THE SIRC REVIEW

RESEARCH • TECHNOLOGY • PUBLIC POLICY

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The SIRC Review: The Need, The Purpose

The SIRC Review is published by the Styrene Information and Research Center, which was established in 1987 by companies involved in the manufacture and use of styrene. The purpose of SIRC is to explore health questions involving styrene and to disseminate information so that appropriate actions are taken if there is evidence of any threat to the health of workers in the industries making or using styrene, or to the general public.

The decision to form SIRC was precipitated by a determination of the International Agency for Research on Cancer (IARC) based in Lyon, France, to reclassify styrene from Group 3 (not classifiable as to its carcinogenicity to humans) to Group 2B (possibly carcinogenic in humans). Upon hearing of this change, the first concern of toxicologists working in the field was that IARC may have uncovered some new data not previously known to us. To our relief, this was found not to be the case. There was no new human or animal data. What had happened is that IARC had changed the way it arrived at its classifications. In the case of styrene, it had reviewed data on genetic toxicity and on a styrene metabolite, styrene oxide. Under IARC's new classification system, this review resulted in the upgrade. The depth of the review which led to this far-reaching decision was not discussed by IARC and has remained a mystery to those of us who have sought further explanation. Thus SIRC considered one of its first tasks to be a thorough examination of the evidence cited by IARC.

This endeavor was the starting point of what has now become a significant body of new research and independent expert reviews of all previous studies on the possible health effects of styrene, conducted by recognized authorities in their respective disciplines. Much of this new evidence was presented for the first time to agencies of the United States Government, to assist in their investigations of various commonly used chemicals and their potential impacts on human health or the environment. SIRC has also monitored the work being done in laboratories abroad and is actively cooperating with the European Chemical Industry Ecology and Toxicology Center and others to help clarify areas where further scientific inquiry appears necessary. One continuing concern of the scientific community, for example, is the relevance of animal data to humans. The work being done by SIRC and the scientists it is working with in Europe and Canada should make a significant contribution to the clarification of this fundamental and troubling question as it pertains to styrene.

Until now, the results of the research and literature reviews conducted under the auspices of SIRC have been conveyed primarily to a small, discrete audience of government and industry scientists. Because of the increasing governmental and public interest in all areas of occupational and environmental health issues, however, as well as the need for industry to be informed more broadly on these issues, it was decided to make the SIRC papers more generally available to interested parties. This was the genesis of The SIRC Review.

The Review will not only document the current research and literature reviews whose results may be of general interest to all with responsibilities in the fields of health maintenance and regulation, but will also serve to examine the various processes by which scientific determinations are translated into public policy. The decision to broaden our review to include this public policy element arose directly from our research. In exploring the basis for IARC's reclassification of styrene, we were surprised to discover not only that the scientific justification left much to be desired, but also that the unquestioning acceptance of IARC's classifications at many different levels of government goes far beyond what IARC itself would recommend. In its preamble to its classifications, IARC specifically warns against using them as a basis for regulation. IARC's director, Lorenzo Tomatis, has also personally warned against doing so in appearances before the Toxicology Forum, whose meetings are attended by numerous government officials with responsibilities for regulation. The reasons are clear. IARC's emphasis is on positive evidence, and it excludes negative evidence, which in many cases —and most likely in the case of styrene—would produce a different, less ominous classification. But perhaps even more significant for those involved with risk manage-

ment is that IARC does not concern itself with likely human exposure levels. Despite these very clear limitations, we found that government/ regulatory officials at both the federal and state levels in the United States and elsewhere often appear totally unaware of them. In response to the resulting widespread misuse or misapplication of the IARC classifications, Doctor Tomatis has responded that this is not IARC's responsibility. At many levels of government, classifications, from IARC or other sources, are simply accepted at face value. In pursuing our inquiries relating to the reclassification of styrene, we found that the impact of these attitudes is quite enormous. Because of the absence of questioning of the IARC classifications, they are used as prima facie justification for subsequent legislation as well as regulation, and generate a quite needless degree of public anxiety. We concluded that this process should be of concern to the scientific community as much as to the governmental and business circles among which it is more normally discussed. Indeed, the Science Advisory Board of the U.S. Environmental Protection Agency has itself expressed its concern that EPA has been "applying its policy judgments in a scientifically unfair manner to the classification of numerous substances proposed for regulation as carcinogens."

For these reasons, we believe The SIRC Review can play a useful role in furthering understanding of the policy process and perhaps contribute to needed improvement in the way this process is applied to regulation of substances perceived to affect human health. In this first issue, we include an illuminating examination of the weaknesses in current carcinogen classification systems used by various public bodies, with particular attention to the process used by the U.S. Environmental Protection Agency. In noting the inherent contradictions in the system presently used by EPA, the author, Dr. Robert J. Moolenaar, makes several specific recommendations for improvement that would "minimize public confusion and avoid loss of credibility". Risk management, he concludes, will serve the public best when the scientific basis is sound and "the scientific evaluations are accurately communicated." Many in the scientific community share Dr. Moolenaar's views and hope that his recommendations will be given serious attention by those in a position to effect the necessary changes.

Also, in this issue, we provide a tour d'horizon of the health issues involving styrene. Compiled by Dr. Daniel P. Boyd, a former director of standards at the U.S. Occupational Safety and Health Administration, it includes contributions by other eminent scientists who have worked in this area and have carefully examined the numerous studies:, It should prove a valuable reference for all involved with these questions. This issue also contains literature reviews on specific issues raised in connection with styrene. Dr. Julian Preston of Oak Ridge National Laboratory discusses the potential mutagenicity of styrene and its metabolites and Professor Martin Alexander of Cornell University explores the evidence on the environmental fate of styrene.

While these contributions all tend to allay any alarm with respect to the health effects of styrene on either workers in the industry or the public at large, they also reinforce the concern felt by many of us in the field of toxicology that the initial causes of the alarm, and the governmental processes which seem designed to strengthen and perpetuate it, bear as urgent a need of investigation as any of the substances themselves. We trust that this first edition of The SIRC Review will prove of interest and welcome suggestions from our readers for future contributions.

Geoffrey C. Granville
Chairman
Science and Technology Task Group
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Authors in this Issue

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Dr. Alexander has authored more than 400 technical papers and reviews and has written several books in the areas of microbiology and microbial ecology. He is associate editor of the *Geomicrobiology Journal*, a member of the editorial board of the *Mircen Journal of Applied Microbiology and Biotechnology* and a consulting editor for Soil Science. He holds a Ph.D. in bacteriology from the University of Wisconsin.

Kenneth M. Bodner is a Project Leader in Epidemiology in the Health and Environmental Sciences Department at The Dow Chemical Company. He has done extensive research on mortality, morbidity, and reproductive outcomes in the Dow workforce, as well as on general methodological problems in epidemiology. He has also consulted on a number of global issues of chemical risk and has developed many of the analytical tools currently in use at Dow.

Mr. Bodner holds a Masters of Science in Public Health degree in biostatistics from the University of North Carolina, Chapel Hill, where he also minored in epidemiology and gained research experience as a trainee in the Occupational Health Studies Group. He has done additional work in physiology at Columbia University and neurobiology at the University of North Carolina.

As research leader and technical coordinator for the Epidemiology Department of The Dow Chemical Company, **Dr. Gregory G. Bond** is responsible for managing the epidemiology research program for Dow Chemical U.S.A. He obtained a Ph.D. in epidemiologic science from the University of Michigan after receiving a B.S. degree in cellular and molecular biology and a masters degree in public health. He has published more than 30 epidemiology research papers in the peer-reviewed literature, including case-control studies of cancers in the brain, kidney, lung and connective and soft-tissues, as well as cohort studies of workers exposed to arsenic, acrylamide, benzene, chlorinated dioxins and the herbicide 2,4-D.

Dr. Bond has also served as an advisor to various government agencies, including the National Center for Health Statistics and the Agency for Toxic Substances and Disease Registries. He is board certified and has been elected a Fellow in the American College of Epidemiology.

Dr. Daniel P. Boyd, a technical consultant to the Styrene Information and Research Center since 1987, has specialized for many years in occupational safety and health issues. He was Director of the Office of Standards Development in the Occupational Safety and Health Administration (OSHA) at the U.S. Department of Labor from 1973 to 1976. He was previously Director of Science and Engineering Training, U.S. Civil Service Commission, and an Associate Professor of Management Science at The George Washington University. After leaving government, he established a consulting practice specializing in occupational safety and health analysis which provides management, education and training services to both industry and government. He has undertaken large-scale epidemiological studies and developed computerized chemical tracking systems and health surveillance systems for a number of industrial organizations.

Dr. Boyd has advanced degrees in chemical engineering and materials science from the University of Maryland.

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Dr. Robert J. Moolenaar has been involved in public policy issues affecting science and public health for many years as an active member of the American Industrial Health Council. He was Chairman of its Scientific Committee from 1982 to 1987. A former Director of the Environmental Sciences Research Laboratory at The Dow Chemical Company, he is currently Project Director for Health and Environmental Sciences at Dow. He is on the Board of Directors of the Toxicology Forum and is a prolific writer, contributing many articles to such journals as *Environmental Toxicology and Chemistry* and *Regulatory Toxicology and Pharmacology*. He holds a Ph.D. in physical chemistry from the University of Illinois at Urbana.

A former instructor in pharmacology and toxicology at the University of Kansas, **Dr. Richard J. Nolan** is now a toxicologist in the Health and Environmental Sciences division of The Dow Chemical Company, working in the Biotransformation Group and in Biomedical Research. An author of numerous publications, his primary areas of interest lie in the analytical use of pharmacokinetics/metabolism in dose response and species sensitivity.

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Dr. R. Julian Preston, Section Head of the Biology Division of Oak Ridge National Laboratory in Oak Ridge, Tennessee, is well known for his work in genetics, cytogenetics and molecular biology. In addition to his academic and research work, he is a member of the Toxicology Study Section of the National Institutes of Health, and a consultant in the areas of genetics and molecular biology to the Radiation Effects Research Foundation. He has also been associated with the U.S. Environmental Protection Agency as a group leader for a gene-tox group studying cytogenetics. He has served on the editorial boards of *Mutation Research Letters*, *Mutation Research*, *Environmental and Experimental Botany* and *Environmental and Molecular Mutagenesis*.

Dr. Preston is an honors graduate of Britain's Cambridge University (genetics) and holds a Ph.D. in radiation biology from Reading University. Since 1970 he has been an adjunct professor at the University of Tennessee's Biomedical Graduate School and was associate director of the school from 1977 to1982.

Styrene: Perspectives on the Carcinogen Question

*Daniel P. Boyd, Ph.D., et al.**
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The authors review and discuss the major studies to date on the possible carcinogenicity of styrene. They begin with the work of the International Agency for Research on Cancer (IARC) which in 1987 changed its classification of styrene from "notclassifiable" to "possibly carcinogenic to humans", despite the lack of any new human or animal data. They point out that several major national and international organizations have disagreed with this classification and have determined that the evidence does not support the classification or regulation of styrene as a carcinogen. They believe that significant studies currently in progress will clarify some of the issues and allow the development of improved risk estimations.

In 1969, IARC initiated a program to evaluate the carcinogenic risk of chemicals to humans and to produce monographs on individual chemicals. The monographs produced by IARC are entitled *Monographs on the Evaluation of the Carcinogenic Risk to Humans*.

IARC first established criteria to evaluate carcinogenic risk to humans in 1971. The criteria were subsequently re-evaluated in 1977, 1978, 1979, 1982, 1983, and again most recently in 1987. For the 1987 meeting, several changes were made in the structure and criteria for the categorizations in comparison to previous meetings. For example, the title for Group 2B was changed from "probably carcinogenic to humans" to "possibly carcinogenic to humans". In addition, criteria were formalized for incorporating genotoxicity data in the categorization process at the 1987 meeting. Subsequent to the changes in the structure and criteria, the 1987 Working Group meeting made overall evaluations of carcinogenicity for all 617 agents that had been previously evaluated in Volumes 1-42 of the IARC Monograph Series. The results of those evaluations, as well as the changes in the categorization process, were published in 1988 as Supplement 7 of the IARC Monograph Series.

For styrene, the 1987 IARC Working Group concluded that the evidence for carcinogenicity in humans was inadequate and that the *evidence for carcinogenicity in animals was limited.* These conclusions were consistent with previous IARC evaluations of styrene, since there was no new human or animal carcinogenicity data to be considered. However, the overall evaluation of styrene was changed at the 1987 meeting from Group 3 (not classifiable as to its carcinogenicity in humans) to Group 2B (possible carcinogenic to humans) as a result of the changes in the structure and criteria for the classifications, which formalized the use of genotoxicity data for "upgrading" the classification. The evidence for activity in genotoxicity tests was considered to be sufficient, and hence the classification for styrene was upgraded from Group 3 to Group 2B despite the fact that there was no new human or animal carcinogenicity data to support the upgrade.

HUMAN DATA

The epidemiologic data on styrene is quite substantial, including eight cohort mortality studies.

The eight studies examined workers

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employed in the reinforced plastics manufacturing industry or in styrene monomer, polymer and styrene-butadiene rubber manufacturing operations. Employees in the reinforced plastics industry were more likely to have been younger and hired more recently, but exposed to higher levels of styrene, frequently measured in the range of 50–100 ppm. Employees of the other operations tended to be older, followed for longer periods of time, and probably exposed to styrene at lower levels. Collectively, the eight studies included nearly 50,000 employees during the time period between 1940 and 1986, thus presenting a substantial amount of data for comparison and evaluation.

A summary of the findings from the studies is presented in Table 1. The two that are most recent, those by Coggon *et al.*, and Matanoski and Schwartz, demonstrated deficits for almost all subtypes of lymphatic and hematopoietic cancer, including some that were markedly diminished.

The study by Okun found no deaths from lymphatic and hematopoietic cancer in a large but young cohort of reinforced plastics boatbuilders with limited follow-up.

Environmental Health Associates reported 5 deaths from leukemia, compared with 4.76 expected, and 9 deaths from the combined category of lymphatic and hematopoietic cancer versus 12.3 expected from among 15,908 workers in the reinforced plastics industry.

Nicholson and colleagues found one leukemia and one lymphoma and concluded that, although their data were not definitive, the data did indicate that an environmental risk from styrene was "not extraordinary."

The differences among the results of these various studies do not seem to be clearly attributable to known dissimilarities among the cohorts or their working conditions. Although some of the cohorts were small and not followed long enough to rule out cancers of long latency, the study of Matanoski and Schwartz provided reasonable follow-up time without showing any unusual outcomes.

Three of the eight studies reported excess deaths and/or incidence from cancer of the lymphatic and hematopoietic systems. Meinhardt and colleagues reported an elevated, but not statistically significant, leukemia mortality among employees from two styrene butadiene rubber (SBR) facilities which they interpreted as suggestive of an association. Five of the six cases were of the myelogenous cell type.

Ott and coworkers found a statistically significant excess incidence of lymphatic leukemia among workers involved in colorant blending, roll compounding or extrusion of plastics. This operation was characterized by complex, multi-chemical exposures, and the possibility that the tumors were induced by styrene is diminished by the absence of any leukemia cases among the subgroup of workers with the highest exposures.

Hodgson and Jones reported a statistically significant excess in lymphoma mortality among workers producing styrene monomer and polymers. It was concluded that all three cases were believed to have been non-Hodgkin's lymphoma.

All three of the foregoing studies that reported excess deaths and/or incidence from cancer of the lymphatic and hematopoietic systems were conducted in occupational groups with mixed chemical exposures including exposure to benzene, a known human leukemogen. Each study points to a different tumor type, making it unlikely that a single environmental factor was involved. Furthermore, none of the studies found evidence for a dose-response relationship, although attempts were hampered somewhat by the lack of precise exposure measurements and the small number of tumor cases available for study. Any determination of styrene carcinogenicity from these three reports would rest on the reliability and interpretation of no more than seven to eight total cases.

Keeping in mind the heterogeneous results of individual studies, the combined weight of the evidence is the ultimate basis for evaluating the potential of any substance to cause cancer in humans. Of the eight studies, five reported either substantially fewer deaths than were expected or that the observed and expected numbers of deaths were equal. As noted previously, the three remaining studies might be said to raise a question on cancer of the lymphatic or hematopoietic system although confounding exposures, different tumor types and other limitations would not support a conclusion that styrene exposure was the cause of these endpoints. To gain a better perspective of this issue, the data from the individual studies were pooled. The pooled results are shown in Table 2. In the major subcategories, lymphoma and leukemia, as well as for the entire category of lymphatic and hematopoietic cancer, only deficits in mortality are seen.

One consideration is the potential of styrene to cause cancer only after a long latency period. It is difficult to speculate on a latency period when there is little evidence to suggest what, if any, cancer types might be associated with styrene. The current data base is probably sufficient to test for cancer after a latency period up to 15 years. Continued follow-up of many of these cohorts could help to extend that period by another 10–15 years. However, any extraordinary risks posed by exposure to styrene should already be evident from these investigations.

In summary, the *substantial epidemiologic data on styrene argue against a carcinogenic role for styrene* at levels of occupational exposure which are drastically higher than those likely to be encountered in the environment. Hence,

SIRC agrees with IARC in concluding that the available human data does not support classification of styrene as a carcinogen.

ANIMAL DATA

A total of nine long-term animal studies have been conducted on styrene, and two additional long-term studies have been conducted on a mixture of styrene and ß-nitrostyrene. These studies are briefly summarized in Tables 3 and 4 for rats and mice, respectively. Although most of the long-term animal studies that have been conducted on styrene to date have deficiencies or limitations, the available data is nevertheless substantial and rather complex. Four of the nine styrene studies showed no increase in tumors of any type in animals exposed to styrene, while the remaining five studies have major flaws or deficiencies which preclude definitive conclusions. The following is a brief assessment of each of the studies.

