THE SIRC REVIEW

RESEARCH • TECHNOLOGY • PUBLIC POLICY

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Chronic Toxicity/Oncogenicity Study of Styrene in CD rats by Inhalation Exposure for 104 Weeks

Uptake of Styrene in the Upper Respiratory Tract of the CD Mouse and Sprague-Dawley Rat

Mortality from nonmalignant diseases of the respiratory, genitourinary and nervous systems among workers exposed to styrene in the reinforced plastics and composites industry in the United States

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Introduction

In publishing this seventh volume of *The SIRC Review*, the Styrene Information and Research Center (SIRC) continues its efforts to present new research studies on styrene health and environmental effects that represent the most current, sound science. A founding mission of SIRC, now in its 13th year, was to expand and improve the scientific database for styrene. The organization continues in its efforts to identify and review research in this area — and to sponsor new research, as needed. Indeed, the three articles in this volume are key studies sponsored by SIRC.

Other research is ongoing and, as this volume goes to press, SIRC and its international partner organizations also have committed to funding major new styrene studies in reproductive and developmental neurotoxic effects, results for which will be available in 2002. While these two studies address what appear to be the last significant data gaps for styrene, SIRC will continue to monitor emerging research needs in order to maintain its position as a definitive resource center for information on styrene.

For this issue, SIRC is particularly pleased to reproduce a report on an important animal carcinogenicity study. In this study, CD rats were exposed to styrene via inhalation for two years (i.e. most of their normal life span). Originally published in *Toxicological Sciences*, the article reports on the most definitive study to date on the potential carcinogenic effect of styrene in rats. Importantly, the study concluded that there was no evidence of increased treatment-related tumors in the rats. The research was conducted at Huntingdon Life Sciences (HLS) in England and funded by SIRC. This work was preliminarily discussed in a past issue of *The SIRC Review* (Volume 5, No. 1).

I am further pleased to report that an article on a companion two-year inhalation study in mice — also conducted at HLS and sponsored by SIRC — has just recently been accepted for journal publication. We look forward to including that report in the next volume of *The SIRC Review*.

Our second article, by Dr. John Morris of the University of Connecticut, reports on his research on styrene uptake in the upper respiratory tract of rats and mice. Published in *Toxicological Sciences*, Morris' research was undertaken to provide nasal dosimetric information in response to olfactory toxicity seen in the aforementioned rat and mouse studies. His work contributes to our increased understanding of the distinctions between species, and forms a basis for further investigation of the underlying metabolic reasons for these effects, to determine their appropriate extrapolation to human health.

Our last article is by two past contributors to *The SIRC Review*, Dr. Otto Wong and Lisa Trent (Volume 2, No.1 and Volume 4, No.1). Their article, which appeared in *Scandinavian Journal of Work, Environment & Health*, reports on their further analysis of styrene-exposed workers in the reinforced plastics industry. Although their original work focused on evaluating potential cancer effects in the workers (and reported no increase in cancer incidence attributable to styrene exposure), their recent work evaluated mortality from non-malignant diseases (i.e., respiratory, genitourinary, and nervous system). Another similar epidemiology study conducted in Europe originally also had reported *no* increases in mortality in their cohort from either cancer or non-cancer endpoints. However, three subsequent articles recently suggested such effects *were* noted in the European cohort. Wong and Trent's research on the U.S. cohort, however, could not replicate the newer European findings, and indicated no relationship between styrene exposure and mortality from respiratory, genitourinary, or nervous system causes.

Future editions of *The SIRC Review* will include the aforementioned report on the chronic mouse study, results of investigations into mode of action, and updated reviews of the research on the reproductive and developmental effects of styrene (especially interpreting its potential to act as an endocrine modulator). We also will eventually report the results of the reproduction and

developmental neurotoxicity studies just now beginning.

In addition to new research, upcoming volumes also will cover the results of several styrene hazard and risk assessment projects currently underway, which will have significant international impact in the months ahead. These include the US Environmental Protection Agency's styrene hazard assessment for its Integrated Risk Information System database, and styrene risk assessments by both the Harvard Center for Risk Analysis and UK Health and Safety Executive (for the European Union).

Our goal is to provide a compendium of the most important new health and environmental research on styrene. As editor, I trust that this material is timely, informative, and useful to our readers, and I welcome comments on our publication.

Keith A. Johnson, DVM, PhD
Editor

Chronic Toxicity/Oncogenicity Study of Styrene in CD rats by Inhalation Exposure for 104 Weeks

George Cruzan, Janette R. Cushman, Larry S. Andrews, Geoffrey C. Granville, Keith A. Johnson, Colin J. Hardy, Derek W. Coombs, Pamela A. Mullins, and W. Ray Brown

ABSTRACT

Groups of 70 male and 70 female Charles River CD (Sprague-Dawley derived) rats were exposed whole body to styrene vapor at 0, 50, 200, 500, or 1000 ppm six hr per day five days per week for 104 weeks. The rats were observed daily, body weights and food and water consumption were measured periodically, a battery of hematologic and clinical pathology examinations were conducted at weeks 13, 26, 52, 78 and 104. Nine or 10 rats per sex per group were necropsied after 52 weeks of exposure and the remaining survivors were necropsied after 104 weeks. Control and high-exposure rats received a complete histopathologic examination, while target organs, gross lesions and all masses were examined in the lower exposure groups.

Styrene had no effect on survival in males, but females exposed to 500 or 1000 ppm had a dose-related increase in survival. Levels of styrene in the blood at the end of a six-hour exposure during week 95 were proportional to exposure concentration. Levels of styrene oxide in the blood of rats exposed to 200 ppm or greater styrene were proportional to styrene exposure concentration. There were no changes of toxicologic significance in hematology, clinical chemistry, urinalysis, or organ weights. Males exposed to 500 or 1000 ppm gained less weight than the controls during the first year and maintained the difference during the second year. Females exposed to 200, 500 or 1000 ppm gained less weight during the first year; those exposed to 500 or 1000 ppm continued to gain less during months 13-18. Styrene-related non-neoplastic histopathologic changes were confined to the olfactory epithelium of the nasal mucosa.

There was no evidence that styrene exposure caused treatment-related increases of any tumor type in males or females or in the number of tumor bearing rats in the exposed groups compared to controls. Based on an overall evaluation of eight oncogenicity studies, there is clear evidence that styrene does not induce cancer in rats.

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females or in the number of tumor bearing rats in the exposed groups compared to controls. In females, there were treatment-related decreases in pituitary adenomas and mammary adenocarcinomas. Based on an overall evaluation of eight oncogenicity studies, there is clear evidence that styrene does not induce cancer in rats.

KEY WORDS:

styrene,inhalation, CRL-CD (Sprague-Dawley) rats, nasal irritation, olfactory epithelium, decreased mammary tumors, decreased pituitary tumors, oncogenicity

INTRODUCTION

Styrene (ethenylbenzene, CAS # 100-42-5) is a clear, colorless liquid with a characteristic pungent odor. In 1993, world-wide production capacity of styrene was 17.8 million tons (Miller *et al*; 1994). Styrene is used in the production of many important commercial polymers including polystyrene, acrylonitrile-butadiene-styrene (ABS), styrene-acrylonitrile (SAN), styrene-butadiene (SB Latex, SB Rubber), and in resins used in the manufacture of numerous glass-reinforced plastic products (e.g., boats, tubs, tanks, etc.). The greatest occupational exposures occur in the reinforced plastics industry. However, in all of the industrial uses of styrene, other chemicals are present in the workplace atmosphere; thus, an understanding of potential styrene toxicity in humans is complicated by the presence of these other chemicals.

Exposure (Miller *et al*., 1994), toxicologic (McConnell & Swenberg, 1994; Phillips & Farmer, 1994; Scott & Preston, 1994), metabolic (Sumner and Fennell, 1994) and epidemiologic (Rebert & Hall, 1994; Coggon, 1994) data on styrene were recently reviewed. Several chronic toxicity/oncogenicity studies of styrene in rats were performed in the 1970s (Table 1), but most are deficient for use in human health hazard assessments due to problems which included route of exposure, dose interval, small group sizes, intercurrent disease, exceeding the Maximum Tolerated Dose, and minimal descriptions of the conduct and detailed results of the studies (McConnell and Swenberg, 1994). Therefore, the Styrene Information and Research Center initiated state-of-the-art subchronic and chronic studies of styrene by the inhalation route.

The thirteen-week inhalation study of styrene in CD rats (Cruzan *et al*., 1997) identified 1000 ppm for six hrs/day five days/week as the predicted Maximum Tolerated Dose in males and females based on increased water consumption and reduced body weight gain. In addition, lesions were seen in the nasal olfactory epithelium at exposure levels of 500, 1000, and 1500 ppm. Based on the behavior of the rats, these concentrations appeared to be irritating during exposure. This report describes the subsequent chronic toxicity/oncogenicity study in rats. A companion chronic toxicity/oncogenicity study in mice was initiated later than the rat study and will be reported separately.

MATERIALS AND METHODS

Test Material.

Commercial styrene was supplied by Shell Chemicals U.K. Ltd. Each batch was analyzed for purity by gas chromatography/mass spectroscopy (GC/MS); the batches were typical of commercial styrene. Analyses of styrene aliquots taken at the start and finish of each drum showed: styrene >99.5 to 99.7%, benzene < 1 to 24 ppm, ethylbenzene 143 to 731 ppm, styrene dimer <1 to 6 ppm, styrene oxide <5 to 6 ppm, and tertiary butyl catechol (inhibitor) 5 to 15 ppm. Drums were stored at 4°C.

Exposure Generation and Analysis System.

Styrene was metered from a sealed reservoir pressurized by nitrogen to drip onto a glass sinter; heated air (60°C) was passed through the glass sinter to vaporize styrene. The air temperature was kept at the minimum required for vaporization to minimize the formation of oxides, dimers, or polymers. The styrene-laden air was diluted with filtered conditioned air and introduced into the 2.43 m^3 stainless steel and glass whole body exposure chambers. Chamber environmental conditions, including styrene concentration, airflow, temperature and humidity, were recorded every thirty minutes during each 6-hour exposure period using an automated system. Analytical determination was by GC using styrene, styrene oxide and styrene dimer standards for identification. Chamber air was analyzed monthly by GC for styrene oxide (limit of detection 0.25 ppm) and styrene dimer (limit of detection ~2 ppm). Nominal and analyzed chamber concentrations of styrene were calculated for each day. Rats were exposed for at least 104 weeks.

Animals and Husbandry.

Virus Antibody Free (VAF) CD (Sprague-Dawley) rats were received from Charles River Portage at approximately 4 weeks of age. Prior to study initiation, selected rats were sampled for hematologic (methods described below) and serologic investigations and macroscopic evaluation at necropsy. Serologic evaluation of sentinel rats was performed every eight weeks throughout the study for pos-

TABLE 1

Summary of Previous Long-Term Studies of Styrene in Rats

Strain	Route of Exposure	Administered Dose	Results Reported by Authors	Reference
SD	Water	125, 250 ppm	Negative	Beliles et al., 1985
SD	Gavage	50, 250 mg/kg/day	Negative	Conti et al., 1988
BDIV	Gavage	500 mg/kg/wk	Negative	Ponomarkov and Tomatis, 1978
F344	Gavage	500, 1000, 2000 mg/kg/day	Negative	NCI, 1979 (a)
F344	Gavage	175, 350 mg/kg/dose (3x/wk) mixture of 70% styrene, 30% β-nitrostyrene	Negative	NCI, 1979 (b)
SD	Inhalation	25, 50, 100, 200, 300 ppm	Increased total and malignant mammary tumors - females- all doses	Conti et al., 1988
SD	Inhalation	600, 1000 ppm	Negative	Jersey et al., 1978
SD	Subcut.	50 mg/anim. (1 dose)	Negative	Conti et al., 1988
SD	Intraperit.	50 mg/ anim. x 4 doses	Negative	Conti et al., 1988

sible rat viral and mycoplasma infections. The rats were housed 5/cage in suspended stainless steel caging when not in the inhalation chambers. During exposures each animal was housed separately. Animal husbandry complied with the requirements of the UK Home Office Animals (Scientific Procedures) Act 1986. Animals were fed pelleted SDS Rat and Mouse Diet No. 1 (Special Diet Services, Witham, Essex, UK) *ad libitum*; tap water was provided *ad libitum*, except during exposures, from water bottles which were rinsed and filled daily and thoroughly cleaned periodically. Analysis of all batches of diet demonstrated them to be within nutritional requirements and to not have chemical or bacterial contamination that would interfere with the study. Drinking water was analyzed every six months and found to be within safe limits for selected chemical contaminants. Animals were housed in air-conditioned rooms with 12-hr light/dark cycles.

Experimental Design.

During the acclimation period, rats were assigned to five groups of 70 males and 70 females by a stratified randomization according to body weight. Four groups of rats were exposed to styrene vapor six hours per day for at least 104 weeks (520 exposures) at target concentrations of 50, 200, 500, or 1000 ppm (Groups 2, 3, 4, and 5, respectively) in 2.43 m^3 inhalation chambers. The remaining group (Group 1) served as a control and were treated to the same inhalation chamber conditions, but were not exposed to styrene. Ten predesignated rats of each sex from each group were sacrificed after 52 weeks of exposure. The animals were individually observed for clinical signs at daily placement and removal from the inhalation chamber; group observations were made during exposures. Body weight was determined weekly for the first thirteen weeks and every four weeks thereafter. Food consumption was measured weekly throughout the study. Overnight water consumption was measured daily during weeks 1, 4, 12, 25, 51, 77 and 103. After dilation with a tropicamide ophthalmic solution, the eyes of all rats were examined using a Keeler indirect ophthalmoscope before exposures began and after 52 and 104 weeks of exposure.

Following an overnight fast, blood was obtained from the periorbital plexus of 10 males and 10 females from each group after 13, 26, 52, 78, and 104 weeks of exposure for hematology and clinical chemistry determinations. For

the first three intervals, the rats sampled were those scheduled for termination after 52 weeks. Hematologic evaluation included packed cell volume, hemoglobin, erythrocyte (RBC) count, white blood cell (WBC) count, reticulocyte count, platelet count and Thrombotest (Ortho ELT 1500). WBC differential count and RBC morphology were evaluated using Wright's stained blood smears. Clinical chemistry evaluation included creatine phosphokinase, sorbitol dehydrogenase, 5'-nucleotidase, phospholipid (Roche Cobas centrifugal analyzer), glucose, alanine aminotransferase, aspartate aminotransferase, total protein, albumin, globulin (by subtraction), urea nitrogen, alkaline phosphatase, total bilirubin, creatinine, sodium, potassium, calcium, inorganic phosphorus, chloride, and cholesterol (Hitachi 737 analyzer). Urine was collected overnight after weeks 13, 26, 52, 78, and 104 and analyzed for pH, volume, osmolality, color, appearance and microscopic material. Dipsticks (Ames Co.) were used for semiquantitative analysis of total reducing substances, glucose, ketones, proteins, bile pigments, urobilinogen, and heme pigments. Protein, creatinine, and glucose were measured using a Roche Cobas centrifugal analyzer.

During week 95, blood was obtained from 5 males and 5 females of each group immediately at the end of the daily exposure of each animal. In the last few minutes of the 6-hour exposure, the chamber door was opened slightly for the removal of each rat separately; blood was collected within 2 minutes of removal from exposure. The concentrations of styrene and styrene-7,8-oxide were determined in each sample by immediate extraction of blood into hexane and analysis of the hexane fraction by capillary gas chromatography based on the method of Kessler *et al*. (1990).

Ten animals per sex per group (except 9 females each in the 0, 500, and 1000 ppm groups because of mortalities before week 52) were euthanized after 52 weeks by exsanguination under deep pentobarbitone anesthesia and necropsied. All surviving animals were euthanized by the same method during weeks 105-107. The following organs from each animal, except for those animals found dead, were dissected free and weighed: adrenal glands, brain, kidneys, liver, lungs, spleen, and ovaries/testes. Tissues were preserved in neutral-buffered formalin; eyes were preserved in Davidson's fixative. Lungs were inflated with neutral-buffered formalin before immersion in fixative. At necropsy, the nasal cavity was flushed with neutral-buffered formalin. The lower jaw was removed and the head was immersed in neutral-buffered formalin.

Following fixation, the head was decalcified in Kristensen's fluid (6 days) and the nasal cavity was sampled at 4 levels based on the description of Young (1981). Tissues for histologic evaluation were routinely processed, embedded in paraffin wax, and 4μm sections were prepared, stained with H&E and examined under the light microscope. The following tissues from the high exposure and control animals were examined: nasal passages, nasopharynx, larynx, trachea, lungs, aorta, heart, thymus, cervical and mandibular lymph nodes, spleen, liver, pancreas, kidneys, urinary bladder, uterus, cervix, ovaries, prostate, seminal vesicles, epididymides, testes, thyroid glands, parathyroid glands, adrenal glands, pituitary gland, salivary glands, skeletal muscle, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, mammary glands, skin, spinal cord, sciatic nerve, brain, femur, sternum/bone marrow, eyes, extra-orbital lacrimal glands, and Harderian glands, as well as other macroscopically abnormal tissue including all masses. In addition to histopathologic examination of the control and high-exposure animals, histopathologic examination of the nasal passages, lungs, liver, kidneys, testes/epididymides, and macroscopic abnormalities was performed on the animals of all lower exposure levels.

The study was conducted in accordance with Good Laboratory Practices (US EPA, 1989; UK HSE, 1989; EC Council, 1986; Japan MITI, 1984; OECD, 1982). Pathology observations were peer reviewed within the testing facility and by an independent pathologist (WRB).

Statistics.

