposures have also been reported (Takeuchi, 1988).

It is also known that various diseases as well as therapeutic drugs result in color vision deficiencies. These include glaucoma (Adams and Rodic, 1982), ethambutol — a tuberculostatic drug (Spekreijse et al., 1991; Wietsma et al., 1995), heroin (Dias, 1990), digoxin — used in the treatment of cardiac dysrhythmia (Duncker et al., 1994; Hobley and Lawrenson, 1991; Young et al., 1991), methazolamide — a carbonic anhydrase inhibitor which is used in glaucoma management (Widengard et al., 1995), meprobamate — used to treat peptic ulcers and spastic colon (Applegate et al., 1986), and chloroquine — used in the treatment of connective tissue disorders (Birch, 1993).

Several disease conditions, drugs and other occupational exposures can result in measurable color vision deficiencies.

SUMMARY – IS STYRENE EXPOSURE RELATED TO COLOR VISION DEFICIENCY?

Although some of the studies in this area are inconclusive because of methodology issues, the following 4 studies provide substantial evidence that styrene exposure is associated with color vision deficiencies:

1) Gobba et al. (1991)

2) Gobba and Cavalleri (1993) – The first group reported in this paper is the same as reported in Gobba et al. (1991), but the second group reported in this study is new.

3) Mergler et al. (1992) and Campagna et al. (1994) used the same data.

4) Eguchi et al. (1995).

The results of the 3 studies that compared color vision (all used the Desaturated D-15 test) in an exposed group compared to a control (non-exposed) group and reported their measured values are summarized in Table 12.

The results of the 3 studies are remarkably similar in terms of the mean exposure of the test group (15 – 18.5 ppm) and in terms of the extent of the CCI difference between the test and control groups.

The mean exposure levels reported in these exposed

TABLE 12.

Summary of studies which showed a color vision difference on the Desaturated D-15 test between a styrene-exposed group and a matched control group.

Study	Mean Styrene Exposure level - ppm equiv	Mean CCI test group	Mean CCI control group	Significance level
Gobba, 1991	16	1.265	1.151	$p<0.01$
Gobba, 1993	15	1.206	1.053	$p<0.001$
Eguchi, 1995	18.5	1.220	1.120	$p<0.01$

TABLE 13.

Summary of two studies which separated the styrene-exposed group into high and low exposure groups, and then tested color vision (Desaturated D-15) in each group compared to a control group.

Study	Exposure group	Exposure level (ppm)	Mean CCI - test group	Mean CCI - control group	Significance level
Gobba, 1991	High	>50, mean not reported	1.297	1.11	$p<0.05$
Gobba, 1991	Low	<50, mean not reported	1.078	1.11	NS
Eguchi, 1995	High	>30, mean of 93	1.332 (0.293)	1.125 (0.126)	$p<0.01$
Eguchi, 1995	Low	<30, mean of 8	1.173 (0.191)	1.118 (1.30)	NS

groups, however, do not indicate the levels at which styrene exposure is associated with color vision deficiency. The exposed groups in these studies include individuals with a large range of exposure levels and, as shown below, only the higher exposure levels have an association with color vision deficiency.

WHAT IS THE THRESHOLD EXPOSURE LEVEL FOR COLOR VISION DEFICIENCY?

Gobba et al. (1991) and Eguchi et al. (1995) show that higher levels of styrene exposure are associated with poorer color discrimination as measured on the Desaturated D-15 test. Both studies split their exposed population according to exposure level and determined a significant difference between the high exposure group and a control group, and both also showed a non-significant difference between the low exposure group and a control group. High exposure levels are associated with color vision deficiency, whereas low exposure is not. A summary of the results is shown in Table 13.

The Gobba et al. study used 50 ppm styrene as the division between high and low exposure groups, the Eguchi et al. study used 30 ppm equivalent (based upon mandelic acid) for the division. The mean exposure levels of the high and low groups were not reported by Gobba et al., but were 93 ppm and 8 ppm respectively in the Eguchi et al. study. Based upon these studies, the minimum exposure level (or duration) at which significant color vision discrimination deficiency occurs cannot be precisely determined.

Eguchi et al. (1995) also reported the standard deviations of the CCI for the high exposure group and of the control group. The difference in the CCI is 0.207 and the standard deviations for the high exposure and control groups are 0.293 and 0.126 respectively. The difference between the two populations is similar in quantity to the standard deviations — indicating a great deal of scatter in the data. Therefore there is considerable overlap between the CCI values for the two groups — it is not possible to establish a single mandelic acid concentration (or derived air exposure level) which would differentiate between the two groups without having large numbers of subjects who are misclassified.

Campagna et al. (1996) performed an analysis of the 128 workers from the Campagna et al. (1994 and 1995) study and the 75 workers from the Gobba et al. (1991) study. After exclusions for contaminating factors, 118 subjects were included for analysis. They statistically analyzed the styrene exposure to CCI relationship by calculating the "change point." This method assumes that for low styrene exposure levels there is no relationship between exposure level and CCI, whereas at higher levels there is a relationship. The change point is the styrene exposure level below which no detectable effect occurs and above which there is a dose-response relationship. The calculated change point was 4 ppm, with an upper limit of confidence interval (5%) of 26 ppm. Based upon these data and statistical methods, this is the level at which the dose-response relationship begins.

It is difficult to place confidence in the results of the Campagna et al. (1996) analysis. The scatter in the data (as discussed above for Eguchi et al.) is very high, resulting in a low correlation coefficient ($r=0.246$, $r^2=.059$) between CCI and environmental styrene exposure. This means that only 5.9% of the variance in CCI is attributable to styrene exposure level. Given the high level of scatter in the data, it is difficult to place confidence in the result of a

TABLE 14.

Mean Desat D-15 performance of styrene exposed and control groups.

Study	Group	CCI	TCDS	TCDS (High-control)
Gobba, 1991	High exposure	1.297	72.6	10.4
	Control	1.11	62.2	—
Eguchi, 1995	High exposure	1.332	74.6	11.6
	Control	1.125	63.0	—

statistical procedure which effectively draws two lines of different slopes through the data and determines the intersection point of those two lines. Also, it has been shown by Gobba et al. (1991) and Eguchi et al. (1995) that low exposure groups do not have color vision deficiencies compared to control groups. The low exposure groups of Gobba and Eguchi were selected for exposures of under 50 ppm and 30 ppm respectively, the mean of the Eguchi low exposure group was 8 ppm. The lack of measured color vision deficiency in these low exposure groups is inconsistent with the Campagna et al. (1996) analysis which calculates the effect to begin at 4 ppm.

FUNCTIONAL SIGNIFICANCE OF COLOR VISION DEFICIENCY

Magnitude of Color Vision Deficiencies

The magnitudes of the measured color deficiencies in the high exposure groups compared to control are presented in Table 14. The high exposure groups in the Gobba et al. (1991) and Eguchi et al. (1995) studies had TCDS values that were respectively 10.4 and 11.6 higher than the control groups to which they were compared. These values represent the increased travel in color space compared to a perfect color arrangement.

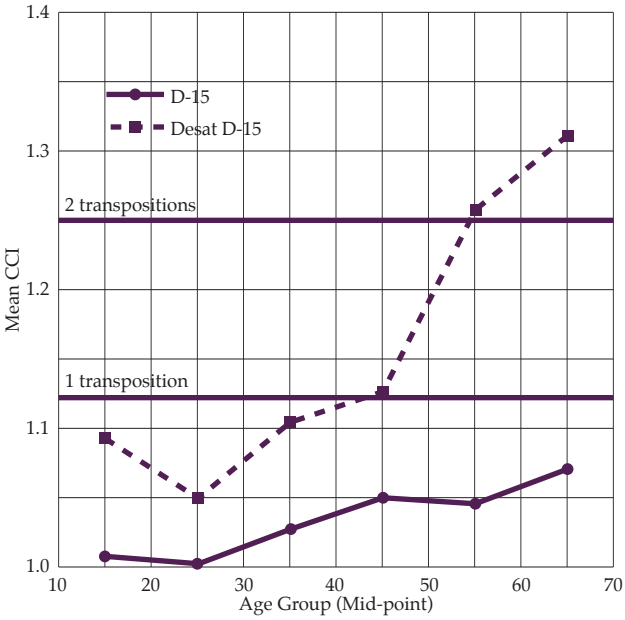
Comparison to Color Vision Deficiency with Age

A means of assessing the level of color vision deficiency would be to compare it to the age normative data provided by Bowman et al. (1984). Figure 3 shows the mean CCI values for the age groups for both the D-15 and the Desat D-15 tests. The CCI values associated with 1 and 2 transpositions are indicated on the graph. Since the CCI is a value representing arrangement relative to a perfect arrangement and each test has the same number of color chips, single and double transpositions result in the same average CCI values for both tests.

The data presented in Figure 3 show that the decreased color discrimination that occurs with age results in more transpositions on the Desat D-15 than on the D-15 test. This is because the somewhat subtle discrimination deficiencies which occur with age are not detected with the D-15 test. This is for reasons discussed earlier in this paper.

FIGURE 3.

Mean CCI scores for D-15 and Desat D-15 as a function of age. From Bowman et al. (1984).



Furthermore, there is a greater magnitude of TCDS deficiency with age measured with the Desat D-15 test than the D-15 for these same reasons. In other words, the same color vision deficiency (i.e., that associated with various age levels) manifests itself with fewer errors on the D-15 test and also the magnitude of the decrease in test performance with increasing color discrimination deficiency is less on the D-15 than on the Desat D-15.

The mean Desat D-15 CCI values for the Gobba and Eguchi control groups were 1.11 and 1.125 respectively. As seen in Figure 3 these approximate the average performances at age 35 and also approximate the performance of a single transposition on the Desat D-15 test. The high exposure groups of Gobba and Eguchi had CCI values of 1.297 and 1.332 respectively. These values approximate the average performances expected of age 65 and represent almost 2.5 transpositions on the Desat D-15.

Only the Desat D-15 test was used to assess the styrene-induced color vision deficiency in the Gobba and Eguchi studies. However, the magnitude of the color deficiency which would have been measured with a D-15 test can be predicted by the model that the increase in color space travel will be, on average, 2.09 times more on the Desat D-15 than on the D-15 (discussed earlier). The increased travel in color space can be related to the average number of color chip transpositions by using the average factors of 7.0 and 14.6 respectively for the Desat D-15 and D-15 tests respectively. Table 15 presents the calculated transpositions on both tests for the Gobba and Eguchi data for styrene deficiency and for the Bowman data on age-related color vision deficiency. The final column shows calculated predicted deficiencies of 0.34 and 0.38 D-15 transpositions based upon the Desat D-15 deficiencies of the Gobba and Eguchi data respectively. These compare favorably to the measured D-15 deficiencies from ages 35 to 65, as also shown in Figure 3.

Figure 3 and Table 15 show that the control groups of Gobba and Eguchi performed very similar to normal 35 year olds on the Desat D-15 and that the high styrene exposure groups performed very similar to normal 65 year olds. Therefore, one way of characterizing the color deficiency associated with high styrene exposure is that it reduces color discrimination approximately the same amount as aging from 35 to 65.

The control groups of Gobba and Eguchi and the age 35 group of Bowman made an average of approximately 1 transposition on the Desat D-15 test. The styrene exposed groups and the age 65 group made an average of approximately 2.5 transpositions. Therefore another way of characterizing the styrene and/or age induced color deficiency is approximately 1.5 transpositions on the Desat D-15 test.

Although Gobba and Eguchi did not use the D-15 test, calculations show that D-15 performances of control and styrene exposed groups would be approximately 0.5 to

TABLE 15.

Projected D-15 results based upon Desat D-15 data.

Study	Group	Desat D-15 TCDS-56	Desat D-15 # transpositions	D-15 TCDS-117	D-15 # transpositions
Gobba, 1991	High exposure	16.6	2.37	7.94*	0.54
	Control	6.2	0.88	2.97*	0.20
	Delta	10.4	1.49	4.98*	0.34
Eguchi,1995	High exposure	18.6	2.66	8.90*	0.61
	Control	7.0	1.00	3.35*	0.23
	Delta	11.6	1.66	5.55*	0.38
Bowman, 84	Age 65	17.5	2.50	8.1	0.55
	Age 35	6.0	0.86	3.4	0.23
	Delta	11.5	1.64	4.7	0.32

*calculated (predicted) TCDS values, all others are experimental data.

0.6 and 0.20 to 0.25 transpositions respectively. Therefore, another way of characterizing the styrene and/or age induced color deficiency is approximately 0.35 transpositions on the D-15 test.

The standard D-15 test is commonly used for occupational screening of job applicants. The hues of the D-15 test were selected so that mild color defectives could pass the test. It is reported that whereas 8% of the male population has a congenital color vision deficiency, only 5% of the male population will fail the D-15 (more than a single place transposition) (Birch, 1993). Benson (1995), writing in Duane's Clinical Ophthalmology, states "It is felt that those who pass the test (D-15) can perform almost all functions in our society that depend on hue discrimination." One transposition on the D-15 test is considered a passing performance. Given the mean calculated D-15 performance of the styrene exposed groups (0.54 and 0.61 transpositions) and the decrease relative to control group (0.34 and 0.38 transpositions) it is unlikely that many of the styrene-exposed subjects would fail to pass the D-15 test.

