Environmental Health Perspectives
Vol. 17, pp. 125–133, 1976

Quantitative Analysis of Styrene Monomer in Polystyrene and Foods Including Some Preliminary Studies of the Uptake and Pharmacodynamics of the Monomer in Rats

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A variety of food containers, drinking cups and cutlery, fabricated from polystyrene (PS) or polystyrene-related plastic, were analyzed for their styrene monomer content. Samples of yogurt, packaged in PS cups, were similarly analyzed and the leaching of styrene monomer from PS containers by some food simulants was also determined.

Blood level studies with rats, dosed with styrene monomer by various routes, illustrated uptake phenomena that were dependent on the dose and route of administration and were also affected by the vehicle used to convey the styrene monomer.

Introduction

Rigid polystyrene (PS) and polystyrene-related plastics which are used as food packaging materials have had a longer history of use than poly(vinyl chloride) (PVC). Some of the physical characteristics of PS, for example, its low impact strength and chemical resistance, have led to the development of other food-use plastics in which styrene is copolymerized with monomers like butadiene and acrylonitrile, to give a flexible rubbery solid. In 1973, the annual production in Canada of styrene-related polymers was over 200 million pounds (*1*) and this was second only to polyethylene types.

The recent concern with the migration of vinyl chloride monomer (VCM) to foodstuffs from rigid poly(vinyl chloride) containers and its subsequent prohibition by the F.D.A. (*2*) has drawn attention to the potential hazards of a large variety of other plastic materials which are used in the food packaging industry. In the assessment of priorities for work with other monomers which are present in plastic food-packaging materials, the factors considered were: physical characteristics of the polymer; chemical structure and reactivity of the monomer; biotransformations of the monomer; existing regulations for monomer content in the polymer; volume of use of the polymer in the food industry; known toxicology of the monomer. In rigid plastics, occluded monomer may be entrapped at high concentrations even when fabrication processes involve thermal elevation and the monomer is extremely volatile like vinyl chloride. Rigid PS food packaging products are very similar to PVC in this respect. Some properties of styrene and VCM, which are given in Table 1, show that the chemical structures of styrene and vinyl chloride are similar, in that the aryl substituent in styrene is replaced by a chlorine atom in VCM. The large difference in boiling points and solubility characteristics (*3, 4*) contribute to the significant differences in analytical methodology and pharmacokinetics of these monomers. Polystyrene was first introduced commercially in 1938, although it was discovered as far back as 1831.

The metabolism of VCM has attracted a good deal of attention recently in view of the postulate that a possible metabolic intermediate, vinyl chloroexpoxide, and not vinyl chloride monomer is the ultimate carcinogen (*5*). The principal urinary metabolites of styrene, which are hippuric acid, mandelic acid, phenyl glycol and benzoic acid (*6, 7*) have also been thought

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Table 1. *Comparison of properties of VCM and styrene.*

Property	VCM	Styrene
Chemical structure		
Molecular weight	62.50	104.14
Boiling point, °C	−13.9	145.2
Solubility	0.27 g in 100 ml of water; about 10 g/100 ml in octanol, vegetable oil, etc.	0.31 g in 100 ml of water, miscible with most organic solvents

to be formed via an epoxide intermediate. Further, the *in vitro* epoxidation of styrene by liver microsoma lenzymes has been demonstrated (*8*).

Existing regulations in the U.S. (*9*) permit up to 1 wt-% (10,000 ppm by weight) of the monomer in food-grade PS, except that when used in contact with fatty foods not more than 0.5 wt-% monomer is allowed. Apparently it is very difficult indeed to remove occluded styrene monomer from the finished plastic. The variety of applications of rigid food grade PS is perhaps greater than that for rigid PVC. Not only is it used in the rigid molded state as food containers (principally dairy products) but is now manufactured in a form suitable for use as reuseable cutlery and, in the foamed state, as drink containers (for hot drinks or alcoholic beverages). It has not been used as a film wrap or as containers for vegetable oils, the reason for the later exclusion being its high permeability to oxygen and the consequent spoilage of the oil.

The acute toxicity of styrene has been well studied (*4*). Liquid styrene is a primary skin irritant causing erythema and blistering. It is extremely irritating to the eyes and mucous membranes and has narcotic properties. In common with other aromatic hydrocarbons, it can cause chemical pneumonitis at the site of contact with pulmonary tissue. At an atmospheric concentration of 800 ppm, human subjects experience immediate eye and throat irritation, drowsiness, depression and weakness. In animals at 5000 ppm (which is close to the saturated vapor pressure of liquid styrene at 20°C), rats experienced coma and death within 1 hr. Very little pathology of any consequence, except for the changes which are usually associated with irritants, has been reported from animal or human studies. There is no published literature on the carcinogenic activity of styrene, although Van Duuren (*10*) has shown that styrene epoxide induces tumors when applied to animal skin. Preliminary tests in our own laboratories have demonstrated that the epoxide is mutagenic but relatively high concentrations (200 μg/ml) are required.

