THE SIRC REVIEW

RESEARCH • TECHNOLOGY • PUBLIC POLICY

Vol. 3, No. 1

SPECIAL REPORT

The Helsinki Symposium on Butadiene and Styrene

Summary of Presentations and Discussions Related to Styrene

Compendium of Abstracts

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Introduction

The Finnish Institute of Occupational Health (FIOH), the International Agency for Research on Cancer (IARC), and the Commission of the European Communities sponsored art International Symposium on Health Hazards of Butadiene and Styrene April 18-21, 1993 in Helsinki, Finland. The Symposium was organized by Drs. Marja Sorsa, FIOH; Kimmo Peltonen, FIOH; and Harri Vainio, IARC. Viveca Bergman, FIOH, served as symposium secretary. Co-sponsors included the US National Institute of Occupational Safety and Health (NIOSH), US Environmental Protection Agency (EPA), the Organization for Economic Co-operation and Development (OECD), and World Health Organization (WHO), International Labor Organization (ILO) and United Nations Environment Programme (UNEP) through the International Programme on Chemical Safety (IPCS).

A total of 80 papers were presented at the symposium; 48 covered styrene and 34 examined butadiene. Twenty-two of the styrene papers resulted from research sponsored by the Styrene Information and Research Center (SIRC) or the European styrene industry. Certain papers from this symposium were pre-selected by the organizers and will be published in the IARC Scientific Publication Series.

The purpose of this issue of the *SIRC Review is* to present a review of all of the work on styrene that was presented and discussed at this meeting. Prior to the symposium, scientists from SIRC member companies were assigned specific topics for review and summarization. At least two scientists were assigned to each session and their reviews comprise the articles in this issue.

The reviewers were:

George Cruzan, Ph.D., DABT; Research Associate, Toxicology, Mobil Oil Corp., Princeton, NJ. After completing his doctoral degree in biochemistry at Purdue University in 1969, Dr. Cruzan was an Assistant Professor of Chemistry at The King's College, Briarcliff Manor, NY, until 1973. He was a Research Biochemist in the Animal Health Department at Rohm and Haas Co. from 1973 to 1976. He was a toxicologist at Rohm and Haas from 1976 to 1980, and has been at the Mobil Environmental and Health Sciences Laboratory since 1980.

Janette R. Cushman, Ph.D., DABT; Senior Toxicologist, Chevron Research and Technology Co., Richmond, CA. Dr. Cushman completed her doctoral degree in toxicology at Utah State University in 1982. Since joining Chevron, she has conducted toxicology studies in their laboratory and supervised the contract toxicology group. In her current position in the Toxicology and Health Risk Assessment group, she provides toxicology support to the Chevron Chemical Co.

Robin C. Leonard, Ph.D.; Principal Epidemiologist, ARCO Chemical Company, Newtown Square, PA. Dr. Leonard received her doctoral degree in human genetics from the Medical College of Virginia in 1978 and completed NIH postdoctoral fellowships in genetic epidemiology at the University of North Carolina, Chapel Hill, and at Johns Hopkins School of Hygiene and Public Health. She joined ARCO in 1991 to design and implement the company's worldwide occupational epidemiology program.

Roland R. Miller, Ph.D., DABT; Project Manager in Toxicology, The Dow Chemical Co., Midland, MI. Dr. Miller obtained his doctoral degree in Toxicology from the University of Michigan in 1978. His research has focused on animal inhalation toxicology and metabolism studies. His assignments have included three years (1986- 1989) as corporate toxicologist in Dow Europe, Horgen, Switzerland.

M. Gerald Ott, Ph.D., Director of Epidemiology, BASF Corporation, Parsippany, NJ. Dr. Ott is a fellow of the American College of Epidemiology and Adjunct Assistant Professor at New York University Medical Center. He began his career as an officer in the Commissioned Corps of the U.S. Public Health Service and has more than 20 years

of experience conducting and publishing occupational epidemiology studies. He received his doctorate in Epidemiologic Science from the University of Michigan in 1982 and has taught occupational epidemiology at Yale University.

Flora Ratpan, Ph.D.; Senior Specialist, Applied and Genetic Toxicology, Novacor Chemicals, Ltd., Calgary, Alberta, Canada. Dr. Ratpan obtained her doctoral degree in genetic to)dcology from the Lvov State Medical School, former U.S.S.R., in 1973. She spent six years at the Research Institute of Toxicology, Epidemiology and Occupational Health, Lvov, Ukraine, before coming to Canada. Dr. Ratpan worked at the Polysar Rubber and Chemical Co., Sarnia, Ontario from 1979 to 1989. She has been at Novacor Chemical Ltd.'s Occupational and Environmental Health Department since 1989, providing toxicological and human health assessment of various petrochemicals.

Symposium Overview

The 48 styrene-related papers presented at the symposium covered a wide range of subjects. This review of the symposium will be divided into the following topics:

- Styrene Exposures and Markers of Exposure
- Human Carcinogenicity Data
- Animal Carcinogenicity Data
- Genotwdcity
- Metabolism and Dose Estimation
- Other Health Effects
- Risk Assessment and Management

The symposium included a report by Dr. Pirkko Pfaffli that the primary occupational exposure to styrene is to workers in the reinforced plastics industries. Exposures there vary from short peaks of very high exposures to periods of very low exposures; in other styrene industries (e.g., monomer production and polymerization) styrene exposures are routinely low. Pfafffi's data from Finland indicated that exposures to reinforced plastics workers have decreased from about 100 ppm in the 1970s to less than 50 ppm (8 hr time-weighted average [TWA]) in the early 1990s. The general population is exposed to very low levels of styrene through food, food packaging, automobile exhaust, and cigarette smoke. Exposures in the general population, except for smokers, are estimated at less than 1 μg/kg/day; smokers may be exposed to up to 3.5 μg/kg/day.

The internal dose of a chemical is more important than the external dose in determining toxic response for an individual, and urinary metabolites may comprise a good method for calculating dose received. The predominant urinary metabolites of styrene, mandelic and phenylglyoxylic acids, are the most commonly used biological markers of exposure. Because there are wide variations in the amount of metabolites excreted by different individuals exposed to the same external concentration, inference of external exposure from the amount of urinary metabolites is very imprecise. In addition, these markers are very dependent on the time the urine sample was obtained relative to when the exposure took place, because of the short half-life of styrene and the pattern of short-term peak exposures.

The International Agency for Research on Cancer (IARC), which in 1987 classified styrene as a possible human carcinogen, reported that preliminary results of its extensive epidemiological study of 40,000 European reinforced plastics workers indicate that styrene presents no obvious carcinogenic risk to workers. Dr. Manolis Kogevinas, who directed the six-country study on behalf of IARC, said results showed no statistically significant increase in any type of cancer as compared to the general population. Further analyses are being conducted on the data to determine if there is any increased risk of leukemia and lymphoma related to styrene exposure among these workers.

Dr. Ernest McConnell, former head of the National Toxicology Program, reviewed all of the long-term animal studies on styrene and styrene wdde. He concluded that each of the studies has deficiencies that limit their usefulness as a definitive study of the carcinogenic effects of styrene in animals. Taken together, these data do not present convincing evidence that styrene causes cancer in laboratory animals. In order to provide data that will be appropriate to assess the carcinogenic potential of styrene in laboratory animals, the Styrene Information and Research Center (SIRC) has embarked on state-of-the-art chronic studies in rats and mice. SIRC reported on two-week mouse and thirteen-week rat inhalation studies, which were conducted to design the planned chronic inhalation studies.

Significant disagreement occurred over the quality of the evidence and significance of the mutagenicity data on styrene and reports of cytogenetic damage in workers exposed to styrene. Dr. Marja Sorsa, symposiurn chairperson, presented her analysis of the mutagenicity and cytogenicity studies that have been reported on styrene. She concluded that, although most of the studies are negative, styrene is mutagenic and does cause cytogenetic damage in hurnans

exposed to greater than 30 ppm (TWA) of styrene.

In contrast, Dr. David Scott of the Patterson Institute for Cancer Research in the United Kingdom, conducted a comprehensive review of the human data on cytogenetics, and reported that styrene did not cause cytogenetic damage in humans. Dr. Scott noted that 30 of 47 studies reported no effects, and in the remaining 17 the effects did not increase with increased exposure to styrene. In new experiments in mice and/or rats exposed to styrene via inhalation for up to 4 weeks at concentrations up to 1000 ppm, there was no increase in chromosomal aberrations. The.lack of response in rats exposed to high levels casts further doubt on whether low levels of styrene could cause increased chromosomal aberrations in humans.

Nolan et al. reported that mice have the greatest capacity to metabolize styrene to styrene oxide (SO) and humans the lowest capacity. They further reported that blood levels of styrene oxide should be higher in rodents, particularly mice, than in humans at any external styrene concentration. Based on comparative metabolic data in rats, mice, and humans, Canady et al. developed a physiologically-based pharmacokinetic (PB-PK) model was developed to describe the distribution and metabolism of styrene and SO in liver, fat, muscle, and visceral tissue. The model predictions were validated by experimental measurements of blood SO in rats and mice given styrene or SO via intraperitoneal (ip), oral, and inhalation routes. Using this model, it was predicted that the area under the curve of blood SO (AUCso) for humans exposed to 50 ppm styrene for 8 hours would be two orders of magnitude lower than the AUCso in laboratory animals in long-term studies where there was no evidence of an oncogenic response.

The Brown and Lindbohm reviews of the reproductive studies of styrene published prior to 1990 indicated that styrene did not exert any specific developmental or reproductive effects. Nine additional studies published since that time did not alter this conclusion. Three papers provided an overview of the studies reporting neurotoxic effects from styrene; above 100 ppm, there are clear indications of pre-narcotic effects on the central nervous system (CNS). The symposium did not provide any additional specific information concerning exposure concentrations in the 50 to 100 ppm range. Gobba and Cavalleri reported that styrene exposure may affect color vision. The report did not address color vision loss as a function of exposure concentration.

A review of the studies reported at the Helsinki Symposium indicates that the data currently available do not support the conclusion that styrene is a carcinogen. More extensive research is necessary before a sound conclusion can be reached about the overall health effects of styrene.

Exposure to Styrene

J.R. Cushman, Ph.D. and R.R. Miller, Ph.D.

Human exposure to styrene occurs by several routes depending on the individual's occupation, place of residence, smoking habits and even eating habits. Practically everyone is exposed to styrene, although nearly all people are exposed at very low levels. Several presentations focused on estimating exposure to occupationally exposed people or people in the general public who do not work in styrene industries.

Pfaffli [#2] reported on occupational exposures to styrene, including the monomer production, polymerization, styrene-butadiene rubber, styrene-butadiene latex, polystyrene molding and reinforced plastics industries. For all of these industries except lamination, air concentrations of styrene routinely average less than 4 ppm, and in many cases are less than 0.5 ppm. He emphasized that exposure levels for laminators in Finland have decreased considerably from around 100 ppm in the 1970s to an average of less than 50 ppm in the early 1990s (8 hr time-weighted average [TWA]). Pekari's report [#29] of monitoring the urine of Finnish workers for the styrene metabolite, mandelic acid, reinforced this conclusion. Nonetheless, styrene exposures frequently remain well above Finland's exposure standard of 20 ppm, primarily due to peak exposures of > 100 ppm for short periods. Controlling peak exposures by automating would be very expensive due to the nature of laminating work, which involves relatively small numbers of complex-shaped articles. The potential importance of short-term, high exposures versus longer-term, low exposures on human health is not understood, and complicates extrapolation of possible health effect findings in reinforced plastics workers to other styrene industries where exposures are significantly lower and less variable.

Styrene has been measured in a variety of foods, but the source of the styrene has been questioned, i.e. was the styrene naturally occurring or due to migration from styrene containing plastic packaging? Van den Berg et al., [#4] hypothesized that styrene measured in table-ready foods could be a natural constituent, be formed by bacteria or molds, or result from chemical changes in other natural constituents during processing. Miller et al. [#5] and van den Berg et al. [#4] reported the results of recent studies in which strenuous efforts were made to ensure that raw foods analyzed for styrene were not contaminated with styrene during collection and handling. Styrene in raw foods was found at highest concentrations in cinnamon (157 to 39,200 ppb). Since styrene (cinnamene) and cinnamic aldehyde are structurally very similar, this finding is not surprising. Considerably lower but measurable concentrations of styrene (0.5 to 8 ppb) occurred in beef, coffee beans, peanuts, wheat, oats, strawberries, peaches, and black currents. Thus, even a diet of raw, unprocessed foods never packaged in styrene-based plastics is likely to contain very low levels of styrene.

In estimating the total exposure to styrene of people who are riot exposed occupationally, several sources of styrene must be considered. Information on styrene concentrations in indoor and outdoor air, water, and food was used by Newhook and Caldwell [#3] to estimate daily styrene intakes in the Canadian general public. Estimated total daily intakes ranged from < 0.185 to < 0.88 μg per kg of body weight per day for various age groups. Additional exposure from smoking 20 cigarettes per day increased the estimate to 2.9 to 3.5 lig per kg body weight per day. Tang and Eisenbrand [#6] made similar calculations of general population exposures in Germany, adding an estimate of styrene migration from plastic packaging into the diet. They concluded that non-smokers may be exposed to up to 8.5 mg per year, or 0.33 lig per kg of body weight per day for a 70 kg person. Smokers may be exposed to up to 12.5 mg per kg per year, or 0.46 μg per kg per day. Both of these reports clearly suggest that exposures to non-occupational sources of styrene are very low.

Markers of Exposure

G. Cruzan, Ph.D. and F. Raptan, Ph.D.

A principle rule of toxicology is that all chemicals will cause adverse effects at some dose level. Therefore, an important focus of toxicological research is to determine the lowest dose of a chemical that will cause adverse effects, so that exposure to this chernical can be kept below the adverse effect level. Because toxic effects occur at the cellular level, knowledge of the dose at the target cell or target organ is crucial. However, in many cases no specific target cell has been identified or the concentration at the target cell is not readily measurable. In lieu of concentration at the target site, health professionals have traditionally used average external exposure as a surrogate of internal dose in humans. When measurements of a compound at the target site are not practical, biomarkers of overall internal dose are sought. Various methods are used to measure internal dose: urine concentrations of parent compound or metabolites, blood concentrations of parent compound or metabolite, reaction products with blood proteins or DNA in selected cells, or cells of altered characteristics.

If toxicity is caused by a metabolite, internal dose of the metabolite is a more critical consideration than external exposure to the parent compound. Rates of metabolism may vary among individuals, and within the same individual, the rate of metabolism may differ among various organs. In addition, the rate of metabolism may not be directly proportional to the external or internal concentration. Since the exact metabolic rate is not known for each individual, it is extremely difficult to estimate exposure concentration reliably for an individual based on a measured level of urinary or blood metabolites. The usual procedure is to obtain exposure measurements and metabolite measurements on a population and calculate a correlation coefficient to develop an equation relating metabolite level to exposure concentration. Normal procedures for monitoring workers include collecting a single urine sample at a given time point. The amount of compound or metabolite present is dependent not only on the amount of exposure, but also the time relationship of exposure to sample collection and the metabolic rate. Peak exposures that might cause toxic effects might be underestimated by a urine sample collected at an inappropriate time.

For styrene, urinary mandelic acid, or the combination of mandelic acid and phenylglyoxylic acid either at the end of the shift or the next morning are often used as a marker for exposure. Many studies have reported styrene exposures using these techniques; unfortunately, the authors use several differing methods of expressing the metabolite concentrations: mg/1 urine, mmo1/1 urine, mg/mg urinary creatinirte, or mmol/mmol urinary creatinine.

Kivisto, Pekari, and Aitio [#22] reported at the symposium that phenylglyoxylic acid was not stable in urine when stored at 6°C. Urine samples stored for up to 70 days at -18°C had no degradation of phenylglyoxylic acid, while those stored at 6°C lost 46°/0 in 1 month.

Aitio, Pekari, Nylander-French, Pfaffli, and Sorsa [#81 measured urinary mandelic acid (MA), phenylglyoxylic acid (PGA), p-hydroxymandelic acid (HMA), and styrene as a means of biological monitoring of styrene exposure. They measured workplace air and urinary markers for 205 workers in 32 workplaces; 114 wore gloves but did not use respiratory protection, 58 had respiratory protection but no gloves, and 33 used both. The mean time-weighted average (TWA) exposure in the workplace air was 37 ppm. In some cases, styrene concentration was measured continuously. Peak styrene concentrations were as high as 300 ppm with many time periods of very low exposure. Because the exposure to styrene varied considerably throughout the day and peak exposures occurred at different times, in 10 of 25 workers the peak urinary concentration of MA + PGA occurred during the work day or within 2 hours after the end of the shift. However, for the remaining 15 workers, the peak urinary concentration of MA + PGA occurred more than 2 hours after the end of the shift. In this particular boat building facility, layup and sprayup were frequently done near the end of the shift to allow curing overnight.

In after-shift urine samples, there was a wide variation in the ratio of MA to PGA; the authors claimed there

was less variation in results in morning-after metabolite samples, but this was not obvious from their data. For workers exposed to an average TWA styrene of 37 ppm, the end of shift urinary MA was 4.1±4.9 mmo1/1 while the next morning sample was 1.5±1.3 mmo1/1. Despite wide variation in urine concentrations, there was a close linear correlation between time-weighted concentration of styrene in air and the mean concentration of MA, PGA and styrene in the urine of workers exposed to styrene primarily via inhalation. There was no linear correlation between time-weighted exposure and urinary metabolites when the styrene exposures were dermal. Based on their data, the authors recommended analysis of morning-after urine samples for MA and PGA as a biological marker for styrene exposure. They reported that morning-after MA + PGA of 1.4 mmol/1 corresponded to a TWA exposure of 20 ppm.

Severi, Pauwels, Van Hummelen, Roosels, Veulemans and Kirsch-Volders Ut61] reported a field study where 52 workers exposed to styrene in the production of fiberglass reinforced styrene polyester resins were monitored for 4 weeks by urinary post-shift mandelic acid and daily personal air sampling. Daily mandelic acid concentrations were 8-504 mg/g urinary creatinine (mean 98 mg/g), while the air monitoring showed daily average exposures of 0-25 ppm. The correlation coefficient between individual air concentration and urinary mandelic acid was 0.71.

Farmer, Tang, Anderson, Sepai, and Bailey [#36] reported two techniques for biomonitoring exposure to styrene oxide (SO) based on SO adducts with hemoglobin. One method, derivatization and gas chromatography-mass spectrometry (GC-MS) analysis of valine adducts, had a lower limit of detection of 10 pmol adduct/g globin. The other method, GC-MS analysis of styrene glycol hydrolyzed from carboxylic amino acid esters, had a lower limit of detection of 15 pmol/g globin. In rats given ip doses of 0-833 prnol SO/kg, there was a linear increase in valine adducts (0-179 pmol/g globin), but the dose-response for the carboxylic acid esters was not linear. Occupational exposure to styrene has been studied on a limited number of samples, but no adducts were detected.

Two papers reported on measurement of styrene-DNA adducts by ^{32}P-postlabeling. The ^{32}P-postlabeling method of determining DNA adducts was originally developed for large polynuclear aromatic hydrocarbons (e.g. benzo-a-pyrene) which are highly lipophilic, form stable adducts, and are not volatile. Reliable and valid application of the ^{32}P-postlabeling assay to other classes of chemicals may require extensive research. The assay typically involves isolation of DNA from the presumed target organ or from peripheral lymphocytes, digestion of the DNA to the 3′-nucleotides, adduct enrichment, phosphorylation at the 5′-position with ^{32}P-ATP and a kinase, adduct separation by thin-layer chromatography, and quantitation of ^{32}P in the various adduct spots.

Cantoreggi, Lutz, and Gupta [#35] reported on their work to develop a sensitive method that could be used to biomonitor workers occupationally exposed to styrene. They used a new chromatographic separation technique to separate DNA adducts formed by *in vitro* reaction of 3H-S0 with DNA. They identified 1 major and 3 minor peaks. Female B6C3F1mice were subsequently dosed with 100 or 300 mg/kg styrene. Preliminary results showed that styrene-DNA adducts could be detected at about the limit of detection, 1 adduct/107 nucleotides. The method is not at present sensitive enough to serve as a biomarker of human exposures.

Vodicka and HemminIci [#31] also used *in vitro* reactions of SO with DNA to develop a ^{32}P-postlabeling assay for monitoring human populations. *In vitro* reactions yielded about 75°/. of the adducts at the N-7 position of guanine, 20`)/0 at the 0-6 position, and 5% at the N-2 position. Although the greatest proportion of the adducts were at the N-7 position, this proved not to be useful in the postlabeling assay, because a very low labeling efficiency of N-7-adducts. N-2 adducts were not useful because they degraded during chromatography. With adjustments to reaction conditions, they were able to get a 10°/0 labeling efficiency for 0-6 adducts. Blood samples obtained from a small group of reinforced plastics workers from the Czech Republic with estimated exposures of 100 to 700 mg/m^3 (25 - 170 ppm) for at least 5 years indicated a slight increase in adducts (1.5±0.3 adducts/10^8 nucleotides vs. 0.5±0.1 for agricultural workers).