1. Ponomarkov and Tomatis (1978)

These researchers conducted oral gavage bioassays on styrene with exposures initiated in utero in two strains of mice, the O_{20} and $C_{57}B1$, and in the BDIV rat. The design of these studies was very unusual in that pregnant animals were given oral doses at day 17 of gestation, and the progeny given weekly doses. This type of study design is dramatically different from accepted international guidelines for conducting animal oncogenicity studies.

(a) O_{20} Mouse Study

In the O_{20} mouse study, both the dams and progeny (45 males and 39 females) were administered doses of 1350 mg/kg. Treatment of progeny was stopped after 16 weeks because of mortality. There were two control groups: one control group (20 males and 22 females) was given weekly doses of 0.1 ml of olive oil for life and the other control group (54 males and 47 females) was untreated. The mean survival time of the male and female O_{20} mice given styrene was significantly less than that of the controls. A statistical increase in lung tumors was observed in male and female mice relative to the olive oil control group and for female mice versus the untreated control group.

It is SIRC's opinion that it is inappropriate to use this study as a basis for classification of styrene as an animal carcinogen, for the following reasons:

(i) MTD exceeded. The dose of styrene given clearly exceeded the maximum tolerated dose as evidenced by the increased mortality in the weanlings and early mortality in the progeny of the styrene-treated mice. The impact of this increased/early mortality on the selection of the progeny for further dosing and on the incidence of spontaneous tumors having high background rates is unknown and can neither be ignored nor discounted. Interpretation of these data is further complicated by the small number of animals involved in the study, and also by the absence of data on litters as well as data on the background incidence of pulmonary tumors in this strain of mice.

(ii) Litter effects. Attention must be drawn to the fact that the data were analyzed as if the progeny were independent entities. The effects of litters were not taken into account. This is clearly inappropriate. Haseman and Hogan of the National Institute of Environmental Health Sciences (NIEHS) have pointed out that fetuses from the same litter do not respond independently since maternal influences and other environmental factors will generally cause litter mates to be more alike than fetuses from different litters (Haseman and Hogan, 1975).

Ponomarkov and Tomatis, the authors of the study, recognized these shortcomings and concluded:

> The increased incidence and early appearance of lung tumors could possibly indicate a carcinogenic effect for styrene in O_{20} mice. This experiment, however, has severe limitations, since the dose used was very high, causing severe toxic effects and early mortality .... Additional studies are needed before a final evaluation of the carcinogenicity of styrene in rodents can be made.

(iii) Treatment-related deaths. The high incidence of early treatment-related deaths was attributed to liver, spleen and lung toxicity. There was no indication that lung tumors were a factor in this mortality; in fact, the first lung tumor in O_{20} mice appeared after 21 weeks (in a male mouse) by which time more than 31 of the 45 treated male mice had died. Because control animals did not exhibit any significant early mortality (the average age of death for all controls was ≥85 weeks), the reported "earlier incidence of tumors" is clearly due to the fact that the treated animals were dying and being examined earlier.

Finally, with respect to the ratio of pulmonary carcinomas/adenomas in female mice, the authors clearly state "the proportion of benign to malignant lung tumors did not appear to differ among the various groups ..."

SIRC concludes this study is inadequate to use as a basis to classify styrene as a carcinogen.

(b) $C_{57}B1$ Mice

In the study with $C_{57}B1$ mice (Ponomarkov and Tomatis, 1978), the pregnant mice were given an oral dose of 300 mg/kg of styrene on day 17 of gestation and the progeny (27 males and 27 females) were given weekly oral doses of 300 mg/kg for life. Two control groups were used; these consisted of 13 female and 12 male progeny dosed with

vehicle alone, and 51 male and 49 female untreated animals. The incidence of liver tumors observed in the treated mice, vehicle-treated controls, and untreated controls were 3 of 24 (12.5%), 1 of 12 (8.3%) and 1 of 47 (2.1%) mice, respectively. The difference between the incidence of liver tumors in the treated male mice (3 of 24) and the pooled control mice (2 of 59) is not statistically significant. Therefore this study provides *no evidence* that styrene is an animal carcinogen.

(c) BDIV Rats

In the study with BDIV rats (Ponomarkov and Tomatis, 1978), the pregnant rats were given an oral dose of 1350 mg/kg of styrene and the progeny (73 males and 71 females) were given weekly oral doses of 500 mg/kg of styrene. Control group animals (36 males and 39 females) were given vehicle alone. SIRC agrees with the authors in concluding that this study provides *no evidence* that styrene is an animal carcinogen.

2. Jersey** et al. **(1978)

Sprague-Dawley rats were exposed to 0, 600, or 1000 ppm styrene vapors 6 hours per day, 5 days per week, for 2 years. Initially, the high exposure group was exposed to 1200 ppm styrene, but early mortality in the males necessitated lowering the exposure concentration to 1000 ppm after 2 months. The study results were complicated by an outbreak of chronic murine pneumonia unrelated to the styrene exposures. This disease had a significant effect on all groups of animals, but it was particularly severe in control and high (1000 ppm) exposure group male rats, resulting in early mortality in those groups. The intercurrent infection and early mortality are confounding variables that severely limit interpretation of some of the results of the study, especially in males.

A statistically significant increase in the incidence of mammary gland adenocarcinomas occurred in female rats in the low (600 ppm) exposure group. The incidence of mammary gland adenocarcinomas was: 1 /85 (1.18%), 7/ 85 (8.23%) and 0/75 (0%) in female rats at 0, 600 and 1000 ppm, respectively. Hence, there was clearly no dose-response relationship since the incidence of mammary gland adenocarcinomas in high exposure group females was not different from controls. Moreover, the incidence of mammary gland tumors in the 600 ppm group females was within the range of normal for historical control groups of female rats of the same strain in the testing laboratory (historical control incidences ranged from 0 to 9.3%).

The incidences of the combined diagnoses of leukemia plus lymphosarcoma (leukemia-lymphosarcoma) were higher in both exposure groups of female rats as well as in the low (600 ppm) exposure group males than was observed in the control groups. However, the differences were not statistically significant and, for females, there was no dose-response relationship since the incidences of leukemia-lymphosarcoma were the same (6 of 85; 7.06%) in both the high and low exposure groups. For males, the incidence of leukemia-lymphosarcoma was actually lower in the 1000 ppm exposure group. (1 of 84; 1.19%) than in the 600 ppm group (5 of 86; 5.81%); however, the assessment of the dose-response relationship in males was confounded by the excessive mortality and intercurrent infections in the control and 1000 ppm groups.

In addition, there have been a number of changes in the accepted procedures for grouping and analyzing laboratory animal tumor data since the Jersey *et al.* study was reported. The National Toxicology Program (NTP) has maintained since 1986 that it is inappropriate to combine leukemias and lymphomas (McConnell *et al.*, 1986).

In general, the study provided no clear evidence of an oncogenic response related to exposure to styrene and, as a result of the various limitations and deficiencies of the study, SIRC concludes that it is *inadequate* for purposes of carcinogenic classifications.

3. Conti** et al. **(1988)

This study is a compliCated study, in which styrene was given to Sprague-Dawley rats via four different routes of administration:

(1) Intraperitoneal injection: 4 injections, 50 mg per injection, two-month intervals between injections, administered to 40 male and 40 female rats.

(2) Subcutaneous injection: A single injection of 50 mg to 40 male and 40 female rats.

(3) Inhalation: Groups of 30 male and 30 female rats were exposed to 25, 50,100, 200 or 300 ppm styrene for 4 hours per day, 5 days weekly for 1 year. Sixty rats of each sex served as controls.

(4) Ingestion: Groups of 40 males and 40 female rats were dosed with 50 or 250 mg styrene/kg, in olive oil, 4-5 days weekly for 1 year.

In all of the above studies, the rats were maintained until death.

Conti *et al.* reported there were no effects in any of the experiments except in the inhalation study, where significant increases in the incidence of mammary tumors in all exposed female groups was noted. The incidence of malignant mammary tumors (MT) was 20, 13, 30, 40 and 30% at 25, 50, 100, 200 and 300 ppm, respectively, with the control group incidence of 10%. For total benign plus malignant mammary tumors (TBMT), the control incidence was

56.7%, with treatment groups having 80, 70, 70.7, 80 and 83.3% incidence at 25, 50, 100, 200 and 300 ppm, respectively. Hence, there obviously was no clear dose-response relationship in this study, since the incidences of both TBMT and MT were essentially the same in each of the five exposure groups. The authors of the report did not specify whether or not the concurrent control group animals were placed in inhalation chambers while the styrene exposures were in progress; this is an important factor in conducting chronic inhalation studies which could have some relationship to the observed differences between control and treatment groups of animals in this study. Furthermore, in view of the high spontaneous incidence of mammary tumors in the strain of animals used, historical control values are needed in order to allow a more definitive interpretation of the results obtained. In fact, ten years earlier Dr. Maltoni pointed out that there was a high incidence of mammary tumors in the rat colony at the Institute of Oncology in Bologna where Conti *et al.* also conducted their study (Maltoni, 1978):

> It should be pointed out [however] that the incidence of mammary tumors is quite high in the colony of rats we employed, that some fluctuation of that incidence is currently observed from group to group, and that the results of the other experiments do not support a correlation between styrene exposure and mammary tumors.

In a 1982 publication which may or may not relate to the Conti *et al.* (1988) study, Maltoni *et al.* (1982) reported no evidence of carcinogenicity in a study of extremely limited scope in which there was no increase in brain tumors in male or female rats given daily oral doses of 50 or 250 mg/ kg of body weight in olive oil 4-5 days per week for 52 weeks.

SIRC finds these studies to provide either *no evidence* or *inadequate* evidence to classify styrene as a carcinogen.

4. Beliles** et al. **(1985)

This was a study in which styrene was administered to Sprague-Dawley rats in their drinking water at levels of 125 and 250 ppm. The rats were exposed for two years, during which time males and females were randomly selected for breeding the F_1 generation, and then returned to the chronic bioassay. The F_1 generation was also exposed to styrene in drinking water and mated at around 110 days of age to derive an F_2 generation. Subsequently, an F_3 generation was also derived. The calculated daily dose of styrene was stated as being 7.7 and 14 mg/kg/day for male rats and 12 and 21 mg/kg/day for females. It may be noted that a saturated solution of styrene in water at 25° C contains 320 ppm.

The authors of the study concluded that the treatment "produced no gross or histologic dose-related changes nor evidence of carcinogenicity. Furthermore, administration of styrene under these conditions produced no deleterious dose-related effects or decrements in reproductive function through three generations."

SIRC concludes that this study provides *no evidence* of carcinogenicity. It is a particularly relevant study because the level of styrene in the drinking water approximated the maximum possible concentration that can be obtained in drinking water due to solubility limitations, and the rats were constantly exposed to styrene over three generations.

5. National Cancer Institute (1979)

The National Cancer Institute (NCI) (1979) conducted a study using Fischer 344 rats and $B_6C_3F_1$ mice.

(a) Fischer 344 Rats

In the study with Fischer 344 rats, oral doses of 0, 1000, or 2000 mg/kg of styrene in corn oil were given by gavage 5 days per week for 78 weeks. Treatment was stopped after 78 weeks and the animals were observed for another 27 weeks. Because of the high incidence of mortality in rats given the 2000 mg/kg dose level, additional animals were added to the study and given oral doses of 0 or 500 mg/ kg of styrene dissolved in corn oil by gavage 5 days per week for 103 weeks. The 2000 mg/kg dose level exceeds the maximum tolerated dose, since there was excessive styrene-related mortality at this dose level and survival was inadequate to evaluate the risk from late developing tumors. The survival of rats at both the 500 and 1000 mg/ kg dose levels was adequate to evaluate their risk from late developing tumors and these dose levels clearly meet current guidelines of an adequate challenge. It is thus of major importance to note that no increase in any tumor type was observed in rats given either 500 or 1000 mg/kg dose level. This study shows *no evidence* of a carcinogenic response.

(b) $B_6C_3F_1$ Mice

In the study with $B_6C_3F_1$ mice, oral doses of 0, 150 and 300 mg/kg body weight of styrene dissolved in corn oil were administered by gavage 5 days per week for 78 weeks. Treatment was stopped after 78 weeks because of the high mortality in the mice given the 300 mg/kg dose level and the animals were allowed to live for an additional 13-week observation period. The only statistically significant increase in tumors observed in this study was an increase in combined alveolar/bronchiolar carcinomas and adenomas in male mice. The observed incidences of these tumors were

0 of 20 (0%) for controls, 6 of 44 (14%) for the low dose, and 9 of 43 (21%) for the high dose. Although there was an apparent increase in tumors in both the low and high-dose males, only the increase in pulmonary tumors at the high dose level was arguably significant ($p = 0.24$). The authors of the NCI report specifically noted the following:
(1) The $B_6C_3F_1$ mice have a high incidence of spontaneous pulmonary tumors,
(2) The incidence of pulmonary tumors in the control males (0%) was unusually low, and
(3) The incidence of pulmonary tumors in the high-dose male mice did not differ from the historical incidence of these tumors in untreated control mice (32 of 271; 12%) maintained at this laboratory for the NCI Carcinogenesis Testing Program.

The NCI report cites a lower incidence of pulmonary tumors in vehicle-treated mice (0/40) but indicated this was based on only two studies and included too few animals for meaningful use as a historical control value. Based on the historical control data, the authors of the NCI study concluded "that under the conditions of this bioassay, no convincing evidence for the carcinogenicity of the compound was obtained in Fischer 344 rats or $B_6C_3F_1$ mice of either sex."

It is SIRC's opinion that this study is uninterpretable, and concludes that the study provides no clear evidence of carcinogenicity.

6. NCI (1978)

In addition to the nine studies discussed above, a carcinogenicity study in rats and mice on a mixture of 30% ß-nitrostyrene and 70% styrene was reported by NCI in 1978. Although exposure was not to styrene alone, there are no good reasons for discounting the study. Fischer 344 rats were orally administered styrene at dosages calculated to be 700 or 350 mg/kg in males and 350 or 175 mg/kg in females. $B_6C_3F_1$ male and female mice were treated with 407 or 203 mg/kg, respectively. Dosing was for 3 days per week for 79 weeks with further observation for 29 weeks (rat) or 14 weeks (mouse). No increase in any tumor type was noted. This study provides *no evidence* of carcinogenicity.

In summary, *the available long-term animal studies provide no clear evidence of a carcinogenic response* related to styrene. This conclusion is supported by a weight-of-the-evidence approach based on the fact that several studies showed no oncogenic response, together with the fact that there was no consistent target organ or effect (i.e., no organotropism) in the various studies that have been conducted. Hence, the results of the long-term animal studies do not warrant classification or regulation of styrene as a carcinogen.

OTHER DATA

The 1987 IARC Working Group formalized criteria for using genotoxicity data for their carcinogenicity classifications, as previously discussed. Moreover, styrene-7, 8-oxide is known to be an intermediate metabolite of styrene. Hence, genotoxicity data as well as metabolism and pharmacokinetic data for styrene and styrene oxide have some degree of relevance to the overall consideration of the carcinogenic classification of styrene.

1. Genotoxicity Data

SIRC has commissioned a well-known independent authority, Dr. Julian Preston of the Oak Ridge National Laboratory, to review the literature on the mutagenicity/genotoxicity of styrene and its metabolites. Dr. Preston's conclusions on mutagenicity are that styrene is either non-mutagenic or very weakly mutagenic, and that the role of styrene oxide as a mutagenic intermediate is uncertain and requires further study if it is to be adequately assessed. With respect to cytogenetic effects, Dr. Preston has concluded that the data are not adequate for drawing any further perspective on the question of styrene's carcinogenic potential. His conclusions are as follows:

> **Mutagenicity**—The potential mutagenicity of styrene and styrene oxide has been the subject of many studies in a variety of assay systems, both prokaryotic and eukaryotic. The conclusions that can be drawn in part reflect this variety of assay systems, and also the fact that, in most, exogenous activation has been utilized, that introduces a source of variation of response. The use of exogenous activation systems also makes it difficult to extrapolate from *in vitro* assays to the potential effect *in vivo*.
>
> Styrene is not mutagenic in *in vitro* assays without metabolic activation. Its response in the presence of activation is equivocal. It is either very weakly mutagenic or non-mutagenic. More studies are necessary if it is deemed to be important to determine mutagenicity under these particular conditions. The specific assay systems used are perhaps not ideal for the study of compounds that are potentially weakly mutagenic and that need to be analyzed with exogenous activation.
>
> Styrene oxide is mutagenic in the absence of metabolic activation in both prokaryotic an-

deukaryotic assays. However, in the presence of metabolic activation, styrene oxide appears to be equally mutagenic or of reduced mutagenicity compared to its effect in the absence of activation. In addition, its effectiveness is enhanced in the presence of inhibitors of detoxification mechanisms. These results indicate that more studies are needed if the role of styrene oxide as a mutagenic intermediate in the metabolism of styrene *in vivo* is to be adequately assessed.

Cytogenetic Effects—The magnitude of any cytogenetic effects of styrene in *in vitro* assays is difficult to ascertain because of the particular protocols and/or tissue culture media used. However, from a consideration of all the available data from *in vitro* assays, it can be concluded that the effectiveness of styrene at inducing chromosome aberrations lies somewhere between marginal and noneffective in rodent cells and cultured human lymphocytes. Styrene does induce sister chromatid exchanges in cultured human lymphocytes, but not in Chinese hamster cells *in vitro*, even in the presence of metabolic activation. This might be the result of different metabolic activation/deactivation capabilities of different species.

