THE SIRC REVIEW

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Environmental Fate
and Effects of Styrene

Ecotoxicity Hazard
Assessment of Styrene

Review of Styrene as a
Potential Endocrine Disruptor

Integrated Risk Information System:
An Overview

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Introduction

As editor, it is my pleasure to bring you this sixth volume of *The SIRC Review*. The articles in this edition cover a variety of topics related to styrene health effects and regulation, and reflect the broad spectrum of issues the Styrene Information and Research Center (SIRC) has addressed since it's inception in 1987.

A fundamental reason for SIRC's formation was to better understand the potential for styrene to cause cancer and/or other health effects; or to cause effects on the environment. While SIRC's initial research efforts addressed the inadequate database on which a determination of carcinogenicity might be made, SIRC also sponsored studies to understand other potential health and environmental effects. This volume focuses on two such important issues: the environmental effects of styrene and the evolving debate over the potential for chemical exposures to cause effects on the endocrine systems of humans and animals.

SIRC's early research acknowledged the need to define the environmental fate of styrene, and its effects upon aquatic biota. Included herein are two important recent articles addressing environmental effects. The first is a comprehensive review of the data on the environmental fate and effects of styrene by a past contributor to *The SIRC Review*, Dr. Martin Alexander of Cornell University (reprinted from *Crit. Rev. Environ Sci. Technol.* 27:383-410, 1997). The second article is by Dr. Janette Cushman et al., and assesses styrene's toxicity to living organisms in the aquatic environment (reprinted from *Ecotox. Environ. Safety* 37:173-180, 1997).

More recently SIRC has had to address the attention directed — both in the United States and abroad — at the premise that endocrine effects, specifically estrogenic effects, may result from exposure to styrene. Several early articles that listed chemicals claimed to cause hormonal or reproductive effects implicated styrene — with little or no scientific basis; and in 1997 styrene was listed as a "probable" endocrine disruptor by the Illinois Environmental Protection Agency (IEPA). SIRC's subsequent productive dialogue with IEPA, including IEPA's summary of a scientific forum on the issue and revised conclusion about styrene, is the subject of an article by John Snyder, SIRC's Deputy Director, and Jeffrey Terry, Manager, SIRC State Government Relations.

After ten years, where is SIRC headed? An activity that will figure prominently in the year ahead addresses SIRC's interest in the U.S. Environmental Protection Agency's (EPA) evaluation of scientific evidence – and the agency's official positions on the potential health effects of a chemical – as reported on their globally-referenced Integrated Risk Information System (IRIS) electronic database. In January 1998[1] styrene appeared on a list of chemicals published by EPA for review under the IRIS program. Recognizing that SIRC's research efforts over the years will contribute to the effectiveness of the styrene review, EPA has asked SIRC to be a participant in the process.

Dr. Chris Bevan of Amoco Corporation, Chairman of SIRC's Science and Technology Task Group, provides helpful background on the IRIS program, its intended use, its components, its limitations, and the general process by which chemicals are assessed. In the future, *The SIRC Review* will provide more specific details on the IRIS review for styrene, as the process moves forward.

The first volume of *The SIRC Review* was published in 1990. Since then this journal has served as a printed forum bringing together the most recent and valid scientific data and articles on styrene and styrene health effects. While SIRC has successfully completed the majority of the scientific work it had identified to address past data gaps, there still will be much to report in coming issues. SIRC's continued hope is that both current and future editions of *The SIRC Review* contribute to the discussion on styrene health and environmental effects from a perspective of sound science.

Keith A. Johnson, DVM, PhD
Editor

[1] 63 Federal Register 75; Jan.2, 1998

Environmental Fate and Effects of Styrene

Martin Alexander, Ph.D.

ABSTRACT

Enormous quantities of styrene are produced each year. Monitoring studies show that the concentrations in air usually are less than 10 μ/m^3 and the levels in waters are usually less than 20 μ/l. The compound is highly reactive in air, and it readily biodegrades under aerobic conditions in soils and waters. Many evaluations have been made of the toxicity of styrene. Based on the results of these toxicological studies, information on the concentrations of the compound in natural environments, and data showing its reactivity in the air, volatilization from soil and water, and rapid biodegradation, styrene is not deemed to cause deleterious effects on nonmammalian species, mammals, or natural communities of organisms as a consequence of environmental exposures, except in the immediate vicinity of a spill.

KEY WORDS

styrene, monitoring, fate, toxicity, biodegradation

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Styrene is a widely used chemical, and as such is of potential environmental concern. In this article, Dr. Martin Alexander reviews the data on styrene occurrence, reactions, biodegradation, and toxicity. He concludes that styrene is unlikely to cause effects at levels found in the environment.

I. INTRODUCTION

Styrene is a widely used aromatic compound, and structurally it is appropriately designated phenylethylene. The melting and boiling points of the pure chemical are -30.6 and 145.2°C. It is soluble in oganic solvents such as acetone, ether, and ethyl alcohol, and its solubility in water at 20°C is 300 mg/l. Its Henry's law constant at 25°C has been calculated to be 2.61 x 10^{-3} atm-m^3/mol (Agency for Toxic Substances and Disease Registry, 1992).

Because of the enormous quantities produced each year, styrene is of potential environmental concern. In 1995, for example, 5.166 x 10^9 kg was manufactured in the United States alone, an amount that ranks this compound as one of the major products of the chemical industry (Kirschner, 1996). It is widely used for the manufacture of polystyrene, plastics, resins, and rubber. It is a solvent for the processing of polymers, and it is often employed as a cross-linking agent in glass fiber-reinforced polyesters used in construction materials and for the making of boats. These industries and activities may release styrene into the air, waters, or both, and it has been found in air near such sources as well as in the effluents from chemical, textile, and latex plants. Occasional spills have resulted in contamination of soils and aquifers, and waters flowing from hazardous waste sites have also occasionally been found to contain

the chemical. In addition, emissions from gasoline and diesel-powered motor vehicles are often appreciable contributors of styrene to the atmosphere. Because of its entry into air, water, soil, and aquifers from accidental spills, leakages, emissions of volatile materials, and combustion processes as well as its toxicity at certain concentrations, considerable attention has been given to its environmental fate and effects.

Nonanthropogenic sources may also contribute to its presence in some environments. Special attention has been given to its occurrence in certain foods, in which its presence is responsible for a distinct and unpleasant off-flavor in seasoned herring roe (Sato et al., 1988) or in cheese, such as camembert or brie, whose manufacture involves ripening resulting from fungal action (Spinnler et al., 1992). It may also appear in fish products to which cinnamic acid is added as a preservative and a spice (Shimada et al., 1992). The conversions in these instances result from microorganisms that synthesize styrene, namely the yeast *Torulopsis candida* in herring roe (Sato et al., 1988), the fungus *Penicillium caseicolum* in cheese (Spinnler et al., 1992) and the fungus *Pichia carsonii* in ground fish (Shimada et al., 1992). In artificial culture media, strains of the yeast *Saccharomyces cerevisiae* can form styrene from cinnamaldehye (Chen and Peppler, 1956), and the fungus *Aspergillus niger* can synthesize the compound from cinnamic acid (Clifford et al., 1969).

II. OCCURRENCE

Measurements made in many regions at all seasons of year have shown the ubiquity of styrene in air. It has been found but not quantified in the atmosphere of a forest in Germany at a distance of approximately 1 km from a city (Jüttner, 1986) as well as in cities in Russia (Ioffe et al., 1977, 1979). The mean concentrations reported in a survey of sites in Netherlands in 1980 ranged from 0.09 to 1.5 $\mu g/m^3$ (0.02 to 0.35 ppb) with maximum concentrations of 0.64 to 27.3 $\mu g/m^3$ (Guicherit and Schulting, 1985). In one Netherlands city, Delft, the average concentrations were less than 0.4$\mu g/m^3$, but values as high as 3 $\mu g/m^3$ (0.7 ppb) were sometimes noted (Bos et al., 1977). Concentrations in other cities have been reported to be 0.4 to 2.3 $\mu g/m^3$ in downtown Los Angeles (Grosjean and Fung, 1984), and the mean levels in three cities in New Jersey were 0.30, 0.47, and 0.55 $\mu g/m^3$ in the summer and 0.64, 0.60, and 1.0 $\mu g/m^3$ in the winter (Harkov et al., 1984). The concentrations in these cities varied on a daily basis, and were generally higher in winter than summer and during pollution episodes. Variations with season and between day and night have been observed also in Los Angeles air (Hartwell et al., 1987a). Even at altitudes of between 350 and 650 m in Tokyo, 0.4 $\mu g/m^3$ of styrene has been detected (Uno et al., 1985). An investigation of several outdoor sites in the United States reported weighted mean values of 0.23 to 4.20 $\mu g/m^3$ at night and 0.18 to 1.90 $\mu g/m^3$ during the day (Hartwell et al., 1987b). The concentrations in a tunnel of the Pennsylvania Turnpike were higher, as expected, because of emissions of gasoline-powered automobiles and diesel trucks, and ranged from 1.1 to 6.6 $\mu g/m^3$ (Hampton et al., 1983). Styrene is also emitted from hazardous and nonhazardous waste sites and landfills, and mean concentrations in the overlying air have been reported to range from 0.47 to 6.5 $\mu g/m^3$ with maximum values of 1.4 to 66 μ/m^3 in waste sites and 1.7 to 1.8 $\mu g/m^3$ with a maximum of 6.48 $\mu g/m^3$ in the air over a sanitary landfill (Harkov et al., 1985; La Regina et al., 1986). Monitoring of the air in Canada gave mean values of 0.09 $\mu g/m^3$ in January to October of 1988 and 0.5 $\mu g/m^3$ in February 1989 to January 1990 for a rural site and from undetectable to 34.2 $\mu g/m^3$ (an industrial site in Vancouver) with an average of 0.59 $\mu g/m^3$ for 10 major urban areas in 1988-1989 (Environment Canada, 1992). In their literature review of volatile organic compounds in the atmosphere, Brodzinsky and Singh (1983) reported that the median value for all sites was 2.3 $\mu g/m^3$ and that 75% of all the analyses gave values less than 7.2 $\mu g/m^3$.

Although these values differ markedly, they do show the maxima that occur, and these maxima and the ranges are important in assessing possible ecological effects.

Many measurements also have been made of concentrations in aqueous effluents from industrial sources, in surface waters at some distances from point-source discharges, and in waters used for human consumption. As expected, effluents from certain industries were early reported to contain the compound. It was thus present in effluents discharged into the Calcasieu River estuary in Louisiana from industrial sources (Pereira et al., 1987) and previously observed in some but not other industrial wastewaters released into a Louisiana River (Keith, 1974). Its presence was detected in effluents of a latex and a chemical plant in Kentucky in 1974 and 1975 (Shackelford and Keith, 1976), in wastewater of a petrochemical company, and at 2.6 $\mu g/l$ in the wastewater of a factory using styrene to make synthetic rubber (Keith, 1974). A 1979 report states that 1 of 63 industrial effluents contained the compound but at a concentration of less than 10 $\mu g/l$ and 1 of 33 effluents contained in excess of 100 $\mu g/l$ (Perry et al., 1979). A number of studies in Canada also showed

styrene in industrial effluents. Thus, approximately 385 and 45 kg were apparently spilled into the St. Clair River in Ontario in 1989 and 1990, and concentrations of 0.4 to 1.1 μg/l were present in some effluents released to that river. From less than 1.0 to as much as 970 μg/l were present in industrial effluents in Sarnia. Furthermore, 70 μg/l was observed in one groundwater monitoring well adjacent to a chemical manufacturing plant in Canada, although none was present in two other wells (Environment Canada, 1992). An individual chemical plant along the St. Clair River was found to discharge 133 kg/day into the river (King and Sherbin, 1986). Coal gasification plants have also been sources, as indicated by the finding of 1.2 and 3.2 μg/l in groundwater from such operations (Pellizzari et al., 1979). Furthermore, approximately 2% of the hazardous waste sites investigated contained styrene in surface and groundwaters; of the water samples that had some present, the geometric mean concentrations in surface and groundwaters were 9.3 and 5.3 μg/l, respectively (Agency for Toxic Substances and Disease Registry, 1992). In addition, waste water is another potential source, as indicated by the fact that, according to a 1988 report from Ontario, 9 of 274 samples of raw waste water from water pollution-control plants had styrene with a maximum level of 1.21 μg/l, 1 of 38 samples of primary effluent from these plants had the compound (15 μg/l), and 1 of 224 samples of secondary effluent was positive with a value of 13.0 μg/l (Environment Canada, 1992).

Few analyses have been made of river and lake waters, except as they may be used directly for human consumption. None was found in a 1988 survey of Canadian Rivers (Environment Canada, 1992), an unspecified level was observed in the Waal River in Netherlands (Meijers and Vanderleer, 1976), none was found in the lower Delaware River except for traces in one sample (Sheldon and Hites, 1978), 0.01 μg/l was present in a sample from the Rhine River in Netherlands (Zoeteman et al., 1980), 1.7 μg/l was measured in the River Tees estuary but none was found in surface waters near other estuaries in England (Law et al., 1991), and 18 μg/l was detected in a sample from the Kanawha River in West Virginia (Dostal et al., 1965). Occasional samples from Lake Erie showed its presence (Konasewich et al., 1978; cited by Agency for Toxic Substances and Disease Registry, 1992). A study encompassing the years 1985-1992 found none in surface waters, however (Environment Canada, 1992). Occasional groundwater samples are known to contain the compound, but the highest concentration in groundwater of the Netherlands was 10 μg/l (Zoeteman et al., 1981). An investigation of 1174 community wells and 617 private wells in Wisconsin revealed only one with a positive sample (Krill and Sonzogni, 1986). Only 0.0047 μg/l was found in a well surrounding a municipal wastewater infiltration system despite the presence of 0.61 μg/l in the influent water (Bedient et al., 1983); these numerical values are open to question because of the sensitivity of analytical methodologies.

Regular analyses of drinking water throughout the United States have generally found that these waters have none present.

The regular analyses of drinking water throughout the United States have generally found that these waters have none present, although very low concentrations are sometimes observed. Styrene was found in the drinking water supply of Evansville, (Kleopfer and Fairless, 1972), Cleveland (Sanjivamurthy, 1978), New Orleans and Pittsburgh (Abrams et al., 1975) and in occasional samples from Philadelphia (Suffett et al., 1980) in the period before 1980, but the concentrations were not determined. Coleman et al. (1984) found 0.024 μg/l in the Cincinnati water supply in 1980, and analyses of 9 Canadian cities along the Great Lakes revealed concentrations in raw water sometimes reaching 0.5 μg/l in the spring, levels that occasionally reached 0.2 μg/l in the winter but values less than 0.2 μg/l in the summer (Otson, 1987). The raw, treated, or tap water of 31 Canadian and U.S. communities along the Great Lakes usually contained values below or near the detection limit (Canadian Public Health Association, 1986). Further evidence for the uncommon occurrence are reports of none present in 4 surveys of 321 sample sites providing drinking water at locations serving less than 10,000 people and 158 sample sites of communities serving more than 10,000 people (Westrick et al., 1984), and the absence of detectable levels of the compound from drinking water samples in three national surveys in the United States involving a large number of water supplies (U.S. Environmental Protection Agency, 1987a). A 1988 survey of municipal water supplies in Canada failed to find concentrations

greater than 0.2 μg/l (Environment Canada, 1992).

Based on these data, it appears that, apart from locations immediately adjacent to an industrial discharge, the concentrations in surface and groundwaters are either extremely low or none is present.

Sediments may also contain the compound. For example, 4.2 μg/kg was found in sediment from the Lower Tennessee River in Kentucky (Goodley and Gordon, 1976), and the chemical was tentatively identified but not quantified in sediment from Tobin Lake in Saskatchewan (Samoiloff et al., 1983). It has also been found but the amount not quantified in the leachates from industrial landfills (Brown and Donnelly, 1988; Kosson et al., 1985). In one instance in which a landfill received industrial and chemical wastes in 1971-1979, styrene was present in the leachates that were collected in 1981-1983 (Kosson et al., 1985). In a 1986 report of a survey of 455 hazardous waste sites, the compound was found in samples of soil from 3.5% of the sites; in samples in which it was present, the geometric mean concentration was 0.53 mg/kg (Agency for Toxic Substances, 1992). The soil at an unspecified site in Canada contained up to 0.2 μg/kg (Environment Canada, 1992). An aquifer in Connecticut was also observed to contain the compound, the source in this instance being drums of styrene that had been buried 1.2 m below the soil surface (Grossman, 1970). On rare occasions, fish in rivers containing styrene may show its presence; e.g., in emerald shiner *(Notropis atherinoides)*, black crappie *(Pomoxis nigromaculatus)*, bluegill *(Lepomis macrochirus)*, pumpkinseed *(L. gibbosus)*, walleye *(Stizostedion vitreum)*, and splake *(Salvelinus fontinalis)*, the concentrations in the last two species ranging from 15 to 100 μg/kg (R. F. Bonner and O. Merz, 1981; cited in Environment Canada, 1992). Rainbow trout *(Oncorhyncus mykiss)* exposed to effluent from a chemical plant were reported to accumulate 17 mg/kg, despite the fact that levels in the effluent were below the limit of detection (Environment Canada, 1992); this finding is highly suspect and probably in error, however, because if the detection limit was 2 μg/l, it would suggest an anomalously high bioconcentration factor of more than 8500.

Styrene is highly reactive in the atmosphere.