A number of methods are available for monitoring worker exposure to styrene. Inherent variability in exposures during the day and individual metabolic rate differences make correlation of biological marker and workplace exposure concentration difficult. In general, there is a good correlation over large groups of workers between average worker exposure and average concentration of biomarker. While levels of a biomarker in an individual may indicate more about the exposure of that individual, workplace exposures currently are controlled by the concentration present in the atmosphere. Controlling exposure by biomarker values would be difficult.

Human Cancer Measurements

M.G. Ott, Ph.D. and R.C. Leonard, Ph.D.

Evaluating the weight of evidence for carcinogenicity in humans involves three steps: (1) separate characterization of the evidence from human and animal studies, (2) combination of the two separate characterizations into an overall weight of evidence for human carcinogenicity, and (3) evaluation of all supporting information that could result in modification of the overall assessment.

Human studies are observational in nature and, hence, are often vulnerable to misinterpretation because of chance, selection and information bias, and confounding. For this reason, it is especially important to identify and assess all relevant studies, including those reporting negative as well as positive findings. To be included in a weight-of-the-evidence risk assessment, each study should address a relevant biologic outcome, provide a reasonable basis for assessing exposures to the agent of interest, and be methodologically sound within its intended purpose.

> . . . the body of evidence does not currently support a casual link between styrene exposure and L+H cancer.

Five International Agency for Research on Cancer (IARC) symposium presenters reported findings from cohort mortality or cancer incidence studies of workers with potential styrene exposure. A sixth presenter, Bodner [#69], reviewed existing evidence for a causal association between styrene exposure and the occurrence of lymphatic and hematopoietic tissue (L+H) cancers. This assessment, based on eight recent cohort studies in three industries (styrene monomer and polymerization production, styrene-butadiene rubber production and reinforced plastics [RP] production), concluded that the body of evidence does not currently support a causal link between styrene exposure and L+H cancer.

The two key human health studies discussed at the symposium were the IARC mortality study of 40,700 workers in the European RP industry [Kogevinas et al., #63] and the update of the American RP industry study of nearly 16,000 workers [Wong et al., #76]. The geographic distribution of employees in the IARC cohort was: Denmark 15,863; Finland 2,085; Italy 7,256; Norway 2,035; Sweden 3,666; and United Kingdom 9,778. Cancer incidence as well as mortality data will eventually be available for the Scandinavian countries. A cited advantage of the IARC study was that confounding exposures to 1,3-butadiene and/or benzene are generally not present in the RPindustry. The hypothesized outcomes of primary interest were L+H cancers.

The IARC cohort included many short-term workers (41% with < 1 year of employment) and also many workers first employed after 1975 (55%). Workers were classified according to potential styrene exposure: high potential exposure (laminators, unspecified and "rotating" jobs (74%); other exposed (painters, laminators in semi-automatic processes, etc. [13%]); non-exposed (10°/0); and unknown (3%). There were 34,556 men and 6,127 women (15%) in the cohort. This cohort is much larger than the American RP cohort (40,683 vs. 15,878). However, since the U.S. cohort is older and followed for a longer time period, the proportionate number of deaths is higher in the U.S. cohort (10.3`)/0 [1,637] vs. 6.7% [2,713]). Lost-to-followup in both cohorts was 3%.

The exposure assessment for the IARC cohort was supported by personal and environmental measurements as well as by mandelic acid (MA) and phenylglyoxylic acid (PGA) measurements for the Italian cohort. Utilizing personal exposure data from Denmark, it was estimated that styrene levels had declined from an average of about 200

ppm in 1965 to a range of 20 - 40 ppm during the late 1980s. These data have not yet been summarized in terms of 8-hour time-weighted average (TVVA) concentrations. A good correspondence was observed between the log(mean MA) and log(mean environmental styrene concentration).

The standardized mortality ratio (SMR) is the ratio of observed to expected deaths, where expected deaths are calculated from general population mortality rates. An SMR above 1.0 indicates higher mortality in the study versus the corresponding general population and conversely, an SMR below 1.0 indicates lower death rates in the study population.

For all causes of death, the SMRs were 0.92 for exposed and 0.77 for nonexposed members of the IARC cohort. For all cancers, the SMRs were 0.90 and 0.75, respectively, and for accidental deaths the SMRs were 1.10 and 0.75. Among selected causes of death, the SMRs for cohort members were as follows: bladder cancer SMR=1.00, breast cancer SMR=0.75 (< 10 deaths in women), L+H cancer SMR=0.96, ovarian cancer SMR=1.48 (<10 deaths), pancreatic cancer SMR=1.05, prostate cancer SMR=1.15, and stomach cancer SMR=1.02. None of these findings was significantly elevated. The lung cancer findings were also unremarkable. A decreasing lung cancer SMR was seen with longer duration of exposure suggesting lifestyle differences between short-term and long-term employees. No data were presented for kidney cancer because of the small number of deaths and the lack of any notable trends.

More detailed analyses were then presented for the L+H cancers. The most interesting findings were noted for time since first exposure (TSE) where the SMRs increased with increasing TSE. It should be cautioned that SMRs in the first 10 years since hire are generally low in industrial cohorts because individuals with pre-existing cancer are unlikely to seek employment—this is indicative of the so-called healthy worker selection effect. For total L+H cancer, the SMR increased with increasing TSE as follows: <10 years SMR=0.67 (15 observed vs. 22.4 expected deaths); 10-19 years SMR=1.09 (24 observed vs. 22.0 expected deaths); and 20+ years SMR=1.40 (11 observed vs. 7.9 expected deaths). For duration of employment, the trends were unimpressive: < 1 year SMR=0.84 (16 observed vs. 19.0 expected deaths); 1-4 years SMR=1.14 (21 observed vs. 18.4 expected deaths); 5- 9 years SMR=0.80 (7 observed vs. 8.8 expected deaths); and 10+ years SMR=0.99 (6 observed vs. 6.0 expected deaths). Detailed SMR analysis for four subcategories of L+H cancer, namely non-Hodgkins lymphoma, Hodgkins Disease, multiple myeloma, and leukemia were unremarkable.

A relatively weak argument for TSE being a more informative indicator of styrene exposure than even employment duration was offered on the basis that exposures in the early years of styrene use were much higher than today's exposures. Based on this argument, a long TSE should correlate with higher styrene intensities. It can also be argued that a 20 year latent period may be required before occupationally-induced cancer deaths occur. On the other hand, since the same companies may have been making wood products before switching to RP products, these mortality findings could also reflect earlier exposure to other chemicals. For example, working with wood has been linked to non-Hodgkins lymphoma occurrence as well as to nasal cancer occurrence. Kolstad (see below) reported an excess of nasal cancer in the Danish RP cohort, which contributed 15,800 workers to the IARC study.

A number of other cancer sites (bladder, esophagus, Liver, larynx, ovary, pancreas, prostate, stomach, and testicle) were noted as being elevated in one or more of the national cohorts, although not elevated in the total IARC cohort. These findings will be further explored utilizing Poisson regression analysis. In fact, some Poisson regression analyses had already been carried out for L+H cancers, but were not presented because of time restrictions. At this time, Dr. Kogevinas interprets the IARC data as showing that styrene is not a potent carcinogen. However, he is presently unwilling to dismiss the findings as totally negative.

Study methods, data on completeness of vital status follow-up, and total number of deaths, but no SMR findings, were presented for the update of the American RP industry study [Wong et al., #76]. Because the update contributed an additional 1,100 deaths for evaluation, the combination of the European and American RP industry studies should add considerably to the available human health effects database.

In a separate poster presentation, Kolstad et al. [#74] described a cancer incidence study among all employees working in the RP industry within Denmark. The study was conducted based on an existing tumor registry covering all of Denmark, and an employment registry covering all persons employed since 1964. A total of 64,000 persons were identified who ever worked for 552 (mostly very small) companies in the Danish RP industry. The 15,800 employees from companies indicating that styrene exposure was likely for at least 50°/0 of their work force were also contributed to the IARC study. Data were summarized in terms of standardized incidence ratios (SIR), which are

calculated in an identical fashion to the SMRs mentioned above. An elevated SIR for L+H cancer (SIR=1.3) was reported for employees of companies with confirmed RP production. Restricting the analysis to employees starting employment between 1964 and 1970 resulted in an SIR of 1.4 based on 71 incident cases. This study is not currently supported by an exposure assessment. Detailed analyses were reported only for L+H cancers; however, summary data for other cancer sites were mostly unremarkable. The overall SIR for kidney cancer was 0.97. An excess of nasal cancer in this cohort was thought to be possibly due to past exposure to wood dusts. For the present, this study presents cancer incidence data not contained in the IARC report. However, once cancer incidence data have been analyzed by IARC, the Danish study will be essentially subsumed within the IARC cohort mortality study.

Mortality studies involving mixed exposures to styrene and 1,3-butadiene were reported by Downs et al. [#721 and Matanoski [#68]. Most data contained in the Matanoski presentation were published in 1992 [Am. J Epidemiol, 136:843-855, 1992]. The Downs et al. study of 1,000 hourly workers assigned to an acrylonitrile-butadiene-styrene (ABS) production facility reported low overall SMRs for cancer and only 2 deaths due to L+H cancer compared to 2.8 expected deaths. No data on environmental concentrations of styrene or 1,3-butadiene were presented. Substantial monitoring of acrylonitrile revealed 8-hour TWAs above 10 ppm in 5% of the samples.

The Matanoski nested case/control study was conducted within a cohort of employees from eight styrene-butadiene rubber (SBR) production facilities. An apparent association was observed between leukemia occurrence and 1,3-butadiene exposure. After inclusion of 1,3-butadiene in the regression model, there was no significant contribution of styrene in explaining the leukemia occurrence. The database underlying this nested case/control study is currently being reconstructed by independent researchers from the University of Alabama. Until that work is completed, the findings of this study are likely to remain controversial.

Investigation of Styrene Carcinogenicity Using Laboratory Animals

G. Cruzan, Ph.D. and R.C. Leonard, Ph.D.

In long-term animal studies, many of the problems inherent in human studies (e.g., selection and information bias, and confounding factors) can be avoided; in particular, exposure to other chemicals can be minimized and an appropriate control group is available for comparison of tumor rates. Therefore, long-term animal tests are very valuable in assessing the carcinogenic potential of chemicals.

McConnell and Swenberg [#64] reviewed eleven long-term animal toxicity/oncogenicity studies on styrene and three on styrene oxide. Each study was reviewed and evaluated for details and adequacy of design, adequacy of reported data, and interpretation. They concluded:

1. None of the studies of styrene or styrene oxide is well-suited for extrapolating the potential carcinogenic activity of either compound to humans, because all have deficiencies in design, conduct, interpretation and/or utilized a less than ideal route of exposure.

2. There is no convincing evidence of carcinogenic activity of styrene in animals. two rat studies, treated females had a higher incidence of breast cancer than controls. In one study, the incidence was increased only in the low dose, while in the other study there was no dose response (all treated groups were equally greater than control). Two mouse studies have been interpreted as showing increased lung tumors. In one study the incidence rate was identical to that of the historical controls; i.e., the study-specific controls had an abnormally low incidence. In the other study, the maximum tolerated dose was clearly exceeded since the investigators stopped dosing after 16 weeks because of deaths in the treated animals.

3. Styrene oxide was carcinogenic to the forestomach of both sexes of rats and mice after gavage exposure, which is not relevant to human exposure. At the low dose level in one sex an increased incidence of liver neoplasms was noted in one of the two studies. No carcinogenic activity was reported after dermal exposure.

Because styrene is metabolized to styrene oxide in ani mals and styrene oxide is carcinogenic in animals, it has been suggested that styrene should be regarded as carcino genic in animals. However, because styrene oxide causes tumors only at the site of exposure and is associated with severe irritation at that site, it is not clear

In that styrene oxide causes tumors by a genotoxic mechanism or that the low concentrations that would exist in cells as a result of the metabolism of styrene would cause tumors. There were two reports investigating the mechanisms of styrene oxide tumorigenicity in the forestomach.

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Cantoreggi and Lutz [#541 investigated whether DNA binding could be responsible for the forestomach tumors induced by gavage administration of styrene oxide (SO). Four hours after an oral gavage dose of [7-^{3}H]SO, forestomach DNA was isolated and purified. No DNA radioactivity was detectable. Expressed in the units of the Covalent Binding Index (CBI = [µmol adduct per mol DNA nucleotide]/[mmol chemical administered per kg body wt.1), it was deduced that the CBI for styrene oxide in the forestomach was less than 2.6. Following an intraperitoneal (ip) dose of [7-^{3}HISO, the CBI in mouse liver was less than 0.6. These CBI values were extremely low in comparison to those obtained for established genotoxic carcinogens.

Following whole body inhalation exposure of rats and mice to [7-^{3}H]styrene in a closed chamber, DNA from lung and liver was isolated, purified, and enzymatically degraded to the 3'-nucleotides. Most of the radioactivity was associated with normal nucleotides, indicating the ^{3}H came from metabolic degradation of styrene and subsequent incorporation into normal DNA constituents. Some

radioactivity was associated with adducts in some of the liver and lung samples; the CBI was calculated to be 0.1 in mouse liver from exposures that resulted in 110 mg styrene absorbed/kg body wt..

These two studies indicate that SO is unlikely to cause forestomach tumors by a purely genotoxic mechanism.

These investigators also examined the effect on cell proliferation in the forestomach of rats, resulting from oral gavage of SO 3 times/week for 4 weeks at doses of 138, 275, and 550 mg/kg/day. They found increased cell proliferation, measured by 24-hour infusion of BrdU, which was not dose related. In addition, they saw less stimulation of cell proliferation from SO than from BHA, although SO is reported to induce forestomach tumors in a higher percentage of animals than does BHA.

Dalbey, Rodriguez, Cope, and Cruzan f#71] measured cell proliferation from oral gavage of SO by pulse labeling with 3H-thymidine. In an initial range-finding study, SO was administered by gavage 3 times/week for 4 weeks at doses of 55 or 550 mg/kg/day; the labeling index (percent of cells with labeled nuclei) was reduced when measured 1 hour after the last SO exposure. After a. dditional range-finding studies showed increased labeling index at 24 hrs after 3 times/week administration of 1000 or 1500 mg/kg/day SO for 4 weeks, a time course study following a single dose of 800 mg/kg SO or 800 mg/kg/day SO 3 times/week for 3 weeks was conducted. The labeling index was determined 1, 6, 15, 24, and 48 hours after the last dose of SO. After 1 hour there was a depression of labeling index; the maximum increase in labeling index occurred at 15 hours after the last dose of SO. The response to SO, as measured by labeling index and morphologic observation, was highly focal and variable; i.e., in a given forestomach there were areas that appeared normal, some that were slightly affected, and some that were severe. Mean values of labeling index do not adequately represent the focal changes in cell proliferation.

These two papers argue against SO genotoxicity playing a strong role in either forestomach tumors from gavage administration of SO or any tumorigenic response from the metabolism of styrene.

The Styrene Information and Research Center is engaged in a research program to conduct state-of-the-art inhalation chronic toxicity/carcinogenicity studies of styrene in rats and mice. Two preliminary studies were presented.

Cushman, Andrews, Cruzan, Miller, and Hardy [#44] exposed CD-1 and B6C3F1 mice to styrene vapors of 15, 60, 250, and 500 ppm six hours/day five days/week for two weeks. For female mice of both strains, mortality was greater at 250 ppm than at 500 ppm. In males the greatest mortality occurred at 500 ppm. Nearly all deaths occurred within the first 4 days of exposure. Liver effects (increased organ weights and centrilobular hepatocyte necrosis) were seen in both strains of mice in the 250 and 500 ppm exposure groups.

Morgan, Mahler, O'Conner, Price, and Adkins (Fund. Appl. Toxico1.20: 325-335, 1993) recently reported similar findings in B6C3F1 mice. They showed an inverted dose-response in both males and females, accompanied by severe liver toxicity. In their studies, males were more susceptible to styrene toxicity than females. As above, nearly all deaths occurred within the first 4 days.

The two studies combined show the following mortality for B6C3F1 mice:

	Males		Females	
	250 ppm	500 ppm	250 ppm	500 ppm
Cushman et al.	1 of 20	8 of 20	10 of 20	0 of 20
Morgan et al.	11 of 25	8 of 27	6 of 65	0 of 65

Cruzan, Andrews, Cushman, Miller, Coombs, and Hardy [#17] reported on a thirteen-week inhalation exposure of Charles River. CD (Sprague-Dawley derived) rats to styrene at concentrations of 200, 500, 1000 and 1500 ppm six hours/day five days/week. Males exposed at 1500 ppm had reduced food consumption and body weight (-10`)/0) compared to controls. Both males and females at 1000 and 1500 ppm had increased water consumption; in females at 1500 ppm water consumption was approximately double that of the controls. No treatment-related effects on hematology, clinical chemistry, urinalysis, organ weights or cell proliferation in liver or lung were reported. Exposurerelated histopathologic changes were confined to minor effects in the olfactory epithelium of both males and females exposed to 500, 1000, and 1500 ppm. The water consumption and olfactory effects attest to the irritating nature of high concentrations of styrene.

The Styrene Information and Research Center is currently sponsoring research to determine whether differential metabolism of styrene explains the differences in toxicity between rats and mice, and at different exposure levels in mice. In addition, SIRC is currently conducting a chronic inhalation study in CD rats, and a thirteen-week inhalation study in mice is expected to start during the third quarter of 1993.

Genotoxicity of Styrene

F. Ratpan, Ph.D., G. Cruzan, Ph.D. and M.G. Ott, Ph.D.

The cancer assessment of chemicals is based not only on human epidemiology and long-term animal cancer studies, but also on other relevant data. The other data includes metabolism, mechanism and genotoxicity. Genotoxicity studies include mutagenicity and cytogenetics. Genetic toxicity measures have been of interest as possible predictors of the carcinogenic potential of an agent. While distinct patterns of chromosomal abnormalities are now being detected for many site-specific cancers in humans, it is not yet known whether these are early or late events in carcirtogenesis. It is also not yet possible to distinguish "chemically-induced" from "spontaneous" tumors via patterns of chromosomal aberrations. Hence, there is no unanimity of opinion on the meaning of cytogenetic testing as an indicator of carcinogenic potential. The mutagenicity of styrene was reviewed at the symposium. The published cytogenetic studies were also reviewed, as well as the presentation of new cytogenetic data on styrene.

Kligerman [#51] presented the results of an extensive genotoxic study of mice and rats exposed to styrene concentrations of 0, 125, 250, or 500 ppm for 14 consecutive days. At concentrations of 250 ppm and 500 ppm, there were small but statistically significant concentration-related elevations of sister chromatid exchange (SCE) frequency in the lungs (11.18 and 11.34 vs 9.04 in control), peripheral blood lymphocytes (11.4 and 11.61 vs 9.4 control), and splenocytes (11.6 and 12.84 vs 10.5 control) of the mice, as well as in the peripheral blood lymphocytes of the rat (13.8 and 14.3 vs 11.03 control respectively). However, no statistically significant concentration-related increases were found in the frequency of chromosome aberrations (CA) or micronuclei in the cultured murine splenocytes or lung cells. DNA strand breakage was not detected in the lymphocytes from exposed rats analyzed using the single cell gel assay. Hence, the results of the study indicate that the styrene is a relatively weak SCE inducer *in vivo* when administered at high doses to rodents by inhalation. No evidence of clastogenicity was found after the inhalation of relatively high styrene concentrations over a 2-week period.

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Kligerman discussed four hypotheses to explain why the CA results for the rodents are negative, whereas for some human monitoring studies the CA results were positive. One is that occupationally exposed humans may be subjected to a higher total dose than rodents in the study; second, the metabolism of styrene in humans and rodents, mice in particular, may be qualitatively and/or quantitatively different; third, human and rodent cells might have inherently different sensitivities to either the induction of DNA damage caused by styrene or the repair of the damage; fourth, the increase in CA frequency observed in some occupationally exposed humans is due to some factor other than styrene in their environment.

Preston [#52] reported the results of a cytogenetic study in male rats exposed to styrene vapors at concentrations of 0, 150, 500 or 1000 ppm for 6 hrs/day, 5 days/week, for 4 weeks. In this study, ethylene oxide at 150 ppm under exactly the same conditions, served as a positive control. Peripheral blood lymphocyte cultures were prepared from each animal at 1, 2, 3 and 4 weeks after the start of exposure and 4 weeks after the termination of exposure. In consultation with Dr. Dick Albertini, the assay was modified to enhance growth of rat lymphocytes by adding human lymphocyte growth factor. Each culture was assayed for SCEs and CAs. Consequently, two types of dose-response curve were available; one with time as the variable, and one with exposure concentration as the variable. No increase in SCE frequencies were observed in cultured rat peripheral lymphocytes at 150, 500 or 1000

ppm of styrene. These concentrations did not result in an increased incidence of chromosome aberrations. These data were consistent with the results of Dr. Kligerman's inhalation study.

