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Urinary elimination of styrene in experimental and occupational exposure

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PEZZAGNO G, GHITTORI S, IMBRIANI M, CAPODAGLIO E. Urinary elimination of styrene in experimental and occupational exposure. *Scand J Work Environ Health* 11 (1985) 371–379. Twenty human volunteers were exposed to styrene vapor at 273–1 654 $\mu\text{mol}/\text{m}^3$ (28.4–172.3 mg/m^3) for a period of 1 to 3 h at rest (15 cases) and during light physical exercise (5 cases). Subsequently 51 workers occupationally exposed to styrene were studied during a workweek (median value 1 138 $\mu\text{mol}/\text{m}^3$, geometric standard deviation 2.23). As expected, the relative uptake averaged about 65 %, and the ratio of the alveolar concentration to the time-weighted average of the environmental concentration averaged about 0.15. Both in the experimentally exposed subjects and in the occupationally exposed workers the urinary styrene concentration showed a linear relationship to the corresponding environmental time-weighted average concentration. The correlation coefficients of the regression lines ranged between 0.88 (occupationally exposed group) and more than 0.93 (experimentally exposed groups). The regression coefficients were closely linked to the amount of styrene taken up and to the exposure times. The findings show that the urinary styrene concentration can be used as an appropriate biological exposure indicator whose meaning differs from that of other suggested indices. As an example, in occupationally exposed subjects performing moderate work the urinary styrene concentration corresponding to the time-weighted average of the threshold limit value is 815 nmol/l , and the 95 % lower confidence limit (biological threshold) is 740 nmol/l .

Key terms: biological monitoring, styrene, urinalysis.

In industry styrene is a widely used solvent which can cause several toxic effects (28). It is a volatile compound entering the body mainly through the lungs or skin (8, 31). General studies have been devoted to its uptake (1, 37), distribution in body tissues (6, 10), and elimination as urinary metabolites (mandelic and phenylglyoxylic acids) (11, 12, 21, 28). It is a substance easily soluble in the blood and biological tissues and therefore probably accumulates with each consecutive exposure (9, 10).

For biological monitoring, determination can be made of the concentration of the parent (1, 5, 36) or metabolized compounds (1, 11, 28) in alveolar or expired air samples, in venous or arterial capillary blood samples, and in urine samples. Determination of its uptake during exposure has also been proposed as an exposure indicator (13).

More than 90 % of the styrene taken up in the organism is excreted in urine as metabolites (28). Elimination via the lungs is less than 3 % (6, 10, 13). Small amounts of styrene are eliminated as such by means of biological fluids (urine, sweat) (7).

In previous studies carried out during experimental and occupational exposure to certain solvents, the

relationship between the time-weighted environmental concentration and the urinary concentration of solvents as such (nonmetabolized amount) has been studied. The results suggest that the concentration of the solvent in a sample of urine (produced during the exposure period) could be considered an appropriate indicator of exposure from which the biological indices corresponding to the time-weighted average (TWA) of the threshold limit value (TLV) could be derived (17, 23, 29).

The main aim of our study was to verify the relationship between the urinary styrene concentration produced during a period of controlled exposure and the corresponding mean environmental concentration in the breathing zone.

Subjects

Experiments were performed in a laboratory on healthy volunteer subjects (stage 1) and directly on the work premises on groups of subjects occupationally exposed (stage 2).

Stage 1

Twenty subjects, 14 males and 6 females (23 to 51 years of age), were exposed, in an open exposure chamber (16), to styrene at different concentrations but constant for each subject under study. The total group was divided into four five-subject subgroups. For each subgroup different exposure means were chosen as fol-

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Figure 1. Scheme applied during the experimental chamber exposure (stage 1) (A) and during occupational exposure (stage 2) (B) to styrene; 1st, 2nd, 3rd and 4th refer to the four subgroups considered. (See the text.) The arrows indicate the collecting times of environmental, alveolar, mixed-expired air, and pulmonary ventilation measurements (arrows in the postexposure period refer to collecting times of alveolar samples only); asterisks refer to collecting time of personal samples (stage 2, B); a = bladder emptying, immediately before exposure; b = urine sample collection; c = venous sample collection; and dotted areas = periods of physical exercise (in A), and lunch break (in B).

lows: subgroup 1: 1-h exposure at rest, subgroup 2: 2-h exposure at rest, subgroup 3: 3-h exposure at rest, and subgroup 4: exposure during light physical exercise and alternately at rest (15-min work load of 50 W on a bicycle ergometer, 15 min at rest, four times each during 2 h of exposure) (figure 1 A).

