

The toxicology of styrene monomer and its pharmacokinetics and distribution in the rat

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WITHEY, J. R. The toxicology of styrene monomer and its pharmacokinetics and distribution in the rat. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 31—40. The pharmacokinetics of styrene after intravenous administration to rats (at doses ranging from 1.3 to 9.3 mg/kg) yielded dose-dependent kinetics which fit a two-compartment mathematical model. The elimination and distribution coefficients all decreased as the dose increased, but the apparent volume of distribution remained the same. Rapid distribution to the major organs was observed, and qualitative kinetics for the elimination from the heart, liver, lung, spleen, kidney and brain all indicate a kinetic behavior and magnitude similar to that observed for the blood. Five-hour exposures of individual rats to atmospheres having styrene concentrations of from 50 to 2,000 ppm yielded uptake blood profiles which showed a continued and increasing absorption of styrene, proportional to the styrene concentration, over the entire exposure period. Postexposure elimination followed a dose-dependent two-compartment model, similar to that observed after intravenous administration, although the initial stage of the elimination was more rapid and the terminal stage much slower than observed in the intravenous case. Tissue concentrations of styrene in heart, liver, lung, kidney, spleen, brain, and perirenal fat showed a different pattern of distribution as the dose increased. At all exposure concentrations, the concentration of styrene in the perirenal fat was 10 times that in any organ. At the lowest exposure concentration the kidney registered the highest concentration, while a higher level of styrene was found in liver, brain and kidney as the exposure level increased, and for these tissues the concentration of styrene was always greater than that in the blood. The pharmacokinetics of styrene after intragastric dosing were dependent on the dosing vehicle. For aqueous solutions a very rapid uptake stage was observed (peak concentrations being observed at < 4 min), while solutions in vegetable oil showed a slower and delayed absorption (with peak concentrations at about 2 h after dosing). The elimination after dosing with aqueous solutions showed a marked similarity to the intravenous kinetics.

Key words: distribution, food, inhalation toxicology, intravenous, pharmacokinetic models, pharmacokinetics, styrene.

The innovators of polystyrene were reported to be Simon and Bonastre in the 1830s, and it was introduced commercially in the 1930s (4). Modified rubbery polystyrene appeared in the late 1950s. Its appearance coincided with the rapid commercial growth of this class of plastics, which was facilitated largely by the ready availability of styrene monomer as a consequence of the search for rubber

substitutes during World War II. The ability of styrene to form graft and copolymers with other monomers like butadiene and acrylonitrile has led to the development of plastics with an extraordinary range of applications.

Our interest in the use of polystyrene plastics for food packaging use arose from the concern which was generated by the association, in 1974, of vinyl chloride monomer with a rare form of liver cancer that ultimately led to the introduction of strict regulations regarding the use of polyvinyl chloride as a food packaging

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material in most countries. Previous reports from this laboratory have presented data on the presence of styrene monomer in polystyrene food packaging and food contents (35, 38).

Largely as a consequence of the concern for personnel exposed to styrene monomer in the industrial workplace, the uptake, distribution and elimination of styrene after exposure in the vapor phase has been the subject of a number of important studies, both in animals (30) and man (7, 39). While acute exposure to styrene in the vapor phase requires concentrations of 10,000 ppm to cause rapid death in animals, at lower concentrations the principal effects have been reported as mucous membrane, eye and skin irritation followed by effects upon the central nervous system which induce nausea, weakness, incoordination and coma (30, 39). As a consequence of these studies, the threshold limit for workroom air has been set at 100 ppm or approximately 420 mg/m³ (3).

More recently, greater attention has been focused on the toxicology of styrene largely as a consequence of its having metabolic characteristics that are similar to those of the well-defined carcinogen, vinyl chloride monomer. Styrene has been shown to be extensively metabolized in animals and man, only about 1 % being expired unchanged (15) and the ultimate metabolites being hippuric, mandelic and phenylglyoxylic acids. It has been shown that metabolism takes place mainly in the liver, and it is stimulated by phenobarbital and inhibited by simultaneously administered toluene (16). The proposed metabolic pathways for styrene, arising from a number of studies (5, 8, 9, 17, 27), was by way of an epoxide intermediate. Since epoxides have been demonstrated to be carcinogenic (33) and the epoxide metabolic intermediate of vinyl chloride has been suggested as the ultimate carcinogen (13), a number of mutagenic and carcinogenic studies have been undertaken with styrene (41).