Anwar and Shamy[# 58] monitored workers exposed to styrene in the reinforced plastics industry in Egypt. Employment ranged from 5 to 22 years. The level of exposure to styrene was estimated from the mandelic acid level (328.3 + 266.2 mg/mg creatinine) in urine samples. Thioether level and RNase activity in urine were also determined. A statistically significant increased incidence of chromosome aberrations including or excluding gaps was observed in the peripheral blood lymphocytes in exposed workers (5.12 + 3.9 vs 2.2 + 1.6 in control, including gaps). Higher levels of thioether and RNase activity were also found among exposed workers. The authors implicate styrene as the causative agent; however, no information on other chemicals in the workplace was given. In addition, since the link between increased levels of thioether and RNase and cancer formation is not yet established, and the correlation of genotoxicity and carcinogenicity is not absolute, the conclusion that these findings support the "idea that the mutagenic and/or carcinogenic effect of styrene exposure is evidenced either on the chromosomal or the biochemical level" is not legitimate. At most, the results of the study indicate that the exposure to chemicals at the Egyptian company for plastics manufacturing is associated with the induction of chromosome aberrations in occupationally exposed individuals.

Norppa and Sorsa *[It* 47] reported that the available data on the genotoxicity of styrene demonstrate that in the absence of metabolic activation, styrene is not genotoxic in *in vitro* assays for gene mutation (bacterial and mammalian system), clastogenicity or DNA damage. This contrasts with the clear genotoxic activity in the same assay of the primary metabolite of styrene, styrene-7,8 oxide, arid indicates that any genotoxic activity of styrene will be dependent upon appropriate metabolic activation. She also reported that most *in vitro* mutagenicity studies of styrene in the presence of a metabolic activating system are negative or equivocal. She reported preliminary results showing that isolated erythrocytes were capable of metabolizing styrene into genotoxic species.

Sorsa also spoke on her experience of using cytogenetic techniques for biomonitoring human populations exposed to styrene. She reported that examination of lymphocytes from the peripheral blood for CA, SCE and micronuclei (MN) indicates that the exposure to styrene above 30 ppm is associated with increased chromosome aberrations. However, potential confounding factors such as smoking may have contributed to the observed effects. Sorsa stated that the human epidemiological studies which fail to demonstrate significant increases in CAs at relatively high styrene exposures may stiffer from inaccurate estimation of the worker exposure concentrations.

On the discrepancies between *in vivo* findings on the chromosome-damaging effects of styrene in rodents and the types of damage in exposed workers, Sorsa commented that the human data are meaningful and some damage could be so severe that although visible as CA at the first metaphase, after treatment it does not allow the cell to undergo the division necessary to express SCE. However, the evident lack of cytotoxicity in all experiments with styrene does not support this assumption, though it is true that most chromosome aberrations are cytotoxic and cells with such lesions generally do not survive more than one cell cycle.

Scott *f*# 59] presented a comprehensive review of 47 published cytogenetic studies of workers exposed to styrene. The lack of a positive dose/response relationship between styrene exposure and chromosome damage, and discrepancies between *in vitro* findings of the cytogenetic effects of styrene on human lymphocytes and the types of damage in exposed workers were discussed. Seventeen out of 47 studies have demonstrated an increased level of chromosome damage; however, there was no positive correlation between exposure levels and increased cytogenetic damage. Although cytogenetic analysis is considered a more appropriate means of evaluating long-term exposure, the studies failed to show a positive correlation between the incidence of chromosome aberrations and length of employment. Doubts were expressed as to whether styrene, or some other clastogens that were present at the work sites, was the causative agent of the cytogenetic damage.

Scott also examined the nature of genetic lesions induced by styrene *in vivo* and *in vitro* in human peripheral lymphocytes. A striking difference in cytogenetic endpoint was demonstrated using these two complimentary systems. *In vitro,* styrene induces a significant increase in SCE/cell at concentrations as low as 0.5 mM; furthermore, styrene oxide induces SCE at lower concentrations than that which induces duumosome aberrations. Therefore, it is reasonable to assume that if styrene were responsible for the chromosome damage observed in lymphocytes, higher incidence of SCE than of CA would be expected in

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cytogenetic studies of subjects exposed to styrene.

However, only two out of ten studies indicated an elevated level of SCE, and ten out of 24 reported an increase in CA. Furthermore, the elevation of SCE was induced at much higher concentrations than the increase of CA. For example, Cammurri et al (1984) reported a greater than three-fold increase in CA for workers employed in workplaces with styrene concentrations of 10 ppm although elevated SCE were reported only at five times greater styrene concentrations (~50 ppm). The type of chromosome aberration (dicentrics and rings) reported in three papers is not expected to be induced by styrene. These types of aberrations have never been demonstrated in *in vitro* studies in human lymphocytes exposed to styrene or styrene o)dde. The conclusion of the review was that the findings are "not consistent with the interpretation that styrene is responsible for the observed positive results in the workers."

Kligerman also recognized that their results do not support the findings that the increased frequencies of CA in epidemiological studies are due to styrene. Thus, it is not surprising that consensus opinion has not been reached as to whether styrene is the causative agent of cytogenetic damage observed in human monitoring studies. In the majority of these studies, estimates of styrene concentration in air were obtained from measurements of styrene metabolites in the urine. However, urinary metabolites are reliable markers only for assessing current styrene exposure, while chromosome aberrations can be derived from past exposures, even years before, to several other genotoxicants at the workplace or elsewhere. Furthermore, authors of most studies monitored workers employed in small workplaces. The subjects may have encountered high peaks of intermittent exposure, which are difficult to assess in terms of biologic impact.

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In conclusion, new studies were presented showing no effect on chromosomal aberrations from exposure to styrene at concentrations up to 1000 ppm for 4 weeks in rats or up to 500 ppm for two weeks in mice. However, there was significant controversy over whether increased chromosomal aberrations observed in reinforced plastics workers are related to styrene exposure.

Metabolism and Dose Estimation

R.R. Miller, Ph.D. and G. Cruzan, Ph.D.

Dose estimation is a critical component of risk assessment. Occupational exposure to styrene may occur via the skin or via inhalation of styrene vapors. Biological indicators of exposure to styrene, including measurement of styrene in blood and breath, as well as the measurement of styrene metabolites in urine, have been studied as techniques to assess the internal dose of styrene. Approximately 90% of an absorbed dose of styrene is known in man to be metabolized to mandelic acid (MA), phenylglyoxylic acid (PGA) or phydroxymandelic acid (HMA), and these metabolites are then excreted in the urine. Urinary metabolite measurements have therefore become the preferred biomonitoring method in field studies of workers potentially exposed to styrene.

Aitio et al. [# 8] reported the results of a study in which urinary excretion of MA, PGA, HMA, and styrene itself, were evaluated as a means for biological monitoring of styrene exposure. The study involved 205 workers in 32 workplaces in Finland. HMA was found to be unsuitable since the concentrations of this metabolite in urine were not quantitatively related to air concentrations of styrene. It was concluded from these studies that the sum of MA and PGA in urine samples collected 16 hrs. after exposure, i.e., the morning after exposure, is the best approach for biological monitoring of styrene. This conclusion was based on the correlation between mean airborne styrene concentrations and mean concentrations of the metabolites in urine. There was no indication of the degree of inter-individual variability in the concentrations of these urinary metabolites. PGA is unstable and samples must be frozen if they cannot be analyzed immediately after collection. No increase in the mean urinary concentrations of MA and PGA was observed during the course of a workweek. However, Gobba et al. [#13] reported results from a similar study in a group of 18 Italian workers, and concluded that there was an increase in the biological exposure indices (blood and urine styrene concentrations, and urinary MA and PGA concentrations) during the work week. In these latter studies, the degree of inter-individual variability was apparent. For example, the pre-shift blood styrene concentrations measured on Monday and Thursday were reported to be 0.18 μg/1 (0.25 mg/1S.D.) and 18.62 μg/1 (18.92 S.D.), respectively. The large standard deviation associated with these measurements shows that there is pronounced inter-individual variability in the biological exposure indices.

Perbellini et al. [#15] compared the blood styrene concentrations in a group of 76 workers exposed to styrene to those measured in a group of 81 "normal people." In this comparison group, styrene was detected in 95% of the blood samples, with a mean styrene concentration of 211 ng/l. At the end of a workshift with an average styrene airborne concentration of 204 μg/1, the mean blood styrene concentration was 1211 μg/l. The blood styrene concentrations were reported to be significantly higher when measured at the end of shift on Thursday, in comparison to the levels measured at the end of shift on Monday. The half-life of styrene in blood was found to be about 3.9 hours.

The metabolic interactions between styrene and ethanol were investigated by Coccini et al. [#16]. One route of detoxification of styrene oxide, the initial intermediate metabolite of styrene, is conjugation with glutathione (GSH). GSH is a nudeophile present in most mammalian tissues and is thought to play a protective role against reactive substances. GSH homeostasis is known to also be affected by high doses of ethanol, and repeated ingestion of ethanol has been shown to enhance the activity of the enzymes that catalyze the conversion of styrene to styrene oxide. The potential interactions of ethanol and styrene were investigated in rats given 5% ethanol (13-14 g/kg/day) and exposed to styrene vapor concentrations of approximately 326 ppm. It was concluded that administration of ethanol at doses sufficient to cause induction of styrene metabolism did not influence the depleting action of styrene on GSH levels in lung or liver.

Co-exposure to styrene and 1,3-butadiene can occur during certain manufacturing processes such as the production of synthetic rubber. Both substances are metabolized to epoxide intermediates as an initial metabolic step. Since the initial metabolic step for the two substances is similar, there is potential for each substance to influence the metabolism of the other when there is a simultaneous exposure to the two. Riser [#121 reported on the metabolic interactions in groups of rats exposed to 1,3-butadiene concentrations as high as 6000 ppm (v/v) and styrene concentrations as high as 500 ppm. The results of the studies indicated that co-exposure to the two substances resulted in a partial inhibition of the metabolism of 1,3- butadiene by styrene, whereas 1,3-butadiene had no influence on the elimination of styrene. The inhibition of 1,3- butadiene metabolism by styrene was competitive at styrene concentrations up to 90 ppm; higher styrene concentrations resulted in very little additional inhibition.

The metabolism of styrene to styrene oxide (SO) occurs via cytochrome P-450 monooxygenase enzymes. In human liver there are at least 10 cytochrome P-450 isozymes that could be effective. Nakajima et al. [#271 reported that of these 10 isozymes, CYP2B7 was the most effective in the metabolism of styrene to SO, followed by CYP1A2, CYP2E1, and CYP2C8. Three other isozyrnes also catalyzed the reaction, but at lower activities than the four mentioned above, while three additional isozymes showed little activity. It was concluded that any hazard from styrene exposure may depend on the specific cytochrome P-450 isozyme profile in the target organ.

There has been substantial concern in recent years about the toxicologic significance of styrene oxide as an intermediate metabolite of styrene. Extensive investigations focusing on species differences in the formation and degradation of styrene oxide (SO) have therefore recently been completed. Nolan et al.[#28] evaluated the activities of the enzymes involved in the formation and degradation of SO in liver tissue samples from rats, mice and humans. The studies indicated that, adjusted for relative liver and body size, the mouse has the greatest capacity and humans the lowest capacity to form SO from styrene. In addition, humans should be more effective in hydrolyzing low levels of SO than rodents. The results indicate that SO levels should be higher in rodents, particularly mice, than in humans at any given external styrene exposure concentration.

Kreuzer et al. [#23] reported on recent toxicokinetic studies of styrene in rats and mice. In both species, the rate of metabolism of inhaled styrene was concentration-dependent, increasing linearly with styrene exposure concentrations up to approximately 300 ppm. More than 95% of inhaled styrene was metabolized, with only small amounts exhaled as unchanged parent compound. Saturation of metabolism (vmax) occurred at styrene exposure concentrations of about 700 ppm in rats and 800 ppm in mice. At styrene concentrations below 300 ppm, there was little bioaccumulation of styrene in fatty tissues since the rate of metabolism was limited by the rate of uptake. Pre-exposure of rats and mice to 150 or 500 ppm on five consecutive days, 6 hrs./day, resulted in no change in the rate of styrene metabolism in comparison to non-pretreated controls. Related studies by Kessler et al. [#21] focused on the toxicokinetics of SO in rats, mice and humans exposed to styrene vapors, as well as in rats and mice given intraperitoneal (ip) or oral doses of SO. Oral administration of 200 mg/kg SO to rats and mice resulted in an area under the blood concentration time curve (AUCso) values of 780 and 11.2 ng*h/m1 in rats and mice, respectively. The systemic bioavailabilities of orally administered SO compared to ip administered SO were less than 4% in rats, and less than Pk in mice. These low bioavailability values via the oral route were thought to be due to fast hydrolysis of SO at the acidic pH in the stomach. In rats, blood SO concentrations increased from about 8 ng/ml to approximately 460 ng/ml with increasing styrene air concentrations ranging from 20 ppm to 800 ppm. Near-maximum blood SO concentrations were attained in rats at 260 ppm styrene; higher styrene exposure concentrations resulted in very little further increase in the blood SO levels. In mice, the blood SO concentrations were very similar to those in rats at styrene concentrations between 20 and 260 ppm. However, at styrene concentrations higher than 260 ppm, the blood SO concentrations increased sharply with exposure concentration to about 6000 ng/ml in mice exposed to 800 ppm styrene. These studies clearly show that there are pronounced species differences in the blood SO concentrations of rats and mice exposed to styrene concentrations higher than 260 ppm. Blood concentrations of SO were

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also measured in a group of 13 workers exposed to styrene concentrations of 10 to 90 ppm over a period of 4-5 hours. SO blood levels of 1 to 5.4 ng/ml correlated linearly with the atmospheric styrene exposure concentrations. On the basis of these results, the authors concluded that a mean SO blood concentration of 1 ng/ml would be expected in humans exposed occupationally to 20 ppm styrene.

Lof and Johanson [#241 measured blood styrene concentrations in two human volunteers exposed to styrene vapor concentrations ranging from 25 to 400 ppm. Metabolic saturation was indicated at styrene exposure concentrations of 200 and 400 ppm. The authors concluded that their results were consistent with previously published observations in rats, indicating the saturation kinetics are attained at styrene exposure concentrations of 200-600 ppm.

Csanady et al. [#18) developed a physiologically based pharmacoldnetic (PB-PK) model to describe the distribution and metabolism of styrene and its metabolite SO in liver, fat, muscle and visceral tissue in groups of rats, mice, and humans after exposure to styrene or SO. The PB-PK model predictions were validated by experimental blood SO measurements in rats and mice given styrene or styrene or SO via ip, oral, and inhalation routes. The model can be used to predict concentration time curves of styrene and SO in various tissues and blood of experimental animals and humans.

The area under the blood concentration time curve of styrene oxide (AUCso) has been proposed as an appropriate dose surrogate to assess the potential health effects of styrene exposure. Because of pronounced species differences in the rates of formation and degradation of SO, pharmacokinetic considerations must therefore be incorporated into human risk assessments as described in a poster by Poole [#30]. Using the PB-PK model presented by Csanady et al., the predicted AUCso levels in humans exposed to 50 ppm styrene for 8 hours were shown to be two orders of magnitude lower than in laboratory animals in long-term animal studies, where there was no evidence of an oncogenic response. This large margin of safety indicates that occupational exposure to styrene is very unlikely to result in carcinogenic effects.

Other Health Effects

Reproductive Toxicity and Neurotoxicity of Styrene

I. REPRODUCTIVE TOXICITY

J.R. Cushman, Ph.D. and G. Cruzan, Ph.D.

In his previous review of the developmental and reproductive toxicity of styrene, Brown concluded that, overall, styrene did not appear to exert any specific effects (1). Lindbohm [#401 appeared to have reviewed the same literature for her presentation at the Symposium. She interpreted the available information some-what more conservatively, stating that although the available information did not clearly or consistently indicate any reproductive or developmental effects due to styrene exposure, possible effects could not be completely discounted.

In his poster, Brown [#43] updated his review with an additional nine reports. These recent studies included two developmental studies in rats that showed no effects or marginal effects related to styrene; in fact, styrene was included as a "negative control." Four additional studies were judged to have limitations (such as small numbers of animals, parameters difficult to measure, high exposure levels) that compromised or limited their interpretation, although the authors reported effects on pup body weight, behavior, neurotransmitter levels, and developmental delay. In another recent study, an increase in sperm head abnormalities occurred at nearly lethal levels of styrene given orally to rats and mice. Rats exposed only through the milk of mother rats that received moderately high oral exposure showed reduced sperm counts and effects in some testicular enzymes. Brown suggested that these studies, although flawed, would justify additional investigation. The only new study in humans was a report that paternal occupational exposure to styrene did not produce an increase in spontaneous abortions or malformations. Together, the updated information is insufficient to alter the conclusion that styrene does not exert any specific developmental or reproductive effect.

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I. NEUROTOXICITY OF STYRENE

R.C. Leonard, Ph.D., J.R. Cushman, Ph.D. and M.G. Ott, Ph.D.

Three papers concerning the neurotoxicity of styrene were presented at the Helsinki symposium. Two of these presentations described human data, and the third described a rat study.

Matikainen [#39] reported on the relationship of several neuropsychiatric symptoms and exposure to styrene in a group of styrene-exposed reinforced plastics workers. One hundred men, aged 20-60 years (mean age 38 years), with duration of employment in the industry between 0.5 and 32 years (mean length 12.8 years) were categorized into exposure groups based on a qualitative description of the job process and a quantitative measure based on atmosphere concentrations of styrene and urinary mandelic acid. The rationale for this method of assessing exposure was not discussed. An "exposure index" (EI) was constructed based on (a) working method, (b) years on the job, (c) hrs/day spent laminating, (d) air concentration and (e) mandelic acid concentration: [(a+b)+cde]/4. The EI ranged from 1 to 15, with an average of 3.8.

The correlation coefficient of urinary mandelic acid and styrene air concentration was reported to be statistically significant. To avoid reporting bias, a group of aluminum workers was given the same questionnaire as the reinforced plastics workers. Both groups had been told that the study was directed at workers exposed to chemicals on the job.

More styrene workers reported symptoms than aluminum workers. Memory disturbances, irritability, and forgetfulness were reported more often by the laminators than the other exposure groups. Workers were divided into two groups based on the exposure index; no reason was provided for the use of 3.5 (as opposed to the average value of 3.8) as the point for dividing the groups. No significant differences were seen in the quantitative electro encephalogram (EEG) classification between those with an EI less than 3.5 and those with an EI greater than 3.5. However, those workers with higher exposure had higher absolute EEG power in alpha and beta bands in the fronto-temporal regions of the brain. The author concluded that (1) these data support that visuoconstructive disturbances are related to hours spent laminating and a higher exposure index; and (2) the data support the presence of neurotoxic effects below 50 ppm.

The study is limited by the unknown nature of the EEG symptoms and the lack of understanding of the significance of these findings. In addition, the author stated that after the passage of seven months, there were few symptoms, suggesting recovery. This study re-investigated some previously studied workers; some of the psychological endpoints "showed some correlation." However, the interindividual variation was not accounted for in the analyses. It is also interesting that different cut-points on the EI were used for different endpoints. The author stated that the data below 20 ppm were "soft."

Gobba and Cavalleri [#45] reported on an association between styrene exposure and color vision loss in 75 workers in the reinforced plastics industry. The analyses appeared to take into account such factors as individual variation, reliability of the test, and appropriate selection criteria for participants. The authors conclude that styrene exposure, as measured by correlations of air levels to urinary mandelic acid, leads to a dose-related color vision loss. This effect, based on data presented, appears to be synergistic with age.

The study is limited in that the data were not presented in such a way as to enable the identification of an exposure level below which no symptoms were detectable. In addition, comments by the author suggested that the differences in color vision between the exposed and unexposed were very subtle, leading to the question of what exactly constituted a clinically meaningful endpoint. Furthermore, earlier work on the relationship of age to color vision loss had led to a coefficient for age in unexposed individuals that was the same as that of the exposed workers in this study. This suggests that some factor or factors operating in the controls was unusual, and that in fact, there is no interaction between age and styrene exposure in color vision loss. Discussions with the author also suggested that there was little control over the timing for obtaining the urine samples, so that the estimates of

exposure may have been based on a period removed from the time at which the effects were measured.

Data from a 13-week ototoxicologic and neurotoxicologic study in rats were presented by Albee et al. [# 42]. This study was designed to address both the absence of a no observed adverse effect level (NOAEL) for ototoxicity in rats, and the issue of whether older rats would show ototoxic or neurotoxic effects similar to those described in an earlier study of very young rats (21 days old). Inhalation exposures of 0, 50, 200, and 800 ppm styrene vapor were given to 42-day old male Fischer 344 rats for 6 hr/day, 5 days/week for 13 weeks. The endpoints were measured by a functional observational battery (FOB), auditory brain stem responses (ABR), electrodiagnostic tests of the peripheral and central nervous systems, and by a comprehensive neuropathologic examination.