Table 2. Styrene monomer content of polystyrene products.

	Styrene Monomer, ppm												
Code[a]	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Mean	±S.D.	C.V., %
1	2094	2043	1882	1993	1824	1819	1970	2006	2038	1913	1958.3	94.9	4.8
2	475	468	479	451	453	459	526	476	475	480	474.2	21.0	4.4
3	460	575	520	505	515	540	470	547	412	482	502.6	47.6	9.5
4	1509	1447	1499	1519	1518	1543	1551	1622	1575	1615	1540.0	53.5	3.5
5	82	90	69	80	84	99	95	91	95	88	87.2	8.8	10.1
6	107	102	107	114	96	114	112	109	114	101	107.6	6.1	5.7
7	1657	1753	1358	1530	407	417	526	548	632	583	941.0	558.0	59.3
8	3664	3451	3463	3660	3892	3453	3628	3379	3775	3304	3566.6	186.2	5.2
9	1044	1173	1180	1220	1113	1135	1132	1116	1154	1085	1135.1	50.3	4.4
10	1357	1318	1397	1259	1293	1344	1506	1545	1537	1490	1404.5	106.3	7.6

[a] Code for types of containers and utensils: (1) yogurt cups, 125-ml capacity, thin semirigid film; (2) polystyrene foam trays (for meat, fruit and vegetable packs), 20 × 14.5 cm; (3) ABS (acrylonitrile-butadiene-styrene copolymer) containers for margarine, ca. 14.5 × 8.5 × 6.0 cm, contains 400 g or 14.1 oz. of margarine; (4) similar to (3), but polymer originates from a different manufacturer; (5) polystyrene foam containers for "take-out" food, various sizes, data obtained for container of ca. 9.5 cm height × 7.0 cm diameter; (6) polystyrene foam cup for hot drinks, ia. 10 cm height × 6.0 cm diameter; (7) rigid polystyrene fork, cutlery about 4 mm in thickness; (8) rigid clear polystyrene cups (for beer), ca. 11.8 cm height × 8.1 cm diameter; (9) rigid polystyrene fork, cutlery about 1.5 mm thickness; (10) rigid knife, cutlery ca. 1.5 mm thickness.

Experimental and Results

The main thrust of our recent work has been to assess the extent to which styrene is present in food-grade PS material and to what extent it leached into foods or food simulants. Analytical methods, which were suggested to us, involved the dissolution of a weighed sample of the plastic in methylene chloride and reprecipitation of the polymer with methyl alcohol. This method, while useful for high concentrations of styrene monomer, had the disadvantage that the lower limit of detection was about 20 ppm. Many other suitable solvents yielded a similar sensitivity which we attributed to the liability arising from the development of the chromatographic peak for styrene on the trailing edge of the solvent peak. Since styrene monomer is a liquid with a boiling point of 145.2°C and polystyrene is relatively soluble in aromatic hydrocarbons, alternative solvents were examined which would allow the gas chromatographic development of the solvent peak after elution of the styrene monomer. Butylbenzene (bp 182°C) and diethylbenzene (mixture of *ortho, meta,* and *para* isomers, boiling range 175–181°C, Eastman Kodak Company, Rochester, N.Y.) were both satisfactory solvents. Diethylbenzene was selected for this study on account of its low cost ($7.54 for 3 kg as opposed to $1200.00 for a similar quantity of butylbenzene).

Method

A 2-g portion of the polymer was placed in a 15 ml centrifuge tube fitted with a ground glass stopper, and 10 ml of diethylbenzene was added. The flask was stoppered and placed in an Eberbach horizontal shaker. The mixture was agitated until all polymer had dissolved or, as was necessary for the ABS polymer, for precisely 16 hr. All samples dissolved fairly readily (within 1 hr) except for the ABS, sample which merely softened and swelled. The total volume was then measured and a 1-ml sample of the solution was placed in a 15-ml septum vial fitted with a Mininert valve. The septum vial was agitated in a horizontal bed shaker-water bath, which was temperature-controlled at 37°C, for 30 min. Then 250 μl of head space vapor was injected into a Tracor MT 220 dual hydrogen flame chromatograph. The chromatographic conditions were as follows: column, 6 ft × 0.25 in. Carbowax 400 on Porasil F (120/150 mesh); column temperature, 123°C; injection port, 200°C; detector, 250°C; gas flow, air, 110 ml/min; hydrogen, 40 ml/min; carrier gas (N_2), 11 ml/min.