Although styrene epoxide has been shown to be a base-substitution mutagen, the evidence for the mutagenicity of styrene itself is doubtful (23, 32). However, at least two studies have reported that styrene was mutagenic, one with hamster

cells (19) and the other with metabolic activation on *Salmonella typhimurium* strains TA 1535 and TA 100 (34). The initiation of at least 11 cancer studies with styrene have been cited, but no results from these have yet been reported. They include one teratological and three generation studies of rats in which styrene was administered via drinking water, by gavage, per-os and by inhalation. A 15-month gavage study with dogs and an inhalation study with rabbits have also been announced (41).

As well as the potential mutagenic, teratogenic and carcinogenic threat posed by exposure to styrene monomer, concern has been expressed with respect to the neurotoxic effects which have been discovered in epidemiologic studies. Effects upon the central nervous system were revealed by abnormalities in the electroencephalographic recordings of workers who were exposed from 6 months to 14 years (29). In another study, visuomotor inaccuracy and poor psychomotor performance was noted in an assessment of the psychological functions of exposed workers (18).

The clinical findings and toxic effects in man after exposure by several routes have been reviewed recently (20). No clear pattern of changes in liver function tests or hematological parameters were noted although it has been suggested that the determination of hippuric and mandelic acid in urine is a good indicator of the severity of exposure (12, 14, 15).

Styrene monomer was apparently well absorbed in the lungs (2, 24), about 70—90 % of that contained in alveolar air being taken up. Åstrand also reported that styrene, at exposure concentrations of up to 350 ppm, continued to be absorbed during the exposure period and produced increasing concentrations of the monomer in arterial blood throughout the exposure. This observation has been confirmed (35) for exposure concentrations of up to 2,000 ppm over a period of at least 5 h. The elimination of styrene via the lung has been reported to occur at a rate with a half-time of 1 to 3 h (31). The half-time for the excretion of styrene via the urine, mainly as metabolites, has been given as 1 to 8 h (15). Styrene monomer has been found to be widely distributed to the major organs of the body and to lipid depots

after intravenous administration and after inhalation exposure (1, 10, 28, 40).

In the present study the pharmacokinetics of styrene were evaluated both for the principal organs and the blood after intravenous dosing at four different levels. The kinetics of the uptake and elimination of styrene in the rat, during and after a 5-h exposure to styrene monomer and at concentrations varying from 50 to 2,000 ppm have also been studied. Distribution studies were also carried out in which the heart, liver, lungs, brain, kidney, spleen, perirenal fat, and blood were analyzed for styrene immediately after exposure. These studies throw some light on previous toxicologic findings in terms of the dose dependence of the observed pharmacokinetics and the relative organ distribution.

MATERIALS AND METHODS

Pharmacokinetics

Murine viral pneumonia free male Wistar rats (Charles River, Boston, Mass., U.S.A.) of between 350 and 400 g were surgically prepared with an indwelling jugular cannula. The methods for administering the dose and for monitoring the styrene concentration in the exposure chamber and in blood and tissues have been described previously (35, 37). Blood concentrations which were measured after the administration of intravenous doses ranging from 1.3 to 9.4 mg/kg of pure styrene monomer were plotted on semilogarithmic paper, and the resulting biexponential curves were analyzed according to well-established procedures for a two-compartment pharmacokinetic model (21).

For the uptake phase, during the exposure to styrene vapor, the blood levels continued to rise at a rate that was proportional to the styrene vapor concentration. It was assumed that the pharmacokinetic model which described these uptake curves was one in which there was a zeroth order uptake of styrene. Since the elimination curves, which were obtained after expo-

sure, gave biexponential plots similar to those obtained after intravenous dosing, the whole pharmacokinetic model was assumed to be of the two-compartment, open-ended type. The integrated form of the rate equation for the central compartment was:

$$C_t^1 = \frac{k_0}{V_1} \left[\frac{k_{21}}{\alpha\beta} - \frac{(\alpha - k_{21}) e^{-\alpha t}}{\alpha(\alpha - \beta)} + \frac{(\beta - k_{21}) e^{-\beta t}}{\beta(\alpha - \beta)} \right], \quad (a)$$

where C_t^1 was the measured blood concentration at any time (t) during the exposure, k_0 was the zeroth order uptake rate, V_1 the volume of distribution for the central compartment, and k_{21} was the rate coefficient for the transfer of styrene from the second to the central compartment.