The high-dose animals showed auditory dysfunction at mid and high frequencies and elevated ABR thresholds at 16 kHz and 30 kHz. No treatment-related lesions were observed in the brain, spinal cord, or peripheral nerves, although neuropathologic examination showed hair-cell lesions in the cochleas of the 800 ppm dose group. This study demonstrated that older rats can experience ototoxic effects if the exposure level is high enough. The NOAEL for ototoxic effects of styrene was 200 ppm in this study.

Risk Assessment and Risk Management

Risk Assessment and Research Needs for Styrene

I. RISK ASSESSMENT

R.C. Leonard, Ph.D. and J.R. Cushman, Ph.D.

Gelbke [#77] elucidated the theme that risk assessment was far more complex than extrapolating directly from animal studies to human risk. This tenet was supported by a presentation that identified several aspects of chemical data that must be included in a risk assessment. These aspects include chemical structure, mutagenicity and carcinogenicity, the impact of differences in susceptibility, epidemiological data, and the generally large differences in the experimental doses and the human exposure under conditions of use. Data were presented to show that for styrene, the information on mechanisms and toxicokinetics is particularly important. The evidence is good that the metabolic mechanisms in rats and mice are quite different, and that humans appear to be more like rats than mice. This suggests that humans metabolize styrene to styrene oxide at a slower rate than do mice, and that humans also detoxify it more rapidly. This is supported by data that show styrene-7,8-epoxide has a low intrinsic potency for DNA-binding. All of this information provides useful data that should be incorporated into any human risk assessment of styrene.

. . . humans metabolize styrene to styrene oxide at a slower rate than do mice, and that humans also detoxify it more rapidly.

The inhalation Reference Concentration (RfC) for a chemical is an EPA estimate of a continuous inhalation exposure to the human population, including sensitive subpopulations, that is likely to be without appreciable risk of deleterious noncancer effects during a lifetime.The RfC for styrene was reviewed by Foureman and Jarabek [#41]. Fouremen stated the RfC was derived from the study of Muth (1984) involving a battery of neuropsychological function tests on a panel of 50 workers grouped into exposure categories by urinary metabolite levels. The analyses indicated a concentration-response relationship for 3 of the 8 tests, including those measuring intellectual function, memory, and visuo-motor speed. The EPA RfC workgroup considered the measurement tools to be reliable and valid, the statistics appropriate, and the dose-response relationship indicative of a genuine effect. In addition, they cited supporting data for a styrene effect on the vestibuloocular reflex. After the application of uncertainty factors because of the shortness of the exposures and variability in the data, EPA derived an RfC of 1 mg/m^3 (230 ppb) for styrene.

Lack of time prevented Foureinan from expanding on the controversial issues, such as the choice of uncertainty factors and the subjective basis for the confidence levels ascribed to the study, database, and the RfC itself. Medium confidence was ascribed to the RfC value. A critical assessment of the data needed to increase the confidence in the RfC also seems necessary, along with discussion of the 5 tests of the Mutti study that showed either no effect or no dose response.

I. RESEARCH NEEDS FOR STYRENE

G. Cruzan, Ph.D.

Dr. Cote, U.S. EPA [#80] pointed out that provisions of the Clean Air Act Amendments of 1990 are designed to minimize health risks from emissions of stationary sources and motor vehides. These provisions have increased the need for research that will provide both methods and data to support risk assessments. For styrene, there are many studies in humans suggesting neurotoxic responses with conflicting effects and no-observed-effect levels. Objective methods need to be developed and standardized for testing effects on cognitive dysfunction.

In addition, studies are needed to relate observed toxic effects to underlying biologic mechanisms in order to extrapolate data from animals to humans. Dr. Cote also pointed out that state-of-the-art chronic inhalation studies are needed for styrene, and that the industry was currently conducting such studies. Lastly, she pointed out that workplace exposure assessments for styrene need further verification and standardization.

Abstracts of Styrene-Related Presentations at the International Symposium on Health Hazards of Butadiene and Styrene

April 18–21, 1993, Helsinki, Finland

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#2

STYRENE ON THE OCCUPATIONAL SCENE

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Styrene (vinylbenzene, ethenylbenzene) is a commercially important chemical used in the production of various polymers. The world annual production of styrene' was estimated at almost 10 million metric tons in the late 1980s after an increase of about 6% per annum since the 1970s. The main production method' of styrene is catalytic alkylation of benzene with ethylene to obtain ethylbenzene, which is then dehydrogenated to styrene.

Of the total styrene production, 60-70% is polymerise[d1,2,]3 to expanded or impact (general purpose) polystyrene, 12% is ter- or copolymerised with acrylonitrite and butadiene to thermoplastics, 13°/0 is used for styrene-butadiene rubber, 8% for styrene-butadiene latexes and a smaller amount for emulsions. Styrene is also used as a reactive solvent for unsaturated polyester resins (6-7%), polymerised with maleic anhydride to yield special-purpose plastics, and used as raw material in various chemical syntheses.

The levels of occupational styrene exposure[3] largely depend on the type of operation involving styrene. No reports are available on styrene exposure in plants producing the styrene raw material. Besides styrene, these processes may give rise to benzene and ethylbenzene exposures. In polystyrene manufacture, the average styrene concentrations are low, less than 5 ppm, but high peak concentrations are possible, especially during batching, and machine cleaning and maintenance. In the reinforced plastics industry, where styrene is used as a solvent-reactant for polyester resins, styrene is a major air contaminant, with mean concentrations of 45 ppm and peak concentrations reaching hundreds of ppm. Other air impurities encountered in the manufacturing of reinforced plastic products include styrene oxide (0.1-0.5 ppm), methyl ethyl ketone peroxide, resin-fibre glass dust and acetone. In styrene-butadiene rubber production, exposures to butadiene, benzene and solvents may also occur.

The highest exposures to styrene occur in industries and operations where it is used more or less as raw material. Lower exposure levels (<1 ppm) are found in secondary processes, where styrene may be formed, for instance, through thermal degradation of polymers. The industry also contributes to the styrene exposure of general population, also with other sources such as building materials, traffic, tobacco smoke and combustion processes.

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1 United Nations. Industrial Statistic Yearbook 1989, Vol. 11, Commodity Production Statistics 1980-1989, New York, 1991.

2 Lieb M, Hildebrand B. Styrol. In Ullmanns Encyldopadie der technischen Chemie, Band 22, Verlag Chemie, Weinheim, 1982:293-309.

3 IPCS International Programme on Chemical Safety. Environmental Health Criteria 26, Styrene, World Health Organiza-

tion, Geneva, 1983:23-31.

#3

STYRENE EXPOSURE FOR THE CANADIAN GENERAL POPULATION

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As part of an environmental health assessment for styrene in Canada, we have derived estimated intakes of styrene via air, water, foods, and cigarettes by the general population. A detailed review of styrene concentrations reported in air, water and foods in Canada was performed. Studies considered comprised both the published literature, and unpublished industry reports and surveys of indoor air and foods conducted under contract for Health and Welfare Canada. Data for ambient air were collected by Environment Canada during 1988 to 1990[1]; the mean concentration of styrene at 18 sites across Canada ranged from 0.09 to 2.35 μg/m³. Indoor air data were from a national pilot study[2] of 757 homes across Canada, in which the mean styrene concentration was 0.28 μg/m³. The drinking water level used was the range of mean concentrations of styrene (0.05-0.25 μg/L) in treated water from 80 supplies in Ontario's 1990 Drinking Water Surveillance Program. For styrene levels in foods, the results of a limited Canadian survey[3] were used, in which samples of 34 food groups (each a weighted composite of individual food items) were collected from retail outlets in Windsor, Ontario in 1992. Styrene was not detected (LOD 0.001 μg/g for liquids and 0.005 μg/g for solids) in any of the food groups.

Based on these monitoring data, in conjunction with standard values for body weights and the intakes of air, water, soil, and food, daily intakes have been estimated from various media and for different age groups. Both air and food potentially contribute similar amounts to the intake of styrene by the non-smoking general population. Estimated intakes via ambient and indoor air combined range from 0.074 to 0.27 μg/kg b.w./day for various age groups (infants, toddlers, school-age children, teenagers, and adults were considered). Based on the LOD, intakes via food are calculated to range from < 0.11 to < 0.58 μg/kg b.w./day, depending on the age group. Estimated intakes via drinking water range from 0.001 to 0.03 μg/kg b.w./day. In contrast, potential exposures via cigarette smoke are much higher (based on the styrene reported in mainstream smoke [10 μg per cigarette][4], and a smoking rate of 20 cigarettes per day), as much as 2.86 μg/kg b.w./day for adults.

1 EC (Environment Canada). 1990. Unpublished data from National Air Pollution Surveillance Program, provided by T. Dann, Pollution Measurement Division, Conservation and Protection, EC.

2 HWC (Health and Welfare Canada). Results of a national pilot survey of airborne volatile organic compounds in Canadian residences. Volume 1. 1992a. Unpublished contract report by Concord Environmental Corporation, for Organic Chemistry Section, HVVC.

3 HWC (Health and Welfare Canada). Windsor area background study: Analysis of food products for target organic and inorganic parameters. 1992b. Unpublished contract report by Enviro-Test Laboratories, for Hazardous Waste Section, HWC.

4 U.S. Department of Health and Human Services. Reducing the health consequences of smoking - 25 years of progress - A report of the Surgeon General. Centers for Disease Control, Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health.

#4

NATURAL OCCURRENCE & ROUTES OF FORMATION OF STYRENE IN FOOD PRODUCTS

van den Berg, F., Maarse, H., van Ingen-Visscher, C.A.

In the literature the occurrence of styrene in food products and beverages is regularly reported. However, in a number of cases it is unclear whether it has to be regarded as a natural constituent or as a contamination.

In order to improve insight into this matter:

- an extensive literature search was carried out
- an inventory was made focused on the mechanisms of the formation of styrene
- the natural occurrence of styrene in 6 selected fresh
- food products was investigated

In this poster the mechanisms of formation in both fresh and processed products will be elucidated. Styrene can be formed:

- by bacteria and moulds
- from carotenoids
- from hydrocarbons
- from aldehydes
- by Maillard reactions
- during autoxidation of methyl arachidonate
- from 2-phenylethanol

The poster reports the results of analyses by means of multidimensional gas chromatography with mass

spectrometric identification in: apple, black current, cauliflower, onion, tomato and wheat. For each product, six samples, originating from different production sites, were analysed separately.

Conclusions:

- The concentration of styrene in the samples of apples, cauliflowers, onions and tomatoes is well below 1 μg/kg.
- The concentration of styrene in the samples of black currants is between 2 and 6 μg/kg.
- The concentration of styrene in the samples of wheat is between 0.5 and 2 μg/kg.
- Although the information from the literature on the occurrence of styrene in these products has been confirmed, the actual concentration in all products investigated is low.

This may be explained, at least in part, by the fact that in our study all precautions possible have been taken to avoid contamination of these products during harvesting, storage and sample preparation in order to safe-guard the reliability of the data obtained.

#5

DETERMINATION OF STYRENE CONCENTRATION IN SELECTED FOODS*

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The purpose of this study was to determine the amount of styrene present in several important raw agriculture commodities. The foods to be analyzed were very carefully collected and handled to prevent contact with styrene or any type of styrene-based polymer. The study included twelve types of foods: beef, chicken, milk, peanuts, pecans, coffee beans, oats, wheat, tomatoes, peaches, strawberries and cinnamon. Styrene measurements were performed using a dynamic heated headspace purge and trap extraction technique followed by quantification by selected ion monitoring capillary gas chromatography/mass spectrometry.

Styrene was detected in 8 of 12 selected food types. The highest concentrations of styrene occurred in cinnamon (157 to 39,200 ppb). This observation is not surprising in view of the close structural similarity between styrene (cinnamene) and cinnamic aldehyde, the principal constituent of cinnamon flavoring. Lower styrene concentrations on the order of approximately (0.3 to 8 ppb) were detected in samples of beef, coffee beans, peanuts, wheat, oats, strawberries, and peaches. No styrene was detected in samples of tomatoes, pecans, milk, and chicken at a detection limit of approximately 0.1 ppb.

The results of the studies indicated that styrene may be a natural constituent of many foods. The occurrence of styrene in processed foods cannot therefore automatically be assumed to be related to contact with styrene-based polymers such as polystyrene.

*Sponsored by the Styrene Information and Research Center, Washington, D.C.

#6

STYRENE IN FOODS AND ENVIRONMENT: ESTIMATION OF HUMAN EXPOSURE IN GERMANY

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In the present paper, data and informations about the occurrence of styrene in foods and environment and the potentially resulting human exposure in Germany are reviewed.

Styrene in some fruits, fruit juices or vegetables might result from its occurrence as a natural compound or from environmental contamination. Fruits and vegetables usually show a styrene content not exceeding 0.1 ppb. In wine, styrene was postulated to be formed by decarboxylation of cinnamic acid. The majority of German wines has been found to contain 1-3 μg/1 styrene. If a concentration of 3 μg/1 is assumed, the annual intake of styrene via wine would not exceed 0.1 mg/person on the basis of an average per capita consumption of 27-29 I wine.

Migration of residual styrene from polystyrene food packaging materials into food appears to be more relevant than styrene of natural origin. Migration is dependent on the residual styrene content, the nature of the packed foods, the contact area, the duration of the contact and some other factors. Butter, oil, margarine, cheese, milk, and dairy products packed in polystyrene packaging materials show styrene concentrations of < 5-25 ppb. The total average

per capita consumption of these food items is 97 kg/year for Germany. On the supposition that all these foods are packed in polystyrene and that an average styrene content of 20 ppb results from migration, the annual styrene intake from these sources would amount to 2 mg/person.

Exposure to styrene from inhaled air has been estimated in the Netherlands to amount to about 6.5 mg/person per year, corresponding to an average atmospheric concentration of 1.5 μg/m^3 styrene[1]. Personal air of smokers has been reported to contain about 2.4 μg/m3 styrene, making up for about 10.5 mg/person per year[2].

On the assumption that the data on inhalative styrene exposure also apply to the German situation, the total annual per capita exposure to styrene for nonsmokers in Germany can be estimated to reach 8.5 mg. For smokers total exposure might be up to 12.5 mg/person. If these assumptions are correct, about 25°/0 of total styrene exposure is attributable to intake through food.

1 Guicherit R, Schulting FL. The occurrence of organic chemicals in the atmosphere of the Netherlands. Sci Total Environ 1985;43: 193-219.

2 Wallace L, Pellizzari E, Hartwell TD, Perritt R, Ziegenfus R. Exposure to benzene and other volatile compounds from active and passive smoking. Arch Environ Health 1987;42:272-9

#7

INTERSPECIES DIFFERENCES IN METABOLISM AND KINETICS OF 1,3-BUTADIENE, ISOBUTENE AND STYRENE

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Investigations on inhalation pharmacokinetics of 1,3-butadiene and its primary reactive intermediate,epoxy-butene,in mice and rats have reasonably demonstrated that species differences observed in butadiene carcinogenicity are related to species differences in metabolism of butadiene. Compared to rats, a higher metabolic rate of 1,3-butadiene in mice, limited detoxification and accumulation of its reactive epoxide intermediate, epoxy-butene, are viewed as major determinants for the higher susceptibility of mice. The detection of alkylation products of epoxybutene and diepoxybutane with guanine residues in liver DNA of mice exposed to butadiene indicates that epoxybutene is further biotransformed to diepoxybutane in this species. This view is supported by a cross-linking activity of butadiene towards DNA and proteins in mice which can be attributed to the bifunctionally alkylating diepoxybutane. The quantitative differences in butadiene metabolism and in biological effectivity of the reactive epoxide intermediates between rats and mice reflect the different enzyme activities involved in butadiene metabolism. The major part of epoxide metabolism (epoxybutene) procedes via glutathione-S-transferase mediated pathways with the result of glutathione depletion and of subsequently increased toxicity and covalent binding of reactive butadiene intermediates. A drastic depletion of tissue non-protein-sulfhydryl content observed in mice but not in rats after acute exposure to butadiene is supportive of this view.

Isobutene (2-methylpropene) is converted by hepatic monooxygenase(s) to the epoxide, 2,2-dimethyloxirane. This epoxide, when appropriately tested, is mutagenic in S.typhimurium TA100 and TA1535. Addition of S9 mix diminishes the mutagenicity of 2,2-dimethyloxirane. The transformation of isobutene to its epoxide *in vitro* by hepatic microsomal enzymes is highest in mice, followed by rats, and finally man.

Styrene is also metabolized via its epoxide (styrene oxide). Observations in humans point to interindividual variations in the stereospecific metabolism of styrene by which a risk assessment on the basis of experimental animal data is complicated.

#8

BIOLOGICAL MONITORING OF EXPOSURE TO STYRENE : ASSESSMENT OF DIFFERENT APPROACHES

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Urinary excretion of mandelic acid (MA), phenylglyoxylic acid (PGA), p-hydroxymandelic acid (HMA), and styrene itself, were evaluated as means for biological monitoring of styrene, and the results were compared to those published earlier, in order to arrive at a biological reference value for styrene-exposed workers, corresponding to the Finnish work-place air standard, 20 ppm (85 mg/m^3).

There was a close linear relationship between the time-weighted concentration of styrene in the air on one hand, and the concentrations of MA, PGA, and styrene in the urine in workers exposed mainly through the lungs (skin exposure excluded by use of protective gloves). Such a correlation was not observed among workers exposed mainly through the skin (using protective masks). Styrene-exposed exhibited elevated urinary concentrations of HMA; these concentrations were, however, not quantitatively related to the air borne styrene concentrations. The levels of styrene were low. More than half of the workers studied excreted peak concentrations of mandelic acid two hours after the cessation of the exposure or later. No increase in the average urinary concentration of MA or PGA was observed during a working week.

We conclude that in the present working conditions, where the highest exposures tend to occur toward the end of the work day, the best approach for biological monitoring is the analysis of the sum of mandelic acid and phenylglyoxylic acid in a urine specimen collected 16 hours after the exposure, i.e., in the morning after the exposure. If the specimens cannot be analyzed rapidly or stored frozen, the analysis of mandelic acid in an after-shift specimen is the best choice, because of the instability of phenylglyoxylic acid in the urine (See Kivisto, et al, this volume). In the present study the sum of MA and PGA in a next-morning specimen corresponding to a time-weighted average (TWA) exposure to 20 cm^3/m^3 was 1.4 mmol/L. The published studies, this included, give 1.2 mmol/L as the best aggregate estimate for this biological exposure index.

#12

TOXICOKINETIC INTERACTIONS BETWEEN 1,3-BUTADIENE AND STYRENE IN RATS

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Styrene and 1,3-butadiene are widely used monomers in the production of synthetic rubber and other resins, especially in form of copolymers such as styrene-butadiene, acrylonitrile-butadiene-styrene. During manufacturing processes co-exposures to 1,3-butadiene and styrene may occur. These compounds can be transformed to their epoxides in the first metabolic step catalysed by cytochrome P-450 dependent monooxygenases. In laboratory animals 1,3-butadiene is carcinogenic. The potential carcinogenicity of styrene is still under discussion. because a series of carcinogenicity studies failed to give unequivocal results.

The aim of this work was to investigate the individual pharmacokinetics of 1,3-butadiene and styrene in Sprague-Dawley rats and to study pharmacokinetic interactions between both chemicals at simultaneous exposure. In order to determine the individual pharmacokinetics, animals were exposed to 1,3-butadiene gas or styrene vapors in closed all-glass chambers using the "closed chamber technique"[1]. To investigate the pharmacokinetic interactions, animals were co-exposed in closed all-glass chambers to mixtures of 1,3-butadiene (20, 100, 500, 1000, 3000, and 6000 ppm (v/v)) and styrene (0, 20, 100, 250 or 500 ppm). Prior to exposure to 1,3-butadiene, rats were preequilibrated with the respective styrene concentrations for 3.5 hours. Atmospheric concentrations of 1,3-butadiene and styrene were determined by withdrawing air samples at about 5 min. intervals and analyzed by gas chromatography. Calulated amounts of the compounds were injected every 30-40 minutes into the exposure chamber to maintain the concentrations within a pregiven range of ±15%). By this technique steady-state conditions were achieved. Under these conditions the amounts taken up equaled the amounts metabolized. For each exposure concentration of 1,3-butadiene and styrene the corresponding metabolic elimination rates were calculated based on the amounts of chemicals injected in the exposure chamber.

Metabolism of both substances was described by a Michaelis-Menten process. The maximal metabolic rates of 1,3-butadiene and styrene were 230 and 220 [μmol/kg/h], respectively. The apparent Michaelis-Menten constants related to the respective average concentrations were 1.2 and 40 μmol/L of tissue]. Furthermore, our results demonstrate that coexposure resulted in a partial inhibition of metabolism of 1,3-butadiene by styrene, whereas the presence of 1,3- butadiene had no influence on the metabolic elimination of styrene. The inhibition was competitive at atmospheric concentrations of styrene up to 90 ppm. The inhibition constant was 0.23 [μmol/L tissue]. Higher concentrations of styrene did result in a small additional inhibition only. This findings could be explained by polymorphism of the metabolizing enzymes.