The subjects went into the exposure chamber immediately after emptying their bladder. At the end of the chosen exposure period a urine sample was collected (for the precautions taken, see the Methods section) from which to measure the styrene concentration (C_u).

Samples of chamber air, alveolar air, and mixed-expired air (the last sample being taken from the subjects of the first three subgroups only) were collected at regular intervals of 20 min. Pulmonary ventilation was measured simultaneously with the expired air sampling. Finally, immediately after the exposure, a venous blood sample was taken (from a brachial vein).

For all the subjects of the four subgroups, the sampling of alveolar air continued for 10 min after the end of the exposure (at 30 and 150 s and then at 10 min) (figure 1 A).

Stage 2

The second stage of the study involved 51 male subjects (average age of the group 44 years) employed at a plastic boat factory (first factory) and in a plastic button factory (second factory). Shifts were 8 h daily (0800–1600, with a half-hour lunch break). The oc-

cupational exposure included, in addition to styrene, also acetone at a concentration of $2801 \mu\text{mol}/\text{m}^3$ (median value) (geometric standard deviation = 1.95) (corresponding to $162.3 \text{ mg}/\text{m}^3$).

Urine was collected from all the subjects at 0800, 1200, and 1600. A total of 101 samples was taken (figure 1 B). In addition, during the workday, the environmental concentration of styrene (breathing zone) was measured by means of personal passive samplers (25).

The complete exposure period was monitored for each subject with four consecutive dosimeters and from calculations of the time-weighted individual value (C_i) from the four determinations.

Methods

Urine collection and styrene urinary concentrations

During stages 1 and 2 the urine was collected from all the subjects in the same manner in 120-ml glass vials (Supelco Vials) with airtight plugs (without silicone). The vials were equipped with a valve (Minimert valve Supelco) enabling a headspace gas sample of styrene to be withdrawn over the urine after partial pressure equilibration. The volume of urine was measured by weight, starting from the urine density.

The subjects voided rapidly in areas known to be unpolluted. (Significant variations in C_u do not occur if collection time is less than 1 min.) The vials were then stored at 37°C for 2 h and shaken periodically

to speed the separation and partial pressure equilibrium of the styrene vapor between the urine and the headspace air over it. After at least 1 h a headspace air sample was analyzed by means of a gas chromatograph (HP 5880 A) plus a mass selective detector (HP 5970 A). The analytical conditions for the gas chromatograph were as follows: cross-linked column 5 % phenyl methyl silicone (internal diameter 0.2 mm, length 25 m), column temperature 150°C, carrier gas helium with a flow rate of 1 ml/min, retention time of styrene 2 min 10 s, sampler volume (kept at 37°C) 0.5 ml. (The headspace air sample was transferred to the gas chromatograph sampler by means of a Hamilton gastight syringe.) The analytical conditions for the mass selective detector were: monitored ion 104, dwell time 50 ms, selected-ion-monitoring window time 0.1 amount mass units, electromultiplier voltage 1 800 V.

From the headspace styrene concentration it is easy to obtain the urinary concentration if the λ urine/air value and the volume of the two phases [urine volume (V_u) and headspace air volume (V_a)] are known. [Once a balance between the two phases of the vial has been reached, the volume of dissolved solvent (x) is divided between V_u and V_a proportionally to λ (Ostwald partition coefficient) (x_1 and x_2 are the two volumes of solvent distributed to V_u and V_a , respectively): $x = x_1 + x_2$ and $\lambda = [(x_1/V_u)/(x_2/V_a)]$ (concentration ratio, by definition). Thus: $x = \lambda \cdot x_2 \cdot (V_u/V_a) + x_2 = x_2 [\lambda \cdot (V_u/V_a) + 1]$, and $(x/V_u) = (x_2/V_u) \cdot [\lambda \cdot (V_u/V_a) + 1]$ (standard in air), where x_2 is the measured amount of styrene in the headspace. The λ urine/air of styrene, at 37°C, is 7.13 (17).]

Determination of venous blood concentration

Similar to what was described for the C_u measurement, in the experimental exposure only, the heparinated venous blood sample (5 ml) was kept at 37°C in similar airtight vials (volume 13.5 ml) and shaken periodically for the determination of the venous blood

concentration (C_v). The analysis of the headspace samples and the blood concentration determination followed the same modalities described with regard to the C_u determination. The λ blood/air value used was 52 (32).

Alveolar and mixed-expiratory air sampling for the styrene concentration

In the experimental exposure only, the alveolar (C_A) and mixed-expired (C_E) air samples were collected every 20 min during the exposure at the same time as the collection of environmental air from the chamber and the pulmonary ventilation measurement (V). Three additional air samples were collected at the end of the exposure (after 30 and 150 s and after 10 min).