Peak areas were obtained with a Hewlett-Packard 3370B electronic integrator. A calibration curve was constructed from data obtained with solutions containing known weights of styrene monomer in diethylbenzene. The detection limit was about 1 ppm.

Ten different samples from each of ten types of containers, drinking and cutlery utensils, fabricated from food-grade polystyrene were assayed for their styrene monomer content. The results are given in Table 2.

Table 3. Styrene content of container and yogurt contents.

Sample	Days to expiration date[a]	Styrene in container, ppm (w/w)	Styrene in yogurt ppb (w/v)
1	6	541	9.7
2	6	526	5.5
3	6	519	9.2
4	6	580	7.0
5	8	676	13.6
6	8	725	12.6
7	1	646	2.5
8	1	723	34.6
9	11	734	12.2
10	11	647	14.3
Mean		631.7	12.12
S.D.		84.7	8.75
C.V., %		13.4	72.2
A	9	1481	82.8
B	−5	1333	135.5
C	9	1855	151.7

[a] This is properly defined as a "durable life data" and for yogurt is about 14 days.

The yogurt cups (1) were supplied unfilled from a food processing plant. Aluminum covers were in place on all samples. A sample of air taken directly from these cups gave styrene vapor concentrations of 4 ppm.

The Styrofoam products, (2), (5), and (6), gave peaks which were eluted prior to styrene and these are probably associated with the blowing agents used in their manufacture. The

Table 4. Leaching of styrene with cold water (24 hr).[a]

Polystyrene sample	Internal surface area, cm^2	Volume of H_2O added, ml	[Styrene], ppm	Total amount leached, μg	Amount leached per unit area, $\mu g/cm^2$
Yogurt cup	107	116	0.0518	6.01	0.0563
Styrofoam cup	199	255		None detected	
Styrofoam cup	109	120		None detected	
ABS container	257	460		None detected	
Beer glass	270	399	0.0112	4.46	0.0165

[a] The limit of detection for aqueous solution was 0.00045 ppm.

Table 5. Leaching of styrene with hot water (24 hr).[a]

Polystyrene sample	Internal surface area, cm^2	Volume of H_2O added, ml	[Styrene], ppm	Total amount leached, μg	Amount leached per unit area, $\mu g/cm^2$
Yogurt cup	107	102	0.3264	33.29	0.3119
Styrofoam cup	199	251	0.0307	7.69	0.0387
Styrofoam container	109	110	0.0202	2.22	0.0204
ABS container	257	450	0.0184	8.28	0.0323
Beer glass	270	380	0.0859	32.63	0.1209

[a] The limit of detection for aqueous solutions was 0.00045 ppm.

Table 6. Leaching of styrene with 50% ethanol–water (24 hr, 100 ml of solvent added).

Polystyrene sample	Surface area exposed to solvent, cm^2	[Styrene], ppm	Total amount leached, μg	Amount leached per unit area, $\mu g/cm^2$
Yogurt cup	97	0.263	25.85	0.266
Styrofoam cup[a]	93	0.120	10.29	0.111
Styrofoam cup[a]	90	0.105	9.89	0.110
ABS container	114	0.090	8.78	0.077
Beer glass	93	0.038	3.69	0.040

[a] The styrofoam containers allowed the solvent to penetrate the walls.

ABS plastic (3) also gave peaks at lower retention times than styrene and these may be occluded acrylonitrile and butadiene. That the peaks obtained from these polymer samples were styrene monomer was confirmed by single ion monitoring on a Hitachi Perkin-Elmer RMS-4 mass spectrometer. It is of interest to note the large value for the coefficient of variation (C.V.) for PS reuseable forks (7) which were taken from the same box and were of the same color but varied widely in styrene content. The styrene content of samples 1–4 appear to fall in one group which is similar to those in code (9), and samples 5–10 have a styrene concentration which is reduced to 1/3.

Analysis of Styrene Monomer in Yogurt

Ten separate samples of yogurt (in 4-oz. PS containers) were analyzed in duplicate for styrene monomer content. These were purchased locally, and no record of their history, apart from the recommended date of expiration, was available. A 1-g portion of the yogurt was placed in a 15-ml septum vial and shaken for 30 min at 60°C. Then 250 μl of head space vapor was injected onto a Hewlett-Packard 5730A gas-phase chromatograph, and the peak area for styrene monomer was measured. Chromatographic conditions were: column, 6 ft $\times$ 0.125 in. diameter, Carbowax 400 on Porasil F (120/150 mesh); Oven temperature, 100°C; inlet temperature, 200°C; detector temperature, 250°C; air flow, 211 ml/min; hydrogen, 37 ml/min; carrier (N_2), 25 ml/min.