If the carcinogenic risk associated with styrene exposure is less than that of 1,3-butadiene, co-exposure to styrene and 1,3-butadiene should result in a smaller carcinogenic risk than exposure to 1,3-butadiene alone, due to the inhibiting effect of styrene on metabolism of 1,3-butadene. A corresponding observation has been made in an epidemiological study[2]. The authors found a 7.6

fold excess risk of leukemia associated with exposure to 1,3-butadiene alone. Co-exposure to both substances did not result in an increase of the excess risk (7.4).

1 Filser JG. The closed chamber technique - uptake, endogenous production, excretion, steady-state kinetics and rates of metabolism of gases and vapors. Arch Toxicol 1992;66:1-10.

2 Matanoski G, Santos-Burgoa C, Zeger S, Schwartz L. Epiderniologic data related to health effects of 1,3-butadiene. In: Mohr U ed. Assessment of Inhalation Hazards 1989:201-214.

#13

KINETICS OF THE URINARY EXCRETION OF STYRENE DURING THE WORKING VVEEK AND ITS IMPLICATIONS IN BIOLOGICAL EQUIVALENT EXPOSURE LIMIT (BEEL) DEFINITION

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One of the most relevant problems in Biological Monitoring (BM) is the definition of effective Biological Limit Values (BLV) for exposure indexes. For many chemicals the BLV arise from the external dose-adverse effects relationship; these values can be called BEELs. Several factors, as body burden, work load, etc., can influence the relationship between external- and internal-dose. We decided to evaluate, in field conditions, the relevance of one of these factors, i.e.-the effect of the increase of styrene body burden during the working-week, on the relationship between environmental styrene and urinary styrene (StU), one of the main exposure index of the solvent. Also blood styrene (StB), urinary mandelic acid (MA) and phenylglyoxilic acid (PGA) were studied.

Eighteen styrene exposed workers were examined. Environmental monitoring was performed by passive personal sampling; styrene levels were measured daily from Monday to Thursday. Biological monitoring was performed twice, on Monday and on Thursday of the same week. Urine samples for StU, AM and PGA measurement were collected at 8 a.m. (pre-shift), at 12 p.m. (end of morning half-shift) and at 5 p.m. (end-of-shift). At 12 p.m., 5 ml of venous blood were collected for StB dosage. Styrene in urine and blood was measured by a gas chromatograph (HP 5880 A) connected with a mass selective detector (HP 5970 A); MA and PGA were measured by gas chromatography.

Environmental levels of styrene ranged 2.8-490.7 mg/m³; no statistical differences among the days were observed, suggesting a fairly constant exposure during the working-week. Pre-shift StU values significantly increased from Monday to Thursday: 0.18 lμg/l (0.25 S.D.) and 18.62 μg/l (18.92 S.D.) respectively ($p<0.001$). A significant increase ($p=0.02$) was also observed for pre-shift MA. These data prove a relevant increase of styrene body burden during the week, as pointed out by the ten-fold increase of pre-shift StU. The relationship between external exposure and biological exposure indexes was studied. For both Monday and Thursday, StU values were significantly related to the external exposure (correlation coefficients -r- were respectively 0.91 and 0.90), but on Thursday the intercept of the regression line was more than twice compared to Monday's one (19.76 vs.8.57), while the slope was similar (0.36 vs. 0.33). The resulting BEELs were respectively 85.97 and 90.71,u/l, based on Monday and Thursday regression lines: the former is similar to the BEEL we have previously proposed (85 μg/1), the latter is more than 5% higher. Similar results were obtained for MA (952.2 vs. 1006.5 mg/1). Even for StB we observed an increase of the mean values during the week, and an increase of the resulting BEEL. Furthermore, the increase of Styrene values in urine and in blood were significantly related ($r= 0.84$), supporting the hypothesis that StU is representative of the internal dose.

Our data confirm the usefulness of StU in the biological monitoring of styrene exposure, but they also prove, in field conditions, an increase of the body burden of the solvent during the working-week influencing the resulting StU values. Similar considerations are also applicable for the other tested exposure indexes. Taking into account the above observations, we suggest the opportunity to define the day of the working-week to be monitored or, at least, that the body burden of styrene is taken into account in the evaluation of the results of biological monitoring of styrene exposure.

#15

BLOOD STYRENE LEVEL IN „NORMAL PEOPLE" AND IN EXPOSED WORKERS AT THE END OF THE WORKSHIFT AND THE MORNING AFTER

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Blood styrene was measured in 81 "normal people" and 76 workers exposed to styrene by using a GC-MS method. Styrene was found in nearly all (95%) blood samples of "normal" people with a mean level of 221 ng/l. Styrene level was not significantly different between the smokers (233 ng/l) and non-smokers (211 ng/l). 95°/0 values were below 512 ng/1.

At the end of the workshift, in workers with an average exposure to styrene of 204 μg/l, blood styrene was 1211 μg/1. Significantly higher levels were found in blood samples of Thursday (1590 μg/1) than in Monday (1068 μg/1). Similar differences were found in blood samples collected the morning after the exposure (60 and 119 μg/1 respectively). Significant correlations between the blood and environmental styrene were found both at the end of the shift and the morning after the exposure (r =0.61 and 0.41 respectively). In workers occupationally exposed to styrene, 16 hours after the end of the work-shift, blood styrene level (94 μg/l) was significantly higher than that found in "normal" people (0.22 μg/1). The half life of blood styrene was 3.9 hours.

#16

STYRENE-INDUCED DEPLETION OF RAT PULMONARY NONPROTEIN SULPHYDRYL CONTENT: INFLUENCE OF ETHANOL*

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Recent studies have indicated morphological and biochemical changes including depletion of pulmonary glutathione (GSH) in the respiratory tract of animals exposed to high concentrations of styrene[1,2]. Although the mechanisms of styrene toxicity and mutagenicity are not well understood, an important role is attributed to the reactive intermediate, styrene oxide[3],which is conjugated with GSH and causes a drastic depletion of GSH contents in mammalian tissues[4]. Pharmacokinetic studies have documented the presence of styrene oxide in the lung of animals exposed to styrene[1]. GSH homeostasis is also affected by high dosages of ethanol and repeated ingestion of ethanol has been shown to enhance the activity of P450 monooxygenases, in particular cytochrome P45011E1, which catalyze the conversion of styrene to styrene oxide[5].

In the present study, pulmonary nonprotein sulphydryl (NPSH) contents and microsomal mixed function oxidase activities were investigated in rats exposed to styrene or to styrene in combination with ethanol. Male Sprague Dawley rats were given a liquid diet for three weeks yielding an ethanol-derived caloric content of 36% (5% w/v ethanol; 13-14 g ethanol/kg/day). Starting from the second week, groups of ethanol-treated animals and pair-fed controls were exposed to styrene vapour (326 ± 47 ppm) 6 hours daily, 5 days a week, for 2 consecutive weeks. In control pair-fed rats, styrene inhalation produced about 50cY0 and 40% depletion of NPSH contents in liver and lung, respectively (average NPSH values in untreated animals were 5.84 ± 0.59 and 1.14 ± 0.06 pm/g for liver and lung, respectively). A reduction of hepatic and pulmonary NPSH levels of the same magnitude occurred in the rats exposed to styrene in combination with ethanol. Compared to pair-fed controls, the animals of the styrene-ethanol group also exhibited significant increases in hepatic cytochrome P450 and b5 contents and aniline hydroxylase activity as well as increased urinary excretion of the styrene metabolites, mandelic acid, and phenylglyoxylic acid, measured during the last day of styrene exposure. Ln rats treated with ethanol alone, liver cytochrome P450 and b5 contents and aniline hydroxylase activity were increased while hepatic and pulmonary NPSH levels did not differ from those of untreated controls. Lung protein contents were not affected by any treatment. It is concluded that subchronic administration of ethanol at doses causing induction of styrene metabolism does not influence the depleting action of styrene on NPSH levels in hepatic and

pulmonary tissues.

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#17

SUBCHRONIC (THIRTEEN-WEEK) VAPOR INHALATION STUDY OF STYRENE IN RATS

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Male and female Sprague-Dawley rats were exposed to 0, 200, 500, 1000, or 1500 ppm, styrene vapor concentrations six hours per day five days per week for thirteen consecutive weeks. The study was conducted to assess the toxicity from repeated exposures and to determine appropriate conditions for a chronic (two-year) vapor inhalation study. Evaluation of toxicity was based on body weights, food and water consumption, hematologic, serum chemistry, and urinalysis evaluations after 4 and 13 weeks, gross necropsy, organ weights and histopathologic examination. Satelite groups of 5 male rats per exposure concentration were sacrificed after 1, 4 and 13 weeks of exposure to evaluate cell proliferation in lung and liver.

There were no exposure-related mortalities in this study. Males exposed to 1500 ppm styrene had reduced food consumption and body weights compared to the controls. Both males and females exposed to 1000 or 1500 ppm had a dose-related increase in water consumption throughout the entire study. There were no treatment-related effects on hematology, serum chemistry or organ weights. Exposure-related histopathologic changes were confined to minor effects on the olfactory epithelium of both males and females exposed to 500, 1000, or 1500 ppm. Styrene did not affect the rate of cell replication in liver or lung at any exposure level during the thirteen weeks of the study.

A chronic vapor inhalation study is currently in progress.

#18

PHYSIOLOGICAL PHARMACOKINETIC MODEL FOR STYRENE AND STYRENE-7,8-OXIDE IN MOUSE, RAT, AND MAN

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Styrene (S), a widely used industrial chemical, is mainly used for production of various plastic polymers. Occupational exposure to vapors of S may occur. In the first metabolic step S is oxidized to styrene-7,8-oxide (SO) by cytochrome P450 dependent monooxygenases. This reactive intermediate is further metabolized by epoxide hydrolases (EH) and glutathione transferases (GST). Concern about the carcinogenic potential of S has been associated with the occurrence of its metabolite SO, which produced forestomach tumors in rats and mice. The area under the curve of styrene oxide in blood (AUC_{SO}), which is in analogy to Haber's rule has been proposed as an appropriate dose surrogate to assess the carcinogenic risk associated with S exposure. The value of AUC_{SO} depends on the balance between bioactivation of S and detoxification of SO. This balance depends on species as well as route of exposure. Several pharmacokinetic models have been developed to describe the metabolism and disposition of styrene and its urinary metabolites in rodents and man, but all are limited to inhalative uptake and none can be used to predict the

body burden of SO. However, a comprehensive model has been developed describing the metabolism of 1,3-butadiene and 3,4-epoxy-l-butene following exposure to gaseous 1,3-butadiene in rodents[1]. The goal of the present study was to modify the physiological pharmacokinetic model presented in the literature to describe the distribution and the metabolism of S and its metabolite SO in liver, fat, muscle and visceral tissue groups of rodents and man following ip, po and inhalative administration of S or SO. The bioactivation of 1,3- butadiene leads to a similar reactive epoxide as in the case of styrene. The 3,4-epoxy-1-butene is further metabolized by GST and EH. This formal analogy, related to the similar biotransformation of styrene and butadiene, indicated to preserve the published model structure, which included already the oxidation of S to SO, the intracellular first-pass hydrolysis of SO catalyzed by EH and the conjugation of SO with glutathione (GSH) described by an ordered sequential Ping-Pong mechanism among GSH, SO and GST. The equations were revised to refer rather to the actual tissue concentration of chemicals than to their air equivalents. Furthermore, ip and po administrations were added too. The model contains four separate compartments as liver, fat, musde and a lumped group of visceral tissues. The partition coefficients describing tissue/blood and blood/air distribution were taken from the literature. Metabolic parameters were taken from *in vitro* or from *in vivo* measurements. The model has been validated: 1) by closed chamber experiments, in which Sprague-Dawley rats and B6C3F1 mice were exposed to S; 2) by steady-state exposure of Fisher rats to S in which blood concentration of S has been measured; 3) by steady-state exposure of Sprague-Dawley rats to S in which blood concentration of SO has been measured; 4) by closed chamber experiments, in which S was administered ip and po to Sprague-Dawley rats and B6C3F1 and the exhaled S was monitored: 5) by experiment in which SO was administered ip and po to Sprague-Dawley rats and B6C3F1 mice and blood concentration of SO has been measured; 6) by own and published human data. The model presented here can be used to predict concentration-time curves of S or SO in different tissues and blood. The forecasted AUC_{SO}-values have been used to estimate the carcinogenic risk associated with an occupational exposure to S.

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#21

TOXICOKINETICS OF STYRENE-7,8-OXIDE IN B6C3F1-MICE, SPRAGUE-DAWLEY RATS, AND HUMANS

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Styrene-7,8-oxide (styrene oxide), the first intermediate metabolite in the biotransformation of styrene, produced forestomach tumors in rats and mice following oral administration. A carcinogenic potential of styrene is therefore linked with the resulting burden of styrene oxide. The first aim of this study was to investigate toxi-cokinetics of styrene oxide in blood of animal strains treated in long-term studies with styrene oxide and styrene. A second one was to determine styrene oxide levels in blood of workers exposed to styrene and to compare the results with those obtained in rodents. The study was based on the hypothesis that in all three species the area under the blood concentration-time curve of styrene oxide (AUC_{SO}) could serve as a dose surrogate which is proportional to the carcinogenic risk of styrene and styrene oxide. Concentration-time courses of styrene oxide were monitored in blood of male B6C3F1 mice and male Sprague-Dawley rats following administration of a single dose of styrene oxide either intraperitoneally (ip) or orally in olive oil (po). In another series of experiments, animals were exposed to constant concentrations of styrene vapor. Furthermore, concentrations of styrene oxide was measured in blood of laminators exposed to styrene vapors. A direct gas chromatographic method was used for the determination of styrene oxide[1].

Quantitative relations between dose of styrene oxide or concentration of styrene and level of styrene oxide in blood have been found. From the blood concentration-time courses of styrene oxide, the AUC_{SO} values (x- ± S.D.) were computed. After ip administration of 200 mg/kg styrene oxide AUC_{SO} was similar in both species, 19700 ± 8700 [ng*h/ml] (n=3) in rats and12200 ± 3400 [nrh/ml] (n=3) in mice. Administration of 200 mg/kg styrene oxide po to rats and to mice led to AUC_{SO}'s rats of 780 ± 290 [ng*h/ml] (n=3) and in mice of 11.2 [ng*h/ml] (n=2). Systemic bioavailabilities of po administered styrene oxide compared with that after ip administration were 3.7 ± 2.2% in rats

and 0.09 ± 0.03% in mice. These low values may result from fast hydrolysis of po administered styrene oxide within the stomach. In vitro, half-life of styrene oxide depended strongly on the pH value, being only 0.4 min at pH 3 and 37°C. In rats exposed to various styrene air concentrations from 20 ppm to 800 ppm under steady state conditions, styrene oxide blood concentrations increased from about 8 ng/ml to approximately 460 ng/ml reaching a plateau at exposure levels above 260 ppm styrene. Styrene oxide blood concentrations were correlated linearly with the rate of the saturable metabolism of styrene. In mice, styrene oxide blood concentrations were similar to those in rats at lower styrene exposure concentrations of 20 and 260 ppm. However, at styrene concentrations above 260 ppm the resulting steady state styrene oxide concentrations showed a sharp dose-related increase to about 6000 ng/ml in mice exposed to 800 ppm styrene.

In 13 workers, blood concentrations of styrene oxide were measured after exposure to styrene over 4-5 h. During this time period the atmospheric styrene concentrations at the workplace ranged from 10 to 90 ppm as determined by personal air monitoring. Styrene oxide levels in blood lying between 1 and 5.4 ng/ml were correlated linearly to the atmospheric styrene concentrations resulting in a styrene oxide/styrene ratio of 0.052 [(ng/ml)/ppm]. On the basis of an exposure to 20 ppm styrene (German MAK-value) a mean styrene oxide concentration of about 1 ng/ml blood could be calculated which was about one order of magnitude lower than the corresponding values found in rodents.

The obtained toxicokinetic data facilitate the calculation of AUC_{SO} in mouse, rat, and man for various routes of exposure to styrene or styrene oxide. The AUC_{SO} values provide the basis for carcinogenic risk estimates of styrene in man.

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#22

ANALYSIS AND STABILITY OF PHENYLGLYOXYLIC AND MANDELIC ACIDS IN THE URINE OF STYRENE-EXPOSED PEOPLE

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The most widely used method for the biological monitoring of exposure to styrene is the urinary excretion of mandelic acid. However, several recent studies indicate that the sum of mandelic acid (MA) and phenylglyoxylic acid (PGA) in urine would best reflect the exposure[1,2],3. A prerequisite for the use of this approach is the stability of PGA in the urine, which has been questioned[1],4.

Therefore, a re-examination of the stability of PGA was undertaken; at the same time, a new liquid chromatographic method to analyse MA and PGA was developed. 'This method uses a modern diode array detection technique in which these compounds can be measured using different wavelengths. This liquid chromatographic method was compared to a gas chromatographic method developed for the analysis of MA, PGA and p-hydroxymandelic acid (HMA). The methods gave results consistent with each other.

These two methods were then used to check the stability of the main metabolites of styrene, especially of phenylglyoxylic acid, in urine samples stored at +6°C or at -18°C for periods up to 70 days.

None of the frozen samples showed any significant decrease in the phenylglyoxylic acid concentration, whereas at +6°C one of the samples showed a reduction of 46% after 1 month.

We conclude that for accurate analysis of PGA it is mandatory to store the samples frozen. Also external quality control samples have to be transported frozen.

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#23

TOXICOKINETICS OF STYRENE IN B6C3F1-MICE AND SPRAGUE-DAWLEY RATS

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The goal of this work was to investigate the toxicokinetics of styrene in rat and mouse strains which were used in

long-term bioassays to enable the calculation of the styrene body burden resulting from various kinds of exposure.

Male rats and mice were exposed, as described earlier[1], in closed all-glass chambers to styrene vapors of initially produced concentrations ranging from 600 to 4,600 ppm or after receiving intraperitoneal (ip) doses of pure styrene from 20 to 340 mg/kg or oral (po) doses of styrene in olive oil from 100 to 350 mg/kg. Concentration-time courses of styrene in the chamber atmosphere were monitored by gas chromatography and toxicokinetics were analyzed by a two-compartment model. In both species, the rate of metabolism of inhaled styrene was concentration dependent. At steady state it increased linearly with exposure concentration up to about 300 ppm. More than 95% of inhaled styrene were metabolized, only small amounts were exhaled as the unchanged compound. At these low concentrations transport to the metabolizing enzymes and not their metabolic capacity was the rate limiting step for metabolism. Consequently, below 300 ppm toxicokinetic behavior of styrene is strongly influenced by physiological parameters as blood flow and especially the alveolar ventilation rate. At exposure concentrations above 300 ppm the rate of metabolism at steady state was limited progressively by biochemical parameters of the metabolizing enzymes. Saturation of metabolism (V_{max}) was reached at atmospheric concentrations of about 700 ppm in rats and 800 ppm in mice, V_{max} being 224 μmol/ (h*kg) and 625 μmol/(h*kg), respectively. The atmospheric concentrations at $V_{max}/2$ were 190 ppm in rats and 270 ppm in mice. Styrene accumulates preferentially in the fatty tissue as deduced from its partition coefficients olive oil:air and water:air which have been determined *in vitro* at 37°C to be 5500 and 15. In rats and mice exposed to styrene vapors below 300 ppm, there was little accumulation since the rate of metabolism was limited by the rate of uptake. This is expressed by the rather low bioaccumulation factor body:air at steady state (Kst*), in comparison to the thermodynamic partition coefficient body:air (Keq) which was determined to be 420. Kst* increased from 2.7 at 10 ppm to 13 at 300 ppm in a rat and from 6.0 at 20 ppm to 13 at 300 ppm in a mouse. Above 300 ppm, Kst* increased considerably with growing concentration since metabolism became saturated in both species. At levels above 2000 ppm Kst* reached its maximum of 420 being equivalent to Keq. Diethyldithiocarbamate, administered ip (200 mg/kg in rats, 400 mg/kg in mice) 15 min prior to styrene exposure, reduced the rate of styrene metabolism to about 10% indicating that most of styrene is metabolized by cytochrome P450-dependent monooxygenases. In order to simulate chronic exposure, rats and mice were exposed to 150 and 500 ppm styrene on five consecutive days (6h/d). On day six, inhalation kinetics were studied. No change in the rate of styrene metabolism was detected compared to non-pretreated controls. The ip administration of styrene to rats and mice resulted in concentration-time courses in the atmosphere of the closed chamber which coincided with those predicted by the applied toxicokinetic model. After po administration of styrene to rats and mice concentration time courses showed considerable inter-animal variability. For obtaining reasonable fits through the data the kinetic model was extended by a first order absorption from the gastrointestinal tract with half-lives of 0.87 h (rat) and 0.41 h (mouse). The parameters describing distribution and metabolism of inhaled styrene were extrapolated allometrically from rat to mouse and from rat and mouse to man. A good agreement with experimentally determined values was obtained.

The presented toxicokinetic data of styrene are of essential importance for computing the burden of the reactive metabolite styrene oxide resulting from exposure to styrene.