The open-circuit apparatus is schematized in figure 2. It had a oneway valve (through which the subject under study breathed), the inspiratory end of which was equipped with a pulmonary ventilation analyzer (ultrasonic spirometer LS 75 Bourns), and the expiratory end of which had a fourway tap allowing the expiratory flow to pass across an alveolar sampler and an expiratory air sampling bag, a plastic bag whose inner wall is an aluminum foil barrier (5 l of volume) which opens outside but is kept closed by means of a oneway valve letting the extra expired air out (Multi-layer gas sampling bag, Calibrated Instruments). The bag was provided with a device to draw the air samples either into a gastight syringe to be analyzed or to the outside directly. The entire expiratory part of the circuit was placed in a metal container (kept at 37°C).

The alveolar sampler was a metal cylindrical device in which an alveolar air sample can be trapped and from which the sample can be partly taken out to be analyzed (22). Alternatively the alveolar sampling can be made by means of an airtight, heated syringe set up on the expiratory end of the circuit (figure 2).

The C_A and C_E measurements were made with the same gas chromatograph and mass selective detector

Figure 2. Apparatus employed to collect alveolar (C_A) and mixed-expired air samples (C_E) during exposure to styrene and to measure the pulmonary ventilation (V). (A = mouthpiece, B = ultrasonic spirometer, C = inspiratory valve, D = expiratory valve, E = gastight syringe to collect alveolar air samples, F = fourway tap, G = aluminum foil bag to collect mixed-expiratory air samples, H = alveolar air sampler, I = outside connection, L = electric resistance + thermostat, M = thermometer, N = heated box)

Table 1. Alveolar concentration: time-weighted environmental concentration ($C_A:C_i$) ratio throughout the experimental exposure (first, second, and third subgroups). The regression equation is the following: $C_A = 0.12 \cdot C_i + 16.2$ (correlation coefficient = 0.85) (with C_A and C_i given in micromoles per liter).

Subgroups	Number of cases	5 min		30 min		60 min		90 min		120 min		150 min		180 min	
		$C_A:C_i$ ratio	SD	$C_A:C_i$ ratio	SD	$C_A:C_i$ ratio	SD	$C_A:C_i$ ratio	SD	$C_A:C_i$ ratio	SD	$C_A:C_i$ ratio	SD	$C_A:C_i$ ratio	SD
1 st + 2 nd + 3 rd 2 nd + 3 rd 3 rd	15 10 5	16.2	4.7	14.4	3.7	15.7	5.8	16.3	6.2	13.2	2.6	14.1	3.9	12.2	3.9

Table 2. Single values of the time-weighted average of the environmental concentration (C_i), the urinary concentration (C_u), (urine sampled immediately after exposure), the venous blood concentration (C_v) (blood withdrawn immediately before the end of exposure), and the uptake of styrene (U) during exposure. The last column shows the regression equation relating C_u and C_i .

Subgroup	C_i ($\mu\text{mol}/\text{m}^3$)	C_u (nmol/l)	C_v (nmol/l)	U ($\mu\text{mol}/\text{time}$)	Regression equation
First					
1	516	69	713	187	$C_u = 0.11 \cdot C_i + 15.2$ ($r = 0.97$)
2	273	43	448	75	
3	1 035	149	1 441	340	
4	1 375	157	1 790	265	
5	1 037	128	1 787	203	
Second					
6	662	76	904	345	$C_u = 0.16 \cdot C_i - 2.6$ ($r = 0.93$)
7	388	64	842	286	
8	917	202	2 289	319	
9	1 654	251	3 225	974	
10	383	48	609	119	
Third					
11	281	45	401	193	$C_u = 0.17 \cdot C_i + 20.4$ ($r = 0.95$)
12	526	106	1 095	206	
13	535	115	1 156	336	
14	916	212	2 005	496	
15	1 495	247	3 045	943	
Fourth					
16	758	188	2 601	935	$C_u = 0.26 \cdot C_i + 21.7$ ($r = 0.95$)
17	547	167	2 194	666	
18	1 096	297	3 210	1 427	
19	1 105	350	4 381	1 401	
20	343	129	1 543	356	

and under the same analytical conditions used for the urine samples.

Time-weighted average of the environmental concentration measurement in the breathing zone

The activated charcoal of passive dosimeters, used in stage 2 only, were deabsorbed with 5 ml of carbon disulfide and then kept at room temperature (20°C) for 1 h and shaken periodically. Finally 0.5 μl of the deabsorption liquid was injected into the gas chromatograph-mass selective detector unit.