III. REACTIONS

Styrene is highly reactive in the atmosphere. It is unlikely that the speed of its transformation can be attributed to direct photochemical or photolytic reactions because such processes are likely to be very slow. This is a result of the fact that it absorbs little light from solar radiation at the wavelengths above 300 nm that reach the lower atmosphere (Santodonato et al., 1980). Experimental studies have indeed shown that natural sunlight does not cause photolytic degradation in a 6-h period of exposure (Kopczynski et al., 1972). The high reactivity is associated with styrene oxidation by hydroxyl radicals (OH·), and a half life in the troposphere because of styrene oxidation by hydroxyl radicals is approximately 3 h (U.S. Environmental Protection Agency, 1987a). Styrene also reacts readily with ozone, most likely by ozone addition to the -$CH=CH_2$ moiety of the molecule (Atkinson and Carter, 1984), and the rate constant for its reaction at 23°C with ozone (at initial ozone concentrations at or below 2.40×10^{13} molecule/cm^3) has been determined to be $2.16 ¥ 10^{-17}$ cm^3/molecule/s (Atkinson et al., 1982). The half-life in the atmosphere because of its reaction with ozone is approximately 9 h (U.S. Environmental Protection Agency, 1984). Other reports suggest that the half life due to photooxidation by ozone and hydroxyl radicals is approximately 13 h (Agency for Toxic Substances and Disease Registry, 1992). In the reaction of styrene with ozone in the dark, benzaldehyde, formaldehyde, benzoic acid, and traces of formic acid are generated (Grosjean, 1985). The reaction with ozone may be far more important in polluted ozone-rich air than the reaction with hydroxyl radicals. It is likely that the speed of its reaction with ozone and hydroxyl radicals is responsible for the very low levels in the atmosphere, except near highways with considerable vehicular traffic (Grosjean, 1985).

When NO_x is present in the atmosphere, sunlight may rapidly degrade the compound. This is suggested by the observation that 90% was destroyed when 4.3 mg styrene per m^3 was irradiated for 6 h at 35°C with NO at 2.1 mg/m^3 (Heuss and Glasson, 1968). In reaction of styrene with NO in sunlight, ozone, peroxybenzoyl nitrate, benzaldehyde, formaldehyde, benzoic acid, and 2-nitrophenol are produced (Grosjean, 1985, Bignozzi et al., 1981).

Not only does the high reactivity lead to low levels of styrene in the atmosphere but it also probably results in limited long-range atmospheric transport, and little or none is carried through the air to waters or soils at some distance from point source emissions (U.S. Environmental Protection Agency, 1987b).

In waters, a key process affecting the fate of the compound is its volatility. Under laboratory conditions, 50% of 2 to 10 mg styrene per liter was lost by volatilization in 1 to 3 h in lakewater samples and in 6 to 7 h in distilled water (Fu and Alexander, 1992). In other studies, the levels in water samples fell from 23 to 3.3 and 0.4 mg/l in 2 h and 7 d, respectively, and from 46 to 12.5 and 1.5 μg/l in 2 h and 10 d, respectively (Lindström and Lindström, 1980). These findings are relevant to surface waters but not to waters at depth. Based on only two data points and assuming first-order kinetics, Zoeteman et al. (1980) calculated a half-life in Rhine River water of 14 h. Volatilization half-lives in rivers or streams have been calculated to range from 0.75 h to 51 d, the values varying with environmental conditions, and to be 6.0 h at 1 m depth in a lake and 2.5 d at 10 m depth in a turbulent lake (U.S. Environmental Protection Agency, 1987a). Other calculated values are 1 to 3 h for the half life in water (U.S. Environmental Protection Agency, 1987b). These various values suggest that much of the styrene that enters surface waters would be lost by volatilization, although the rate of loss would be slow from deep, stagnant waters.

Few data exist for volatilization from soil, but the loss undoubtedly would be slower in soil, even surface soil, than in waters. For example, even in 1.5-cm deep samples of a loamy soil, only 26% of the 2 mg/kg added volatilized in 31 d (Fu and Alexander, 1992). The transfer to the air would be far slower and less extensive from soil at some depth.

Abiotic reactions are not likely to result in rapid or appreciable styrene transformation in waters, whether the process be hydrolytic, photolytic, or oxidative (U.S. Environmental Protection Agency, 1984). As in the atmosphere, because the molecule does not significantly absorb wavelengths of light above 300 nm, appreciable photochemical degradation is unlikely in natural waters (Santodonato et al., 1980). This has been confirmed by experimental observations in laboratory tests with distilled water. However, styrene in sea water is converted in the light to organic products, the major one being styrene oxide (Toole and Crosby, 1988).

Sorption to solids in soils, subsoils, aquifers, and sediments is of considerable importance because it governs the mobility and may influence the toxicity and biodegradability of organic molecules. In the case of styrene, sorption by soil and aquifer solids is rapid, and more than half may be bound within less than 6 h. Even with samples from a sandy aquifer, more than 85% is sorbed in 78 h. Such retention by particulate matter is particularly marked in organic matter-rich soils (Fu and Alexander, 1992). On the other hand, much of the recently sorbed styrene is readily desorbed, and 61.0 and 66.7% of styrene that had been allowed to sorb for 3 d was removed in 16 d from soil and aquifer solids, respectively. Much but not all of the nondesorbed chemical can be extracted with methanol. It thus appears that sorbed styrene exists in three forms: readily desorbed, not desorbed but easily extractable, and not desorbed and not easily extracted (Fu et al., 1994). When the strong sorptive capacity that is evident in at least some sandy aquifers is exhausted, the compound becomes evident in the water phase (Roberts et al., 1980).

A chemical that is not appreciably sorbed can be highly mobile. Mobility is particularly important because it can result in exposure of humans, animals, or plants that exist far from the point of chemical release. Mobility in soil is approximated from the soil sorption constant (normalized for carbon content of soil), K_{oc}, which in turn is calculated from the soil sorption coefficient, K_d. From the values of K_{oc}, which have been variously estimated to be 567, 900 (U.S. Environmental Protection Agency, 1987a), and 260 (Agency for Toxic Substances and Disease Registry, 1992), it would seem that styrene is moderately mobile in soil and will leach vertically in reasonable periods of time. This is consistent with the behavior in laboratory experiments, as discussed above. Of particular relevance are field measurements of mobility. In one such investigation, styrene-containing effluent from a municipal waste-treatment plant was pumped into a well 15 m deep in a confined aquifer that was approximately 1.5 m thick. The injected water preferentially moved to an observation well 8 m away. The data showed that the compound was present in the observation well by 11 days (i.e., it had moved at least 8 m) and that the concentration in the observation well reached that in water in the injection well by 50 d and remained high at 86 d, when the study terminated (Roberts et al., 1980). More extensive movement was observed at a site in Connecticut in which were buried two styrene drums at a depth of 0.3 to 1.2 m. The chemical migrated downward into the underlying aquifer and then moved laterally and was present in six domestic wells. In this instance, the maximum lateral movement was greater than 90 m. However, 2 years after the source of contamination was removed, styrene was no longer detected (Grossman, 1970). Similar extensive underground movement was reported following a train derailment in Tennessee in which more than 15,000 liters of styrene was spilled. The chemical moved downward into the underground

aquifer and, because of its low specific gravity, formed a layer on top of the groundwater. The groundwater containing the compound in aqueous solution moved from the spill site and was observed 232 m away. As a result, three springs and three wells were contaminated (Crawford and Ulmer, 1994).

Lateral transport in water is thus a reality. The extent of dispersal will be governed by the rate of water movement, the rate and extent of volatilization in surface waters, and the rate of biodegradation. As discussed below, the microbial transformation may be rapid, provided that the temperature is favorable, the compound is in aqueous solution, and the concentration of inorganic nutrients is sufficient for rapid microbial degradation.

IV. BIODEGRADATION

Biodegradation by aerobic microorganisms may lead to extensive or complete destruction of the compound in soil. In an early study, it was found that 97 and 87% of 8-^{14}C-styrene added to soil at levels of 2.0 g/kg was converted to $^{14}CO_2$ in 16 weeks in a landfill soil and sandy loam, respectively. Because $^{14}CO_2$ was not produced in sterile soil, the conversion is microbial (Sielicki et al., 1978). Examination of Figure 4 in that paper shows that the active biodegradation commenced after 2 to 4 weeks, and 8 to 42% was converted to CO_2 each week. Gas chromatographic analysis of two other soils was used to confirm that the degradation destroyed the compound added at 800 mg/kg (Ye and Cao, 1988). Although the latter authors state that the transformation is first order, their data belie the claim.

Biodegradation by aerobic microorganisms may lead to extensive or complete destruction of the compound in soil.

In contrast with the high levels added to soil in the two previous studies, Fu and Alexander (1992) used a range of concentrations, extending from as low as 5 μg up to 4 g/kg. As determined by the amount metabolized, the rate increased with increasing concentration and was even rapid at 4 g/kg. At concentrations up to 1.0 mg/kg, the rate was 0.22 to 0.41% mineralized per hour; i.e. the rate in terms of actual amount degraded was directly correlated with concentration. Even at levels of 0.5 to 4.0 g/kg of soil, 0.032 to 0.043% was destroyed per h. Below 100 mg/kg of soil, aerobic degradation was detectable even very shortly after the chemical entered the soil, but it only was evident after a short period at higher concentrations. The rate of degradation was proportional to concentration at 1.0 mg/kg or lower, suggesting first-order kinetics at the low concentrations. The rate of microbial transformation varied in different soils and was notably slow in an acid silt loam (pH 4.87). Near the soil surface, volatilization and biodegradation occurred simultaneously, but the chief loss mechanism in soil at some depth below the surface is probably chiefly microbial, provided conditions favor biological activity (Fu and Alexander, 1992).

Inasmuch as styrene is quickly sorbed, the effect of sorption on biodegradation was investigated. Microorganisms undoubtedly use desorbable styrene as it moves from the particulate matter into aqueous solution, but some microorganisms apparently metabolize part, but not all, of the compound that remains bound to soil particles. Some evidence exists that the styrene that persists in soil may somehow become sequestered so that it becomes progressively less available to the species in soil responsible for its metabolism; reaching a definitive conclusion on sequestration as a cause of declining bioavailability is complicated because of possible oligomerization or polymerization of the compound (Fu et al., 1994).

Some field measurements also suggest the importance of biodegradation. For example, percolation of wastewater containing styrene through soil in an investigation of groundwater recharge resulted in greater than 92% removal of the compound from the water present at 18 and 30 m depths (Bouwer et al., 1984). In the bioremediation of a site contaminated as a result of the overturning of a tank truck containing 19,000 l of styrene, the concentration in the underlying groundwater was reduced to less than 0.5 mg/l in 14 weeks (Kuhlmeier, 1988, 1989). The groundwater in this bioremediation received 50 mg of H_2O_2/l to provide O_2 to the microorganisms.

Biodegradation also occurs in surface waters. This has been demonstrated in the laboratory with samples of lake water to which the compound was added to give 2.5 μg to 1.0 mg/l, and from 10 to 20% of the compound was mineralized in 3 weeks. The transformation required several days for acclimation of the indigenous microorganisms (Fu and Alexander, 1992).

Evidence exists for both persistence and rapid biodegradation in aquifers or groundwaters. Persistence

is evident from the monitoring of a confined aquifer into which was pumped styrene-containing wastewater. The compound was present from day 11 to the end of the monitoring on day 86 (Roberts et al., 1986). Although the aquifer continued to receive styrene-containing water during the test period, the appearance of a styrene-utilizing microflora should have resulted in a marked decline in concentration, but this did not occur. Persistence is also shown by the presence of styrene in an aquifer for at least 2 years after its leakage from styrene-containing drums buried below ground (Grossman, 1970). The longevity could have resulted from anaerobic conditions in the aquifer, the existence of styrene as a poorly available nonaqueous liquid, or the paucity of N and P in the water, but the information needed to reach a conclusion was not collected. The observation that removal of the source of the contamination from the buried drums led to undetectable levels in 2 years or less in the water suggests biodegradation in the field (Grossman, 1970), although transport away from the sampling site may account for the contamination-free water.

Laboratory tests have provided more definitive results attesting to aerobic biodegradation in subsurface solids and groundwater. For example, Wilson et al. (1983) found that 2.3 to 12.0% of styrene added at 600 to 800 μg/l to cores of subsurface samples from above and below the water table was metabolized per week. The loss was confirmed as biological because the transformation was abolished by autoclaving. Rapid and extensive aerobic degradation also occurred in samples of ground water amended with 1 mg/l. The conversion was slower in aquifer sand, and only 1.09 and 1.51% was degraded to CO_2 in 3 weeks in samples amended to give 20 and 100 μg/kg, respectively (Fu and Alexander, 1992). In other laboratory studies, 18% of styrene-carbon was found to be converted to CO_2 in 50 d in samples of aquifer solids (Fu et al., 1994), the concentration in samples of a sandy aquifer fell from 1.47 to 0.019 mg/l in 17 d (Battermann and Werner, 1984), and it declined from 70 mg/l to below the detection limit in 25 h in groundwater samples (Kuhlmeier, 1988). Caution needs to be exercised in interpreting the rapid loss in laboratory tests to biodegradation because the published reports rarely present information to interpret the rapid disappearance as arising from biodegradation or volatilization. The latter process, as discussed above, may be very rapid in water samples tested in the laboratory but will not be significant in actual aquifers.

Laboratory tests have provided more definitive results attesting to aerobic biodegradation in subsurface solids and groundwater.

Degradation is also rapid in sewage when O_2 is available. Samples of municipal sewage supplemented with 1.0 mg/l degraded the chemical linearly, with more than 50% of the C being converted to CO_2 in 33 d, and the oxidation began very shortly after first addition of the chemical. The process is microbial because CO_2 was not produced in sterilized sewage (Fu and Alexander, 1992). In 5 d, 65% of the compound (probably added at 3 mg/l) was oxidized in fresh water inoculated with samples of domestic wastewater (Price et al., 1974). Other early tests also show biodegradation in samples of sewage (Bridié et al., 1979b, Ludzack and Ettinger, 1960; Pahren and Bloodgood, 1961), but the value of some of these data is limited because of short incubation periods or volatilization of the compound.

Microorganisms often develop as dense growths or films on solid surfaces. These biofilms may be particularly active in biodegradation when O_2 is present. Such a biofilm, when provided with a mixture of organic compounds, in 11 weeks destroyed 99% or more of the styrene in solution at initial concentrations of 81 and 280 μg/l, but the oxidation was less extensive when the water was supplemented with 8 or 21 μg/l (McCarty et al., 1984).

Under anaerobiosis, biodegradation sometimes does not occur, at least not in the time periods that have been used. Anaerobiosis is a frequent consequence of microbial activity in soils, subsoils, aquifers, and waters containing large quantities of readily utilizable organic materials, either synthetic or natural; the resident populations consume all the O_2 to bring about the decomposition, and the entry of O_2 from the overlying air is too slow to meet the large microbial demand. A laboratory study indeed showed that a considerable part of the styrene added to O_2-deficient aquifer solids or soil at 200 mg/kg disappeared in the first 5 d, but then the rate of its disappearance either slowed or stopped so that the concentration was only slightly lower or was the same at 260 d as at 5 d; after 260 d, more than 40% of styrene added to aquifer material and approxi-

mately 30% in soil remained. The absence of further degradation did not result from a fall in pH or the lack of nitrate to serve as an electron acceptor but could have resulted from the accumulation of breakdown products that inhibited metabolism. Similar data were obtained with an acid soil (pH 4.87) and an organic soil in which, after some degradation had occurred in the first 10 d, no further transformation was evident from 10 to 172 d. Phenylethanol, phenylacetic acid, phenylacetaldehyde, and benzoic acid were formed and persisted for some time in these soils, but low-molecular-weight products were not detected in the aquifer solids (Fu and Alexander, 1996). The persistence of styrene under anaerobic conditions is important because of the many locations in nature that are devoid of O_2.

The failure of styrene to be destroyed under anaerobic conditions is not unexpected. Thus, a microbial biofilm attached to solid surfaces failed to destroy the compound anaerobically in a 12-week period (McCarty et al., 1984), and a methane-forming, anaerobic biofilm also was inactive (Bouwer and McCarty, 1984). Similarly, an anaerobic, methane-producing mixture of microorganisms did not convert styrene to methane (Schink, 1985). Moreover, the rate of degradation was found to be slow in samples of waterlogged soil and aquifer sand (Fu and Alexander, 1992; Ye and Cao, 1988).

On the other hand, some mixtures of microorganisms are able to metabolize styrene anaerobically, although the conversion does not lead to methane. Under these circumstances, phenylethanol, phenylacetaldehyde, phenylacetic acid, ethylbenzene, 1-phenylethanone, toluene, and a variety of aromatic, carbocyclic, and aliphatic products are formed, and all of the styrene is metabolized, albeit slowly (Churchman and Grbic-Galic, 1987, Grbic-Galic et al., 1990).

Despite the importance of knowing the fate of styrene in anaerobic environments, the data are too few to permit generalizations. Clearly, the possibility exists for extensive but slow destruction based on tests with laboratory cultures, but the few studies with environmental samples suggest that conditions in nature may preclude degradation or may result in very slow decomposition.

Another circumstance that may result in persistence is associated with thresholds. At very low concentrations, typically at levels below 10 μg/l, microorganisms may not get sufficient energy from the little substrate-carbon diffusing into the cell per unit time to allow them to multiply or possibly even to survive. Thus, if the specific microbial populations that are capable of degrading styrene in water are unable to compete effectively with neighboring microorganisms for the other organic nutrients present, they may not multiply and thus destroy the chemical. The findings that the rate of aerobic styrene degradation of 2.5, 10, and 100 μg/l in lake water, and even of 20 and 100 μg/kg in aquifer sand, declined (on a percentage basis) suggest that a threshold exists at still lower concentrations; i.e., that biodegradation would not occur or would be extremely slow. No evidence has been obtained for a threshold in soil, however (Fu and Alexander, 1992). The threshold phenomenon in laboratory tests and in nature has been discussed fully by Alexander (1994).