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#24

EXPOSURE-DEPENDENT KINETICS OF INHALED STYRENE IN MAN

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The major metabolic pathway of styrene is the oxidation to styrene-7,8-oxide which is subsequently hydrated to styrene glycol. Styrene oxide is genotoxic, binds covalently to macromolecules, and is a carcinogen in laboratory animals. At high styrene exposure levels metabolic saturation can be expected. This would cause nonlinear relationships between the ambient air concentration of styrene and the body burden of styrene and styrene oxide. The aim of this study was to investigate the occurrence of nonlinear lcinetics of styrene in man.

Two volunteers were exposed to styrene vapor (2 h; 50 W) at four different occasions (25, 75, 200, and 400 ppm).

Styrene concentration was measured in arterialized capillary blood during and after exposure by head-space gas chromatography. The maximum metabolic rate of styrene (V_{max}) was estimated by fitting the observed blood data to a physiologically-based pharmacokinetic (PB-PK) model[1].

Metabolic saturation was indicated at the higher exposure concentrations (see figure*, the line is a linear extrapolation of the AUC at 25 ppm). The PB-PK modelling suggests a V_{max} of 2,9 mmol/hour which is in close agreement with that obtained by extrapolation from rat and mouse experiments. Our results are also in agreement with the observation in rats that saturation kinetics are reached at an exposure concentration of 200-600 ppm styrene' and with data from styrene exposed workers where the excretion of mandelic acid and phenylglyoxylic acid in urine appears to reach a plateau above 150 ppm styrene[4].

Our results indicate that at very high styrene exposure levels (8 h x 400 ppm) the AUC of styrene is two times higher than predicted from low exposure levels by linear extrapolation. The opposite relationship is expected for styrene oxide, as indicated by animal experiments. The degree of binding to plasma proteins and DNA in mice after high intraperitoneal doses of styrene was only 40-60% of that seen after low doses[5]. In rats exposed to styrene for 3 h the concentration of styrene oxide in blood levelled off at exposures above 400 ppm[6].

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*Figure not illustrated

#27

CHARACTERIZATION OF HUMAN CYTOCHROME P450 ISOZYMES RESPONSIBLE FOR STYRENE METABOLISM

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Human cytochrome P450 isozymes responsible for styrene metabolism were investigated with vaccinia virus-mediated cDNA expression system in cultured cells. The metabolism of styrene was studied by measuring the rate of styrene glycol formation. In ten human P450 isozymes, CYP2B7 was the most effective in the formation of styrene glycol, followed by CYP1A2, CYP2E1 and CYP2C8; CYP3A3, CYP3A4 and CYP3A5 also catalyzed the metabolism, but their activities were much smaller than those of the first four isozymes mentioned above; CYP2A6, CYP2C9 and CYP2D6 showed little activity toward the metabolism.

The metabolism of styrene in human liver and lungs was also investigated by measuring the rate of styrene glycol formation. There were at least two P450 isozymes with different Km value for styrene metabolism in human livers. Inter-individual differences were seen in the metabolism of styrene at a high substrate concentration; the lowest activity was 0.82 nmol/mg protein/min and the highest one 3.57 nmol/mg protein/min. Styrene was also metabolized in human lungs, but the average activity was 100 times less than that in human livers.

Styrene metabolism was compared between human CYP1A2 and mouse CYP1A2 which are categorized in the same P450 family, but slightly different in the amino acid sequence. The activity of human CYP1A2 was 3 times that of mouse CYP1A2, suggesting that slight difference in amino acid sequence significantly affects the activity. In other words, the use of human P4,50 isozymes is important to know the exact information for the metabolism in human.

In conclusion, a large number of human P450 isozyrnes are able to catalyze the oxidation of styrene to styrene glycol. Styrene is oxidized through a DNA reactive epoxide

intermediate; therefore, any hazard from styrene exposure may depend on the P450 isozymes profile in the target organ.

#28

COMPARISON OF THE KINETICS OF STYRENE AND STYRENE OXIDE METABOLISM MEASURED IN VITRO USING RAT, MOUSE AND HUMAN TISSUES*

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To help evaluate the hazard associated with styrene and its toxic metabolite, styrene oxide (SO), the activity of the enzymes involved in the formation (monooxygenase, EC 1.14.14.1) and destruction (epoxide hydrolase, EC 3.3.2.3 and glutathione-S-transferase, EC 2.5.1.18) of SO were measured in male Fischer and Sprague-Dawley rats, in male B6C3F1 mice and in samples of human liver tissue. The affinity of hepatic monooxygenase for styrene was similar in the rat, mouse and human with the apparent Michaelis constant (K_m) ranging from 0.05 - 0.09 mmolar while the V_{max} for this enzyme in rodent liver was 4 to 7 fold greater than in human liver (V_{max}, of 9 to 13 versus 2 nMoles/min/mg protein). The affinity of hepatic styrene epoxide hydrolase activity for SO was much greater in human liver (K_m 0.01 mmolar) than in rat or mouse liver (K_m = 0.13 - 0.74 mmolar) although the V_{max} for this enzyme was similar in all three species (~1 5 nMoles/min/mg protein). In human liver, glutathione-S-transferase activity was much lower than epoxide hydrolase activity. In contrast, glutathione-S-transferase activity (V_{max}, of 39 - 47 nMoles/min/mg) was 2 to 3 fold higher in rodent liver than styrene epoxide hydrolase activity. Interpretation of the high glutathione-S-transferase activity in rodent liver is uncertain because hydrolysis, rather than conjugation, is the primary pathway for SO detoxification in vivo. Prior inhalation exposure to styrene [3 daily 6-hr to 600 ppm (rat and mouse) or 4 daily 6-hr to 1000 ppm (rat)] had no effect on styrene monooxygenase activity. In contrast, 4 daily 6-hr exposures at 1000 ppm styrene increased hepatic styrene epoxide hydrolase activity 1.6 fold in rats. The activity of these three enzymes was 2 to 15 fold higher in the rodent liver than the lung. In summary, the V_{max} for styrene monooxygenase activity, adjusted for relative liver and body size, suggests that the mouse has the greatest capacity and humans the lowest capacity to form SO from styrene. The relative activities of the monooxygenase to epoxide hydrolase indicates that SO levels should be higher in rodents than human for a given styrene exposure.

*Sponsored by the Styrene Information and Research Center.

#29

EXPOSURE TO STYRENE IN FINLAND IN 1984-1991 AS ASSESSED BY BIOLOGICAL MONITORING

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Biological monitoring of styrene has been carried out in Finland for about twenty years. To estimate possible changes in styrene exposure we collected and analyzed the data of mandelic acid concentrations in post shift urine samples accumulated in the Laboratory of Biomonitoring of the Institute of Occupational Health and in the Regional Institutes of Kuopio, Lappeenranta, Oulu and Turku in 1984-1991, altogether 6619 results, 2999 workers, and 484 work places. The standard industrial classification (SIS)[1] was used for the grouping of similar industrial activities.

After the first year of the follow-up period the armual number of analyses decreased, remaining rather stable until 1991 when there was a further decrease. The mean level of all urinary mandelic acid determinations increased for the first three years reaching the maximum, 4.0 mmo1/1, in 1986, thereafter a decreasing trend has been detected to a level of 1.9 mmol/I in 1991. Exposure to styrene has been detected in 26 of 98 possible classified industrial classes. Out of 6619 samples 732 could not be linked to a certain work place and/or a standard industrial class. The two main classes were plastic products manufacture (class=21, 2715 samples) and transport equipment manufacture, mainly boat building, (class=27, 2060 samples). The mean concentration of mandelic acid in urine exceeded 2.9 mmo1/1, corresponding of exposure to 20 cm^3/m^3 of styrene[2], in three out of the five industrial classes with largest number of analyses in 1989 and in two out of the five in 1990. In 1991 there were no large groups of workers with the mean level of urinary mandelic acid reaching the value 2.9 mmo1/1.

As a conclusion, a decreasing trend in the styrene exposure level has been found during the years 1984-1991 in

Finland. The timing of this trend started after the change of the Finnish hygienic limit value to styrene exposure from 50 to 20 cm^3/m^3 in 1987 followed by the change in the limit value for biological monitoring analysis, mandelic acid in urine, from 7 to 3.2 mmo1/1.

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2 Pekari K, Nylander-French L, Pfaff P, Sorsa M, and Aitio A, Biological monitoring of exposure to styrene - Assessment of different approaches, submitted.

#30

APPLICATION OF PHARMACOKINETICS AND MACROMOLECULAR BINDING IN A RISK CHARACTERIZATION OF THE PUTATIVE CARCINOGENIC POTENTIAL OF STYRENE

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The weight of the evidence from currently available epidemiology studies and long-term animal studies does not show a carcinogenic response related to styrene (S). This absence of an exposure related oncogenic response in man or experimental animals precludes the use of conventional methods of quantitative risk estimation. Nevertheless concern has been raised about a putative carcinogenic potential due to formation of styrene-7,8- oxide (SO) as an intermediate metabolite of S. In order to address this concern data were generated on 1) species differences in relative rates of formation and degradation of SO in tissues and 2) the level of interaction with DNA.

Toxicokinetic investigations showed that while rodents have a greater capacity than humans to form SO from S, humans are more effective than rodents at hydrolyzing the oxide. Internal SO concentrations in plasma are thus higher in rodents than humans at equivalent dose of S. A physiologically based pharmacokinetic (PB-PK) model, developed to describe these interspecies differences, shows that the internal dose of SO in rodents at exposure concentrations of S where there is no evidence of a systemic oncogenic response, is at least two orders of magnitude higher than in man exposed to 50 ppm S for 8 hours. This "margin of safety approach" indicates that occupational exposure to S is unlikely to result in carcinogenic effects.

Using data on pharmacokinetics and macromolecular binding a different approach attempted to provide a quantitative risk estimation for S. Such calculations show that the highest possible theoretical life time risk for occupational exposure is in the order of 6 in 10^5 to 1 in 10^6. These estimates conceded as being worst case (probably largely overestimating the possible risk), support the conclusion that occupational exposure to S does not represent a carcinogenic hazard.

#31

DNA ADDUCTS OF STYRENE AS A MARKER FOR BIOMONITORING OF HUMAN EXPOSURE

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Styrene oxide, used as model compound, was reacted with DNA and the adducts were characterized by th^e 32P-post-labeling assay. Using all the main 2-deoxyguanosine 3'-monophosphate adduct standards available, including N-7, N^2- and O^6-adducts, it was shown that O^6-dGMP adducts were recovered in higher yields than N^2-dGMP adducts, while N-7 adducts were not determined because of poor labeling. Both O^6 and N^2 adducts were shown to be resistant to the action of nuclease P1.

Styrene oxide-modified DNA with known amount of O^6 adducts, based on HPLC separation, was diluted in steps of 10-fold with non-modified DNA and the O^6-adducts were quantified. The labeling efficiencies were higher at low substrate concentration (about 10% at 0.9 fmol as compared to 1.5°/0 at 900 fmol) due to exhaustion of radioactive ATP from the reaction mixture.

These quantitative data and further optimization of the postlabeling method in many parameters enabled the analysis of white blood cell 06-adducts from lamination workers exposed to relatively high concentrations of styrene for at least 5 years. Preliminary data from few individuals indicate about 2-fold increase of O^6-adducts in styrene-exposed workers (1.51±0.30 adducts/10^8 dNp) as compared to controls (0.56±0.06 adducts/10^8 dNp) employed in agriculture. It is unclear whether samples of the controls actually exhibited styrene-adducts or, perhaps more likely, unspecific background radioactivity.

#35

DETERMINATION OF STYRENE-DNA ADDUCTS WITH 32P-POSTLABELING

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Styrene 7,8-oxide (SO), the main intermediate metabolite of styrene (S), has been shown to form DNA adducts *in vitro.* The goal of this study was to develop a sensitive assay for DNA adducts which could be used to biomonitor individuals occupationally exposed to S. The method chosen was the ^{3}2P-postlabeling technique, which allows the analysis of microgram amounts of DNA with high sensitivity. The assay typically involves isolation of DNA from an organ of interest (or from peripheral lymphocytes), digestion to the 3'-nucleotides, adduct enrichment, phosphorylation at the 5'-position with ^{3}2P-ATP and a kinase, adduct separation using thin-layer chromatography (TLC) and quantitation of 32P in the various adduct spots.

In a first set of experiments, [7-^{3}H]SO was reacted *in vitro* with DNA. Adducts were enriched with nuclease P1, ^{32}P-labeled and purified by high-salt C_{18} reverse-phase TLC. After elution from this layer, adducts were separated by two-dimensional PEI cellulose TLC. One major, three minor and several trace adducts were detected[1]. The efficiency of the kinase reaction depended upon the ATP concentration. Labeling efficiencies of over 50% (based on [^{3}H]SO-labelled DNA) could be achieved at high ATP concentration.

The possibility of using S-adducts as a biomarker for exposure was explored in liver DNA of female B6C3F1 mice treated with S at dose levels which gave rise to detectable adduct formation using [^{3}H]S[2]. Preliminary results show that S-nucleotide adducts are measurable by postlabeling, although the detection of S-derived spots is difficult because of a high background. So far, the limit of detection was of the order of 1 adduct/10^{7} nucleotides.

It is concluded that the postlabeling assay is suitable for the detection of SO-derived adducts *in vitro* and, at high styrene dose levels, also *in vivo.* Further development is needed, however, for the application of the assay to a biomonitoring of occupational exposure.

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2 Cantoreggi S, Lutz, WK. Covalent binding of styrene to DNA in rat and mouse. Carcinogenesis 1993: 14; 355-360

#36

BIOMONITORING EXPOSURE TO STYRENE OXIDE BY GC-MS ANALYSIS OF ITS ADDUCTS WITH HAEMOGLOBIN

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Exposure to styrene oxide results in the formation of an N-terminal phenylhydroxyethylvaline derivative in haemoglobin (Hb). An analytical method for this adduct has been developed using a modified Edman Degradation technique employing the reagent pentafluorophenyl isothiocyanate. The resulting pentafluorophenylthiohydantoin is purified by solid phase chromatography, converted to the acetyl or trimethylsilyl (TMS) derivative and detected by gas chromatography-mass spectrometry (GC-MS) selected ion recording (SIR). Quantitation is achieved using an internal standard prepared by reacting c18-styrene oxide *in vitro* with Hb. The lower limit of detection of the assay is 10pmol adduct/g globin, and the yield of thiohydantoin produced in the procedure (determined by radiochemical means) accounted for 5.2°/o of total globin alkylation *in vitro* by styrene oxide.

Styrene oxide also reacts with carboxylic residues in Hb, yielding phenylhyroxylethyl esters. Mild hydrolysis of the globin cleaves these esters, yielding 1-pheny1-1,2-ethanediol (styrene glycol). The yield of this product accounted for 15% of total globin alkylation *in vitro* by styrene oxide. A GC-MS method to detect styrene glycol in globin hydrolysates, with a limit of detection of 15pmol/g globin has been developed to yield a second estimate of the bound dose of styrene oxide. The liberated styrene glycol is converted to its TMS derivative and quantitated by SIR using c18-styrene (from hydrolysis of c18-styrene oxide reacted globin) as internal standard.

The dose-response relationship of adduct formation with N-terminal valine and carboxylic acids in Hb was studied in rats given ip doses of 0-833μmol styrene oxide/kg body weight. A linear increase in adduct levels (0-179pmol/g globin) was observed for the valine adduct, but the dose-response relationship for the car boxylic acid

esters was not linear. Occupational exposure to styrene has been studied on a limited number of samples, but no adducts have so far been detected.

#39
NEUROTOXICITY IN STYRENE EXPOSED WORKERS

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There is a general agreement that styrene can cause neurotoxic disturbances[1]. Clinical neurotoxic syndromes are, however, uncommon. In the literature, there are number of case reports linking occupational styrene exposure and neurological disease, but the evidence behind the occupational etiology is at best vague. Although overt clinical intoxications from occupational exposures are rare, there is no uncertainty about the ability of styrene to cause disturbances in the functions of the central nervous system. Both acute and chronic exposure can cause neurotoxic adverse effects.

In chronic occupational exposure, increased symptomatology, and neurophysiological and psychological changes have been reported. An increase in abnormal EEGs, when urinary mandelic acid concentration exceeded 4,6 mmo1/1, has been reported. In a recent study, quantitative EEG (QEEG) data showed a significant increase in abnormal QEEG classifications in workers with higher exposure[2]. When the workers were divided into two groups, based on the long-term exposure data, those with higher exposure had higher absolute EEG power in alpha and beta bands in the fronto-temporal regions of the brain. Higher exposure was also related to reported neurological symptoms. Memory disturbances, headache and limb pain were especially associated with exposure. In psychological studies, prolonged reaction times and a decrease in neurobehavioral performance have been reported.

The central point of interest in styrene neurotoxicity is not its capability to cause central nervous system disturbances, but the level of exposure where alterations can be expected. Neurobehavioral alterations have been reported in several studies where the 8 hour time-weighted air concentration has exceeded 100 cm^3/m^3. Changes in psychomotor functions have been reported with levels exceeding 35 cm^3/m^3, and visuomotor inaccuracy with levels above 50 cm^3/m^3. Diminished verbal learning has been reported even with lower exposure levels. There are, however, reports showing no evidence of central or peripheral nervous system involvement below exposure levels of 100 cm^3/m^3. This discrepancy is explained by differences in the sensitivity of the methods applied, and by the difficulties to determine long-term exposure levels. Based on the available literature, it seems logical to consider the recent trend to lower the administrative levels of exposure to be in the right direction.

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#40
EFFECTS OF STYRENE ON THE REPRODUCTIVE HEALTH OF WOMEN

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Styrene monomer and its metabolite, styrene oxide, have been suspected of being hazardous to reproduction. The developmental and reproductive toxicity of styrene has been examined in several animal stud[ies]1,2. Styrene has been shown to cross the placenta in rats. The concentrations in fetal blood were only slightly lower than those in maternal blood. Some studies have suggested increases in embryonic, fetal and neonatal death as well as skeletal variations in the offspring of styrene-exposed animals at high doses. However, other investigations have failed to show any embryoto>dcity or fetotoxicity.

Epidemiologic investigations on the effects of styrene exposure on the reproduction have been conducted among women employed in the reinforced plastics industry and in the polystyrene processing indus[try]1,2. The results of these studies have been contradictory. Two early reports have suggested associations of styrene exposure with spontaneous abortions and congenital malformations. In the follow-up studies, for which more complete data were available, no risk was observed among exposed workers. A more recent investigation indicated an excess of spontaneous abortions in women whose work included the processing of polystyrene. Another study, however, showed no increased risk for infant deaths, malformations, or low birth weight among the offspring of workers in these industries. A weak association has been noted

between high exposure in the reinforced plastics industry and low birth weight. Reduced fertility has also been reported among styrene exposed laminators.

Some earlier reports have suggested that exposure to styrene induces menstrual disturbances. In two more recent well-documented studies, styrene exposure in the reinforced plastics industry was not associated with any menstrual disorders.

The findings of experimental and epidemiologic studies on the effects of styrene exposure on reproduction are contradictory. Some studies have indicated that styrene exposure may be related to adverse pregnancy outcome or menstrual disorders. However, the validity of most of these investigations is weakened by methodological shortcomings. The available evidence cannot be used to draw any firm conclusions.

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#41

DERIVATION OF THE U.S. ENVIRONMENTAL PROTECTION AGENCY'S (EPA) INHALATION REFERENCE CONCENTRATION (RFC) FOR STYRENE

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Abstract not printed, at authors' request.

#42

STYRENE: OTOTOXICOLOGIC AND NEUROTOXICOLOGIC EVALUATION OF RATS*

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Styrene previously has been shown to cause significant hearing loss (ototoxicity) in 3-week-old rats exposed to 800 ppm or higher for 14 hrs/day, 7 days/week for 3 weeks (Pryor, 1987). A no-observed-adverse-effect level (NOAEL) was not determined, and the potential of styrene to cause ototoxicity in more mature rats from occupationally relevant exposures was not evaluated. Consequently, 42-day-old male Fischer 344 rats were exposed to 0, 50, 200 or 800 ppm styrene vapor for 6 hrs/day, 5 days/wk, for 13 weeks. Ototoxicity and neurotoxicity endpoints were evaluated by a functional observational battery (FOB; similar to EPA guidelines), auditory brainstem responses (ABRs), electrodiagnostic tests of the peripheral and central nervous systems, and by a comprehensive neuropathologic examination.

No treatment-related changes were observed in the FOB, including the qualitative assessment of auditory startle. Although clinically normal, rats exposed to 800 ppm were found to have auditory dysfunction at mid and high frequencies (16 and 30 kHz, respectively) but not at lower frequencies (4 and 8 kHz). ABR thresholds were elevated 40 dB at 16 kHz and 25 dB at 30 kHz. Electrodiagnostic tests of the visual pathway and the caudal nerve showed slight differences that were considered not to be adverse consequences of exposure. Neuropathological examination revealed hair-cell lesions in the cochleas of 800 ppm exposed rats; no treatment-related lesions were observed in the brain, spinal cord or peripheral nerves. The NOAEL for this study was 200 ppm. Although ototoxicity in rats was clearly linked to styrene exposure, the ototoxic potential of styrene in other species (and humans) has yet to be determined.