For the elimination kinetics after the animal was removed from the exposure atmosphere the monitored blood concentration followed the biexponential equation:

$$C_t^1 = Ae^{-\alpha T} + Be^{-\beta T}, \quad (b)$$

where T was the time from the instant the subject was removed.

The semilogarithmic plot of blood concentration against time was treated by the method of residuals so as to resolve the curve into a linear terminal phase of slope β and the ordinate intercept of B and a linear initial phase of slope α and the ordinate intercept of A. The hybrid rate coefficients α and β were the same for the uptake phase, equation a, and for the elimination curve, equation b. It was also possible to derive the relationships for the preexponential coefficients, A and B, which are given in equations c and d:

$$A = \frac{k_0 (k_{21} - \alpha)}{V_1 (\beta - \alpha) \alpha} (1 - e^{-\alpha T}), \quad (c)$$

$$B = \frac{k_0 (k_{21} - \beta)}{V_1 (\beta - \alpha) \beta} (1 - e^{-\beta T}). \quad (d)$$

The individual rate coefficients for the two-compartment model describing the elimination kinetics were also derived. For the rate coefficient for the transfer from the central to the second compartment:

$$k_{12} = \alpha + \beta - k_e - k_{21}. \quad (e)$$

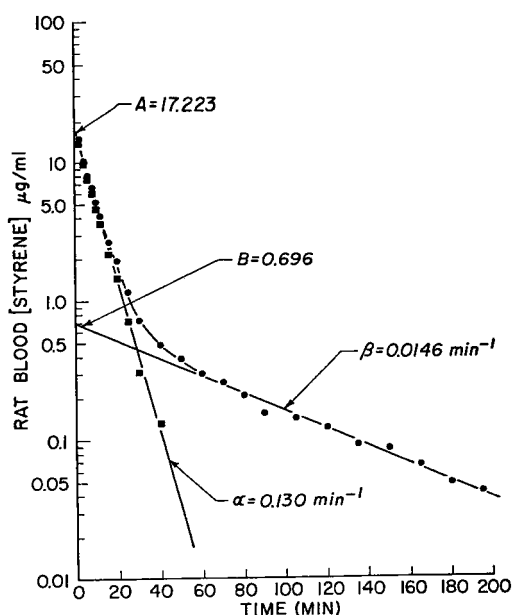


Fig. 1. Typical semilogarithmic plot of blood data after the intravenous administration of a 9.4 mg/kg dose of styrene monomer to rat. The residual plot is also illustrated and was obtained by the method of Mayersohn and Gibaldi (21). The symbols are defined in the text.

The rate coefficient for the transfer from the second to the central compartment:

$$k_{21} = \frac{a\beta A (1 - e^{-\beta T}) + B (1 - e^{-aT})}{Aa (1 - e^{-\beta T}) + B\beta (1 - e^{-aT})}, \quad (f)$$

and for the coefficient for the elimination from the central compartment:

$$k_e = \frac{a\beta}{k_{21}}. \quad (g)$$

All of the parameters on the right-hand side of equation f were derived from the

semilogarithmic plot of the blood concentration against time with the use of data obtained after the animal was removed from exposure and with the value $T = 0$ inserted. The subsequent use of these parameters in equations e and g allowed the evaluation of k_e and k_{12} .

Since the value of A when $T = 0$ was determined from the graphic treatment of the elimination data and the values of a , β and k_{21} were also known, the value of k_0/V_1 was calculated from equation c. Thus values for all of the constants on the right-hand side of equation a could be used, together with different values for t , to derive theoretical values of C_1^t for the uptake phase. The limiting value of C_1^t was calculated for each exposure by the setting of $t = \infty$, and the time taken to reach 99 % of this value was also derived from equation a.