Quantitative data were analyzed by Bartlett's (1937) test for homogeneity; if the data were heterogenic, a logarithmic transformation was performed to see if a more stable variance could be obtained. Data without significant heterogeneity were analyzed by one-way analysis of variance, and Williams' test (1971, 1972) for dose-response. The Kruskal-Wallis (Kruskal and Wallis, 1952, 1953) analysis was performed for non-parametric data. For organ weight data, the final body weight was used as a covariate in the analysis of covariance.

Mortality was analyzed using log ranks method (Mantel, 1966).

Tumor incidence was analyzed using methodology described in the International Agency for Research on Cancer (IARC, 1980). Other pathologic data were analyzed using Fisher's (1973) exact test.

RESULTS

Exposure conditions

Average analyzed concentrations (standard deviation) of styrene were 0, 50 (2.6), 202 (6.4), 501 (15.8), and 997 (36.0) ppm. These measured concentrations were within 1% of those intended; therefore concentrations are referenced by target level in this publication. Nominal concentrations (the amount of styrene used daily divided by the daily chamber airflow) were 49, 194, 506, and 1019 ppm. No styrene oxide or styrene dimer was detected in any of the exposure chambers. Chamber airflow averaged 647, 644, 646, 647, and 646 liters/minute for Groups 1-5 (0, 50, 200, 500, and 1000 ppm), respectively. Mean chamber temperature and humidity were 21.6, 21.4, 21.3, 21.0 and 20.3 °C and 57, 56, 60, 67, and 67% for Groups 1-5, respectively. Increased chamber humidity and slightly decreased chamber temperature were consistently observed with increasing exposure level and were attributed to the animals' reaction to styrene exposure.

Sentinel Animals

Serological analysis of blood samples obtained from the sentinel rats prior to study initiation, at 8 week intervals during the study, and from 2 rats per sex per group at the terminal sacrifice were all negative for the antibodies examined. There was no evidence of any infectious disease at any stage of this study.

Survival and Observations

During week 61, 8 males in the 1000 ppm group and 6 males in the 500 ppm group received a massive dermal exposure of styrene due to a technical problem which resulted in liquid styrene dripping into the exposure chambers in a discrete location at the start of exposure. All died or were sacrificed within the next two weeks and

TABLE 2

Survival of CRL-CD Rats Exposed to Styrene Vapors for 24 Months

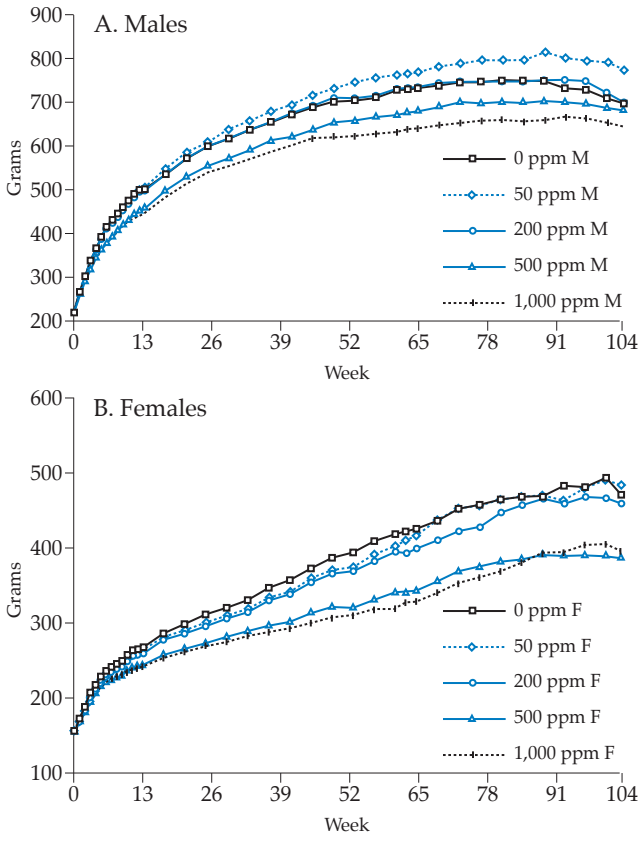
Exposure Concentration	Percent Surviving at Week[a]:										
	26	52	65	70	75	80	85	90	95	100	104
Males											
0	100	98	95	92	88	83	78	70	68	62	58
50	98	97	93	93	92	87	82	73	68	62	60
200	100	100	97	93	93	92	88	82	70	60	50
500	100	97	94	93	91	85	83	78	70	63	56
1000	100	97	96	96	94	90	87	71	69	67	60
Females											
0	98	98	92	88	85	80	78	70	63	55	48
50	97	95	93	85	82	77	72	68	60	52	47
200	100	98	97	87	83	78	75	72	63	58	48
500	98	98	97	97	93	92	85	80	75	73	67
1000	98	98	97	95	93	93	93	93	93	87	82

[a]Based on 60 per sex per group designated for 24 months of study; does not include deaths in subgroups (10/sex/group) scheduled for sacrifice at 52 weeks. After week 61 Group 4 (500 ppm) survival of males based on 54 rats; Group 5 (1000 ppm) males based on 52 rats. Survival of females exposed to 500 or 1000 ppm significantly greater than control.

were not included in the mortality or tumor analysis. Inhalation of styrene had no effect on survival of male rats (Table 2). There was a dose-related increase in survival of female rats (Table 2) exposed to 500 or 1000 ppm relative to control females.

There were no effects of styrene exposure on appearance or behavior of rats at exposure concentrations up to 1000 ppm except during exposures. Rats exposed to styrene at 500 and 1000 ppm exhibited signs indicative of its irritating properties: salivation with restlessness, which lasted up to 30 minutes after the start of each exposure, followed by hunched posture for the remainder of the exposure period. In general, effects were most evident on Mondays with a gradual diminution by Friday.

FIGURE 1



Mean Body Weights of CRL-CD Rats Exposed to Styrene Vapor for 2 years.

Salivation, increased urination, and/or water loss through the respiratory system may have been sufficient to cause increased humidity and slightly lower temperature in the exposure chambers for Groups 4 and 5 (see above). This increased humidity was not seen if the chambers were operated with rats present without styrene exposure or styrene exposure without rats present, based on studies conducted during the weekend when styrene exposure of the test animals were not conducted (data not shown). When body weights were measured before and after a six hour exposure period on Monday and on Friday of week 46, weight loss during exposure was increased in all exposure groups in males and at 1000 ppm in females. In addition, exposed rats consumed more water than controls in the first hour after exposure when measured during week 46. These findings are consistent with increased fluid loss during exposure.

Body weight and Food and Water Consumption

Males exposed to 500 ppm and 1000 ppm styrene gained less weight than the controls (Fig 1A), primarily during the first year of the study and consumed less food during the first 26 weeks (data not shown). After one year males at 500 ppm had gained 10% less weight than controls and males at 1000 ppm had gained 17% less. During the next six months the weight differences remained the same. Over the last six months the styrene-exposed males lost less weight than the controls, thus the weight differences were less at the termination of the study. Males exposed to 50 ppm styrene gained more weight than the controls throughout the study; after 104 weeks they had gained 15% more.

Females exposed at 200, 500 or 1000 ppm gained significantly less weight than the controls during the first year (Fig. 1B): 10, 29 and 34% less, respectively. Those exposed to 500 or 1000 ppm continued to gain less weight through 78 weeks. From week 91 to the end of the study females exposed to 500 ppm weighed less than those exposed to 1000 ppm. Females exposed to 500 and 1000 ppm consumed ~10 % less food throughout the study than did the controls.

In males exposed to 500 or 1000 ppm, there was a dose-related increase in water consumption throughout the study (Table 3; 121% and 127% of control consumption). Similar increases in water consumption were evident in females exposed to 500 or 1000 ppm only during the first 6 months (Table 3). Increased water consumption (112% of control consumption) was recorded in both males and females exposed to 200 ppm during the first month of the study. At week 39, visual observation of

TABLE 3

Water Consumption and Urinalysis of CRL-CD Rats Exposed to Styrene Vapors for 24-Months

Exposure Concentration (ppm)	Weekly Water Consumption (ml) at Week of Study						Urinalysis Week 26		Urinalysis Week 104	
	1	12	25	51	77	103	pH	Osmol[a]	pH	Osmol
Males										
0	200	259	270	238	229	246	7.0	1183	6.9	923
50	205	269	289	255	280	408*	6.9	1195	6.5	881
200	211*	276	287	257	237	290*	6.2*	1912*	6.6	894
500	229*	288*	310*	274*	276*	334*	6.2	1894*	6.6	1141
1000	242*	300*	328*	288*	304*	346*	6.0*	2011*	6.3*	1230
Females										
0	146	191	214	258	282	339	6.1	1450	6.2	788
50	148	203	222	269	300	389	6.3	1748	6.2	629
200	165*	207	228	273	297	313	5.9	1933	6.0	876
500	171*	213*	233*	258	290	318	5.8*	2318*	6.0	851
1000	219*	263*	277*	276	292	329	5.6*	2289*	6.0	1225*

[a]Osmol, Osmolality, mOsm/liter
*Significantly different from control, p<.05

drinking behavior post exposure and overnight placement of hoppers beneath the water bottles did not find evidence of unusual spillage or other loss of water due to interference with the water bottles by the rats.

Hematology, Clinical Chemistry, Urinalysis, and Ophthalmology

There were no hematology or clinical chemistry differences between treated and control rats that were of toxicologic significance (data not shown). Minor elevation of serum phosphorus levels, noted inconsistently, may have been related to effects of styrene exposure on water balance.

Urine pH was decreased in a dose-related manner in rats exposed to 200, 500 or 1000 ppm (Table 3); this is presumed to be due to the excretion of increasing amounts of mandelic and phenylglyoxylic acids, the normal urinary excretion products of styrene metabolism. Increased osmolality may have been related to water balance.

Styrene and Styrene Oxide in Blood

Levels of styrene in blood at the end of a six hour exposure during week 95 were proportional to exposure concentration (Table 4). No styrene or styrene oxide was detected in the blood of control rats. Although styrene was found in the blood of rats exposed to 50 ppm, no styrene oxide was found (limit of detection 10 ng/ml). Levels of styrene oxide in the blood of rats exposed to 200 ppm or greater were proportional to styrene exposure concentration, although the increase was to a much lesser extent than for styrene.

Organ Weights

Using body weight as a covariate, there were no toxicologically significant differences in organ weights between treated and control rats at the interim or terminal sacrifices (data not shown). Because of the body weight differences, expected lower liver and kidney weights and increased relative brain and gonad weights were found.

TABLE 4

Blood Levels of Styrene and Styrene Oxide (SO) in CRL-CD Rats Immediately after Exposure to Styrene Vapor During Week 95

	Styrene Conc. in blood		SO Conc. in blood		
Exposure Conc. (ppm)	ng/ml±sd	ng/ml/ppm sty in air	ng/ml±sd	ng/ml/ppm sty in air	Blood conc. SO/S[a]
Males					
0	BLQ[b]		BLQ	—	
50	430 ± 80	8.6	BLQ	—	
200	2780 ± 490	13.9	66 ± 40	0.33	0.023
500	12490 ± 2390	25.0	116 ± 22	0.23	0.009
1000	33210 ± 2800	33.2	185 ± 14	0.19	0.005
Females					
0	BLQ BLQ	—			
50	290 ± 40	5.8	BLQ	—	
200	1950 ± 620	9.8	28 ± 10	0.14	0.014
500	9460 ± 2740	18.9	92 ± 27	0.18	0.010
1000	29680 ± 4730	29.7	153 ± 15	0.15	0.005

[a]SO/S = SO concentration/styrene concentration.
[b]BLQ = below limit of quantitation; limit of quantitation for styrene = 100 ng/ml; for SO = 10 ng/ml;

Pathology

At the week 52 interim necropsy, no treatment-related macroscopic changes were observed in any tissue. At the terminal necropsy, it was observed that more male rats exposed to 500 and 1000 ppm had testicular masses than the other groups. In addition, at the terminal sacrifice, fewer females in the 500 and 1000 ppm groups had enlarged pituitary glands and subcutaneous masses (Table 5). Pale foci/areas of the lung were seen in a greater number of females exposed to 1000 ppm styrene than in the other groups. Microscopically these were seen as foamy alveolar macrophages and/or cholesterol cleft granulomata. These lesions are occasionally seen as spontaneous findings in rats and their presence, in low numbers, in this study was considered to be incidental.

Histologically there was no increase in the number of tumor-bearing rats in the treated groups compared to controls. In addition, there was no evidence that styrene exposure caused treatment-related increases in any tumor types in male or female rats (Table 6 [page 16]). There were treatment-related decreases in pituitary tumors and mammary tumors in females.

The testes masses noted macroscopically were all interstitial cell adenomas; the incidences were: 3.3, 3.3, 3.3, 7.4, and 11.5% of the rats for the 0, 50, 200, 500, and 1000 ppm groups, respectively. Testicular proliferative lesions were assessed using the current STP/ARP/AFIP criteria (McConnell *et al.*, 1992). Although the trend test was positive, the differences were judged to be incidental because: 1. none of the incidences were significantly different from control by pairwise comparison, 2. the incidence in all groups was within the historical control range of 12 studies conducted in this strain of rats at this laboratory (0-13.5%), and 3. there was no treatment-related increase in non-tumor findings typically associated with chemically-induced interstitial cell tumors.

TABLE 5

Incidence of Macroscopic Observations of CRL-CD Rats Exposed to Styrene for 24 Months

	Males					Females				
Observation	0	50	200	500	1000	0	50	200	500	1000
Subcutaneous Mass(es)	8	11	6	12	6	41	31	30	30	26
Brain Ventral Depression Due to Pituitary Mass	18	17	23	15	14	39	41	34	24	24
Pituitary Enlarged	22	19	25	18	15	47	44	41	31	29
Lungs Pale Foci	1	2	5	3	6	3	3	5	4	10
Testes Mass(es)	0	1	1	4	6					

Specifically, there was no increase in interstitial cell hyperplasia or seminiferous tubular atrophy.

In females, there were treatment-related decreases in pituitary tumors and mammary tumors. The incidences of adenocarcinomas of the pituitary were similar among exposed and control rats, but the incidences of adenomas decreased in a dose-related fashion. Fewer than 50% (24 of 49) of the females exposed to 1000 ppm styrene that survived for two years had pituitary adenomas, while 75% (21 of 28) of the control survivors had adenomas. Furthermore, the pituitary tumors in the females exposed to 1000 ppm styrene were smaller than those in the controls. In 10 of 36 females exposed to 1000 ppm which developed pituitary tumors, the tumors were not observed at necropsy, while this was the case in only 2 of 51 control females with pituitary tumors.

Similarly, fewer females exposed to styrene developed mammary adenocarcinomas than control rats. The incidences were: 33, 22, 15, 8, and 3% of rats with adenocarcinomas for 0, 50, 200, 500, and 1000 ppm groups, respectively. Incidence of mammary adenocarcinoma was based on the total population in each group even though mam-

TABLE 7

Major Treatment-Related Histopathological Changes in the Olfactory Areas of the Nasal Passages in CRL-CD Rats Exposed to Styrene Vapor for up to 104 Weeks

	Concentration (ppm)									
	Males					Females				
Finding	0	50	200	500	1000	0	50	200	500	1000
Atrophic and/or degenerative changes in epithelium	0	11	13	18	38	0	3	7	15	29
Prominent Bowman's glands in epithelium	0	4	16	25	36	0	2	14	26	47
Atrophy/dilatation/hypertrophy/hyperplasia of Bowman's glands	0	1	3	15	29	0	0	1	5	11
Ulceration/necrosis/erosion of olfactory epithelium	0	1	0	2	6	0	0	2	2	1
Epithelial hyperplasia/regenerative hyperplasia of olfactory epithelium	0	2	0	2	2	0	0	0	0	8
Atrophy of olfactory nerve fibers in dorsal median meatus	0	0	0	1	1	0	0	0	0	2
Number of rats examined	60	36	27	29	52	60	30	29	41	60

TABLE 6

Incidence of Neoplasia in CRL-CD Rats Exposed to Styrene Vapors for 24 Months

	Exposure Conc.				
	0	50	200	500	1000
Males					
Lymphoid/Multicentric	(7)[a]	(6)	(3)	(1)	(5)
Lymphoblastic/lymphocytic lymphoma (malignant)	0	3	1	0	2
Pleomorphic lymphoma (m)[b]	3	0	0	0	0
Histiocytic lymphoma (m)	4	1	0	0	2
Myeloid leukemia (m)	0	1	2	0	0
Mesothelioma (m)	0	1	0	1	1
Lungs	(60)	(60)	(60)	(54)	(52)
Pulmonary adenoma (b)[c]	0	0	1	0	0
Liver	(60)	(60)	(60)	(54)	(52)
Hepatocellular adenoma (b)	0	1	0	0	1
Hepatocellular carcinoma (m)	0	0	0	1	0
Pancreas	(60)	(14)	(16)	(8)	(52)
Exocrine adenoma (b)	0	1	4	0	0
Islet cell adenoma (b)	9	10	8	7	9
Islet cell carcinoma (m)	0	3	1	1	1
Mixed islet cell/exocrine adenoma (b)	1	0	2	0	0
Testes	(60)	(60)	(60)	(54)	(52)
Interstitial cell tumor (b)	2	2	2	4	6
Thyroid Glands	(60)	(2)	(5)	(1)	(52)
Follicular adenoma (b)	5	0	1	0	5
Follicular carcinoma (m)	0	0	2	0	1
C cell adenoma (b)	9	1	1	0	11
C cell carcinoma (m)	1	0	1	0	0
Ganglioneuroma (b)	1	0	0	0	0
Adrenal	(60)	(34)	(36)	(30)	(52)
Pheochromocytoma (b)	9	7	4	4	4
Pheochromocytoma (m)	2	1	0	0	0
Cortical carcinoma (m)	1	1	1	0	1
Ganglioneuroma (b)	1	0	0	0	0
Pituitary Gland	(60)	(21)	(30)	(25)	(52)
Adenoma (b)	31	17	28	24	20
Adenocarcinoma (m)	0	1	0	0	1
Adenoma in pars intermedia (b)	1	0	0	0	0
Mammary Glands	(60)	(3)	(1)	(3)	(52)
Adenocarcinoma (m)	0	0	0	1	0