Styrene oxide, a primary metabolite of styrene, and other styrene metabolites are considerably more effective than styrene at inducing aberrations and SCE in *in vitro* cell systems. This is an interesting observation, but does not allow for speculation of the effects of styrene *in vivo* since it is not clear how effectively styrene is metabolized, and what proportion of the metabolites, or styrene itself, actually reaches the cells that are analyzed, namely peripheral lymphocytes. In addition, the balance between activation and deactivation will affect the amount of the different metabolites, and will be highly variable among individuals. This point is exemplified by *in vivo* studies with mice and Chinese hamsters. Styrene appears to induce SCE in mouse bone marrow cells, but is ineffective at inducing either aberrations or SCE in Chinese hamster bone marrow cells. It could well be that an increased level of epoxide hydratase in Chinese hamsters causes a more rapid metabolism of styrene oxide, thereby reducing the effectiveness of styrene through the 7,8-oxide. For humans the kinetics of metabolism have not been studied directly, and so whether the response would be expected to be similar to Chinese hamsters or mice cannot be accurately determined. However, Ramsey and Anderson (1984) provide some data that suggest that inhalation pharmacokinetic models for rats could be used to extrapolate to man.

The studies involving the induction of chromosome aberrations and SCE in human peripheral lymphocytes following exposure in the workplace to styrene (as well as to a variety of other chemicals) are generally considered to be the most relevant for determining the potential risk of styrene exposure. Unfortunately, the published data fall rather far short of allowing any risk estimation. Not only are the studies oten fraught with technical problems (inadequate protocol, small sample size, only superficial control matching, and uncontrolled sources of variation) but the exposure information on chemicals other than styrene is completely lacking. In addition, the potential cytogenetic effects of these other agents is not determined even though *in vitro* and *in vivo* data are sometimes available. The studies reported cannot be used to show that styrene alone can induce chromosome aberrations in human peripheral lymphocytes *in vivo*, and, although the SCE assays have some of the same problems, the results are negative, i.e. no increase in SCE for "styrene exposed" groups compared to controls.

SIRC has also evaluated a DNA adduct study reported by Nordqvist *et al.* (1985). The authors reported that very low levels of radioactivity were covalently bound to DNA following intraperitoneal administration of styrene and styrene oxide. However, even the authors considered these data to be "uncertain due to the low amount of radioactivity" bound to the DNA. In view of the tenuous nature of these findings, they cannot be used to support a carcinogenic classification for styrene.

SIRC considers the mutagenicity and genotoxicity data to be highly important. However, the currently available information on styrene does not permit any meaningful additional insights with respect to the classification and regulation of styrene as a possible human carcinogen.

2. Metabolism and Pharmacokinetic Data

Styrene is known to be biotransformed to styrene-7, 8-oxide via microsomal monooxygenase enzymes (Figure 1). Once formed, styrene oxide is in turn either hydrolyzed via ep-

oxide hydratase to styrene glycol (phenylethylene glycol) or conjugated with glutathione. Several studies have confirmed that styrene metabolism is highly dose (exposure concentration) dependent. In humans as well as in rodents, styrene metabolism is saturated at styrene exposure concentrations in excess of 200 ppm. As a result, toxic effects that occur at high exposure concentrations in excess of 200 ppm cannot be directly extrapolated to low exposure concentrations. This fact is highly important in making human risk estimations based on laboratory animal data.

The toxicological significance of styrene oxide as an intermediate metabolite of styrene remains uncertain. There is concern about styrene oxide because of results from long-term animal studies. A total of seven long-term animal studies have been conducted on styrene oxide; five of those studies involved administration of the material at high doses by gavage (stomach intubation), while in the other two studies it was applied to skin as a 5% or 10% solution in either acetone or benzene. Results of the styrene oxide long-term studies are tabulated in Table 5 and Table 6 for rats and mice, respectively. The gavage studies consistently showed increased incidences of hyperplasia and neoplasia in the forestomach, but there was no convincing evidence of treatment-related tumors in any other organ or tissue in those studies. Hence, there was no indication of systemic oncogenic response in the chronic oral studies.

The significance of the rodent forestomach lesions for human risk estimation remains uncertain, as noted by the authors of the most recent study (Lijinsky *et al.*, 1986):

> The relationship of induction of forestomach lesions to assessment of human cancer risk from exposure to styrene oxide is difficult to determine. Since humans lack a forestomach, it is not possible to say which organs might be at risk .... Although the outcome of this study can be considered reliable, extrapolation from a small group of 50 animals of either sex or species to a potentially large human population is not easy, in the absence of either knowledge about the route of human exposure or more information about the distribution, metabolism, and activation of this compound in animals or humans.

The two skin application studies did not result in the development of skin tumors when styrene oxide was applied three times per week to the shaved backs of mice throughout their lifetime. The absence of an oncogenic response in the skin studies, together with the fact that humans are certainly not exposed to high oral doses of either styrene or styrene oxide, makes it extremely difficult to assess the potential human health hazards from styrene oxide exposure. Moreover, the results of the styrene oxide gavage studies are of even greater uncertainty when making risk estimations for individuals in which styrene oxide occurs only at low levels as a transient, intermediate metabolite of styrene. SIRC is currently sponsoring metabolism and pharmacokinetic studies which will be extremely important in assessing the toxicologic significance of styrene oxide as an intermediate metabolite of styrene.

In view of the uncertainty about the significance of the styrene oxide gavage studies for assessing human health hazards from styrene oxide exposure as well as the even greater uncertainties associated with human risk estimations for styrene oxide as an intermediate metabolite of styrene, it is inappropriate to classify or regulate styrene as a carcinogen based on metabolism concerns.

REGULATORY DECISIONS

Subsequent to the IARC reclassification of styrene in 1987, the carcinogenic classification of styrene has been considered by several important national and international organizations:

A. European Community

The European Communities, consisting of 12 member countries (France, W. Germany, Italy, England, Spain, Greece, Belgium, Holland, Portugal, Denmark, Ireland and Luxembourg), has enacted common legislation for classification and labeling of chemicals. In 1988, the regulatory authorities responsible for classification and labeling of chemicals in the European Community conducted a thorough evaluation of styrene carcinogenicity data. This evaluation included an assessment of the styrene data by the Specialized Experts for Carcinogenicity, Mutagenicity and Teratogenicity, a group of highly respected independent scientists from universities in the various member countries. Despite the 1987 IARC reclassification, the Specialized Experts concluded in 1988 that the available information did not warrant classification of styrene as a carcinogen. Hence, styrene is not being classified, labeled or regulated as a carcinogen in the European Community countries.

B. OSHA/NIOSH

In the United States, both the Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH) have recently evaluated the carcinogenicity data for styrene. In a notice and comment rulemaking conducted during 1988-1989, OSHA proposed to regulate styrene as a carcinogen. 53 Fed. Reg. 20960, 21202-21203 (June 7, 1989). However, after a lengthy hearing, OSHA concluded that:

> The current evidence of styrene's carcinogenicity does not support its classification in the final rule as a carcinogen. (54 Fed. Reg. at 2430, Jan. 19, 1989)

Among the comments filed in this OSHA air contaminants rulemaking, NIOSH noted in its October 1988 submission:

> There seems to be little basis from experimental animal investigations or epidemiologic studies to conclude at this time that styrene is carcinogenic.

Thus, both OSHA and NIOSH concluded in 1988 that the available information did not warrant regulation of styrene as a carcinogen. This is particularly important here in light of OSHA's statutory mandate. Section 6(b)(5) of the Occupational Safety and Health Act, 29 U.S.C. § 655(b) (5), specifically requires that:

> The Secretary, in promulgating standards dealing with toxic materials or harmful physical agents under this subsection, shall set the standard which most adequately assures, to the extent feasible, on the basis of the best available evidence that no employee will suffer material impairment of health or functional capacity even if such employee has regular exposure to the hazard dealt with by such standard for the period of his working life.

Hence, in setting a 50 ppm time-weighted average Permissible Exposure Level (PEL), OSHA concluded that exposure to 50 ppm for 8 hours per day, 5 days per week, for a working lifetime will not result in any adverse health effects to employees occupationally exposed to styrene.

C. EPA Office of Drinking Water/Science Advisory Board

In November 1985, the Environmental Protection Agency (EPA) issued a proposal to establish recommended maximum contaminant levels (RMCLs) for 59 chemicals under the Safe Drinking Water Act (SDWA). 50 Fed. Reg. 46936 (Nov. 13, 1985). As part of this rulemaking, EPA solicited comments on whether styrene should be classified in EPA's Group C (possible carcinogen). *Id.* at 47004-47005. That proposal was never finalized and the Agency did not formally classify styrene as a carcinogen.

Prompted by 1986 amendments to the SDWA, in May 1989, EPA issued a new proposal to regulate styrene and 37 other chemicals. In an unusual step, EPA proposed alternative dual maximum contaminant levels (MCLs) for styrene. 54 Fed. Reg. 22062 (May 22, 1989). These alternative proposals were based on proposed alternative carcinogen classifications of styrene as a Group B2 probable carcinogen or Group C possible carcinogen. In its 1989 proposal, EPA referred to the OSHA and NIOSH positions, clearly indicating that EPA was open to reconsidering the possible carcinogenicity of styrene.

As part of its pre-proposal preparation, EPA prepared a draft criteria document reviewing the toxicological literature on styrene. A panel of the EPA Science Advisory Board reviewed the draft criteria document and concluded that there was insufficient evidence to support a Group B2 classification. The EPA Office of Drinking Water is currently considering the conclusions of the Science Advisory Board as well as extensive public testimony on the proposed regulation. A final decision on the styrene carcinogenicity classification is not expected until late in 1990.

STUDIES IN PROGRESS

SIRC is currently sponsoring metabolism and pharmacokinetic studies which will be extremely important in developing a better understanding of the toxicologic significance of styrene oxide as an intermediate metabolite of styrene. The relative rates of formation and degradation of styrene oxide in rats and mice are being determined, and this data together with *in vitro* metabolism constants for rats, mice and human beings will allow the development of physiological pharmacokinetic model. Important results to date include the quantitation of styrene oxide blood levels in rats given the same high oral doses of styrene oxide (550 or 275 mg/kg) as were used in the chronic study by Lijinski *et al.* (1986). Following oral administration of 550 mg/kg, peak peripheral blood levels of styrene oxide were as high as 13 μg/ ml. Despite that fact, there was no convincing evidence of a systemic oncogenic response in the Lijinski *et al.* studies since the treatment-related tumors were localized to the site of administration (forestomach), as previously discussed. The peripheral blood levels of styrene oxide following a high oral dose of styrene (500 mg/kg), on the other hand, never exceeded 0.5 μg/ml, and thus were far lower than occurred after administration of styrene oxide. Since the metabolism of styrene was saturated following the 500 mg/kg oral dose, it is unlikely that styrene oxide concentrations greater than 0.5 ug /ml would be found in animals given a larger dose of styrene. This data together with other *in vivo* and *in vitro* information is being used to validate a physiologic pharmacokinetic model which will be very important in assessing the potential human health hazards from exposure to styrene.

SIRC is sponsoring an update of the 1978 Environmental Health Associates cohort mortality study. This study will provide additional follow-up on the cohort consisting of 15,908 male and female employees from 30 manufacturing plants in the reinforced plastics industry.

EUROPEAN STUDIES

The styrene industry in Europe is currently sponsoring a number of studies on styrene through the European Chemical Industry Ecology and Toxicology Centre (ECETOC). The ECETOC studies include additional metabolism and pharmacokinetic investigations, focusing on styrene oxide as an intermediate metabolite of styrene following both oral and inhalation exposure. Additional ECETOC studies include protein (hemoglobin) binding and DNA binding assays with both styrene and styrene oxide. Final results of the studies are expected in mid-1990.

NTP STUDIES

The National Toxicology Program (NTP) is currently investigating the carcinogenic potential of styrene in rats and mice. NTP is conducting these studies because they have concluded that the available long-term animal studies on styrene are "... equivocal because of small numbers of animals, the use of obviously toxic doses of styrene, unusually low spontaneous tumor incidences in control groups, and poor survival of control and test animals due to infections."

These new NTP studies will provide important additional perspective about the carcinogenic potential of styrene.

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Table 1

SUMMARY OF FINDINGS FROM EIGHT STUDIES OF STYRENE WORKERS

Author	All Leukemia O	All Leukemia E	*Malignant Lymphoma* HD O	HD E	NHL O	NHL E	All O	All E	All Cancer of Lymphatic and Hematopoietic O	All Cancer of Lymphatic and Hematopoietic E
Meinhardt *et al.*	6.0	3.5	1.0	1.4	2.0	2.4	5.0	3.8	11.0	8.3
Ott *et al.*	6.0	3.4	NR	NR	NR	NR	3.0	0.6	3.0	0.9
Hodgson and Jones	0.0	0.3	NR	NR	NR	NR	3.0	0.6	3.0	0.9
Coggon *et al.*	3.0	6.0	1.0	2.4	1.0	4.2	2.0	6.6	6.0	14.9
Matanoski and Schwartz	17.0	18.7	5.0	6.7	5.0	10.2	13.0	16.9	40.0	47.1
Okun *et al.*	0.0	1.7	NR	NR	NR	NR	0.0	2.1	0.0	4.2
Environ. Health Assoc.	5.0	4.8	3.0	2.4	0.0	2.6	3.0	5.0	9.0	12.3
Nicholson *et al.*	1.0	0.8	NR	NR	NR	NR	1.0	1.3	2.0	2.0

NR = Not reported
O = Observed number of deaths.
E = Expected number of deaths.

Table 2

POOLED RESULTS FROM EIGHT STUDIES OF STYRENE WORKERS

Cancer Type	Obs	Exp	SMR	95% CI
Total Lymphatic and Hematopoietic Cancer	84.0	98.4	85.0	68–106
All Lukemia	38.0	39.1	97.0	69–133
Malignant Lymphoma				
All	34.0	41.5	82.0	51–106
Hodgkin's Disease	13.0	12.9	101.0	54–173
Non-Hodgin's	10.0	19.5	51.0	25–94

Obs = Observed number of deaths.
Exp = Expected number of deaths.
SMR = Standardized mortality ratio = (Obs/Exp) X 100.
95% CI = 95% confidence interval about the SMR.

Table 3

SUMMARY OF LONG-TERM STUDIES WITH STYRENE IN THE RAT

Strain	Route	# Animals Per Group	Exposure Level	Schedule	Duration	Tumor Site/Type	Comments	Reference
BDIV	Gavage	73M 71 F	500 mg/kg Weekly	Weekly	120 weeks	–no significant increase		Ponomarkov &Tomatis, 1978
Fischer	Gavage	50 M 50 F	500, 1000 2000 mg/kg	5/wk	103 weeks –1 wk obs (low dose) 78 weeks –27 weeks observation (high doses)	–no significant increase	–high mortality in top dose	NCI, 1979
Sprague-Dawley	Gavage	40 M 40 F	50, 250 mg/kg	4-5 d/wk	52 weeks –lifetime observation	–no significant increase	–increased mortality in top dose group	Conti *et al.*, 1988
Sprague-Dawley	Drinking water	50 M 70 F	125, 250 ppm	ad lib	2 years	–no significant increase	–no increased mortality –decreased H_2O consumption –decreased terminal body weight in high dose females	Beliles *et al.*, 1985
Sprague-Dawley	Inhalation	30 M 30 F	25, 50, 100 200, 300 ppm	4 hr/d 5 d/wk	52 weeks lifetime observation	–non-dose related increase is mammary tumors in females		Conti *et al.*, 1988
Sprague-Dawley	Inhalation	40 M 40 F	0, 25, 50, 100 200, 300 ppm	4 hr/d 5 d/wk	52 weeks	–no increase in brain tumors	–only brain tissue examined for tumors	Maltoni *et al.*, 1982
Sprague-Dawley	Inhalation	85 M 85 F	600, 1000 ppm[a]	6 hr/d 5 d/wk	M-18.3 mo. F-20.7 mo. –observation until 24 mo.	–non-dose related increase is adenocarcinomas of mammary gland in females; non-stat. signif. increase in combined leukemias and lymphomas in both sexes	–murine pneumonia in control and high dose males –decreased body weights in top close groups –decreased body weights in 600 ppm group for first 263 days in males	Jersey *et al.*, 1978
Fischer[b]	Gavage	50 M 50 F	350, 700 M 175, 350 F	3 d/wk	79 weeks –observation until week 108	–no significant increase		NCI, 1978

[a]**1200 ppm for first two months.**
[b]**Test material was 30% β-nitrostyrene and 70% styrene**

Table 4

SUMMARY OF LONG-TERM STUDIES WITH STYRENE IN THE MOUSE

Strain	Route	# Animals Per Group	Exposure Level	Schedule	Duration	Tumor Site/Type	Comments	Reference
0_{20}	Gavage	45 M 39 F	1350 mg/kg	weekly	16 weeks –observation for lifetime	–increase in frequency and earlier appearance of spontaneously occuring lung tumor	–treatment suspended after 16 weeks due to toxicity –extremely high mortality	Ponomarkov and Tomatis, 1978
$C_{57}B_1$	Gavage	27 M 27 F	300 mg/kg	weekly	120 weeks	–non-statistically significant increase in liver tumors in males		Ponomarkov and Tomatis, 1978
$B_6C_3F_1$	Gavage	50 M 50 F	150, 300 mg/kg	5 d/wk	78 weeks –observation until week 91	–significant increase in lung tumors in high dose males compared to concurrent but not historical controls –non-statistically significant increase in hepatocellular ademona in females	–dose-related increase in mortality in males –dose-related decrease in weight gain in females	NCI, 1979
$B_6C_3F_1$ [a]	Gavage	50 M 50 F	203/407 mg/kg	3 d/week	78 weeks –observation until week 92	–non-dose related increase in lung tumors of male in low dose group only		CI, 1978