Distribution and organ kinetic studies

Six rats were dosed intravenously with styrene (4 mg/kg) and then killed, by cervical separation, at 8, 15, 30, 45, 60 and 90 min after dosing. Samples of the blood, heart, brain, liver, spleen, and kidney were then analyzed for their styrene content as described previously (37). These data were then plotted on semilogarithmic paper and allowed the approximate rate data for the elimination of styrene from each organ to be obtained.

Five rats were simultaneously exposed at each of five vapor concentrations of styrene for 5 h. They were then removed, killed by cervical separation, and samples of the blood, heart, lung, liver, spleen, kidney, and brain were removed and analyzed for their styrene content. The values which were obtained for each organ were

Table 1. Kinetic coefficients and parameters for the elimination of styrene monomer after intravenous administration. The symbols are defined in the text.

Dose (mg/kg)	Rat weight (g)	Parameter estimates				Estimated rate coefficient			Volume of distribution (ml)
		A (µg/kg)	α (min ⁻¹)	B (µg/kg)	β (min ⁻¹)	k_{21} (min ⁻¹)	k_e (min ⁻¹)	k_{12} (min ⁻¹)	
1,337	434	2.364	0.260	0.223	0.0512	0.0692	0.192	0.0496	224.3
4,911	412	5.621	0.192	0.363	0.0255	0.0356	0.138	0.0444	276.2
6,695	427	10.110	0.122	0.465	0.0134	0.0181	0.0897	0.0271	262.3
9,359	389	15.359	0.0975	0.464	0.0109	0.0134	0.0790	0.0159	228.8

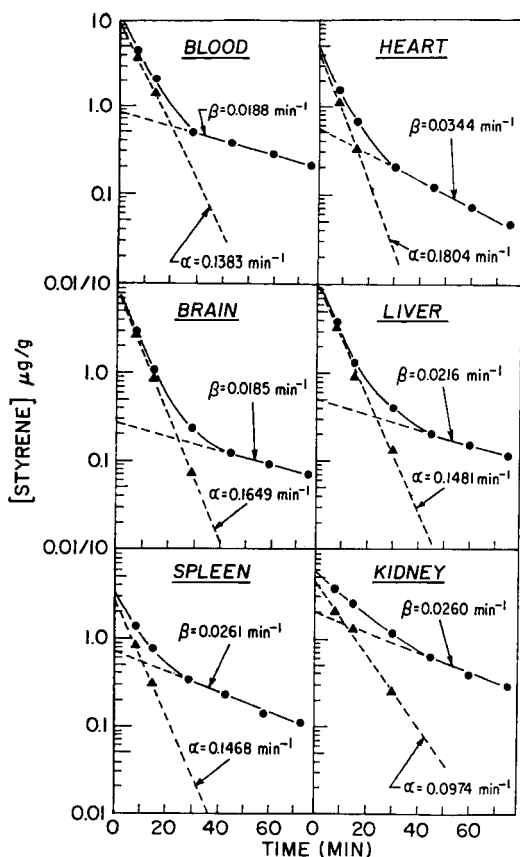


Fig. 2. Elimination of styrene monomer from the principal organs of rats after the intravenous administration of a 4 mg/kg dose of styrene monomer.

then divided by the determined concentration in the blood, and a statistical analysis was performed so as to compare the variance within and across the different exposure levels with Barlett's test (6). Bonferoni t-tests were also performed to ascertain whether the relative concentration of styrene in each organ to that in the blood varied significantly from unity as the exposure level increased (22).

RESULTS

An example of a semilogarithmic plot of blood concentration against time after the administration of an intravenous dose of

styrene is shown in fig. 1, and the mean values, obtained from individual kinetic coefficients at each dose level, are summarized in table 1. Semilogarithmic plots of the styrene concentration against time, which were obtained for each organ after the administration of the intravenous dose, are presented in fig. 2, and the derived rate parameters are also given.

The uptake profile of the concentration of styrene in rat blood during a 5-h exposure to different concentrations of styrene vapor is shown in fig. 3. An example of a typical semilogarithmic plot of rat blood concentration against time after the animal was removed from the exposure chamber is illustrated in fig. 4, and the mean values of individually determined rate parameters are given in table 2. Theoretical uptake curves for a 24 h exposure, which were predicted from the kinetic data obtained from the elimination studies, are shown in fig. 5, and the calculated values of the maximum blood concentration for each exposure level and the time needed to achieve 99 % of this level are given in table 3. The mean tissue levels of styrene, which were also determined after a 5-h exposure of five rats, are illustrated in fig. 6.