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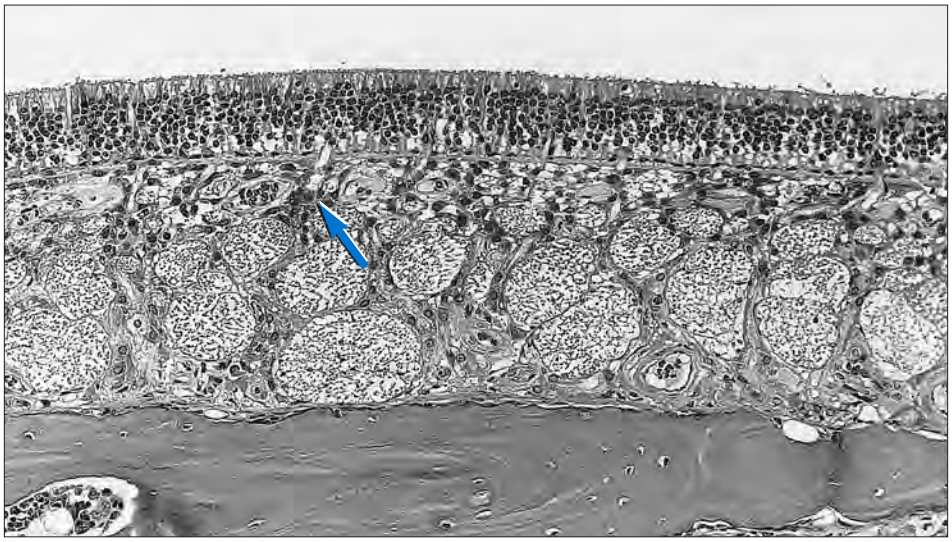
TABLE 6 *continued*

Incidence of Neoplasia in CRL-CD Rats Exposed to Styrene Vapors for 24 Months

	Exposure Conc.				
	0	50	200	500	1000
Females					
Lymphoid/Multicentric	(2)	(2)	(0)	(0)	(4)
Pleomorphic lymphoma (m)	0	2	0	0	0
Histiocytic lymphoma (m)	1	0	0	0	2
Lg. gran. lympho. lymphoma (m)	1	0	0	0	1
Mesothelioma (m)	0	0	0	0	1
Lungs	(60)	(60)	(60)	(60)	(60)
Squamous cell carcinoma (m)	0	1	0	0	0
Liver	(60)	(60)	(60)	(60)	(60)
Hepatocellular adenoma (b)	0	1	1	0	2
Hemangiosarcoma (m)	0	1	0	0	0
Pancreas	(60)	(2)	(4)	(4)	(60)
Exocrine adenocarcinoma (m)	0	0	0	0	1
Islet cell adenoma (b)	2	2	4	2	0
Thyroid Glands	(60)	(4)	(1)	(1)	(60)
Follicular adenoma (b)	3	0	0	0	4
C cell adenoma (b)	9	0	0	0	8
C cell carcinoma (m)	0	1	0	0	0
Adrenals	(60)	(51)	(49)	(43)	(60)
Pheochromocytoma (b)	0	0	0	2	0
Cortical adenoma (b)	1	0	1	0	0
Cortical carcinoma (m)	0	1	1	0	0
Pituitary Gland	(60)	(49)	(42)	(37)	(60)
adenoma (b)	45	42	35	29	31
adenocarcinoma (m)	6	5	4	5	6
Mammary Glands	(60)	(44)	(43)	(38)	(59)
Adenoma (b)	1	1	0	2	1
Adenocarcinoma (m)	20	13	9	5	2
Fibroadenoma (b)	21	16	13	18	17
Fibroadenoma with epithelial atypia (b)	9	10	5	5	3
Adenoacanthoma (m)	1	0	0	0	0

[a]Number in parentheses indicate the number of animals examined for each tissue type.
[b](m) = malignant.
[c](b) = benign.

FIGURE 2



Normal olfactory epithelium in the dorsal region of the central septum showing pseudostratified columnar epithelium with underlying Bowman's glands (see arrow). Control rat (0 ppm styrene) at 2 years, H and E, x250.

mary tissue was not examined from the 50, 200 and 500 ppm females unless macroscopically abnormal (16, 17, and 22 rats, respectively) because mammary tumors are seldom found microscopically when not seen macroscopically. The incidence of mammary fibroadenomas was lower among styrene-exposed females than among control females, although there was no obvious dose-response. Among survivors, the percent of females with fibroadenomas was 38, 64, 58, 61, and 33% for groups exposed to 0, 50, 200, 500, and 1000 ppm respectively. However, among females which died during the study, fewer of those exposed to styrene had fibroadenomas; the incidences were: 52, 36, 29, 47, and 27%, respectively.

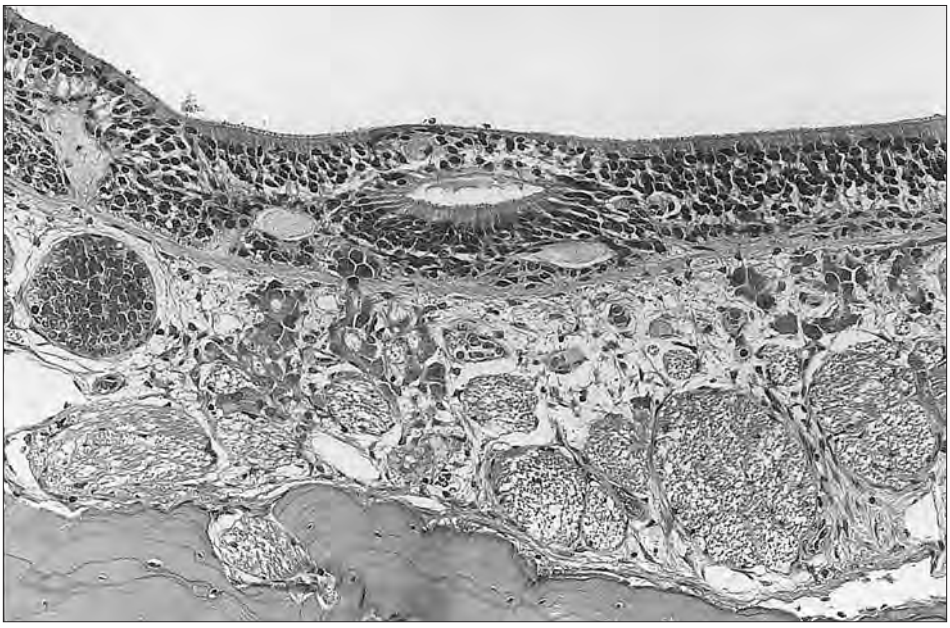
Nonneoplastic treatment-related histopathological findings in rats exposed for 52 or 104 weeks were confined to the olfactory epithelium of the nasal passages (Table 7). The changes included atrophic and/or degenerative changes in the olfactory epithelium and changes in the underlying Bowman's glands compared to the control rats (Fig. 2). The atrophic/degenerative changes were classified as: 1) Focal disorganization with rosette formation (Fig. 3a,b); 2) atrophy/apparent cell loss where there was an apparent cell loss from the olfactory epithelium, but no decrease in the overall height of the epithelium (Fig. 3b,c); 3) Atrophy/degeneration where there was a decrease in the height of the olfactory epithelium resulting from a decreased cellularity (Fig. 3e). Prominent Bowman's glandular elements were present in the olfactory mucosa only in areas of atrophy with apparent cell loss (Fig. 3c). Changes in the underlying Bowman's glands were not seen in rats exposed for 12 months, but were present in rats exposed for 24 months (Fig. 3d,e). These changes comprised atrophy, which was generally confined to the glands in the dorsal median meatus; hypertrophy; hyperplasia and dilatation . Hyperplasia of the basal cells in the olfactory epithelium was seen in rats killed after 12 months of exposure, but was not noted in older rats.

Other changes in the olfactory areas which occurred in only a few rats exposed for up to 24 months included ulceration, necrosis (Fig. 3f), erosion and epithelial hyperplasia/regenerative hyperplasia. Atrophy of the olfactory nerve fibers also was seen in occasional rats exposed to 500 or 1000 ppm styrene for up to 24 months. In rats exposed to 500 or 1000 ppm styrene, there was an increase in the degree of eosinophilic inclusions in the olfactory epithelium. The presence of eosinophilic inclusions is a common incidental finding in Sprague-Dawley rats (Monticello *et al*., 1990).

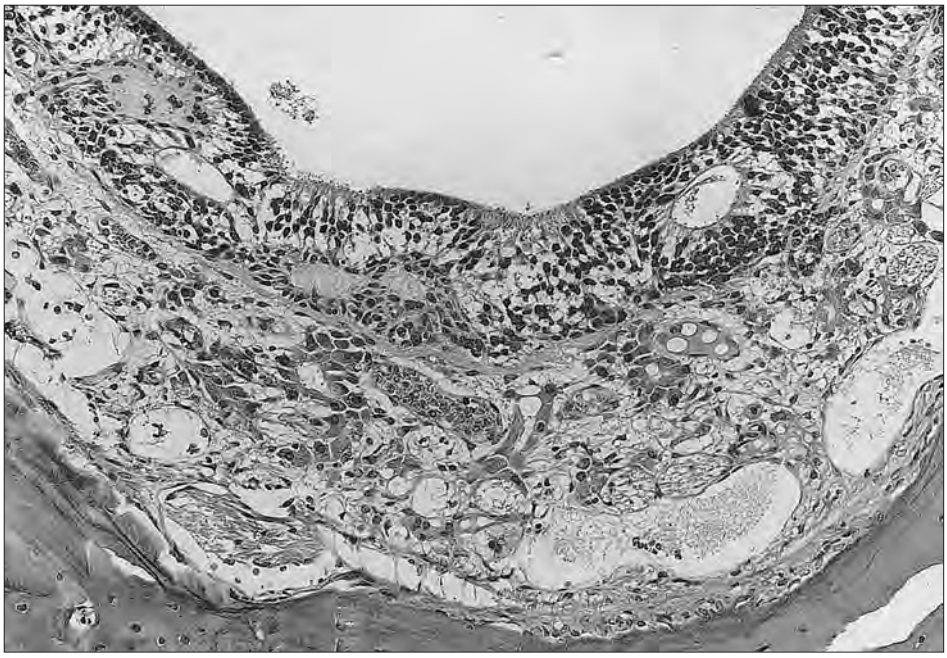
The distribution of lesions was related to exposure concentration. In general, the lesions of rats exposed to styrene for 12 months were focal, whereas those in rats exposed for 24 months were more extensive. In rats exposed to 50 ppm for 12 months, lesions were seen only in the anterior areas of the dorsal median meatus and

FIGURE 3

Photomicrographs of nasal lesions in styrene-exposed rats.



3A.
Olfactory epithelium of the dorsal median meatus of a rat exposed at 1000 ppm for one year showing disorganization with rosette formation, H and E, x250;



3B.
Olfactory epithelium showing disorganization with rosette formation and atrophy with apparent cell loss in a rat exposed to 1000 ppm for 2 years, H and E, x250;

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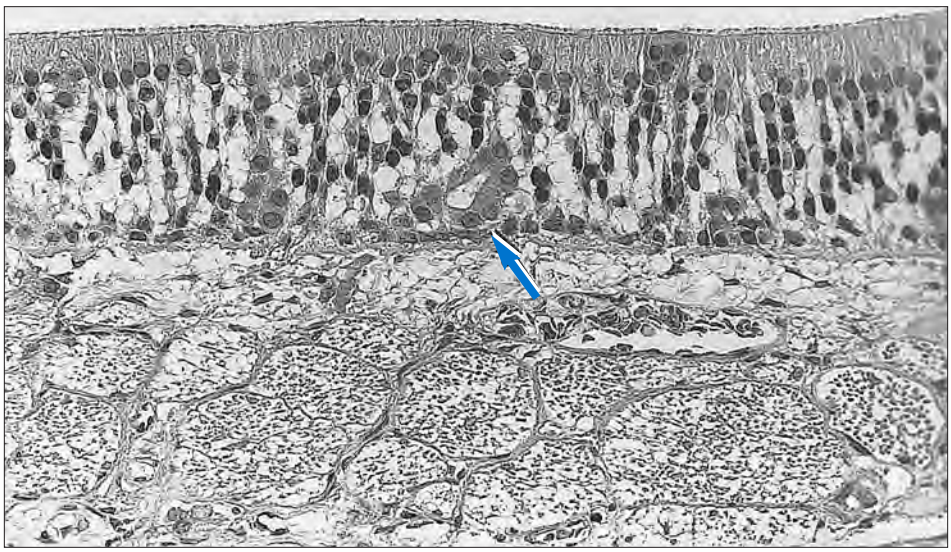
FIGURE 3 *continued*

3C.
Atrophy with apparent cell loss from the olfactory epithelium. Prominent Bowman's glandular elements are present within the epithelium (see arrow) in a rat exposed to 1000 ppm for 2 years, H and E, x250;



3D.
Olfactory epithelium showing atrophy with apparent cell loss and prominent Bowman's glandular elements within the epithelium in a rat exposed to 1000 ppm for 2 years. The submucosal Bowman's glands show dilatation (see star) and hypertrophy (see arrow), H and E, x250;

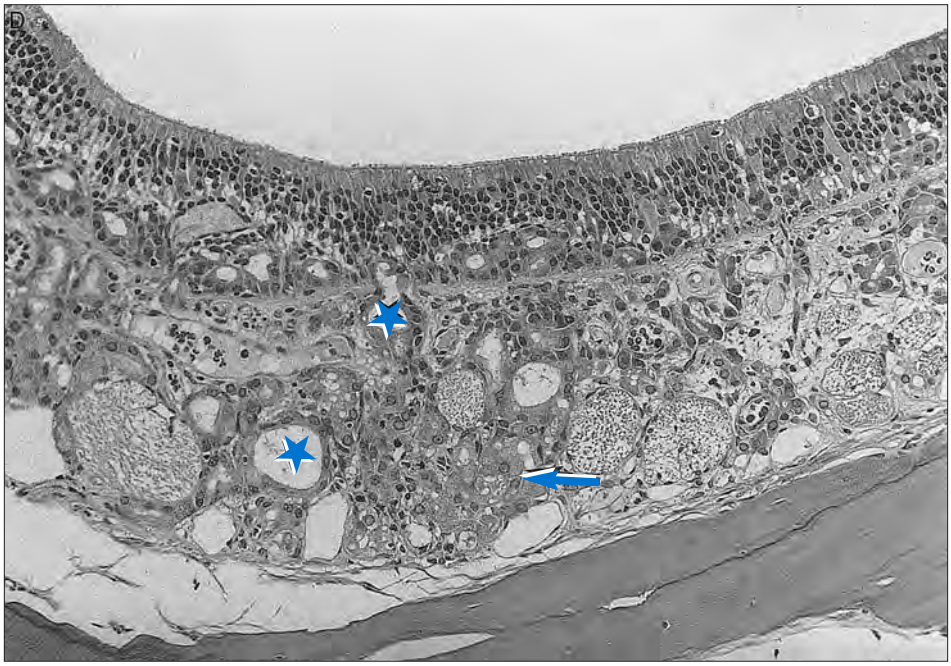


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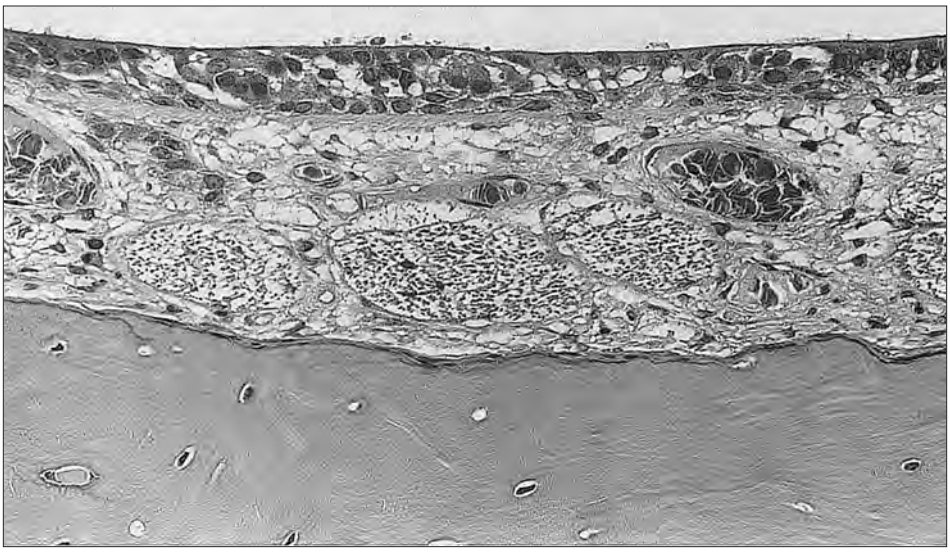
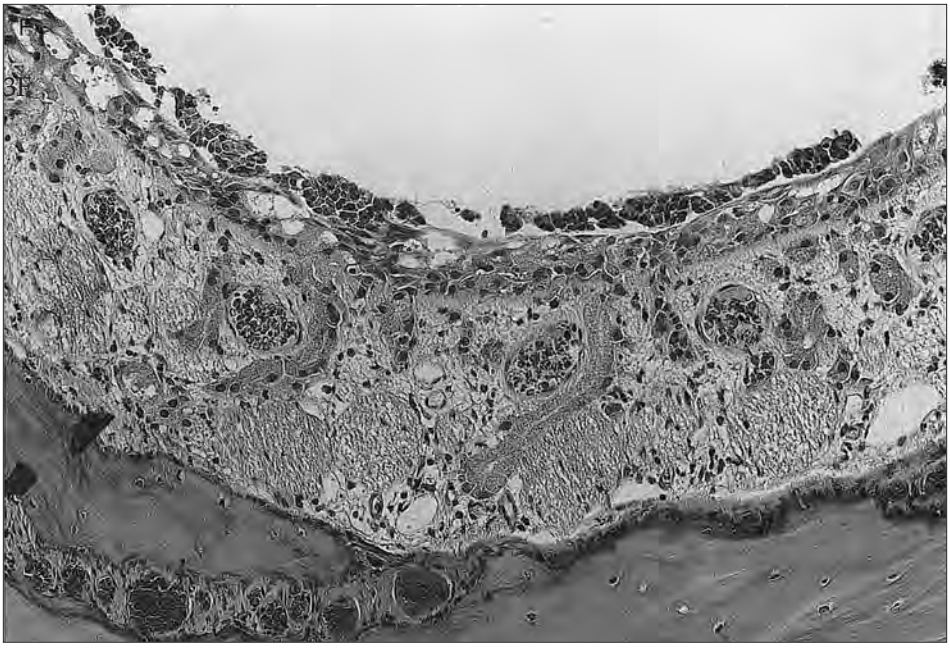
FIGURE 3 *continued*



3E.
Olfactory epithelium of the dorsal region of the central septum showing an extensive area of degeneration with decreased height of epithelium and atrophy of underlying Bowman's glands in a rat exposed to 1000 ppm for 2 years, H and E, x500;



3F.
Olfactory epithelium of the dorsal median meatus showing necrosis with exfoliation of the epithelium in a rat exposed at 1000 ppm for 2 years, H and E, x250.

adjacent central septum (Figure 4a). After 24 months, the affected area was extended to include the median aspect of the dorsal leaflet of ethmoturbinate 3 (Fig. 4b). As the dosage increased, the distribution of the lesions extended towards the most posterior ethmoturbinates. In rats exposed to 1000 ppm, all ethmoturbinates were affected (Fig. 5a,b). In rats exposed to 1000 ppm for 12 months, the lesions were focal, whereas after 24 months, the lesions were more extensive although unaffected portions of the olfactory epithelium were present.