[a]**Test material was 30% β-nitrostyrene and 70% styrene.**

Table 5

SUMMARY OF STYRENE OXIDE CARCINOGENICITY STUDIES USING RATS

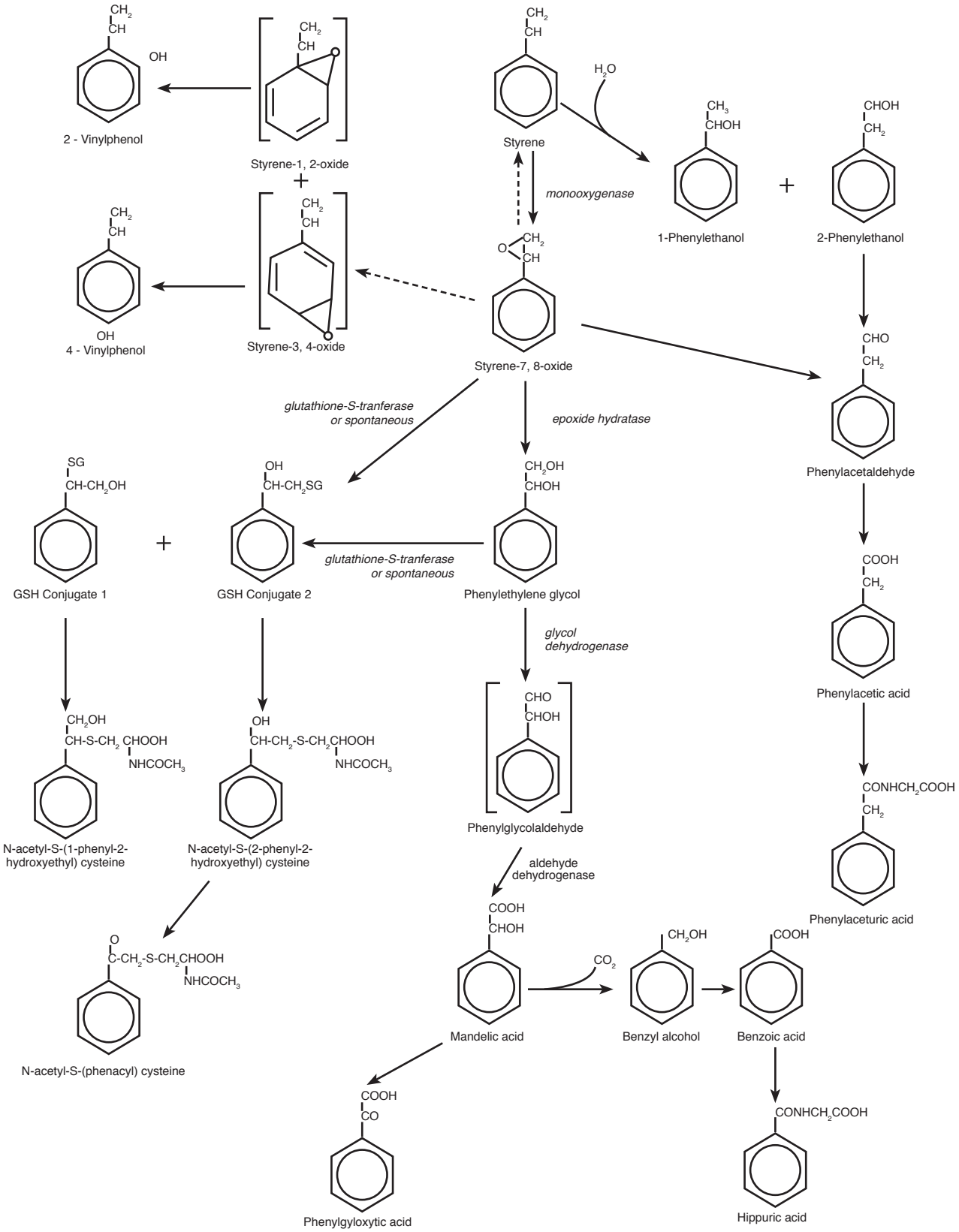
Strain	Route	# Animals Per Group	Exposure Level	Schedule	Duration	Tumor Site/Type	Comments	Reference
Sprague-Dawley	Gavage	40 M 40 F	50, 250 mg/kg/d	4-5 d/wk	52 weeks –104 weeks observation	–dose-related increase in forestomach tumors (papillomas and squamous cell carcinomas)	–non-neoplastic forestomach lesions in treated animals	Maltoni *et al.*, 1979
Sprague-Dawley	Gavage	40 M 40 F	50, 250 mg/kg/d	4-5 d/wk	52 weeks –lifetime observation	–dose related increase in forestomach tumors (papillomas and squamous cell carcinomas)	–non-neoplastic forestomach lesions in treated animals	Conti *et al.*, 1980
BDIV	Gavage	42 M 60 F	100-150 mg/kg/d	once per week	96 weeks –24 week observation	–forestomach papillomas and squamous cell carcinoma		Ponomarkov *et al.*, 1984
Fischer 344	Gavage	52 M 52 F	275, 550 mg/kg	3 per week	–forestomach papillomas and squamous cell carcinomas	–forestomach papillomas and squamous cell carcinoma	–non-neoplastic forestomach lesions in treated animals	Lijinsky, 1986

Table 6

SUMMARY OF STYRENE OXIDE CARCINOGENICITY STUDIES USING MICE

Strain	Route	# Animals Per Group	Exposure Level	Schedule	Duration	Tumor Site/Type	Comments	Reference
C_3H	Dermal	30–40	5, 10%	3 per week	lifetime	none		Weil *et al.*, 1963
Swiss-Millerton	Dermal	30 M	10%	3 per week	lifetime	none		Van Durren *et al.*, 1963
$B_6C_3F_1$	Gavage	52 M 52 F	375, 750 mg/kg	3 per week	104 weeks –3-4 weeks observations	–forestomach papillomas and squamous ceel carcinomas in treated animals –increased liver neoplasms in low dose males	–non-neoplastic forestomach lesions in treated animals	Lijinsky, 1986

FIGURE 1 METABOLIC FATE OF STYRENE



THE MUTAGENICITY ASSAY SYSTEMS

The following sections provide brief descriptions of prokaryotic and eukaryotic mutagenicity assay systems. More detailed information can be found in the references provided.

Prokaryotes

A variety of prokaryotic mutagenicity assays have been utilized for assessing the potential genotoxicity of chemical agents. The one most frequently used is that with *Salmonella typhimurium* often referred to as the Ames assay (Kier *et al.* 1986). The principle of the assay is that mutagenicity is detected as a reversion from histidine auxotrophy (his^-) to prototrophy (his^+). Such reversion can be due to base substitutions (detected by, for example, strains TA1537, TA1538 or TA98). In addition, the involvement of metabolic activation in converting a non-mutagen into a potential mutagen can be assessed by incubating the test agent and the bacteria in the presence of an activating system. A variety of such metabolic activating systems have been employed, but the one most frequently used is the S9 soluble fraction from rodent livers that have been induced by phenobarbital, methylchloranthrene or Arochlor 1254.

The sensitivity of the assay will be influenced in part by the particular strains used, the control frequency for a particular strain, and the S9 inducing agent. A variety of minor protocol variations have been described, particularly for the assay of specific chemical classes, but these will not be discussed here since they were not used in the testing of styrene and its metabolites.

For the analysis of the data discussed in this report, a positive response is considered to be as defined by Dunkel *et al.* (1984) —"a positive response requires a dose-related increase with at least two doses being greater than, or equal to, twofold background, unless the background was less than ten (revertants) in which case a threefold increase is.required." A borderline response is considered to be "a test in which only a single dose was equal to or greater than twofold (or threefold) background."

A similar assay involving *Escherichia coli* strain K12(WP2) has also been fairly frequently used for mutagenicity testing. Reversion from tryptophan auxotrophy (trp^-) to prototrophy (trp^+) is the end-point, with metabolic activation being the same as for *S. typhimurium.* Additional mutations have been incorporated into strain WP2 to increase its sensitivity to mutation induction—most specifically $uvrA^-$ and $recA^-$.

Eukaryotes

There are in excess of one hundred different assay systems that have been used to test the potential genotoxicity of chemical agents. The only ones described here are those that were utilized in the assessment of the potential mutagenicity of styrene and its metabolites. However, cytogenetic assays in mammalian *in vitro* and *in vivo* test systems are not included as they are the subject of a separate report.

Yeast

Saccharomyces cerevisiae and *Schizosaccharomyces pombe* have been used in genotoxicity testing, with point mutations and gene conversion (at many different loci) being the endpoints analyzed. It should be noted that the results on gene conversion, based upon several large comparative studies, have been somewhat equivocal.

Drosophila melanogaster (fruit fly)

The most frequently used Drosophila assay involves the analysis of sex-linked recessive lethals, detected by a decrease in male offspring in the test system. This is a sensitive assay for detecting mutations, and since it is an *in vivo* system does not involve the use of exogenous metabolic activation. However, in order to provide good statistical power, it is essential that a large number of X-chromosomes be analyzed (for a discussion see Lee *et al.* 1983).

Mammalian cells in vitro

Several assays involving rodent and human cells *in vitro* have been used to determine the potential mutagenicity of chemical agents. In the assessment of the mutagenicity of styrene and its metabolites only the Chinese hamster HGPRT-assay has been used. In this assay, forward mutations at the hypoxanthine guanine phosphoribosyl transferase (hgprt) locus can be detected by resistance to the DNA base analogs thioguanine (TG^R) or azaguanine (AZ^R); wild-type cells ($hgprt^+$) are sensitive to these analogs. In the case of the studies on styrene and its metabolites Chinese hamster V79 cells were employed, although chinese hamster ovary (CH_O) cells have also been frequently used. There are several features of the protocol that have to be considered: among these are the interval between treatment and addition of selective agent (expression time) must allow for maximal mutant frequency and cell killing should not be greater than about 20% (See Li *et al.*, 1988). Exogenous metabolic activation systems can be incorporated into the assay; the most frequently used being the S9 fraction of induced rat liver.

R.J.P.

The Potential Mutagenicity of Styrene and its Metabolites

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Styrene has been used extensively in a wide range of industrial processes for about 40 years, and it is estimated that about 6 million tons are produced annually throughout the world. The major use is in the production of plastics and polyester resins. Thus, there is the possibility of occupational human exposure that requires a consideration of the possible adverse health effects that would arise from such exposure. The health effects that are frequently assessed are somatic (generally cancer) and genetic. This report will not discuss data on the possible carcinogenicity of styrene, but will concentrate on mutagenicity studies, and particularly on published data on assays that measure point mutations. The conclusions drawn relate to mutagenicity and are not interpreted in terms of potential for carcinogenicity, as it is not considered that this is a legitimate approach.

The published literature on the mutagenicity of styrene and its metabolites is reviewed for each of the assay systems described on the facing page, although it will be apparent that the great majority is for the Ames assay. The studies are considered from the point of view of adequacy of the protocol and statistical significance of the results [a fairly complete review of the toxicology, including a discussion of mutagenicity, can be found in Bond (1989)].

Dr. Preston discusses the published data on mutagenicity with respect to styrene and its metabolites, the adequacy of the data and their statistical significance. He concludes that styrene is not mutagenic in *in vitro* assays unless there is metabolic activation. Even when there is activation, however, styrene remains nonmutagenic or is only very weakly mutagenic. The metabolite styrene oxide is mutagenic without activation, but with activation its mutagenicity either does not change or is reduced. Overall, styrene is not mutagenic, but the role of styrene oxide as a mutagenic intermediary in the metabolism of styrene *in vivo* cannot be adequately assessed from existing evidence.

PROKARYOTES

1. Salmonella typhimurium

Although there is quite an extensive literature on the mutagenicity testing of styrene and its metabolites in *Salmonella typhimurium* strains, with and without metabolic activation, there is a repetitiveness about the studies. However, this has the advantage that it is possible to compare the conclusions from several different laboratories, and perhaps reach a consensus.

Milvy and Garro (1976) studied the induction of mutations by styrene, styrene oxide (the primary metabolite of styrene) and other metabolites in several strains of *S. typhimurium:* TA1535 and TA100 that detect base substitutions and TA1537, TA1538 and TA98 that detect frame-shifts. All assays were conducted in the absence of exogenous metabolic activation. Styrene and styrene oxide were tested at the single concentration of 5µl/plate. Styrene was not mutagenic in any of the strains, whereas styrene oxide was mutagenic in TA1535 and TA100. The other metabolites tested were all negative for mutagenicity.

Vainio *et al.* (1976) studied the mutagenicity of styrene and styrene oxide in *Salmonella* strains TA1535, 1537, 1538, 98 and 100 in the absence of presence of S9 (Clophen C induced rat livers). Styrene was not mutagenic in the absence of S9, but mutagenic in TA1535 and 100 in the presence of S9. However, there is no enhancement in the presence of S9 and 3,3,3-trichloropropene (TCPO, an inhibi-

tor of epoxide hydratase) or diethylmaleate (an inhibitor of glutathione-S-transferase). This is difficult to explain, but perhaps indicates a problem defining styrene as being truly mutagenic in the presence of S9. The authors suggest that styrene could be a direct-acting mutagen, but this is certainly not supported by their own data. Styrene oxide was equally mutagenic in TA1535 and 100 with or without activation. The mutagenicity of styrene oxide with S9 activation was enhanced in the presence of TCPO or diethylmaleate. This indicates that epoxide hydratase and glutathione S-transferase are involved in a detoxification mechanism.

An additional note on these studies concerns the possible influence of toxicity on the results and the interpretation. The authors state that styrene and styrene oxide were toxic to all strains at high concentrations (10^{-6} moles/plate and above for styrene, and 10^{-4} moles/plate and above for styrene oxide). As they correctly concluded "This high toxicity made the mutagenicity determinations at this range of concentration difficult," and "the toxicity of both styrene and styrene oxide makes the construction of reliable dose-response curves rather difficult." Thus, these data should be considered preliminary and generally inconclusive.

de Meester *et al.* (1977a) conducted a similar study with the same strains, and included an S9 fraction from Arochlor 1254 induced rat liver as a metabolic activating system. They also found that styrene was not mutagenic to strains TA1535, 1537, 1538, 98 and 100 in the absence of S9, tested from 1 nmole to 100 μmole/plate. In the presence of S9, styrene was positive in strain TA1535 but over a concentration range (1 μmole-11 μmole/plate) at which appreciable toxicity to the bacteria was observed. Interestingly mutagenicity was not observed with TA100 that also detects base substitutions. Styrene oxide was mutagenic in TA1535 and TA100 in the absence or presence of S9 at concentrations above 100 nmole/plate.

de Meester *et al* (1977b) tested styrene, styrene oxide and styrene glycol in strains TA98, 100, 1530 and 1538 in the presence or absence of S9 from Arochlor 1254 induced rat liver. The complete data are not presented and so no critical review can be provided. The authors' conclusions are that styrene was non-mutagenic in the absence of S9, but mutagenic with S9 in TA1535. Styrene glycol was not mutagenic in any of the strains tested up to 10 mg/plate.

Stolz and Witney (1977) studied the mutagenicity of styrene and styrene oxide in *Salmonella* strains TA1535, 1537, 1538, 98 and 100. The S9 fraction used was from Arochlor induced livers from rats and hamsters. Styrene was not mutagenic in any of the strains even in the presence of S9 up to a concentration of 1 mg/plate [higher than that used by de Meester *et al.* (1977a, b)]. The lack of mutagenicity in the presence of S9 is in contrast to the results of Meester *et al.*, and no explanation can be offered, although differences in metabolic conversion might be a factor. Styrene oxide was mutagenic in strains 1535 and 100, in agreement with the data of Milvy and Garro (1976, discussed above). The authors state that no conclusion on the potential carcinogenicity of styrene can be made from their results. This is a correct conclusion, and indeed can be applied to other similar results described above (de Meester *et al.*, 1977b).

Styrene is not mutagenic in *in vitro* assays without metabolic activation. Its response in the presence of activation is equivocal. It is either very weakly mutagenic or non-mutagenic.

Further information on the metabolism of styrene is provided by Watabe *et al.* (1978). They studied the mutagenicity of styrene in TA100 (the tested strain found to be the most sensitive to styrene oxide) in the presence of S9 from phenobarbital or methylchloranthrene induced rat livers. Metabolic activation was also conducted in the presence of TCPO an inhibitor of epoxide hydratase, in order to enhance the accumulation of styrene oxide. In summary, they found that styrene at doses of 3 or 6 mM was mutagenic only in the presence of S9, and only when TCPO was included with the S9 fraction from methyl chloranthrene-induced animals. Styrene oxide was mutagenic in the absence of S9. The importance of this observation is that any mutagenicity of styrene when metabolized could be expected to be greatly influenced by epoxide hydratase levels.

Busk (1979) conducted a careful study on the mutagenic potential of styrene and styrene oxide in *Salmonella* strains TA1535, 1537, 1538, 98 and 100. Styrene oxide was mutagenic in TA1535 and 100 in the presence or absence of S9, prepared from Arochlor 1254 or Clophen C induced rat

livers. This observation seems to be a general one throughout the reported studies. Styrene (over a dose range of 10^{-3} - 15 μmoles/plate) was non-mutagenic in the absence or presence of S9. In addition, a negative response was also obtained when the metabolic activation was conducted in the presence of TCPO or an inhibitor of the glutathione conjugation pathway (diethyl maleate). This result is very different from that of Watabe *et al.* (above), and serves to emphasize the fact that responses observed in the presence of exogenous metabolizing systems can be very variable not only in terms of level of response, but also whether or not there really is a mutagenic response.

de Meester *et al.* (1981) repeated some of their earlier studies (1977a, b) but used gaseous exposures. Styrene at 24% (v /v; styrene/air) was not mutagenic to TA1530, 1535 or 100 in the absence of S9. The authors report an increase in reversion for TA1530, but the increase is below the accepted criterion for a positive response of equal to or greater than twice the control frequency. In the presence of S9 there is an increase in mutagenicity for strains TA1535 and 100 as reported previously (1977a, b), and for 1530, in contrast to these earlier studies. Additional studies with TA1535 were performed, varying the styrene concentration from 2.5 to 13.3 ppm. By applying the criterion for a positive response of twice background, it can be concluded that styrene was mutagenic at 10.6 and 13.3 ppm in the absence of S9. The authors state that a dose-effect relationship was observed—this is not supported by their data (de Meester *et al.,* 1981, Figure 3). In the presence of S9 (100 μl S9 /ml) a positive response was observed at 8.0 and 10.6 ppm, but not at 13.3 ppm. In addition, if the concentration of S9 is varied from 25-250 μl S9 /plate, at a styrene concentration of 24% (v/v, styrene/air), there is either a reduction in revertants or no change when compared to styrene exposures in the absence of S9. Styrene was mutagenic in this experimental series in the absence of S9, but at a level that was only slightly above the twice background criterion. All the data in this study are reported as histograms, with no statistical analysis included, and so the conclusions should be considered as equivocal.