The limit of detection was about 1 ppb (w/v), as determined from a calibration curve obtained from known styrene concentrations in milk. The styrene content of the PS containers was also determined. The results are given in Table 3. The values obtained from three previous samples, labeled A, B, and C, are also given. It is evident that the larger sample group gave lower styrene concentrations in the container and yogurt as compared to the three other samples which were analyzed.

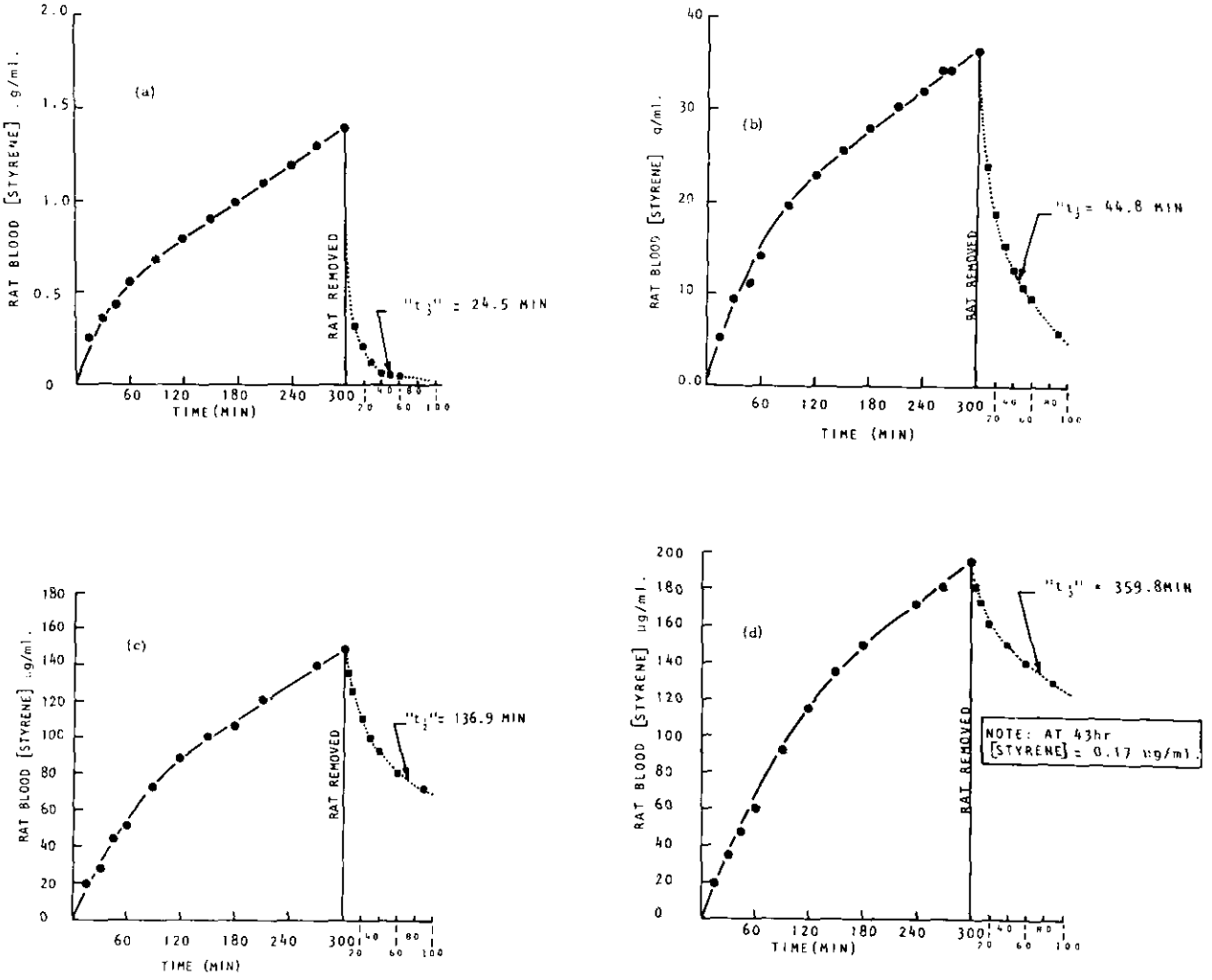

FIGURE 1. Styrene monomer, vapor-phase exposure data: (*a*) 236-g male Wistar rat exposed to 45 ppm styrene monomer, 5 hr; (*b*) 316-g male Wistar rat exposed to 520 ppm styrene, 5 hr; (*c*) 281-g Wistar rat exposed to 2800 ppm styrene, 5 hr.