DISCUSSION

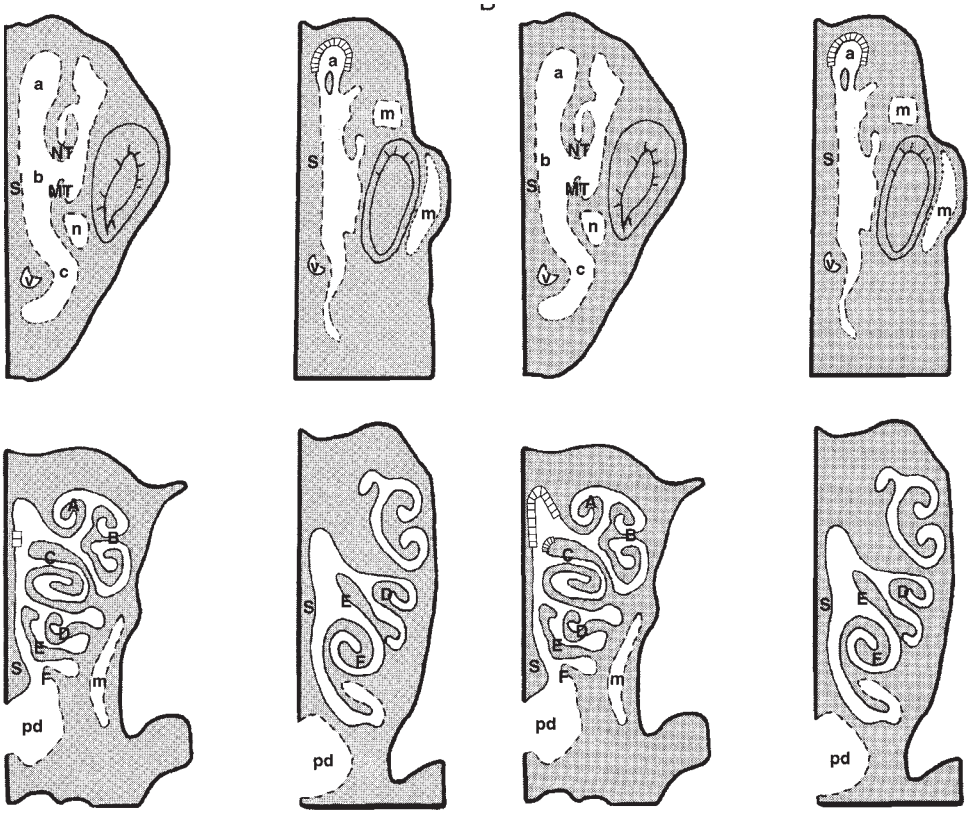
Exposure to styrene by inhalation at concentrations up to 1000 ppm for 104 weeks resulted in decreased weight gain and changes in the olfactory epithelium in male and female CD (Sprague-Dawley) rats. In females, there were also increased survival and decreases in pituitary and mammary tumors.

Seilkop (1995) reported that decreased body weight in female F344 rats was correlated with decreased incidence of pituitary and mammary tumors. While decreased weight may have had some effect on survival and tumor incidence in the present study, other factors seem to be involved. Females exposed to 500 and 1000 ppm had

FIGURE 4

Schematic diagram of cross sections of the nasal passages to show the anatomical features in rat and the areas affected by exposure to 50 ppm styrene:

A. 1 year **B.** 2 years



a, dorsal median meatus; b, medial median meatus; c, ventral median meatus; S, central septum; NT, nasoturbinate; MT, maxilloturbinate; A, ethmoturbinate I (lateral scroll); B, ethmoturbinate 2 (dorsal and ventral scrolls); C, ethmoturbinate 3 (dorsal and ventral scrolls); D, ethmoturbinate 4; E, ethmoturbinate 5; F, ethmoturbinate 6; n, nasolachrimal duct; pd, nasopharyngeal duct; (—), olfactory epithelium; (- - -), respiratory epithelium; (□□), area affected.

equally reduced body weight gains throughout the study; however, more females exposed to 1000 ppm survived (49 of 60) than those exposed to 500 ppm (40 of 60) and both groups had greater survival than in the control group (29 of 60). In addition, only those exposed to 1000 ppm, not 500 ppm, had reduced incidence of pituitary tumors. Thirdly, there was a dose-related decrease in mammary adenocarcinomas at all dose-levels. It is not clear if Seilkop's correlations were based on mammary adenocarcinomas or by combining unrelated adenocarcinomas and fibroadenomas. In this study they were treated separately. Among styrene-treated females there was also a decrease in fibroadenomas compared to the control group, but there was no dose-response.

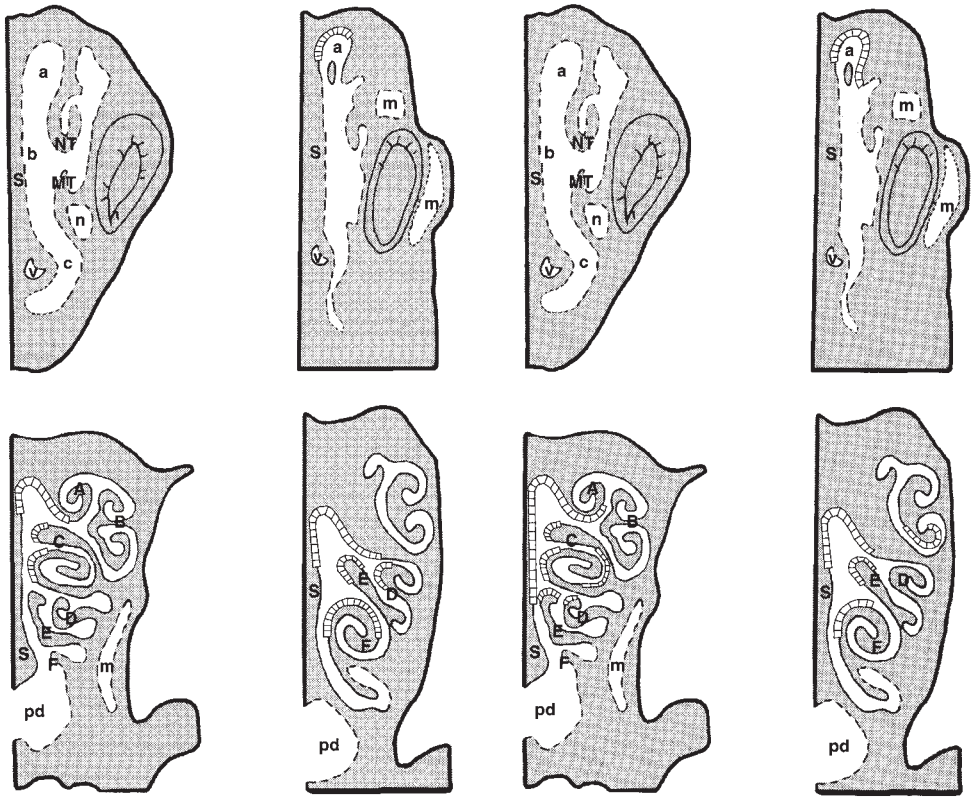
For body weight changes, the No Observed Adverse Effect Level (NOAEL) was 200 ppm. A NOAEL was not established for effects on the nasal epithelium since effects were seen at all exposure concentrations. Although there was little progression in the severity of the lesion between one year and two years of exposure, effects after one year of exposure were seen at lower concentrations than after 13 weeks (Cruzan *et al*., 1997). In the thirteen week study, lesions were seen in the olfactory epithelium of rats exposed to 500 ppm, but not in those exposed to 200 ppm. After one year of exposure to 200 ppm, there were lesions in the olfactory epithelium of males and females, and in males exposed to 50 ppm. There was progression of the area affected with contin-

FIGURE 5

Schematic diagram of cross sections of the nasal passages to show the anatomical features in rat and the areas affected by exposure to 1000 ppm styrene:

A. 1 year

B. 2 years



a, dorsal median meatus; b, medial median meatus; c, ventral median meatus; S, central septum; NT, nasoturbinate; MT, maxilloturbinate; A, ethmoturbinate I (lateral scroll); B, ethmoturbinate 2 (dorsal and ventral scrolls); C, ethmoturbinate 3 (dorsal and ventral scrolls); D, ethmoturbinate 4; E, ethmoturbinate 5; F, ethmoturbinate 6; n, nasolachrimal duct; pd, nasopharyngeal duct; (—), olfactory epithelium; (- - -), respiratory epithelium; (□□), area affected.

ued exposure; after one year the lesions were focal, but after 2 years more extensive areas were affected although portions of the ethmoturbinates were unaffected. The significance of these animal findings for human risk assessment is unclear since Odkvist *et al*. (1985) found no increase in nasal symptoms or histology score in 11 reinforced plastics workers exposed to 47-59 ppm styrene for 1 to 16 years compared to 25 referents.

This chronic study supports the lack of liver toxicity in rats from styrene exposure as demonstrated in the subchronic study (Cruzan *et al*., 1997) and other inhalation studies at similar exposure levels (Roycroft *et al*., 1992; Jersey *et al*., 1978) and disputes suggestions of liver toxicity in rats reported by Srivastava *et al*. (1982) and Vainio *et al*. (1979).

Ohashi *et al*. (1986) reported degenerative changes in the respiratory epithelium of the nasal septum and trachea of rats exposed to 150 or 1000 ppm styrene for 3 weeks. No such changes were found in either our thirteen week study (Cruzan *et al*; 1997) or in this study in rats exposed at levels of 50 to 1000 ppm for up to 2 years. Coccini *et al*. (1997) reported damage to trachea, type II and Clara cells in CRL-CD rats exposed to 300 ppm styrene 6 hrs/day 5 days/week for 2 weeks or by 3 daily intraperitoneal injections of 40 or 400 mg/kg. No such changes have been seen following exposure to styrene for 13 weeks at levels up to 1500 ppm (Cruzan *et al*., 1997) or up to 1000 ppm for up to 2 years in the present study. Coccini and coworkers did not indicate if they inflated the lungs with formalin. Failure to distend the lungs with fixative makes interpretation more difficult and may have contributed to the different results.

Previous metabolic data of styrene in rats was obtained after a single exposure. The levels of styrene and SO were measured after an extended period of exposure (week 95) for comparison of metabolism after chronic exposure to previously published acute exposure, and to assess whether saturation of metabolism had occurred at the highest exposure concentration. The blood levels of styrene were about as predicted by the Csanady *et al*. (1994) model up to 500 ppm, but at 1000 ppm, were only half that predicted by the model. The blood styrene levels were very similar to values reported by Andersen *et al*., (1984) in Sprague-Dawley rats following a single six-hour exposure, but were up to 50% less than those reported following a single five hour exposure in Wistar rats (Withey and Collins, 1979). While complete saturation of metabolism was not demonstrated in this study (i.e., the blood SO concentration continued to increase with each increasing exposure concentration) some degree of saturation was shown. Over the range of 200 to 1000 ppm, blood styrene concentration increased 12 fold in males while blood SO concentration increased only 3 fold; in females blood styrene increased 15 fold while blood SO increased 5 fold.

Although six chronic toxicity/oncogenicity studies were conducted on styrene in the 1970s and one in the early 1980s, none is fully acceptable under current standards. Some studies were deficient in design; some had problems with intercurrent disease; and none was conducted according to Good Laboratory Practices (McConnell and Swenberg, 1994). Styrene was given by gavage, alone or as 70% mixture with β-nitrostyrene, in four studies using Sprague-Dawley, Fischer 344 or BD IV rats at doses of 50 to 2000 mg/kg/day five days per week for 12 to 24 months (Table 1). No treatment-related increases in tumors were reported. Styrene was given in the drinking water at 125 and 250 ppm for two years to Sprague-Dawley rats with no increases in tumor incidences.

Conti *et al*. (1988) exposed groups of 30 male and 30 female Sprague-Dawley rats to 25, 50, 100, 200, or 300 ppm styrene 4 hours per day 5 days/week for 52 weeks during 1974-5, with observation for an additional 52 weeks. They reported total mammary tumor incidences of 57, 80, 70, 77, 80, and 83%, respectively, and malignant mammary tumor incidences of 10, 20, 13, 15, 40 and 30%, respectively. Even though there is no obvious dose-response in these data, the authors concluded the increased mammary tumors were styrene-related. Although the historical control incidence for this laboratory was not given in the paper, Dr. Maltoni (1978) had previously written "It should be pointed out that the incidence of mammary tumors is quite high in the colony of rats we employ, that some fluctuation of that incidence is currently observed from group to group, and that the results of the other experiments do not support a correlation between styrene exposure and mammary tumors." It has also been pointed out by others that the frequency of spontaneously developing mammary tumors is high in female Sprague Dawley rats (van Zwieten *et al*., 1994). In another inhalation study (Jersey *et al*., 1978), the authors concluded that styrene did not cause increased mammary tumors in female Sprague-Dawley rats exposed to 600 or 1000 ppm styrene for 89 weeks and observed until 104 weeks. In that study, females exposed to 600 ppm had a greater incidence of malignant mammary tumors (8%) than the concurrent controls (1%), but not greater than historical controls (mean = 6%, range = 0-9%). In addition, females exposed to 1000 ppm did not have an

TABLE 8

Mammary Tumor Results in Female Rats Treated with Styrene[a]

Strain	Route of Exposure	Administered Daily Dose	Lifetime Dose (g/kg)	Reported Result	Reference
SD	Inhalation	25 ppm	1.9	↑	Conti *et al.*, 1988
SD	Inhalation	50 ppm	3.9	↑	Conti *et al.*, 1988
SD	Inhalation	100 ppm	7.7	↑	Conti *et al.*, 1988
SD	Water	125 ppm	9.9	=	Beliles *et al.*, 1985
SD	Inhalation	50 ppm	11.6	=	This study
SD	Gavage	50mg/kg/day	13.2	=	Conti *et al.*, 1988
SD	Water	250 ppm	14.9	=	Beliles *et al.*, 1985
SD	Inhalation	200 ppm	15.3	↑	Conti *et al.*, 1988
SD	Inhalation	300 ppm	23	↑	Conti *et al.*, 1988
F344	Gavage (m)	175 mg/kg/3x	42	=	NCI, 1979 (b)
SD	Inhalation	200ppm	45	=	This study
BDIV	Gavage	500 mg/kg/wk	53	=	Ponomarkov, 1978
SD	Gavage	250mg/kg/day	66	=	Conti *et al.*, 1988
F344	Gavage (m)	350 mg/kg/3x	84	=	NCI, 1979 (b)
SD	Inhalation	500 ppm	115	↓	This study
SD	Inhalation	600 ppm	115	=	Jersey *et al.*, 1978
SD	Inhalation	1000 ppm	192	=	Jersey *et al.*, 1978
SD	Inhalation	1000 ppm	230	↓	This study
F344	Gavage	500 mg/kg/day	264	=	NCI, 1979 (a)
F344	Gavage	1000 mg/kg/day	396	=	NCI, 1979 (a)
F344	Gavage	2000 mg/kg/day	792	=	NCI, 1979 (a)

[a]Based on percent of female rats with any mammary tumor.

increased incidence of malignant mammary tumors (0%). There was also no increase in mammary fibroadenomas (71, 75, and 74%, respectively).

