

Pharmacokinetics of inhaled styrene in rats and humans

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RAMSEY, J. C. and YOUNG, J. D. Pharmacokinetics of inhaled styrene in rats and humans. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 84—91. The pharmacokinetic profile of inhaled styrene was examined in rats exposed to levels of 80, 200, 600 or 1,200 ppm for periods of up to 24 h. At levels up to 200 ppm for 6 h, styrene was cleared from the blood according to a two-compartment linear pharmacokinetic model, but at levels of 600 ppm and above the clearance was saturated. In going from 80 to 1,200 ppm (a 15-fold increase), the area under the blood concentration/time curves (AUC) increased by a factor of 112. Fat tissue was shown to comprise the second compartment of the two-compartment pharmacokinetic model. It is suggested that saturation of styrene clearance is due mostly to saturation of the metabolic capacity for styrene. In humans exposed to 80 ppm of styrene for 6 h, styrene was cleared from the blood according to a two-compartment linear pharmacokinetic model similar to that for rats. A maximum blood concentration of 0.9 $\mu\text{g/ml}$ was reached at the end of the exposure. Most of the inhaled styrene was excreted in the urine as phenylglyoxylic and mandelic acids, and only a small amount as styrene in the expired air. Simulation of the pharmacokinetic model showed that no continued accumulation of styrene would occur during repeated, daily 8-h exposures to 80 ppm. These data reveal that the rat is a reasonable pharmacokinetic model for styrene in humans. At levels of exposure up to 200 ppm, styrene is cleared from the body very efficiently and will not continue to accumulate upon repeated exposure. But at levels of styrene sufficiently high to saturate the metabolic clearance capacity, the integrated dose (measured by the AUC) will be much greater than expected based on exposure levels alone. Therefore, the extrapolation of toxicity observed at high levels of styrene exposure to that expected at low levels may not be justified.

Key words: humans, mandelic acid, metabolism, pharmacokinetics, phenylglyoxylic acid, rats, styrene.

In the investigation of the potential toxic hazard of a chemical to man, it is helpful to have knowledge of its pharmacokinetic behavior not only in humans, but also in the animal species from which toxicity data is extrapolated to man. Although previous studies with styrene in rats and humans (1, 5, 8, 9) have investigated some of its absorption and distribution charac-

teristics, none of these studies were specifically designed to elucidate the pharmacokinetics of inhaled styrene. Therefore, the studies included in this report were designed to determine the pharmacokinetic model of inhaled styrene in rats and humans. This information allows correlation of blood concentration with exposure levels, prediction of blood concentration on repeated exposures, and comparison of the pharmacokinetic profile of styrene in rats with that in humans.

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MATERIAL AND METHODS

Rats

Groups of 51 male rats were subjected to either 80, 200, 600, or 1,200 ppm of styrene. Twenty-one of the rats in each experiment were removed from the exposure chamber at 6 h for the determination of the clearance kinetics of styrene. We left the remainder of the rats in the chambers for periods of up to 24 h in order to establish whether plateau blood levels would be obtained. At selected time points (shown in figs. 1, 2 and 4) rats were killed and samples of blood and fat were obtained for styrene analysis.

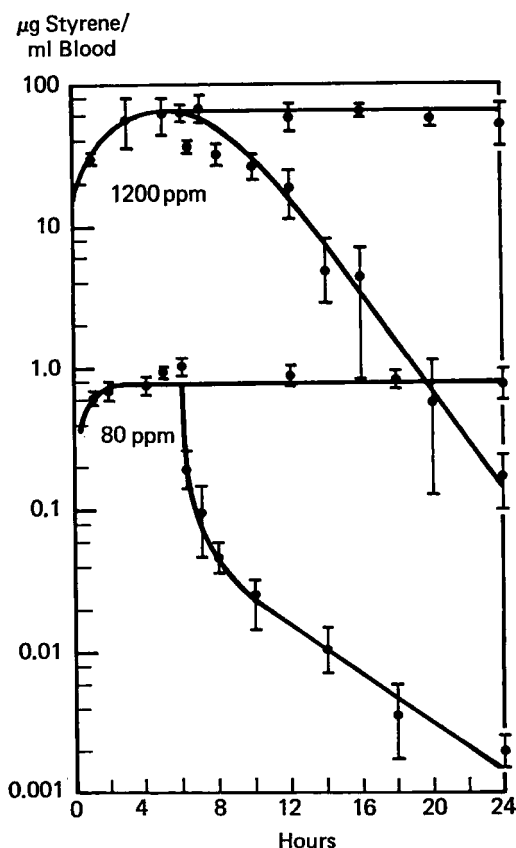


Fig. 1. Blood styrene concentration in rats exposed to 80 or 1,200 ppm.