Subjective Awareness of Color Discrimination Deficiency

Mergler et al. (1991) studied the relationship between subjective visual complaints and measured visual deficiencies. They used a group of 116 former assembly workers from a microelectronics plant with suspected work-related illnesses. Most of the subjects had been obliged to quit their work because of health problems. The workers had been exposed to a wide variety of organic solvents including trichloroethylene, 1-1-1 trichloroethane and chlorofluorocarbons. The subjects had been exposed for a mean of 6.5 years, and it also had been an average of 6.5 years since they were last employed at the site.

Questionnaires were used (yes/no) to determine which subjective symptoms the subjects experienced and how concerned (minor, moderate, major) they were about the symptom. The prevalence of symptoms in the group were 83.6% blurred vision, 55.2% dark vision, and 42.1% color vision difficulty. The degree of reported concern was more minor for color vision than for blurred or dark vision.

The mean CCI (Lanthony Desaturated D-15 test) for those who reported minor or moderate symptoms of color deficiency was significantly higher than those who reported no or minor concern (1.70 vs. 1.35, $p<0.01$). This shows a subjective awareness of the color vision deficiency. However, the mean CCI index of the exposed groups of Gobba et al. (1991), Gobba and Cavalleri (1993), and Eguchi et al (1995) were 1.265, 1.206, and 1.220 respectively — all lower than the mean CCI of the group of subjects who reported no or minor concern. Therefore, since the CCI of the styrene exposed workers in these studies was lower than the CCI of the subjects with little or no concern about color vision, it should be concluded that the level of color vision deficiency in the styrene studies would likely not be subjectively noticed by the subjects.

RECOVERY FROM COLOR VISION DEFICIENCY

Pratt-Johnson (1964) reported a single case of a 48 year old male who reportedly had a styrene-induced retrobulbar neuritis. After 20 days of removal from styrene and administration of vitamin B compound and nicotinic acid, the visual acuities and field deficiencies were reported to be significantly improved. Examination at two months and six months showed progressive improvement, with complete resolution of the scotomas at one year. Color vision findings were not reported. Kohn (1978) suggests that the likelihood of nutritional amblyopia cannot be ruled out in the case, since vitamin B and nicotinic acid were administered during recovery. If clinically significant retrobulbar neuritis were to result from styrene exposure, a much larger literature on the subject would be expected since the Pratt-Johnson (1964) report.

Chang (1990) reported on 11 male patients with severe n-hexane induced polyneuropathy. All patients were removed from exposure and followed for four years. Motor deficits continued to worsen in five of the cases, sensory functions were regained earlier than motor functions. All patients regained full motor capabilities within 1-4 years after cessation of exposure. Only two patients had a mild abnormal color vision finding (color plate testing) — the color defect remained after four years. Maculopathy was observed on ophthalmoscopic examination. The color vision deficiency was supposedly related to the maculopathy, but it cannot be ruled out that these were congenital color vision disorders. If the color vision deficiency was n-hexane induced, then it was about the only function which did not recover during the four year period.

Color vision recovery or improvement has been shown when the color deficiencies have been due to alcohol (possibly the result of vitamin A deficiency) (Kapitany et al., 1993) and ethambutol (Nasemann et al., 1989).

Zdzieszynska and Gos (1995) studied 75 employees who were exposed to petroleum-based products in comparison to 30 non-exposed controls. They measured the FM100 test on both groups before and after a two week vacation. The performances of test and control groups before and after a two week vacation are shown in Table 16.

The distribution of FM100 scores in both the exposed and control populations improved — making it difficult

TABLE 16.

FM100 scores (% of group) of a petroleum products exposed group and a control group before and after a two week vacation (Zdzieszynska and Gos, 1995).

	Exposed group		Control group	
FM100 score	Before vacation	After vacation	Before vacation	After vacation
<16	4%	16%	13%	20%
17-100	67%	68%	70%	73%
>100	29%	16%	17%	7%

to establish a conclusion about reversibility. It might be that rested subjects perform better on the color tests or the improvement might be related to learning on the test.

In a study of 116 former workers who had been exposed to various industrial solvents (including trichloroethylene, 1-1-1 trichloroethane and chlorofluorocarbons), Mergler et al. (1991) determined that significant color vision deficiency was present (mean CCI on Lanthony Desaturated D-15 test of 1.40) — even though it had been 6.5 years on the average (range 1-16 years) since the subjects had been last exposed to the work environment. Most of the subjects had quit work because of health related problems. However, even though this was a group with relatively severe health problems, it appears that the color vision deficiency had persisted for several years. Color vision findings at cessation of employment are unavailable, therefore it cannot be determined whether recovery had occurred.

In a sub-group of 39 workers from their study on styrene exposure, Gobba and Cavalleri (1993) measured color vision immediately before and after a one month summer holiday. They found no difference between the before (mean CCI = 1.23) and after (mean CCI = 1.20) measurements, indicating that the color deficiency persisted for a one month period. However, they also state that "Nevertheless, preliminary evidence (data not shown) suggests that color impairment is potentially reversible after some months of exposure to substantially reduced levels. This point is still being assessed."

Mergler et al. (1995) studied 118 styrene-exposed workers at three plastics plants. Recommendations were made to reduce exposure at job-sites with the highest exposures. Exposure parameters (environmental styrene and end-shift mandelic acid) and various nervous system tests, including Desat D-15, were performed on all workers at the beginning of the study (T_0) and two years later (T_2). Complete measurements were made on 118 workers at the beginning of the study, 75 workers were still under employment after two years and 57 of these returned for testing. At only one of the three plants were improvements in exposure level accomplished during the two years, at the other two plants there was either no change in exposure or increased exposure (at some worksites). Spearman's correlation was used to test the relationships between T_0-T_2 differences in end-shift mandelic acid and T_0-T_2 differences in nervous system tests. Significant relationships were found between mandelic acid changes and color testing (Spearman's Rho: $p<0.001$), simple reaction time ($p<0.05$) digit symbol forward ($p<0.05$), tension and fatigue scale ($p<0.05$), and number of reported symptoms ($p<0.05$). The strongest relationship was with color vision. Since the change in mandelic acid was closely associated with changes in color vision (lowering of mandelic acid was associated with improved color vision scores), the authors conclude that color vision may be particularly sensitive on a short term basis to changes in exposure. These results also show that color vision improves over a two year period when exposure is reduced.

The Gobba and Cavalleri (1993) and Mergler et al. (1995) studies provide the most pertinent data related to recovery of color vision deficiency. The Mergler et al. (1995) study shows improvements in color vision testing are significantly associated with exposure level improvements over a two year period. Since Gobba and Cavalleri (1993) showed no improvements after a one month vacation, it appears that the time course for improvements is between one month and two years.

PHYSIOLOGICAL/ANATOMICAL BASIS OF COLOR VISION DEFICIENCY

Acquired dyschromatopsia could theoretically be the result of either changes in optical filtering (such as yellowing of the crystalline lens) or the result of neural processing of color vision. Mergler, Blaine and Lagace (1987) studied 23 workers (13 moderate exposure and 10 high exposure) exposed to solvents (none was styrene). Using the FM 100 test they determined that 11 of the 23 workers had dyschromatopsia and that it occurred in a significantly greater proportion of the high exposure group. The primary finding of interest here, however, was that they also performed biomicroscopy to identify abnormalities of the ocular media and funduscopy to examine the optic nerve head, macula and fundus background. They determined

that there were no observed abnormalities which would explain the dyschromatopsia. This lack of observable changes to the anatomy or physiology of the eye lead to the conclusion that the color vision reducing effects of solvents are neurally mediated and not due to observable structural disorders of the eye or filtering effects of the optical media.

If the effects are neural, retrobulbar optic neuritis might be evident in cases of over-exposure. Pratt-Johnson (1964) reported a single case of a 48 year old male who had worked at a glass fiber factory and was exposed to styrene for a period of 5 years. The visual findings were a significant decrease in his central vision as a result of a central scotoma (loss of visual field in the central area). The author concluded that this was a case of styrene-induced retrobulbar neuritis. After 20 days of removal from styrene and administration of vitamin B compound and nicotinic acid, the scotomas were reported to be significantly improved. Examination at two months and six months showed progressive improvement, with complete resolution of the scotomas at one year. The patient also smoked one pack of cigarettes/day, and vitamin supplement treatment began at the same time as styrene exposure was stopped. Kohn (1978) suggests that the likelihood of nutritional amblyopia cannot be ruled out in the case. Kohn examined 345 workers who were exposed to average styrene levels of 5 ppm, but ranging to 200 ppm. He found no evidence of optic neuritis or retrobulbar neuritis. His only finding was conjunctival irritation in 22% of the population. He observed that the irritation occurred commonly in those who had exposure levels above 50 ppm, but he provides no data to verify this observation. The evidence of styrene-induced retrobulbar neuritis is not strong.

Zdzieszynska and Gos (1995) studied 75 employees who were exposed to petroleum-based products in comparison to 30 non-exposed controls. They critically evaluated and classified the macula (central retina) in each subject and determined that exposed workers had more noticeable macular deviations from normal compared to the control group. They do not state, however, whether the experiment was double blind. Their observations, if valid, show retinal changes. Since the retina is neural tissue, this is a neural change.

Aliyeva et al. (1986) determined that workers exposed to styrene and tetrachloroethylene had retinal blood vessels (arteries and veins) which were significantly larger (dilated) than a group of non-exposed workers.

Skoog and Nilsson (1981) studied the acute effects of intravenous styrene in monkeys upon the electroretinogram. The electroretinogram is a non-invasive recording of retinal electrical activity resulting from light stimulation. They determined that there was a very significant effect of styrene upon the amplitude of the c-wave portion of the ERG. The c-wave is generated by potential changes in the pigment epithelium of the retina and/or the photoreceptors (which are embedded in the pigment epithelium). This is an indication of a neural change in the retina. It is possible that this may be the result of the effects of solvents as membrane stabilizers. It is difficult, however, to project how these short term effects on the c-wave in monkeys apply to long term effects in man.

Mirzoyev and Sultanov (1989) measured electroretinograms on 102 workers exposed to 2-3 times the maximum permissible (Russian) level in comparison to a control group of 50 administrative workers. The results indicate a significantly lower amplitude of both the a-wave ($p<0.01$) and the b-wave ($p<0.01$) and a longer latency ($p<0.01$) in the exposed group compared to the control group. The a-wave is from electrical activity in the photoreceptors whereas the b-wave is from electrical activity in the first or second neuron in the retina. The reduction in the a-wave is suggestive of an insufficiency in the choroidal circulation. The study lacks controls for other substances such as alcohol or tobacco.

Mergler and Blaine (1987) used the Desat D-15 and FM100 test to study color vision deficiency in a group of 23 solvent exposed workers. All individuals manifesting a color vision deficiency showed a blue-yellow deficiency, however some workers also showed a red-green deficiency. Mergler, Belanger et al. (1988) determined that exposed styrene workers showed a higher incidence (35%) of complex dyschromatopsia (blue-yellow and red-green deficiency) compared to non-exposed workers who showed primarily blue-yellow deficiency. Moreover their data suggest that complex deficiency (R/G and B/Y) was more prevalent among highly exposed groups than lesser exposed groups for whom only B/Y deficiency was measured. Campagna et al. (1995) reported that, of 31 styrene-exposed workers with dyschromatopsia, 22 were blue-yellow, 1 red-green, 2 mixed, and 6 indeterminate. Eguchi et al. (1995), Gobba et al. (1991) and Gobba and Cavalleri (1993) all likewise found that most of the styrene-induced deficiencies were B/Y, although some were complex (including R/G also). None of the deficiencies was just R/G.

According to Kollner's rule, blue-yellow deficiency reflects changes in the external retinal layers whereas red-green disorders reflect changes in the internal retinal layers or optic nerve. Although the rule holds fairly well, all cases of dyschromatopisa do not adhere to it (Hart, Ruddock). Mergler, Belanger et al. (1988) suggest that the early stages of solvent exposure may result in B/Y defi-

ciency which progress to also include R/G deficiency. There are not sufficient data to demonstrate whether Kollner's rule applies to solvent-based color vision deficiency, however assuming that it does, this would indicate that the neural effects begin distally in the external layers of the retina and then progress proximally to include the internal retinal layers and optic nerve.

A retinal basis for the color vision deficiency is also supported by the fact that other substances have been shown to mediate color vision deficiencies via a retinal mechanism. These include glaucoma, digoxin (Duncker, 1994), ethambutol (Nasemann et al., 1989) and methazolamide (Widengard et al., 1995). Glaucoma likely mediates deficiency by physical damage to retinal neurons in the nerve fiber layer or in the optic nerve head, digoxin likely mediates its affect by hyper-polarization of cell membranes, and methazolamide likely has effects upon membrane ion flow.

The site of styrene-induced color vision deficiencies is almost certainly retinal, with considerable evidence supporting the idea that the deficiencies begin in the external (distal) portions of the retina and progress towards the internal layers of the retina and perhaps to the optic nerve.

OTHER CONSIDERATIONS

The measured color vision deficiency in styrene exposure studies is small and the scatter in the data relating styrene exposure level to CCI is very high. The large amount of scatter in the data might represent a large range of human variance in sensitivity. However, it is also possible that the large amount of scatter is due to other factors that have not been controlled in these studies. Future research should aim to determine if the scatter is the result of human variance in sensitivity to styrene or whether it is due to uncontrolled variances in study design.