Styrene oxide was mutagenic in strains TA1530, 1535 and 100 in the absence of S9 at a concentration of 24% (v/v). In the presence of S9 there was a reduction in revertant frequency.

Dunkel *et al.* (1985) conducted an extensive study on the mutagenicity of 63 chemicals, including styrene in 5 strains of *Salmonella* (TA98, TA100, TA1535, TA1537 and TA1538) and E. coli WP2 uvrA (see section on *E. coli).* Four different laboratories participated in the study, and several exogenous metabolic activation systems were included (S9 - preparations from uninduced and Arochlor 1254-induced F344 rats, B6C3F1 mice and Syrian hamsters). The dose range for styrene was 0.3 to 333.31.μg /plate. Negative responses were reported for all *Salmonella* tester strains at all doses with or without metabolic activation. This is a significant result because of the extent of the data, and the fact that four laboratories reached identical conclusions using the same test protocols.

El-Tantawy and Hammock (1980) studied the mutagenicity of several epoxides, including styrene oxide, in *Salmonella.* Styrene oxide was mutagenic in TA1535 and 100 but not in TA1537 or 98. *It is important to note that there was a reduction in mutagenicity in the presence of S9. The reduction varied according to the source of the S9 and was generally related to epoxide hydratase activity. This further points to the difficulty that can be encountered when using exogenous metabolic activation systems as a surrogate for in vivo responses.*

The consequences of metabolic detoxification on the mutagenicity of styrene oxide was further studied by Yoshikawa *et al.* (1980). Styrene oxide was mutagenic in the absence of S9 in TA100. However, this activity was reduced in the presence of S9, and almost completely absent when 2 mM glutathione was added. The concentration of styrene oxide was shown to be reduced in the presence of glutathione. These results show the importance of detoxification in the magnitude of the mutagenic response, and additionally show that glutathione-S-transferase is possibly more important than epoxide hydratase. This latter conclusion is different from that in other studies, but the role of glutathione-S-transferase had not really been considered in these earlier studies (see above).

Watabe *et al.* (1982) further considered the effectiveness of metabolites other than styrene oxide as possible mutagenic intermediates in the metabolism of styrene. The synthesized 1-vinylbenzene-3,4-oxide that is a very short-lived compound (4.3 sec in aqueous solution). This compound was very mutagenic to TA100 but non-mutagenic to TA98. It was considerably more mutagenic than styrene oxide. This is a potentially interesting result, but at this point provides little information on the mutagenicity of the 3.4-oxide following exposure of *Salmonella to* styrene because there is no information on its production in the Salmonella/microsome assay system.

2. Escherichia coli

There are only three published studies on the potential mutagenicity of styrene and/or styrene oxide in *E. coli.* It is not really possible to provide a definitive comparison between results with *Salmonella* and *E. coli,* but they are discussed here for completeness.

Dunkel *et al.* (1985) in an extensive study of the mutagenicity of 63 carcinogens and noncarcinogens (described above under *Salmonella)* tested styrene in the *E. coli* WP2 uvrA assay system. Four laboratories utilised an identical protocol and included 6 different metabolic activation systems. Three laboratories reported negative responses with and without metabolic activation (for all six S9 systems); the fourth laboratory reported a borderline response without activation, and for three activation systems (rat liver S9, mouse liver S9 and hamster liver S9, all three being Arochlor-induced). The reason for this minor discrepancy is not discussed, although the authors concluded (correctly it would appear when all their data are considered) that styrene was negative for mutagenicity with or without activation of *E. coli.*

Sugiura *et al.* (1978) conducted a study that was designed to determine structure-activity relationships between styrene oxide and a series of substituted styrene oxides (3,4-dimethyl-, p-methyl-, m-methyl-, p-bromo, and m-chloro-). The end point studied was reversion from trp^- to trp^+. The strains were WP2 (repair proficient) and repair deficient strains WP2($uvrA^-$), CM571($recA^-$ and WP100 ($uvrA^-$, recA). Styrene oxide was mutagenic in all four strains, and was more effective in the repair deficient strains suggesting that styrene oxide-induced DNA damage is repaired by an excision repair process, as would be the case for a DNA adduct. These observations were confirmed by Sugiura and Goto (1981) together with the finding that the primary site of alkylation was the N7 position of guanine.

Conclusions: Prokaryotes

The published data on the mutagenicity of styrene and styrene oxide in prokaryotes (almost exclusively *Salmonella)* can be summarized fairly succinctly, although the differences in response in different laboratories does not allow for a unique conclusion.

The variety of responses of the effect of styrene in prokaryotic systems makes definitive conclusions difficult. The consensus is that styrene is not mutagenic in *Salmonella.* The most significant data appear to be those of Dunkel *et al.* (1985) who reported a clear-cut negative response in a four laboratory study using a standard protocol. In the presence of S9, styrene appears to be either very weakly mutagenic in strains that detect base substitutions or non-mutagenic. Again the data of Dunkel *et al.* (1985) are of particular significance, since they report a negative response in four laboratories using six different exogenous metabolic activation systems derived from three species (induced or non-induced). In other studies the variability in response in the presence of S9 (not in its absence) serves to highlight the difficulty of utilizing exogenous activation as a "model" for *in vivo* effects. In addition, detoxification mechanisms are known to be acting *in vivo,* and so a weak mutagenic response *in vitro* in the presence of S9 (if indeed it is induced) would be of little significance for predicting an *in vivo* response.

Styrene oxide appears to be mutagenic in the absence of exogenous metabolic activation in *Salmonella* strains that detect base substitutions. In the presence of S9 there is either no change or a reduction in mutagenicity. Inhibition of epoxide hydrolase or glutathione-S-transferase either enhances or maintains the effectiveness of styrene oxide. Again the variability in response where S9 is included adds to the difficulty of predicting *in vivo* effects from *in vitro* assays utilising exogenous metabolic activation systems.

EUKARYOTES

1. Yeast

There is one report of the mutagenicity of styrene in yeast (Loprieno *et al.,* 1976) with point mutations and gene conversion as end-points. The studies were conducted with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe in vitro* and in a host-mediated assay. For concentrations up to 100 mM of styrene, there was no increase in forward mutations at five adenine loci in *S. pombe* without or with metabolic activation (purified mouse liver microsomes). Similarly, a negative response was obtained for gene conversion at the ade_2 and trp_3 loci of *S. cerevisiae,* with or without activation.

Styrene oxide over a concentration range of 0-20 mM produced a positive response for point mutations and gene conversion in the absence of metabolic activation.

For the host-mediated assay the yeast *(S. cerevisiae* or S. *pombe)* were injected i.p. into Swiss albino mice, and styrene at a single dose of 1000 mg /kg was administered. The yeast was sampled at 1, 3, and 6 hours after styrene injection. Gene conversion was increased at all sampling times, with a maximum of 5 times background at 6 hours. There was no increase in point mutations at any sampling time. It should be noted that gene conversion has not been a very consistent indicator of mutagenicity; it has shown a positive response for a variety of chemical agents (particularly non-carcinogens) when other assays have shown a negative response.

These data, since they are derived from only one study, cannot be used to draw definitive conclusions on the mutagenicity of styrene in yeast, but they do add to the overall data base for eukaryotic assays.

2. Drosophila

A single study by Donner *et al.* (1979) was sufficiently complete for assessing the mutagenic potential of styrene and styrene oxide in Drosophila. The end-point was sex-linked recessive lethals. Styrene exposure was by feeding at 200 ppm for 24 hrs and styrene oxide by feeding or by vapor at 200 ppm in an exposure chamber. The study was small in size and so the conclusions must be considered as non-conclusive. Both styrene and styrene oxide increase the frequency of mutations over the control values. There is an increase in effectiveness when phenobarbitone was administered before styrene or styrene oxide, and also when TCPO (an inhibitor of epoxide hydrotase) was used as a pretreatment to styrene oxide exposure.

An abstract by Sorsa *et al.* (1978) also reports the effects of styrene and styrene oxide on the induction of sex-linked recessive lethals in *Drosophila melanogaster.* The chemicals were administered by feeding for 24 hours. Styrene (LC_{50}, 550 ppm) was seven times more toxic than styrene oxide. Male flies treated with three concentrations (not given) of styrene and styrene oxide were mated twice to *Base*-females during days 1-3 and 4-6 after treatment. There was no significant increase in X-linked recessive lethals with either styrene or styrene oxide. However, the methods and data are insufficiently reported for definitive conclusions to be drawn.

The results of Wirtz and Powers (1983) on the induction of sex-linked recessive lethals in *Drosophila* reared in glass and plastic vials will not be considered here, because it is not possible to determine if styrene exposure even occurs.

3. Mammalian cells in vitro

Loprieno *et al.* (1978) assessed the mutagenicity of styrene and styrene oxide at the HGPRT locus in V79 Chinese hamster cells. An S10 metabolic activation system was included in the studies with styrene. Styrene was nonmutagenic at a concentration of 51 mM in the presence or absence of S10 (6-thioguanine selection). Styrene oxide was mutagenic at concentrations of 4.2, 8.5 and 17 mM in the absence of S10 (selection with 6-thioguanine or 8-azaguanine).

Bonatti *et al.* (1978) conducted a study of the induction by styrene oxide of forward mutations in V79 Chinese hamster cells at the HGPRT locus (azaguanine or thioguanine sensitivity to resistance). The concentrations of styrene oxide were 8.5 or 17 mM and treatment was for 1 hr. There was an increase in mutants under both conditions for selection at both concentrations. The study also showed that the time used for mutant expression is very important for maximizing the potential response.

Beije and Jenssen (1981) studied the mutagenicity of styrene and styrene oxide by using the isolated perfused rat liver as a metabolizing system. Mutant induction by the perfusate was assessed in V79 Chinese hamster cells (6-thioguanine resistance). Exogenous metabolism by S9 was used for comparative purposes. The levels of styrene and styrene oxide in the liver perfusate were determined in order to better understand the potential for styrene oxide to induce a mutagenic response *in vivo.* This series of experiments is somewhat complicated to interpret, but the results can be generally summarized as follows.

Styrene is not mutagenic without activation. In the presence of S9 the authors state that there is "a very weak effect" when the results from several experiments were combined. The number of mutant colonies is very small, generally less than 1 per 10^5 cells plated (when corrected for survival) and only 10^5 cells were seeded on each of six plates per treatment group. These data have to be considered as inconclusive. Using the liver perfusion system the authors concluded that styrene is either very weakly mutagenic (two experiments) or non-mutagenic (one experiment). However, the control frequency in the two "positive" experiments is very high, and the induction of mutants only very slight (less than two-fold). These results have to be considered as inconclusive.

Styrene oxide is not mutagenic in the liver perfusion assay. This result is expected from the analysis of the liver perfusate, since it was shown that the levels of styrene oxide were very low even a short time after exposure. Also when styrene was added to the perfusion system, the amount of styrene oxide produced was only about 2% of the initial styrene concentration, indicating that styrene oxide is not likely to be a major mutagenic metabolite *in vivo.* This assay system further shows that responses *in vivo* are not readily modeled *in vitro.*

Conclusions: Eukaryotic Assays

The mutagenicity of styrene is inconclusive. A positive response was reported with Drosophila, but a negative response is observed in mammalian cells *in vitro* with S10 or S9, and is marginally or non-mutagenic in the liver perfusion/Chinese hamster cell assay. Clearly more data are needed before any firm conclusions can be drawn. Such data should include a careful assessment of the metabolism in different systems and organisms, and how these might relate to the situation *in vivo,* particularly in man.

There is very clearly a need for caution in making predictions of the potential mutagenicity of any agent (in this case styrene) in humans from data obtained *in vitro* in cell assay systems with or without exogenous metabolic activation. The data for styrene discussed in this report present a good argument for this opinion.

Styrene oxide appears to be mutagenic *in vitro* in the

absence of metabolic activation. However, it is less clear whether or not it is mutagenic when metabolized *in vitro* or *in vivo:* negative with the liver perfusion system for mutations at the HGPRT locus in Chinese hamster cells; positive in Drosophila for sex-linked recessive lethal induction. The potential for differences in metabolism in different species is a plausible explanation for the observed responses.

OVERVIEW

The potential mutagenicity of styrene and styrene oxide has been the subject of many studies in a variety of assay systems, both prokaryotic and eukaryotic. The conclusions that can be drawn in part reflect this variety of assay system, and also the fact that, in most, exogenous activation has been utilized, that introduces a source variation of response. The use of exogenous activation systems also makes it difficult to extrapolate from *in vitro* assays to the potential effect *in vivo.* This summary represents a consensus drawn from the literature reviewed.

Styrene is not mutagenic in *in vitro* assays without metabolic activation. Its response in the presence of activation is equivocal. It is either very weakly mutagenic or nonmutagenic. More studies are necessary if it is deemed to be important to determine mutagenicity under these particular conditions. The specific assay systems used are perhaps not ideal for the study of compounds that are potentially weakly mutagenic and that need to be analyzed with exogenous activation.

Styrene oxide is mutagenic in the absence of metabolic activation in both prokaryotic and eukaryotic assays. However, in the presence of metabolic activation styrene oxide appears to be equally mutagenic or of reduced mutagenicity compared to its effect in the absence of activation. In addition, its effectiveness is enhanced in the presence of inhibitors of detoxification mechanisms. These results indicate that more studies are needed if the role of styrene oxide as a mutagenic intermediate in the metabolism of styrene *in vivo* is to be adequately assessed.

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The Environmental Fate of Styrene

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Dr. Alexander reviews the research and substantive monitoring data on the fate of styrene in water, soil and the atmosphere. He concludes that the transport of styrene in nature is "very limited" because of its volatility from soils and surface waters, its rapid destruction in air, and its biodegradation in soils and surface and ground waters. The most probable source for any human exposure is the atmosphere, especially urban air, where values up to 6.0 ppb have occasionally been recorded. However, because styrene is highly reactive and rapidly destroyed by ozone and hydroxyl radicals, it is unlikely to be transported to any significant extent, or to be a source of styrene in waters or soils. There is little possibility of styrene occurring in drinking water or entering the food chain.

This review of the data on the environmental fate of styrene in water, soil and the atmosphere (addressed in that order) was prepared at the request of the Styrene Information and Research Center, which was particularly interested in any occurrences of styrene in drinking water. As I will discuss, there is already a vast amount of monitoring data on this subject, indicating that styrene is highly unlikely to occur in drinking water, from whatever source. Water of any kind is a very minor source of potential exposure. Of the three elements, air is the most likely source of exposure. The data are generally firm, the trends are evident, and no major questions exist about the methodologies or the quality of the data.

The solubility of styrene in water is reported to be 280 mg /L at 15°C and 300 mg/ L at 20°C (Verschuren in Sax, 1987).

WATER

Although it is thus quite soluble in the water column of surface waters, styrene is quite prone to become adsorbed to aquatic sediments and probably particulate matter in the water. Indeed, the styrene *concentration* in the exposed bottom sediment and sediment suspended in natural waters is expected to be higher than in the water column (USEPA, 1987A), and styrene has been detected at 4.2 ng/g in sediment from the lower Tennessee River (Goodley and Gordon, 1976) and at an unstated concentration in a Canadian lake (Samolloff *et al.,* 1983) Nevertheless, the *amount* of styrene in bottom and suspended sediments is unlikely to exceed 10% of that in most surface water (USEPA, 1987A).

Because of the relatively small amount of styrene absorbed in suspended or bottom sediment, removal of the chemical from surface water by adsorption and transport in surface waters through moving particulate matter are not significant in most surface waters (USEPA, 1987A).

Styrene is rapidly lost from surface waters by volatilization. The half-life for loss is reported as 1 to 3 h (USEPA, 1987B), 45 min to 51 days based on assumed environmental conditions, 6 h at 1 meter to 60 h at 10 meters in a turbulent lake (USEPA, 1987A), 3 days from a pond to 13 days from an oligotropic lake (USEPA, 1985), and 24 h at a depth of 1 meter (Santodonato *et al.,* 1980). These estimates suggest rapid loss from surface waters, although loss in deep, stagnant water and groundwater is probably slow or of no consequence when compared to other mechanisms of loss.

At conditions prevailing in natural waters, styrene is not subject to abiotic hydrolysis or abiotic oxidation (Santodonato *et al.,* 1980; USEPA, 1987). Nevertheless, apart from a single observation that it did not degrade in the dark (Toole and Crosby, 1988), experimental data are lacking. The half-life for the reaction of styrene with peroxy radicals in waters is long (more than 800 days), and this re-

action is of no environmental importance (USEPA, 1985). In addition, the absence of O_2 in many groundwaters precludes any O_2- requiring reactions.

It has been proposed that styrene decomposition in water by direct action of sunlight is of no consequence (Santodonato *et al.,* 1980; USEPA, 1985). This is because styrene absorbs UV light at wavelengths below 300 nm while the sunlight reaching the earth's surface has UV at wavelengths greater than 300 nm. However, except for the study of Toole and Crosby (1988), experimental data are lacking. In the Toole *et al.* study, styrene (1.1 mg/L) at pH 8 was not degraded in simulated sunlight in distilled water. However, under the same conditions, styrene contained in seawater was converted to organic products, mainly styrene oxide. On the other hand, the absence of light in groundwater precludes light-requiring processes.

During the chlorination of water, epichlorohydrin (1-choloro-2, 3-epoxypropane) may be formed from styrene (USEPA, 1987A).