Some mixtures of microorganisms are able to metabolize styrene anaerobically, although the conversion does not lead to methane.

Many microorganisms can metabolize styrene in pure culture in laboratory media, and based on the sources from which they were obtained, such organisms appear to be ubiquitous. With few exceptions, the isolated species are bacteria, but this does not necessarily mean that the aerobic process is chiefly or solely bacterial because the scientists conducting these studies were usually using isolation techniques that favored bacteria. Even among the bacteria, nearly all of the isolates are aerobes, but a few anaerobic bacteria have been obtained. Hartmans et al. (1990b) found 14 aerobic bacterial strains and 2 fungi in soil and water able to use the molecule as sole C (carbon) and energy source. Species of *Pseudomonas* (Jain et al., 1987; El-Aalam et al., 1993; Baggi et al., 1983; Ye and Cao, 1988; Inoue and Horikoshi, 1989), *Corynebacterium*, *Arthrobacter* (Ye and Cao, 1988), *Rhodococcus* (Warhurst et al., 1994), *Bacillus* (Srivastava, 1990), *Xanthobacter* (Hartmans et al., 1989), and *Nocardia* (Ottengraf et al., 1986) can grow at its expense, thereby using it for both C and energy. Fungi are also readily obtained (Cox et al., 1993). Some microorganisms grow in media with high concentrations (Inoue and Horikoshi, 1989), and some will tolerate up to 37 mg/L (Cox et al., 1993a) or can be adapted to grow at high concentrations in aqueous media with the chemical (Weber et al., 1993). Some can degrade styrene in a two-phase system containing water and a

separate organic phase (El-Aalam et al., 1993) or remove styrene from air passed through solids containing the organisms (Cox et al., 1993b; Ottengraf et al., 1986). The described bacteria that can grow anaerobically on styrene and use it as C and energy source are strains of *Enterobacter* and *Clostridium* (Grbic-Galic et al., 1990). Some bacteria have also been found to grow on breakdown products of styrene. Several bacteria metabolize the compound without getting any or obtaining little of the carbon and energy they need for multiplication; e.g., *Nitrosomonas europaea* (Keener and Arp, 1994), *Brevibacterium* sp. (Hou et al., 1983), and possibly *Methylosinus trichosporium* (Higgins et al., 1979) and *Mycobacterium vaccae* (Burback and Perry, 1993).

The products formed by several of these bacteria and the initial steps in styrene metabolism have been characterized. In assessing this information, it is important to consider that a product formed in culture or by microbial enzymes may have no environmental significance; this is true because many products never are excreted by the microorganisms into the surrounding environment, the product may be destroyed in nature as quickly as it is formed so none can be detected, or the biodegradation in nature is by organisms metabolizing the compound in a different way than observed for a particular isolate. In this context, it is important to refer to the studies with actual environmental samples in which, as discussed above, breakdown products have not been detected except under anaerobic conditions.

A frequent product of the metabolism of styrene by aerobic bacteria is styrene oxide. Such a conversion has been established for *Nocardia corallina* (Furuhashi et al., 1986), *Brevibacterium* sp. (Hou et al., 1983), *M. vaccae* (Burback and Perry, 1993), *N. europaea* (Keener and Arp, 1994), *M. trichosporium* (Higgins et al., 1979), *Bacillus* sp. (Srivastava, 1990), *Pseudomonas putida* (O'Connor et al., 1995), and *Escherichia coli* bearing genes cloned from *Pseudomonas fluorescens* (Marconi et al., 1996). *P. putida* also can transform styrene to 1,2-dihydroxy-3-ethenyl-3-cyclohexene (Bestetti et al., 1989). Other products that have been found are 2- and 1-phenylethanol, phenylacetaldehyde, phenylacetic acid (Hartmans et al., 1989; Shirai and Hisatsuka, 1979; Sielicki et al., 1978; Utkin et al., 1991a), and occasionally 2-hydroxyphenylacetic acid (Baggi et al. 1983; Utkin et al., 1991b; Rustemov et al., 1992) and acetophenone (Utkin et al., 1991b). Based on these and other findings, the following metabolic pathways appear to be operative under aerobic conditions (Warhurst and Fewson, 1994).

1. A stepwise conversion to 1-phenylethanol [$(RCHOH)CH_3$] and acetophenone [$RCOCH_3$].
2. An initial epoxidation to styrene oxide followed by the stepwise conversion of styrene oxide to phenylethanediol [$RCH(OH)CH_2OH$] and 2-hydroxyphenylacetic acid [RCH(OH)COOH; synonym, mandelic acid].
3. A sequential conversion of styrene to 2-phenylethanol [RCH_2CH_2OH], phenylacetaldehyde [RCH_2CHO], phenylacetic acid [RCH_2COOH] and 2-hydroxyphenylacetic acid.
4. A reduction to ethylbenzene [RCH_2CH_3].
5. An hydroxylation and reduction of the ring to yield 1,2-dihydroxy-3-ethenyl-3-cyclohexene.
6. Styrene oxide, alternatively, could be directly converted to 1- or 2-phenylethanol or phenylacetaldehyde.

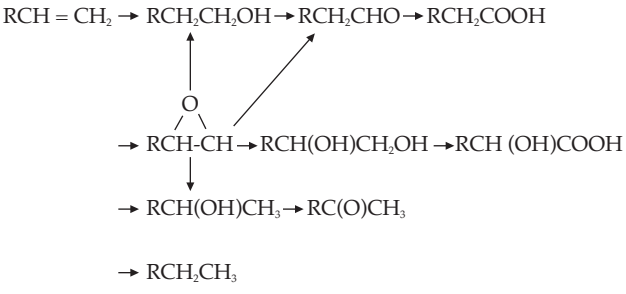
The organisms that use styrene as carbon and energy source for growth cleave the aromatic ring, and the products of ring cleavage are further metabolized. The initial phases of aerobic metabolism of the side chain are shown in Fig. 1.

Some of the enzymes responsible for the conversion have been characterized to a greater or lesser extent. These include styrene monooxygenase that catalyzes the conversion of styrene to its oxide, styrene oxide isomerase which converts styrene oxide to phenylacetaldehyde, phenylacetaldehyde dehydrogenase which oxidizes this aldehyde to phenylacetic acid (Hartmans et al., 1989; 1990a), and styrene oxide reductase that leads to the formation of 2-phenylethanol from the oxide (Marconi et al., 1996).

An entirely different mode of attack is used by *Rhodococcus rhodochrous*. This bacterium hydroxylates the ring by a dioxygenase to form 3-vinylcatechol without acting on the two-carbon side-chain until after the aro-

FIGURE 1.

Initial steps in the aerobic metabolism of the side chain of styrene (R = phenyl).



matic ring is cleaved (Warhurst et al., 1994).

In addition to these pathways of aerobic bacteria, the many products formed by the mixed culture of anaerobic bacteria have prompted a postulated metabolic sequence (Grbic-Galic et al., 1990). Most of the initial steps that were proposed are similar to those of the aerobes. Moreover, data in support of the anaerobic pathways have not been obtained.

V. TOXICITY

Many studies have been conducted of the effects of styrene on animals, both non-mammals and mammals. However, the value of many of these tests is quite limited because they were conducted in systems in which the styrene rapidly volatilized, and measurements were not made of the concentrations that remained and to which the animals were actually exposed for the full duration of the test period. Hence, the toxicity probably is greater than the data suggest, but the authors of many of the reports did not give sufficient information on methods to assess which tests are thus compromised. Hence, the reader must evaluate some of the data, especially those showing little effect except at very high concentrations, with care.

Fish have been of especial interest. The early studies of Pickering and Henderson (1966) gave LC_{50} values (concentrations to kill 50% of the animals) in 24 h tests of 57-63 mg/l for fathead minnows *(Pimephales promelas)*, 25 mg/l for bluegills *(Leponis macrochirus)*, 65 mg/l for goldfish *(Carassius auratus)*, and 75 mg/l for guppies *(Lebistes reticulatus)*, the values changing little if at all in 48-and 96-h tests. Somewhat later 24- to 96-h tests with juvenile fathead minnows provided LC_{50} values of 32 mg/l (Mattson et al., 1976). Other tests with goldfish gave median tolerance limits of 25 mg/l, 12 mg/l causing no mortality (Jensen, 1978), and in a test in which concentrations were determined before and after exposure, the LC_{50} in a 24-h test period of goldfish was 26 mg/l (Bridié et al., 1979a). The LC_{50} values for lake emerald shiner *(Notropis atherinoides* subsp. *acutus)* and sheepshead minnow *(Cyprinodon variegatus)* were reported to be 31 and 30 mg/l in one study (Dow Chemical Co., 1989), but the value for 24-to-96 h exposures of sheepshead minnow in another report was 9.1 mg/l (Heitmuller et al., 1981). In the latter instance, no effect was evident at 5.1 mg/l. Tests in two different laboratories of golden orfe *(Leuciscus idus)*, a freshwater fish, provide LC_{50} values of 17 and 66 mg/l, and 9 and 45 mg/l were without effect (Juhnke and Lüdemann, 1978). In contrast with the high concentrations needed for harm, the LC_{50} value for rainbow trout *(Salmo gairdneri)* in a 96-h test was found to be 2.5 mg/l (Qureshi et al., 1982).

The range of concentrations thus reported for toxicity to fish may result from care being taken in some but not in other assays to minimize volatilization or to monitor styrene remaining in the water during the period of exposure. In contrast, Machado (1995) exposed fathead minnows to styrene in an apparatus with essentially no openings and, in addition, regularly measured the styrene content of the water. The LC_{50} values after exposures of 24, 48, 72, and 96 h were 12, 12, 11, and 10 mg/l, and the no-observed-effect concentration (NOEC) was found to be 4.0 mg/l; i.e., mortality or abnormalities were not observed. At 7.6 mg/l, some behavioral abnormalities were evident at 6 h, and a few of the fish were dead at 72 h.

The range of concentrations thus reported for toxicity to fish may result from care being taken in some but not in other assays.

A study of sea urchins *(Paracentrotus lividorus* and *Psammechinus microtuberculatus)* revealed that styrene at 10 mg/l, which was the lowest concentration evaluated, caused abnormalities in the eggs and of the embryos that developed (Pagano et al., 1978).

Careful assessments have been made of the effects on two amphipods, *Pontoporeia affinis* and *Hyalella azteca)*. These amphipods reside at the bottom of bodies of water. In this study, which was conducted with a test system in which the compound quickly volatilized, measurements were made of the amounts that remained with time. When exposed to initial levels of 69 mg/l, the animals died in a few hours. When the water initially contained 23, 35, and 46 mg/l, the swimming of the amphipods stopped immediately; after several days, however, the activity rose to many times the normal level. At the time swimming resumed, and even enhanced swimming, the concentration in the water was 4.4 mg/l. At the time at which the formerly increased swimming activity returned to normal, the concentrations were 0.4, 0.6, and 1.0 mg/l in the three treatments (Lindström and Lindström, 1980).

In contrast, Putt (1995a) assessed the acute toxicity to

the amphipod *H. azteca* in a closed system to minimize volatilization, and determinations were also made of concentrations in the water. The compound had no effect on mortality in 96 h at 4.1 mg/l or lower levels, but the numbers of survivors were reduced at 24 h or longer periods by 7.4 mg/l, and 20 and 85% of the amphipods were dead at 96 h in the presence of 7.4 and 13 mg/l, respectively. The LC_{50} was calculated to be 9.5 mg/l.

Effects on water fleas, *Daphnia magna*, have also been measured. Early reports give: LC_{50} values of 27 and 23 mg/l for 24- and 48-h tests respectively, and NOEC values in 48 h of 6.8 mg/l (LeBlanc, 1980); LC_{50} and NOEC values in 24 h of 255 and 130 mg/l, respectively (Bringmann and Kühn, 1977); LC_{50} values of 59 mg/l in a 48-h assay (Qureshi et al., 1982); and values for EC_{50} and EC_0 (effects concentration, in which evaluations were made of immobilization of *D. magna*, the EC_0 representing the lowest level caused no detectable harm) of 182 and 105 mg/l (Bringmann and Kühn, 1982). Because these reports provide no documentation that loss by volatilization was prevented or determined, all of the values are questionable. On the other hand, Putt (1995b) exposed water fleas in a laboratory system designed to minimize volatilization and also determined the amounts remaining in the water. At 1.9 mg/l or lower concentrations, none of the daphnids had become immobilized in 24 or 48 h, immobilization referring to the absence of movement after gentle prodding of the animals. Immobilization was not evident at 24 h, but 5% of the daphnids were no longer mobile at 3.3 mg/l. All the animals were immobilized by 7.4 mg/l in 24 h. From the data, a 48-h EC_{50} value of 4.7 mg/l was calculated.

Assays of the toxicity to brine shrimp *(Artemia salina)*, as measured by cessation of movement, have given LC_{50} values after 24 and 48 h of 68 and 52 mg/l respectively (Price and Conway, 1974). Here, too, the data must be considered questionable because of possible volatilization of the chemical. The same question applies to reports that styrene at 100 mg/l was not toxic to the protozoan *Chilomonas paramecium* (Bringmann et al., 1980) and that 256 mg/l had no effect on the protozoan *Entosiphon sulcatum* (Bringmann and Kühn, 1980).

Only a single soil animal has been subject to assessment, *Eisenia foetida.* The test was made using an artificial solid substratum composed of sand, clay, and peat moss, and the responses observed were length of time it took for earthworms placed on the surface to completely burrow into the solid substratum, mortality, lethargy, and worm weight. Analyses indicate that much of the compound was lost during the first 7 d. Although Garvey (1995) chose to present the toxicity data from his assay based on the mean concentration found during the test period (i.e., the mean of styrene concentrations at days 0 and 7), I have used a somewhat different approach, namely to consider the minimum concentration to which the worms were exposed: the animals were surely exposed to that and unknown higher concentrations, whereas the mean of the initially added and final amounts does not provide an adequate assessment. Using such an approach, the data show that styrene at 34 mg/kg of solid substratum had no effect, whereas a concentration of 65 mg/kg or some unknown higher amount killed half of the worms. Thus, the NOEC value may be assumed to be 34 mg/kg, if not higher.

Because of concern with possible effects on humans in an occupational setting, numerous studies have been conducted with mammals as test species. Such information should be considered as a means for predicting possible injury to higher organisms in natural ecosystems. However, the exposures are typically to far higher levels than are encountered outside of the workplace, and environmentally realistic concentrations are not part of these assessments.

Rats that drank water containing 125 or 250 mg styrene per liter for 2 years showed no effect on survival, body or organ weights, fertility, food consumption, clinical chemistry, or histopathology, although water consumption was reduced (Beliles et al., 1985), and rats given 20 doses of styrene by stomach tube at 0.1 g/kg body weight exhibited good weight gains and no pathological lesions (Spencer et al., 1942). The LD_{50} by oral exposure has been found to be 350 mg/kg of body weight for mice (Bond, 1984) and 5000 mg/kg for rats (Wolf et al., 1956). Mice given 150 mg/kg (5 d/week) for 78 weeks had slightly depressed body weights among the females but not among the males, and 20% of the females and 8% of the males died. Of the rats exposed to 500 mg/kg (5 d/week) for 78 weeks, 12% died (National Cancer Institute, 1979). The survival of pregnant rats was not affected if they were given 1.0 mL of styrene orally for 10 consecutive days, but body and organ weights were initially reduced and then increased to approach normal weights; fetal abnormalities were largely absent (Chernoff et al., 1990). The compound given orally at 20 mg/d for 5 d had no influence on body weight or weights of the thymus, but the weights of the adrenal, spleen, and liver were reduced somewhat and immunological responses were evident (Dogra et al., 1989). At doses of 133 mg/kg body weight per day in 132 feedings in a 185-d period, styrene had no discernible influence, but 400 mg/kg affected growth, liver and kidney weight, blood

counts, and histopathology (Wolf et al., 1956). The chemical given orally at 200 mg/kg per d for 60 d caused no biochemical and histological changes in rat testicles, but 400 mg was injurious (Srivastava et al., 1989). The compound given to rats orally at 90 or 150 mg/kg twice per day from days 6 to 15 of gestation was not teratogenic and induced no embryological or fetal changes (Murray et al., 1978). In a summary of the literature, the Agency for Toxic Substances and Disease Registry (1992) gives these no-observed-adverse effects levels (NOAELs) following oral exposure of rats (all in mg/kg/d): 270 and 100 for neurological effects (daily exposure for 7 and 14 d, respectively), 300 for developmental effects (daily exposure for 10 days), 133 for hepatic and renal effects (exposure of 5 d/week for 6 months), 200 and 35 for reproductive effects (exposure of 6 d/week for 60 d and 90-d continuous exposure, respectively), and 21 for systemic effects (exposure of 7 d/week for 105 weeks). It was also concluded that the NOAELs from inhalation exposure were (in mg/m^3): 2600 for developmental effects, 570 for renal effects, 1300 for reproductive effects, and 640 for respiratory systemic effects. Additional studies indicate higher NOAELs for some of these endpoints. Thus, oral and inhalation exposure of mammals only produced deleterious changes at far higher concentrations than are anticipated to be found in the water, food, or air of mammals in natural habitats.