Humans

Four human male volunteers were exposed to styrene at a concentration of 80 ppm for 6 h. The volunteers were essentially at rest while they were in the exposure chamber. Styrene concentration in the blood was measured during and after the exposure and in the expired air after the exposure. The volunteers took complete physical examinations before and after the exposure, and the results were normal in every respect. No neurological or behavioral measurements were made in this experiment, which was designed solely to determine the pharmacokinetic model for styrene in humans.

Styrene analysis

The concentration of styrene in blood and fat samples was determined by gas chromatography/mass spectrometry of a hexane extract of the samples. Details of the analytical methodology have been described by Karbowski and Braun (7).

RESULTS

Rats

Fig. 1 is a semilogarithmic graph of the concentration of styrene in the blood of those rats exposed to 80 or 1,200 ppm. At an exposure level of 80 ppm the blood concentration rose quickly to about 0.8 $\mu\text{g/ml}$ at 6 h. Then, in those rats that were removed from the chamber, it was cleared in a biphasic log-linear manner that was consistent with a two-compartment linear pharmacokinetic model. The solid line shown in fig. 1 for the uptake and clearance at 80 ppm is the theoretical blood concentration predicted by that model. However, at 1,200 ppm the blood concentration rose to about 64 $\mu\text{g/ml}$, and the clearance kinetics of those rats removed from the chamber at 6 h was no longer linear, but had the appearance of a saturated clearance process. In going from 80 ppm to 1,200 ppm, the exposure

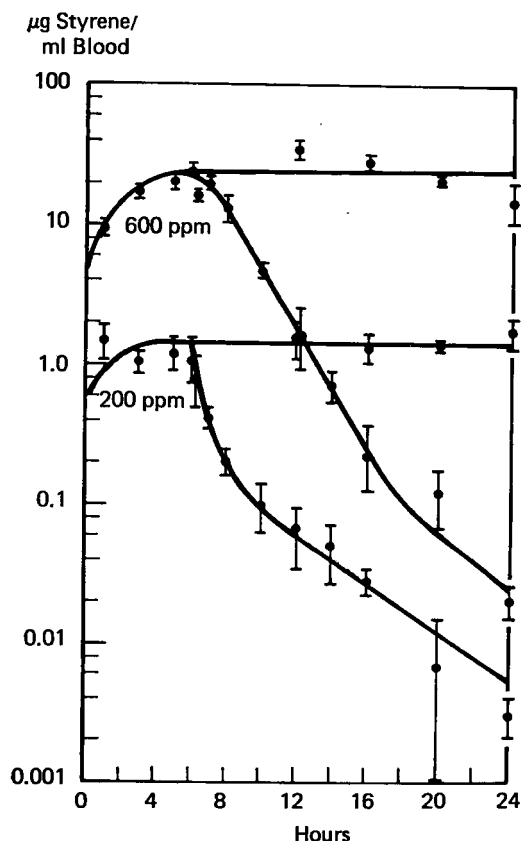


Fig. 2. Blood styrene concentration in rats exposed to 200 or 600 ppm.

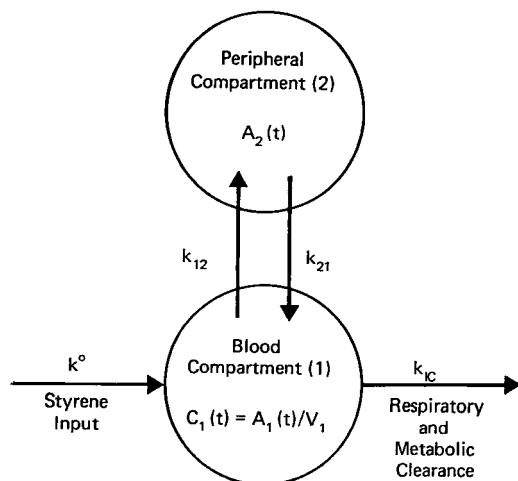


Fig. 3. Two-compartment linear pharmacokinetic model describing the uptake, distribution, and clearance of inhaled styrene in rats and humans.

level was increased by a factor of 15, but the blood concentration was increased by over 80 times. Thus, there is a transition from linear to saturated styrene clearance somewhere between 80 and 1,200 ppm. Also, at neither exposure concentration did the blood concentration continue to rise, but it reached a maximum in each case.