Table 7. Data for vapor-phase exposure data of rats to styrene monomer.

[Styrene], ppm	[Styrene] in blood at 5 hr, μg/ml	Terminal elimination $t_{1/2}$ min
45	1.4	24.5
520	36	44.8
1200	148	136.9
2800	195	359.8

Leaching Studies

Since some of the PS products which we had examined for styrene monomer content were designed to be used for contact with hot foods, individual containers were filled with either hot or cold water, capped with saran wrap, and allowed to stand for 24 hr. The aqueous solution was then analyzed for styrene monomer content. The results, shown in Tables 4 and 5, are presented in terms of the styrene monomer concentration extracted into the water, the total

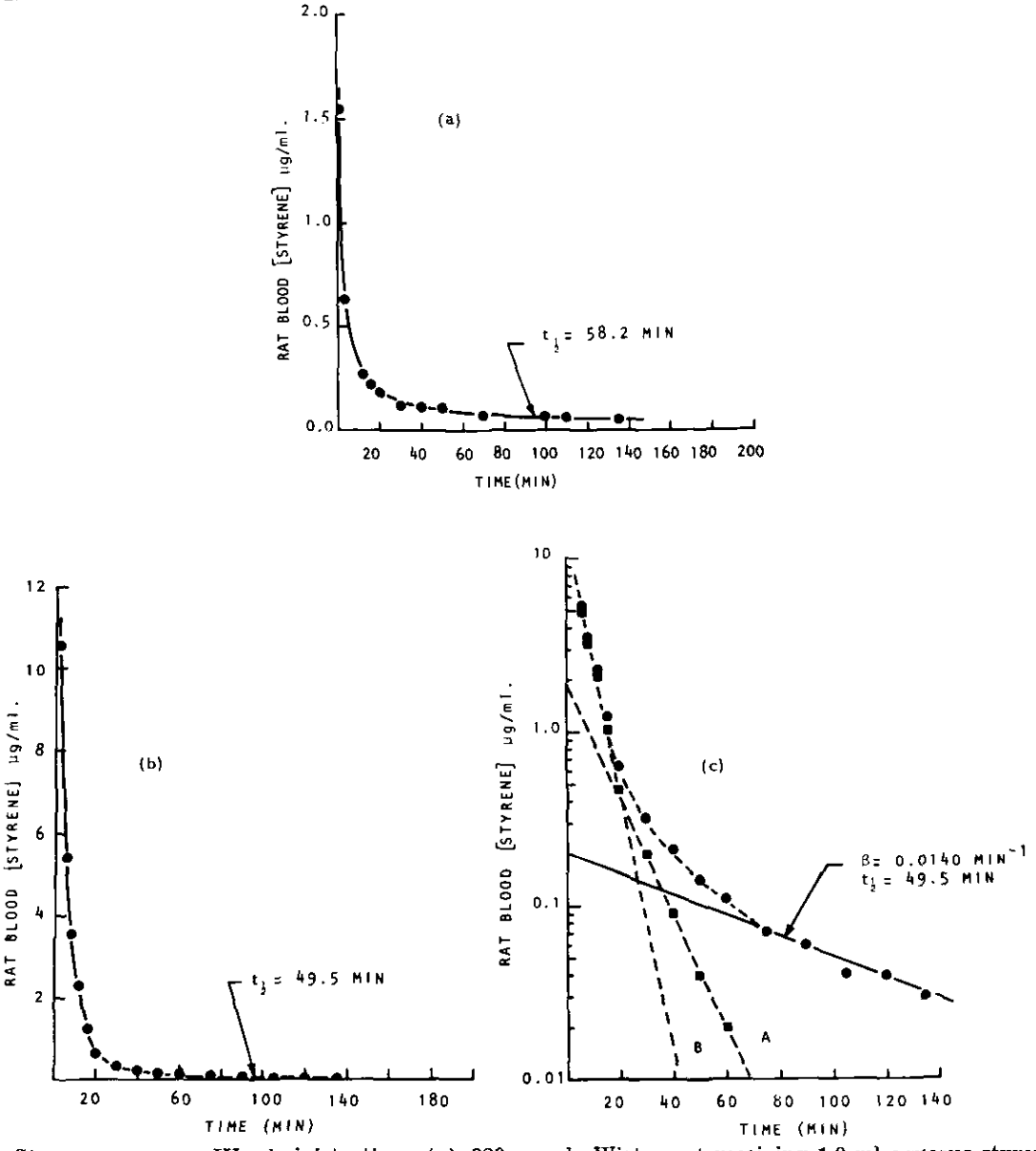

FIGURE 2. Styrene monomer, IV administration: (*a*) 320-g male Wistar rat receiving 1.0 ml aqueous styrene containing 254 μg/ml (total IV dose 254 μg); (*b*, *c*) 328-g male Wistar rat receiving 2 μl pure styrene (total IV dose 1808 μg).

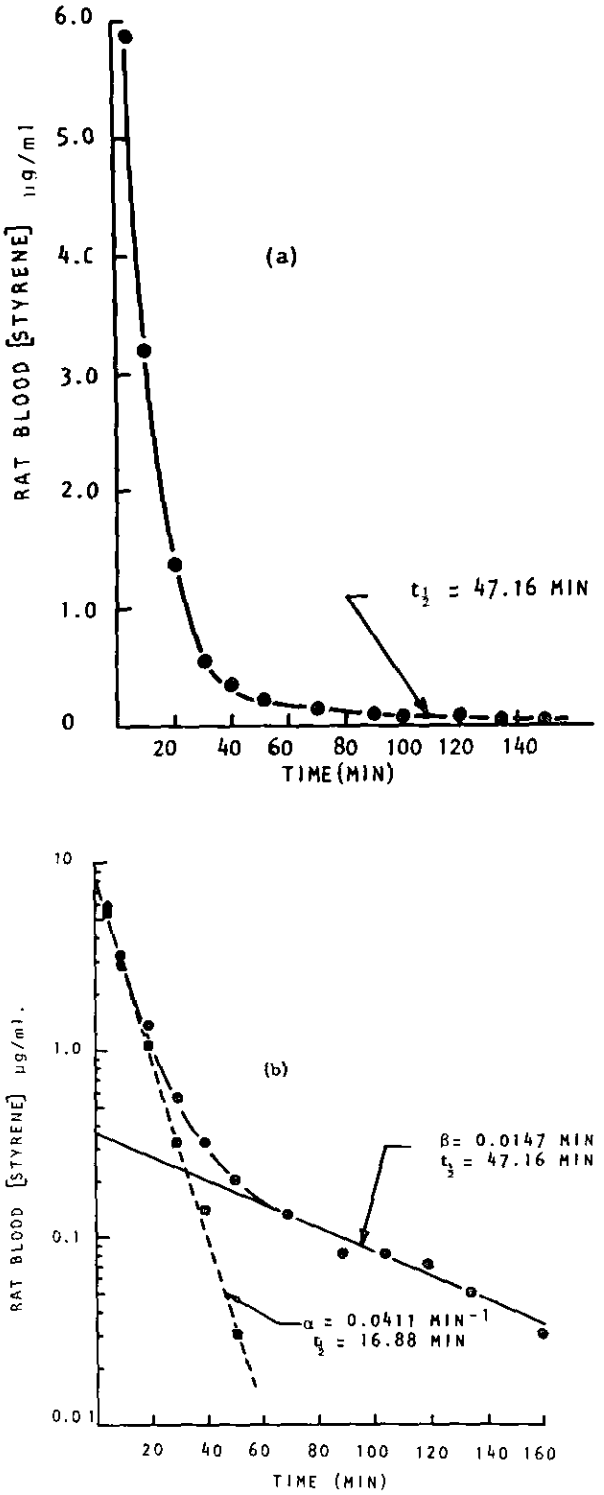

FIGURE 3. Styrene monomer in aqueous solution, intragastric administration: (*a*, *b*) 338-g male Wistar rat receiving aqueous solution of styrene, total dose 3.147 mg in 10 ml.

amount of styrene monomer leached, and the amount leached per unit surface area in contact with liquid.

Khamidullin et al. (*11*), in a communication published in 1967, actually did a toxicological study with water containing material which had been leached from dye-cast beakers constructed of rigid PS. These beakers contained 0.15% (1500 ppm) of residual styrene monomer and the aqueous extracts, which were obtained by totally immersing the beaker in boiling water which was then allowed to stand for 24 hr, contained 0.2 mg/l (0.2 µg/ml) of the monomer. Fresh extracts were used to refill the animal's drinking cups each day for a period of 11 months. Fifteen test and ten control albino rats were used in this study. Functional disturbances of a number of systems and histological lesions of certain internal organs were observed. Their principal findings in the test animals were: reduced acetyl and butyryl cholinesterase levels; a reduction in organ weights (hearts, liver, kidney, and spleen); the splenic pulp contained considerable amounts of iron and hemosiderin; the liver showed collagen fibers along some of the capillaries and, in the kidneys, a medium degree of hyperemia of the cortext and medulla with destruction of the tubules; the proliferation of connective tissue and slight round cell infiltration. These authors concluded that this form of PS container could not be recommended for use in the food industry.