Animals in nature may also be exposed to styrene by inhalation, and thus the data on inhalation toxicology are important. The LC_{50} following inhalation of styrene was found to be 11.8 g/m^3 for rats exposed for 4 h and 21.0 g/m^3 for mice exposed for 2 h (Shugaev, 1969). The respiratory rate of mice was reduced by 50% at concentrations of 0.67 g/m^3 (Alarie, 1973). An early study reports that 126 to 264 inhalation exposures in a 148- to 360-d period produced no gross changes or effects on growth, body or organ weights, or histopathology in guinea pigs exposed to 3.0 g/m^3, rabbits exposed to 9.3 g/m^3, or rhesus monkeys exposed to 6.0 to 6.3 g/m^3, although eye and nasal irritation was noted in rats and guinea pigs exposed to air containing 6.0 to 6.3 g/m^3 (Wolf et al., 1956; Spencer et al., 1942). The nasal and tracheal changes in rats exposed to 728 mg/m^3 of styrene by inhalation for 4 h/d (5 d/week) for 3 weeks were gone by 12 weeks (Ohashi et al., 1986), which was the only time of examination except immediately following the treatment. Morphological changes in the nasal mucosa are also induced in rats exposed to 128 mg/m^3 of styrene in air for 8 weeks (Ohashi et al., 1985), but styrene at 565 mg/m^3 in the air caused no change in renal function in rats exposed for 7 h/d (5 d/week) for 13 weeks (Viau et al., 1987). Rats and rabbits exposed to 1.3 or 2.6 mg/m^3 for 7 h/day (from day 6 to 15 and day 6 to 18 of gestation in rats and rabbits, respectively) showed no teratolological abnormalities (Murray et al., 1978), and the sperm of mice inhaling the same concentrations for 5 days, 6 h/d, was normal (Salomaa et al., 1985). Styrene in the air at 2100 mg/m^3 provided three times to rats 5 h/day or in a single 24-h exposure decreased glutathione levels and cytochrome P_{450} oxidative metabolism (Elovaara et al., 1990). The summary by the Agency for Toxic Substances and Disease Registry (1992) gives these NOAELs (all in mg/m^3) following inhalation exposure: 2600, 2600, and 3200 for developmental effects in rats, rabbits, and hamsters, respectively; 1300 for reproductive effects in mice; 640 for effects on rat respiratory system and 570 for the rat renal system. Thus, to bring about a harmful response in mammals, concentrations far higher than are found in outdoor air would be required.

> . . .to bring about a harmful response in mammals, concentrations [of styrene] far higher than are found in outdoor air would be required.

With one exception, toxicity to higher plants has yet to be determined. That one exception is from a study in which it was found that 0.05% styrene (vol/vol) (400 mg/l volume percent) induced cytogenetic effects in the root-tip cells of onion *(Allium cepa)*, specifically chromosome breakage (Linnainmaa et al., 1978). Thus, high concentrations are needed to affect root-tip cells.

The observations with algae may have some relevance to higher plants, however. This is particularly true of green algae because they, like rooted plants, are eukaryotes. In this connection, the report of Bringmann and Kühn (1980) that *Scenedesmus quadricauda*, a green alga, is not inhibited even at 200 mg/l is difficult to accept because of the absence of any indication of losses of the compound from the cultures during the test. In contrast, a study of *Selenastrum capricornutum* was performed in a closed system to minimize loss of styrene by volatiliza-

tion and, in addition, the concentrations during the bioassay were measured. Styrene had no effect on multiplication of the alga at 0.063 mg/l, growth was inhibited somewhat at 0.28 and 0.68 mg/l, the effect was pronounced at 2.0 mg/l, and the organism failed to multiply at 7.0 mg/l. The alga was inhibited but not killed at the highest level because growth resumed when the chemical was removed. The EC_{50} was calculated to range from 0.56 to 3.9 mg/l, depending on the age of the culture. However, the lower value for the EC_{50} may be incorrect because of variability in algal growth during the early phase of the study (Hoberg, 1995).

Microcystis aeruginosa, which is photosynthetic but is considered as a cyanobacterium by some microbiologists because it is a procaryote and a blue-green alga by others, is reported to be inhibited by 67 mg/l (Bringmann and Kühn, 1978), but these data must be considered as equivocal owing to the absence of information on volatilization of styrene from the cultures. Similarly, the reports that styrene inhibition of the bacterium *Mycabacterium vaccae* occurred at 460 mg/l (Burback et al., 1994) and that streptomycetes grew on agar containing 2% of the compound (Grbic and Munjko, 1977) must be considered with skepticism because of the possible rapid loss of the compound from the cultures. Nevertheless, styrene at 4.0 g/kg was readily degraded aerobically in soil (Fu and Alexander, 1992), and some bacteria, such as *Pseudomonas putida*, although initially sensitive, can grow and degrade the molecule even in water that is saturated with styrene (Weber et al., 1993) demonstrating that some microorganisms have a high tolerance. Similarly, *Spirillum volutans* is not affected by 636 mg/l (Qureshi et al., 1982). On the other hand, *Rhodococcus* sp. and a strain of *P. putida* are inhibited by 180 mg/l (Hartmans, 1995), the yeast *Exophiala jeanselmei* fails to grow above 37 mg/l (Cox et al., 1993a), 10 mg/g of soil suppressed *Azotobacter chroococcum* although 50 mg/g did not affect *Cellulolomonas* and various fungi and yeasts (Cao and Ye, 1991), and 5 mg/l reduced light emission by the luminescent bacterium *Photobacterium phosphoreum* (Qureshi et al., 1982). Thus, the sensitivity of heterotrophic microorganisms varies appreciably. Yet, these concentrations exceed appreciably the concentrations that have been observed in water and soil.

Although a compound may be present in waters and soils at a level that is nontoxic, it could be assimilated by an organism and reach internal concentrations that are harmful. Hence, assessing bioconcentration is important. Bioconcentration is expressed as the ratio of the concentration of a compound in living organisms (e.g., mg/kg of tissue) to that in the surrounding environment (e.g., mg/kg of water) at equilibrium. The higher the value, the greater is the extent of concentration. Although experimental data are sparse, a bioconcentration factor of 13.5 has been reported for goldfish, *Carassius auratus* (U.S. Environmental Protection Agency, 1987b). The octanol-water partition coefficient (K_{ow}) is a good predictor of bioconcentration, and the log K_{ow} has been calculated to be 2.87 and determined to be 2.95 (Banerjee and Howard, 1988). The low bioconcentration factor for fish and the modestly high K_{ow} suggest that the compound probably does not accumulate appreciably in aquatic organisms. Moreover, because styrene is probably metabolized and excreted and because its level in waters and soils are typically very low, bioconcentration to cause injury is unlikely. However, one contradictory report raises some question in a plant effluent having styrene below the levels of detection, rainbow trout (*O. mykiss*) accumulated 17 mg/kg (Environmental Canada, 1992). Nevertheless, the absence of evidence for bioaccumulation in any other instance suggests this to be a rare and anomalous observation. Moreover, it is surprising that such a high level would be found in trout if the concentration in the effluent was below the limit of detection.

VI. CONCLUSIONS

The concentration of styrene in air does not exceed 66 $\mu g/m^3$ in air even at waste-disposal sites, levels are far lower in air not adjacent to waste sites, and the concentration rarely exceeds 20 $\mu g/l$ in water.

Styrene is highly reactive in air. As a result, transport through the atmosphere for appreciable distances and its entry into water and soil in significant amounts from point-source emissions to the atmosphere are unlikely.

The compound is quite volatile, and it is usually readily biodegraded in waters and soils under aerobic conditions. As a result, distant transport through waters or through soils is unlikely when O_2 is present. Although it is sorbed by soil, part is readily desorbed and can move vertically and enter ground waters; however, except when it exists in anaerobic environments, at very high concentrations, or in a nonaqueous-phase liquid, it will not be transported considerable distances because of its biodegradability. It also probably will not be discharged from sewage-treatment plants using aerobic processes because of the volatility and biodegradability. Moreover, concentrations probably would never be high enough to inhibit biodegradation, except near the site of a spill.

Little information exists on the fate of styrene in

anaerobic environments. Although the potential for anaerobic biodegradation exists, the few data available suggest that the compound may persist in subsoils, anoxic aquifers, septic tanks, or sludge. Information on the fate of the chemical in such circumstances would be helpful in further assessing ecological and health effects.

Products of aerobic metabolism of styrene in laboratory cultures have been identified. However, no evidence exists that any of these compounds appear in natural environments in detectable amounts. Any products generated anaerobically may persist, however.

Many early studies of toxicity of styrene are of little value because volatilization was not minimized during the test period or the residual concentrations were not determined. Nevertheless, more careful assays have shown that the concentration present in air, waters, and soil are, except in the immediate vicinity of a spill, too low to exert a deleterious effect on nonmammalian species or mammals exposed through the air they breathe or the food or water they consume or on microorganisms. Moreover, the volatility and biodegradability would nearly always keep the exposure level below that required for toxicity. Furthermore, the properties of the compound make bioconcentration to yield harmful levels unlikely. Data on plants are lacking. At concentrations in water below 10 μg/l, it is possible that styrene may persist, but because of the far higher concentrations needed for toxicity, it is unlikely that these levels have importance to human health or to populations of organisms in nature.

The volatility and biodegradability would nearly always keep the exposure level below that required for toxicity.

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Ecotoxicity Hazard Assessment of Styrene

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The ecotoxicity of styrene was evaluated in acute toxicity studies of fathead minnows *(Pimephales promelas)*, daphnids *(Daphnia magna)*, amphipods *(Hyalella azteca)*, and freshwater green algae *(Selenastrum capricornutum)*, and a subacute toxicity study of earthworms *(Eisenia foetida)*. Stable exposure levels were maintained in the studies with fathead minnows, daphnids, and amphipods using sealed, flowthrough, serial dilution systems and test vessels. The algae were evaluated in a sealed, static system. The earthworms were exposed in artificial soil which was renewed after 7 days. Styrene concentrations in water and soil were analyzed by gas chromatography with flame ionization detection following extraction into hexane. Test results are based on measured concentrations. Styrene was moderately toxic to fathead minnows, daphnids, and amphipods: fathead minnow: LC_{50} (96 hr), 10 mg/liter, and NOEC, 4.0 mg/liter; daphnids: EC_{50} (48 hr), 4.7 mg/liter, and NOEC, 1.9 mg/liter; amphipods: LC_{50} (96 hr), 9.5 mg/liter, and NOEC, 4.1 mg/liter. Styrene was highly toxic to green algae: EC_{50} (96 hr), 0.72 mg/liter, and NOEC, 0.063 mg/liter; these effects were found to be algistatic rather than algicidal. Styrene was slightly toxic to earthworms: LC_{50} (14 days), 120 mg/kg, and NOEC, 44 mg/kg. There was no indication of a concern for chronic toxicity based on these studies. Styrene's potential impact on aquatic and soil environments is significantly mitigated by its volatility and biodegradability.

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INTRODUCTION

Styrene (cinnamene, ethenylbenzene, phenylethylene, styrol, styrolene, and vinylbenzene; CAS No. 100-42-5) is primarily used in the production of polymers and copolymers, including polystyrene, copolymers with acrylonitrile and butadiene, and a variety of other resins. The annual worldwide production capacity of styrene was estimated to be over 16 million tons in 1992 (Miller et al., 1994).

Styrene is commonly shipped by barge and vessel over fresh and marine bodies of water. Despite an extensive amount of monitoring of surface water, ground water, and drinking water, styrene has rarely been detected and then at very low levels (part per billion or part per trillion) (Alexander, 1990, 1997; ATSDR, 1992; Environment Canada, 1993; HSE, 1996).

Because styrene is volatile (vapor pressure, 4.5 mm Hg at 20°C), is less dense than water (specific gravity, 0.9045 at 25°C), has limited water solubility (≤300 ppm at 20°C), and has a high Henry's Law Constant (2.61×10^{-3} atm • m^3/mol) (Verschueren, 1983; ATSDR, 1992), results of previously reported aquatic toxicity studies conducted under static open-air (uncovered) conditions without analytical verification of styrene concentrations have been variable. Although these tests may be considered to be more environmentally realistic, they tend to only

match those exposure scenarios where the loading, loss rates, and dissolution kinetics match the exposure conditions in the laboratory. Hence, the static open-air tests only apply to a small fraction of the possible exposure scenarios, and they tend to underpredict a volatile chemical's inherent aquatic toxicity. Typically, inherent toxicity tests are accomplished with flowthrough or static-renewal exposures whereby test organisms are exposed to measured constant concentrations of test substance. Thus, the Styrene Information and Research Center undertook the conduction of a series of aquatic and soil toxicity tests to better characterize styrene's potential environmental hazards. Acute toxicity studies were conducted on a freshwater fish (fathead minnows), an invertebrate zooplankton (daphnids), and an epibenthic organism (amphipods) under flowthrough conditions, in sealed test vessels, to maintain consistent test water concentrations. A phytoplankton test with unicellular green algae was conducted under static conditions in sealed flasks. The earthworm test was conducted using partially covered vessels which permitted fresh air for test organism survival, but also allowed the loss of styrene from the soil by volatilization. The studies were conducted according to EPA, OECD, and/or EEC published guidelines and in accordance with EPA Good Laboratory Practice regulations.

METHODS

Chemical

Styrene is a clear, colorless liquid that is slightly soluble in water (approximately 300 mg/liter). The styrene used in these studies was obtained from Shell Canada Limited and had a 99.929% purity.

Biological Tests

The following test guidelines were followed for the toxicity tests: fathead minnow, OECD Guideline 203 and EC Guideline L383A-C.1; daphnids, OECD Guideline 202 and EC Guideline L383A-C.2; green algae, U.S. EPA 40 CFR 797.1050 as amended in the Federal Register on 20 May 1987; earthworm, OECD Guideline 207. The methods used to test amphipods generally met the guideline

TABLE 1

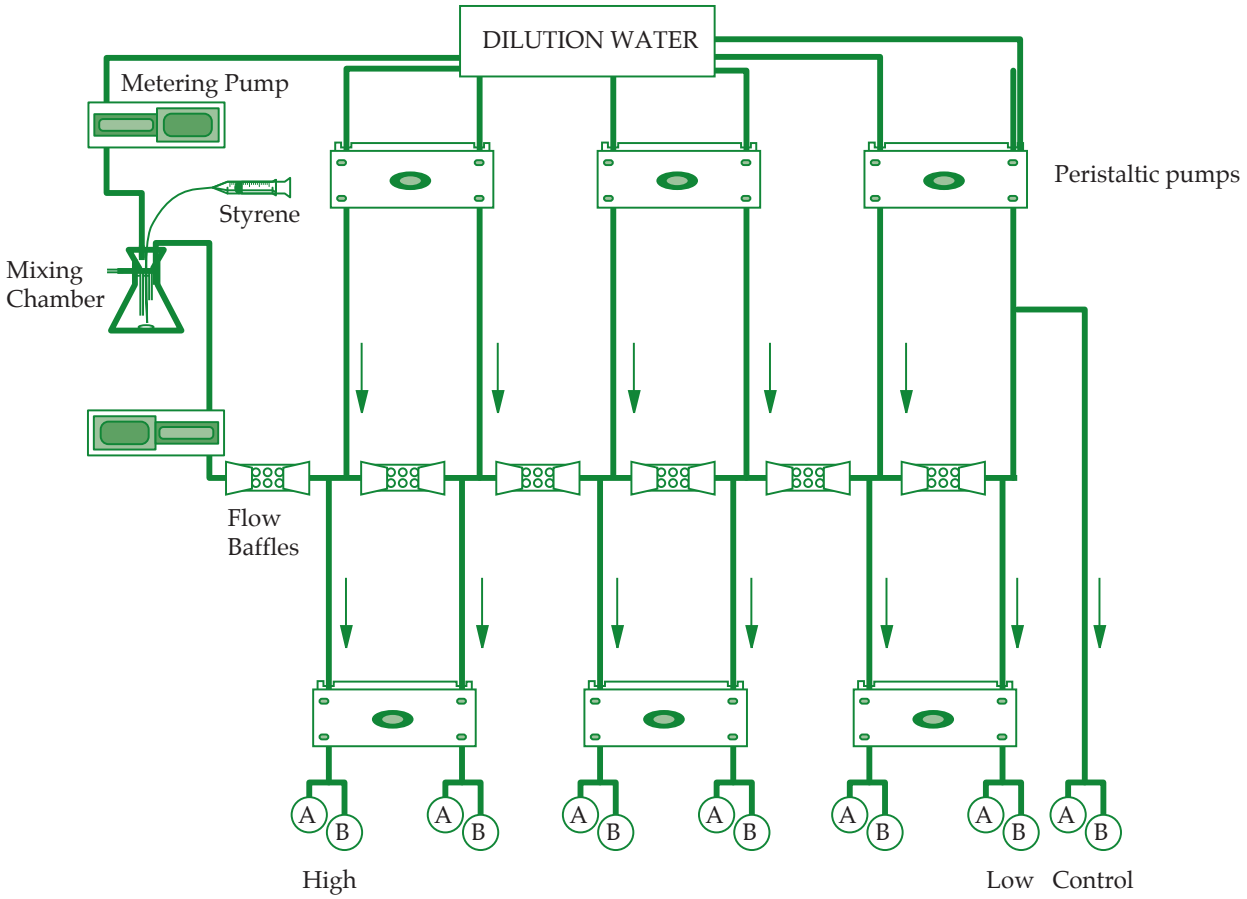
Culturing and Specific Conditions Used in Styrene Toxicity Tests

Species	Age/size	Temperature (°C)	Photoperiod	Hardness (mg/liter)[a]	Total alkalinity (mg/liter)	Specific conductance (μm hos /cm)	Dissolved oxygen (mg/liter)/ moisture (%)[b]	pH
Fathead minnows *(Pimephales promelas)*	Mean, 37 mm; range, 29–46 mm	22	16 hr light, 8 hr dark	35–36	21–23	140	7.4–9.3	6.9–7.2
Daphnids *(Daphnia magna)*	≤24 hr	20–21	16 hr light, 8 hr dark	170–180	110–120	500	5.8–8.4	7.5–8.0
Amphipods *(Hyalella azteca)*	7–14 days	22	16 hr light, 8 hr dark	36–40	20–22	140	6.1–8.4	7.0–7.6
Green algae *(Selenastrum capricornutum)*	3 days since previous transfer	24–25	24 hr light	—	—	500	—	Initial, 7.6; 96 hr, 8.5–9.4
Earthworms *(Eisenia foetida)*	0.31–0.40g	20	24 hr light	—	—	—	25–28	6.1–6.5

[a] Total hardness as $CaCO_3$.
[b] Dissolved oxygen is presented for flowthrough tests; moisture is presented for the earthworm test.