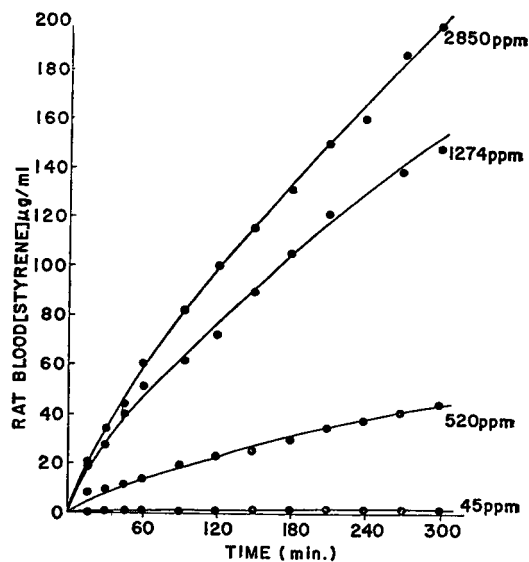


Fig. 3. Blood concentrations of styrene monomer during the pulmonary exposure of rats to different concentrations of styrene monomer.

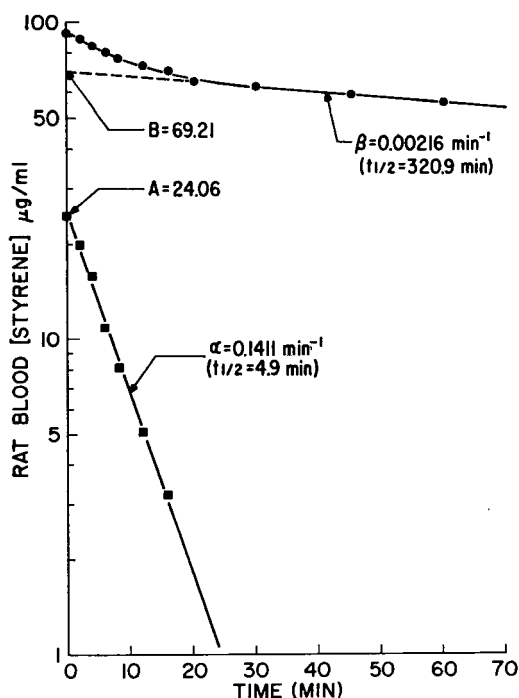


Fig. 4. Typical elimination curve for styrene monomer after the exposure of a rat to an atmosphere of 2,417 ppm for 5 h. The residual curve is also shown and the symbols are defined in the text.

DISCUSSION

The value of pharmacokinetic studies as a prerequisite or adjunct to chronic dosing studies has been pointed out in a number of recent publications (11, 36). A knowledge of the extent and rate of uptake of a xenobiotic material, when administered by various routes, in addition to the quantitative determination of its distribution to the major organs was considered to be of value to the interpretation of dose-response curves and to the understanding of toxicologic mechanisms.

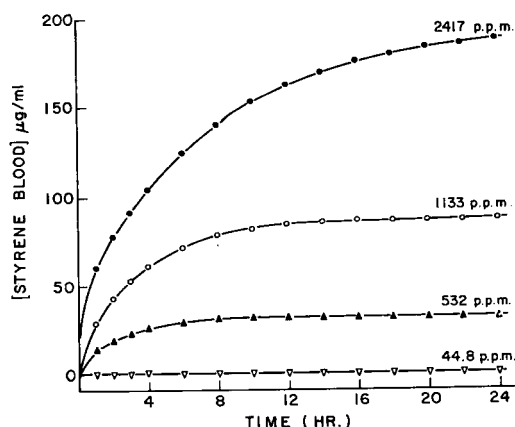


Fig. 5. Theoretical uptake curves for styrene in the rat during a 24-h exposure to atmospheres which contained different concentrations of styrene monomer.