The analysis of styrene in vegetable oils by the head space technique yielded data with a very much lower sensitivity, the lower detection limit being about 1 µg/ml even when the shaker bath temperature was elevated to 60°C. The probable reason for this is that the high solubility of styrene coupled with its high boiling point results in a very low partial vapor pressure for styrene in the head space. A similar detection limit was found for styrene in 50% ethanol–water mixtures as a consequence of the chromatographic peak for styrene being swamped by the trailing edge of the ethanol peak. However, ultraviolet absorption spectrophotometry, at 245 nm gave a sensitivity of better than 0.09 µg/ml for styrene in 50% ethanol–water (cells of 1 cm path length in a Beckman DBGT spectrophotometer). The results of some leaching studies, conducted in a similar manner to those with water are given in Table 6.

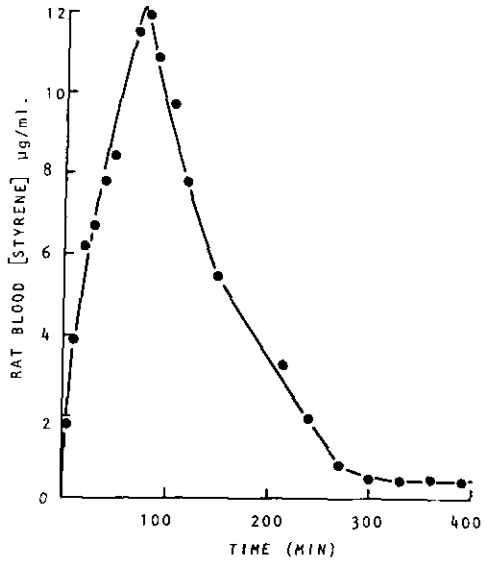

FIGURE 4. Styrene monomer in vegetable oil, intragastric administration; 270-g Wistar rat receiving total dose of 32.61 mg in 2 ml.

Uptake and Pharmacodynamic Studies with Rats

Vapor-Phase Exposures

Studies similar to those conducted for VCM (*12*) were carried out. The exposure chamber which was used, was a modified Leach-Laskin chamber in which styrene vapor was generated by means of a stream of air passed through a reservoir of liquid styrene held at 20°C. The rats were male Wistar strain, surgically prepared at least 48 hr prior to use, with an indwelling jugular cannula which was exteriorized on the dorsal side. For vapor exposure studies, the rat was restrained in a specially designed cage. Cannulated rats were exposed to chamber vapor concentrations of about 50, 500, 1200, and 3000 ppm for 5 hr. The rats were then removed, and their blood concentrations monitored for a further 2 hr. The results are shown in Figure 1. It was at once apparent that a pharmacodynamic situation very different to that observed for VCM prevailed. Whereas a plateau equilibrium concentration, directly proportional to the exposure concentration was observed for VCM (*12*), in this case the blood levels continued to rise in a linear fashion after the initial 90–120 min of exposure. Although the elimination after the exposure was not followed down to the limits of estimation, the evidence, revealed by the terminal points on each of the elimination curve, indicated a progressively slower elimination rate with increasing exposure concentration. This observation is more pronounced than the similar observation for VCM, for which the penetration of the VCM into more deep-seated tissues and the consequent overflow of physiological dams was suggested as an explanation. When the elimination phase data were plotted semilogarithmically, it was plainly evident that the "terminal" half-life represented the slow rate component of an elimination process which was at least biexponential. It would appear that, at higher exposure concentrations, the mathematical kinetic model is represented by a series of equilibria between several compartments. The observed terminal elimination rate would then be a function of a series of consecutive rate processes, and the observed half-life would then represent the half-life of the slowest or rate-limiting step. The physiological model, at low exposure concentrations, would also be considered as the blood compartment and organs with a good blood supply. At higher exposure concentrations the physiological model, like the mathematical model, would change due to the involvement of deep-seated lipid depots which could sequester styrene monomer as the blood concentration increased.