An extensive amount of monitoring has been conducted in the United States to determine styrene levels in drinking waters, and in surface and groundwater that serve as sources of drinking water. In the National Screening Program for Organics in Drinking Water involving samples collected in 1977-81, styrene was not found in finished drinking water obtained from 102 surface water supplies and 12 groundwater supplies (Boland, 1981). A survey of 945 sample sites, both random and non-random, of municipalities divided into populations of greater than and less than 10,000 people failed to find a single site containing styrene (Westrick *et at.,* 1984). Of 1174 community wells and 617 private wells analyzed for chemical contamination in Wisconsin, 1790 contained no detectable levels of styrene, and only 1 was positive (Krill and Sonzogni, 1986). The detection limits in these analyses were 0.3 or 0.5 μg/L. Similarly, a survey of information from 1970-1985 on drinking water from the Great Lakes demonstrated that most water samples did not contain detectable levels of styrene, or the levels reported were near the detection limit. A few of the samples from 31 United States and Canadian drinking waters and 24 Great Lake sites had very low levels (Canadian Public Health Association, 1986). Earlier analytical studies, however, have reported detecting styrene in unspecified concentrations in occasional samples from the Philadelphia water treatment plant (Suffett *et al.,* 1980), in Cleveland drinking water (Sanjivamurthy,1987), in the drinking water from the Ohio River of Evansville, Indiana (Kloepfer and Fairless, 1972), at 0.024 μg/L in the Cincinnati water supply (Coleman *et al.,* 1984) as well as in groundwater in the Netherlands (Zoeteman *et* at., 1981). A 1987 review by EPA states that styrene was not detected in three national surveys of drinking water nor in a large number of samples from state and local surveys. The same report states that 272 sites in Kansas, Missouri and Nebraska analyzed in 1982 were free of styrene, although the drinking water from three of eight cities (analyzed in another study) using surface water had levels that were not quantified (USEPA 1987A). It is worth emphasizing the extremely low levels of styrene that can now be detected and identified; e.g., the level of 0.024 μ/L reported by Coleman *et al.* (1984) represents 24 parts per trillion. This mass of information led to the EPA conclusion that styrene is not likely to occur in drinking water (USEPA, 1987A).

This mass of information led to the EPA conclusion that styrene is not likely to occur in drinking water.

Analyses have also been conducted of other waters. Samples from the Delaware River between Trenton, NJ and Marcus Hook, PA collected in 1976-1977 were nearly always free of detectable levels of styrene, although one sample contained trace amounts (Sheldon and Hines, 1978). Styrene at unquantified levels has also been found in the lower Tennessee River (Gordon and Goodley, 1971) and in the Waal River in the Netherlands (Meijers and Vanderveer, 1976). The Rhine River may have concentrations of 0.01 to 0.1 μg/L (Pagga, 1987; Zoeteman *et al.,* 1980).

Industrial waste waters have been shown to contain styrene. These include waste water from a petrochemical company in unquantified concentrations and at 2.6 μg/L in the waste water of a factory that used styrene in the manufacture of synthetic rubber. Other industrial waste waters tested in that survey were styrene free (Keith, 1974). EPA reports styrene's presence in effluent from a latex plant, a chemical plant, and a textile plant in Kentucky (Shackleford and Keith, 1976) and at levels below 10 μg/L in unspecified industrial waste waters (Perry *et al.,* 1976).

SOIL

The half life for the volatilization of styrene at the soil surface has been estimated to be about 1 min, but the rate of

volatilization becomes slower with increasing depth (USEPA, 1987A).

The mobility of styrene through soil is of critical importance because transport of the chemical downward from the upper zones could result—in the absence of degradation or volatilization—in its presence in groundwater, and the subsequent mobility of the chemical through groundwater could result in the spread of the chemical. Such movement is inversely related to the degree of its adsorption to soil or aquifer solids. The greater the adsorption, the less the transport. For a substance like styrene, the degree of adsorption is related to the organic matter content of the soil; the more organic matter present, the more styrene retained. The effect of soil organic matter in adsorbing (and slowing movement) styrene in water passing through soil is similar to that of a solvent like n-octanol; therefore, the relative amount of styrene in a mixture of n-octanol and water (the so-called octanol-water partition coefficient, K_{ow}) is often used to estimate the relative mobility of a chemical. The relative mobility can also be estimated from the soil (or sediment) sorption constant which is :

$$\frac{\text{[amount of chemical retained per gram in soil]}}{\text{[amount remaining per gram of water]}}$$

and is normalized for organic matter level (K_{oc}. The value of K_{oc} represents the amount of chemical in a soil (sediment)-water mixture that is retained by the soil (or sediment), normalized to reflect the influence of different amounts of organic matter in the soil or sediment. The value of K_{oc} for styrene has been calculated to be 900 (estimated from K_{ow}) and 567 (estimated from aqueous solubility) (USEPA, 1987A). These values of K_{oc} place styrene in a class of chemicals having low mobility (Swann *et al.,* 1983). When compared to a chemical that is not adsorbed, as a rule, it thus would move only slowly through soil into groundwater.

During this slow vertical transport, it would also be subject to biodegradation. The extent of entry of styrene into groundwater from overlying soil would then be a balance between the rate of its movement—which is believed to be slow—and the rate it is destroyed by microorganisms in soil. If the biodegradation is rapid relative to the slow rate of movement through the soil, styrene will not reach the groundwater.

Styrene does occasionally reach groundwater, however. This movement is evident in a study of groundwater recharge using the effluent from a municipal activated-sludge treatment plant. Styrene was present in the effluent that was pumped into a well dug to allow for the groundwater recharge, and it was also present at a site 8 meters from the injection well (Roberts *et al.,* 1980). In a municipal waste water infiltration system in Massachusetts, the styrene level fell from 605 ng/L in the influent water to a level of 4.7 ng/L in an adjacent well (Bedient *et al.,* 1985). Styrene originally present in drums buried below the surface has also been observed to leach downward into underlying aquifers and also to migrate laterally at least 90 meters (Grossman, 1970). Styrene has also been found (in unspecified concentrations) in the leachate from an industrial landfill in New Jersey that had received wastes several years earlier (Kosson *et al.,* 1985).

The instances where styrene demonstrated movement and persistence may have resulted from (a) relatively rapid mobility through surface or subsurface soils and aquifers having little organic matter or through cracks, fissures or macropores in the solid matrix or (b) low biodegradative activity.

BIODEGRADATION

The process of biodegradation results in significant destruction of styrene provided conditions are suitable for the active microorganism. The major unsuitable conditions are probably associated with hazardous waste sites and possibly anaerobiosis. It is likely that the mixture of high concentrations of organic and inorganic toxicants at hazardous waste sites precludes significant microbial activity, and the information available on the effect of anaerobiosis suggests that the microbial destruction is slow or may not occur.

Biodegradation is largely (or entirely) a microbial process in soils, groundwater aquifers, and surface and waste waters. That biodegradation is of major importance is evident in the findings that styrene is not destroyed in sterilized subsurface materials (sterilization killing microorganisms) (Wilson *et al.,* 1983) and is not mineralized in sterile soil (Sielicki *et al.,* 1978), although these conversions occur in nonsterile subsurface materials and soil, which contain an abundance of microorganisms. Mineralization is the conversion of an organic chemical to CO_2.

A variety of data confirm the biodegradability. A typical test involves measurement of the disappearance of O_2, as aerobic microorganisms use up O_2 in solution when they metabolize the chemical. Often the test involves adding a small amount of sewage, which is rich in microorganisms, to a solution containing styrene and inorganic nutrients to support microbial growth. In a typical test, 42% of the theoretical amount of O_2 was consumed in 5 days, and the val-

ue rose to 80% if the microorganisms were earlier adapted or acclimated to styrene degradation (Bridie *et al.,* 1979). A similar assay of O_2 disappearance showed that 65% of the amount of O_2 needed to destroy styrene was consumed in 5 days in freshwater inoculated with sewage (Price *et al.,* 1974). Although the initial concentration is not clearly stated in this investigation, if the value were 3.0 mg / L, the data suggest a rate (assuming linear kinetics) of 0.6 mg/L/ day in the first 5-day period.

The conversion of styrene to CO_2 with an inoculum of sewage microorganisms added to water was also measured in these early investigations. The data indicate a low conversion—18% in 10 days in one study (Ludzack and Ettinger, 1960) and 6 and 23% in 17 days and 10, 11, and 12% in 36 days in 5 tests in a second study of 5 samples (Pahren and Bloodgood, 1961). The low conversion was attributed in one instance to the fact that part of the styrene did not go into solution, and the insoluble chemical floating on the water may have volatilized before it was acted on by the microorganisms (Pahren and Bloodgood, 1961).

	Landfill Soil		Sandy loam	
Activity per week	5.0*	2.0	5.0	2.0
Percent mineralized	5.5	8.0	17.0	42.0
mg mineralized /kg soil	280.0	160.0	850.0	840.0

*g styrene/kg soil.

Microorganisms that destroy styrene may act on it as a consequence of their using the organic molecule as a source of carbon to sustain their multiplication. This is evident in the ability of individual microorganisms (Grbic and Munjko, 1977) or mixtures of microorganisms (Sielicki *et al.,* 1978) to use it as the sole carbon source to support multiplication. When aerobic microorganisms use an organic chemical as a carbon source for their growth, usually the compound is almost entirely converted to CO_2. In contrast, some microorganisms metabolize organic molecules without using them as nutrients; in these instances, organic products accumulate. This type of incomplete, non-growth-linked conversion is suggested by the fact the enzymes of a bacterium (grown in the absence of styrene) are able to convert the molecule to an organic product that accumulates. The product was styrene oxide (Hou *et al.,* 1983). Based on the extensive conversion of 8-^{14}C-labeled styrene to CO_2 it is likely that the dominant process in most aerobic environments is the growth-linked transformation.

Based on the extent of O, utilization, it has been suggested that styrene is essentially completely converted to CO_2, with no major accumulations of organic products (Kuhlmeir, 1988). Such a conclusion is premature because of the lack of precision in these indirect tests. Even the conversion of most of the ^{14}C in 8 ^{14}C-styrene to $^{14}CO_2$ (Sielicki *et al.,* 1978) does not confirm the lack of accumulation of organic products because only one of the eight carbon atoms was labeled.

Biodegradation is especially rapid in soils that are aerobic. In laboratory studies, 95 and 87% of the ^{14}C-styrene added to soil at 2.0 g/kg was converted to CO_2 in 16 weeks in a landfill soil and a sandy loam soil, respectively. About 75% was converted to CO_2 in 16 weeks if these soils received 5.0 g/kg (Sielicki *et al.,* 1978). From Fig. 4 of the publication of Sielicki *et al.,* 1978, one can calculate these rates of biodegradation during the period of maximal activity that follows the initial lag or acclimation phase.

Another laboratory study of styrene disappearance in a soil-groundwater system found the total disappearance of styrene (initial concentration of 70 mg/ L) in 25 h at 20°C (Kuhlmeir, 1988). This author used first-order kinetics to calculate a rate constant for styrene disappearance, but the use of first-order kinetics are unwarranted given the concentrations of the available data and theoretical considerations.

A field study of the rapid infiltration of secondary-treated municipal waste water from Phoenix for groundwater recharge also suggests the susceptibility to biodegradation. The concentration of styrene was measured in the waste water applied to the spreading basin and also in the renovated water taken from monitoring wells. In the first period of percolation of the styrene-containing waste water through the soil, the decline of styrene as a result of the infiltration exceeded 92%, and the decline in the second period was 98%. Although the chemical could have been lost by volatilization or its adsorption by soil particles, biodegradation is undoubtedly a significant factor as well (Bouwer *et al.,* 1984).

A field study designed to enhance biodegradation in soil (i.e., bioremediation) provided evidence that styrene in soil and groundwater had been reduced to 50 μg/L after bioremediation at a site where an accident resulted in a tanker spill of 4900 gallons of styrene. In the bioremediation, activated sludge was used as an inoculum, and H_2O_2, was injected into the groundwater to provide O_2. The minimum temperature for the bioremediation was reported to

be about 10°C (Kuhlmeir, 1988).

Biodegradation is also a significant mechanism of styrene loss from aerobic subsurface materials. This is evident from an EPA study of the disappearance of styrene added at 600 to 800 μg/L to microcosms made up of uncontaminated samples of subsurface material from sites in Oklahoma and Louisiana. The core samples were taken just above and just below the water table, at depths of 2.1 to 4.8 meters. The percentages of the initial concentration degraded per week were 2.3, 3.8, 4.3 and 12.0%. Although these degradation rates are slow compared to those noted above for surface soils, they do result from microbial action alone since styrene did not disappear from autoclaved material (Wilson *et al.,* 1983). It is noteworthy that the activity was still appreciable even in samples at pH 4.2 and 4.3. The paper by Wilson *et ed.,* (1983) does not state the number of points used to calculate these values and does not state whether there was an acclimation period or whether biodegradation was first order, zero order or followed some other kinetic model; hence, the calculation of half lives from these data (calculated as 4 to 22 weeks by USEPA (1987B) for subsurface aquifers) is inappropriate and misleading. Batteman and Werner (1984) demonstrated that styrene initially present in material from a sandy aquifer at 1.47 mg /L declined to 0.019 mg /L as a result of microbial degradation in laboratory tests. In a similar type of laboratory inquiry, styrene was added to samples of a pristine aquifer core taken in Oklahoma at a depth of about 7 meters. The initial concentration was about 400 μg / L in the aqueous phase. Inoculation of two core samples with different bacteria resulted in destruction of 86 and 93% of the styrene in 6 weeks. Uninoculated microcosms were not examined (Jain *et al.,* 1987).

Although the data above suggest extensive biodegradation, long persistence has been recorded in the field by Grossman (1970), who reported that styrene originating from a drum buried below ground persisted in the bedrock aquifer for at least 2 years.

In environments dominated by solids with large surface areas, as in aquifers and soils, microorganisms may develop in masses that cling to the surfaces. The activity of these types of growths has been investigated using bacteria that adhere to glass beads that simulate the solids of aquifers. Such biofilms are able, when O_2 is present, to destroy more than 99% of styrene in the presence of a second and major organic nutrient. In the study, the concentration was reduced from 7.6 μg/ L in the influent to less than 0.05 μg/ L in the effluent, and models were developed to predict the kinetics of such reactions (Bouwer and McCarty, 1984). Evaluations of the same type of model ecosystem showed that 90, 25, 99, and more than 99% of the styrene in an influent containing 8, 21,81 and 280 μg/L was destroyed in 11 weeks (McCarty *et al.,* 1984).

Biodegradation undoubtedly also occurs in surface waters and the upper portions of aquatic sediments when O_2 is present. The statement that biodegradation is probably slower in natural water than is observed when sewage is the inoculant (USEPA, 1987A) is probably true because of the low concentrations in waters of nitrogen (N) and phosphorous (P), since N and P often are in insufficient concentration for rapid microbial decomposition of even low levels of organic compounds. However, if the N and P levels are high, as in eutrophic waters, the rate of biodegradation probably will be rapid once the acclimation period of the microorganisms is over.

The absence of O_2 poses a special problem. Many hydrocarbons are only slowly degraded, or are not degraded at all, under anaerobiosis. This presumed resistance of styrene to anaerobic attack is supported by findings that biofilms developed on glass beads, which were active aerobically, did not give detectable styrene degradation (7.9 μg/L) anaerobically (Bouwer and McCarty, 1984). The effect was the same at these low levels and even at 280 μg/ L since no chemical loss was evident anaerobically in 12 weeks (McCarty *et al.,*1984). On the other hand, microbial mixtures containing methane-producing bacteria are able to destroy styrene anaerobically to produce a series of organic products and CO_2, and individual bacteria also can destroy styrene anaerobically (Churchman and Grbic-Galic, 1987). The information is yet too limited to conclude with certainty whether anaerobic degradation, which for specific compounds only begins after a long lag period, occurs in soils, landfill sites, anoxic groundwater or sediments.

An acclimation (also called lag or adaptation) period often precedes the active phase of degradation. This acclimation period is of great importance because little or none of the compound is destroyed in the time interval, although the subsequent rate of biodegradation may be very rapid. The period may be as short as a few hours, or it may be as long as several months; of particular concern is the inability to predict its duration or to model it for predictive purposes. From published figures, the acclimation period may be 1 to 4 weeks in aerobic soils receiving 2 or 5 g of styrene per kilogram of soil (Sielicki *et al.,* 1978) or only 2 days in aerobic waters amended with styrene and an inoculum of sewage microorganisms (Pahren *et al.,*1961). Acclimation may occur anaerobically as well, but experience with other synthetic compounds suggests that anaerobic acclimation will be slow; however, data on styrene are not available. Nevertheless, in a system receiving styrene continuously—or

when styrene additions are intermittent but not sufficiently spaced in time to allow for de-acclimation to occur—the acclimation period (aerobically and presumably anaerobically) occurs only after the first addition, and the subsequent rate is uniformly more rapid.

Because of the critical role of biodegradation in destroying styrene, its toxicity to microorganisms assumes special relevance. The occurrence of such toxicity would slow or prevent the microbial destruction. However, the available information argues against microbial inhibition being a problem in waters, soils, or sediments. Thus, to inhibit a common bacterium *(Pseudomonas putida),* a green alga *(Scenedesmus guadricauda),* and a flagellated protozoan required 72, greater than 200, and greater than 256 mg/L, respectively (Bringmann and Kuhn, 1980). Two other bacteria were also found to tolerate high styrene levels (Inoue and Horikoski, 1989). Tests of individual species of microorganisms in pure culture revealed no toxicity to algae at 500 mg/L or to fungi at 1000 mg/L, and 4 of 5 streptomycetes were able to grow in media containing, and thus tolerated, 20,000 mg/L (Grbic and Munjko, 1977). It is not clear how meaningful such numerical values are because they exceed the water solubility of styrene but they do indicate that some microbial species are very tolerant of the chemical. Of special relevance is the finding that styrene becomes toxic to species degrading the chemical in a soil-groundwater system only at levels exceeding 100 mg/L (KuhLmeir, 1988). Toxicity to microorganisms is thus highly unlikely at environmentally relevant concentrations, except at sites of catastrophic spills or in hazardous waste sites where the contaminant load is locally high.