FIGURE 1

Diagram of the closed-system, continuous-flow, serial diluter used during the exposures of fathead minnow, daphnids, and amphipods to styrene.



for testing daphnids: U.S. EPA 40 CFR 797.1300. All tests were conducted according to TSCA Good Laboratory Practices (U.S. EPA, 1989). The test conditions and water quality parameters are presented in Table 1. Fathead minnows, daphnids, amphipods, algae, and earthworms were from culture facilities at Springborn Laboratories (Wareham, MA).

The fathead minnow, daphnid, and amphipod tests were conducted in a closed, flowthrough system designed to minimize volatilization of styrene from water. Styrene was stirred with dilution water in a primary mixing chamber placed in an ultrasonic water bath, and then additional dilution to provide a 60% dilution factor was accomplished by a continuous-flow serial diluter (Benoit *et al.*, 1982) equipped with closed-system modifications (Sousa *et al.*, 1995) (Fig. 1). Exposure vessels were held in a temperature-controlled water bath. Dissolved oxygen, temperature, and pH were measured daily in each replicate during the tests. Hardness, alkalinity, and conductivity were measured in one replicate at each concentration on Day 0. All treatment levels and control solutions were tested in duplicate with 10 organisms per vessel.

Algae were tested in closed Erlenmeyer flasks, 12 replicates per treatment level. Culture medium was algal assay procedure medium prepared with sterile deionized water with an additional 500 mg/liter of sodium bicarbonate added to provide a sufficient dissolved carbon

source for cell growth in a closed system. A 200-mg/liter stock solution of styrene was diluted to prepare the test solutions. The temperature was measured daily, and pH and conductivity of the media in the flasks were measured at initiation and at 96 hr. The light intensity was 300–400 footcandles. Each flask was completely filled to capacity (no headspace). The flasks were inoculated with approximately 1.0 x 10^4 cells/ml and placed on orbital shakers. Three flasks from each treatment level were opened daily for cell counts using a compound microscope and hemacytometer and then discarded. Two additional flasks at 30 mg/liter of styrene were used for analytical chemistry purposes only. To determine potential recovery of the algae subsequent to the 96-hr recovery, a composite sample from three replicates was removed from the highest test concentration which most severely inhibited algal growth. The algal subculture was inoculated into fresh culture medium containing no styrene. Subcultures were microscopically observed every other day for up to 9 days or until there was at least a 10-fold increase in cell density. If recovery was observed, the test material would be considered algistatic, where if no recovery was observed, the test material would be considered algicidal.

The earthworm test was conducted using 1-liter glass vessels covered with plastic wrap with small holes to allow for air exchange and secured with an elastic band. The vessels were positioned in a water bath to maintain appropriate test temperature. The artificial soil consisted of 70% industrial sand, 20% U.S. Pharmocopia kaolin colloidal powder, and 10% sphagnum peat moss. This soil preparation was similar to sandy loam. Soil moisture was measured on Days 0, 7, and 14. Styrene was mixed in a 1:2 soil:water slurry which was then added to artificial soil in a covered Hobart mixer and mixed for 10 min. Four replicates of 10 earthworms each were tested per treatment level and control. Burrowing time was recorded on Day 0 and mortality and observations (including lethargy and blisters) were recorded on Days 7 and 14. Group weights of the live earthworms were measured on Days 0 and 14. On Day 7, after observations were performed, the live earthworms were returned to freshly prepared soil medium containing the same target concentrations of styrene.

Chemical Measurements

Styrene concentrations were measured in both replicate test solutions of each treatment level and control at 0, 48, and 96 hr of exposure for the fathead minnow test, 0 and 48 hr for the daphnid test, 0 and 96 hr for the amphipod test, and 0, 24, and 96 hr for the algae tests. In the earthworm test, styrene concentration was measured in freshly mixed soil on Day 0, aged soil and freshly mixed soil on Day 7, and aged soil on Day 14.

Styrene concentrations were measured using a gas chromatograph with flame ionization detection. For the method validation study prior to the initiation of the definitive studies,recoveries from freshwater samples fortified with styrene with nominal concentrations of 0.05 to 100 mg/liter averaged 93.9 ± 4.85% with a limit of quantitation of 0.024 mg/liter. Recoveries from soil fortified with styrene at 10 to 2000 mg/kg averaged 84.2 ± 10.5% with a limit of quantitation of 1.22 mg/kg. Samples were extracted a single time with hexane, approximately 2 min for water samples and 1 hr for soil samples. Instrument conditions were as follows: column, RTX 624, 60 m x 0.53 mm i.d., 3.0- μm film thickness; gas flows: helium, 30 ml/min; hydrogen, 31 ml/min; air, 260 ml/min; temperatures: injector, 300°C; column, 50.0 to 140°C; ramp, 10.0 degrees per minute; detector, 350°C; injection volume: 4 μl; attenuation: 2°; threshold: 2; peak width: 0.070; retention time: styrene, 8.7 to 8.9 min.

Statistics

For the fathead minnows, daphnids, and amphipods, the median lethal concentration (LC_{50}), median effect concentration (EC_{50}), and 95% confidence limits were calculated from the mean measured concentrations and the corresponding mortality data using a computer program (Stephan, 1982). Based on the data set, the most appropriate statistical method (binomial interpolation, moving averages, or probit) for determining the LC_{50} was selected. The no-observed-effect concentration (NOEC) was the highest measured concentration of test substance tested at or below which there were no toxicant-related mortalities or physical or behavioral changes. For the algae test, the effect concentrations which reduced cell density of 10, 50, and 90%, respectively (EC_{10} ,EC_{50} , and EC_{90} values) were calculated from the mean measured concentrations and the corresponding cell density data. The EC values and their 95% confidence limits were determined by linear regression of response (percentage reduction of cell density compared with that of the control) versus mean measured concentration over the range of treatments where a clear concentration–response relationship was observed. Four linear regressions were estimated based on untransformed data, untransformed response versus logarithm-transformed concentration, probit-transformed response versus untransformed concentration, and probit-transformed response versus logarithm-transformed concentration. The regression that best fit

the data was selected based on the highest coefficient of determination (r^2). This regression was then applied to estimate the EC_{50} values and their 95% confidence limits, using the method of inverse prediction (Sokal and Rohlf, 1981). The highest mean measured concentration that caused no statistical adverse effect in cell density at 96-hours ($P \leq 0.05$), the NOEC, was determined using Williams' test (Williams, 1971, 1972) after checking the data for normality with the Shapiro– Wilks' test (Weber *et al*., 1989) and for homogeneity of variance using Bartlett's test (Horning and Weber, 1985).

For the earthworm test, the LC_{50} values were determined as indicated above (Stephan, 1982). Fisher's exact test ($P < 0.05$) was used to identify treatment levels with significantly reduced earthworm survival compared to control survival. Untrans-formed burrowing time data were first tested for normality using the Shapiro–Wilks' test and for homogeneity of variance using Bartlett's test. Burrowing times and percentage weight changes were compared using the Williams' test.

RESULTS

Fathead Minnow Test

In the fathead minnow test, the measured concentrations of styrene in water were 51 to 69% of nominal concentrations (Table 2). Styrene was moderately toxic to fathead minnows, with an LC_{50} of 10 mg/liter and a NOEC of 4 mg/liter at 96 hr (Table 3). One hundred percent mortality was observed at 24 hr for the highest concentration tested (31 mg/liter, mean measured concentration). Loss of equilibrium and erratic swimming behavior were observed in fish exposed to 7.6, 11, and 19 mg/liter.

Daphnid Test

Measured concentrations were 9.1 to 28% of nominal concentrations for the daphnid study (Table 2). Styrene was moderately toxic to daphnids, with an EC_{50} of 4.7 mg/liter and a NOEC of 1.9 mg/liter at 48 hr (Table 3). In the daphnids, 100% immobilization was observed at 7.4 and 14 mg/liter. At 3.3 mg/liter, some of the daphnids

TABLE 2

Styrene Concentrations Tested in Aquatic Toxicity Tests with Styrene

Fathead minnow		Daphnid		Amphipod		Green algae	
Nominal[a]	Measured[a,b]	Nominal	Measured	Nominal	Measured	Nominal	Measured
0	ND[c] (<0.11–<0.13)	0	ND(<0.22–<0.23)	0	ND(<0.21–<0.26)	0	<0.011[d]
3.9	2.0±0.4	3.9	0.39 ± 0.04	3.9	0.19 ± 0.08	0.073	0.023 e
6.5	4.0 ± 0.2	6.5	0.59 ± 0.25	5.4	1.0 ± 0.17	0.24	0.063 ± 0.0073
11	7.6 ± 0.6	11	1.9 ± 0.07	11	1.9 ± 0.21	0.81	0.28 ± 0.13
18	11 ± 0.6	18	3.3 ± 0.53	18	4.1 ± 0.17	2.7	0.68 ± 0.069
30	19 ± 0.6	30	7.4 ± 0.65	30	7.4 ± 0.64	9.0	2.0 ± 0.025
50	31 ± 0.8	50	14 ± 0.94	50	13 ± 0.67	30	7.0 ± 0.87
						100	28 ± 3.3

[a] Data presented in mg/liter.
[b] Mean concentration ± standard deviation, based on duplicate measurements at 0, 48, and 96 hr for the fathead minnow test, 0 and 48 hr for the daphnids, 0 and 96 hr for the amphipods, and 0, 24, and 96 hr for the algae.
[c] ND, not detected. Detection limit in parentheses.
[d] Measured concentrations were <0.011 mg/liter at 0 and 96 hr. At 24 hr, 0.076 mg/liter was measured; however, this finding is considered to be due to contamination during handling prior to analysis since levels below the detection limit were measured at 0 and 96 hr.
[e] Mean concentrations were 0.023 mg/liter at 0 and 24 hr and <0.011 mg/liter at 96 hr.

TABLE 3

Toxicity of Styrene in Fathead Minnows, Daphnids, Amphipods, Algae, and Earthworms

Species	LC or EC	Time interval						
		24 hr	48 hr	72 hr	96 hr	7 days	14 days	NOEC [a]
Fathead minnow *(Pimephales promelas)*	LC_{50} [b]	12 (11–14)c	12 (11–14)	11 (10–13)	10 (9–12)	—	—	4.0
Daphnid *(Daphnia magna)*	EC_{50} [b]	5.0 (3.3–7.4)	4.7 (3.3–7.4)	—	—	—	—	1.9
Amphipod *(Hyalella azteca)*	LC_{50} [b]	>13	>13	>13	9.5 (7.4–13)	—	—	4.1
Algae	EC_{10} [b]	0.92 (0.03–11)	0.05 (0.003–0.49)	0.42 (0.13–1.3)	0.13 (0.03–0.59)	—	—	
(Selanastrum	EC_{50} [b]	3.9 (0.22–66)	0.56 (0.05–6.0)	1.4 (0.46–4.3)	0.72 (0.15–3.2)	—	—	0.063
capricornutum)	EC_{90} [b]		16 (1.3–510)	6.7 (0.65–88)	4.9 (1.6–15)	3.9 (0.88–19)	—	—
Earthworm *(Eisenia foetida)*	LC_{50} [d]	—	—	—	—	160 (130–200)	120 (99–150)	44

[a] NOEC is the no-observed-effect level.
[b] Based on mean measured concentrations of styrene expressed in mg/liter of water.
[c] 95% confidence limits.
[d] Based on mean measured concentrations of styrene expressed in mg/kg dry weight of soil.

were lethargic and on the bottom of the test vessel.

Amphipod Test

Measured concentrations were 4.8 to 26% of nominal concentrations for the amphipod study (Table 2). In amphipods, the EC_{50} at 96 hr was 9.5 mg/liter and the NOEC was 4.1 mg/liter, indicating that styrene was moderately toxic (Table 3). At 96 hr, 85% mortality was observed at the highest concentration tested (13 mg/liter). There were no sublethal effects observed among surviving amphipods.

Algae Test

For the algae test, analysis of the 200-mg/liter stock solution used to prepare the test solutions found a measured concentration of 71 mg/liter (36% of the nominal level) despite measures designed to minimize losses (Table 2). In the test solutions, measured concentrations were 22 to 31% of nominal levels. Analyses of the 30-mg/liter test solution not inoculated with algae and those inoculated with algae were similar (mean of 6.6 mg/liter versus mean of 7.0 mg/liter), indicating that the presence of algae did not have a major impact on measuring styrene concentrations in the test. Styrene was highly toxic to Selenastrum, with an EC 50 based on cell density at 96 hr of 0.72 mg/liter and NOEC of 0.063 mg/liter (Table 3). At test termination, cell fragments and bloated cells were observed in the 0.68-, 2.0-, and 7.0-mg/liter groups while only cell fragments were observed at the highest treatment level, 28 mg/liter. Cells exposed to the remaining treatment levels were observed to be normal. Since algal growth was severely inhibited at the measured concentration of 7.0 mg/liter, an aliquot was removed from this treatment level to determine the potential algicidal or algistatic effects of styrene. The three remaining replicates were combined and diluted with fresh algal medium to yield a nominal concentration of 0.24 mg/liter which was equivalent to the highest nominal concentration at which no growth inhibition was observed during the 96-hr exposure. After 4 days, the observed growth indicated that styrene had an algistatic, rather than an algicidal, effect at the 7.0-mg/liter level.

TABLE 4

Styrene Concentrations, Burrowing Times and Weight Changes in the Earthworm Toxicity Test

Nominal concentration (mg/kg)Measured concentration (mg/kg)a Mean measured concentration (mg/kg)b		Mean burrowing time (min ± SD)c		Mean weight change (% ± SD)d			
Day 0 (fresh)	Day 7 (aged)	Day 7 (fresh)	Day 14 (aged)				
0	<1.5	<1.5	<1.6	<2.0	—	9.3 ± 0.96	–11 ± 6.3
125	17	2.4	29	<1.5	23	7.8 ± 2.2	–10 ± 4.7
250	34	14	55	<1.8	44	11 ± 2.8	–16 ± 8.3
500	48	34	82	<4.2	65	13 ± 4.6*	–32 ± 13*
1000	200	65	150	<6.6	180	— [e]	–7.4 ± 56
2000	600	280	410	<22	500	— [e]	NA [f]

[a] Total amount of styrene (mg) measured per kilogram (dry weight) of soil.
[b] Mean of the measured concentrations in freshly prepared mixtures of soil on Day 0 and Day 7.
[c] Time required for all 10 earthworms to completely burrow into the soil at the study initiation (Day 0). d ((Mean weight, Day 14 – mean weight, Day 0)/(mean weight, Day 0)) × 100.
[e] Earthworms exhibited avoidance behavior and were covered with soil after approximately 1 hr to avoid dehydration.
[f] Not applicable to 100% mortality of group.
* Statistically different from control values based on Williams' test; $P \leq 0.05$.

Earthworm Test

In the earthworm study, the measured concentrations of freshly prepared soil were 13 to 25% of nominal concentrations (Table 4). Toxicity of styrene to earthworms is summarized in Tables 3 and 4. Styrene was slightly toxic to earthworms, with an LC_{50} value at 14 days of 120 mg/kg; this value is slightly lower than the LC_{50} at 7 days (160 mg/kg) when the earthworms were placed into freshly mixed soil. The NOEC was 44 mg/kg. Of the earthworms exposed to ≥65 mg/kg that died, 50 to 89% of those that died in each group were missing and had presumably decomposed, which suggests that the earthworms had died shortly after test initiation. One earthworm exposed to 180 ppm had white blisters. When placed on treated soil at 180 and 500 mg/kg, earthworms did not burrow and were covered with exposure medium after approximately 1 hr to avoid dehydration. The earthworms in the 500-mg/kg treatment level were observed to have a swollen clitellum (three to four times normal size) prior to being covered with exposure medium. There was a statistically significant increase in burrowing time and mean weight change at the 65-mg/kg treatment level.

DISCUSSION

The measured concentrations of styrene were generally consistent between replicates and sampling intervals. The fathead minnow, daphnid, and amphipod studies were all conducted in the same flowthrough system. Modifications were made to flow rates and test vessels in the dilution system used in the fathead minnow test in order to accommodate the daphnia and amphipod tests. Presumably, these changes affected the extent to which styrene concentrations were maintained in the initial mixing chamber and flowthrough system. Measured concentrations of styrene in water were 51 to 69% of nominal for the fathead minnow test whereas measured concentrations for the daphnid and amphipod tests ranged from 4.8 to 28% of nominal. The variable recoveries, measured versus nominal, reinforce the need to use measured concentrations as opposed to nominal when conducting studies of dilute aqueous concentrations of sparingly soluble, volatile, reactive, biodegradable, and/or adsorbable chemicals. Analysis of test concentrations ensures that accurate and reproducible treatment–response data will be obtained in the test. In the earthworm study, the difference between the measured concentrations of styrene for the freshly prepared soil (13 to 25%) and the nominal

levels was likely primarily due to the loss of the test material due to volatilization during the mixing and handling process, but adsorption, biodegradation, and polymerization may have played lesser roles. Similarly, volatility most likely contributed significantly to the difference in concentrations between the freshly prepared soil and aged soil (7 days old), since the exposure vessels were only partially covered.