*Sponsored by the Styrene Information and Research Center.

#43

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF STYRENE: A REVIEW UPDATE

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I have previously reviewed the world literature on the developmental and reproductive toxicity of styrene[1]. This presentation will summarize that survey and examine results published since late 1989. My previous conclusions were: The developmental toxicity of styrene has been tested in several mammalian species, with no evidence for teratogenicity. Reports of embryonic, fetal, and neonatal death, and perhaps skeletal variations, are convincing

only at doses which are maternally toxic. Styrene induces defects in chick embryos at lethal doses, which does not suggest specific developmental toxicity. Initial human studies suggested associations with central nervous system (CNS) malformation and spontaneous abortion but were disproved by subsequent investigations. One study has reported a 4r/o decrease in the birth weight of children born to women exposed to high levels of styrene and other solvents but this was not statistically significant. There are no reports of styrene affecting the fertility of animals. Some data suggest an effect on the estrous cycle in rats and the menstrual cycle in women but data are inadequate, and there are conflicting human studies. A small study suggests testicular pathology and reduction in sperm coimts in rats treated with a high oral doses, but others have not observed testicular pathology. An increase in abnormal sperm in men exposed to styrene has been suggested in one study, but the data are weak. High-level inhalation exposure of rabbits induces changes in brain dopamine which may affect the hypothalamus/pituitary. Abnormal pituitary secretion in women exposed to styrene has also been suggested, and may be connected with the putative effects on the menstrual cycle. Overall, styrene did not appear to exert any specific developmental or reproductive toxicity.

In two recent studies, styrene was used as a "negative control" for developmental toxicity. In one, no treatment effects were observed[2], in the other, maternally toxic levels (1.1g/kg/day) had no effect on fetal weight, death, skeletal or brain morphology, but dilated renal pelvis was reported, in the left kidney only[3]. 300 (but not 50 or 60) ppm styrene on pregnancy days 7-21 in the rat had significant effects on pup body weight, weanling neurotransmitter levels or behaviour[4,5,6]. Lactational exposure to 25 or 50 ppm (7h/d, 6d/w) caused developmental delay and behavioral effects . Lactational exposure of rats to 400 (but not 200) mg/kg/d styrene reduced sperm counts and affected some testicular enzyme levels[8]. Near-lethal oral styrene caused sperm head abnormalities in rat and mouse[9]. Paternal occupational exposure to styrene had no effect on spontaneous abortion or malformation[10]. Reports[4-7] have significant flaws, but together they justify further studies. Otherwise, there remains little indication of especial toxicity of styrene to reproduction or development.

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6 Kishi R et al. Toxicol Lett 1992;63:141-6.

7 Shigeta S et al. J Toxicol Sci 1989;14:279-86.

8 Srivastrava S et al. Drug Chem Toxicol 1992;15:233-44.

9 Simula AP Priestly BG Mutrt Res 1992;271:49-58.

10 Taskinen H et al. Scand J Work Environ Hlth 1989;15:345-52.

#44

TWO-WEEK STYRENE VAPOR INHALATION STUDY WITH MICE

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Male and female B6C3F1 and CD1 mice (20/sex/strain) were exposed whole-body to styrene vapor concentrations of 0, 15, 60, 250 or 500 ppm for 6 hours/day, 5 days/week, for 2 consecutive weeks. The purpose of the study was to determine if there are sex or strain differences in the sensitivity of mice to inhaled styrene.

Exposure-related deaths occurred at both the 250 and 500 ppm exposure concentrations. For female mice, mortality for both strains was greater at 250 ppm than at 500 ppm. The mortality in male mice of both strains was highest in the groups exposed to 500 ppm. Nearly all deaths occurred during the first 4 days of exposure. There were no treatment-related effects on weight gains as a result of the exposures. The liver weights of male and female B6C3F1 and female CD1 mice were statistically significantly greater than those of control mice. Exposure-related histopathologic changes were seen in the lungs (leucocytosis, single cell necrosis in the bronchiolar epithelium) and livers (centrilobular hepatocyte necrosis and associated changes) of mice of both strains in the 250 and 500 ppm exposure groups. These changes were generally most pronounced in male mice exposed to 500 ppm and in female mice exposed to 250 ppm. The incidence of microscopic

lesions was in general higher in B6C3F1 mice than in CD1 mice similarly exposed. Minor liver changes were observed in a small proportion of mice exposed to 60 ppm, but no adverse effects were found at 15 ppm, the low exposure concentration.

The basis for the unusual dose-response of female mice is unknown and is currently being investigated.

#45
STYRENE EXPOSURE AND COLOR VISION LOSS

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The knowledge on the eye effects of styrene was so far limited to few cases of optic neuropathy in heavily exposed workers. We decided to evaluate whether this was the result of a weak oculotoxic effect or, rather, of an insufficient attention on this matter. Among eye functions, color vision was evaluated because: a) color vision loss seems to represent an early indicator of optic nerve toxicity, and b) such an early effect was observed in workers exposed to other solvents. Color vision was assessed by the Lanthony 15 Hue Desaturated panel (D-15d). The test was performed at the work place, at the beginning of the workday, before exposure. Lighting conditions were standardized. The D-15d outcomes can be expressed as a score; our results are presented as Color Confusion Index (CCI), i.e. the ratio between subject's score the and the optimal score: 1 represents the best score, while each error increases CCI values. The reproducibility of D-15d was evaluated in field conditions in 10 subjects: based on 10 repetitions, within-day and day-to day coefficients of variation (CV) were 0.5% and 2.8°/0 respectively. Seventy-five workers, representing the styrene exposed workforce in seven Fiberglass-Reinforced Plastic factories (FRP), were examined. Occupational exposure was evaluated by personal passive sampling and urinary styrene (StU) measuring: airborne styrene levels ranged 3.2-549.5 mg/m3. The FRP workers were compared to 60 referents not exposed to solvents. Congenital dyschromatopsias, diseases or drugs interfering with color vision, excessive alcohol consumption and/or tobacco smoke were ruled out by questionnaire. Color vision in normal population is related to the age, therefore the correlation between age and CCI was evaluated in both exposed and referents: a significant correlation was observed in the two groups, but the slope was higher in styrene workers: CCI=0.4 x age + 56.26 ($r=0.44$; $p<0.01$) compared to referents: CCI=0.3 x age + 55.89 ($r=0.37$; $p<0.01$), suggesting a synergistic effect between styrene exposure and age on color vision loss. To evaluate styrene effects on color vision excluding age interference another group matched for this factor (± 3 years) was selected. Alcohol and tobacco may interfere with color vision, and therefore exposed and referents were matched also according to alcohol consumption (± 10g/die) and cigarettes smoke (± 10 sig/die). Thirty-seven couples of exposed and paired referents were examined. Environmental styrene levels ranged 4.6- 354 mg/m3. In the exposed workers a significant loss of color vision was observed: CCI=1.23 (0.2 SD) vs 1.05 (0.07 SD) respectively, $p<0.01$. A dose-effect relationship between styrene exposure and color vision loss was evaluated by classifying the whole group of styrene-exposed according to the results of environmental monitoring: subjects whose exposure exceeded TLV-TWA ACGIH (213 mg /m3) had CCI values significantly elevated ($p<0.05$) compared to workers with lower exposure. Furthermore, a significant correlation ($r=0.27$; $p=0.02$) was observed between end-of shift StU and CCI values; similar results were also obtained for airborne styrene. The reversibility of the effect of styrene on color vision was evaluated by examining a group of 39 workers just before their one month vacation and on the first working day back, before exposure, but no tendency toward a restoration was observed: CCI values were respectively 1.23 (0.19 DS) vs 1.20 (0.21 DS), p= not significant.

As a conclusion, our results show that styrene exposure can induce a dose-related color vision loss; this effect seems synergistic with age. Styrene induced color vision loss is not reversible after one month of exposure interruption, and is significantly related to end-of-shift urinary excretion of the solvent, confirming that StU may be a useful index for the biological monitoring of styrene exposed workers.

#47
GENETIC TOXICITY OF 1,3-BUTADIENE AND STYRENE

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1,3-butadiene and styrene (vinyl benzene) share similar bi-

ological properties in the sense that both of these important compounds of the polymer industry require metabolic activation into an epoxide form to be capable of covalent binding to DNA.

Styrene-7,8-oxide, the active metabolite of styrene, has been shown to be genotoxic in most experimental test systems and for various genetic endpoints. It is also a rodent carcinogen. The genotoxicity of styrene, even with an exogenous metabolic system used, or *in vivo*, is less clear although mainly positive responses have been obtained[1]. In human lymphocyte cultures, red blood cells are able to activate styrene and positive responses have been obtained in various chromosomal endpoints[2].

Cytogenetic damage has repeatedly been reported also in worker studies mainly from reinforced plastics industry, where ambient styrene concentrations may be high (50-100 ppm); while negative findings are mostly associated with lower exposure levels[3]. In reference to the variability in exposure kinetics, individual responses and biologically relevant doses in different studies, no concordance is to be expected.

1,3-butadiene is metabolized into two reactive forms, 1,2-epoxy-3-butane and further to 1,2: 3,4-diepoxybutane, both of which are genotoxic in various test systems, the lowest effective dose of the latter being 1-2 orders of magnitude higher than that of the respective monoepoxide[4].

In vivo data on butadiene with mice and rats shows a species-specific difference of response in various cytogenetic endpoints. The lowest effective concentrations in rats are about 2-3 orders of magnitude higher than in mice.

The cytogenetic techniques for biomonitoring are not sensitive enough to detect human effects in worker studies below 3 ppm ambient butadiene levels typical for the manufacturing industry.

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#51

CYTOGENETIC STUDIES OF RODENTS EXPOSED TO STYRENE BY INHALATION

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By exposing animals to potentially genotoxic chemicals using the same route of exposure and analyzing the same endpoints as used in human epidemiological studies, we believe that rodent models can be used to aid in risk assessment. Data on the *in vivo* genotoxicity of styrene are equivocal. To evaluate the clastogenicity and sister chromatid exchange (SCE)-inducing potential of styrene under carefully controlled *in vivo* conditions, female $B_6C_3F_1$ mice and female F-344 rats were exposed by inhalation for 6 hours/day for 14 consecutive days to nominal concentrations of either 0, 125, 250, or 500 ppm styrene. One day after the final exposures, murine peripheral blood, spleen, and lungs were removed, and the cells were cultured for the analysis of chromosome breakage, micronuclei (MN) induction using the cytochalasin B-block method, and SCE. Peripheral blood smears were scored for MN in normochromatic erythrocytes. For the rat, peripheral blood Lymphocytes were cultured for the analyses of SCE, chromosome aberrations, and MN in cytochalasin B-induced binucleated cells. Bone marrow smears were also made from the femurs of the rats for analysis of MN in the normochromatic erythrocytes. There were small but statistically significant concentration-related increases in the SCE frequencies of the murine lung cells, splenic and peripheral blood Lymphocytes, as well as in the peripheral blood Lymphocytes of the rat. These increases were due to an increase in the median rather than an increase in a few high frequency cells. In the murine tissues, no statistically significant dose-related increase in chromosome aberration or MN frequencies were observed. For the rat bone marrow and peripheral blood, there does not appear to be a marked increase in chromosome aberration or micronucleus frequencies, although analysis is not yet completed. Lymphocytes from exposed rats were also analyzed using the single cell gel assay for the detection of DNA strand breakage under alkaline conditions. No statistically signif-

icant concentrated related effects were observed. These results indicate that styrene is a relatively weak SCE-inducer *in vivo* when administered to rodents by inhalation, and lend support to epidemiological studies that demonstrate an increased frequency of SCEs in peripheral blood lymphocytes of occupationally-exposed workers. However, the absence of clastogenicity after the inhalation of relatively high styrene concentrations over a 2-week period is not consistent with epidemiological findings that implicate styrene as a clastogen after relatively low exposures.

[This abstract has been reviewed by the Health Effects Research laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents reflect the views and policy of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.]

#52

THE INDUCTION OF CHROMOSOMAL ALTERATIONS BY STYRENE*: IN VIV*O AN*D IN VITR*O STUDIES

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A broad range of studies on the clastogenic potential of styrene and its primary metabolite, styrene oxide, following *in vivo* or *in vitro* exposure have been reported in the literature. However, it is apparent that these have not allowed for an unequivocal determination of the effectiveness of styrene to induce chromosome aberrations and/or sister chromatid exchanges (SCE) in humans under occupational exposure conditions (see abstract by Scott, D.). In contrast, it is rather clear that styrene oxide, administered as such, is clastogenic in animal models and *in vitro* cellular assays.

Of particular importance with regard to describing any potential risk to humans from exposure to styrene are studies *in vivo*, either directly on humans occupationally exposed or animal models. The reported studies on the induction of chromosome aberrations and SCE in human peripheral lymphocytes following exposure to styrene in the workplace fall rather far short of allowing any risk estimation. In a number of cases the studies are technically flawed; for example: small sample size, superficial control matching and uncontrolled sources of variation. In particular, exposure information on chemicals other than styrene in the workplace environment is lacking, and in a number of instances these other agents have been shown to be clastogenic *in vivo* and/or *in vitro* assays.

Animal studies have their own set of confounding factors, although as a general conclusion it would appear that styrene exposure does not induce chromosomal alterations or SCE in mouse lymphocytes following sub-chronic exposures. In order to enhance the database for the cytogenetic effects of styrene exposure, we have conducted a carefully controlled study with rats. Fischer 344 male rats were exposed to styrene by inhalation for 6 hrs/day, 5 days/week, for 4 weeks at concentrations of 0, 150, 500 or 1000 ppm, with ethylene oxide at 150 ppm using the same exposure conditions as a positive control. Reciprocal blood cultures were set up at 1, 2, 3 and 4 weeks after the start of exposure and 4 weeks after the termination of exposure for each animal. SCE and chromosome aberrations are currently being analyzed and the full data set will be presented. This particular experimental design will provide two types of dose response curve; one for time as the variable, and one with exposure concentration as the variable. It is anticipated that this study will provide unequivocal data on the clastogenicity of styrene *in vivo.*

#54

DNA BINDING AND STIMULATION OF CELL DIVISION IN STYRENE AND STYRENE-7,8-OXIDE TOXICITY

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A. It was investigated whether DNA binding could be responsible for the forestomach tumors induced by the mutagen styrene 7,8-oxide (SO). [7-^{3}HISO was administered by oral gavage to rats. After 4 hours, forestomach DNA was isolated and purified. No DNA radioactivity was detectable. Expressed in the units of the Covalent Binding Index, CBI = (μmol adduct per mol DNA nucleotide)/(mmol chemical administered per kg body wt.), a maximum possible DNA-binding potency CBI<2.6 was deduced. Upon comparison with the carcinogenic potency of other forestomach carcinogens, a purely geno-toxic mechanism of tumorigenic action of SO in the forestomach appears unlikely. Carcinogenesis 1992, 13: 193-197.

The effect of SO on cell proliferation in the forestomach was compared with the nongenotoxic forestomach carcinogen t-butyl-hydroxyanisole (BHA). Rats were treated for 1 month with SO and BHA under conditions which gave rise to carcinomas in the forestomach (84°/0 and 28%, respectively). Cell proliferation was assessed with BrdU-incorporation and immunohistochemistry. A similar increase of the labeling index was found in all treated groups. The rats treated with BHA showed, in addition, strong hyperplastic lesions in the prefundic region. This was not seen with SO despite the higher tumor incidence. It is concluded that the mechanism of forestomach tumorigenesis by SO includes both elements of mitogenesis and DNA binding, the latter being below the limit of detection reached in the experiments described above. Cancer Res 1993; 53: in press.

C. Covalent binding of [7-^{3}H]styrene (S) to DNA *in vivo* was investigated to answer the question whether DNA-adduct formation could form a mechanistic basis for tumor induction in a carcinogenicity bioassay. [7-^{3}H]S was administered by inhalation in a closed chamber to rats and mice. DNA from liver and lung was purified to constant specific radioactivity and enzymatically degraded to the 3'-nucleotides. HPLC fractions with the normal nucleotides contained most of the radioactivity. In some DNA samples from mouse liver and rat lung, adducts were detected at CBI=0.1. Upon comparison with covalent binding index (CBI) values and carcinogenic potencies of established genotoxic carcinogens it is concluded that the DNA-binding potency of S is so low that unrealistically high dose levels would be required to produce a level of DNA damage which could become responsible for a detectable increase in tumor incidence. Carcinogenesis 1993; 14: 355-360.

#58

CYTOGENETIC AND BIOCHEMICAL CHANGES IN WORKERS EXPOSED TO STYRENE

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The aim of this study is to determine the cytogenetic and biochemical changes due to styrene exposure in the reinforced plastics industry. Blood and urine samples were collected from 18 styrene exposed workers from the Egyptian Company for Plastics Manufacturing which is located in Victoria, Alexandria. Samples were also collected from 18 age and sex matched control subjects from the administrative department of the same factory. Chromosome aberrations were analyzed in blood lymphocytes of workers and control subjects. All the subjects included in the study were non smokers. The duration of employment ranged from 5-22 years (14.3 ± 4.4). In order to monitor exposure to styrene, urinary mandelic acid level was measured using a standard colorimetric method. The levels of thioether and the activity of RNase in urine were also determined colorometrically. The level of mandelic acid was significantly higher in the exposed workers (328.3 ± 266.2 mg/gm creatinine) compared with that of the controls (53.09 ± 21.60 mg/gm creatinine) ($p < 0.05$). The number of chromosomal aberrations was significantly higher in workers (5.12 ± 3.9) compared with controls (2.2 ± 1.6) ($P < 0.05$). Higher levels of thioether and RNase activity have been found among exposed workers. The present findings support the idea that the mutagenic and/or carcinogenic effect of styrene exposure is evident either on the chromosomal or the biochemical level.

#59

CYTOGENETIC STUDIES OF WORKERS EXPOSED TO STYRENE: A REVIEW

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Seventeen out of 47 published cytogenetic studies (24 on chromosome aberrations, 11 on micronuclei, 12 on sister chromatid exchanges [SCE]) of peripheral blood lymphocytes of 945 workers in industries in which there is exposure to styrene have reported significant increases in chromosome damage compared with non-exposed controls.

The positive or negative outcome of the different studies is unrelated to the extent of exposure to styrene. For example, chromosome aberration (CA) induction has been claimed at breathing air levels of only 10 ppm and urinary mandelic acid (MA) levels of <50 mg/1, whereas other studies have given negative results at 180 ppm and >1000 mg/I. In the 17 investigations that gave positive results there is no convincing evidence of a positive dose

response relationship, in spite of wide ranges of exposure and different methods of measurement (e.g. 40-fold ranges of urinary MA levels, 15-fold ranges of breathing air levels, 10- fold ranges in years of exposure). Thus, from these various cytogenetic studies there is no quantitative relationship between styrene exposure and chromosome damage.

There are also serious discrepancies between *in vitro* findings on the chromosome-damaging effects of styrene or styrene oxide in human lymphocytes and the types of damage in exposed workers. For example, lowest effective concentrations of styrene or styrene oxide for SCE induction *in vitro* are 3-4 times lower than for CA, whereas only 2/12 SCE studies, but 10/24 CA studies of styrene workers gave positive results. Several worker studies have reported elevated levels of chromosome-type aberrations (dicentrics and rings) whereas such aberrations have never been reported in *in vitro* studies.

These findings are not consistent with the interpretation that styrene is responsible for the observed positive results in the workers. There are several other known chromosome-damaging agents in workplaces in which styrene is present.

#61

BIOMONITORING OF WORKERS OCCUPATIONALLY EXPOSED TO STYRENE

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Abstract not printed, at authors' request.

#62

INDUCTION OF SINGLE-STRAND BREAKS IN DNA IN LEUCOCYTES AFTER OCCUPATIONAL EXPOSURE TO STYRENE

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The aim of the present study was to investigate whether exposure to styrene below 20 ppm causes an increase in DNA single-strand breaks (SSB). Exposure to styrene during one 8-h workshift was monitored by personal air sampling. Styrene exposure was also assessed by analyzing styrene in blood and urine and mandelic acid in urine. The biological samples were collected prior to shift, at end of shift and on the following morning prior to shift. Smoking significantly increased the level of SSB. Multiple linear regression analysis indicated that the amount of SSB was doubled after 8-h exposure to 18 ppm styrene. Smoking 20 cigarettes/day increased the SSB levels by about 50%. In conclusion, our study indicates that SSB monitoring may be a sensitive indicator of an exposure to a genotoxic compound.