When all the groups of female rats in these eight studies exposed to styrene are analyzed by cumulative lifetime dose (Table 8), there is no indication of a dose-response for increased mammary tumors. In fact, it is only the lowest exposures tested, and all in the same study, that report increased mammary tumors.

Exposure to styrene has not been associated with treatment-related increased incidences of tumors at any other sites in rats. Based on an overall evaluation of eight chronic/oncogenicity studies, there is clear evidence that styrene does not induce cancer in rats.

ACKNOWLEDGEMENTS

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Uptake of Styrene in the Upper Respiratory Tract of the CD Mouse and Sprague-Dawley Rat

John B. Morris

ABSTRACT

Inspired styrene is an olfactory toxicant in the mouse and rat. To provide nasal dosimetric information, upper respiratory tract uptake efficiency (UE) of styrene was measured in the surgically isolated URT of the urethane-anesthetized CD mouse and Sprague Dawley rat throughout a 45 minute exposure. In the first studies the effect of inspiratory flow rate on styrene UE was examined. At flows of 12, 24 or 70 ml/min average UE of 17%, 9.8% and 4.1% were observed in the mouse. For the rat, UE averaged 14%, 9.1% and 5.7% at flow rates of 70, 150 and 400 ml/min, respectively. In the second study, UE was measured at inspired concentrations of 5, 10, 25, 50, 100 or 200 ppm at a flow rate of 12 ml/min in the mouse and 70 ml/min in the rat in both naïve and metyrapone (150 mg/kg s.c.) pretreated animals. In the rat steady state UE decreased with increasing exposure concentration, averaging between 24 and 10% efficient at 5 to 200 ppm ($p<0.0001$). Metyrapone pretreatment resulted in statistically significant reductions in UE with steady state UE averaging 10-14% at 5-200 ppm. Metyrapone pretreatment abolished the concentration dependence. In naïve mice styrene UE did not maintain a steady state, but steadily declined during exposure. The mechanisms of the non-steady state behavior are not known, but appear to be due to a styrene metabolite as evidenced by the fact that steady state UE was observed in metyrapone-pretreated mice. In the mouse UE averaged between 42 and 10% efficient at 5 to 200 ppm ($p<0.0001$). Metyrapone pretreatment resulted in statistically significant reductions in UE, with steady state UE averaging 20-10% at 5-200 ppm. As in the rat, metyrapone pretreatment abolished the concentration dependence. In toto, these data provide strong evidence that inspired styrene is metabolized in nasal tissues in the rat and mouse and that a metabolic basis exists for the observed inspired concentration dependence of UE.

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INTRODUCTION

Styrene is a clear pungent liquid that is used extensively in the polymer industry. Annual production of styrene in the US in 1993 was estimated to be in excess of 5 million tons (Miller et al., 1994). The current ACGIH TLV is 20 ppm. The OSHA PEL is 100 ppm, however, the styrene industry and OSHA have adopted a voluntary standard of 50 ppm. The EPA RfC is 1 mg/m^3 (0.24 ppm) and is based upon neuropsychological effects. Styrene is a

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sensory irritant. RD50 values in mice of 157, 586 and 980 ppm have been reported (Alarie, 1973; Alarie, 1981; DeCeaurriz et al., 1981).

Recent rodent inhalation toxicity studies have revealed that styrene is an olfactory toxicant. In both the rat and mouse subchronic exposure leads to olfactory mucosal degeneration with the lesions being most severe in the dorsal medial portions of the nasal cavity (Cruzan et al, 1997, 1998). Subchronic (13 week) inhalation studies provided a NOEL of 200 ppm and an LOEL of 500 ppm for styrene induced olfactory degeneration in the rat. In the mouse 13 week exposure to 50 ppm results in olfactory degeneration suggesting the mouse olfactory mucosa is more sensitive to this vapor than is the rat olfactory mucosa. It is noted however, that 1 year or 2 year exposure to 50 ppm styrene results in olfactory degeneration in the rat (Cruzan et al., 1998). The mechanism(s) responsible for styrene induced nasal injury or the apparent heightened sensitivity of the mouse are not known.

Styrene is CYP450 substrate (Sumner and Fennell , 1994). Styrene oxide is the initial product of the metabolic pathway. The nose expresses high levels of CYP450 (Thornton-Manning, 1997) suggesting nasal tissues might be capable of metabolizing styrene. Specfic data on nasal mucosal sytrene metabolism are not currently available, however. The role of metabolism in the olfactory injury is unknown (Cruzan et al., 1998), however the pattern of injury - olfactory mucosal damage in the absence of marked respiratory mucosal injury- is typical of that observed for toxicants that are metabolically activated in olfactory tissue.

Quantitative inhalation risk assessment for styrene requires knowledge of the nasal dosimetry of this vapor. The proposed study was designed to provide such information. Towards these ends the effect of inspiratory flow rate on nasal styrene uptake efficiency (UE) was examined. Such data are useful in the formulation of mathematical UE simulation models (Morris et al., 1993; Plowchalk et al., 1997; Frederick et al., 1998; US EPA, 1984). In addition, styrene UE was measured over a wide range of inspired concentrations (5-200 ppm) in normal animals and in animals pretreated with the CYP450 inhibitor metyrapone. These studies were performed to provide insights into the metabolism of inspired styrene in nasal tissues over a range of inspired concentration. Our previous studies have shown that this inhibitor reduces nasal UE of two structurally similar CYP450 substrate vapors - xylene and bromobenzene (Morris, 1993). All studies were performed in both the Sprague-Dawley rat and CD-1 mouse to provide comparative information between the two species that have been most recently utilized in styrene inhalation toxicity testing.

MATERIALS AND METHODS

General design

This study contained two phases. In the first phase, the effect of inspiratory flow rate on upper respiratory tract (URT) UE was examined. The URT is defined as all regions of the respiratory tract anterior to and including the larynx. These studies were performed at flow rates equivalent to 50, 100 and 275% of the predicted minute ventilation of each species. These flows have been used in our previous studies (Morris, 1997). Only one exposure concentration was used - 50 ppm, corresponding to the voluntary occupational standard for styrene. In the second phase studies, UE efficiency over a wide range of concentrations was examined (5, 10, 25, 50, 100 or 200 ppm). In addition the effect of the CYP inhibitor metyrapone on UE was examined at each of these concentrations. Only one flow rate was used for these studies. Because only moderate UE were anticipated a flow rate of 50% of the minute ventilation was selected to maximize the observed values. Data from animals in the low flow rate group of the first phase studies were included in statistical analysis of the second phase to enhance statistical power. Metyrapone was administered s.c. at a dose of 150 mg/kg (25 mg/ml in distilled water) 30 minutes prior to UE measurement. The dosing regimen was used in our previous studies on URT UE of CYP450 substrate vapors (Morris, 1993).

Animals and reagents

Specific pathogen free male Sprague Dawley rats (Crl:CDBR, 125-150 g on arrival) or CD-1 mice (Crl:CD-1 (ICR) BR, 19-21 g on arrival) were obtained from Charles River laboratories (Wilmington, MA). Animals were housed over hard wood bedding in filter top cages in animal rooms maintained at 22-25 °C with a 12-hr light-dark cycle (lights on 6:30 AM). Animals were acclimated for 1 week prior to use and were used within 3 weeks of arrival. At the time of use body weights averaged approximately 30g and 220 g for mice and rats, respectively. Styrene (99% pure) and metyrapone were obtained from Sigma Chemical Company (St. Louis, MO). All other reagents were obtained from local suppliers and were the highest purity available.

Exposure Protocols

For exposure the URT was isolated by the method used

previously in this laboratory (Morris, 1990). All procedures were performed after the onset of urethane anesthesia (1.3 g/kg ip). The URT was isolated by insertion of a polyethylene tube in the trachea in an anterior direction such that its tip lay at the larynx. The animal was then placed in a nose-only chamber and chamber air was drawn through the isolated URT for 45 minutes under the flow conditions described below. Immediately after the end of exposure each animal was killed by exsanguination.

UE Measurement

To measure URT UE, vapor concentration was measured in chamber air (i.e. air entering the URT, Cin) and in air that had been drawn through the URT (Cex – exiting air concentration). UE was calculated from the difference between these two concentrations by the formula (Cin-Cex)/Cin and expressed as a percent. Exiting air concentrations were measured every 3 minutes during the 45 minute exposure. Values obtained between 15-45 minutes were used to assess UE. In our previous studies steady UE values were not observed until 15 minutes of exposure, therefore values obtained at earlier exposure times were not used to estimate UE (Morris, 1999). It is recognized that this is an arbitrary decision, but the results are not altered if the data from 9-45 or 12-45 minutes are utilized.

Measurements were made under unidirectional inspiratory flow conditions at flow rates of 70, 150 or 400 ml/min in the rat and 12, 24 or 70 ml/min in the mouse. These flows have been used previously in our laboratory (Morris 1997) and correspond to approximately 50, 100 and 275% of the predicted minute ventilation of each species (Guyton, 1947).

For each animal, Cin was measured immediately before and again immediately after Cex determination using the same flow conditions as used for Cex determination. The average value of these chamber concentrations was used to calculate UE. The ratio of the "before" and "after" chamber concentrations provides an indirect index of the steadiness of the chamber vapor concentrations during the exposure. This ratio averaged 98.0 $\pm$ 6.6 % (mean $\pm$ SD) for the mouse studies and 99.4 $\pm$ 6.5 % (mean $\pm$ SD) for the rat studies. Since the before and after samples were separated by 45 minutes these ratios suggest that chamber air levels changed by 0.05% or less per minute during the exposure (assuming a linear change during the 45 minute exposure period).

Air sampling and analysis

A schematic of the air sampling system is provided in Morris (1999). The sampling system used for drawing air samples consisted of stainless steel tubing with a stainless steel T. Polyethylene tubing was used to connect the sampling system to either the chamber sampling port (inspired air analysis) or the animal endotracheal tube (exiting air analysis). Air was drawn off one arm of the T and through an 8-port two loop (0.5 ml) gas sampling valve (connected to a gas chromatograph) via heated tubing at a flow rate of 7 ml/min. The other arm of the sample line T was connected to a vacuum source to control total air flow rates at the desired level (e.g. 12, 24 or 70 ml/min in the mouse). Air flow rates were controlled with rotameters which were calibrated in the sample line with a bubble meter. Air samples were injected into the chromatograph from the gas sampling valve every 3.0 minutes to provide continuous sampling.

Airborne styrene concentrations were measured in a Varian model 3600 gas chromatograph equipped with a flame ionization detector. A 15 m DB-Wax megabore column (J&W Scientific, Folsom, CA) was used with a column oven temperature of 100 °C and a carrier gas (N2) flow rate of 30 ml/min. Styrene peaks eluted 0.45 minutes after injection onto the column. Peak heights (Varian model 4290 integrator) were converted to concentrations on the basis of a standard curve that was prepared for each vapor. For this purpose 4 ul aliquots of styrene standard (dissolved in methanol) were injected into teflon gas sampling bags (Cole-Parmer, Niles, IL) which were then filled with 0.8 l of clean air. After at least 1 hour to allow for evaporation, air was drawn from the bags through the sample train used for UE measurement and into the GC gas sampling valve for analysis.

Chamber conditions and vapor generation

Total air flow rates through the 1.2 l stainless steel Jaeger-NYU directed flow nose-only inhalation chamber (CH Technologies, Westwood NJ) were maintained at 10 l/min with clean filtered heated humidified air. Chamber air temperature averaged 40 °C and airborne water content exceeded 30 mg/l corresponding to greater than 70% relative humidity at 37 °C. Chamber walls, inlet tubing, and sample tubing were heated to prevent condensation. A hot air gun was used to warm the animal and also to minimize condensation in the endotracheal tubing.

Chamber atmospheres were generated with a syringe pump system (Model 355, Sage Instruments). Pure styrene was fed into a J tube maintained at 80 °C. Air (0.6 l/min) was passed through the tubing and into the chamber diluting air line. Chamber concentrations were controlled by changing the styrene delivery rate. The chamber was operated for at least 45 min prior to

measurement of deposition to allow for equilibration.

Nominal styrene concentrations for these studies were 5, 10, 25, 50, 100 and 200 ppm. The measured concentrations were (mean ± SD) 5.8 ± 0.9, 10.9 ± 0.9, 25.5 ± 2.3, 52.0 ± 5.1, 104 ± 10 and 202 ± 25 ppm.

Mathematical Analysis

All data are presented as mean ± SD unless otherwise indicated. Linear relationships were assessed and compared by linear regression analysis. Groups of data were analyzed by t-test, single- or multi-factorial analysis of variance followed by Newman-Keuls test. Statistical tests were performed with Statistica software (Statsoft, Inc., Tulsa OK).

RESULTS

Flow Rate Study

The measured exposure concentration was 54 ± 6.5 ppm. UE tended to decline somewhat during exposure in the rat and mouse, with the rate of decline being greater in the latter species. Linear regression analysis was performed on the relationship between UE and exposure time for each animal to calculate the slope, ie the rate of change in UE during 15-45 minutes of exposure. A slope of zero would indicate the maintenance of a steady state. In the mouse, the average slope values were negative at all flow rates, however for no flow rate were the average values significantly different from zero ($p > 0.05$ t-test). Among all mice (regardless of flow) the values averaged –0.116 ± 0.23 %/min (n=15). In the rats both negative and positive slopes were observed, the overall average being –0.03 ± 0.23 %/min (n=15) a value not different from zero ($p > 0.05$ t-test).

An average UE efficiency was calculated for each animal by averaging the UE efficiencies obtained between 15-45 minutes of exposure. These values were then compared by two factor ANOVA with the factors being flow rate and species (Table 1). A significant effect of flow rate was observed, no difference between species was detected and no interaction between flow rate and species was detected. Newman-Keuls test revealed that UE efficiency was significantly different among all three flow rates, with UE efficiency decreasing as flow rate increased.

Concentration Dependence Study

In this study UE was measured in naive (not pretreated) or metyrapone pretreated rats or mice at inspired concentrations of 5, 10, 25, 50, 100 or 200 ppm (nominal).

Mice. Shown in Figure 1 is the average UE during the exposure in naive and metyrapone pretreated mice at an inspired concentration of 5 ppm. UE efficiency steadily diminished during 15-45 minutes of exposure in the naive mice but remained steady in the metyrapone-pretreated mice. This pattern was observed at all exposure concentrations.

As in the flow rate study, linear regression analysis was performed on the UE versus time relationship for each animal and the resulting slope values for the animals in each group were averaged to statistically evaluate steady state UE behavior. The average slope values in every naive exposure group were negative ranging between –0.44 and –0.10 %/min depending on the concentration. In contrast, in the naive mice, average slope values were both negative and positive and ranged between -0.02 and +0.09, except for the 200 ppm group in which the slope averaged –0.175 ± 0.16 (n=5). This latter value was not statistically different from zero.

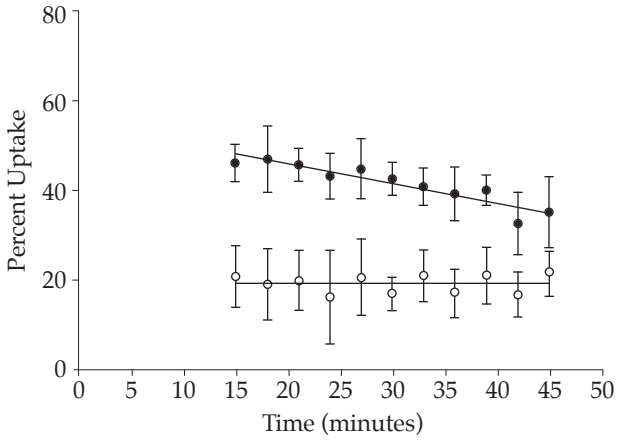
TABLE 1

Effect of Inspiratory Flow Rate on URT Styrene Uptake Efficiency in the Mouse and Rat

	Inspiratory flow rate		
	Low	Mid	High
MOUSE	17.2^{a} ± 4.5 (5)	9.8^{b} ± 1.9 (5)	4.1^{c} ± 4.2 (5)
RAT	14.1^{a} ± 4.8 (5)	9.1^{b} ± 4.0 (5)	5.7^{c} ± 0.7 (5)

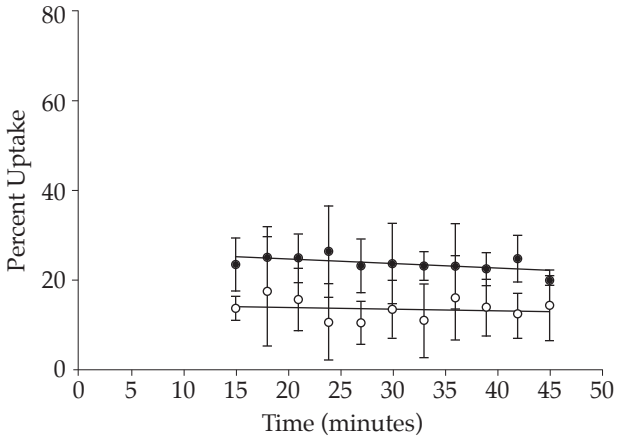
Average uptake efficiency between 15-45 minutes expressed as percent. Numbers in parentheses refer to the numbers of animals (n). Data are reported as mean ± SD. The low, mid and high flow rates correspond to approximately 50%, 100% and 275% of the predicted minute ventilation for each species. The actual flows for the rats and mice were 70, 150 and 400 ml/min and 12, 24 and 70 ml/min, respectively. Data were analyzed by two factor ANOVA which detected a significant effect of flow rate ($p=0.00005$), no difference between species ($p=0.60$) and no interaction between flow rate and species ($p=0.34$). Each group was then compared by Newman-Keuls test, groups with differing superscripts differ significantly ($p<0.05$) from each other.