Variance in human sensitivity can be studied by making other psychological and physiological measures on the subjects. In particular, those with poorer color discrimination should be studied to determine why their performance was poor.

There are several ways in which other variances could be better controlled. It is likely that a record of styrene exposure and urinary metabolites for longer periods of time would provide a better measure of styrene exposure. The measure of color vision could also be tightened by giving the Desat D-15 test multiple times (probably using more than one copy of the test) in order to smooth out performance variations and obtain a truer measure of the subjects' color discrimination ability. It is also possible that the FM100 test would give a better assessment of color discrimination ability. Better attention should also be given to matching certain characteristics of the control population to the styrene-exposed group. For example, since performance on the Desat D-15 test requires critical observation skills, it is possible that it may be influenced by motivation, experience, skill and/or comprehension. These factors should be controlled in future studies or their influence upon Desat D-15 performance may be studied independently.

It is also possible that a better relationship between styrene exposure level and color discrimination could be obtained in monkey experiments with much better control on the level of styrene exposure. With animal studies, however, relevance is always an issue.

SUMMARY

The effects of styrene, as an independent solvent, upon color vision have been studied much more than those of other solvents. Perchloroethylene exposure has been shown to have effects upon color vision. Conclusions about the effects of other solvents upon color vision cannot be made. Several disease conditions, drugs and other occupational exposures can result in measurable color vision deficiencies.

Styrene exposure at higher concentrations, but not lower concentrations, is associated with color vision deficiency. While four studies have shown that styrene exposure is related to color vision deficiencies (Gobba et al., 1991; Campagna et al., 1994; Gobba and Cavalleri, 1993; and Eguchi et al., 1995), only two of them (Gobba et al., 1991 and Eguchi et al., 1995) split their styrene-exposed population according to exposure level. These two studies determined a significant difference between the high exposure group and a control group, but no color vision difference between the low exposure group and control. The mean exposure levels of the high and low groups were not reported by Gobba et al., but were 93 ppm and 8 ppm (equivalents of mandelic acid levels) respectively in the Eguchi et al. study. Based upon these studies, the minimum exposure level (or duration) at which significant color vision discrimination deficiency occurs cannot be precisely determined, but is between 93 ppm and 8 ppm based upon the Eguchi data.

Research shows that the level of styrene-induced color vision deficiency is likely not subjectively noticeable. The control groups of Gobba and Eguchi made an average of approximately one transposition on the Desat D-15 test. The high styrene-exposed groups made an average of approxi-

mately 2.5 transpositions. Therefore one way of characterizing the styrene-induced color deficiency is as approximately 1.5 transpositions on the Desat D-15 test. Although Gobba and Eguchi did not use the D-15 test, calculations show that D-15 performances of control and styrene exposed groups would be approximately 0.20 to 0.25 and 0.5 to 0.6 transpositions respectively. Therefore, another way of characterizing the styrene and/or age induced color deficiency is approximately 0.35 transpositions on the D-15 test. It is unlikely that many of the styrene-exposed subjects would fail to pass the D-15 test which is more commonly used for occupational color vision screening.

Eguchi et al. (1995) also reported the standard deviations of the CCI for the high styrene-exposure group and of the control group. The difference in the CCI is 0.207 and the standard deviations for the high exposure and control groups are 0.293 and 0.126 respectively. The difference between the two populations is similar in quantity to the standard deviations. Therefore, there is considerable overlap between the CCI values for the two groups. It is not possible to establish a single mandelic acid concentration (or derived air exposure level) which would differentiate between the two groups without having large numbers of subjects who are misclassified.

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Update on the Research Program of the Styrene Information and Research Center

By George Cruzan, PhD, DABT

ABSTRACT

A principle purpose of the Styrene Information and Research Center (SIRC) is to clarify any potential health effects to humans from exposure to styrene. Inconsistencies and deficiencies in many of the numerous studies of styrene that have been conducted over the past 50 years, as well as new developments in techniques and understanding of mechanisms, have made definitive conclusions difficult to establish. This is particularly noticeable in attempts to assess the carcinogenic potential of styrene, which has been subject to many different interpretations.

In 1991, the Science and Technology Task Group of the Styrene Information and Research Center published an article in *The SIRC Review* (vol. 2, pp. 56-60) outlining the research plan for clarifying the carcinogenicity issue for styrene. Research was to be carried out under the sponsorship of SIRC, the European Chemical Industry Ecology and Toxicology Center (ECETOC), or the European Styrene Steering Committee (SSC). This article provides a status report of that research program. The major focus has been on research to assess the carcinogenic potential of styrene, but other areas of the SIRC research program are also discussed. As with any research program, the studies conducted by SIRC have answered many questions, but have also raised some new questions. SIRC is actively pursuing additional research to answer the new questions.

Styrene is a material with a rich, but often conflicting, database of studies in humans and experimental animals. SIRC continues to sponsor research and ongoing projects, such as the mouse bioassay, in the belief that complete understanding of the data and mechanism will lead to scientifically based regulations.

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THE RESEARCH PROGRAM - CARCINOGENICITY POTENTIAL

Numerous authoritative and regulatory bodies have evaluated the carinogenic potential of styrene, with inconsistent results. The differences stem from different interpretations of data and different evaluation/classification criteria.

As described in the EPA Proposed Guidelines for Carcinogen Risk Assessment, the purpose of a carcinogen risk assessment is to construct a total case analysis including tumor responses in humans and laboratory animals, mode of action, and metabolism. SIRC has evaluated the existing data on styrene and has conducted an active research program to add information in areas needing clarifications, including studies of tumor responses in humans and animals, metabolic determinations and physiologically-based pharmacokinetic (PBPK) modeling, cytogenetics, and biomarkers of exposure.

EPIDEMIOLOGY STUDIES

By 1990, eight cohort mortality studies of workers involved in the manufacture or use of styrene were reported in the literature (reviewed by Bond et al., 1991). Collectively, they involved nearly 50,000

workers employed between 1940 and 1986. Although these studies did not identify styrene as a potent carcinogen, the average follow-up of exposed individuals was too short to rule out increased cancer risks after more than 15 years from first exposure. Increased incidences of lymphatic and hematopoietic (LH) cancer, compared to normal population rates, were reported in some of these studies, but none of these studies concluded that styrene exposure was responsible for the potential increases in these tumors.

In order to extend the latency period, SIRC sponsored the updating of the largest cohort of U.S. workers in the industry with the highest exposures, those involved in the manufacture of reinforced plastics and composites (RPC). Wong et al. (1994) reported on the update of 15,826 workers employed for at least six months in the RPC industry in the U.S. during 1948-1977, with an average followup of 19.6 years (307,932 person years). They reported finding no relationship between several indices of styrene exposure (time since first exposure, duration of exposure, average exposure, cumulative exposure, or job category) and any LH cancer deaths. In addition to no increase in overall LH cancer deaths, there was no increase in any subcategory, including leukemia. Two statistical analyses (Cox regression models) were run; one using only cumulative exposure as a variable and the other with both cumulative exposure and duration of exposure as variables. Neither analysis showed styrene exposure to be a significant risk factor.

IARC also recognized the deficiencies in the epidemiologic data and sponsored a large study of reinforced plastics workers in Europe. Although this was not sponsored by SIRC, the European SSC or ECETOC, it is included here for completeness.

The IARC study (Kogevinas et al., 1994a, b) reported on 40,688 workers employed in the European RPC industry (no minimum employment period). This study was a combination of eight cohorts from six European countries. Each cohort had a different follow-up period ranging from 1945-1990. Overall, there was an average follow-up of 13 years (539,479 person years). There was no increase in deaths from LH cancers in this cohort; however, the authors reported that LH cancer deaths showed an increasing trend with time since first exposure and average styrene exposure. Although the trend test was statistically significant, none of the Standard Mortality Ratios (SMRs) for the different exposure categories were significantly increased. LH cancer deaths did not show an increasing trend with either duration of exposure or cumulative exposure to styrene.

Several additional factors in this study indicate styrene is not likely a causative factor: deaths from LH cancer were higher among workers with unspecified tasks than among laminators (workers with the highest potential exposures), the leukemia deaths did not show a statistically significant increasing trend with average exposure or time since first hire, the increase was seen only in the Danish subcohort (a higher percentage of short-term workers, a larger proportion hired more recently, a younger population, no individual exposure assessments, and shorter follow-up), rather than across the whole cohort.

Thus, except for the Danish subcohort, for which there is no styrene exposure assessment or job history, American (15,826) and European (24,821) cohorts of RPC workers showed no increase in LH cancer deaths in relation to styrene exposure, and had fewer than expected LH cancer deaths.

No additional epidemiology studies are planned by SIRC.

Three recent articles on the IARC cohort (Welp et al., 1996a,b,c) have suggested increases in deaths from nonmalignant causes (genitourinary system and nervous diseases and mental disorders, but not respiratory diseases) with increasing styrene exposure, although the standardized mortality ratios were less than 1.00. SIRC will reexamine the SIRC cohort (Wong et al., 1994) for any relationship between these causes of death and styrene exposure.

ANIMAL STUDIES & LONG-TERM CARCINOGENICITY STUDIES

Prior to 1991, a total of 11 long-term animal studies by the oral or inhalation routes had been reported on styrene or a mixture of styrene and b-nitrostyrene. Inhalation studies, the most likely route of exposure due to the high vapor pressure of styrene, were conducted using rats (Conti et al., 1988; and Jersey et al., 1978). Oral gavage studies have been reported for rats (Conti et al., 1988; National Cancer Institute, 1979a; and Ponomarkov and Tomatis, 1978) and for mice (NCI, 1979a; and Ponomarkov and Tomatis, 1978). Oral gavage studies of a mixture of 70% styrene and 30% b-nitrostyrene have also been conducted in rats and mice (NCI, 1979b). Styrene in the drinking water has been evaluated in rats (Beliles et al., 1985). These studies, except for the Beliles drinking water study, were all conducted prior to 1980 (the Conti studies were conducted in 1974-1977 although not published until 1988). The design of the studies often differed markedly from currently accepted methodology with

consequent deficiencies and difficulty in interpretation. Factors such as inappropriate dose levels or dosing regimen, inadequate numbers of animals in the treatment or control groups, and intercurrent disease processes all complicate interpretation of these studies. In addition, none of these studies were conducted according to OECD or EPA Good Laboratory Practice requirements.

A weight-of-the-evidence evaluation of these studies indicates that there is no clear indication of a carcinogenic response. However, concerns have been expressed by several groups who have reviewed the styrene animal carcinogenicity studies. The US EPA, IARC and the Canadian government were concerned with the potential for increased mammary tumors in female Sprague-Dawley rats in the Conti et al. (1988) inhalation study and with the potential for increased lung tumors in mice in the Ponomarkov and Tomatis (1978) and NCI (1979a) oral gavage studies.

Thus, SIRC, EPA, and the National Toxicology Program (NTP) agreed that state-of-the-art chronic inhalation studies of styrene were needed in rats and mice to clarify these questions. Subchronic studies demonstrated that mice are much more susceptible to styrene toxicity than rats (Cruzan et al., 1997); liver and lung toxicity were seen in mice. The results of the recent rat inhalation study (SIRC, 1996), and the ongoing mouse inhalation study, which were designed and conducted in accordance with EPA and OECD guidelines, under Good Laboratory Practice requirements, and with an independent peer review of histopathology, are critical in resolving the question of animal carcinogenicity.

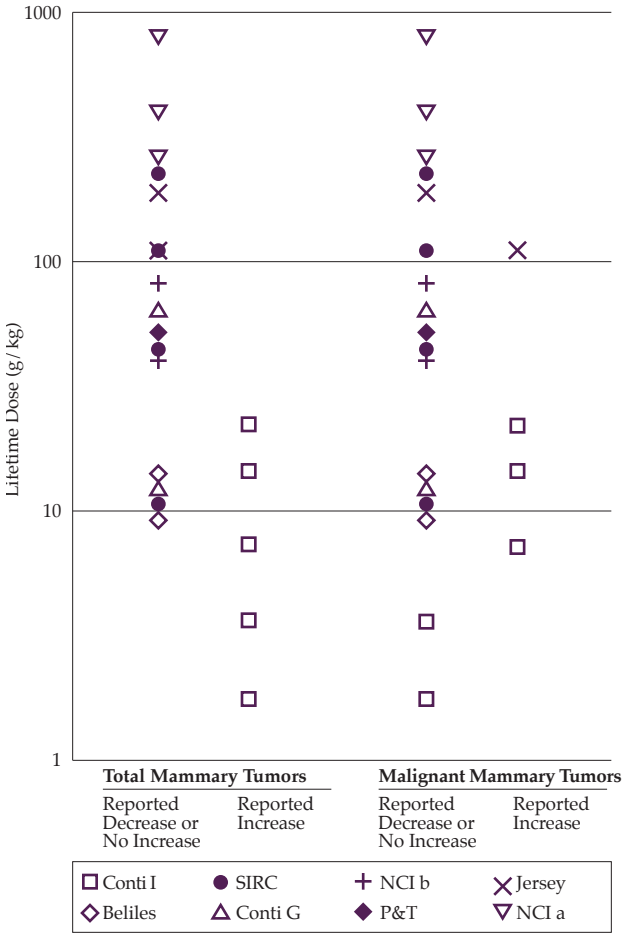
In the SIRC rat study (1996), groups of 60 male and female CD (Sprague-Dawley derived) rats were exposed to 0, 50, 200, 500, or 1000 ppm styrene vapor six hrs/day five days/week for 24 months. There were no treatment-related increases in tumor incidences in this, the only rat study performed according to GLPs and current study design criteria. Factors implemented in the study design and conduct included: sentinel animals to monitor spontaneous infectious disease; study designed to EPA and OECD guidelines; large group sizes, interim necropsies (10 additional rats/sex/group) after 12 months and thorough clinical pathology evaluations throughout the study; thorough characterization of test article and chamber atmospheres; thorough histopathologic examination; GLP compliance and external GLP monitor; and external peer review of pathology. The study demonstrated that styrene does not induce mammary tumors in female rats. In this study there was a dose-related decrease in mammary and pituitary tumors in females.