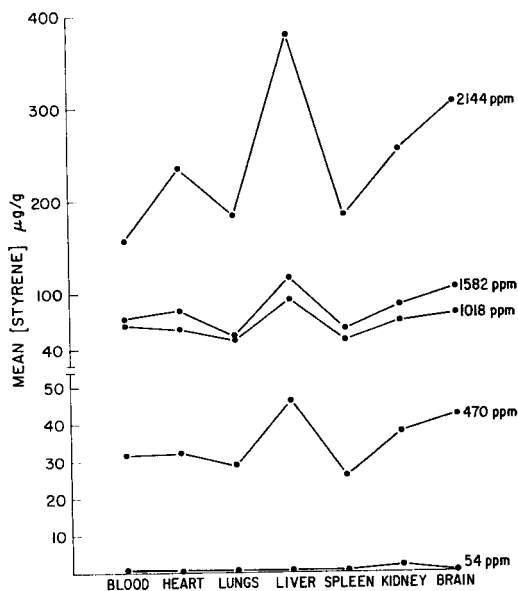


Fig. 6. Organ distribution of styrene in rats after a 5-h exposure to atmospheres which contained different concentrations of styrene monomer.

Table 2. Kinetic parameters for styrene monomer in rat after a 5-h inhalation exposure. The symbols are defined in the text.

Styrene (ppm)	A (µg/ml)	α (min ⁻¹)	B (µg/ml)	β (min ⁻¹)	k_{12} (min ⁻¹)	k_e (min ⁻¹)	k_{21} (min ⁻¹)
44.8	0.75	0.11	0.22	0.014	0.018	0.088	0.018
532	5.32	0.20	22.75	0.005	0.031	0.037	0.140
1,133	12.41	0.11	54.58	0.004	0.025	0.019	0.072
2,417	24.04	0.14	69.21	0.002	0.014	0.022	0.107

Table 3. Calculated C_t^t equilibrium levels and time to reach 99 %.

Styrene (ppm)	Limiting styrene blood ($\mu\text{g/ml}$)	Time to 99 % equilibrium (h)
44.8	0.9677	4.0
532	29.69	9.0
1,133	87.36	17.0
2,417	194.36	33.5

The pharmacokinetic data derived after the intravenous administration of the dose gave some indication of the way in which styrene monomer was distributed and eliminated in the rat. The values for the derived kinetic parameters and rate coefficients, given in table 1, showed that all of the rate coefficients decreased as the dose increased. Of especial importance was the observation that k_e , the rate coefficient for the elimination from the central compartment, was affected the most over the dose range used in this study, and this may be a consequence of the saturation of metabolic pathways, as has been previously demonstrated by Ikeda and Imamura (15). The apparent volume of distribution was about 230 ml, or about 10 times the blood volume of a 400-g rat, and was suggestive of extensive distribution to organs outside the blood compartment.

The semilogarithmic curves which were obtained from organ concentration data (fig. 2) indicated that distribution occurred rapidly and that the elimination kinetics were very similar for each organ. It was somewhat surprising that the data points which were obtained for the blood concentration in different animals should give kinetic values which closely approximated those given in table 1, which were obtained from multiple samples from a single animal that had received a similar dose.

The elimination kinetics, which were observed after a 5-h exposure at different concentrations of styrene vapor, gave biexponential semilogarithmic plots similar to those illustrated in fig. 4. Dose dependent kinetics were also evident from the data reported in table 2 and at each dose level the kinetic coefficient for the terminal phase, β , was smaller than that for

the initial phase, α , by a factor which varied from 10- to 100-fold as the dose was increased. The hybrid rate coefficient α appeared to remain fairly constant (half-time ~ 5 min) over the dose range. The individual rate coefficients for the model, equations e, f and g, showed a trend in which k_e decreased as the dose increased and was similar to the trend revealed in the intravenous studies. No pronounced variation for the values of k_{12} or k_{21} was evident although k_{12} was slightly greater than k_{21} throughout the dose range; this finding suggests a potential for the accumulation of styrene in the tissues.

The uptake of styrene during a vapor phase exposure, as revealed by the blood level curves shown in fig. 3, showed that the blood concentration continued to rise throughout the exposure. Similar observations have been recorded for human exposure (2). The application of a two-compartment model with a zeroth-order input of styrene to the central compartment was compatible with the observed biexponential curves, which were obtained for the elimination phase after the exposure ceased and with the high oil to blood partition coefficient for styrene of 130 (26). The theoretical uptake curves, shown in fig. 5, not only revealed that their shape was similar to those in fig. 3, but that the value of C_t^t at $t = 300$ min closely approximated the value of C_t^t , which was measured at the beginning of the elimination phase.