In polluted air in cities, the ozone concentrations may sometimes be sufficiently high for ozone to destroy styrene more readily than hydroxyl radicals.

Evidence exists that some chemicals that are biodegradable at mg/L concentration may persist at low μg/L or lower levels. Other chemicals are biodegraded even at 0.0001 μg/L. However, it is not now possible to categorize which chemicals exhibit a threshold and which do not. This information comes largely from the studies of the author of this report. Based on the studies of individual microbial species in culture media, it is assumed that species that exhibit no threshold for biodegradation of these very low concentrations are, in fact, growing on a second organic compound and simultaneously destroying the substance of interest. In this context, the ability of microorganisms attached to glass beads as biofilms to destroy more than 99% of styrene provided at 7.6 or 7.2 μg/L (in the vicinity of or below the threshold) is particularly noteworthy (Bouwer and McCarthy, 1984; McCarthy *et al.*, 1984) because it suggests that a threshold may not exist if styrene-metabolizing microorganisms can compete with species using other organic compounds in the same environment. In this study, the second (and major0 organic nutrient was acetate at 1.0 mg/L.

It is possible that a compound that represents no risk—because of low hazard or low exposure—is converted to a product of high risk. The risk of the product can be high because it is intrinsically toxic or because it is environmentally more mobile or more subject to biomagnification. The products may be generated by photochemical reactions or other abiotic reactions (which are dealt with elsewhere in this report) or during the microbial transformation of the product.

Individual microorganisms or mixtures of aerobic microorganisms in culture media, when growing on styrene, convert it to 2-phenylethanol, phenylacetic acid (Sielicki *et al.,* 1978; Shirai and Hisatsuka, 1979), 2- hydroxyphenylacetic acid (Baggi *et al.,* 1983), and low-molecular- weight styrene oligomers (Sielicki *et al.,* 1978). Under certain conditions, microorganisms may also form 1,2-dihydroxy-3-ethenyl-3-cyclohexane (Bestetti *et al.,* 1989) and possibly styrene oxide (Hou *et al.,* 1983). Under aerobic conditions in surface and ground-waters, soil and sediments, these products would be destroyed, but they could persist long enough to pose a risk.

Under anaerobic conditions, a range of products is expected, and this is what has been found in mixtures of bacteria. The products that have been identified include 2- phenylethanol, phenylacetaldehyde, phenylacetic acid, benzoate, phenol, ethylbenzene, tolurene, and ethyl and methylcyclohexane (Churchman and Grbic-Galic, 1987). In contrast with the behavior of organic products in aerobic environments, organic products of metabolism do persist in nature under anaerobiosis.

Possible organic products of styrene biodegradation have not been sought in nature. It is likely that some are present. However, in waters in which the styrene level is itself low because little was introduced, such products probably are at too low levels to represent a substantial risk. However, if the styrene concentration is high, organic products may be produced and pose a risk.

AIR

A large amount of information exists on the concentrations of styrene in the atmosphere, especially in cities. The concentrations are usually below 1.0 ppb (by volume) but higher values have also been found, and levels of 5.0 ppb or higher in urban air have been recorded. The amounts rise during pollution episodes and are affected by the season of year (being higher in winter than in summer in New Jersey cities, for example) and whether it is night or day (Harkov *et al.*, 1984; Hartwell *et al.*, 1987A, 1987B). Levels in downtown Los Angeles ranged from 0.1 to 5.5 ppb (Grosjean and Fung, 1984). The geometric means in three cities in New Jersey ranged from 0.07 to 0.24 ppb in 1981 and 1982 (Harkov *et al.*, 1984). The available data for atmospheric concentrations in the United States through 1983 have been summarized and evaluated, some locations having no detectable level of styrene (Brodzinsky and Singh, 1983).

Data are also available from other countries. In Delft, the Netherlands, average styrene levels measured in 1975 were less than 0.1 ppb with maximum values of 0.7 ppb (Bos *et al.*, 1977), but further analyses in the Netherlands in 1980 gave mean values for the test areas of 0.02 to 0.35 ppb and maximum concentrations of 0.15 to 6.40 ppb. In one study, the styrene in the atmosphere was attributed to exhaust gases from vehicular traffic and the production of plastic resins and synthetic rubber (Guichert and Schulting, 1985). In another study, automobile exhaust was believed to be the source of the unquantified styrene in cities of the USSR (Ioffe *et al.*, 1977, 1979). The atmosphere at altitudes of 35 and 650 meters over Tokyo contains 0.1 ppb (Uno *et al.*, 1985). Styrene has also been found in forests in Germany, but the chemical in the forest may have originated in a nearby city (Juttner, 1986).

The air in highway tunnels may be rich in styrene, the hydrocarbon undoubtedly coming from gases emanating from gasoline- and diesel-powered automobiles and trucks (Hampton *et al.*, 1983).

Styrene may also be emitted to the air around hazardous waste sites. The mean concentrations in the air have been reported to range from 0.11 to 1.53 ppb with a maximum concentration reaching 15.5 ppb. Sanitary landfills receiving municipal and nonhazardous industrial wastes have mean concentrations at locations in New Jersey of 0.23 to 0.41 ppb and maximum values of 1.09 to 1.52 ppb. The concentrations vary appreciably on a daily basis (Harkov *et al.*, 1985; LaRegina *et al.*, 1986).

Indoor air in homes also may have appreciable styrene concentrations (Debortoli *et al.*, 1986). The average and median concentrations in one study involving 2125 data points were 1.41 and 0.31 ppb, respectively (Shah and Singh, 1988).

Styrene is very reactive in the atmosphere, especially in the presence of ozone and hydroxyl radicals. This high degree of reactivity probably results in a marked destruction of the compound in air. Hydroxyl radicals are major reactants, but in polluted air in cities, the ozone concentrations may sometimes be sufficiently high for ozone to destroy styrene more readily than hydroxyl radicals (Grosjean, 1985). Rate constants have been calculated for the reaction between ozone and styrene (Grosjean, 1985; Atkison *et al.*, 1982; Atkinson and Carter, 1984), and the styrene half-life in the troposphere resulting from its oxidation by ozone has been estimated as greater than or equal to 9 h (USEPA, 1987A). The products of this oxidation are benzaldehyde, formaldehyde, benzoic acid and traces of formic acid (Grosjean, 1985).

The oxidation of hydroxyl radicals yields benzaldehyde, and a high percentage of styrene may be converted to benzaldehyde by cleavage of the aliphatic moiety of the molecule. Rate constants for this oxidation have also been determined (Bignozzi *et al.*, 1981; Grosjean, 1985), and reaction mechanisms have been proposed (Sloane and Brudzinsld, 1979). The estimated half-life of styrene resulting from this oxidation is about 3 h (Hendry and Kenley, 1979; USEPA, 1987A).

Because styrene absorbs little light at the wavelengths of sunlight reaching the earth's surface, it probably is not rapidly destroyed by direct action of the sun (Santodonato *et al.*, 1980). The indirect consequences of photochemical reactions involving other atmospheric constituents are appreciable, however, and they may remove styrene through these reactions (Grosjean, 1985). Physical removal of styrene by wet or dry deposition is probably not important in view of its rapid destruction by other means (USEPA, 1987B).

Because of the rapid rate of its destruction in the atmosphere, styrene is probably not transported to a significant extent in air. Styrene in air is thus not a likely source of the compound in waters or soils at a distance from the place where the compound is emitted into the atmosphere.

ECOLOGICAL EFFECTS

The acute toxicity of styrene to a number of animals has been assessed. The effects have been expressed by the various researchers in terms of LC_{50}, TL_m or EC_{50}, which designate the concentrations of a chemical in water to kill 50% of the test animals, the median tolerance limit (the concentration of chemical in water at which 50% of the test animals survive), and the concentration that causes a modification in some sublethal physiological response of 50% of the test animals, respectively. Values of TLm reported for fish are 57, 25, 65, and 75 mg/L for fathead minnows *(Pimephales promelas),* blue gills *(Lepomis macrochirus),* goldfish *(Camassius auratus),* and guppies *(Lebistesreticulatus),* respectively, in 24-h tests conducted in soft water. Tests of 48 and 96 h revealed no differences in the concentrations that are tox-

ic. When tested in hard water, the TL_m value for fathead minnows is 63 mg/L (Pickering and Henderson, 1966). The LC_{50} values for sheepshead minnows *(Cyprinodon variegates)* is 9.1 mg/L, the values being the same in 24, 48, 72, and 96 h test periods. At 5.1 mg/L styrene had no effect on sheepshead minnows (Heitmuller *et al.,* 1981). The LC_{50} value for lake emerald shiner *(Notropis atherinoides* subspecies *acutus)* is 31 mg /L, and 8 mg /L is considered to be "safe" (Dow Chemical Co., 1989).

The TL_m values for brine shrimp *(Artemis salina)* are 68 and 52 mg/L in 24 and 48 h tests, respectively. For the water flea *(Daphnia magna),* the EC_{50} value was fond to be 182 mg/L, and 105 mg/L had no detectable acute toxicity (Bringmann and Kuhn, 1982).

In view of the high concentrations required for acute toxicity and the far lower concentrations found in surface waters, acute effects on aquatics animals are unlikely.

A chemical may enter an aquatic animal and be concentrated within its tissues. For fish, such bioconcentration may result from transport of the compound through gills and other membranes. The extent of accumulation is commonly expressed as the bioconcentration factor, which is the ratio of the concentration (typically in μg/ g) of the chemical in the surrounding water (μg of compound p logarithm of the *n*-octanol/water partition coefficient (log K_{ow}) is often a good indication of bioconcentration, and values of log K_{ow} are variously calculated or experimentally determined to be 2.87, 2.95, and 2.41 (Banerjee and Howard, 1988; Yalkowsky *et al.,* 1983). These values suggest that bioconcentration of styrene is unlikely. Calculation of the bioconcentration factor from values of K_{ow} and water solubilities of styrene give factors of 150 and 25, respectively (USEPA, 1987A). However, the actually measured bioconcentration factor for goldfish *(C. auratus)* is 13.5 in water containing 1.0 mg of styrene per L (Ogata *et al.,* 1984). These low values suggest that bioconcentration is unlikely to be appreciable (USEPA, 1987A).

Although few species have been tested, the movement of styrene through aquatic food chains to result in elevated concentrations in higher animals is not likely under most circumstances. Nevertheless, in the immediate vicinity of migrating styrene at a catastrophic spill, undesirable levels in fish used for human consumption would result. (Environmental Protection Service, 1984).

Microorganisms are important in waters and soils because they convert organic forms of nitrogen, phosphorus, and other elements to the inorganic forms. These transformations are necessary for the regeneration of nutrients needed for the continued development of aquatic plants and animals. In view of the fact that the concentrations of styrene needed for such inhibition are nearly always far higher than those found in waters and soils, suppression of the microbial regeneration of essential nutrient elements is highly unlikely.

For the same reason, a suppression of the algae important to primary productivity in surface waters is not likely.

OVERVIEW

For the purpose of calculating daily intake, EPA proposes 0.00 and 0.25 ng/L for high and low values for exposure levels in drinking water and 0.00 and 3,300 $\mu g/m^3$ in air. From these values, EPA estimates a daily intake for adults of 0 to 0.5 μg via water and 66,000 μg for air (USEPA, 1987A). Given the available literature, the high values for exposure levels from water may be too high. However, the estimates suggest that air is by far the prime route of exposure in urban areas.

The most convincing argument for water being a very minor source and air the major source for exposure comes from the monitoring information. The data are firm, the trends are evident, and no major questions exist about the methodologies or the quality of the data.

Styrene has a short persistence near the soil surface because of its volatility and biodegradability, but its mobility could result in its being transported downward to subsurface sites where the rates of volatilization and biodegradation would be slow.

Transport of styrene in nature is very limited because of its volatility from soils and surface waters, its rapid destruction in air, and its biodegradation (except at spill and hazardous waste sites, and possibly anaerobically) in soils and surface and groundwaters. Mobility associated with spills and through fissures and soil macropores could be a problem.

Based on theoretical arguments, it is likely that styrene is not appreciably affected by photochemical or abiotic chemical reactions in surface and groundwater, soils, and sediments, but experimental verification is lacking.

The large amount of monitoring information on atmosphere levels provides a strong case of urban air pollution (concentrations usually less than 1.0 ppb, but occasional values of up to 6.0 ppb). Levels around waste sites and industrial facilities may be above 1.0 ppb also. However, the rapid reaction of styrene with hydroxyl radicals and ozone would make unlikely the presence of the compound at some distance.

Biodegradation is a major means of styrene loss in oxygen-containing surface and groundwaters, soils, sediments, and waste waters. Such conversions may be slow or

may not occur at sites of hazardous-waste disposal, adjacent to spills, or when oxygen is absent.

Landfill sites, the immediately adjacent groundwaters, and other polluted waters are typically anaerobic. Information on anaerobic biodegradation is lacking, and the little data available suggest that styrene may persist. The practical problem may be lessened because humans are usually exposed to the water after it has become aerobic, but the biodegradation may not then be sufficiently rapid to prevent significant human (or ecological) exposure. Theoretical and experimental information indicates that hydrocarbons like styrene, if destroyed anaerobically, are only metabolized after an acclimation period that may last several months and are then converted to a variety of organic products, which may also persist.

Some compounds are not biodegraded, even aerobically, at significant rates, when initially present below certain concentrations. When present in surface waters, styrene is below these threshold concentrations.

The continued presence of styrene at a site in nature, or its reasonably frequent introduction, would result in an increase in size of the population of microorganisms degrading it, leading to a shortening and disappearance of the acclimation period and more rapid biodegradation. The effect of such long-term presence or repeated introduction has not been determined.

The presence of styrene in oxygen-containing waste waters is probably neither a health nor an ecological problem because of its volatility and biodegradability. This view is not supported by experimental data with actual waste waters. Its presence during anaerobic waste treatment may represent a problem—except as exposure is preceded by the chemical moving through an anaerobic zone before exposure occurs.

The toxicity of styrene to aquatic animals is not a concern because the levels for toxicity are far greater than those that occur in environments where higher animals live. There is little likelihood of significant bioconcentration through a natural food chains. Moreover, it is highly unlikely that aquatic phytoplankton and higher aquatic plants are affected at the concentrations in surface waters.

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Carcinogen Classification Systems: A Time for Change

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The identification of a substance as a "possible", "probable" or "reasonably anticipated to be" human carcinogen sounds many alarm bells. It raises the concerns of the public, of regulators and health officials, and of corporate executives whose companies manufacture or use the substance. It can have significant health, economic, financial and legal consequences. Yet the national and international systems used to classify carcinogenicity are confusing, contradictory, unreliable—and often arbitrary. The U.S. Environmental Protection Agency and its Science Advisory Board have recognized the deficiencies in EPA's own classification process and are seeking ways to improve it. Dr. Moolenaar discusses the problems and recommends ways to make the classifications more accurate and more meaningful to those who depend on them.

Ever since the cranberry scare of the 1950s, carcinogens have occupied a prominent position in the public mind. In the early days, chemical carcinogens were identified by direct observation of cancer incidence as a result of chemical exposure in the human population. Only a handful of chemicals had been identified as carcinogens, and a simple yes or no answer was all that was required to deal with the available data base.

In recent years, the situation has become much more complicated. There are still only about 25 known human carcinogens,[1] and, with appropriate testing, handling, and disposal procedures now in place for industrial chemicals, the list of proven human carcinogens important in commerce is not likely to grow. Significantly, however, there are many substances (natural and synthetic) that have shown an indication of carcinogenic activity in animal studies. In fact, about half those tested in long-term bioassays have shown an indication of carcinogenic activity and the list (now over 1000) is still growing.

The nature of the carcinogenicity data available on chemicals is highly variable. For some substances there are multiple bioassays showing carcinogenic activity, whereas for others there may be only one positive study. The study design is sometimes considered adequate, whereas for other studies it isn't. The evidence for carcinogenicity from a long-term bioassay can be "equivocal" or "clear." Epidemiologic data of varying significance may also be available. Thus, today, instead of having a simple list of carcinogens, classification systems have been devised to describe the results of evaluating this very diverse spectrum of data. Initially the schemes were utilized primarily as an index to the literature on chemical carcinogenesis. Today they have become the means to express conclusions from the first step in the risk assessment process—hazard identification—and they play a prominent role in public policy.

In a broad context, the basic purpose of carcinogen classification systems developed by industry, governmental agencies, or research scientists is the same: to summarize results from the evaluation of a complex array of data in order to identify and communicate hazard to humans resulting from exposure to the substances.

Three organizations have published classification systems that have gained prominence in the United States: the International Agency for Research on Cancer, Lyon, France (IARC), the Department of Health and Human Services Annual Report on Carcinogens (ARC), and the Evironmental Protection Agency (EPA). In Europe, an additional system developed by the European Community (EC) is being used as a basis for labeling chemicals. As the EC proceeds to harmonize health and safety regulations, their classificiation system will probably gain wider recognition in the United States.

Because the philosophical underpinnings of evaluation, assessing relevance to humans, and the communication of hazand to humans are all central to the

basic purpose of carcinogen classification, the prominent systems will be compared with respect to these three critical elements.

I. Philosophical Underpinnings

Although all of the systems rely heavily on animal data, they are intended to classify substances in terms of potential human hazard. Thus, substances described as known or possible carcinogens are intended to be read as known or possible human carcinogens. The various systems are quite consistent in categorizing compounds for which a causal relationship between exposure and *human* cancer has been observed. Such substances are placed in the "highest" category of known human carcinogens.