In order to elucidate further the transition from linear to saturated clearance kinetics, we exposed rats to two intermediate exposure levels, 200 and 600 ppm. As shown in fig. 2, the blood concentration at 200 ppm was almost directly proportional to that at 80 ppm, and the clearance of styrene exhibited typical two-compartment linear clearance characteristics. However, at 600 ppm the maximum blood concentration was approximately 25 $\mu\text{g/ml}$, four times higher than expected based on the results at 80 ppm. And at 600 ppm the clearance was typical of that of a saturated system. These data demonstrate that somewhere between 200 and 600 ppm, the clearance of styrene in the rat becomes saturated.

The diagram for the two-compartment model for inhaled styrene in rats is shown in fig. 3. In this model, the input of styrene into the blood was assumed to occur at a constant and continuous rate over the entire 6-h exposure period. It was also assumed that 66 % of the inhaled styrene was absorbed (1, 2). This fraction, combined with the average minute volume of rats and the concentration of styrene in inspired air, result in a constant input rate of 2.85 mg/h. The constants k_{12} and k_{21} are the mass transfer coefficients between the central and peripheral compartments. The apparent first order clearance rate constant, k_{1c} , accounts for the removal of styrene both by expiration and by metabolism. After the observed blood data were fitted to this model, the best pharmacokinetic parameter estimates at 80 ppm resulted in half-life values of 0.26 ± 0.04 h for the rapid (α) clearance phase and 3.6 ± 2.76 h for the slower (β) clearance phase. The apparent volume of distribution of the first compartment was approximately 5.3 l/kg.

The styrene concentration in fat in those rats exposed to 80 or 1,200 ppm is shown

in fig. 4. The concentration of styrene in fat was typical of the pattern to be expected from the second (peripheral) compartment of the two-compartment pharmacokinetic model. The solid line representing the uptake and clearance of styrene in fat at 80 ppm in fig. 4 is the theoretical concentration of styrene in the peripheral compartment, if it is assumed that 10 % of the body weight is fat. These measurements indicate that fat comprises most of the peripheral compartment in the two-compartment model for styrene. Styrene was highly concentrated in fat relative to blood; the ratio of the concentration of styrene in fat to that in blood was approximately 40:1 at 80 ppm and 80:1 at 1,200 ppm.

The pharmacokinetic model and the best parameter estimates were used to simulate the blood concentration of styrene in rats exposed to 80 ppm for 8 h per day over a 5-d week with a 2-d clearance period at the end of the week. The results are shown in fig. 5. After only 1 d of exposure, the maximum blood concentration was over 98 % of the theoretical steady state value of 0.8 $\mu\text{g}/\text{ml}$, and no continuous increase in concentration was indicated.

Humans

The concentration of styrene in blood of humans during and after 6-h of exposure

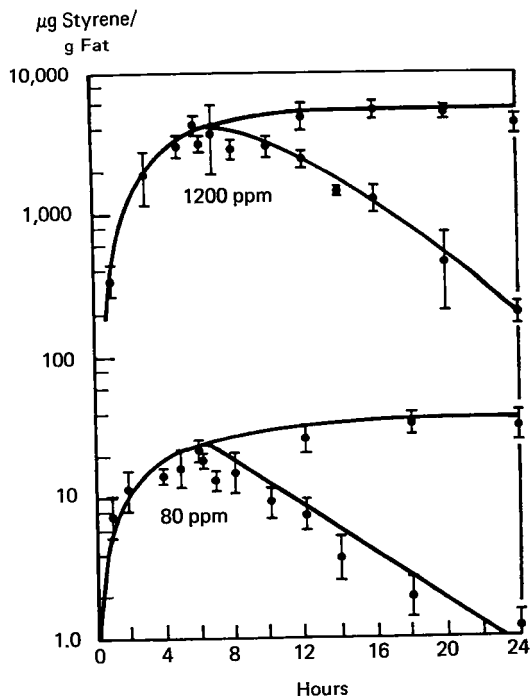


Fig. 4. Fat styrene concentration in rats exposed to 80 or 1,200 ppm.