Figure 1 demonstrates that 12 groups of rats in six different studies exposed to greater amounts of styrene than the highest dose reported by Conti et al. (1988) to cause increased mammary tumors did not have an increased incidence of these tumors. Thus, a complete weight-of-the evidence evaluation demonstrates that styrene does not induce cancer in rats.

In the on-going SIRC mouse study, groups of 50 female CD-1 mice were exposed to 0, 20, 40, 80, or 160 ppm six hrs/day five days/week for 22.6 months, while similar groups of males were exposed to the same con-

FIGURE 1.

Mammary Tumor Results for Female Rats Exposed to Styrene.



centrations for 24 months. Increased incidences of benign adenomas in the lung have been seen in treated mice. There was not a consistent dose-response. In females treated at 160 ppm, there was also an increase in malignant adenocarcinomas. The pathologic evaluation of tissues is not yet completed. The lung tumors appeared to be a late occurring event since no increase in lung tumors was seen through 18 months of exposure. A final report of the study findings is anticipated by late 1997. No increase in lung tumors was seen in a screening assay in sensitive A/J mice (Brunnemann, 1992). The question of carcinogenic activity in mice is unresolved at this time.

A research program is being implemented to understand the significance of the mouse lung tumors for human risk assessment. The plan is a joint effort between SIRC and the European Styrene Steering Committee and will include research activities aimed at identifying the risk of lung cancer in humans exposed to styrene. The basic data for the risk assessment will include identification of the target cell, toxic agent, comparative metabolism of the toxic agent in mouse, rat and human tissue, mode of action, and human exposure assessment.

SIRC plans no further long-term animal studies beyond completion of the mouse study.

Structure/Activity Relationships

The effects of chemicals on living organisms are determined by their chemical structure. Materials having similar structures may have similar toxicologic properties. Thus, carcinogenicity data on materials similar to styrene give some insight into the carcinogenic potential of styrene.

Chronic inhalation of vinyltoluene (32-35% p-methylstyrene; 65-71% m-methylstyrene) did not result in increased tumors in either rats or mice (NTP, 1990). Similarly, p-methylstyrene (>97% para) was not carcinogenic in rats or mice in chronic studies by gavage administration (Conti et al., 1988). In addition, ethylene and propylene, less closely related monomers that are also metabolized to epoxides similar to styrene, were also negative in one inhalation study in rats (Hamm et al., 1984) and two negative studies each in rats and mice (Quest et al., 1984; NTP, 1985; Ciliberti et al., 1988), respectively.

Thus, other monomers containing a single reactive olefinic moiety have not demonstrated carcinogenic effects in laboratory animals. This adds support to the lack of carcinogenic response following exposure to styrene.

No additional research on compounds structurally related to styrene is planned by SIRC.

Metabolism/Pharmacokinetic Data

The metabolism of styrene in animals and man is known to proceed largely through styrene-7,8-oxide (SO) to mandelic and phenylglyoxylic acids, which are subsequently excreted in the urine. Several studies have confirmed that metabolism of styrene is highly dose (exposure concentration) dependent. In humans, as well as in laboratory animals, the ability to metabolize styrene becomes saturated beginning around 200 ppm styrene. As a result, toxic effects observed at dose levels higher than 200 ppm cannot be directly extrapolated to low exposure concentrations. This indicates the need for great care in assessing risks to humans from low level exposures based on effects seen in laboratory animals only at high exposure levels.

Studies sponsored by SIRC and ECETOC have demonstrated that humans have the least capacity to form SO from styrene and the greatest capacity to remove SO by further metabolism (Mendrala et al., 1993). In rats, the blood SO level is about 1% of the blood styrene level (Mendrala et al., 1993), while in humans it is about 0.5% of the blood styrene level (Korn et al., 1994). Comparative data in mice have not been published, but the SO level would be expected to be much higher. A physiologically based pharmacokinetic (PBPK) model (Csanady et al., 1994) has been constructed which can more accurately estimate the dose of styrene and SO delivered to target organs in each of the different species, including humans.

In more recent research sponsored by SIRC, Sumner et al. (in press) demonstrated a faster excretion of styrene metabolites in F344 rats and CD-1 mice than B6C3F1 mice following a single six hour inhalation exposure at 250 ppm, consistent with a greater liver injury in B6C3F1 mice. No difference in excretion was seen following three or five days of exposure, consistent with the absence of ongoing acute liver necrosis beyond the first three days of exposure.

In research at Purdue University sponsored in part by SIRC, lung toxicity from intraperitoneal administration of styrene has been demonstrated (Gadberry et al., 1996). Important differences in metabolism, especially by multiple cytochrome P450 isozymes may be important in the contribution of toxicity in liver and lung (Carlson, 1996).

Further research on lung toxicity and metabolism in mice is anticipated.

Genotoxicity

Genetic toxicology studies focus on the interaction of chemical and physical agents with the process of heredity. Short-term tests for genotoxicity were developed to study mechanisms of chemically induced DNA damage,

and to assess the potential genetic hazard of chemicals to humans. A series of *in vitro* and *in vivo* assays are currently available to assess the various kinds of genetic damage that could potentially be caused by environmental agents. Because genotoxicity studies measure genetic endpoints and the theory that carcinogenic responses may result from mutational responses in somatic cells, positive results in genotoxicity studies are often cited as supporting evidence for the carcinogenicity of a chemical. In recent years, the use of short-term tests for predicting carcinogenic potential has been more seriously questioned.

Mutagenicity

The potential mutagenicity of styrene and its metabolites, especially SO, have been studied extensively in a variety of assay systems. SO is mutagenic in a number of *in vitro* mutagenicity studies; however, activity was reduced in the presence of glutathione or metabolic activating systems, indicating ready metabolic clearance. The *in vitro* studies indicate that styrene is not mutagenic in the absence of metabolic activation. The response to styrene in assay systems which incorporate metabolic activation is equivocal; a few studies have reported weak activity, but most studies are negative.

At present, the extrapolation of results of short-term *in vitro* mutagenicity tests to human risk assessment is unclear. Thus the significance of positive mutagenicity findings from SO in *in vitro* studies is unclear. No additional work is planned by SIRC in this area.

A complete weight-of-the evidence evaluation demonstrates that styrene does not induce cancer in rats.

Cytogenetics

There are a wide variety of assay systems to evaluate potential cytogenetic effects (i.e., effects on chromosome structure and number) of chemicals. The assays examine chromosomal aberrations (deletions of parts of chromosomes or rearrangement of chromosomes), sister chromatid exchanges (apparent reciprocal exchanges between two chromatids of a single chromosome) and aneuploidy (gains or losses of one or more chromosomes). The cell types studied in these assays may be obtained from humans or animals exposed *in vivo* (e.g., peripheral blood lymphocytes), or cultured cells from unexposed humans or animals may be exposed to test chemicals *in vitro*.

There is substantial controversy regarding the human health significance of cytogenetic assays. Most experts maintain the results of cytogenetic assays cannot be used directly to estimate adverse health effects that might arise from exposure to a particular agent because:

(a) the unsuitability of the cell type for direct extrapolation (i.e., from differentiated circulating cell to somatic cells that might be involved in cancer),

(b) the end-point does not have known biological consequences (e.g., sister chromatid exchanges), or

(c) specific alterations that might be related to genetic or somatic adverse effects are not measured.

Nevertheless, some regulatory officials interpret cytogenetic effects as supporting evidence for carcinogenic potential.

A large number of cytogenetic studies have been carried out using styrene or SO in experimental animals. All *in vitro* studies of styrene using human lymphocytes have reported increased chromosomal aberrations and sister chromatid exchanges, while those using Chinese hamster lung or ovary cells were generally negative. *In vivo* studies of chromosomal aberrations and micronuclei were generally negative, but most studies of sister chromatid exchange were positive. Since 1991, both the US EPA and SIRC conducted cytogenetic studies at high inhalation exposure concentrations. In the SIRC study, rats were exposed to styrene at up to 1000 ppm six hrs/day five days/week for four weeks. No increases in chromosomal aberrations or sister chromatid exchanges were found (Preston and Abernathy, 1993). In the EPA studies, Kligerman et al. (1992, 1993) exposed rats and mice at up to 500 ppm six hours/day for fourteen consecutive days. Increased sister chromatid exchanges were found but no increases in chromosomal aberrations or micronuclei were seen in rats or mice.

Cytogenetic studies of reinforced plastics workers, with relatively high styrene exposures, have yielded mixed results. An evaluation of those studies by Scott and Preston (1994) concluded that 18 of the 52 studies reported positive cytogenetic effects (sister chromatid exchange, chromosomal aberrations or micronuclei) but the data are not compatible with the conclusion that styrene is the responsible agent. A lack of dose-response, contradiction by well-conducted animal experiments at much higher levels, and effects inconsistent with *in vitro*

experiments lead to this conclusion.

No further research in this area is planned by SIRC.

Styrene-7,8-Oxide Mechanism Studies

The generally accepted regulatory view is that any carcinogenic potential of styrene should be attributed to styrene-7,8-oxide (SO). Five long-term studies in which SO was given by gavage (stomach intubation) have resulted in cellular damage and repair in the forestomach, as well as increased hyperplasia and tumors of the forestomach, but not in other tissues (reviewed in Dalbey et al., 1996). On the other hand, two skin application studies of SO did not result in increased skin tumors. SO is mutagenic in a number of *in vitro* mutagenicity studies; however, activity was reduced in the presence of glutathione or metabolic activating systems, indicating ready metabolic clearance.

Because SO has some genotoxic properties and causes tumors, it has been assumed by some that SO acts by a genotoxic mechanism and therefore SO generated by the metabolism of styrene will have the same carcinogenic effects as SO administered by gavage. Studies sponsored by ECETOC examined the biological reactivity of SO as measured by the binding of styrene and styrene oxide to macromolecules. Very low levels of 0.02 and 0.075 picomoles hydroxyphenylethyl adducts per gram hemoglobin per micromole of styrene have been demonstrated following intraperitoneal injection in rats and mice, respectively (Osterman-Golkar et al., 1995). The level of hemoglobin adducts from exposure to SO was about 4-6 times that from exposure to styrene. Christakopoulos et al. (1993) reported a very low level of 0.025 picomoles hydroxyphenylethyl adducts per gram hemoglobin per ppm-hour in workers with an average exposure of 75 ppm styrene.

Very low levels of reaction products of styrene with DNA have been reported in the livers of mice, but not rats. Cantoreggi and Lutz (1993) reported tightly bound radioactivity in the livers of mice exposed to 90 mg/kg in a closed inhalation chamber; the level of radioactivity corresponded to 5 to 9 adducts per 10^8 nucleotides. Oral administration of radiolabeled-SO to rats resulted in tightly bound radioactivity in the DNA of forestomach corresponding to about 1 adduct per 10^7 nucleotides (Lutz, et al., 1993). Neither styrene oxide nor styrene are very reactive toward DNA.

Oral administration of SO to rats results in cell damage and in increased cell proliferation. Gavage administration three times per week for four weeks at doses of 137, 275, and 550 mg/kg/day caused increased cell proliferation (Lutz et al., 1993; Cantoreggi et al, 1993). Subsequent work demonstrated increased cell proliferation from gavage administration of 50 mg/kg or greater, with a plateau above 250 ppm and a NOEL of 20 mg/kg (Dalbey et al., 1996). These data indicate that the forestomach tumors from gavage administration of SO are secondary to tissue damage, not from direct DNA damage, and suggest a threshold for the effect.

Future work on biomarkers of exposure and mechanism of action are anticipated to focus on lung reactions in mice.

Future work on biomarkers of exposure and mechanism of action are anticipated to focus on lung reactions in mice.

A key change in cancer risk assessments advocated in the new EPA proposed Guidelines for Carciogen Risk Assessment (1996) is the development of an understanding of the mode of action of tumor formation in animal studies and how that relates to human risk. SIRC and SSC will be looking at genotoxic and non-genotoxic mechanisms in mice and to what extent those mechanisms function in humans.

THE SIRC RESEARCH PROGRAM - NON-CARCINOGENIC ENDPOINTS

Neuro-epidemiology

Worker exposure standards such as the previous ACGIH TLV and the 1989 OSHA PEL (later voided) were set at 50 ppm based on the avoidance of acute neurotoxic effects of styrene. The literature upon which these evaluations are made was reviewed by Drs. Rebert and Hall (1994). Their evaluation indicated that, almost universally, the reports of styrene-related neurotoxic effects at levels below 50 ppm were false positives due to statistical error, the action of some other factor, or misinterpretation of the data. They concluded that there was no scientific basis for a reduction in the current U.S. exposure standards of 50 ppm TWA and 100 ppm STEL.