The kinetic model also showed that the blood levels should reach limiting values but, as was evident from the data in table 3, the higher the exposure concentration the longer it would take for the limiting or plateau concentration to be reached. It should be pointed out that this model took no account of the pulmonary kinetics which would perturb the shape of the uptake curve in the initial stages to give lower values of C_t^t than those predicted by the model. The physiological and physicochemical parameters which could influence the exchange of alveolar vapor with environmental atmosphere that contains a known concentration of a xenobiotic substance have been described as the respiration rate, the tidal volume, the relative solubility of the substance in the blood and tissues of the animal, and as the cardiac

output and blood flow per unit volume in the various tissues (25).

The concentrations of styrene in the important tissues in animals that were killed immediately following a 5 h exposure to styrene at different vapor concentrations are shown in fig. 6. A statistical analysis of these data revealed that at the lowest exposure concentration the levels in the kidney were higher than for any other organ. As the exposure concentration increased, the distribution to the liver and brain was significantly higher than for the other organs, including the blood. In fact, for the majority of these analyses, the ratio of the styrene concentration in organs to that in blood was significantly different ($p < 0.05$) and suggested that the blood styrene concentration would not be appropriate as a measure of the toxicologic insult after vapor phase exposure to styrene.

The concentration of styrene found in the perirenal fat, in another set of animals than those used in the main study, ranged from 2,500 $\mu\text{g/g}$ after a 5-h exposure to 2,100 ppm of styrene to 8.9 $\mu\text{g/g}$ after a similar exposure to 53 ppm. These values were between 8 and 10 times greater than those for any other organ and reflected the high affinity of styrene for lipid depots as a consequence of its solubility and pharmacokinetic characteristics.

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QUESTIONS AND ANSWERS

Questions to Dr. WITHEY

- Dr. SMITH: From your pharmacodynamic studies on inhalation, when do you consider the best time to sample urine or blood to reflect the level of exposure? Do you think this could be of use in human exposure?
- Dr. WITHEY: I think that the blood concentration of styrene is a very poor index of exposure. Not only does the pattern of distribution to the major organs appear to change with the exposure level in the atmosphere, but some organs have significantly higher and some significantly lower concentrations than that in blood.
- Prof. ASTRAND: There is a great difference between the rat and the human with respect to the amount of body fat. Would the two-compartment model fit the human if you especially consider this difference?
- Dr. WITHEY: It is possible that a different fat distribution could affect the amount of uptake (i.e., total body burden). It is unlikely that the pharmacokinetic model would be affected. One must, however, be very careful in relating the pharmacokinetics to the physiological model.
- Mr. KENDALL: What was the maximum level of the migration of styrene monomer to the food encountered by Dr. Withey?
- Dr. WITHEY: 436 parts per billion in a wall sample of sour cream. [*Bull. environ. contam. toxicol.* 19 (1978) 86—94].
- Dr. RINZEMA: Levels of styrene monomer in the samples tested are excessively high as compared to what I know about the European market. Levels are usually around 700 ppm or lower in polystyrene based packaging materials in Europe. Where were your samples especially selected? Maybe these were made from old styrene polymers. Since then the monomer level may have come down in Canada, too.
- Dr. WITHEY: Our samples of the polystyrene containers were collected by the Field Operation Group of the Food Directorate, H.P.B., Canada, from food packagers. These packages were taken "off-line" in food packaging plants at the same time that our samples of foods in polystyrene packages were obtained. A few samples (e.g., disposable cutlery) were obtained from local stores. All samples were obtained in February or March of 1976.
- Dr. KAMM:
1. Regulations for food-contact articles were established in most European countries many years ago.
 2. The amount of residual monomers in final products, especially in the case of vinyl chloride, are much lower in Europe today.
- Dr. WITHEY:
1. I am aware of the European situation.
 2. The figures that were cited for the vinyl chloride content of rigid food containers were from data obtained prior to the knowledge that vinyl chloride was a carcinogen and to the subsequent regulations that were legislated.