to 80 ppm is shown in fig. 6. The rapid rise in blood concentration leveled off to a final concentration of 0.9 $\mu\text{g}/\text{ml}$ at the end of the 6-h exposure. The concentration then declined rapidly in a manner consistent with the two-compartment

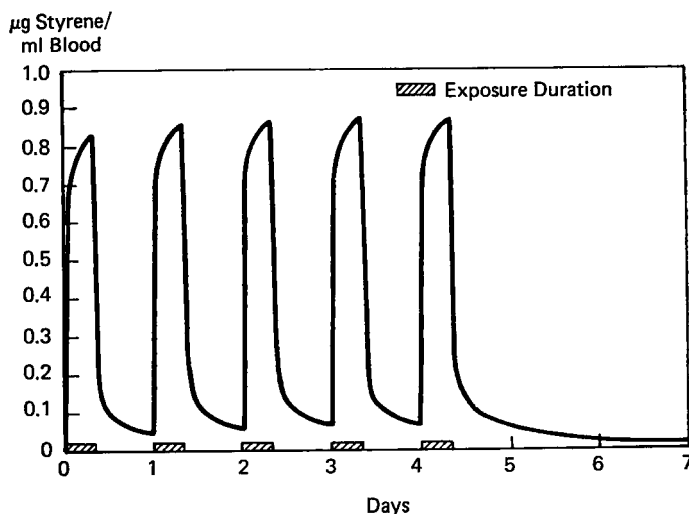


Fig. 5. Simulated blood concentration of styrene in rats for five daily 8-h exposures to 80 ppm.

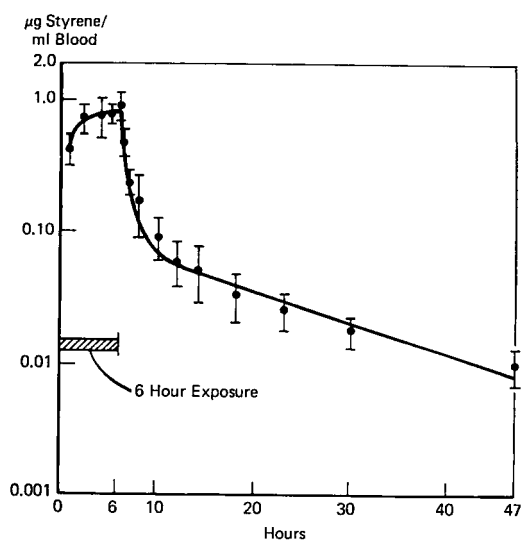


Fig. 6. Blood styrene concentration in humans exposed to 80 ppm for 6 h.

Table 1. Areas under curves (AUC) (0–24 h) for the styrene blood concentration in rats exposed to 80, 200, 600 or 1,200 ppm for 6 h.

Exposure concentration (ppm)	AUC (µg.h/ml)	Normalized	
		Exposure concentration	AUC
80	4.87	1.0	1.0
200	8.97	2.5	1.8
600	152	7.5	31
1,200	546	15	112

linear model, as was shown previously in rats. The solid line in fig. 6 is the theoretical blood concentration obtained from the model parameters, and it demonstrates an excellent fit to the observed data.

In the case of humans, the average minute volume at rest and the absorption of 66 % (1, 2) of the inspired styrene yield an assumed uptake rate of approximately 100 mg/h. The best fit of the blood data to the two-compartment model yielded the following parameter estimates: a half-life value of 0.58 ± 0.08 h for the rapid (α) clearance phase and 13.0 ± 0.7 h for the slower (β) clearance phase. The average multiple correlation coefficient for the four individuals was 0.98.

We used the pharmacokinetic parameters for humans to simulate five daily 8-h exposures to 80 ppm styrene, followed by a 2-d clearance period over the weekend. The results of the simulation are shown in fig. 7. After one 8-h exposure the blood concentration was within 95 % of the predicted steady-state value of $0.9 \mu\text{g/ml}$.

The concentration of styrene in expired air after the exposure is shown in fig. 8. The general shape of the concentration versus time curve was similar to that in blood. By computing the area under the curve, we calculated that an average of approximately 60 μmoles of styrene were cleared from the body in expired air during this time period.