Color Discrimination

Several researchers have reported slight diminutions in the ability of reinforced plastics workers to discriminate

subtle color differences. These were reviewed by Dr. James Sheedy; his review is the first article in this issue of *The SIRC Review*. He concluded that at higher, but not lower, concentrations styrene exposure is associated with color vision deficiency. The existing data do not permit the assessment of a NOEL. In one study the cohort was split between those with greater than 30 ppm (mean 93 ppm) and those with less than 30 ppm (mean 8 ppm); in another study, the cohort was split between those with exposures greater than 50 ppm and those with less than 50 ppm. In both studies increased color confusion was seen in the higher exposed group, but not in the lower. The differences were slight, not likely to be subjectively noticeable and would be within the range of normal on occupational color vision screening.

Irritation/Toxicity to Respiratory System

Repeated exposures of rats and mice to styrene vapors results in concentration and time dependent changes in the olfactory epithelium of the nasal passages (Cruzan et al., 1997). In rats, effects were seen after 24 months of exposure at 50 ppm in males and 200 ppm in females. In mice, effects were seen in males and females after 12 months of exposure to 20 ppm. Effects were seen after 13 weeks of exposure in rats at 500 ppm and in mice at 50 ppm. Although not systematically studied in humans, diminished sense of smell has not been a prevalent worker complaint even among those exposed to levels greater than 50 ppm.

SIRC has initiated a study to determine the time-course of nasal lesion development and reversibility in mice. It is anticipated that some studies in workers may be conducted.

Ecotoxicity

SIRC has recently completed acute ecotoxicity tests on a number of key organisms using flow-through or renewal systems with careful analytical determination of styrene concentrations throughout the tests (Cushman et al., in press). The data indicate that styrene, like other monoaromatic compounds is moderately toxic to fathead minnows (96 hr LC_{50} = 10 mg.l), daphnids (48 hr EC_{50} = 4.7 mg/l), and amphipods (96 hr LC_{50} = 9.5 mg/l). Styrene was highly toxic to green algae (96 hr EC_{50} = 0.72 mg/l); the effect was algistatic, not algicidal. Styrene was only slightly toxic to earthworms (14 day LC_{50} = 120 mg/kg soil). These studies were performed in systems that minimized styrene evaporation; effect levels were calculated based on levels of styrene in these systems determined analytically. Styrene's potential impact on aquatic and soil environments is significantly mitigated by its volatility and biodegradability. Styrene is found in the environment only at low levels. There are adequate margins of safety for realistic exposures of aquatic species to styrene and no adverse effects are expected to aquatic species or soil unless styrene is spilled directly into a low-energy water body or onto soil.

A comprehensive review of the environmental fate and effects of styrene has been completed by Dr. Martin Alexander, Cornell University, and has been submitted for publication. No further ecotoxicity work is currently planned by SIRC.

CONCLUSION

Interest among regulators remains high in evaluating the carcinogenic potential of styrene. Styrene is a material with a rich, but often conflicting, database of studies in humans, experimental animals and genotoxicity, mechanistic and metabolic studies. SIRC continues to sponsor research and ongoing projects, such as the mouse bioassay, in the belief that complete understanding of the data and mechanism will lead to scientifically based regulations.

We have identified the current areas of ongoing research: understanding the mechanism of styrene effect on olfactory epithelium in rodents, its relevance for human exposure limits, effects of styrene on human respiratory system, and relevance of lung effects in mice exposed to styrene.

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OECD SIDS Process and Styrene

ONE OBSERVER'S VIEWS

By Geoff Granville

SIDS, the acronym for "Screening Information Data Set," is a program which the Organization for Economic Cooperation and Development (OECD) has been coordinating for the past 10 years. OECD member countries agreed in the 1980s that a collaborative approach to the testing and evaluation of chemical substances was consistent with its broader objective of ensuring high-quality economic growth across its membership. The SIDS program allows member countries to share the results of a package of mammalian and environmental toxicology tests on a series of substances agreed to be of high priority. The data package generated under the SIDS program comprises an agreed upon series of physical, chemical and toxicological studies which allow a risk manager to make an initial assessment of the likely health and environmental hazards that could be presented by the substance in question. This program was developed in response to the public demand for more data on man-made substances whilst keeping a controlling hand on the evaluation and general management of testing requirements.

Over the past two years, it was appreciated by those involved in the SIDS program that testing was by itself not enough; results of the tests needed to be uniformly discussed and evaluated in order to allow the OECD process to lead to a conclusion about the relative safety of the substance in question. Out of this concern was born another OECD forum: the SIDS Initial Assessment Meeting, or SIAM. The fourth meeting of this forum (SIAM4) was held in May 1996 in Tokyo and styrene was one of the many substances discussed.

One might well ask why a well-researched substance like styrene should be the subject of a "SIDS" approach - surely it has more than enough data readily available? The answer to this is not straightforward, but relates to recent developments by the European Union (EU) on the subject of existing chemicals regulations. Under these requirements, European Industry has been targeted to conduct further toxicity testing, if a formal assessment conducted by an EU country and endorsed by the EU recommended such a test. As a result, industry in Europe became very concerned that it could be placed at a competitive disadvantage versus other geographical areas if it had to test substances both for OECD SIDS as well as for EU purposes. Based on this concern, an agreement was reached within Europe that the results of EU assessments became part of the EU input to the SIAM process. The Tokyo meeting was the first at which the results of recent EU assessments were discussed; benzene, 1,3-butadiene, trichloroethylene and styrene were all on the agenda.

> Another expert assessment has been drafted on styrene...and again there have been differences of opinion.

The United Kingdom (UK) was the country which assessed styrene. The summary of this draft assessment is presented elsewhere in this volume of *The SIRC Review*, but briefly the UK concluded styrene was unlikely to present a carcinogenic hazard to human beings, and it was unlikely to be a neurotoxic hazard at levels below 100 ppm in the air. Readers will be interested to know there were disagreements with the UK assessment in some areas, particularly with respect to the treatment of neurotoxic and genotoxic endpoints. Disagreements came from

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the Scandinavian countries and Germany in particular, but also from the USA and Canada. The recent availability of the negative rat bioassay completed by SIRC was of importance in lowering concerns about potential carcinogenicity, but it was appreciated SIRC's ongoing mouse bioassay needed to be completed and assessed before drawing any final conclusions. An additional toxicology study (an *in vivo* mutagenicity study, such as an unscheduled DNA synthesis study in rodent liver) was recommended by the UK as part of a broader package of risk assessment-related investigations.

Another expert assessment has been drafted on styrene (although completion is pending inclusion of the SIRC chonic mouse study), and again there have been differences of opinion. No serious concerns were raised about potential carcinogenicity, but there were many questions about the interpretation of the neurotoxicity and genotoxicity data.

It seems that with any industrially-significant substance with an extensive database, there are always more questions than answers. The SIAM4 meeting spent some time later in the session to discuss how to address "data-rich" substances such as styrene, and OECD will be considering modifying its processes as a result. At this time it is not known what form such modifications will take, but it is obvious that a comprehensive assessment on any industrially important substance with a complex database will be time consuming and involve a wide range of toxicological expertise.

A modest testing program for styrene has been suggested by the EU regulatory community, and European Industry will have to address how to carry out these studies. The genotoxicity study may well be helpful in better understanding whether styrene has any *in vivo* genotoxicity.

Chemical assessment procedures are becoming more and more international and sophisticated in scope. International Agency for Research on Cancer (IARC) and U.S. Environmental Protection Agency (EPA) assessments were once the only broadly available sources of scientific review, but this situation is changing. The addition of extra players in the assessment arena will result in additional levels of confusion and in further calls for better coordination. The philosophies underpinning hazard and risk assessment procedures will continue to be analyzed, criticized, and slowly improved upon as we attempt to maintain an approach to managing hazardous substances that is consistent with our current state of knowledge. In time, we will begin to address questions of "potency" and "risk" to a much greater extent than is currently the case, and move away from the relatively simplistic, but still very detailed, "hazard assessment" approach. The EPA has started to move this way in their Proposed Guidelines for Carcinogen Risk Assessment.

With respect to styrene, which is generally accepted around the world as being an highly valuable chemical substance, the future seems similar to the past....research will continue, risk assessors and bureaucrats will monitor the results, and additional regulatory changes will be enacted after prolonged debate with those impacted by these regulations. One simple reality will not change - that the hazard of a chemical substance is related to both its intrinsic (i.e. toxicological) hazards, and to the levels of exposure that can occur for human beings and in the general environment. Therefore the direction forward of styrene's regulatory status will comprise a continual pressure on the allowable levels of exposure for both workers and the general public. This is the same scenario for most other widely-used chemical substances, but the speed and timing of future exposure reductions depends on three basic factors: (i) the scientific database on styrene, (ii) how persuasive industry is in national and international forums in gaining acceptance for its interpretation of the data, and (iii) the level to which the public voices its concerns about chemicals in general and styrene in particular. One thing is certain - by virtue of its ongoing research program and its access to expert scientists, SIRC will continue to play a major role in helping to shape the regulatory future for styrene.

The SIAM4 meeting was notable in that it marked the first time the OECD SIDS program discussed data-rich substances, and therefore OECD now has to consider how to deal with such substances in future. In addition it is noteworthy that the UK presented a risk assessment that was broadly consistent with SIRC's position on styrene. Disagreements on how to safely handle chemicals used in industry will be with us for many years to come, and this subject has now found yet another forum in which to be discussed. Finally, and on a more humorous note, the reader cannot help but be impressed with the generous sprinklings of acronyms that seem to appear in conjunction with any chemical assessment procedure; one wonders how many more acronyms will have to be learned as a result of future assessments processes.

Risk Assessment Under the EU Existing Substances Regulation

By Steve D. Williams

CHEMICAL REGULATION IN THE EU

The Dangerous Substances Directive (67/548/EEC) is the basic vehicle for regulating chemicals in the European Union (EU). Initially it set out harmonized requirements for the classification, packaging and labeling of dangerous substances. Its sixth amendment of 1981 introduced the requirement for the premarketing notification of new substances, specifically those substances not listed on the European Inventory of Existing Chemical Substances (EINECS). Formal risk assessment procedures for chemical substances were developed in the EU to cover notified new substances in 1992. Under the Existing Substances Regulation (ESR), EU risk assessment practices are extended to cover Existing Substances (i.e. those listed on the EINECS inventory).

The EU Existing Substances Regulation (EEC No. 793/93) "on the evaluation and control of the risks of existing substances" mandates the collection of information about chemical substances manufactured or imported into Europe. For selected "priority chemicals" this information is used to assess their risks to man and the environment in line with the risk assessment regulation [Regulation (EC) No. 1488/94]. Styrene was one of 42 substances identified in an initial priority chemicals list.

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The EU Existing Substances Regulation... mandates the collection of information about chemical substances manufactured or imported into Europe. For selected "priority chemicals" this information is used to assess their risks to man and the environment...

DATA COLLECTION UNDER THE ESR

ESR requires all importers or manufacturers of at least 10 tonnes/year of EINECS listed substances to submit a data file covering some commercial and summary technical information in a special electronic format (HEDSET). Where production or import volumes were > 1000 tonnes/year/manufacturer, data submission had to be completed by June 1995. More simple data sets have to be submitted by June 1998 for all imports or production > 10 tonnes by June 1998.

These industry submissions have been incorporated into a data base (IUCLID) by the European Commission's European Chemical Bureau. Whilst the confidential version of the IUCLID database is available only to EU regulators, a non-confidential version is now publicly available in CD ROM format.

PRIORITY SUBSTANCE SELECTION

With more than 2,000 substances within the IUCLID database already, objective selection of substances for risk assessment was expected to present difficulties. In an attempt to make selection more objective, a computer based screening method to screen the IUCLID database was developed on behalf of the EU Commission for prioritizing substances. Although this system (the IPS method) has been tried in the selection of priority substances, to date

final selections have been based on member state nominations and the availability of a member state to act as rapporteur.

Once the member states have agreed on the selection of substances, a priority list is published in the Official Journal of the European Communities. Two priority lists have been published to date and these cover about 70 substances. A third list is under consideration and is expected to be confirmed by member states for publication during 1997.

DATA FOR THE RAPPORTEUR

Companies that made HEDSET submissions for a priority substance have to provide full reports of tests together with details of worker exposure and environmental releases to the rapporteur. Whilst the duty to provide such extra information falls on all companies that made a HEDSET submission, in practice this is generally fulfilled by a lead company from the industry, in conjunction with other companies. In many cases the industry group has provided a "shadow" risk assessment for the rapporteur too.

If the test data set does not meet the minimum required by regulators to carry out a risk assessment (the EU new substance notification "Base Set") then industry is mandated to carry out tests to fill the gaps in the "Base Set". The nature of the test work and its timing is agreed with the rapporteur in advance.