Styrene was moderately toxic to fathead minnows, daphnids, and amphipods, with LC_{50} ,EC_{50} , and NOEC values between 1 and 10 mg/liter. The acute LC_{50} results for the fathead minnow and daphnid tests were lower than values reported previously for other studies of these species tested under static conditions without analytical confirmation of styrene concentrations, i.e., 29–32 (Mattson *et al.*, 1976) and 46.4–59.3 mg/liter (Pickering and Henderson, 1966) for fathead minnows and 23 (LeBlanc, 1980) and 59 mg/liter (Qureshi *et al.*, 1982) for daphnids. A recent static-renewal study of rainbow trout (*Oncorhynchus mykiss*) in which styrene concentrations were analyzed found a 96-hr LC_{50} value of 4.12 mg/liter (Exxon, 1993). Styrene was slightly less toxic to amphipods than to daphnids. Lindstrom and Lindstrom (1980) reported that amphipods (*Pontoporeia affinis*) which were initially immobilized by styrene at 23 to 46 mg/liter in a system which permitted rapid volatilization began to swim again when the concentration was 4.4 mg/liter. The test findings indicate that mortality in aquatic vertebrate and invertebrate species occurs early with exposure to styrene, based on a comparison of first day mortality with final test day mortality. There is no pattern of cumulative toxicity in any of these studies. These findings demonstrate that acute tests are satisfactory for assessing styrene's toxicity in these environments, considering the transitory exposure to styrene expected in aqueous and benthic environments as a result of its volatility and biodegradability (Fu and Alexander, 1992; Fu *et al.*, 1994; Alexander, 1997). Hence, no long-term or chronic toxicity studies should be required for the hazard assessment of styrene in these environments.

In the static closed system used in this test, styrene was highly toxic to *Selenastrum*, with an EC_{50} based on cell density at 96 hr of 0.72 mg/liter and NOEC of 0.063 mg/liter. In previous studies using open vessels, Bringmann and Kuhn (1980) reported that growth of *Scenedusmus quadricauda* was not inhibited at a nominal concentration of 256 mg/liter of styrene and Munjko and Grbic (1977) reported no toxicity to 14 species of algae at a nominal concentration of 500 mg/liter. Presumably, due to volatilization, the actual concentrations of styrene were considerably less than the reported nominal values.

The 96-hr algae test can be considered to be a chronic test because the test measures sublethal treatment-related growth effects instead of mortality or immobilization as in the acute fish and invertebrate tests. Therefore, the 96-hr NOEC can also be designated the chronic NOEC (Nabholz, 1991). The estimated chronic NOECs for fathead minnows, daphnids, and amphipods derived by multiplying acute NOECs by a factor of 0.1 and the algae 96-hr NOEC are greater than styrene concentrations reported for rivers, streams, and drinking water supplies in the U.S., Canada, and Europe (Alexander, 1990, 1997; ATSDR, 1992; Environment Canada, 1993; HSE, 1996). Although styrene has been measured in industrial facility effluents, effluent levels also have been below the algae NOEC with few exceptions. Because effluents discharged into receiving waters are rapidly diluted and volatilization will also occur, it is expected that there are adequate margins of safety for realistic exposures to styrene and no adverse effects are expected to algae or other aquatic species in receiving waters. Styrene was slightly toxic to earthworms. The LC 50 value at 14 days (120 mg/kg) was slightly lower than the LC 50 at 7 days (160 mg/kg), most likely due to the additional exposure when the earthworms were placed into freshly mixed soil at 7 days. The burrowing avoidance behavior and blister observed on one earthworm appear to be consistent with chemical irritancy. Styrene, like many other monoaromatic hydrocarbons, is an irritant to biological membranes (NIOSH, 1990). The fact that the 7-day LC_{50} was less than twice the 14-day LC_{50} illustrates the fact that most mortality occurred early in the study. Hence, toxicity appeared not to be cumulative.

Currently, Canada and The Netherlands have published soil criteria for styrene. Under the Dutch Soil Cleanup (Interim) Act, the following threshold levels for styrene have been set for background, moderate soil contamination, and immediate cleanup: 0.05, 0.5, and 50 ppm, respectively (Beyer, 1990). The Interim Canadian Environmental Quality Criteria for Contaminated Sites have set the following limits for styrene for agricultural, residential/parkland, and commercial/industrial uses: 0.1, 5.0, and 50 mg/kg, respectively (CCME, 1991). Significant findings of increased mortality and burrowing time, and reduced body weight at ≥65 mg/kg but not 44 mg/kg in the 14-day subacute earthworm study, appear to support the Dutch immediate cleanup criteria level of 50 mg/kg for the protection of soil living organisms in sandy loam soil. Styrene's bioavailability and its mobility are influenced by its sorption partition coeffi-

cient (K_{OC} of log 2.43–2.73 [Howard, 1989]) and the type of soil that is exposed to the chemical. Fu and Alexander (1992) and Fu *et al.* (1994) found that soils with greater proportions of organic matter and clay adsorb more styrene than soils like the sandy loam used in the study which contain less of these soil constituents. Hence, styrene's bioavailability and toxicity to earthworms as well as to other soil-dwelling organisms may be greatly mitigated by the type of soil exposed which may make the Dutch immediate cleanup level overly protective for some soil types. Two parameters are needed for a scientifically sound ecological hazard assessment of styrene: exposure and toxicity. Environmental exposure will greatly depend on the specific situation under which the chemical release occurs, whereas toxicity is considered to be an inherent chemical property of styrene as it is with other chemicals. In this evaluation of the ecotoxicity of styrene, the use the inherent toxicity values is based on concentrations of styrene measured under exposure conditions that minimize loss due to volatility. As such, these values will not underpredict styrene's toxicity as earlier studies may have done. However, these results can overpredict styrene's toxicity in any ecological hazard assessments where factors that govern styrene's environmental fate [volatility (Howard, 1989), photooxidation (Howard *et al.*, 1991)] and biodegradability (Fu and Alexander, 1992; Fu *et al.*, 1994) potential have not been accounted for. Nevertheless, these toxicity values should form the basis for the initial hazard assessment for possible exposure scenarios, as well as for product registration and labeling that establish the chemical's toxicity relative to other chemicals.

CONCLUSION

Using a sealed, flowthrough exposure system, styrene was moderately toxic to fish, daphnids, and amphipods. There was no indication of a concern for chronic toxicity based on a lack of cumulative toxicity. Styrene was highly toxic to green algae based on effects on cell density, but the effects were algistatic rather than algicidal. Styrene was slightly toxic to earthworms. It is expected that styrene's availability to earthworms would be significantly influenced by soil type. 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, except if styrene is spilled directly into a low-energy water body or onto soil. Styrene's volatility and biodegradability would significantly limit its environmental persistance and mitigate styrene's potential to produce adverse environmental impacts.

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Review of Styrene as a Potential Endocrine Disruptor

by the Illinois Environmental Protection Agency

INTRODUCTION

John O. Snyder, Jeffrey S. Terry

ISSUE BACKGROUND

In response to the increased interest in chemicals that possibly could affect the endocrine (hormone) systems of humans and wildlife, the Illinois Environmental Protection Agency (IEPA) developed one of the first documents by a regulatory agency focusing on the issue of endocrine disruptors. Issued in February 1997, their draft *Endocrine Disruptor Strategy* (EDS) was the agency's first step in identifying potential endocrine disrupting chemicals.

IEPA performed a literature search and review of existing data, producing a list of 74 chemicals that were identified as "known," "probable," or "suspect" endocrine disruptors. Chemicals for which strong evidence of endocrine-disrupting effects in intact animals was considered to exist were placed in the "known" category. A chemical was listed in the "probable" category based on a preponderance of evidence (in both intact animals and in bioassays) suggesting that the chemical can disrupt the endocrine system. Finally, chemicals lacking good evidence in intact animals or for which only assay evidence exists were placed in the "suspect" category.

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The Illinois Environmental Protection Agency developed one of the first documents by a regulatory agency focusing on the issue of endocrine disruptors. SIRC was one of the initial industry groups to ask the agency to more closely examine the data on a specific chemical. That review prompted a scientific forum that examined not only the styrene data, but the issue of endocrine disruption in general.

Styrene monomer was one of 30 of the 74 chemicals listed as a "probable" endocrine disruptor.

IEPA clearly defined the fact that the EDS document was truly a strategy, and was only a preliminary assessment. Recognizing that additional information could contribute to the understanding of the endocrine disruptor issue, the agency encouraged input from other agencies, industry, and interested parties.

REVIEW PROCESS

This was the first listing of styrene monomer as a potential endocrine disruptor by a regulatory agency. Prompted by concerns about styrene's listing as a "probable" endocrine disruptor when the data did not support such a conclusion, the Styrene Information and Research Center (SIRC) was an early respondent to the EDS. SIRC met with and updated IEPA staff on the body of scientific data on styrene, and provided recent reviews of the data by well-recognized experts in the field of reproductive and developmental effects. In discussions with IEPA staff SIRC scientists relayed their conclusion that, based on the available data, a "probable" endocrine disruptor listing was not warranted for styrene, and that the data did not indicate a concern for styrene as an endocrine disruptor. From these discussions with IEPA, it was agreed that the agency and SIRC would work together on a thorough review of the styrene data to

better understand its potential as an endocrine disruptor.

SIRC subsequently helped IEPA develop a process to review styrene's existing scientific database to determine whether or not, based on existing data, styrene could have the potential to adversely affect the hormone system of humans or animals. An initial step of the review was to agree on a set of parameters that would define evidence of "adverse" health effects in the current scientific data. At the time, the U.S. Environmental Protection Agency's (USEPA) Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) had been characterizing an endocrine disruptor as a substance that may adversely affect the normal function of the androgen, anti-androgen, estrogen, anti-estrogen, or thyroid body mechanisms. IEPA and SIRC agreed to evaluate the styrene data for potential endocrine disrupting effects using those EDSTAC parameters.

Both IEPA and SIRC also recognized the benefit of receiving impartial third-party input into the evaluation process. The concept of a scientific issues forum was endorsed by IEPA and SIRC, and three experts were identified and contacted regarding their willingness to participate: Dr. Nigel Brown, University of London; Dr. Tim Zacharewski, Michigan State University; and Dr. Christopher Borgert, Applied Pharmacology and Toxicology, Inc.

On October 24, 1997, a Scientific Issues Forum was held in Chicago, Illinois, with participants including representatives from IEPA and SIRC, as well as the three panelists. IEPA also invited representatives from other regulatory agencies, environmental and industry groups, and academics.

Given the fact that this was the first such public forum following publication of the EDS, in addition to a styrene weight-of-evidence review by Dr. Brown, Dr. Zacharewski led a broader discussion of recent developments in the endocrine disruptor issue. The agenda also included an update on the progress of EDSTAC by Dr. Borgert, an EDSTAC member. Dr. Borgert reported on EDSTAC's development of recommendations to the USEPA on how to prioritize chemicals to determine potential endocrine disruptors.

REPORTING

Following the October 24 forum, IEPA produced a meeting summary entitled "Proceedings Report for Scientific Issues Forum on Endocrine Disruptors," which provides full details of the styrene discussion, as well as the discussions on the broader endocrine issue and EDSTAC. The Proceedings Report follows this Introduction, as does the agency's two-page summary of the styrene review and conclusions. Other attachments referenced in the Proceedings Report, such as the forum agenda, participant list, and copies of correspondence from the panelists have not been included, but are available from either SIRC or IEPA.

IEPA's updated conclusion on styrene states that *"styrene is of low concern as a potential endocrine disruptor."*

CONCLUSIONS

SIRC's primary goal in promoting the styrene data review was to help IEPA and the regulatory and scientific communities better understand that, based on the currently accepted definition of an endocrine disruptor, the existing scientific evidence does not support styrene's consideration as a "probable" endocrine disruptor. Based on the panelists' comments, IEPA's updated conclusion on styrene states that *"styrene is of low concern as a potential endocrine disruptor."* The Agency stated that its weight-of-evidence evaluation for styrene is based on IEPA's current understanding of mechanisms and the existing database. IEPA also noted that there are data gaps in the research on styrene, and that the endocrine disruptor issue as a whole is still developing in the policy arena.

IEPA added that while it currently has no plans to update or reissue the EDS:

> *"…should revisions be undertaken in the future IEPA would no longer consider styrene as representative of a 'probable' endocrine disruptor, and would not anticipate including it as a chemical of any significant priority in future versions of the EDS. Unless additional evidence to the contrary emerges, the Agency will focus its continuing activities regarding endocrine disruptors on priority chemicals."*

The Proceedings Report serves as a way to record and communicate this conclusion on styrene monomer, as well as IEPA's progress in updating its understanding of the chemicals identified in the EDS.

The review process itself, as developed by SIRC and IEPA, has been recognized as a template for a thoughtful, cooperative way in which government and industry can

advance the scientific understanding of the endocrine disruptor issue. Other groups have requested chemical-specific reviews of endocrine disruptor data. As a result of the styrene review, IEPA has an appropriate and effective way to more thoroughly consider the best existing scientific data on the endocrine disrupting potential of chemicals identified in its EDS; at least until the USEPA process is developed further.

The styrene monomer data review, scientific forum, and subsequent Proceedings Report by IEPA produced the most current assessment of the chemical's existing database relative to the endocrine disruptor issue and have helped clarify specific scientific questions about styrene's potential to affect the endocrine system.

PROCEEDINGS REPORT:

For Scientific Issues Forum On Endocrine Disruptors

Prepared by Illinois EPA (April 2, 1998)

The Illinois Environmental Protection Agency (IEPA or Agency) and the Styrene Information and Research Center (SIRC) co-sponsored a Scientific and Technical Issues Forum regarding IEPA's Endocrine Disruptor Strategy on October 24, 1997 at the James R. Thompson Center in Chicago, Illinois. The purposes of this Forum were to evaluate certain basic science issues regarding endocrine disruption in general, to evaluate the endocrine-disrupting potential of Styrene in particular, to investigate whether activities and work products of the national Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) could be utilized by or incorporated into the Illinois Endocrine Disruptor Strategy, and to become familiar with endocrine disruption testing methodologies under consideration by the EDSTAC. Invited presenters included Dr. Nigel Brown, University of London, and an expert in Styrene toxicity, Christopher Borgert, Ph.D., Applied Pharmacology and Toxicology, Inc., and a member of the EDSTAC, and Tim Zacharewski, Ph.D., Michigan State University, also a member of the EDSTAC and an expert in endocrine disruptor testing. Guests representative of various interest groups and governmental agencies were also invited (Attachment 1) *[not included]*. This report is a summary of the IEPA/SIRC Forum. A copy of the agenda is included as Attachment 2 *[not included]*.

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BACKGROUND

In February 1997, the IEPA issued a document entitled Endocrine Disruptor Strategy (EDS). The EDS was produced in response to a growing concern that chemical compounds in the Illinois environment could be capable of disrupting the endocrine (hormonal) system, potentially affecting the normal development, growth, and reproduction of humans and wildlife.

The EDS was developed as a first step in identifying chemicals that might have the potential to impact the endocrine system. The Agency performed an initial literature search and review of existing data, resulting in a list of 74 chemicals which were identified as either "known," "probable," or "suspect" endocrine disruptors. The preface to the EDS noted the preliminary nature of the document, and proposed working with other state and federal agencies, as well as with industry, to obtain more precise data on potential endocrine-disrupting chemicals. IEPA encouraged interested parties to review and comment on the EDS.

Among the first of several groups to respond to IEPA was the Styrene Information and Research Center, an industry-sponsored non-profit organization that has spent ten years developing the body of science on the potential health effects of the chemical Styrene. In initial discussions and meetings with SIRC, Styrene industry scientists expressed their interpretation that the available data do not support IEPA's preliminary listing of Styrene as a "probable" endocrine disruptor. As a result of these discussions, IEPA agreed to work with SIRC representa-

tives to more thoroughly evaluate the data on Styrene.

In the process of reviewing the Styrene data, the Agency believes a productive protocol has been identified that can be used to address future chemical-specific reviews under the EDS. Currently, IEPA has no plans to revise or reissue the EDS, or immediate plans for regulatory initiatives related to this issue. However, in acknowledgement of the Agency's offer to work with other agencies and industry as noted in the preface to the EDS, IEPA will, if warranted, undertake chemical-specific reviews of data as a way to better understand the potential for chemicals tentatively identified in the EDS to act as endocrine disruptors. The Scientific Issues Forum that is the subject of this Proceedings Report is the culmination of the review process for Styrene.

INTRODUCTIONS AND OPENING REMARKS

Roger Kanerva, IEPA's Environmental Policy Advisor, welcomed the participants, offered some introductory remarks, and presented an overview of the agenda to be covered (as described above). He further remarked that the Agency would be seeking input on the various basic science issues regarding endocrine disruption, that the process the Agency had been developing with SIRC to further evaluate the endocrine-disrupting potential of Styrene would be the prototype of the procedure for the entities who might request review of other chemicals included in the EDS list, and that the discussions and outreach efforts engaged in by the Agency with both the SIRC and the Chemical Industry Council of Illinois have been undertaken in furtherance of the objective contained in the EDS of initiating and maintaining both policy and technical dialogues with interested publics. Mr. Kanerva then initiated the discussion of basic science issues.