#63

IARC MULTICENTRIC COHORT STUDY OF WORKERS EXPOSED TO STYRENE

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Increased risks for leukemia and lymphomas have been suggested in studies of workers exposed to styrene in the rubber and plastics industry. A historical cohort study was conducted in Denmark, Finland, Italy, Norway, Sweden, and the United Kingdom involving 40,683 workers employed in the reinforced plastics industry, where high exposure to styrene occurs. Exposure to styrene was reconstructed through job histories, environmental and biological monitoring data and production records of the plants in the study. Cause specific national death rates were used as reference. Among exposed workers, no excess was observed for mortality from all causes (2195 deaths, standard mortality ratio [SMRI 95, 95°/0 confidence interval [CIL 91-99), from all neoplasms, lung cancer or from other com-

mon epithelial cancers. Mortality from neoplasms of the lymphatic and hematopoietic tissues was not elevated (50 deaths, SMR=96, 95% CI 71-126) and was not consistently associated with length of exposure. The rate of mortality from leukemias and lymphomas increased with time since first exposure. Among subjects who had been exposed for more than one year, a two-fold risk was observed 20 years after first expsoure (eight deaths ; SMR 197; CI85-387). These results are inadequate to exclude the possibility that styrene causes leukemia and lymphoma.

#64

STYRENE/STYRENE OXIDE: RESULTS OF ANIMAL CARCINOGENICITY STUDIES

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Fourteen long-term toxcity studies were reviewed in an effort to evaluate the potential carcinogenic activity of styrene and styrene oxide in animals. Each study was reviewed and evaluated for details and adequacy of design, adequacy of reported data, and interpretation. The results of this review are:

1. There is no convincing evidence of carcinogenic activity of styrene in animals even though it has been studied using several species of animals and by several routes of exposure: inhalation, gavage, drinking water, and intraperitoneal and subcutaneous injection.

2. Styrene oxide was carcinogenic to the forestomach of both sexes of rats and mice after gavage exposure at all doses tested, including 50 mg/kg/day. An increase in liver neoplasms was observed in male mice in one of these studies. No carcinogenic activity was observed in mice after dermal exposure (skin paint). The relevance of the gavage studies to humans is limited because: a) the gavage route is less than ideal for extrapolation to human risk from inhalation or dermal exposure; b) xenobiotics often cause neoplasms at this location in high concentration; and c) neoplasms at sites distant to the site of exposure were found in only one sex/species.

3. None of the studies of styrene or styrene oxide reported here are well suited for extrapolating potential carcinogenic activity of either compound to humans, because all have deficiencies in design, conduct, irtterpretation and/or utilized a less than ideal route of exposure. A chronic state-of-the-art inhalation study is needed to evaluate this aspect of hazard assessment.

#68

CANCER EPIDEMIOLOGY AMONG STYRENE-BUTADIENE RUBBER WORKERS

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1,3-butadiene is a basic component of styrene-butadiene rubber (SBR) as well as several other synthetic rubbers. Several plants with similar designs were built in the 1940's in the U.S. and Canada for the purpose of producing SBR. Workers within these operations could have been exposed to butadiene as well as styrene. A nested case control study of lymphohematopoietic cancers examined the association of each cancer within this group and exposures by job. Initially, exposure for each job was estimated by engineers and industrial hygienists from the industry. Each person was given a cumulative score using these rarddrigs for each job, times, the length of time in the job and summing the estimates for all jobs held. The results indicated that leukemia was associated with exposure to butadiene but not styrene. Exposure to jobs in operation services, laboratory, and utilities, was also associated with an increased odds ratio for leukemia. The best logistic was one which used both jobs and butadiene exposure scores.

Further work has indicated that while the estimated exposures by job are similar in rank to the actual values measured in recent years, the relative ranks between are not the same and the values differ by plant. We have considered various reasons for differences in exposure values by plant and by job and plant. Variation in methods of exposure assessment and evaluation of the impact of these on associations between cancer and butadiene will be discussed.

#69

OCCUPATIONAL STYRENE EXPOSURE AND LYMPHOPOIETIC CANCERS: A REVIEW OF EPIDEMIOLOGICAL EVIDENCE

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Eight recent cohort studies are used to assess the risk of lymphatic and hematopoietic cancers (LHC) from occupational exposures to styrene in three industries: styrene monomer production and polymerization, styrene-butadiene rubber production (SBR), and reinforced plastics manufacturing (RP). The body of research, which represents 50,000 workers and 47 years of followup, fails to support a causal association.

General mortality and mortality from neoplasms are at or below expected levels in all studies. In aggregate, 114 deaths from LHC were reported, compared to 119 expected based on national population rates, giving a small net deficit (standardized mortality ratio [SMR]=96, 95% confidence interval KI1=79-115). The subtypes of leukemia (46 deaths, SMR=97, 95°/0 CI=71-130) and lymphoma (65 deaths, SMR=95, 95°/0 CI=73-121) also show slightly diminished risk. The diverse mixture of cell types among the "positive" studies makes a single agent etiology unlikely.

None of the studies individually or collectively shows a clear association of disease with increasing duration or intensity of styrene exposure, or with latency (time since initial exposure). The RP cohorts are characterized by short duration, high intensity exposures and short follow-up. SBR and monomer/polymer cohorts have low or moderate exposures of long duration, lengthy followup, and numerous confounding exposures. Updates of these investigations currently in progress promise to contribute valuable information to this issue.

#71

CELL PROLIFERATION IN EPITHELIAL CELLS OF RAT FORESTOMACH FOLLOWING ADMINISTRATION OF STYRENE OXIDE BY GAVAGE*

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Styrene oxide (SO) chronically administered by gavage causes forestomach tumors in rats and mice. The mechnism of tumorigenesis is unclear. Could it be related to increased cell proliferation in epithelial cells of the forestomach? Four range-finding studies were conducted in a limited number of rats to help set doses of SO for a more definitive dose-response study on cell proliferation. SO (0, 55, or 550 mg/kg) was given by gavage to male F-344 rats 3x/wk for 3 weeks. One hour after the last dose, rats were injected ip with 3[H]thymidine (0.50 mCi/g). One hour after this pulse label, stomachs were removed and fixed in formalin. Autoradiograms were prepared from paraffin-embedded, H&E stained sections. Labeling index (LI), the percent of epithelial cells with 3H-labeled nuclei, unexpectedly decreased rather than increasing. To obtain an increased LI, the dose of SO was increased and the effect of number of doses was also evaluated. Two additional groups of rats were given 1000 or 1500 mg SO/kg 3x/wk and LI was to be measured at intervals up to 25 doses of SO. At each interval, 3[H]thymidine was injected at 24 hr rather than 1 hr after gavage with SO. About half of the rats died within the first week of dosing with 1500 mg/kg; this group received only 13 doses. Mean LI in this group increased to —417% of control after one dose and decreased progressively to —267% of control after 13 doses. Stomachs had massive acute inflammation and necrotizing fibrosing ulcerations. Results were similar with 1000 mg SO/kg up to 25 doses, but no animals died and changes in LI and histopathology were less pronounced. A 3rd set of rats was dosed only once with 550 mg SO/kg and LI was measured 24 hr later. Focal acute inflammation and necrosis were present and mean LI was marginally increased rather than decreased. (LI decreased at 1 hr after 550 mg/kg in 1st study.) The observed effect on LI seemed to be dependent on the time after the dose of SO. Therefore, a final study was designed to determine the time-course of changes in LI after single and multiple doses of SO. Also, the thickness of the 5 stomach layers in the fixed tissues was measured as an index of the severity of the morphologic changes. One group of rats was dosed once with 800 mg SO/kg and LI was determined at 0, 1, 6, 15, 24, and 48 hr after dosing. A second set of rats was dosed 9 times and LI was measured at the same intervals in relation to the 9th dose. Vehicle and untreated controls were provided. SO given by gavage produced an initial depression in LI of basal epithelial cells at 1 hr after dosing followed by a dose-related increase. The increase peaked at ~15 hr after dosing. Increased thickness of the epithelium and submucosa followed the changes in LI. After several doses of SO, mean LI continued to peak at 15 hr following a given dose. However, the response was highly focal and variable. Some areas in the same stomach had decreased LI while other areas of the stomach had markedly increased LI. Therefore, mean values of LI do not adequately represent the focal changes in cell proliferation.

*Work sponsored by Styrene Information and Research Center, Washington, DC

#72

CAUSE-SPECIFIC MORTALITY IN A COHORT OF 1000 ACRYLONITRILE-BUTADIENE-STYRENE (ABS) WORKERS

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Concerns arose in the 1970s of the possible human carcinogenicity of acrylonitrile, and in the 1980s of butadiene. Mortality among 1037 male wage workers at an ABS plastics/rubber facility in Louisiana was observed from 1950 to 1984. Work durations of short- and long-term were used as surrogates for exposure. Selected characteristics of these 2 groups are shown in the table below.

These results do not support the contention that the ABS chemicals are human carcinogens.

		Short-term	Long-term	Total
RACE	Number white	108	254	362
	Non-white	38	133	171
	Unknown	302	202	504
	Total Number	448	589	1037
AVERAGE YEARS FOLLOWED		17.3	22.3	20.1
PERSON-YEARS OF FOLLOW		7750	13145	211895
AVERAGE AGE AT HIRE		29.2	29.7	29.5
AVERAGE YEAR OF HIRE		1966.0	1960.6	1963.1

Major mortality findings are as follows:

	Short-term:			Long-term			Total		
Cause	Obs	SMR	CI	Obs	SMR	CI	Obs	SMR	CI
All causes	27	55	36-81	58	63	48-82	85	60	48-75
All cancer	7	75	30-154	12	66	34-116	19	69	42-1118
Digestive Sys.	1	42	1-235	4	86	24-221	5	71	23-167
Respirat. Sys.	4	114	31-291	3	43	9-126	7	67	27-138
Genitour Sys.	0	0	0-476	2	142	17-512	2	92	11-3311
Lymphopoietic	0	0	0-378	2	112	13-404	2	72	9-261
Circulator. Sys.	7	38	15-78	31	87	59-124	38	70	50-97
External Causes	9	86	39-164	11	62	31-110	20	71	43-109
Suicide	3	181	37-530	3	114	24-333	6	140	51-304

#73

DIFFERENT APPROACHES TO ESTIMATE THE CARCINOGENIC RISK OF STYRENE BASED ON ANIMAL STUDIES

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Styrene is an important chemical of wide industrial use, particularly in the manufacture of polymers and reinforced plastics. Environmental and occupational exposures to styrene occur predominantly via inhalation. The carcinogenic risk of styrene in experimental animals has been investigated in eleven long-term studies, which failed to give unequivocal results. In humans, epidemiologic studies did not prove styrene to be a carcinogen. However, concern about the carcinogenic potential of styrene has been related to the occurrence of styrene-7,8- oxide (styrene codde) as a reactive intermediate metabolite of styrene which produced increased incidences of forestomach tumors in rats and mice following oral administration. The aim of our work was to estimate the carcinogenic risk of styrene based on animal studies. The underlying assumption was that styrene oxide is the ultimate carcinogen. The area under the blood concentration-time curve of styrene oxide (AUC_{SO}) has been used to calculate the mean concentration over the life time (C_{SO}) as a dose surrogate for styrene exposure.

The first approach has established a link between tumor probabilities or "highest possible risk" (worst case) calculated from two rodent bioassays with styrene and styrene oxide, respectively, and the C_{SO} related to the exposure scenarios. For extrapolation from high to low doses a linear dose-response relationship was used. The estimated highest possible life time risk and the cancer risk associated with an exposure to 20 ppm styrene (8 h/d, 5 d/w, 48 w/a and 40 a) for humans was estimated to be 1.7 10^{-5} and 3.6-10^{-5}, respectively.

The second approach was based on a reactivity parameter, i.e., the hemoglobin binding of styrene oxide to the N-terminal valine measured in rats following exposure to styrene[1]. This reactivity parameter served to predict adduct levels in humans. Then, the predicted adduct levels following exposure to styrene were compared with those arising from exposure to ethylene oxide. Assuming the ratios of adduct level to carcinogenic risk of ethylene oxide and styrene oxide to be identical, the carcinogenic risk estimated earlier for ethylene oxide was projected to styrene. The estimated carcinogenic lifetime risk associated with an occupational exposure to 20 ppm styrene for a working life was 5.9-10^{-5}.

The third approach relied on the experimentally determined binding of styrene oxide to liver DNA. The correlation between the covalent liver DNA binding index (CBI)[2] and the carcinogenic potency in the liver was used to estimate in rats the risk of liver tumors due to styrene exposure. Considering the proportion of C_{SO} in rat and man, the result was scaled to the human exposure condition mentioned above. The estimated liver tumor probability was 1.4 •10-6.

These small cancer risk estimates for occupational exposure to styrene might explain why epidemiologic investigations have failed to give unequivocal proof of the carcinogenic potency of this compound. The estimated risks are distinctly smaller than the risk estimated for ethylene produced endogenously in the human organism. Possibly, the risk estimates might be validated in long-term studies with rats. However, even groups of 100 animals per dose might be to small for detecting a significant increase of the tumor incidence in inhalation studies with styrene.

1 Osterman-Golkar S, Ehrenberg L, Segerback D, Hallstrom I. Evaluation of genetic risk of alkylating agents. 11 Hemoglobin as a dose monitor. Mutat Res 1976; 34: 1-10.

2 Lutz W. In vivo covalent binding of organic chemicals to DNA as a quantitative indicator in the process of chemical carcinogenesis. Mutat Res 1979; 65: 289-356.

#74

RISK OF MALIGNANT NEOPLASMS OF THE LYMPHATIC AND HAEMATOPOIETIC TISSUES IN EMPLOYEES OF THE DANISH REINFORCED PLASTICS INDUSTRY

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This study was set up to evaluate the incidence of malignant neoplasms in workers of the reinforced plastics industry, in which high exposure levels of styrene have been reported. Special emphasis was given to the risk of malignant neoplasms of the lymphatic and haematopoietic tissues.

We identified 552 Danish companies assumed to have produced reinforced plastics at some point in time since the 1960s and having records on all employees ever employed between 1964 and 1988, a total of 64,000 persons. Three quarters of the companies employed less than

20 persons and less than half of the companies were still in operation in 1988.

The production of reinforced plastics of each company was assessed independently by two dealers of polyester resins supplying the industry back to the early 1960s. In addition, information on the production was obtained from 372 employers by mailed questionnaires. A wide spectrum of reinforced plastic products was reported such as boats, containers, panels, and polymere concrete. Most companies produced by hand lay-up and only a few by hot or automatic processes.

The population was followed up for cancer incidence from 1965 to 1989. A total of 1970 male cancer cases and 360 female cancer cases occurred within 750,000 person years, the numbers did not exceed the expected numbers based on national rates. Among the men we observed a small significantly increased risk of malignant neoplasms of the lymphatic and haematopoietic tissues (standardized mortality ratio[SMR1 1.2, CI 1.0-1.4, 169 observed); no excess was seen in the female employees.

Production of reinforced plastics was confirmed for 386 companies, 82 companies were unknown and 84 had never produced reinforced plastics according to the dealers of polyester resins. Men employed in the 386 companies producing reinforced plastics showed an SMR of malignant neoplasms of the lymphatic and haematopoietic tissues of 1.3 (1.1-1.5, 117 observed). In male employees of the 84 companies with no reinforced plastics production the risk ratio did not differ from unity (SMR 1.0, 0.7-1.4, 39 observed). The highest risk (SMR 1.9, 1.1-2.4, 13 observed) was found in employees of companies unknown by the dealers. When the analysis was confined to men employed in companies with confirmed production of reinforced plastics and starting employment between 1964 and 1970, a period with styrene exposure levels documented higher than during later periods, the SMR was 1.4 (1.1-1.8, 71 observed). Risk ratios close to 1 were observed for employees starting employment during the subsequent years.

This data indicates a small increased risk of malignant neoplasms of the lymphatic and haematopoietic tissues in employees of the reinforced plastics industry.

#76

AN UPDATED COHORT MORTALITY STUDY OF WORKERS POTENTIALLY EXPOSED TO STYRENE IN THE U.S. REINFORCED PLASTICS INDUSTRY

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Vital status and cause-specific mortality ratios (SMRs) are being updated for an additional 12 years for a cohort of workers in the reinforced plastics and composites industry with exposure to styrene monomer and other chemicals. The mortality experience of this cohort has previously been described.[1] The cohort in the original study consisted of 15,908 male and female employees who worked for at least six months between 1948 and 1977 in 30 manufacturing plants in the reinforced plastics and composites industry. The total number of observed deaths in the original study (499), was identical to the expected number of 498.8 (S1VIR=100). Neither mortality from cancer of all sites, nor any specific site, was significantly elevated.

The objectives of the current study are to (1) reduce the percentage with unknown vital status (originally 16`)/0), (2) provide data for analyses using longer length of employment and time since first exposure, and (3) increase statistical power of the study by extending the observation period. The study protocol and follow-up procedures are being audited against Good Epidemiology Practices (GEP) Guidelines[2] by an independent auditor.

Updated vital status among cohort members was established through the use of the Social Security Administration's Death Master File, the National Death Index, a retail credit bureau, and company records. As of December 31, 1989, approximately 1,750 deaths were identified, an increase of nearly 3-fold since the original study. The percentage lost to follow-up has been reduced to below 10°/0.

The substantial increase in the number of deaths and the extended follow-up have improved the statistical power considerably, particularly for hematologic malignancies, and have made finer stratifications in the analyses possible. Mortality data based on cause-specific SMRs are being analyzed by gender, length of employment, time since first exposure, process, department, and various exposure indices.

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1 Wong O. A cohort mortality study and a nested case-control study of workers potentially exposed to styrene in the reinforced plastics and composites industry. Br J Ind Med 1990;47:753-62.

2 Epidemiology Resource and Information Center (ERIC), Chemical Maunfacturers Association. Guidelines for good epidemiology practices for occupational and environmental research. Washington, D.C., 1991.

#77

EVALUATING THE CARCINOGENIC RISK OF CHEMICALS: STYRENE AND BUTADIENE AS EXAMPLES

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The simple paradigm that animal carcinogens are human carcinogens and that a possible human health risk from exposure to a carcinogen can be estimated by linear extrapolation from animal data is now recognised to be much too simplistic and inaccurate. The overall process of carcinogenic risk assessment is complex involving many different aspects (chemical structure, mutagenicity and carcinogenicity, the impact of different rates and routes of exposure, metabolism and kinetics, interspecies differences in susceptibility, epidemiological data, and above all the generally large differences in the experimental doses and the human exposure under conditions of use). For each chemical a careful study of the basic hazard information should be complemented by mechanistic studies to improve the overall risk assessment.

The two chemicals under discussion are interesting examples of how information on mechanisms alters the assessment of risk. For styrene, long-term animal experiments and epidemiological data did not show a carcinogenic effect. Styrene itself is not genotoxic, but is metabolised to a genotoxic metabolite, styrene-7,8-oxide. This metabolite gives concern with regard to a possible carcinogenicity also of the parent compound styrene. The mechanistic studies demonstrate that a major element in the risk assessment is the substantial differences in the rate of metabolism between species and the low intrinsic potency of styrene-7,8-oxide for DNA-binding, which can be confirmed by estimation of the rate of macromolecular binding.

For butadiene, there is clear evidence of carcinogenicity in animals, but not in man. Butadiene is also not itself genotoxic and its mutagenicity and carcinogenicity are the consequence of metabolic conversion to genotoxic metabolites. The conclusion from mechanistic studies is similar to that from styrene, namely that there are large differences in the kinetics of the metabolism between species.

The implications for assessing the hazard to human health and for the quantitative assessment of risk will be discussed, drawing on the data presented during the conference and the experience of risk assessment of other chemical carcinogens.

#80

RESEARCH NEEDS FOR 1,3-BUTADIENE & STYRENE

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The Clean Air Act Amendments of 1990 represent a major shift in focus of U.S. air pollution regulations. Provisions to control both stationary source emissions and motor vehicle emissions were greatly strengthened. These new provisions have increased the need for research that will provide both methods and data to support risk assessment of chemicals targeted for regulation. The research needs discussed here focus on 1,3-butadiene and styrene. Three key research areas are noted below.

Hazard identification research is needed to develop, refine and validate methods for identifying potential human hazards for endpoints that are currently not well defined. In particular, butadiene's potential for gonadal toxicity and styrene's potential for neurotoxicity are not well understood. Better techniques need to be developed to determine causal relationships between environmental pollutants and adverse health outcomes.

Dose-response research, which develops pharmacokinetic and biologically based dose-response models, is also needed. These models will elucidate: 1) the relationship between exposure (i.e., applied dose), dose at the site of toxic action (i.e., target dose), and biological effects; and 2) the basic biological mechanisms responsible for the observed effects. Understanding of underlying biologic mechanisms is crucial to the extrapolation of research results (e.g., extrapolation of data from animals-to-humans and high-to-low dose). This type of information can significantly improve assessment of the cancer, as well as noncancer risks, posed by butadiene and styrene.

Exposure assessment research defines the route, magnitude, frequency, and duration of human exposure to environmental pollutants. Research needs in this area focus on the development and validation of biological markers for exposure, effects and susceptibility in human populations.

Research in these three areas will significantly reduce uncertainties in the assessment of public health risk posed by 1,3-butadiene and styrene.