FIGURE 1



Shown is the average uptake efficiency (expressed as a percent) obtained at each time point of the naive (closed circles) and metyrapone-pretreated (open circles) mice. The exposure concentration averaged 6.3 + 0.8 (mean + SD) among the 6 naive and 6 metyrapone-pretreated mice. Data are presented as mean + SD. The linear regression lines (uptake efficiency versus time) are shown

FIGURE 2



Shown is the average uptake efficiency (expressed as a percent) obtained at each time point of the naive (closed circles) and metyrapone-pretreated (open circles) rats. The exposure concentration averaged 5.4 + 0.9 (mean + SD) among the 7 naive and 6 metyrapone-pretreated rats. Data are presented as mean + SD. The linear regression lines (uptake efficiency versus time) are shown.

Slope data among groups were compared by two factor ANOVA with the factors being concentration and metyrapone pretreatment. This analysis detected a significant effect of metyrapone ($p=0.002$), no effect of styrene concentration ($p=0.31$), and no interaction between styrene and metyrapone ($p=0.34$). Since a significant effect of concentration was not detected, data from all groups were averaged. In the 32 pretreated mice the slopes averaged 0.014 ± 0.212 % per minute, a value not different from zero. In the 35 naive mice, the slopes averaged 0.224 ± 0.272 % per minute, a value significantly different from zero ($p=0.0005$, t-test). In toto, these results indicate that steady state UE of styrene was maintained from 15-45 minutes of exposure in the metyrapone-pretreated, but not naive, mice.

The average UE between 15-45 minutes of exposure are shown in Table 2. These values were compared among the groups. It should be noted however, that since the naive mice did not maintain steady state, the precise relationships among groups are time-dependent. For this reason only generalized conclusions are drawn. Data were compared by two factor ANOVA with the factors being styrene concentration and metyrapone pretreatment. A significant effect of exposure concentration, metyrapone pretreatment and a significant interactionwere detected. The interaction demonstrates that metyrapone had statistically differing effects at the different exposure concentrations. Groups were then compared by Newman-Keuls test. In the naive animals UE efficiencies at exposure concentrations of 5, 10, 25 or 50 ppm were significantly higher than at 100 or 200 ppm. In contrast, UE were similar at all exposure concentrations in the metyrapone-pretreated animals. Among the 32 metyrapone-pretreated mice UE averaged 15.9 ± 5.5 %. Thus metyrapone pretreatment abolished the inspired concentration dependence of UE. The UE in all metyrapone-pretreated animal groups were similar to those observed at the high concentrations (100, 200 ppm) in the naive animals. At the two lowest exposure concentrations (5 or 10 ppm) UE averaged significantly higher in the naive than metyrapone-pretreated animals. Direct comparisons between these groups should be made with caution because the metyrapone-pretreated animals demonstrated steady state UE and the naive animals did not, however these results indicate a generalized diminished UE of metyrapone-pretreated animals (see also Figure 2).

TABLE 2

URT Styrene Uptake Efficiency in the Mouse

(Average uptake efficiency between 15-45 minutes expressed as percent)

	Inspired Styrene Concentration (ppm)					
MOUSE	5	10	25	50	100	200
Naïve	41.7 ± 4.0 [a] (6)	29.7 ± 4.4 [b] (6)	23.7 ± 8.3 [b,c] (6)	17.8 ± 4.4 [c] (6)	12.5 ± 7.3 [d] (6)	9.6 ± 6.6 [d] (6)
Metyrapone	19.4 ± 5.6 [c,d] (6)	18.8 ± 2.7 [c,d] (6)	16.7 ± 5.8 [c,d] (5)	16.1 ± 4.8 [c,d] (5)	9.8 ± 4.5 [d] (5)	13.2 ± 4.3 [d] (5)

Numbers in parentheses refer to the numbers of animals (n). Data are reported as mean ± SD (n), naïve animals received no pretreatment, metyrapone animals received 150 mg/kg metyrapone, s.c. 30-60 prior to exposure.

Data were analyzed by two factor ANOVA which detected a significant effect of metyrapone ($p=0.00005$), of styrene concentration ($p=0.00001$) and an interaction between metyrapone and styrene concentration ($p=0.000002$). Each group was then compared by Newman-Keuls test, groups with differing superscripts differ significantly ($p<0.05$) from each other.

Measured exposure concentrations for the mouse studies were: 6.3 ± 0.9, 10.5 ± 1.0, 23.8 ± 2.1, 51.6 ± 2.5, 106 ± 11, and 205 ± 30 ppm (mean ± SD).

The average uptake rate (ug/min) can be calculated from the inspired concentration, flow rate and UE. In naive animals the uptake rate ranged from approximately 0.1 to 1.0 ug/min at inspired concentrations of 5 to 200 ppm, respectively.

Rats. Shown in Figure 2 is the average UE during the exposure in naive and metyrapone pretreated rats at an inspired concentration of 5 ppm. UE diminished slightly 15-45 minutes of exposure in both the naive and metyrapone-pretreated rats, but the decline was not statistically different from zero. Linear regression analysis was performed on the UE versus time relationship for each animal and the resulting slope values for the animals in each group were averaged to statistically evaluate steady state UE behavior. Both positive and negative average slope values were observed in the naive and metyrapone-pretreated rat groups. In no case were the average slopes different from zero indicating that steady state UE was maintained in these groups. Slope data among groups were compared by two factor ANOVA with the factors being concentration and metyrapone pretreatment. This analysis detected no significant effect of metyrapone ($p=0.70$), no effect of styrene ($p=0.93$), and no interaction between styrene and metyrapone ($p=0.96$). In toto, these results indicate that, unlike the mice, steady state UE of styrene was maintained from 15-45 minutes of exposure in the rat.

The average UE between 15-45 minutes of exposure are shown in Table 3. These values were compared among the groups by two factor ANOVA with the factors being styrene concentration and metyrapone pretreatment. A significant effect of exposure concentration, metyrapone pretreatment and a significant interaction were detected. The interaction demonstrates that metyrapone had statistically differing effects at the different exposure concentrations. Groups were then compared by Newman-Keuls test. In the naive animals UE at exposure concentrations of 5 or 10 ppm were significantly higher than at higher concentrations. An effect similar to that observed in the mice (see Table 2). In contrast, UE were similar at all exposure concentrations in the metyrapone-pretreated animals. Among the 36 metyrapone pretreated rats UE 9.7 $\pm$ 4.4 %. Thus metyrapone pretreatment abolished the inspired concentration dependence of UE in rats as it did in mice (see Table 2). The UE in all metyrapone-pretreated animal groups were similar to those observed at the high concen-

TABLE 3

URT Styrene Uptake Efficiency in the Rat

(Average uptake efficiency between 15-45 minutes expressed as percent)

	Inspired Styrene Concentration (ppm)					
RAT	5	10	25	50	100	200
Naïve	23.7 ± 2.9 [a] (7)	22.4 ± 4.2 [a] (6)	15.3 ± 3.4 [b] (6)	13.1 ± 4.7 [b] (9)	8.7 ± 4.6 [b] (7)	10.1 ± 3.8 [b] (7)
Metyrapone	13.6 ± 5.0 [b] (6)	10.5 ± 4.1 [b] (6)	9.9 ± 5.0 [b] (6)	7.6 ± 3.9 [b] (6)	7.7 ± 3.9 [b] (6)	8.5 ± 3.1 [b] (6

Numbers in parentheses refer to the numbers of animals (n). Data are reported as mean ± SD (n), naïve animals received no pretreatment, metyrapone animals received 150 mg/kg metyrapone, s.c. 30-60 prior to exposure.

Data were analyzed by two factor ANOVA which detected a significant effect of metyrapone (P=0.000001), of styrene concentration (p=0.00001) and an interaction between metyrapone and styrene concentration (p=0.007). Each group was then compared by Newman-Keuls test, groups with differing superscripts differ significantly (p<0.05) from each other.

Measured exposure concentrations for the rat studies were: 5.4 ± 0.8, 11.2 ± 0.8, 27.1 ± 1.0, 52.1 ± 6.5, 103 ± 10, and 198 ± 19 ppm (mean ± SD).

trations in the naive animals. At the two lowest exposure concentrations (5 or 10 ppm) UE averaged significantly higher in the naive than metyrapone-pretreated animals.

Uptake rates (ug/min) averaged approximately 0.35 to 6 ug/min at exposure concentrations of 5 to 200 ppm, respectively.

DISCUSSION

Under constant velocity inspiratory flow conditions styrene was removed from the airstream in the nasal passages of the rat and mouse with moderate efficiency. UE ranged between 4-42% depending on the inspired concentration and inspiratory flow rate. The styrene UE observed in the study at exposure concentrations of 25 ppm or higher were similar to those observed in the rat and hamster for xylene and bromobenzene at similar inspired concentrations (Morris, 1993). The blood:air partition coefficients for xylene and bromobenzene (25 and 40, respectively) are also similar to that of styrene (40, Andersen et al., 1984). As for xylene and bromobenzene (Morris, 1993), styrene UE was strongly dependent on the inspiratory flow rate with diminished UE being observed at high flow rates in both the rat and mouse. This behavior has been observed for numerous vapors in many laboratory species (Morris, 1994).

Prolonged uptake of inspired metabolized vapors is dependent upon the ability of the nose to remove vapors from nasal tissues via the bloodstream and via metabolism. Uptake due to bloodstream clearance is a first order process and, in the absence of toxicity, increases linearly with increasing exposure concentration (Morris, 1994, 1999). In contrast, uptake via metabolic clearance is first order at low concentrations but zero order (saturable) at high inspired concentrations (Morris et al., 1993; Morris 1994, 1999). At sufficienty high concentration uptake will be dominated by the blood clearance process. Even though metabolism may be occurring at Vmax, it may account for only a small fraction of the total amount of vapor being removed via the bloodstream.

Styrene is a known CYP450 substrate (Sumner and Fennell, 1994) and nasal tissues express CYP 450 (Thornton-Manning, 1997). That styrene UE was diminished by the CYP450 inhibitor metyrapone (Tables 2 and 3) provides strong evidence that styrene is metabolized, in situ by CYP450, and that this process serves to enhance

uptake. For comparative purposes it is noted that metyrapone exerts similar effects on nasal UE of xylene and bromobenzene (Morris, 1993). Similar results have also been obtained with carboxylesterase inhibition and inspired ester vapors (Morris, 1990; Morris and Frederick 1995), alcohol dehydrogenase inhibition and isoamyl alcohol vapor (Morris, 1993) , aldehdye dehdyrogenase inhibition and acetaldehyde vapor (Stanek and Morris, 1999).

In both the rat and mouse styrene UE was concentration dependent. Enhanced UE at low compared to high exposure concentrations has been observed for other metabolized vapors including propanol (Morris and Cavanagh, 1986) and acetaldehyde (Morris and Blanchard, 1992). For the latter vapor, comparison of the nasal metabolic potential (as measured by the *in vitro* Vmax for nasal aldehyde dehydrogenase) with the uptake rates suggested the diminished UE was due to metabolic capacity limitation. Specifically, diminished UE were observed at inspired concentrations that were sufficiently high that uptake rates (ug/min) greatly exceeded nasal Vmax. Subsequent studies utilizing the aldehyde dehydrogenase inhibitor cyanamide, demonstrated that the concentration dependence on acetaldehyde UE was, indeed, attributable to local metabolism (Stanek and Morris, 1999).

In both the rat and mouse, nasal UE of styrene was significantly lower at inspired concentrations of 50, 100 or 200 ppm than at 5 or 10 ppm. The concentration dependence of styrene UE was abolished by metyrapone. In fact, metyrapone diminished UE at 5 or 10 ppm to the level observed at 100 or 200 ppm in the naive animals. These results provide strong evidence that the concentration dependence attributable to local metabolism. These results suggest that at inspired concentrations of 50 ppm or more nasal tissue concentrations are sufficiently great that metabolism is no longer first order. Data on nasal styrene metabolism are needed to comprehensively evaluate the precise kinetics of the concentrations dependence.

It is not likely that the inhibitory effects of metyrapone on styrene UE were due to some unanticipated side effects. First, were metyrapone to induce non-specific changes in UE then diminished UE would be anticipated at all exposure concentrations. This was not observed, metyrapone diminished UE only at low inspired concentrations. It is at low concentrations that the enhancing effects of local metabolism would be expected to be fully manifested. Second, and more importantly, metyrapone is without effect on nasal UE of acetone, a vapor which is not significantly metabolized by nasal tissue CYP450 (Morris, 1993).

When measured by the technique used in the current study, nasal UE of most vapors rapidly attains a steady state which is maintained for prolonged periods (Morris, 1994, 1996;1999; Medinsky et al 1999). This behavior was observed for styrene in naive and metyrapone-pretreated rats. Steady state UE was not observed in naive (non-pretreated) mice but was observed in metyrapone-pretreated mice, suggesting a CYP450 metabolite is responsible for the non-steady state behavior in this species.

The mechanism(s) responsible for the non-steady state behavior are not known. Under steady state condition the rate at which vapor is removed from the airstream is exactly balanced by the rate at which it is removed from the air:tissue interface by diffusion and clearance by metabolism and/or the bloodstream (Morris, 1994; 1999). The continual decline in UE may be due to several factors including a continual decline in metabolism rate, a continual decrease in the perfusion rate and/or a continual thickening of the air blood barrier. Perhaps a mouse specific CYP450 metabolic product inhibits metabolism thus diminishing UE as the exposure progressed. Hynes et al. (1999) has noted differences between the rat and mouse in pulmonary metabolism of styrene. However, the non-steady state behavior, as quantitated by the change in UE (%/min), was similar at all exposure concentrations of styrene (5-200 ppm), and UE is not dependent on metabolism at the high exposure concentrations (Table 2) making this possibility seem unlikely. Acrolein vapor also demonstrates non-steady state UE and moreover, co-exposure to acrolein induces non-steady state UE behavior for other vapors including acetone and acetaldehyde (Morris, 1996, 1997). Recent studies have suggested acrolein induces this response via stimulation of nasal sensory nerves and release of substance P and induction of a neurogenic edema (Morris et al, 1999). Perhaps a styrene metabolite initiates a similar sensory neuronal response in the mouse although the lowest reported RD50 in mice (157 ppm, Alarie, 1973) is considerably higher than the lowest concentration which exhibited non-steady state behavior (5 ppm) in the current study.

The non-steady state UE behavior observed in the mouse but not the rat may be reflective of physiological/toxicological response differences between species or may reflect a proportionately greater metabolism rate and/or the formation of a different metabolite in the mouse. The mouse is more sensitive than the rat to styrene-induced olfactory injury (Cruzan et al., 1997). Because the kinetics of styrene UE differed in the rat and

mouse (steady state vs non-steady state) it is not possible to directly compare the UE data between these species to assess species differences in metabolism. However, it does appear that metyrapone has a much greater effect on UE in the mouse than rat, reducing UE by ~20% at 5 ppm in the mouse (Table 2) compared to only ~10% at 5 ppm in the rat (Table 3). This greater effect in mice, if real, may be due to a greater metabolic capacity in the mouse than rat.

The distribution of enzymatic activity throughout nasal tissues is important in influencing inspired vapor metabolism (Morris et al., 1993). In the rat only ~10% of the inspired airstream passes through the olfactory-line ethmoturbinates (Kimbell et al., 1993). Thus, regardless of the amount of enzyme expressed in this area of the nose, metabolic clearance in the olfactory mucosa alone can not exceed ~10% of the inspired burden. Presumably regional airflow patterns are similar in the mouse, however, we are not aware of any direct information in this regard. A proportionately greater styrene metabolic capacity in the mouse than rat may, therefore, reflect a proportionately greater expression of total enzymatic activity and/or a differing anatomical distribution of that activity. Further studies are needed to clarify these possibilities.

In summary, the current study revealed styrene vapor was scrubbed from the airstream in the nasal passages of the rat and mouse with moderate efficiency. In both species UE was inhibited by treatment with CYP450 inhibitor metyrapone providing strong evidence that styrene is metabolized in nasal tissues *in situ* and that this process serves to enhance UE. In both species UE was concentration dependent with diminished UE efficiencies being observed at inspired concentrations exceeding 50 ppm. The concentration dependence was abolished by pretreatment with metyrapone suggesting it is due to metabolic saturation and/or capacity limitation at high inspired concentrations.

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Mortality from nonmalignant diseases of the respiratory, genitourinary and nervous systems among workers exposed to styrene in the reinforced plastics and composites industry in the United States

Otto Wong and Lisa S Trent

ABSTRACT

Objectives

Mortality from diseases of the nervous system and nonmalignant diseases of the respiratory and genitourinary systems was examined for workers exposed to styrene.

Methods

Altogether 15 826 styrene-exposed workers in 30 plants in the reinforced plastics and composites industry were included. Vital status was ascertained through 31 December 1989. Individual exposure estimates were developed based on job functions, existing industrial hygiene data, process changes, engineering controls, work practices, and the use of personal protective equipment. Analyses were based on cause-specific standardized mortality ratios (SMR) and the Cox proportional hazards model. Mortality data were analyzed by latency, duration of exposure, average exposure, cumulative exposure, and process category.

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Mortality from nonmalignant genitourinary diseases, nonmalignant respiratory diseases, and diseases of the nervous system among 15 826 US workers exposed to styrene in the reinforced plastics and composites industry was examined in our investigation. We found no relationship between styrene exposure and any of these causes of death.