DESCRIPTION OF BASIC SCIENCE ISSUES

Thomas C. Hornshaw, Ph.D., Manager of the Toxicity Assessment Unit of IEPA's Office of Chemical Safety, noted that certain basic science issues regarding endocrine disruption had emerged either during the development of the EDS or in discussions with various interested parties following publication of the Strategy. He further noted that these issues would need to be addressed by the Agency and/or interested parties as the Agency continued to implement actions identified in the Strategy, and also as the Agency continued to refine the list of chemicals addressed by the Strategy. The issues identified by Dr. Hornshaw included:

- Can adverse effects be agreed upon which can be presumed to be attributable to endocrine disruption? (For example, increased number of resorptions or decreased sperm counts in reproduction tests, altered sex hormone ratios, changes in mammary development or milk secretion.)
- Can indicator effects be agreed upon which can suggest the potential for endocrine disruption to cause adverse effects? (For example, androgen and estrogen receptor binding, hormone-responsive cell proliferation *in vitro*, altered sex hormone ratios, induction of heat shock proteins.)
- Can criteria be developed to distinguish between adverse effects attributable to direct toxicity to an endocrine organ verses endocrine-mediated adverse effects, or is it not necessary to differentiate between these two types of effects (i.e., is direct toxicity to an endocrine organ by definition endocrine disruption)?
- Can criteria be developed to distinguish between maternal toxicity verses endocrine-mediated adverse effects on the offspring (i.e., is maternal toxicity sufficient reason to rule out effects on offspring as true endocrine disruption effects)?
- Can a weight-of-evidence procedure be developed to assess the quality and quantity of data available regarding the endocrine-disrupting capability of a chemical?
- Can a ranking procedure be developed to characterize the hazard to human and environmental health due to various endocrine disruption endpoints?

Dr. Hornshaw then inquired of the panel members whether they were aware of literature or guidance relevant to these issues. While there was general agreement that these were key issues to be addressed, it was noted that these were also issues under discussion by the EDSTAC, and that it might be useful to continue to monitor the progress of any documents generated by the EDSTAC for help with these issues. There being no further discussion or questions, Dr. Hornshaw concluded his portion of the Forum.

STYRENE STUDIES AND DATA

Dr. Brown began the discussion of the evidence for and against the endocrine-disrupting potential of Styrene by noting that there is not a uniform definition of endocrine disruption agreed upon by the EDSTAC or other bodies at this time. He remarked that USEPA's intent for the screening and testing program being developed by the EDSTAC includes determination of whether a chemical can interfere with estrogen, androgen, and thyroid hormone signals, at a minimum. Therefore, if one can rule out estrogenic and anti-estrogenic, androgenic and anti-

androgenic, and thyroid and anti-thyroid effects for a chemical, then there is very little chance that that chemical is an endocrine disrupting chemical. Dr. Brown then proceeded to evaluate each of these endpoints. He also attempted to address the evidence presented by the Agency for endocrine disrupting effects of Styrene in its supplemental table to the EDS where possible (included as Attachment 3) [*not included*].

1 Estrogenic and Anti-estrogenic Effects - Dr. Brown began the discussion of estrogenic and anti-estrogenic effects by stating that the "gold standard" test of such effects is the uterine weight assay in immature/ovariectomized rodents, and that there are no such data available for Styrene (there are data which show that the uterine weight of adult rodents is not affected by Styrene). He then cited animal studies in which Styrene has been shown to have caused no adverse effects for several estrogen-related endpoints, including:

- male food consumption and growth rate in reproduction/developmental toxicity tests (which he believes is one of the most sensitive endpoints for endocrine disruption);
- female sex behavior;
- uterine histology and biochemistry;
- pregnancy loss;
- male mating behavior;
- luteinizing hormone (LH) levels in males;
- sex organ development;
- sexual differentiation; and
- pubertal development/vaginal opening.

Dr. Brown then listed three estrogen-related endpoints for which there were questionable results for Styrene:

- estrous cycle changes and spontaneous abortions - several reports from former Soviet Union countries claim estrous cycle irregularities and/or increased frequency of spontaneous abortions in workers exposed to Styrene (and other chemicals), however, these studies are poorly reported and are not verifiable, and they are contradicted by well conducted studies in Scandinavia and the United States.
- pituitary gland weight/function - certain studies in animals show that pituitary gland weight and function are affected in newborns exposed to Styrene, however, this effect appears to be secondary to the known neurotoxicity of Styrene; and
- prolactin release - diminished prolactin release has been shown in rats exposed to Styrene, and has been shown to be diminished or unaffected in two human studies, however, these studies do not show concurrent increase of LH (as would be expected with estrogen exposure), and it is therefore likely that this effect is also related to pituitary gland effects which are secondary to neurotoxicity.

Finally, Dr. Brown discussed one study in which an adverse effect had been shown on spermatogenesis in rats with exposure to Styrene. This study reported decreased sperm production with high doses of Styrene, but the significance of this effect is unknown since there was no effect on the fertility of the rats.

Dr. Brown's conclusion, based on the weight of the evidence, was that Styrene does not cause estrogenic or anti-estrogenic effects, since 9 of 13 estrogen-related endpoints for which there are data are negative, 3 are questionable, and 1 is positive but of unknown significance. He then added a note of caution that none of the cited studies had been specifically designed to test for estrogenicity or anti-estrogenicity.

2. Androgenic and Anti-androgenic Effects - Similar to the discussion of estrogenic and anti-estrogenic effects, Dr, Brown began the discussion of androgenic and anti-androgenic effects by describing the "gold standard" test for such effects, the Hershberger assay, in which the weights of male accessory sex glands are determined in immature male rodents following exposure to the test substance. As was the case for estrogenic and anti-estrogenic effects, there are no data for Styrene for this assay, although male accessory sex glands have not been shown to be affected by Styrene exposure in adult animals. Dr. Brown then explained that there are four other tests which measure androgenic and anti-androgenic effects, and noted that Styrene has tested negative in three of them:

- serum hormone levels (rodents and humans);
- *ex vivo* hormone synthesis; and
- reproductive tract defects (rodents and humans).

Regarding the last test, pubertal development, Dr. Brown stated that there are no data on anal-genital distance in rodents, but that pubertal development has been indirectly measured by noting sexual function in animals in standard toxicology testing.

Dr. Brown's conclusion regarding the potential for Styrene to cause androgenic and anti-androgenic effects is that this chemical probably causes neither effect based on the weight of the evidence. He did caution that the data are limited, however, as with the estrogenic and anti-estrogenic effects, again noted that none of the studies has been designed to test specifically for androgenic or anti-androgen effects.

3 Thyroid, Anti-thyroid, and Other Effects - Dr. Brown stated that there are no agreed screening endpoints at this time for effects on thyroid gland, adrenal gland, or

hypothalamic-pituitary axis function. Regarding thyroid and anti-thyroid effects, Dr. Brown noted that there are no data at all for Styrene. There is one Russian study reporting a questionable change in *ex vivo* adrenal metabolism, in which an effect was seen at low doses but not at higher doses, and which has not been replicated. Dr. Brown mentioned the data on effects of prolactin release (as noted in the discussion of estrogenic and anti-estrogenic effects) as being probably a marker of neurotoxic, rather than endocrine, effects on the hypothalamic-pituitary axis.

4 Overall Conclusions - Dr. Brown's opinion, based on the weight of the evidence, was that Styrene is not estrogenic or anti-estrogenic, probably not androgenic or anti-androgenic, lacks data for thyroid and anti-thyroid effects, and that the positive reproductive/developmental toxicity data, reduced litter size, is not reliable as an indicator and probably a result of maternal toxicity, and this result has been countered by three-generation reproduction studies which show no effects from Styrene exposure. Given this overall evidence, his opinion was that Styrene is not an endocrine disruptor.

Dr. Brown then responded to several questions/comments regarding Styrene from Dr. Hornshaw:

- Were the effects on adrenal metabolism at low doses but not at higher doses an example of an "inverted U" dose-response curve? Dr. Brown responded that it is difficult to say since the study has not been replicated.
- A particular concern of the Agency is the modification of the estrous cycle in occupationally exposed women, especially since a potential mechanism, altered prolactin production, has been demonstrated also. Dr. Brown agreed that this effect should be of concern, but noted that the one Russian study which reported this effect lacked detail and specificity. He further mentioned that estrous cycle modulation is subject to many influences, making it difficult to draw conclusions based on this effect.
- How difficult and expensive would it be to conduct a study of estrous cyclicity in laboratory animals, since this endpoint hasn't been evaluated in animals? Dr. Brown responded that this would not be that difficult or expensive, and might be worthwhile to clarify the issue.

There being no other questions, the discussion of the EDSTAC progress began.

UPDATE ON EDSTAC

Dr. Borgert began the discussion of the EDSTAC by commenting that the Committee had made much progress in the development of the battery of screening assays in the past couple of months. Since USEPA had decided that estrogenic, androgenic, and thyroid effects should be evaluated, these three hormones were the focus of the screening battery. The previously described "gold standard" assays (the uterine weight and Hershberger tests with immature rodents) were high on the Committee's list of candidates for the screening battery. Also under consideration were a variety of short-term *in vitro* assays for hormone receptor binding, receptor activation, and cellular growth in hormonally responsive cells, and possibly some *in vivo* assays of hormonal function.

Since USEPA had decided that estrogenic, androgenic, and thyroid effects should be evaluated, these three hormones were the focus of the screening battery.

Regarding the battery of testing assays, Dr. Borgert explained that the Committee felt that the standard two-generation reproduction test already used in chemical-testing protocols should be included since this assay examines 26 estrogenic, 8 androgenic, and thyroid endpoints already. It is possible that this assay could be amended to include more sensitive endpoints, and this would not require large changes to the current protocol. Dr. Borgert also noted that the Committee may also recommend that short-term tests examining mechanisms of activity be added to the testing battery.

Dr. Borgert then reported on several other issues being deliberated by the Committee. There has been much work done on setting priorities for the list of up to 70,000 chemicals to be screened, although the Committee has not decided on a recommendation yet. Responding to a question from Dr. Hornshaw about whether the Committee has developed a procedure for determining which high priority chemicals might go directly to the testing phase, Dr. Borgert said he wasn't sure how such discussions were leaning, but that the decision would not be based solely on effects data; other considerations might include production volume, fate and transport data, exposure potential, and likely other criteria. Dr.

Borgert then discussed two issues which had not been resolved by the Committee yet. Regarding positive and negative screening level results, it has not been decided how many positive assays would send a chemical into the testing battery, since some tests have more than one endpoint and some endpoints are redundant or not totally independent. Dr. Borgert thought, however, that there would not be criteria to determine what is *not* an endocrine disruptor, only to relegate a chemical to low priority or to a "hold box" for further action. Part of the problem in deciding how to interpret positive and negative assay results has been how to balance the issue of false positive verses false negative probabilities. Regarding the other undecided issue, how to determine the full weight of evidence on whether a chemical is an endocrine disruptor, Dr. Borgert stated that *in vivo* data will be rated higher then *in vivo* data. Again, a big part of the problem is how to weight the probability of false positive and false negative results; for example, if seven screening assays are all negative and each has been conducted with a probability of false positive results of 5%, then is the overall rate of false positive of 0.54% an acceptable result? Finally, in response to a question from Dr. Hornshaw on the use of existing test data in the screening and testing process, Dr. Borgert responded that this issue will be considered in the priority-setting process, and that not a lot of thought had gone into deciding how to sort out "the good, the bad, and the ugly" among existing study results, other than that any study used would have to have been appropriately conducted. Since there were no further questions, the discussion of testing methodologies then began.

TEST METHODS FOR ENDOCRINE DISRUPTORS

Dr. Zacharewski opened his discussion of endocrine disruptor testing methodologies by noting that four scientific meetings devoted to identifying, prioritizing, and assessing endocrine disruption have been held over the past two years: at Kiawah, South Carolina, regarding animal assays; at Duluth, Minnesota, regarding endocrine disruption in aquatic species; and at Weymouth, England, regarding Europe's strategy for addressing endocrine disruption in humans and wildlife. He noted that a consensus emerging from these efforts is that a tiered testing procedure will be the most efficient way to evaluate the many chemicals for which testing will be necessary. The screening tier would involve a combination of structure-activity relationship, *in vitro*, and short-term *in vivo* evaluations. Results from the first tier would be used (probably with a higher priority to the *in vivo* assays) to decide which chemicals would need further testing in tier 2, longer-term *in vivo* tests, and tier 3, multi-generation tests. He also noted that any positive results from *in vitro* studies would need to be verified by *in vivo* data, since the widely accepted definitions of endocrine disruption all refer to adverse health effects in intact organisms or their offspring.

Dr. Zacharewski then began a discussion of *in vitro* test procedures. The biggest advantage of such tests, in general, is that they are relatively simple and thus capable of high throughput. There are several general types of *in vitro* tests available, each with advantages and disadvantages. The main types include hormone receptor binding assays, assays which measure the expression of hormonally-responsive genes, and assays which measure the proliferation of hormonally-responsive cells. Of these general test types, the last two can provide the most information, and also suggest which *in vivo* studies need to be done. Dr. Zacharewski noted that there are two potentially large problems which are emerging regarding the use of *in vitro* assays in the screening program required by federal law: laboratory validation of the various test methodologies has not yet occurred, and the time to conduct such validation is getting very short if the screening program is to start on schedule; and certain of the gene expression assays are patented and including them in the screening program may present legal difficulties.

In keeping with one of the main themes of the Forum, Dr. Zacharewski also researched the literature for the results of *in vitro* assays with Styrene, and found very little. The one assay completed using cell proliferation methodology was negative for Styrene, while the one study using gene expression as the endpoint was equivocal. Dr. Zacharewski also searched for structure-activity relationship information, which suggested that endocrine disruption potential was unlikely for Styrene. It was his opinion, based on the data he has researched and the information presented by Dr. Brown, that the evidence for endocrine disruption for Styrene was weak.

Dr. Peter Orris, from the University of Illinois at Chicago, then asked Dr. Zacharewski how to test mixtures and how to prioritize further testing (especially for pyrolysis products). He responded that this issue had been discussed by the EDSTAC, but not in great detail because there is still too much uncertainty. Dr. Borgert further responded that the EDSTAC has discussed the issue but has not reached a consensus at this point, and that it is most likely that the EDSTAC will recommend testing of certain "common" mixtures which are consistently found in the environment,

food, etc. Dr. Hornshaw commented that one such mixture of great interest to IEPA is the suite of chemicals commonly found in fish filets from Illinois waters, which includes several chemicals on the Endocrine Disruptor Strategy list. The discussion then turned to the closing remarks.

CLOSING REMARKS

Mr. Kanerva briefly summarized the genesis of the IEPA's EDS, noting that the Agency wanted to be prepared to carry out its duties of protecting Illinois' citizens and environment, including threats from endocrine disruptors. He explained that in order to do this, it was necessary to develop a list of chemicals for the Agency to use in checking existing data on occurrence and potential for exposure. Since there have been questions and challenges concerning the listing of several chemicals on the list, the question facing the Agency is what process should be used to evaluate additional data and how best to use this new data to modify the list (for example, information on potency, or lack of potency, of the endocrine activity of a chemical, which was not considered in the development of the list). Mr. Kanerva acknowledged that the Agency is trying to deal with this next step in the process.

Mr. Mark Homer, of the Chemical Industry Council of Illinois (CICI), said that there would be some support for a weight-of-the-evidence approach among his members. He suggested that the Agency pull together a group of interested parties to develop such an approach. He also recommended that the Agency should focus only on definite endocrine disruptors and, for chemicals for which the evidence is unclear, those for which a significant volume of the chemical is produced, stored, or used. Finally, he recommended that the Agency develop more explicit, qualitative criteria for endocrine disruptors.

Dr. Borgert suggested that an appropriate weight-of-evidence procedure would be to review all data for a chemical and give more weight to study types similar to those currently being considered by the EDSTAC. In response, Dr. Hornshaw asked how many of the chemicals on the IEPA list would have such data, especially the "gold standard" assays. Dr. Borgert did not have an estimate, but he thought an in-depth search for such data would be worth the effort, and might find sufficient evidence for 75% of the list.

Betsy Shirley, of the SIRC, stated that SIRC could find acceptable any of the review approaches discussed, but they would like for Styrene to be taken off the IEPA's list until the formal review of Styrene is concluded. Dr. Mark Johnson, USEPA, noted at this point that there are other risks of Styrene exposure that are more sensitive and that any conclusion of the Styrene review may not impact on regulatory programs which are based on the most sensitive risk.

Mr. Homer then recommended that the Agency compile another document that replaces the current list with a weight-of-evidence discussion for each chemical. Mr. Kanerva responded that this suggestion is a possibility, and that the Agency is considering taking this approach. He then noted that the Agency has more information on Styrene now than at the time of the development of the list, but that the Agency cannot reach closure on Styrene at this point. He also encouraged a continuing exchange of information regarding the Endocrine Disruptor Strategy between all interested parties.

There being no further questions or comments, Mr. Kanerva noted that the Agency may try to seek candidates for the weight-of-evidence panel to follow up on the suggestions just received. He then thanked all the participants for their efforts, requested written summaries of the presentations and opinions on the endocrine disruption potential of Styrene from the invited panel members, and concluded the Forum.

POST-FORUM ACTIONS

The Agency has identified a number of individuals representing various interests who would potentially be available to participate in a weight-of-evidence panel. The Agency is still exploring this option. Written summaries of presentations and opinions on the endocrine disruption potential of Styrene have been received from Drs. Brown, Borgert and Zacharewski, and are included as Attachment 4, 5, and 6, respectively [*not included*].