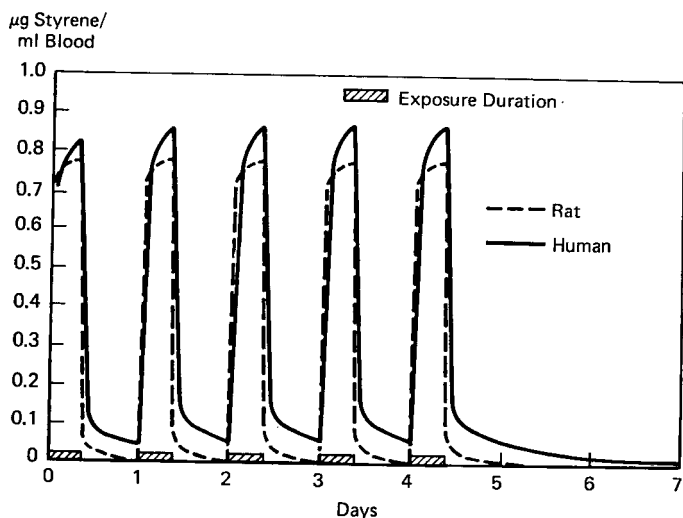


Fig. 7. Simulated blood concentration of styrene in humans for five daily 8-h exposures to 80 ppm.

The average accumulated quantity of both mandelic and phenylglyoxylic acids excreted in the urine during and after the 6-h exposure was 2,320 μ mole. The quantity of styrene excreted in the expired air compared to the quantity of styrene excreted in the urine, as these two metabolites revealed that, during the post-exposure period, a total of only 2 to 3 % of the styrene cleared from the body was cleared as styrene in the expired air, and the remainder was cleared by metabolism.

DISCUSSION

These studies show that, at concentrations of up to at least 80 ppm, inhaled styrene is cleared from the body of both rats and humans in a manner that is consistent with a two-compartment linear pharmacokinetic model. The pharmacokinetic parameters describing the uptake, distribution, and clearance of styrene in rats and humans are quite similar, as is shown in fig. 9. This figure is a superposition of the predicted blood concentration of styrene in rats and in humans exposed to 80 ppm styrene for 8 h per day for a 5-d week. At least 95 % of the predicted maximum concentrations of styrene in blood of 0.8 and 0.9 μ g/ml in rats and humans, respectively,

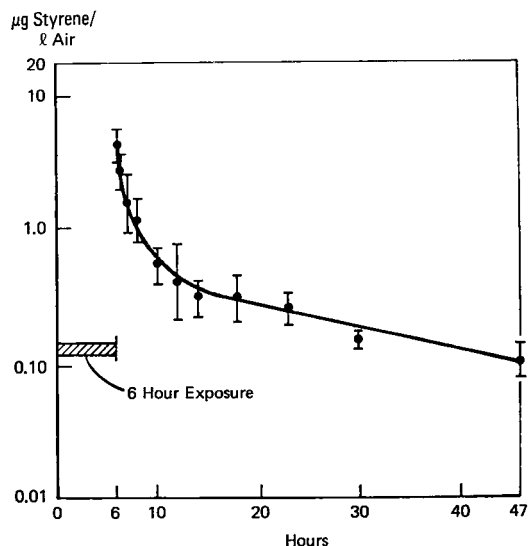


Fig. 8. Styrene concentration in expired air of humans after 6-h exposure to 80 ppm.

are reached after only one 8-h exposure.

The major urinary metabolites of styrene reported in this experiment, mandelic and phenylglyoxylic acids, are the same as those reported by other investigators (3, 4, 6). Since only 60 μ mol of styrene were cleared from the body unchanged in expired air during the post-exposure period, when 2,020 μ mol were excreted as urinary metabolites, it is ap-

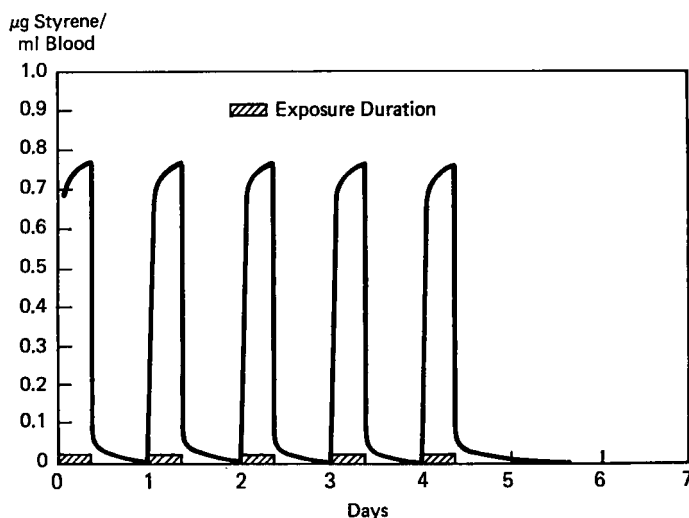


Fig. 9. Simulated blood concentration of styrene in rats and humans for five daily exposures to 80 ppm.