It would be difficult to conceive of a suitable vapor-phase concentration level at which to conduct continuous exposure studies since, ultimately, blood levels would be reached (sooner or later depending on the exposure concentration) at which the animal would become comatose and die. Rowe et al. (*13*) showed that this eventuality arises after about 10 hr at 2500 ppm and 1 hr at 5000 ppm for the rat. The reason for the absence of a blood level equilibrium for styrene is probably that the oil-water partition coefficient for styrene approaches infinity (styrene being only partially soluble in water but miscible in all proportions with most organic oils). Thus the typical Ostwald solubility curve which was obtained for VCM (12) was not observed for styrene. It is not inconceivable that the animal body behaves as a "sink" for styrene monomer until the lipid portion of the animal body either becomes satur-

ated (although death would probably occur prior to this event) or the tissues are equilibrated at the same concentration as the exposure atmosphere. Blood concentration and half-life data for the terminal elimination phase are given in Table 7.

Intravenous Dosing

Styrene was introduced IV by two methods: as styrene in aqueous solution and as the pure substance. The results are presented in Figure 2. Both the data from the dose of aqueous and pure styrene gave a linear terminal phase with half-lives which agreed well (58.2 and 49.5 min). It was evident that the blood level curves in both cases were at least biexponential with a relatively rapid initial phase. The residual plot shown in Figure 2c, derived after the pure styrene dose, gave a strong indication that a triexponential function probably fits these data.

Intragastric Dosing

Rats which had been deprived of food overnight were dosed by gastric intubation with an aqueous solution of styrene and a solution in vegetable oil. The data obtained following the aqueous dosing (Fig. 3) were very similar to those obtained in IV dosing. In fact, the semilogarithmic plot again indicated a biexponential character with the terminal phase having a half-life of 47.2 min, which approximates that observed after an IV dosing.

For the dose administered in vegetable oil a very different type of curve was observed, as shown in Figure 4. Relatively high levels of styrene persisted for more than 7 hr after dosing as opposed to 2 hr for the aqueous solution. The slower uptake and persistence of blood levels of styrene after the administration of the dose in vegetable oil probably arises from the persistence of styrene with the oil in the gut, which gives rise to a consequently prolonged absorption phase.

It is a pleasure to acknowledge the technical assistance of Mr. P. Collins and Dr. R. Ripley of the Additives and Pesticides Division, Department of Health and Welfare Canada, who arranged for the collection of polystyrene containers.

REFERENCES

1. Plastics Fabricating Industry, Catalogue 47-208 Annual, Statistics Canada, 1973.
2. Prior-sanctioned polyvinyl chloride resin, termination of notice of proposed rulemaking. Federal Register 40: No. 171, 40529 (Sept. 3, 1975).
3. Van Oettingen, W. F. The Halogenated Hydrocarbons of Industrial and Toxicological Importance. Elsevier, Amsterdam–London–New York, 1964.
4. Gerarde, H. W. Toxicology and Biochemistry of Aromatic Hydrocarbons. Elsevier, Amsterdam–London–New York, 1960.
5. Hefner, R. E., Watanabe, P. G., and Gehring, P. J., Studies on the fate of inhaled vinyl chloride monomer (VCM) in rats. Environ. Health Perspect. 11: 85 (1975).
6. El Masri, A. M., Smith, J. N., and Williams, R. T. The metabolism of alkylbenzenes: phenyl acetylenes and phenylethylene (styrene). Biochem. J. 68: 199 (1958).
7. Ohtsuji, H., and Ikeda, M., The metabolism of styrene in the rat and the stimulatory effect of phenobarbital, Toxicol. Appl. Pharmacol. 18: 321 (1971).
8. Leibman, K. C., and Ortiz, E., Epoxide intermediates in microsomal oxidation of olefins to glycols, J. Pharmacol. Exptl. Therap. 173: 242 (1970).
9. Code of Federal Regulations 1974, Food and Drugs, Parts 10–129, Section F, p. 498.
10. Van Duuren, B. Carcinogenic epoxides, lactones and haloethers and their mode of action. Ann. N.Y. Acad. Sci., 163: 633 (1969).
11. Khamidullin, R. S., Feldman, N. G., and Vendilo, M. V. Evaluation of the toxicity of materials made from suspended polystyrene PS–S. Hyg. Sanit., For. Sect., 33: 331 (1967).
12. Withey, J. R., Pharmacodynamics and uptake of vinylchloride monomer administered by various routes to rats, J. Toxicol. Environ. Health 1: 381 (1976).
13. Rowe, V. K., et al. The response of laboratory animals to monomeric styrene, J. Ind. Hyg. Toxicol. 24: 295 (1942).