Regarding the endocrine disruption potential of Styrene, the Agency has reviewed the available evidence both pro and con and the opinions of the three invited panelists. The Agency still has two major unanswered concerns regarding the weight-of-evidence for Styrene to cause endocrine disruption. First and foremost of these concerns are the data gaps still present for studies under consideration for the EDSTAC screening battery. Of greatest concern is the lack of "gold standard" immature male and female rodent assays and any thyroid assay data. The Agency is also concerned about the relative paucity of data on androgenic and anti-androgenic effects, especially in light of altered testicular function reported in rats and decreased sperm counts reported in rats and occupationally exposed men (see Attachment 3). The Agency's concerns regarding the missing data on estrogenic and androgenic effects is tempered by the lack

of effects reported in a three-generation drinking water study, as noted by Dr. Brown, and the concerns regarding the total lack of thyroid data are somewhat relieved by the apparent lack of thyroid effects reported for occupationally exposed workers.

The Agency's second major concern involves a basic weight-of-evidence issue: how to balance several reports giving conflicting evidence? More specifically, the Agency has wrestled with the question of whether the results from several sub-optimal studies reporting relatively consistent results can be out-weighed by one well-conducted study reporting an opposite result. In this case, although there is some lingering uneasiness, the Agency is discounting the studies reporting effects on menstrual cycling and increased spontaneous abortion from the former Soviet Union in the weight-of-evidence evaluation.

Finally, in reviewing the opinions of the three panelists, the Agency finds it noteworthy that none of the panelists have concluded that Styrene is *not* an endocrine disruptor. Instead, their responses are phrased such that the conclusion of an examination of the weight-of-the-evidence is that Styrene is of low concern as a potential endocrine disruptor. Given the Agency's own review and the information presented regarding Styrene's potential for endocrine disruption, the Agency has come to the same conclusion, that Styrene is of low concern as a potential endocrine disruptor. Unless additional evidence to the contrary emerges, the Agency will focus its continuing activities regarding endocrine disruptors on other priority chemicals.

The summary of IEPA's review process for evaluating Styrene which is attached (Attachment 7) may be viewed as a "template" for future chemical-specific reviews, although the need for holding a public forum will be decided on a case-by-case basis. Until such time as the EDS may be revised or updated, this "Review Process for Further Evaluation of the Categorization for Styrene Pursuant to IEPA's Endocrine Disruptor Strategy" summary, and future summaries, will serve as a way to record and communicate IEPA's progress in updating its understanding of the chemicals identified in the EDS. When appropriate, such overview summaries also will identify IEPA's updated opinion on a chemical's potential to act as an endocrine disruptor, resulting from a weight-of-the-evidence review process. IEPA may issue such overviews as technical bulletins or similar communications, as a means of publicly providing updates on the progress with the EDS document.

ATTACHMENT 7 [1]

Review Process for Further Evaluation of the Categorization for Styrene Pursuant to IEPA's Endocrine Disruptor Strategy

The following summary outlines the Illinois Environmental Protection Agency's (IEPA) interpretation of an evaluation process of the data on the chemical styrene, relative to its listing in IEPA's February 1997 Endocrine Disruptor Strategy (EDS). This process is being proposed by IEPA as an appropriate means for working with other groups which may wish to initiate chemical-specific evaluations for EDS listings.

1. Request for Evaluation - In March 1997, the Styrene Information and Research center (SIRC) contacted IEPA to convey alternative opinions on IEPA's decision to list styrene as a "probable" endocrine disruptor in IEPA's EDS document. In particular, SIRC noted the existence of a substantial body of data which they felt suggested styrene was not of concern as an endocrine disruptor.

2. Initial Information Exchange - In initial meetings, SIRC representatives updated IEPA staff on the body of scientific data on styrene, as well as recent reviews of the data by well-recognized experts in the field of reproductive and developmental effects. IEPA staff noted the preliminary nature of their own review of styrene data. As a result of these discussions, IEPA concluded that a more thorough evaluation of the styrene data was justified, and agreed to review literature references that SIRC might choose to submit.

3. Submission & Review of Data - SIRC submitted data on styrene relative to reproductive and developmental effects to IEPA, including a 1991 review (and 1995 update) written by Dr. Nigel Brown, University of London. IEPA staff reviewed the data submitted by SIRC.

The weight-of-evidence evaluation for styrene is based on IEPA's current understanding of mechanisms and the existing database.

4. Scientific Issues Forum - In developing a process for effective chemical-specific reviews, IEPA staff recognized the benefit of receiving impartial third-party input. Both IEPA and SIRC endorsed the concept of a scientific issues forum, and three experts subsequently were identified and contacted regarding their willingness to participate: Dr. Nigel Brown, University of London; Dr. Tim Zacharewski, Michigan State University; and Dr. Christopher Borgert, Applied Pharmacology and Toxicology, Inc. As this was the first such public forum following publication of the EDS, in addition to a styrene weight-of-evidence review by Dr. Brown, Dr. Zacharewski lead a broader discussion of recent developments in the endocrine disruptor issue. The agenda also included an update on the progress of the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) by EDSTAC member Dr. Borgert. EDSTAC will be advising the U.S. Environmental Protection Agency on how to prioritize chemicals to determine potential endocrine disruptors. A Scientific Issues Forum was held in Chicago, Illinois on October 24, 1997. In addition to representatives from IEPA, SIRC, and the three panelists, IEPA invited representatives from other regulatory agencies, academics, as well as environmental and industry groups.

5. Conclusions of Scientific Forum - Following the October 24 forum, IEPA produced a meeting summary entitled "Proceedings Report for Scientific Issues Forum on Endocrine Disruptors," which provides full details of the styrene discussion, as well as discussions on the broader endocrine issue and EDSTAC. IEPA also requested that the three forum panelists each provide a summary of their conclusions of the weight-of-evidence forum discussion, to be attached to the "Proceedings Report" document. Although their written comments provide their detailed interpretations of the strengths and weaknesses of the available data, following are the basic conclusions of the three participants:

[1] Review Process Summary, Attachment to *Proceedings Report: For Scientific Issues Forum on Endocrine Disruptors*, Prepared by Illinois EPA, April 2, 1998

- *Dr. Borgert* - "Given the toxicological data set regarding reproductive and developmental effects of styrene, I would conclude that it is unnecessary to maintain any significant concern for potential endocrine disrupting properties of styrene at this time. Should new information become available, it would be appropriate to review those data in light of the total toxicological data set on styrene at this time."
- *Dr. Brown* - "If styrene had an ability to disrupt the endocrine system in any significant way, it is my view that the activity would be reflected in consistent effects on reproduction and/or development, which the current data do not show, even at very high exposures. It would seem, then, that the current weight-of-evidence leads to the conclusion that styrene is of low concern as a potential endocrine disruptor."
- *Dr. Zacharewski* - "Therefore, based on the evidence presented, the Illinois Environmental Protection Agency may wish to reconsider listing styrene in the Endocrine Disruptor Strategy."

6. *Summary and IEPA Conclusions* - The responses of the three forum panelists support a weight-of-the-evidence conclusion that styrene is of low concern as a potential endocrine disruptor. Although the body of research on styrene is extensive, limited data gaps still exist. Furthermore, the issue of endocrine disruptors is still very new, and screening and testing parameters - let alone a firm definition of an endocrine disruptor - remain to be agreed upon. The weight-of-evidence evaluation for styrene is based on IEPA's current understanding of mechanisms and the existing database, both of which are subject to change upon receipt of new or improved data. Given those considerations, IEPA has come to the same conclusion reached by the three scientists: based on the weight of the available data, styrene is of low concern as a potential disruptor.

IEPA currently has no plans to update or reissue the EDS. However, should revisions be undertaken in the future IEPA would no longer consider styrene as representative of a "probable" endocrine disruptor, and would not anticipate including it as a chemical of any significant priority in future versions of the EDS. Unless additional evidence to the contrary emerges, the Agency will focus its continuing activities regarding endocrine disruptors on priority chemicals.

For more information or documentation on the styrene evaluation, or on other chemical-specific reviews under the EDS, please contact Tom Hornshaw, IEPA at (217)785-0830.

Integrated Risk Information System: An Overview

Chris Bevan, Ph.D.

The Integrated Risk Information System (IRIS) is an electronic database of health effects information on chemicals developed by the U.S. Environmental Protection Agency (EPA). Used internationally as a reference, IRIS provides agency determinations on oral and inhalation exposure levels, as well as carcinogen classifications. Following a recent pilot program implementing a new IRIS review process, EPA is now reviewing a new group of chemicals, including styrene. EPA has engaged the Styrene Information and Research Center (SIRC) as an active participant in the styrene review process. This article provides general background on IRIS, its uses, the components of an IRIS listing, limitations of that information, details of past and current IRIS review processes, and information on accessing the database.

INTRODUCTION

In January 1998, the EPA announced it was beginning a review of styrene to update information on the chemical in the agency's IRIS electronic database. Styrene was one of 17 chemicals for which EPA announced it would begin an IRIS review in 1998, this following completion of an EPA pilot program implementing a new IRIS chemical review process. One goal of this new process is for the agency to actively engage external participation in the review, such as seeking industry input. In that spirit, EPA invited SIRC to participate in, and contribute to, the styrene review.

As the styrene IRIS review commences, this article is intended to briefly outline – generically – the IRIS database, its components and intended uses; the review process itself; as well as identify the limitations of the IRIS format.

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BACKGROUND ON THE IRIS DATABASE

Decisions regulating the use, manufacture, and disposal of chemical substances can have far-reaching economic and societal effects. These regulatory decisions are often based on assessments of the risks that chemical substances may pose to human health. The chemical assessment information used by the EPA can be found on a publicly accessible data base called the Integrated Risk Information System, or IRIS. The database summarizes the EPA's scientific positions on the potential health effects that may result from chronic (i.e. lifetime) exposure to a chemical. Currently, IRIS contains health effects information on over 500 specific substances.

Originally developed in 1985 for internal EPA use, IRIS was made externally accessible in 1988, so that the public could be aware of the scientific thought process and supporting research on which the EPA bases its risk assessments for regulatory decisions.

HOW IRIS IS USED

Outside of the EPA, information in IRIS is referenced by federal, state, local, and international regulators in setting regulatory standards for chemicals. This is particularly true for agencies that lack the resources to conduct their own assessments. The IRIS reference concentration, for example, is widely used by state agencies as a basis for setting fenceline exposure limits for chemicals emitted by a manufacturing facility. IRIS also is used as one basis for listing chemicals for consideration under California's Proposition 65. Academics, industry, environmental groups, and the public also use the database.

COMPONENTS OF THE IRIS DATABASE

The core of an IRIS listing for a chemical consists of three

chronic health hazard summary sections: the reference dose (RfD) for non-cancer health effects resulting from oral exposure; the reference concentration (RfC) from effects resulting from inhalation exposure; and the carcinogen assessments for both oral and inhalation exposure. All of these terms are commonly used for judging the effects of lifetime exposure to a given chemical substance.

For non-cancer toxicity, the RfD and RfC values are estimates of a daily oral or inhalation exposure to the human population (including sensitive subgroups) that are believed to be without an appreciable risk of certain deleterious effects during a lifetime. These values are based on determination of a critical effect from a review of all toxicity data, and a judgement of the necessary uncertainty and modifying factors based on a review of the available data. The IRIS files contain the following information pertaining to the RfD or RfC:

1 Reference dose or concentration summary tables
2 Principal and supporting studies
3 Uncertainty and modifying factors used in calculating the RfD or RfC
4 A statement of confidence in the RfD or RfC
5 EPA documentation and review
6 EPA scientific contacts
7 Complete bibliographies for cited references.

Currently, the cancer assessment involves a judgement in the form of a weight-of-evidence classification of the likelihood that the chemical substance is a human carcinogen. These classification categories are: human carcinogen (Group A); probable human carcinogen (Group B); possible human carcinogen (Group C); not classifiable (Group D); or non-carcinogenic (Group E).

However, EPA has proposed new (but not yet promulgated) cancer assessment guidelines[1] in which those classifications would be replaced with general descriptor headers, accompanied by more detailed narrative text on a chemical.

The IRIS file also contains the type of data used as the basis of the cancer classification. The dose-response assessment in this section is a quantitative estimate of the potential activity or magnitude of a substance's carcinogenic effect, usually expressed as a cancer unit risk. A cancer unit risk is an estimate of the increased likelihood that an individual will develop cancer when exposed to a substance over a lifetime. Exposure is based on a concentration of either one microgram per liter ($1\mu/L$) in drinking water for oral exposure, or one microgram per cubic meter ($1\ \mu/m^3$) in air for continuous inhalation exposure. Generally, a slope factor for dietary use is also given. The unit risk is an upper-bound estimate of cancer risk for humans, based on milligram of chemical substance, per kilogram body weight, per day.

In addition to the RfD, RfC, and carcinogenicity sections, IRIS files may contain a summary of an Office of Drinking Water's 'Drinking Water Health Advisory' and/or a summary of EPA regulatory actions on a chemical substance. The only purpose of these supplemental sections is to serve as accessory information to the consensus health information, in order to provide a broader profile of a substance. These supplemental sections should not be used as the sole or primary source of information on the current status of EPA substance-specific regulations.

FIGURE 1

Four steps in conducting a risk assessment (NRC, 1983)

1 Hazard Identification
Gathering and evaluating data on the health effects of a chemical and on the conditions of exposure under which injury or disease are produced.

2 Dose-Response Assessment
Describing the quantitative relationship between the amount of exposure to a substance and the extent of toxic injury or disease.

3 Exposure Assessment
Describing the nature and size of the population exposed to a substance and the magnitude and duration of their exposure.

4 Risk Characterization
Integrating data and analysis of the first three components of the risk assessment process to determine the likelihood that humans will experience any of the forms of toxicity associated with a substance.

[1] U.S. Environmental Protection Agency (1996) Proposed Guidelines for Carcinogen Risk Assessment. Fed. Reg. 61, 17959-18011

[2] U.S. Environmental Protection Agency (1998) Integrated Risk Information System(IRIS) Announcement of 1998 Program; Request for Information, Fed. Reg. 63, 75

LIMITATIONS OF THE INFORMATION IN IRIS

As characterized by EPA,[2] the summaries of health information in IRIS support the first two steps of the risk assessment process, as outlined by the National Research Council's (NRC) 1983 publication (see Figure 1). [3]

The limitations of IRIS, therefore, are in the absence of exposure assessment and risk characterization. These components are critical to a true *risk* assessment, versus the *hazard* assessment provided by IRIS. Furthermore, IRIS does not provide interpretation of the hazard assessment of health effects information in a format that is readily understood by the general public or directly representative of public health effects. Lacking both exposure assessment and risk characterization, there also is no quantitative way for either the regulator or the lay person to evaluate the potential risk – if any – from exposure to a chemical.

The limitations of IRIS, therefore, are in the absence of exposure assessment and risk characterization.

THE ORIGINAL IRIS REVIEW PROCESS

The IRIS review process has undergone considerable change since it started in 1985. In the past there were two EPA workgroups, the Carcinogenic Risk Assessment Verification Endeavor (CRAVE) and the Oral Reference Dose/Inhalation Reference Concentration (RfD/RfC) Work Group, that developed the health hazard information for IRIS. Each group consisted of EPA scientists from a mix of pertinent disciplines and represented a variety of EPA Program Offices. The work groups also served as the EPA's final review for the risk assessment information. When the work groups reached a consensus on the health effects information and the dose-response assessments for a particular chemical substance, the information was added to IRIS.

REVISIONS TO THE IRIS PROCESS

To address perceived limitations and problems with the program, IRIS underwent considerable external and internal review from various perspectives. Concerns addressed included the basic concept of the database, the quality and scope of its contents, and how IRIS can best meet the information needs of the public, as well as of the agency. Several areas were identified as deficiencies in the IRIS process.

In particular, the IRIS review did not involve a formal external peer review process, nor was there any public involvement in the IRIS process. Because of the lack of public involvement, the summaries of the toxicological data did not necessarily include all relevant studies. Moreover, the judgements and decisions leading to the conclusions were not always clear and documented.

In 1996, EPA initiated a Pilot Program for IRIS in response to comments, from both within and outside the Agency, about needed improvements to IRIS. The Pilot Program focused on improving the efficiency, documentation, and public input of the review process that preceded the IRIS data base entries. A diagram of the IRIS Pilot Program is shown in Figure 2.

Eleven chemicals of diverse nature of effects were nominated for the pilot program. Of the chemical substances that were nominated for review under the Pilot Program, most are in the last stage of the process. Based on EPA's evaluation of the success of the Pilot Program, the agency will now use the IRIS process diagrammed in Figure 2 for all future reviews.

There are several noteworthy features of this new process:

1 EPA will solicit scientific information from the public by a *Federal Register* notice

2 A comprehensive "Toxicological Review," which combines the cancer and non-cancer assessments, will become the preferred document for IRIS entries

3 Use of external peer review of the Toxicological Review and health summaries, replacing the former CRAVE and RfD/RfC Working Groups.

In improving efforts to solicit public input, EPA has made a concerted effort to involve affected parties in the review process. Industries using a given chemical, for

[3] National Research Council (NRC). (1983) Risk Assessment in the Federal Government: Managing the Process. Committee on the Institutional Means for Assessment of Risks in Public Health, Commission on Life Sciences, NRC. Washington, DC; National Academy Press

example, are being encouraged to work closely with EPA in identifying appropriate scientific data for consideration, and their comments and input. EPA has welcomed participation by outside parties in the review process, as a way to lighten the agency's burdensome workload, and ultimately expedite the IRIS process.

ACCESSING IRIS

The IRIS database is maintained by the EPA National Center for Environmental Assessment, within the Office of Research and Development. IRIS can be accessed either through their website at www.epa.gov/iris, or through any of the database systems accessed through the National Library of Medicine, such as TOMES or Grateful Med.

FIGURE 2

Pilot process for consensus development