The collation of exposure and environmental emissions data is potentially less straightforward, particularly for chemicals that have a large number of downstream users. Industry have made the point to rapporteurs that whilst the regulatory duty falls on those who submitted HEDSETs to provide data to the rapporteur, in many cases if data exist for downstream uses of the chemical under review they are in the hands of the producer's customers, or their customer's customers and the producer cannot be expected to provide such data. In the case of styrene for instance, there has been good cooperation between the styrene producers and trade associations and companies involved in downstream sectors such as styrene-butadiene rubber (SBR) manufacture and unsaturated polyester resin manufacture and use. It is impractical to expect all companies in a widely dispersed sector such as polyester resin processing to provide data (there are thousands of such workplaces in Europe) and it is hoped that regulators will be willing to accept indicative data from the sector as being representative of current practices.

THE DRAFT RISK ASSESSMENT REPORT

The rapporteur is responsible for reviewing data on the health and environmental effects and on physico-chemical hazards such as flammability. The task involves determining the critical effects and their No Adverse Effect Levels or No Adverse Effect Concentrations. The outline for carrying out the technical appraisals involved in the risk assessment is given in the Technical Guidance Document produced over the last three years by the EU Commission and Member States with industry input. So far the draft Technical Guidance Document has been used as the basis of risk assessment. The final version is expected to be published shortly by the EU Publications Office in Luxembourg.

In summary, the effects data for the substance are compared with the available exposure data and this is used as a basis for deciding if there may be unacceptable risk to man or the environment. This process (Risk Characterization) is carried out for physico-chemical, health and environmental effects in relation to the different exposure contexts. The key exposure contexts considered are:

- workplace exposure
- consumer exposure
- environmental exposure
- man exposed indirectly via the environment (e.g. ambient air or food chain exposure)

Where a substance has many uses with different exposure patterns associated with each use (e.g. worker exposure in styrene production in contrast to worker exposure to styrene in glass reinforced plastics boatbuilding) a separate comparison is needed for each situation.

The conclusions are proposed in terms of three possible outcomes as defined in the Risk Assessment regulation. These conclusions are:

(i) There is a need for further information and/or testing.

(ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

(iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

EU AND OECD DISCUSSION OF THE RISK ASSESSMENT REPORT

Draft reports from rapporteurs are initially considered by an EU risk assessment committee which may be attended by industry delegates. If approved by this group the Risk

Assessment Report is sent to an Organization for Economic Cooperation and Development (OECD) meeting as part of the EU contribution to the OECD Existing Chemicals program. Although OECD are reviewing the hazard of the chemical and not carrying out a risk assessment, comments from OECD delegates are fed back to the rapporteur and considered together with the EU Member States comments.

There is then a full technical discussion of the report at an EU technical meeting. The aim is to produce a clear and scientifically based risk assessment report for subsequent publication. No substance has yet concluded this part of the procedure and so there is no experience of the full review process. It is expected that some elements of the debate will be controversial for some substances, reflecting the breadth of scientific (and potentially political) attitudes to be found within Europe. Once a risk assessment report is agreed technically it is proposed for approval by the Member States' policy committee. Once approved, a short summary version of the report and its conclusions will be published in the Official Journal of the European Communities.

IMPLICATIONS OF A FINAL RISK ASSESSMENT REPORT

The ESR risk assessment is an umbrella review of all aspects of a substance at all stages in its life cycle. It is carried out under the overall supervision of the EU Commission's Environmental Directorate (DGXI). Whilst the conclusions from the review are presented under the ESR, the regulatory competence for risk reduction measures [e.g. hygiene limits can be controlled by other regulatory bodies at the Commission (e.g. DGV for labor affairs) or by Member State governments]. The Risk Assessment Report will therefore often be highlighting areas that will need to be followed up by other agencies.

Where risk reduction is called for by the risk assessment, new regulatory focus will be applied in relation to measures such as changes to environmental emission limits, hygiene limits, product labeling and material safety data sheets. In extreme circumstances a substance might be recommended for a total or partial ban, such as a ban on its use in consumer products.

As the risk assessment reports and their conclusions are public documents they will be available as information for the news media and interested members of the public. The intention is that these will be scientific and open assessments so that the basis of decisions is clear to all. As one of the three possible conclusions for the process is that there is "no need for risk reduction measures beyond those that are already being applied," it should be expected that for many chemicals the risk assessment should confirm the safe nature of many current practices and uses of the substance.

Given the complexity and variability of the chemical world, it is hoped that the process will retain some flexibility to deal with substances on a case by case basis.

EU RISK ASSESSMENT IN PRACTICE

The EU ESR program is an ambitious one and already the process is running behind schedule as full and careful scientific reviews of substances are a time consuming exercise. Industry supports the open nature of the process and is working hard to provide information to regulators and to enter into debate upon the scientific issues raised during the course of reviews. It is important that the risk assessments are based upon objective facts and that the outcome is not clouded by political considerations.

The need for real exposure and emissions data are underscored by the fact that if there are no specific or satisfactory data available, rapporteurs will fill the gap with computer predicted exposure data. In the absence of data, environmental exposure is predicted by an EU sponsored environmental fate program called EUSES. Similarly, occupational exposures may be predicted by a UK government program called EASE. Other modeling software is used, too. The conservative nature of many of these models means that it is very much in industry's interests to demonstrate the way things are in the real world.

As experience is built up as the program develops, it is hoped that lessons learned will be incorporated into the Technical Guidance Document. However given the complexity and variability of the chemical world, it is hoped that the process will retain some flexibility to deal with substances on a case by case basis where this is justified.

EU RISK ASSESSMENT OF STYRENE

As noted, styrene was amongst the 42 substances named in the first priority list with the United Kingdom acting as rapporteur. The European industry, with BP Chemicals as lead company, has liaised with the twin arms of the UK government rapporteur (Health and Safety Executive and Department of the Environment) to provide information for and comment on the styrene draft document. Europe's Styrene Steering Committee (SSC), the Styrene Information and Research Center (SIRC) and the trade association groups representing styrene users have also aided in the process.

The UK rapporteur accepts that with the exception of an acceptable skin sensitization study in animals the necessary "base set" data are available. Because widespread human experience of skin exposure to styrene over many years has given no significant clinical evidence of allergic dermatitis, the rapporteur is proposing that a new animal test cannot be justified.

To complete the data set required for full review of genotoxicity according to the EU testing strategy and to give further assurance, the UK have indicated they would like an in vivo mutagenicity test carried out in a tissue other than bone marrow. Industry have agreed to carry this work out in advance of the formal risk assessment findings being published.

The UK have prepared a comprehensive risk assessment draft and this has been circulated to Member States. After consideration at an EU technical meeting, the styrene document was tabled at the OECD meeting in May 1996. UK government advised the EU technical meeting in October 1996, that the styrene risk assessment should not be completed until the results of recent and current SIRC toxicology studies were available and taken into account in the risk assessment. Whilst SIRC has provided the rat carcinogenicity and ecotoxicity studies for the rapporteur, the mouse carcinogenicity study is still in progress. EU debate on the styrene risk assessment is therefore not expected to resume before late 1997, at which time the mouse carcinogenicity study data will become available.

By the time the styrene discussion resumes, it may be expected that the EU debate will have been concluded for at least five other priority substances, so the practices of the EU risk assessment process may be better defined than for at present. It is hoped that clear and open scientifically based risk assessment will emerge from the program, providing information and guidance to all interested parties.

Summary of the EU Styrene Draft Risk Assessment By the United Kingdom Health and Safety Executive

As part of the Organization for Economic Cooperation and Development (OECD) and European Union (EU) efforts at risk assessment and risk reduction activities (see separate articles in this issue Granville, p.40, Williams, p.42), the United Kingdom developed a draft risk assessment on styrene. This draft, completed in February 1996, was circulated to member OECD countries. The draft, along with those of several other chemicals, was discussed at an OECD meeting in Tokyo in May 1996. Further discussions of the risk assessment of styrene are occurring in Europe although definitive discussion will be postponed until receipt of a final report on SIRC's chronic mouse inhalation study. The UK will consider comments from OECD and EU countries as it completes it risk assessment on styrene. Results of the SIRC-sponsored chonic mouse inhalation study also will be considered, when available.

The draft risk assessment evaluated exposures, environmental releases, environmental fate and effects, and human health effects in detail. These were summarized in a Screening Information Data Set (SIDS) Initial Assessment Report. Because the report is still in draft form and due to its length, the entire report is not reproduced here, but is available through the Styrene Information and Research Center (SIRC) or the UK Health and Safety Executive (HSE). The following article is a summary by SIRC of the Risk Characterization section of the report. A future edition of *The SIRC Review* will cover the environmental discharge, exposures, fate and effects sections of this assessment.

The overall conclusions of the draft risk assessment were that while the studies in animals and humans suggest that the mutagenic activity of styrene *in vitro* is not expressed *in vivo*, a further *in vivo* study in a second tissue is required for complete reassurance, and that more information on the contribution made from polymeric products to consumer exposure was needed, as well as more information on exposures arising from consumers use of resin kits.

> Taken together, animal and human data suggest that styrene does not exhibit carcinogenic activity of significance to human health.

They also concluded that there was at present no need for further information and/or testing or for risk reduction measures beyond those already being applied.

RISK CHARACTERIZATION

General aspects

A substantial amount of human data is available on the toxicokinetics of styrene and overall, the pattern of toxicokinetic data is qualitatively similar in animals and man. Styrene has been shown to be well absorbed by inhalation (59 – 88%) in man. For the purposes of risk assessment, a conservative assumption of 100% absorption via the inhalation route will be made.

There is one study describing absorption of styrene through the skin in volunteers (Berode, 1985). The rate of dermal absorption, measured after exposure of one hand of each volunteer dipped into liquid styrene for 10-30 minutes, was 60 $\mu g/cm^2/hr$. This information will be used to calculate dermal uptake for assessing the risks to

This summary was prepared by the Styrene Information and Research Center

workers and to consumers using resin filler kits.

There is no information on absorption via the oral route in humans. However, a study in rats indicates that oral doses of styrene prepared in corn oil or aqueous suspension are well absorbed (90%). For the purposes of risk assessment, a conservative assumption of 100% absorption via the oral route will be used when assessing the risks to man via the environment.

Assessment of available data indicates that styrene is generally of low acute toxicity in experimental animals (with the exception of the mouse) and humans. Single exposure to styrene has the potential to produce depressant effects on the central nervous system (CNS) at high concentrations of 800 ppm in animals and humans. Liquid styrene is irritating to the skin and both the liquid and vapor are irritating to the eyes. There are inadequate animal sensitization data. However, given that widespread exposure to styrene has led to only one reported possible case of skin sensitization, this extensive human experience indicates that styrene is not a skin sensitizer. Similarly, there has been extensive inhalation exposure in humans which has resulted in only two case reports of asthma, each of which has unconvincing aspects to it. This suggests that styrene is not a respiratory sensitizer.

Styrene is generally of low toxicity following repeated exposure in the rat and other species which have been tested except the mouse. In the rat, a no observable adverse effect level (NOAEL) of 200 ppm is consistently observed in inhalation studies. Respiratory tract irritation occurred at concentrations of 500 ppm and above and histopathological damage to the auditory system was observed at 800 ppm. The mouse has been shown to be appreciably more susceptible to toxic effects of styrene, deaths and marked liver toxicity were observed at 250 ppm and above. In limited studies conducted by the oral route, a NOAEL of 130 mg/kg/day was determined in the rat. No dermal studies are available, although low systemic toxicity would be predicted with the possible exception of the mouse.

In humans, a large number of studies have been conducted in workers exposed to styrene. These have generally not produced clear evidence of adverse effects. This provides considerable reassurance of the absence of notable deleterious effects arising from repeated occupational exposure to concentrations typical of common industries in which styrene is used.

Styrene is mutagenic *in vitro*, requiring metabolic activation in order to express its activity. There is evidence from contemporary *in vivo* micronucleus and cytogenetic studies to indicate that styrene has no mutagenic effects in the bone marrow. Overall, consideration of the genotoxicity studies in humans indicated that there is no good evidence for an association between increases in cytogenetic markers in lymphocytes of workers and exposure to styrene and there is no good evidence that styrene causes or has the potential to cause genetic damage in humans.

Complete reassurance is to be sought from a further well-validated *in vivo* mutagenicity study in a second tissue.

Although not providing conclusive proof, the available animal evidence suggests that styrene does not possess carcinogenic activity of relevance to human health. In large well-conducted studies in humans, cancer mortality was investigated in the reinforced plastics and composites (RPC) industry with relatively high exposure to styrene and no significant exposures to other chemicals. Taken together, animal and human data suggest that styrene does not exhibit carcinogenic activity of significance to human health.

There are no adequate studies which address fertility in animals exposed to styrene. Overall, it appears that styrene does not exhibit any developmental toxicity in animals that raises concern for humans. A range of epidemiological studies, particularly focussing on developmental effects, has been conducted but most of these investigations have been too small to be conclusive. Nevertheless, the available human data provides no evidence for styrene exposure-related increases in spontaneous abortions, congenital abnormalities, birth weight or menstrual disorders. Overall, there is no evidence of an effect of styrene on human reproduction.