Where the systems differ is in how they deal with the evaluation of the relevance or predictive value of the experimental data to human hazard. Basically, two procedures have evolved, and they have been described as evaluating 1) the strength of evidence *for* carcinogenicity, and 2) the weight of evidence *of* carcinogenicity.

Strength of Evidence. The procedure based on strength of the evidence for carcinogenicity starts with a presumption: "In the absence of adequate data on humans, it is biologically plausible and prudent to regard agents for which there is sufficient evidence of carcinogenicity in experimental animals as if they presented a carcinogenic risk to humans."[2]

The systems differ in how they deal with evaluation of the relevance or predictive value of the experimental data to human hazard.

Following this approach, experimental data are evaluated to arrive at conclusions concerning whether or not carcinogenic activity was reliably observed in experimental animals. Further evaluation of additional experimental studies is not necessary to reach conclusions concerning carcinogenicity in humans, since carcinogenic activity in animals is presumed to be predictive of humans. The combined strengths of all the positive animal studies are added together according to predetermined rules, often using such terms as *inadequate* (no valid positive studies), *limited* (one valid positive study), or *sufficient evidence of carcinogenicity* (two valid positive studies) to describe the result. Historically, animal bioassays and human epidemiology have been the major contributors to strength-of-evidence evaluations. More recently, genotoxicity data have also played a role. The key is that for animal bioassays and genotoxicity data, only results that demonstrate or suggest carcinogenic activity have been part of strength-of-evidence evaluations. Studies that do not show carcinogenic activity are ignored since they do not add to the strength of evidence *for* carcinogenicity.

Weight of Evidence. The other approach has been described as a weight-of-the-evidence approach to evaluation of cancer hazard to man from an array of data. Rather than rest upon a presumption of human cancer risk from all substances showing carcinogenic activity in animals, this approach makes a judgement concerning human hazard on a case-by-case basis. This approach has been recommended by the U.S. Office of Science and Technology Policy (OSTP). It recognizes the value of the IARC "principle," but follows it with the statement, "However, this presumption is evaluated along with other relevant information (Principle 25) in making a final judgement concerning human carcinogenicity (Chapter 6, Section II, Part B) and should not foreclose further inquiry into the human relevance of animal carcinogens."[3]

Weight-of-evidence evaluations rest upon weighing indicators of lack of carcinogenicity along with indicators showing carcinogenic activity to arrive at an overall conclusion. Such indicators include identification of metabolic products likely to be responsible for the carcinogenic activity, evaluation of pharmacokinetic information to estimate the dose of the active substances at the target tissue as a function of administered dose, species-specific processes, genotoxicity, and other parameters that suggest biochemical mechanisms important to the expression of carcinogenic activity. Observations from the bioassay itself often provide clues concerning why carcinogenic activity is observed in a long-term animal experiment. These include the enhancement of commonly occurring tumors versus formation of rare tumors, consistency across species, organ toxicity in association with tumor formation, etc. Such clues provide guidance for evaluating the relevance of the animal response to humans under human exposure conditions.

Evaluations of this type are especially important in predicting potential results in humans based on a combination of human epidemiologic studies, animal bioassays, and mechanistic information. For example, if a chemical showed carcinogenic activity in only one animal species but a lack of activity in others, and mechanistic informa-

tion supported the uniqueness of the positive animal responder, the weight of evidence would suggest lack of carcinogenic activity in humans. Such a conclusion is not possible under a strength-of-evidence system. Thus, the value of the weight-of-evidence system is that conclusions drawn from it reflect a more complete evaluation of the total data base available for any particular chemical.

At a recent EPA Workshop on Carcinogen Risk Assessment, Dr. Richard Hill, of the EPA's Office of Pesticides and Toxic Substances, very successfully illustrated the two approaches. He used the image of a beam balance with a single pan to describe the strength-of-evidence approach, and a balance with a double pan to describe the weight-of-evidence approach. The "positive" pan of the balance with the double pan is identical to the single pan of the strength-of-evidence system, but the weight-of-evidence approach also includes a "negative" pan of the balance that may or may not significantly impact the overall evaluation, depending on the nature or weight of the "negative" data.

The bottom line is that weight-of-evidence provides a far superior basis for evaluation of a carcinogenic hazard to humans. It uses scientific data and understanding to evaluate the predictive value of experimental data to describe human hazard rather than relying on a presumption. A comparison of the philosophical underpinnings of the four systems in terms of their basis for evaluation is shown in Table 1.

Since the IARC and ARC procedures are based on strength of the evidence *for* carcinogenicity and not a complete weight-of-evidence evaluation, they are best regarded as beginning points for identifying chemicals that may pose a carcinogenic hazard to humans. The rest of the data, i.e., the data indicating whether or not the carcinogenic activity observed in animal studies is predictive of human hazard, needs to be evaluated before one is ready to complete the hazard identification step of risk assessment and consider quantitative dose response leading to full assessment and management of carcinogenic risk.

II. Relevance to Humans

As described above, the ARC and IARC systems rely solely on the strength-of-evidence procedure, so further evaluation is needed to determine how and whether the experimental data are relevant to humans. These systems are inadequate to fulfill the hazard identification requirements of a risk assessment.

The EPA classification system is described as a weight-of-evidence system. Does that mean the EPA system is the model that should be followed for the identification of carcinogenic hazard to humans?

EPA is definitely on the right track. But the agency is plowing new ground and the ground is pretty rough. As its weight-of-evidence system has evolved, there has been no pre-existing pattern to follow. The transition from strength-of-evidence to weight-of-evidence evaluation has not been easy. EPA recognizes that there are problems with its current approach to classification, and has begun the public-information-gathering process that may lead to revision of the agency's cancer risk assessment guidelines or to the way the guidelines are currently being implemented.

Let's take a look at the EPA procedure in more detail. It is well known that there are several critical judgments that must be made in evaluating the meaning of experimental data for human hazard.

TABLE 1

Evaluation Basis for Four Classification Systems

System	Evaluation Basis
IARC	Strength-of-evidence
ARC	Strength-of-evidence
EPA	Weight-of-evidence
EC	Weight-of-evidence

The major judgments concern the translation of animal data to humans and the extrapolation from extreme and frequently unusual conditions of exposure used in animal studies to much lower exposure levels in humans. The general approach that EPA advocates is that, where there are no data or scientific understanding to use as a guide, a policy choice must be made.

For identification of chemical carcinogens a "worst-case assumption" or "default option" is chosen. The default option is basically to rely on the IARC presumption and embrace a simple strength-of-evidence approach. However, if relevant data exist, EPA is committed to a weight-of-evidence evaluation on a case-by-case basis. This means EPA must evaluate the complete array of data that may be available on a substance and make a scientific judgment concerning human hazard.

How does this actually work within the context of the EPA guidelines?

EPA follows three steps in hazard identification:

1. The literature is searched for positive studies (i.e.,

studies that indicate that chemical exposure is associated with carcinogenic activity in humans or animals). This might be described as a first step in hazard identification, and is equivalent to the evaluation carried out under the strength-of-evidence approach.

2. A tentative classification is made based on the positive studies. If there is an animal study showing carcinogenic activity, and it has been successfully replicated (i.e., there are a total of two or more positive animal studies), the compound is tentatively classified as a "probable" human carcinogen. If there is only one positive study, it is generally classified as a "possible" human carcinogen.

3. All other relevant data are analyzed to determine whether the classification should be changed. The classification could go "up" or "down," depending on the nature of the other relevant data. This is where the weight-of-evidence approach is supposed to take over.

One of the difficulties with the EPA procedure becomes immediately clear. At step 2, before the complete data base has been reviewed and evaluated, the presumption or default option (probably or possibly carcinogenic to humans) is invoked.

The residual impact of the IARC philosophy on the EPA procedure can be seen at this point. Carcinogenic activity in animals may have been observed, but, by assigning a tentative classification at this stage, it is clear that human risk has been presumed without further evaluation. By way of contrast, the weight-of-evidence approach would, at this point, only indicate carcinogenic activity in animals, and would proceed to evaluate other relevant data that may show how or why the activity was produced in certain experiments. Ultimately, a judgment would be made concerning how to interpret the data in terms of human hazard. Finally, the substance would be placed in an appropriate category.

The second difficulty with the EPA approach arises in step 3. EPA has not described how to carry out this step in the guidelines. However, individual hazard assessment documents provide an indication of how this step is carried out. The July 1987 Update to the Health Assessment Document for Dichloromethane sets forth three criteria that must be met before concluding that the positive animal studies lack predictability for humans:

- Epidemiology data show the substance is not carcinogenic to humans.
- The biochemical pathway responsible for tumors in animals is not present in humans.
- The mechanism of carcinogenic action in animals is unlikely to occur in humans.

The first two of these require proof of a negative, something that science cannot do. No epidemiology study can prove a substance is not carcinogenic in humans. One can always postulate that longer or higher exposures may show an effect. Nor is it possible to prove the absence of a biochemical pathway. One can only show that it is below some limit of analytical detection. Thus, the first two criteria will never be met.

The value of the weight-of-evidence system is that conclusions drawn from it reflect a more complete evaluation of the total data base available for any particular chemical.

That leaves criterion 3, which deals with mechanism of action. Since the word "unlikely" is used, meeting this requirement is theoretically possible. However, most cancer experts would agree that, at the molecular level, we do not know the detailed mechanism by which cancer is produced, either naturally or through exposure to chemicals. Thus, criterion 3, although theoretically possible to use, has become nearly impossible to meet in practical terms.

Herein lies a fundamental problem. We may never be absolutely sure about carcinogenic hazard in humans from data in animals. We will always have some degree of uncertainty. However, scientists can probably agree that certain experimental observations tend to increase confidence that carcinogenic activity in animals is or is not likely to be predictive of human response. The goal of carcinogen classification should be to express that degree of confidence as clearly as possible.

The bottom line is that EPA's current classification procedure still emphasizes the presumption of human hazard from animal carcinogens, and sets up very high hurdles to allow scientific data to negate the presumption. Although this type of conservatism may be appropriate at the regulatory stage, it seems inappropriate in carcinogen classification, where objective communication of the results of scientific evaluation should be the goal. This is unfortunate because it means many very important data are lost from consideration in the risk management process.

The EC system and thought process apparently have not been spelled out in detail. However, it appears to utilize mechanistic data to a greater extent than do the systems common to the United States. In Europe, the occurrence of neoplasms at sites and in animal strains where

tumors are well known to occur spontaneously with a high incidence are, in some instances, considered of little relevance to humans. In addition, special consideration is given for nongenotoxic compounds where tumors are apparently produced by a secondary mode of action, and thresholds are likely. For example, ethylenethiourea and di(2-ethylhexyl) phthalate were not considered to be possible human carcinogens by EC. Both are believed to cause tumors in animals by mechanisms believed not to be predictive for humans. Using the strength-of-evidence approach, IARC categorized them as "possible human carcinogens," and the ARC lists them as "reasonably anticipated to be" human carcinogens.

III. Communication of Hazard

Classification systems have become important communication vehicles, often summarizing several man-years of work, to transmit a judgment on the potential for carcinogenic risk to humans resulting from exposure to chemical substances. The communication is from the scientists evaluating the data to the one who must make decisions concerning acceptable risk on behalf of individuals or on behalf of the general public. Often, the titles of the categories constitute the critical element of the message. Since the decisions could have significant health and/or economic consequences, the message must be as accurate and concise as possible.

The judgments communicated by the classifications are often presented in the form of a single adjective, e.g., *probable* or *possible* carcinogenic to humans. Since terminology does play a significant role in what is comunicated, it may be useful to compare category descriptors used in prominent classification systems (see Table II).

There seems to be general agreement on the descriptor for the top category (known), on the criteria for placing chemicals in it, and also on which chemicals belong in that category. However, after the top category, most similarities end. In fact, some chemicals regarded as *reasonably anticipated* to be in the ARC, *probable* by EPA, and *possible* by IARC , are not even categorized as carcinogens by EC.

A particularly confusing situation arises when the criteria for two systems are similar but the titles are very different. This situation arises in the IARC, EPA, and ARC systems, where the terms *probable, possible,* and *reasonably anticipated* to be encompass very nearly the same criteria.

A further complication arises in the choice of terms used to describe a particular "class" of carcinogens. The terms probable and possible convey a certain meaning to the general public in terms of the probability of an outcome. The scienctists who evaluate the data place substances in a category based on the criteria associated with the category, but may not think in terms of the impact of the title of the category on communicating their conclusions. Thus, there may be a discrepancy between what the scientists would like to communiate based on their evaluation of the data and what is actually communicated based on the category title.

IMPACT OF CARCINOGEN CLASSIFICATION

The potential regulatory impacts of classifying substances as carcinogens are obvious. Arguments in tort litigation based on agency or expert group classifications may be a factor in the outcome of legal actions. However, a potentially more significant impact occurs in corporate and personal decisionmaking. Such decisions are often made without the benefit of agency experts and input from scientific professionals.

The identification of a substance as a *possible, probable,* or *reasonably anticipated* to be human carcinogen sounds a warning bell for corporate executives who manufacture or

TABLE II

Terminology of Four Classification Systems

System	Descriptors for Classification			
EPA	Known	Probable	Probable	Possible
EC	Known	Strong Presumption	Possible	—
IARC	Known	Probable	Possible	Not classifiable
ARC	Known	Reasonably anticipated to be	Reasonably anticipated to be	—

use chemicals and for private citizens who may be exposed to them. The simplest response is to substitute another chemical to accomplish the same purpose, and thus avoid complications from exposure to a "carcinogen." Thus, classification of a substance in formal systems often produces effects driven by concerns for regulation, litigation, and health outcomes even before such activities become reality.

Product switching to avoid the hassle of dealing with a "carcinogen" is certainly understandable. Even for compounds for which the weight of evidence suggests that human carcinogenicity is unlikely (despite activity in animals), the hassle can be considerable. However, the situation may become more complicated. Special attention should be given to the carcinogenicity data as well as other data available for the substitute. If adequate studies are not available, it is likely that they will be required in the future. If past experience holds, about half the compounds tested in the future will show carcinogenic activity in animal studies. One may be faced with the cost of switching (and maybe the cost of carcinogenicity testing), and a few years later find it necessary to deal with another "carcinogen."

Considerations such as these emphasize the need for understanding the nature of current classification systems and the need for improvements. If the interpretation of carcinogen classification could be confined to the regulatory process, application to public policy could be better managed. However, when we have 200 million "risk managers," the need for objective classification systems that result in clear and accurate communication becomes clear.

IMPROVING CARCINOGEN CLASSIFICATION

It is clear that improvements are needed in carcinogen classification. The scientific evaluation of the relevance of experimental data to human hazard must be an important part of the assessment leading to classification. Harmonization among the various schemes would be desirable. It is not surprising that confusion exists regarding what to do about carcinogens when there is inconsistency in both the basis for evaluation of data and how to package the intended message in broadly understood terms.

Continuing the present situation is likely to lead to needless disruption of the economy in the United States, with little health benefit. Ultimately, it could lead to the loss of credibility of carcinogen classification systems and loss of the benefits a credible system could provide. Although some have advocated abandoning classification systems, I believe it would be better to improve the systems rather than let them die.

But how can this be done? Existing systems have a lot of history associated with them, dating back to days when much less was known about the limitations of animal data.

IARC made a change in 1987 by downgrading the title of category 2B, the largest category, from *probable* to *possible* human carcinogen, presumably emphasizing the limitations of animal data in predicting human hazard.

Four ARCs (the fifth is pending) have been published with no change in criteria, and there is little indication changes will be made. It will continue to be a problem since the term *reasonably anticipated to be* seems so definite and the evaluation so limited.

EPA has announced plans to review the agency's carcinogen risk assessment guidelines, and changes in classification will be considered. The EPA initiative should be encouraged and supported as a high priority effort.

Europe may be in the best position, since the EC system is new, and current understandings in science can be more readily incorporated into that system without the encumbrance of history. Developments in Europe must be followed closely. We can learn from them.

What can industry do to improve the way carcinogens are identified in the United States? Several actions come to mind:

- Utilize the creative, problem-solving talents in industry to devise a useful classification system for chemical carcinogens. It must make use of all data available for each chemical, include the results of an evaluation of the predictive value of that data for human cancer hazard, and accurately communicate the results so that risk managers, including the general public, will understand the conclusions of the evaluation.
- Support EPA in the revision of their Cancer Risk Assessment Guidelines. The process will probably take one to two years, and there will be opportunity for public input. Discussion of the basic concepts with interested university scientists and encouraging their advice to EPA would also be helpful.
- Work toward harmonization of classification systems so that all of them will have a common basis for classification, will include evaluation of the relevance of experimental data to human hazard, and will accurately communicate the results. This will not happen overnight, and will require significant changes in the systems prominent in the United States.
- In the interim, before harmonization occurs, distinguish clearly between classification systems based on weight-of-evidence evaluations, which evaluate the predictive value of the experimental data to human response, and those based on strength-of-evidence evaluations,

which do little more than count the number of positive bioassays. This would minimize public confusion and avoid loss of credibility of classifications. If systems based on partial evaluations continue to exist, every effort should be made to identify them in order to avoid confusion between partial and complete evaluations.

The American Industrial Health Council, in co-operation with several other industry associations, is actively working toward improving the scientific basis for carcinogen classification Hazard identification is the first step of carcinogen risk assessment, and it is important that the process start on a solid scientific basis. Risk management will serve the public best when the scientific basis is sound and the scientific evaluations are accurately communicated.

Dr. Moolenaar's views appeared in the October 1989 issue of Chemical TIMES & TRENDS, a publication of the American Industrial Health Council. They are reprinted here by permission.

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[1] IARC has about 50 entries under known human carcinogens, but about half are production processes, e.g., furniture and cabinet making, or mixtures such as soots or coal tars.

[2] IARC Monographs on the Evaluation of Carcinogenic Risk to Humans, Supplement 7, 1987, p. 22

[3] Office of Science and Technology Policy. Chemical Carcinogens: A Review of the Science and Its Associated Principles. February 1985.