parent that styrene is cleared mostly (at least 97 %) by the metabolic route.

In the case of rats exposed to 200 ppm, the uptake and clearance of styrene were described by the same two-compartment linear pharmacokinetic model as at 80 ppm, and no continuous accumulation of styrene would be expected at this exposure concentration. However, at exposure levels of 600 and 1,200 ppm of styrene, the maximum blood concentrations and the shape of the clearance curves of styrene demonstrate that the elimination of styrene became saturated.

Since the area under a curve (AUC) of blood concentration versus time is the product of the concentration of styrene $\times$ time, it is a measure of the integrated dose and can serve as a relative measure of the toxicologic impact that might be expected from different exposure levels. Table 1 shows a comparison of the AUC for rats at all four exposure levels. The data in the last two columns of table 1 have been normalized to the 80 ppm exposure level.

With an increase of 2.5 in exposure concentration, from 80 to 200 ppm, the AUC increased by a factor of 1.8, somewhat less than the expected value of 2.5. However, at 600 ppm, where the exposure level was increased by a factor of 7.5, the AUC increased by a factor of 31, or almost four times more than expected. It is in this range of exposure levels (between 200 and 600 ppm) that the transition from linear to saturated clearance of styrene occurs. At 1,200 ppm, where the exposure concentration was increased by a factor of 15, the AUC increased by 112 times or approximately 8 times in excess of the expected value. These relationships demonstrate that, although the rat can metabolize styrene very effectively at levels of up to at least 200 ppm, the metabolic clearance capacity can be overwhelmed (saturated) at higher exposure levels.

The data in fig. 4 show that fat comprises the peripheral compartment in the two-compartment model for styrene. Since the ratio of the concentration of styrene in fat to that in blood increased from approximately 40:1 at 80 ppm to approximately 80:1 at 1,200 ppm, the capacity of fat for styrene was apparently not satu-

rated, even at 1,200 ppm. Since styrene is cleared from the body mostly by metabolism, the saturation of styrene clearance is more likely due to saturable metabolic clearance rather than to saturation of storage capacity in fat tissue.

In summary, these data show that styrene is cleared from the body in both rat and man in a nearly identical manner, and that the rat provides a reasonable pharmacokinetic model for styrene in humans at exposure levels of up to at least 80 ppm. In both species, styrene is cleared from the body mainly by metabolism (and to a much lesser extent by expiration) at a rate that is directly proportional to its blood concentration. This linear clearance process predicts that styrene will not continue to accumulate on repeated exposure unless the uptake rate is high enough to saturate the metabolic clearance mechanism. This saturation occurs at exposure levels above 200 ppm in the rat. When this clearance capacity becomes saturated, the toxic consequences may be disproportionately greater than expected on the basis of exposure levels alone.

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QUESTIONS AND ANSWERS

Questions to Dr. RAMSEY

- Prof. IKEDA: Will you give me your guess of what concentration might be a metabolic breaking point in human exposure to styrene vapor?
- Dr. RAMSEY: My guess is that metabolic saturation probably occurs at greater than 200 ppm in humans. And since exposure of humans to 300 or 400 ppm causes unpleasant symptoms such as irritation, these might be practical upper exposure limits.
- Dr. LEHTNIEMI: How have you eliminated absorption of styrene via skin, especially at high ppm levels (800—1,200 ppm)? Could it affect scope and saturation? How great do you estimate the amount absorbed via skin at 100 ppm, 200 ppm and 1,000 ppm is? Stores in keratin?
- Dr. RAMSEY: We have not considered skin absorption of styrene. Probably skin absorption at high levels would have a negligible effect on excretion kinetics and saturation levels. I think the amount absorbed through the skin at these levels is less than a few percent of that absorbed via inhalation.