Results

For diseases of the nervous system, the SMR was 0.56 [95% confidence interval (95% CI) 0.31—0.95]. Mortality from nonmalignant genitourinary diseases was not increased (SMR 0.87, 95% CI 0.46—1.50). Latency, duration of exposure, average exposure, cumulative exposure, and process category showed no association between styrene exposure and these 2 types of disease. A small increase in mortality from nonmalignant respiratory diseases was found (SMR 1.21, 95% CI 0.98—1.47), mainly due to "other nonmalignant respiratory diseases" (SMR 1.40, 95% CI 1.04—1.84). The highest increase occurred for short exposure duration (SMR 1.79 for <1 year's exposure) or low exposure (SMR 2.15 for <10 ppm-years); there were no increased risks in the high exposure categories. The Cox proportional hazard model revealed no association between styrene exposure and the diseases.

Conclusions

No relationship was found between mortality from any of the diseases examined and any of the styrene exposure indices. The findings were compared with those reported in a European study of styrene-exposed workers.

KEY TERMS
cohort study, epidemiology, occupational health.

INTRODUCTION

Currently there are 2 large-scale mortality cohort studies of workers exposed to styrene, the European study conducted by the International Agency for Research on Cancer (IARC) and its collaborators and the United States (US) study by Applied Health Sciences. The IARC study was based on a multicentric investigation consisting of several cohorts from Denmark, Finland, Italy, Norway, Sweden, and the United Kingdom (1, 2). It consisted of 40 683 workers in the reinforced plastics industry in these 6 European countries. According to the investigators, no excess was observed for mortality from all causes, all cancers, lung cancer, or other major epithelial cancers (1, 2). Mortality from neoplasms of the lymphatic and hematopoietic tissues was also not elevated. In the 1993 or 1994 report (1, 2), Kogevinas et al reported no increased mortality from mental disorders [standardized mortality ratio (SMR) 1.07], diseases of the nervous system (SMR 0.79), diseases of the respiratory system (SMR 0.81), or diseases of the genitourinary system (SMR 0.97).

In 1996, 3 papers based on the IARC study were published by Welp et al (3—5), reporting mortality analyses from nonmalignant respiratory diseases, nonmalignant genitourinary diseases, diseases of the nervous system, and mental disorders. For nonmalignant respiratory diseases, Welp et al (3) drew the following conclusion: "Mortality from pneumonia was associated with intensity of exposure to styrene, but this may have been due to chance. Mortality from bronchitis, emphysema, and asthma was not associated with styrene exposure [p 499]". With regard to nonmalignant genitourinary diseases, it was concluded that mortality "increased as the average intensity of exposure increased [p 226]" (4). The authors also commented that "This finding indicates that other data should be scrutinized [p 223]." Finally, Welp and her colleagues (5) reported that "mortality from diseases of the central nervous system increased with time since first exposure, duration of exposure, average level of exposure, and cumulative exposure to styrene [p 623]." The increase in mortality from the central nervous system was primarily influenced by the increased mortality from epilepsy. Welp et al stated that "mortality from epilepsy increased monotonically with all styrene exposure indicators, while associations for degenerative diseases of the central nervous system were generally weaker [p 623]". The authors concluded that "These findings suggest that, in addition to the known acute effects, exposure to styrene may contribute to chronic diseases of the central nervous system [p 623]".

The other large-scale mortality study was based on workers in the United States (6). Mortality of a cohort of 15 826 male and female workers exposed to styrene at 30 participating plants in the reinforced plastics and composites industry in the United States has recently been updated and reported by Wong et al (7). The primary analyses were conducted using the University of Pittsburgh OCMAP program, which provided a standard set of causes of death. Except for nonmalignant respiratory diseases, causes of death discussed in the 1996 papers by Welp et al were not part of the standard OCMAP analysis, and, therefore, not presented for the US cohort in the Wong et al (7) report. The present report summarizes new analyses for mortality from diseases of the nervous system, nonmalignant diseases of the respiratory system, and nonmalignant diseases of the genitourinary system in the US cohort.

SUBJECTS AND METHODS

The cohort consisted of 15 826 male and female employees at 30 reinforced plastics plants in the United States. To be included in the cohort, an employee must have worked in areas with styrene exposure for a minimum of 6 months between 1 January 1948 and 31 December 1977. The vital status of the cohort was ascertained through 31 December 1989. Sources of vital status information included personnel records maintained at the participating plants, the Death Master File of the Social Security Administration, the National Death Index of the National Center for Health Statistics, and the data base of a commercial retail credit bureau. With the use of information from these sources, death certificates were obtained from individual state health departments. Causes of death were coded according to the revision of the International Classification of Diseases (ICD) in effect at the time of death.

Person-years of observation started after 6 months of exposure to styrene and ended on the date of death or 31 December 1989, whichever was earlier. For those lost to follow-up (unknown vital status), person-years were counted up to the last date of contact. Person-years were classified by age (5-year groups), gender, and calendar years (5-year groups). Expected deaths were based on US national age- gender- cause- race- and year-specific death rates, and cause-specific standardized mortality ratios

(SMR) were computed using the University of Pittsburgh OCMAP computer program. Since mortality rates for the causes of death included in our report are not part of the regular mortality rates in the OCMAP program, the rates were requested from the University of Pittsburgh. Because race information was missing from employment records for most of the cohort, the entire cohort was assumed to be white (7).

The Cox proportional hazards model was also used in the analysis. One important advantage of the Cox model was that the actual exposure data could be used instead of grouped data. An additional advantage was that only internal cohort data were used, whereas SMR values were based on comparisons with an external population. The actual computation was performed using SAS PHREG software. The independent variables used in these models included age, gender, duration of exposure, average exposure, and cumulative exposure.

The 30 reinforced plastics plants in the study manufactured various products, including sheet molding compounds, bulk molding compounds, tanks, pipes, ducts, boats, panels, auto-parts, trays, and small miscellaneous parts. The first year of styrene exposure at the individual plants ranged from 1948 to 1968. At the time of the original epidemiologic study, a parallel exposure assessment investigation was conducted (6, 7).

The exposure classification scheme used in the study was developed in several stages. First, for each plant, a list of job titles was generated based on employment records collected in the study. This list consisted of job or department (or location) titles or both as they appeared in the personnel records. The initial list consisted of a large number of entries, since the same job or department title could have been recorded slightly differently over the years. With assistance from the participating plants, these duplications were consolidated.

The consolidated list (by plant) was provided to the industrial hygiene team for exposure assessment. Individual plants were visited by a field survey team, and a detailed industrial hygiene assessment was conducted at each plant to measure current (around 1980) exposure levels of styrene and other substances. Information on job functions, work practices, past industrial hygiene measurements, process changes and modifications, engineering controls, and personal protection equipment at each plant was also obtained. Using the information collected, the industrial hygienists developed a list of 19 job categories with homogeneous exposures. Each job title was assigned to 1 of the 19 job categories.

A job-exposure matrix was developed for each plant, and a current 8-hour time-weighted-average (8-hour TWA) and an exposure range were assigned to each job category. Based on information on changes over time, historical TWA values were likewise estimated. Job categories in which typical TWA estimates for styrene were low included finish and assembly (5 ppm), storage and shipping (5 ppm), office and other nonproduction (2 ppm), injection molding (4 ppm), field service (5 ppm), preform production (7 ppm), and pultrusion (5 ppm). Job categories with moderate TWA values (20—45 ppm) included molding compound production, gel coating, and winding. A typical styrene exposure in the spray-up or lay-up process category was 60 ppm, with a range of 5 to 120 ppm that reflects considerable differences among facilities and the nature of specific work activities. In terms of job titles, on the average, laminators were exposed to the highest levels (8-hour TWA of 80 ppm). Overall, the average 8-hour TWA values for the majority of jobs was 10 ppm or less.

Two quantitative styrene exposure indices were developed. Based on the employment history of each cohort member (through the end of 1977) and the exposure estimates derived from the job-exposure matrix for that particular plant, a TWA was assigned to each job in a worker's employment history. A cumulative exposure in ppm-years, calculated as the sum of the products of the TWA and duration of exposure of each job, was developed for each cohort member. In order to compare the results to those in the IARC study, an average exposure in parts per million (cumulative exposure divided by duration of exposure) was also calculated for each cohort member.

In addition, based on a consideration of both exposure estimates and processes and job activities, 6 process categories were created, each with a distinct and relatively homogeneous exposure profile to styrene in combination with other chemicals. The 6 process categories are as follows, along with examples of their component job categories: (i) open-mold process (examples: spray-up/lay-up, winding, gel coating, stringing and fitting, laminating), (ii) mixing and closed-mold process (examples: cutting, weighing, pressing, mixing, pultrusion, inject molding, casting), (iii) finish and assembly (examples: finishing, storing and shipping, repairing), (iv) plant office and support (examples: general and nonproduction, quality control, office and others), (v) maintenance and preparation (examples: maintenance, utility, mold preparation), and (vi) supervisory and professional (examples: supervisors' and engineers' tasks).

TABLE 1

Descriptive statistics of the cohort of workers exposed to styrene in the reinforced plastics and composites industry.

	Workers	
Description	N	%
Total cohort	15 826	100.0
Men	11 958	75.6
Women	3868	24.4
Duration of employment (years) as of 31 December 1977		
0.5–0.9	3712	23.5
1.0–1.9	3528	22.3
2.0–4.9	4326	27.3
Å5.0	4260	26.9
Cumulative exposure (ppm-years) as of 31 December 1977		
<10.0	3778	23.9
10.0–29.9	4119	26.0
30.0–99.9	4210	26.6
Å100.0	3719	23.5
Average intensity of exposure (ppm) from hire to 31 December 1977		
<5.0	2970	18.8
5.0–9.9	3702	23.4
10.0–19.9	3736	23.6
20.0–59.9	3161	19.9
Å60.0	2257	14.3
Vital status as of 31 December 1989		
Alive	13 651	86.2
Dead	1628	10.3
With death certificates	1586	97.4
Without death certificates	42	2.6
Unknown	547	3.5

Table 1 summarizes the descriptive statistics of the cohort. In terms of follow-up, the vital status of only 547 cohort members (3.5%) remained unknown at the end of 1989. Of the 1628 workers identified to have died, death certificates were obtained for all but 42 (2.56%). These 42 deaths were included in the overall SMR calculations, which have been reported previously (7), but not in the cause-specific SMR calculations. In terms of exposure, close to one-quarter of the cohort (23.5%) had more than 100 ppm-years of cumulative exposure, and one-third (34.2%) had an average exposure of 20 ppm or higher. It should be pointed out that the employment histories were not updated after 1977, the closing date of the original study. Therefore, all work and exposure assignments were truncated in 1977.

TABLE 2

Observed deaths (O) in the entire cohort and the standardized mortality ratios (SMR) with their 95% confidence intervals (95% CI) for selected causes by time since first exposure to styrene.

Cause of death	<10 years		10–19 years		20+ years		Total	
	O	SMR 95% CI	O	SMR 95% CI	O	SMR 95% CI	O	SMR 95% CI
Diseases of the nervous system	1	0.10 0.00–10.57	6	0.68 0.25–1.49	7	1.13 0.45–2.33	14	0.56 0.31–0.95
Epilepsy	—	0	.1	1.00 0.02–5.59	1	2.84 0.07–15.84	2	0.73 0.09–2.66
Nonmalignant respiratory diseases	11	0.65 0.32–1.17	40	1.27 0.90–1.73	46	1.44 1.05–1.92	97	1.21 0.98–1.47
Pneumonia	3	0.44 0.09–1.31	8	0.84 0.36–1.65	12	1.38 0.71–2.41	23	0.92 0.58–1.38
Bronchitis, emphysema and asthma	6	1.26 0.46–2.75	7	0.96 0.38–1.98	10	1.65 0.79–3.03	23	1.27 0.80–1.91
Other nonmalignant respiratory diseases	2	0.39 0.04–1.44	25	1.74 1.13–2.57	24	1.41 0.90–2.10	51	1.40 1.04–1.84
Nonmalignant genitourinary diseases	1	0.23 0.01–1.30	8	1.45 0.62–2.86	4	0.79 0.21–2.03	13	0.87 0.46–1.50
Nephritis	—	0	.2	1.70 0.20–6.17	—	0	2	0.54 0.06–1.96

RESULTS

Table 2 shows the observed deaths, SMR values, and 95% confidence intervals (95% CI) for selected causes of death for the entire cohort and by latency (time since first exposure). For diseases of the nervous system, there were 14 deaths in the entire cohort, significantly fewer than the 24.7 expected. The corresponding SMR was 0.56 (95% CI 0.31—0.95). Only 2 deaths in the cohort were attributed to epilepsy, comparable to the 2.7 expected (SMR 0.73, 95% CI 0.09—2.66). For diseases of the genitourinary system, there were 13 observed deaths, comparable to the 14.8 expected (SMR 0.87, 95% CI 0.46—1.50). There were only 2 deaths from nephritis, slightly fewer than the 3.7 expected (SMR 0.54, 95% CI 0.06—1.96). Altogether 97 deaths were coded as nonmalignant respiratory diseases, more than the expected 80.2. However, the increase was not statistically significant (SMR 1.21, 95% CI 0.98—1.47). Within the broad category of nonmalignant respiratory diseases, 23 deaths were from pneumonia, comparable to the 24.9 expected (SMR 0.92, 95% CI 0.58—1.38). For the subcategory "bronchitis, emphysema and asthma," there were 23 deaths, slightly more than the 18.1 expected (SMR 1.27, 95% CI 0.80—1.91). There was a statistically significant increase in mortality for the subcategory "other nonmalignant respiratory diseases." A total of 51 deaths in the subcategory were observed, compared with 36.3 expected (SMR 1.40, 95% CI 1.04–1.84). Thirty-six of these deaths (71%) were from "chronic airway obstruction, not otherwise specified" (9th ICD 496), which included "chronic nonspecific lung disease," "chronic obstructive lung disease," and "chronic obstructive pulmonary disease, not otherwise specified."

Although not shown in table 2, an analysis was also

TABLE 3

Observed deaths (O) in the entire cohort and the standardized mortality ratios (SMR) with their 95% confidence intervals (95% CI) for selected causes by duration of exposure to styrene.

Cause of death	<1.0 year		1.0-1.9 years		2.0-4.9 years		5.0-9.9 years		10.0+ years	
	O	SMR 95% CI	O	SMR 95% CI	O	SMR 95% CI	O	SMR 95% CI	O	SMR 95% CI
Diseases of the nervous system	1	0.18 0.01-1.05	4	0.82 0.22-2.11	3	0.47 0.09-1.38	2	0.46 0.05-1.68	4	1.03 0.28-2.64
Epilepsy	1	1.36 0.03-7.60	0	—	0	—	1	2.59 0.06-14.46	0	—
Nonmalignant respiratory diseases	20	1.41 0.86-2.18	17	1.25 0.73-2.01	22	1.18 0.74-1.79	20	1.29 0.79-2.00	18	0.97 0.57-1.54
Pneumonia	3	0.62 00.12-1.83	6	1.36 0.50-2.97	6	0.98 0.36-2.14	3	0.65 0.13-1.92	5	0.98 0.32-2.3
Bronchitis, emphysema and asthma	6	1.95 0.71-4.26	3	0.99 0.20-2.90	4	0.94 0.25-2.41	6	1.68 0.61-3.66	4	0.96 0.26-2.46
Other nonmalignant respiratory diseases	11	1.79 0.89-3.21	8	1.34 0.58-2.65	12	1.49 0.77-2.61	11	1.54 0.77-2.76	9	0.99 0.45-1.88
Nonmalignant genitourinary diseases	1	0.34 0.01-1.93	0	—	3	0.82 0.17-2.41	3	1.10 0.22-3.22	6	2.04 0.74-4.44
Nephritis	0	—	0	—	1	1.02 0.02-5.73	0	—	1	1.94 0.04-10.82

performed separately by gender. All 14 deaths from diseases of the nervous system in the cohort occurred among the male workers, compared with 19.5 expected (SMR 0.71, 95% CI 0.39—1.20). Among the women, no death was attributed to diseases of the nervous system, whereas 5.1 were expected. Another cause of death for which the 2 genders differed was nonmalignant respiratory diseases. For the men, the SMR for nonmalignant respiratory diseases was 1.24 (84 observed, 95% CI 0.98—1.53), whereas that for the women was 1.04 (13 observed, 95% CI 0.55—1.78). For other causes of death, mortality was similar between the 2 genders.

Mortality analysis by latency (time since first exposure to styrene) is also presented in table 2. The increase in mortality from nonmalignant respiratory diseases was the most prominent 20 years after the first exposure (SMR 1.44, 95% CI 1.05—1.92), whereas there was no increase within the first 10 years (SMR 0.65, 95% CI 0.32—1.17). In the group with a latency of 10—19 years, the SMR for nonmalignant respiratory diseases was 1.27 (95% CI 0.90—1.73).

Table 3 depicts mortality by duration of exposure. Short-term workers with less than 1 year of exposure had the highest mortality from nonmalignant respiratory diseases (SMR 1.41, 95% CI 0.86—2.18), whereas for those with at least 10 years of exposure there was no increase (SMR 0.97, 95% CI 0.57—1.54). No significant increase was observed for any specific causes of death in any of the groups by duration of exposure. No pattern by length of exposure was evident for any specific causes of death in table 3. In particular, among those with 10 or more years of exposure, no increased mortality was seen with

TABLE 4

Observed deaths (O) in the entire cohort and the standardized mortality ratios (SMR) with their 95% confidence intervals (95% CI) for selected causes by cumulative exposure to styrene.