Animal and human data indicate that styrene is not genotoxic *in vivo* (although complete reassurance is to be sought by a further well-validated *in vivo* study in a second tissue) or carcinogenic. It is possible to establish that there is a level of exposure at which there will be no significant adverse effects on health. There is an enormous number of studies on the health effects of styrene in humans but no convincing evidence of any significant health concerns under normal occupational exposure conditions has been found. The changes that have sometimes been reported cannot be considered to be adverse health effects, and overall the extensive human data are reassuring when considered against data on other organic substances. The database is of mixed quality and a precise threshold for significant health effects cannot be easily identified from human studies. Loss of co-ordination and balance, probably related to known CNS depressant effects of styrene, and respiratory tract irritation are observed in workers exposed to hundreds of ppm

styrene. At or below 100 ppm, there is no indication that styrene is likely to be injurious on single or repeated exposure. The animal data support this conclusion, with a NOAEL of 200 ppm in repeated exposure studies in the rat. A histopathological change in the olfactory epithelium indicating respiratory tract irritation was observed at concentrations of 500 ppm and above. At a higher concentration (800 ppm), damage to the auditory system (hair cell loss), with associated functional impairment, was observed.

For the purposes of risk assessment an exposure of 100 ppm (433 mg/m^3) will be taken as an indicator level. While there is no clear evidence of health effects being expressed immediately above this level, where such exposures do occur, closer attention should be paid to them. Exposure to 100 ppm for 8 hours implies, assuming 100% absorption and a 70 kg worker breathes in 10 m^3 in a working day, a dose of 60 mg/kg bw/day.

EXPOSURE

Workers

The risks to workers in the styrene industry are presented in three sections, concerned with risks following exposure via the inhalation and dermal routes and, where appropriate, combined exposure. The reason for presenting the assessment in this way is because exposures in most of the styrene industry are low, with the exception of maintenance procedures (when appropriate protective equipment should be worn) and, more broadly, in the RPC industry and during the use of unsaturated polyester resins.

The numbers of people potentially exposed differ widely. There are approximately 1100 - 1300 operators exposed to styrene in the monomer and polymer industries in the EU. There are no precise figures for RPC production and unsaturated polyester resin use, but throughout the EU thousands of small and medium sized companies employ many thousands of workers between them.

Inhalation

While information was obtained on workplace exposure within the UK, from industry and from HSE, only limited exposure information has been obtained for the EU. However, companies with plants in the UK and the EU indicate that exposures are similar.

Exposure data provided by UK industry indicate that during production of styrene, polyester, polyester resin and styrene-butadiene or styrene-butadiene latex, 8-hour time-weighted average (TWA) personal exposures are below 20 ppm, with short-term exposures being below 50 ppm. There is evidence that high atmospheric concentrations occasionally occur during maintenance procedures but respiratory protective equipment would be expected to be used in such circumstances, limiting personal exposures. Most exposure can be readily controlled by total enclosure and local exhaust ventilation.

In RPC manufacture and unsaturated polyester use, high personal exposures to styrene of over 100 ppm 8-hour TWA can occur, mainly due to the lack of good local extraction ventilation at the workplace, poor working practices or a combination of both.

If inhalation exposure in the workplace is 50 ppm (210 mg/m^3) 8-hour TWA maximum, then assuming a 70 kg worker breathes in 10 m^3 of air in a working day and all the styrene is absorbed, a maximum body burden of 30 mg/kg bw/day is estimated. At 100 ppm the burden will be 60 mg/kg bw/day. Contrasting these levels of airborne exposure (and the implied levels of body burden) with the toxicity data above, there appear to be no significant health risks to workers.

Contrasting these levels of airborne exposure (and the implied levels of body burden) with the toxicity data above, there appear to be no significant health risks to workers.

However, there is evidence that in some circumstances in the RPC industry and during maintenance work in other industries that potential exposure levels are significantly above 100 ppm (8-hour TWA). This gives rise to some concern that health effects (CNS effects and irritation) may be experienced.

Within the EU, the manufacturers and users of styrene including the RPC industry have drawn up a "Charter for a Voluntary Code of Practice" to achieve as a minimum requirement at the workplace a "maximum styrene exposure" not higher than 50 ppm 8-hour TWA by 1996.

Dermal

There is one study describing absorption of styrene through the skin in volunteers (Berode, 1985). The rate of

dermal absorption, measured after exposure of one hand of each volunteer dipped into liquid styrene for 10-30 minutes, was 60 $\mu g/cm^2/hr$. For a worst case situation, where personal protective equipment is not worn, exposures to the hand and forearm are assumed to correspond to an area of exposed skin of 2000 cm^2.

If a constant rate of absorption and exposure for a full 8-hour working day are assumed in this worst case (which assumes, in effect, that the full area is dipped in liquid styrene for an 8-hour working day), then the total estimated exposure for an individual is 960 mg/day. Assuming a weight of 70 kg, a body burden of 13.7 mg/kg bw/day would be achieved.

However, there is experimental evidence which suggests that the dermal route is not of great significance in any case. Eight workers in the reinforced plastics industry were monitored for levels of styrene in blood and urinary metabolites of styrene after lamination work involving exposure to styrene (Brooks et al., 1980). The effects of no protection and wearing a respirator and/or gloves impermeable to styrene were compared. There was no significant difference in levels of styrene in blood or metabolites in urine between the shift when gloves were worn and the shift when no protection was worn. Skin exposure clearly contributed a small amount to the total body burden of styrene in this study. This suggests that the estimate of dermal exposure based on the rate of absorption found in the Berode study above is clearly a worst case and over-estimates the real contribution of dermal uptake to body burden during occupational use in the RPC industry.

In conclusion, dermal absorption of styrene will not carry a significant risk of systemic ill-health effects. However, to prevent skin irritation and for reassurance, appropriate gloves should be worn.

Combined routes of exposure

Inhalation exposure to 50 ppm (8-hour TWA) which achieves a body burden of 30 mg/kg bw/day, together with skin contact to the worst case extent proposed above, contributing 13.7 (14) mg/kg bw/day, gives a total body burden via lungs and skin of approximately 44 mg/kg bw/day. This body burden would be less than that at which no significant health effects are predicted following occupational exposure. At 100 ppm 8-hour TWA the inhaled body burden is predicted to be 60 mg/kg bw/day, combined with the worst case skin uptake the total body burden would be 74 mg/kg bw/day.

However, for the reasons stated above, the dermal route of exposure is not believed to contribute significantly to the total body burden of styrene and is readily prevented with appropriate protective equipment, which should be worn. The main concern must therefore be for inhalation exposure, with perhaps a marginal dermal contribution. Up to and at 100 ppm there appears to be no significant health risk to workers. Significantly above this level, there is a concern for irritation and CNS effects. These levels may be reduced significantly by appropriate work practice and respiratory protective equipment.

Consumers

Risks from long term low level exposure

The airborne concentration levels implied by the uptake from polymeric sources (5 $\mu g/m^3$; 1.15 ppb) and from passive smoking (on average, 0.53 $\mu g/m^3$; 0.12 ppb) are well below any levels of concern for irritation or CNS effects.

Estimated worst case daily exposures for consumers from:

Polymeric materials	1.9	μg/kg bw/day
Container emissions	0.1	μg/kg bw/day
Chewing gum	0.04	μg/kg bw/day
Passive smoking	0.2	μg/kg bw/day
Heavy smoker	23.0	μg/kg bw/day

This is well below the indicator level of 100 ppm (60 mg/kg bw/day) or the level for any other claimed effect. Consequently, there are no concerns for inhalation or oral uptake of styrene from long term, low level emissions at the levels implied above. However, for reassurance, more information to support the exposure calculations for emissions from polymeric materials are needed.

Risks from specific events or activities

The amount inhaled for the activity of fitting a new carpet is 2 mg. Assuming 100% absorption, the inhaled dose for a 70 kg individual is estimated to be 29 μg/kg bw/day. Given that the levels of styrene exposure drop rapidly following installation and that this dose is well below the 100 ppm indicator level and the level for any other claimed effect, the exposure arising from this activity does not give rise to concern.

The exposures arising from use of resin filler kits are of potentially greater concern, implying exposures of above 1000 ppm of styrene and inhaled doses of 157 and 114 mg/kg bw/day for a 70 kg individual. Although exposures will be sporadic and short term, these levels could give rise to irritation and CNS effects. However,

the predicted exposures are critically dependent upon assumptions made using the various models and must be treated with some caution. In conclusion, there is a cause for concern in this area and a need for more information on exposure.

Man exposed indirectly via the environment
Most of the environmental exposure to styrene is predicted to be from the air. For the purposes of risk assessment, therefore, the potential contamination of food and drinking water will be ignored.

Intake from local exposure (worst case) μg/kg bw/day	= 22.8
Intake from regional exposure (worst case) μg/kg bw/day	= 0.67

These doses are considerably below the level for any claimed effect.

Combined exposure
The uptakes arising from long term, low level consumer and environmental sources (occupational and resin kit sources are of a different order and are not included in this calculation) are predicted to be 1.7 mg/day and 191 μg/day (implying dose rates of 24 μg/kg bw/day and 2.7 μg/kg bw/day for a 70 kg individual, assuming 100% absorption) at the local and regional environmental levels respectively. These are considerably below the levels of any claimed effect.

SUMMARY

World wide production of styrene was approximately 14 million tons in 1992. The European Chemical Industry Council (CEFIC) have estimated styrene production and use in Western Europe in 1993 as 3.7 million tons. Styrene is used primarily as an intermediate in the chemical industry. Specific uses include as the monomer in the production of polystyrene and styrene copolymers, styrene-butadiene rubbers and in unsaturated polyester resins.

For the aquatic compartment the few measured levels of styrene do not give rise to concern when compared with the predicted no-effect-concentrations (PNECs) derived for aquatic organisms, sediments and micro-organisms in waste water treatment plants. This is also true for the concentrations calculated from information about releases from the use of styrene. The concentration calculated from releases during production would give rise to concern if the default sizes of waste water treatment plant (WWTP) and receiving river flow were used. However taking into account the sizes of actual treatment plants and rivers receiving discharges from production plants reduces the concentration to below the concern level. Thus the conclusion of the assessment is that there is at present no need for further information or testing.

> The exposure via environmental routes and the combined exposure with long term low level consumer emissions are considerably below the level for any claimed effect.

No information on the toxicity of styrene to organisms in soil is available. Using the methods of the Technical Guidance to extrapolate from aquatic to terrestrial organisms and comparing the values obtained with levels calculated in soil leads to a conclusion of no concern.

For the atmosphere, styrene has a short tropospheric lifetime and is not expected to contribute to the depletion of stratospheric ozone or global warming. It may contribute to the formation of tropospheric ozone.

Animal and human data indicate that styrene is not genotoxic *in vivo* (although complete reassurance is to be sought by a further well-validated *in vivo* study in a second tissue) or carcinogenic. It is possible to establish that there is a level of exposure at which there will be no significant adverse effects on health. There is an enormous number of studies on the health effects of styrene in humans but no convincing evidence of any significant health concerns under normal occupational exposure conditions has been found. The changes that have sometimes been reported cannot be considered to be adverse health effects, and overall the extensive human data are reassuring when considered against data on other organic substances. The database is of mixed quality and a precise threshold for significant health effects cannot be easily identified from human studies. Loss of co-ordination and balance, probably related to known CNS depressant effects of styrene, and respiratory tract irritation are observed in workers exposed to hundreds of ppm styrene. At or below 100 ppm, there is no indication that styrene is likely to be

injurious on single or repeated exposure. The animal data support this conclusion, with a NOAEL of 200 ppm in repeated exposure studies in the rat. A histopathological change in the olfactory epithelium indicating respiratory tract irritation was observed at concentrations of 500 ppm and above. At a higher concentration (800 ppm), damage to the auditory system (hair cell loss), with associated functional impairment, was observed.

In the occupational setting, the numbers of people potentially exposed differ widely. There are approximately 1100 - 1300 operators exposed to styrene in the monomer and polymer industries in the EU. There are no precise figures for RPC production and unsaturated polyester resin use, but throughout the EU thousands of small and medium sized companies employ many thousands of workers between them.

Occupational exposure data indicate that 8-hour TWA exposure levels in industries other than the RPC industry are well below 50 ppm. In the RPC industry the majority of exposures are at or below 100 ppm (8-hour TWA). If inhalation exposure in the workplace is 50 ppm (210 mg/m^3) 8-hour TWA maximum, then assuming a 70 kg worker breathes in 10 m^3 of air in a working day and all the styrene is absorbed, a maximum body burden of 30 mg/kg bw/day is estimated. At 100 ppm the burden will be 60 mg/kg bw/day. Contrasting these levels of airborne exposure (and the implied levels of body burden) with the toxicity data above, there appears to be no significant health risks to workers.

However, there is evidence that in some circumstances in the RPC industry and during maintenance work in other industries that potential exposure levels are significantly above 100 ppm (8-hour TWA). This gives rise to some concern that health effects (CNS effects and irritation) may be experienced. In the case of the RPC industry, continuing adoption of good work practice including the use of respiratory protective equipment should control exposures to 50 ppm 8-hour TWA or less. In the case of maintenance work, respiratory protective equipment would be expected to be provided to prevent excessive personal exposures.

Within the EU, the manufacturers and users of styrene including the RPC industry have drawn up a "Charter for a Voluntary Code of Practice" to achieve as a minimum requirement at the workplace a "maximum styrene exposure" not higher than 50 ppm 8-hour TWA by 1996.

For consumers, there are no concerns for inhalation or oral uptake of styrene from long term, low level emissions. However, for reassurance, more information to support the exposure calculations for emissions from polymeric materials are needed.

The exposures arising from use of resin filler kits are of potentially greater concern, implying exposures of above 1000 ppm of styrene and inhaled doses of 157 and 114 mg/kg bw/day for a 70 kg individual. Although exposures will be sporadic and short term, these levels could give rise to irritation and CNS effects. However, the predicted exposures are critically dependent upon assumptions made using the various models and must be treated with some caution. In conclusion, there is a cause for concern in this area and a need for more information on exposure.

The doses received by man via environmental routes, based on reasonable worst case calculations of environmental levels indicate that most of the exposure occurs via the air. The exposure via environmental routes and the combined exposure with long term low level consumer emissions are considerably below the level for any claimed effect.

The EPA's Proposed Guidelines for Carcinogen Risk Assessment

POTENTIAL APPLICATION TO STYRENE

By C. N. Park, PhD and K. A. Johnson, DVM, PhD

INTRODUCTION

In 1986, the U.S. Environmental Protection Agency (EPA) published Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986). These guidelines were intended to ensure consistency and technical quality in evaluation of data relative to carcinogen risk assessment for use in risk communication and risk management decisions. In the decade since the EPA Cancer Risk Assessment Guidelines were issued, there have been considerable advances in the understanding of carcinogenic mechanisms and in the understanding of the pharmacokinetics of chemicals in test species as well as humans. Thus, the EPA is in the process of updating those guidelines and published the Proposed Guidelines for Carcinogen Risk Assessment on 23 April 1996 (U.S. EPA, 1996a). The process used to update the guidelines has involved considerable peer review and discussion, primarily through two workshops (U.S. EPA, 1994; U.S. EPA, 1996b). There was also a public review comment period that ended in August, 1996. After review of the comments, the EPA will publish the final guidelines that will be used in forthcoming carcinogen risk assessments. The proposed update of the EPA cancer risk assessment guidelines contains a number of changes which could make the risk assessment process more flexible and incorporate more science.

The proposed update of the EPA cancer risk assessment guidelines contains a number of changes which could make the risk assessment process more flexible and incorporate more science.

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1986 GUIDELINES

The 1986 guidelines focused on cancer hazard (the ability of a chemical to cause cancer without regard to other factors) and were largely based upon the results of controlled animal studies or human epidemiology studies, if available. Although the agency asserted their's was a weight-of-the-evidence approach, except for a few cases, their evaluations have generally been made on the basis of animal data, and in the past, have largely been a strength-of-the-evidence approach. That is, if there were two "well run" positive studies, the compound was viewed as being a potential carcinogen. Generally, positive studies in two animal species were regarded as strong evidence although two studies with similar results in the same species were also considered evidence of carcinogenicity. Based upon the data, a chemical was assigned a classification using letters A through E. The basic definitions for these classifications were: A was a known human carcinogen; B was a probable human carcinogen (and sub-divided in B1 and B2); C was a possible human carcinogen; D was regarded as not classifiable and E was regarded as not carcinogenic. Once a chemical was classified into one of the first three (A-C) categories, regulatory risk assessment procedures were limited under the 1986 guidelines to using a linearized multi-stage (LMS) calculation in almost all cases to produce estimates of risk for a given dose. The LMS model assumes that there is no

threshold, i.e. no safe dose, and that tumor incidence is linearly proportional to dose; thus for any dose, no matter how small, there is some risk.

PROPOSED GUIDELINES

The proposed guidelines break down the carcinogen risk assessment process into three steps, each of which results in technical documents which are later integrated into a risk characterization. These three steps in the process are described below:

HAZARD IDENTIFICATION

The first step in the evaluation process, hazard identification, answers the question of whether a particular chemical has the intrinsic potential to cause cancer. In addition to human studies, if available, and animal studies, the new guidelines allow for the incorporation of mode of action information, physicochemical properties, route specific information and the use of structure activity information in arriving at hazard identification conclusions. This should provide an integrated picture that makes use of all available data. For example, exposure to unleaded gasoline resulted in kidney tumors in rats, but extensive information on the mode of action in rats and information on the unlikely possibility of that same pathway in humans resulted in unleaded gasoline not being listed as a possible or probable human carcinogen (U.S. EPA, 1991). When these additional data are not available, default assumptions are generally defined and discussed.

The guidelines also explicitly allow for route specific classification; for example some heavy metals (nickel, chromium, cadmium) are classified as potential carcinogens by the inhalation route but not by the oral route.

The proposed new classification departs from the historical alphanumeric boxes; A, B1, B2, C, D and E, in favor of a more flexible narrative description. This narrative description is supposed to more fully describe the carcinogenic potential of compounds rather than simply assign a letter classification. The description should also incorporate more of the supportive non-tumor data.

Although the emphasis is on moving away from simplistic letter designations, there will still be a small number of "slots" into which chemicals will be placed; albeit with the accompanying narrative description. The classifications proposed in the guidelines are "known/likely," "not likely" and "cannot be determined," although the use of modifying descriptors is acknowledged (e. g., descriptors suggested for "cannot be determined" are suggestive, conflicting, inadequate, or no data).

Comments to the agency on the new classification system have been largely supportive concerning the narrative descriptions, but there have been suggestions on the specific classifications. For example, The Society of the Plastics Industry, Inc., and the Chemical Manufacturers Association, among others, have suggested that known and likely be split apart into two categories, reflecting the very large difference in supportive data between those compounds which are known human carcinogens on the basis of human data and those which are presumed to be potential human carcinogens on the basis of animal data. Additionally, EPA requested comments on whether there should be a separate fourth category for "cannot be determined - suggestive evidence." Comments on this subject have been split, although the predominant response from industry trade associations has been to not add this additional category. The concern has been that this would be a catchall category for all compounds with any suggestive data.

DOSE RESPONSE ASSESSMENT

There are a number of significant changes in the way the agency proposes to change dose response assessment, moving away from the current linearized multi-stage (LMS) default. The most significant change is in the clear differentiation of the observed dose response region from the region of extrapolation. It should be noted that most animal carcinogenicity bioassays are conducted at dose levels that greatly exceed the levels to which humans are likely to be exposed. This is done to accentuate potential carcinogenic effects but extrapolation to the lower levels then becomes problematic. The first step in the proposed dose response methodology is to calculate a benchmark dose (BMD), a dose corresponding to a certain increased cancer incidence; e.g. 10%. This is felt to represent the bottom end of the observable dose region for most bioassays and is used as a point of departure for low dose extrapolation. The methodology entails using non-linear statistical models to estimate the dose at which there is a 10% increased incidence of tumors. Historically, this dose has been called an ED_{10} (Effective Dose at 10%; the dose corresponding to a 10% effect), but is being referred to as a benchmark dose, or BMD, in the context of the risk assessment guidelines. A variation on this is to calculate a lower statistical confidence limit on the dose which causes the 10% effect (the LED_{10}). Whether or not a lower confidence limit should be included in the calculation has been discussed at some length. The original guidelines did not include a lower bound and a subsequent EPA

workshop supported that position (U.S. EPA, 1994), and recommended that the ED_{10}, rather than the LED_{10}, be used as the benchmark dose. A recent workshop discussing BMD's for cancer and non-cancer endpoints did not reach consensus although the majority appeared to recommend against a lower bound. The rationale for not using the lower bound is that it adds undue complexity to a simple concept, and muddies the interpretation relative to the resulting values.

The second step in dose response assessment, low dose extrapolation, will then use one of two methodologies. For those compounds with a demonstrated mode of action which leads to non-linear low dose behavior (e. g., unleaded gasoline), a margin of exposure (MOE) approach will be used. Rather than argue about existence of thresholds, the agency chooses to refer to non-linearity at low doses. The approach for these compounds which have sufficient mechanistic data to warrant a MOE approach will likely be similar to the traditional uncertainty factor methodology used to calculate reference doses or acceptable daily intakes, in which acceptability will be judged relative to the magnitude of the MOE, with a MOE of 100 being generally acceptable for compounds with a complete data base.

For chemicals lacking the evidence to support a MOE approach, the BMD_{10} will be divided by 1000, 10000 or 100000 approximating upper bounds corresponding to risk levels of 10^{-4}, 10^{-5} and 10^{-6} using the linearized multi-stage method under the current system. Regulatory values such as $BMD_{10}/1000$, incorporating the lower statistical bound on the BMD will be numerically almost identical to a 10^{-4} upper bound on risk calculated from the LMS model. But, like the LMS model, these confidence limits cannot be interpreted in the usual statistical sense due to the conservative assumptions embedded in the other components of the calculation.

A further change in cancer risk assessment is the potential for reliance on ancillary endpoints representing markers of toxicity. Examples might include histopathologic changes consistent with a proliferative but non-neoplastic response (i.e., hyperplasia), or adduct formation (chemical interaction with DNA) as a more sensitive indicator of the tumor dose-response curve. It is anticipated that such markers might be used to extend the observed dose response region down to lower doses. The concern is that these more sensitive indicators might be used as a substitute for the tumor dose response curve, with the same extrapolation factors, e.g. 1000, 10000, thereby lowering the resulting regulatory levels. Use of ancilliary data for the purposes of defining the endpoint (i.e., threshold) for the dose response curve will likely be a useful methodology in some cases, but they should not be simply substituted for the tumor incidence data with the usual extrapolation factors added.

> Although the guidelines appear to be very different from the 1986 version, the impact will likely be evolutionary rather than revolutionary.

EXPOSURE ASSESSMENT

The final technical stage in the risk assessment process is exposure assessment. This is not directly addressed in the 1996 proposed guidelines as guidelines for exposure assessment had previously been published (U.S. EPA, 1992). As with the other sections it culminates in a technical document that describes the data, uncertainties and assumptions and methods used in calculating the exposures for different exposure scenarios.

RISK CHARACTERIZATION

The above three steps are summarized in narrative technical papers that define and integrate the data, uncertainties, and default assumptions used to arrive at their conclusions. These are then integrated into a risk assessment. The goal is to produce risk assessments that have "transparency in environmental decision making, clarity in communication, and consistency in core assumptions and science policies." The risk assessment may be broken down by route of exposure. Although the language in the guidelines is not consistent, it appears that the intent is that if a MOE approach appears valid, but is not conclusively demonstrated, then the risk characterization will present results in terms of both the MOE approach as well as the linear approach.

STYRENE AND THE PROPOSED GUIDELINES

Undoubtedly, the new guidelines will be used when the carcinogenic risk of styrene is eventually assessed by the agency. The new guidelines could affect styrene in a number of ways. First, in hazard identification, although

a number of long term bioassays have been done for styrene, none completed to date have conclusively demonstrated a carcinogenic response in rats. Thus a weight-of-the-evidence approach to hazard identification as presented in the proposed guidelines would tend towards the conclusion that styrene is not intrinsically a carcinogen in rats. This is opposed to a strength-of-the-evidence criterion as in the 1986 guidelines, in which some assessments have considered styrene to induce mammary tumors in rats (the question has always been on the "well run" studies part of the evaluation).

Previous assessments have also questioned the effect of styrene on lung tumors in mice. The SIRC-sponsored mouse inhalation study is in the process of thorough microsopic examination of all tissues with a final report due in late 1997. A preliminary report based on the microsopic examination of lungs only indicated elevated incidence of lung tumors in exposed mice and also non-neoplastic effects in the mouse lung airways. However, lack of similar findings in rats exposed to much higher levels or lung tumors in numerous epidemiology studies highlights questions on the mode of action in mice and the relevance of these findings to humans.

The rich data base for styrene provides data for many of the other endpoints illustrated in arriving at an integrated assessment of carcinogenic potential. These include metabolism and pharmacokinetic data, genotoxicity, structure activity relationships, and studies on the mechanism of styrene oxide toxicity and tumor induction. However, like the animal bioassay data, these studies may provide inconclusive or disparate results that need to be recognized when determining an integrated assessment.

In the dose response evaluation of styrene, it is difficult to determine a BMD since a definitive tumor response has not been induced in animal studies to date. It is also difficult to know which method to use for extrapolation to low exposure levels. A likely mechanism of any potential carcinogenicity of styrene is by metabolism to styrene oxide, which induces tumors in the rat forestomach when given by gavage. However, recent data suggests that this may be the result of cell damage and resultant reparative proliferation with only a minor component due to reaction of styrene oxide with DNA. Thus, styrene oxide-induced forestomach tumors had a non-linear (threshold) behavior; thereby implying that an uncertainty factor (MOE) approach for evaluating exposures to styrene will be protective of public health. This approach has also been used for a number of nongenotoxic animal carcinogens in Europe; e.g. perchloroethylene, trichloroethylene, methylene chloride, etc.

SUMMARY

The proposed update of the EPA cancer risk assessment guidelines contains a number of changes which could make the risk assessment process more flexible, incorporating more science. How much impact the revised guidelines will have, however, depends upon how they are implemented. The new guidelines allow for a wider use of mechanistic data which could change the classification and the low dose extrapolation process. The key will be in how high a burden of proof is required to incorporate this mechanistic information.

Although the guidelines appear to be very different from the 1986 version, the impact will likely be evolutionary rather than revolutionary. First, because the new guidelines reflect scientific thinking that has already been in practice in the agency and second, because the incorporation of mechanistic data will be on a case by case basis and will likely affect only the relatively small number of compounds for which sufficient data exists. The question of how much data is "sufficient" to overcome default methodology is, and will continue to be, a controversial one.

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