Cause of death	<10.0 ppm-years		10.0-29.9 ppm-years		30.0-99.9 ppm-years		Å100.0 ppm-years	
	O	SMR 95% CI	O	SMR 95% CI	O	SMR 95% CI	O	SMR 95% CI
Diseases of the nervous system	3	0.49 0.10-1.45	2	0.32 0.04-1.18	6	0.87 0.32-1.90	3	0.53 0.10-1.54
Epilepsy	1 0.03-7.96	1.42	0	—	1 0.03-7.7	1.38	1	0
Nonmalignant respiratory diseases	28	1.64 1.09-2.37	17	0.94 0.54-1.51	29	1.25 0.84-1.80	23	1.04 0.66-1.57
Pneumonia	4	0.72 0.19-1.84	7	1.18 0.47-2.44	9	1.26 0.57-2.40	3	0.47 0.09-1.38
Bronchitis, emphysema and asthma	8	2.07 0.89-4.08	5	1.24 0.40-2.89	4	0.76 0.20-1.95	6	1.22 0.44-2.65
Other nonmalignant respiratory diseases	16	2.15 1.23-3.49	5	0.63 0.20-1.48	16	1.52 0.87-2.47	14	1.33 0.72-2.23
Nonmalignant genitourinarydiseases	1	0.30 0.01-1.67	4	1.13 0.30-2.90	3	0.71 0.14-2.07	5	1.34 0.43-3.13
Nephritis	—	0	1	1.05 0.02-5.88	1	0.97 0.02-5.45	—	0

the exception of nonmalignant genitourinary diseases. Six such deaths were observed among those with 10 or more years of exposure (SMR 2.04, 95% CI 0.74—4.44). However, the increase was not statistically significant. A similar analysis based on length of employment was also performed (not shown). The results were very similar, indicating that length of employment and length of exposure were the same or very similar for most of the cohort members.

The results of the analysis by cumulative exposure are shown in table 4. For mortality from diseases of the nervous system, no increase or pattern was seen with regard to cumulative exposure. For mortality from nonmalignant respiratory diseases, a significant increase was observed among those with less than 10.0 ppm-years of exposure (SMR 1.64, 95% CI 1.09—2.37), whereas no statistically significant increase was evident among those with more than 100.0 ppm-years of exposure (SMR 1.04, 95% CI 0.66—1.57). The increased mortality from nonmalignant respiratory diseases among those with a cumulative exposure of less than 10.0 ppm-years came from the 2 subcategories "bronchitis, emphysema and asthma" (SMR 2.07, 95% CI 0.89—4.08) and "other nonmalignant respiratory diseases" (SMR 2.15, 95% CI 1.23—3.49). Only the latter was statistically significant. There was a nonsignificant increase in mortality from nonmalignant genitourinary diseases among the workers with Å100 ppm-years of exposure (5 observed, 3.7 expected, SMR 1.34, 95% CI 0.43—3.13).

Table 5 shows the mortality analysis by average intensity of styrene exposure. The category nonmalignant respiratory diseases was significantly elevated among work-

ers in the lowest average exposure group (<5.0 ppm). The increase came from "bronchitis, emphysema and asthma" (SMR 2.24, 95% CI 1.07—4.12) and from "other nonmalignant respiratory diseases" (SMR 1.81, 95% CI 1.03—2.94). There was no significant increase in mortality from any of the causes of death examined in any higher categories of average intensity of styrene exposure. Altogether 2257 workers were exposed to an average intensity of more than 60 ppm throughout their employment in the reinforced plastics and composites industry. In this group of workers, there was only 1 death from diseases of the nervous system (SMR 0.50), and no death from epilepsy. In the same group, for nonmalignant respiratory diseases, the SMR was 1.10 (95% CI 0.40—2.39), and that for "other nonmalignant respiratory diseases" was 1.64 (95% CI 0.44—4.20). For nonmalignant genitourinary diseases, the SMR was 0.94 (95% CI 0.02—5.26).

The mortality analysis by major industrial processing categories is presented in table 6. A worker was classified into a specific processing category if he or she spent at least 2 years in that category. Therefore, a worker could be classified into more than 1 category. No significant increase in mortality from any cause of death included in table 6 was found for any of the industrial processing categories.

In addition to the indirect method of standardization (SMR values), the data were also analyzed using the Cox proportional hazards model (tables 7 and 8). The following 4 causes of death were selected for this analysis: diseases of the nervous system, nonmalignant genitourinary

TABLE 5

Observed deaths (O) in the entire cohort and the standardized mortality ratios (SMR) with their 95% confidence intervals (95% CI) for selected causes by average exposure to styrene.

Cause of death	< 5.0 ppm		5.0—9.9 ppm		10.0—19.9 ppm		20.0—59.9 ppm		Å60.0 ppm	
	O	SMR 95% CI	O	SMR 95% CI	O	SMR 95% CI	O	SMR 95% CI	O	SMR 95% CI
Diseases of the nervous system	2	0.35 0.04–1.26	4	0.61 0.16–1.57	5	0.79 0.25–1.84	2	0.48 0.05—1.74	1	0.50 0.01—2.81
Epilepsy	0	—	0	—	2	2.95 0.35—10.68	0	—	0	—
Nonmalignant respiratory diseases	33	1.69 1.16—2.37	16	0.75 0.43—1.23	26	1.26 0.82—1.85	16	1.17 0.67—1.91	6	1.10 0.40—2.39
Pneumonia	7	1.16 0.46—2.39	4	0.61 0.16—1.51	6	0.95 0.35—2.08	4	0.93 0.25—2.38	2	1.10 0.13—4.00
Bronchitis, emphysema and asthma	10	2.24 1.07—4.12	3	0.62 0.12—1.82	4	0.85 0.23—2.18	6	2.03 0.74—4.41	0	— D
Other nonmalignant respiratory diseases	16	1.81 1.03—2.94	9	0.94 0.43—1.79	16	1.71 0.98—2.78	6	0.97 0.35—2.11	4	1.64 0.44—4.20
Nonmalignant genitourinary diseases	2	0.57 0.06—2.05	5	1.27 0.41—2.97	4	1.05 0.28—2.70	1	0.39 0.01—2.20	1	0.94 0.02—5.26
Nephritis	0	—	2	1.97 0.23—7.14	0	—	0	—	0	—

TABLE 6

Observed deaths (O) and the standardized mortality ratios (SMR), with their 95% confidence intervals (95% CI) for selected causes by major processing categories.[a]

Cause of death	Open mold process (N=1386)		Mixing and closed mold process (N=1225)		Finish and assemble (N=2273)		Plant office and support (N=1474)		Maintenance and preparation (N=1192)		Supervisory and professional (N=507)	
	O	SMR 95% CI	O	SMR 95% CI	O	SMR 95% CI	O	SMR 95% CI	O	SMR 95% CI	O	SMR 95% CI
Diseases of the nervous system	0	—	0	—	2	0.50 0.06-1.80	3	1.02 0.21-2.99	4	1.07 0.29-2.75	0	—
Epilepsy	0	—	0	—	0	—	1	3.60 0.09-20.05	0	0	0	—
Non-malignant respiratory diseases	7	1.21 0.31-2.26	7	1.32 0.48-2.50	15	0.96 0.53-2.72	11	1.06 0.53-1.58	18	1.16 0.53-1.90	5	0.97 0.69-1.84
Pneumonia	0	—	1	0.61 0.15-4.44	6	1.33 0.01-3.44	4	1.31 0.48-2.90	4	0.88 0.35-3.35	2	1.23 0.24-2.27
Bronchitis, emphysema and asthma	3	2.38 0.02-4.72	3	2.61 0.49-6.96	4	1.13 0.54-7.63	1	0.41 0.48-2.90	4	1.10 0.01-2.28	1	0.85 0.30-2.81
Other non-malignant respiratory diseases	4	1.47 0.10-3.10	3	1.22 0.40-3.76	5	0.67 0.25-3.57	6	1.26 0.21-1.56	10	1.40 0.46-2.75	2	0.86 0.67-2.57
Non-malignant genitourinary diseases	1	0.99 0.02-5.55	1	0.95 0.02-5.30	4	1.47 0.40-3.78	4	2.20 0.60-5.63	2	0.77 0.09-2.81	0	—
Nephritis	0	—	0	—	1	1.82 0.04-10.14	0	0	1	1.85 0.04-10.34	0	—

[a] Workers could be included in more than one category.

diseases, nonmalignant respiratory diseases, and pneumonia. The independent variables included in the models in table 7 consisted of age, gender, and cumulative exposure. For diseases of the nervous system, gender was not included in the model because all 14 deaths from this cause occurred among the male workers. As indicated in table 7, cumulative exposure to styrene was not associated with an increased risk of mortality from any of the diseases examined. The models in table 8 included 2 additional exposure indices as independent variables: duration of exposure and average intensity of exposure. No significant increase in mortality from any of the 4 causes of death was seen in relation to any of the 3 styrene exposure indices: duration, average intensity, or cumulative exposure.

DISCUSSION

This report is based on data from a previous study of workers in the reinforced plastics and composites industry in the United States (6, 7). The analysis presented in the report was stimulated by the recent papers of Welp et al (3—5), which reported mortality from diseases of the nervous system, nonmalignant respiratory diseases, and nonmalignant genitourinary diseases, as well as some of the subcategories within these 3 broad categories, in a study of workers exposed to styrene in Europe. Most of these diseases have not been reported previously in the US cohort.

For the broad category of nonmalignant respiratory diseases, Welp and her colleagues (3) found a significant deficit for the IARC study overall (SMR 0.81, 95% CI 0.67—0.96). However, they (3) found a positive trend by average intensity for mortality from pneumonia (P<0.01) and a significantly elevated risk of 6.10 (95% CI 1.44—25.8) for an average intensity of >200 ppm.

In the US cohort, we found a nonsignificant increase in mortality from nonmalignant respiratory diseases (SMR 1.21, 95% CI 0.98—1.47) (table 2). The increase was due primarily to a significant increase in "other nonmalignant respiratory diseases" (SMR 1.40, 95% CI 1.04—1.84). When mortality from "other nonmalignant respiratory diseases" was examined by various indices of styrene exposure, it was found that the increase occurred among workers with a short length of exposure, low average exposure, or low cumulative exposure. As discussed in our previous reports (6, 7), the increase was probably not related to exposure to styrene, but more likely to the low socioeconomic class, smoking, or life-style factors charac-

TABLE 7

Analysis of selected causes of death based on the Cox proportional hazards model, with age, gender, and cumulative exposure as independent variables.

Cause of death (N)	Independent variable	b-coefficient	SD	P-value
Diseases of the nervous system	Age (years)	0.019	0.024	0.41
(N=14)	Cumulative exposure (ppm-years)	-0.001	0.001	0.75
Nonmalignant genitourinary diseases	Age (years)	0.028	0.023	0.24
(N=13)	Gender	-0.193	0.660	0.76
	Cumulative exposure (ppm-years)	0.000	0.001	0.94
Nonmalignant respiratory diseases	Age (years)	0.084	0.008	0.00
(N=97)	Gender	-0.954	0.298	0.00
	Cumulative exposure (ppm-years)	-0.001	0.001	0.14
Pneumonia	Age (years)	0.078	0.017	0.00
(N=23)	Gender	-0.644	0.551	0.24
	Cumulative exposure (ppm-years)	-0.004	0.002	0.14

teristic of short-term workers. The observation that most of the deaths (70%) from "other nonmalignant respiratory diseases" were chronic obstructive pulmonary diseases tended to confirm the role of cigarette smoking.

For pneumonia, specifically, in the US cohort we did not find any relationship with any of the exposure indices. The pneumonia SMR values were 0.98 and 0.47 for workers with Å10.0 years of exposure and Å100.0 ppm-years of cumulative exposure, respectively. In terms of the average intensity of exposure, the SMR values were 1.16, 0.61, 0.95, 0.93, and 1.10 for <5, 5.0—9.9, 10.0—19.9, 20.0—59.9, and Å60.0 ppm, respectively. None of the SMR values were significantly elevated, and there was no upward trend. Thus the pneumonia finding from the IARC study could not be confirmed in the US study.

Similarly, in the IARC study, Welp and her colleagues (4) reported that mortality from nonmalignant diseases of the genitourinary system increased with increasing average intensity of exposure. In the US cohort, there were only 13 deaths due to nonmalignant genitourinary diseases, slightly fewer than the 14.8 expected. When the data were examined by duration of exposure, cumulative exposure, or average intensity of exposure, no significantly elevated SMR values or upward trends were found. Therefore, the results of nonmalignant genitourinary diseases from the US cohort were not consistent with the findings reported by Welp et al (4) in the IARC study.

Of the 3 categories of diseases reported by Welp et al in 1996, it appears that these authors considered the finding of diseases of the nervous system much more definitive than the other 2 categories. They (5) stated that "Mortality from diseases of the central nervous system (27 deaths) increased with time since first exposure, duration of exposure, average level of exposure, and cumulative exposure to styrene [p 623]". However, the statistical tests conducted by Welp and her co-workers indicated that there was no significant upward trend for time since first exposure (P_{trend}=0.32) or average exposure (P_{trend}=0.37). Welp and her colleagues (5) also concluded that "Mortality from epilepsy increased monotonically with all styrene exposure indicators [p 623]". Again, the trend tests reported (5) indicated that there were no significant trends for average exposure (P_{trend}=0.32) or cumulative exposure (P_{trend}=0.07). On the other hand, the trend between mortality from epilepsy and time since first exposure was highly significant (P_{trend}=0.008), due to an extremely high risk ratio of 485.9 (95% CI 1.19—9,999) based on only 1 observed death for the category "20 or more years after first exposure". Thus, except for epilepsy and time since first exposure, there was no significant upward trend in the exposure-response analyses in the IARC study.

In the US cohort there was no increased mortality from diseases of the nervous system in general or from epilepsy in particular. When mortality was analyzed in relation to various indices of exposure to styrene, no pattern was found. For US workers with more than 10 years of styrene exposure, the SMR for diseases of the nervous system was 1.03, and that for epilepsy was 0 (no epilepsy death occurred in the group). Similarly, in the US cohort, among persons with the highest cumulative exposure (Å100.0 ppm-years), the SMR for diseases of the nervous system was 0.53, and that for epilepsy was 0. Thus data from the US cohort did not support the findings on mortality from diseases of the nervous system in the IARC study reported by Welp et al (5).

Thus the results between the IARC study and the US cohort were considerably different for diseases of the nervous system. In fact, the results for diseases of the nervous system were considerably different across countries within the IARC study. An 1994 internal IARC report provided a detailed mortality analysis by country (8). Of the 40 deaths due to central nervous system diseases, 30 were from Denmark (N=13) or the United Kingdom (N=17), with the remaining 10 from Italy (N=3), Finland (N=1), Norway (N=3), and Sweden (N=3). Table 1—22 in part III of the 1994 IARC report (8) shows that the SMR for central nervous system diseases for Denmark was 1.10 (13/11.82) and that for the United Kingdom was 0.52 (17/32.57). The increase in Denmark could not have been explained by higher exposure since exposure levels in the United Kingdom were consistently higher than those in Denmark (figure 1 in reference 5). Furthermore, according to Welp et al (5), for central nervous system diseases "an inconsistently increasing risk by average exposure was observed in Denmark, while there was no increase in the other five countries [p 629]".

Mortality from central nervous system diseases or epilepsy has not been associated with chronic occupational exposures. On the other hand, persons with the following conditions are known to have a high risk of epilepsy: birth trauma (inadequate oxygen supply to the brain), perinatal infection, anoxia (postrespiratory or postcardiac arrest), infectious diseases (meningitis, encephalitis), inherited disorders or degenerative diseases (phenylketonuria or tuberous sclerosis), head injury or trauma, metabolic disorders (hyperglycemia or hypoparathyroidism), and cerebrovascular accident. Unfortunately, none of these potential confounding risk

factors were available for analysis in the IARC study. Alcohol consumption is a known risk factor of central nervous system diseases. The Danish cohort consisted of a disproportionally higher percentage of short-term workers. In the IARC study, 20 of the 28 deaths due to cirrhosis of the liver occurred among subjects with less than 1 year of employment (2). Thus alcohol consumption could have been a potential confounding factor among the Danish workers.

In summary, mortality from nonmalignant genitourinary diseases, nonmalignant respiratory diseases, and diseases of the nervous system among 15 826 US workers exposed to styrene in the reinforced plastics and composites industry was examined in our investigation. We found no relationship between styrene exposure and any of these causes of death. The increased risks reported in the IARC study could not be replicated in our study. Apart from some of the reasons already discussed, there are at least 2 additional possible explanations for the observed discrepancies.

First, exposures among the US workers appeared to be lower than those among their European counterparts. More than 40% of the US workers were exposed to average levels below 10 ppm (table 1). Only 14% were exposed to average levels above 60 ppm. A great majority of the US workers were employed in the reinforced plastics and composites industry for a short period of time (70% for <5 years). Half of the US workers (50%) had cumulative exposures of less than 30 ppm-years. Only 24% of the US workers had cumulative exposures of more than 100 ppm-years. On the contrary, exposures in the IARC study appeared to be much higher. Although the number of workers were not reported, judging by the exposure categories used, it appeared that a considerable number of workers in the IARC study were exposed to average levels above 200 ppm (3, 4) and many accumulated more than 750 ppm-years (5). Thus exposure levels among the US workers might not have been high enough to produce the risks reported in the IARC study.

Second, the numbers of deaths in some of the analyses in the US study were small. For example, there were only 2 deaths each from epilepsy and nephritis. Even for the broader categories "diseases of the nervous system" and "nonmalignant genitourinary diseases," there were 14 and 13 deaths, respectively. The numbers were made even smaller in some of the subcohort analyses stratified by exposure category. The statistical power to detect a modest increase in risk was low in some of the analyses.

Therefore, in comparing the results between the US and the IARC studies, differences in exposure levels and statistical power between the 2 studies should be taken into consideration. Hopefully, future updates of the 2 studies and studies from other locations will provide further insights.

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