Results

Stage 1

The average concentration in the exposure chamber (C_i) ranged from 273 to 1 654 $\mu\text{mol}/\text{m}^3$ [the conversion factor from milligrams to micromoles: 1 mg = 9.6 μmol (of styrene)] for the various subjects. During the exposure the chosen values for each subject were maintained at a relatively constant level (SD 23 $\mu\text{mol}/\text{m}^3$).

The alveolar concentration value (C_A) was also found to be consistent for all the subjects up to the third exposure hour with a $C_A:C_i$ ratio close to 0.15 (table 1).

The relative uptake (R) (measured from C_i and C_E) [$R = 1 - (C_E/C_i)$] was 0.68 [coefficient of variation (CV) $\pm 2.9\%$] in the subjects of the first three subgroups (at rest). In the subjects of subgroup 4 (for which no C_E measurements were made), the relative uptake was determined from the $C_A:C_i$ ratio values [$R = -0.86 \cdot C_A:C_i + 79.3$ (9)], it was 0.64 (CV $\pm 2.5\%$).

The mean pulmonary ventilation ($\dot{V}$) [under standard conditions (0°C, 101.3 kPa, dry)] was 6.3 l/m (CV $\pm 13\%$) in the subjects at rest and 14.8 l/m (CV $\pm 21\%$) in the subjects of subgroup 4.

Alveolar clearance (Cl_A) [under standard conditions (0°C, 101.3 kPa, dry)] for styrene, where $Cl_A = \dot{V} \cdot R$, was found to be 4.2 l/m (in the first three subgroups) and 9.4 l/m (in subgroup 4) (CV ± 7.1 and $\pm 21\%$, respectively).

The amounts absorbed (total uptake) during the exposure time ranged from 75 to 1 427 μmol (table 2).

The TWA environmental concentrations (C_i) and the urinary (C_u) and blood (C_v) concentrations obtained for the 20 subjects at the end of exposure are shown in table 2, together with the uptake (U) and exposure time. The C_u values displayed a statistically significant linear relationship to the corresponding C_i (or U), and the C_A or C_v values were also statistically significant:

$$C_A = 0.12 \cdot C_i + 16.20$$

(correlation coefficient 0.85) ($N = 85$),

$$C_v = 2.9 \cdot U + 431$$

(correlation coefficient 0.86) ($N = 15$), and

$$C_u = 0.23 \cdot U + 47.3$$

(correlation coefficient 0.83) ($N = 15$),

where C_A and C_i are expressed in micromoles per cubic meter and C_v and C_u in nanomoles per liter and U in micromoles.

The mean alveolar concentration (C_A) of the entire group of subjects was $112 \mu\text{mol}/\text{m}^3$. During the first 10 min following the end of the exposure the C_A values at 30, 150, and 600 s were 19, 16, and $11 \mu\text{mol}/\text{m}^3$, respectively, corresponding to a decrease of 83, 86, and 90 % of the average end-exposure value ($108 \mu\text{mol}/\text{m}^3$).

Stage 2

The same relationship between C_i and C_u observed experimentally also existed for the group of subjects occupationally exposed to styrene vapor (figure 3):

$$C_u = 0.32 \cdot C_i + 154.2 \quad (N = 101, r = 0.884),$$

where C_i and C_u are expressed in micromoles per cubic meter and nanomoles per liter, respectively.

Discussion

The starting values of the relative uptake (R) and relative alveolar concentration ($C_A:C_i$) of styrene depend on their λ blood : air ratios; our results ($R \approx 0.66$, $C_A:C_i \approx 0.15$) are consistent with those of previous investigations (1, 3, 10, 15, 37, 38). The R and $C_A:C_i$ curve (1) during exposure depends on the possibility of the biological tissues to store solvent and on the rate of its biotransformation; our results, in accordance with those already published (1, 13) show a practically constant curve for R and $C_A:C_i$ throughout the entire exposure time (therefore at least up to the end of the third hour) in the range of the environmental concentration values chosen (273 – $1654 \mu\text{mol}/\text{m}^3$ or 28.4 – $172.2 \text{ mg}/\text{m}^3$).

The main aim of this report was, however, to determine if correlation relationships existed between styrene exposure standards (C_i) and the concentration of styrene in samples of urine produced throughout the exposure time (C_u).

Previous attempts at analyzing styrene as such in urine were unsuccessful due to the inaccuracy of the

Figure 3. Scatter diagram relating the time-weighted average of the environmental concentration (in the breathing zone) (C_i) (x axis) and the urinary concentration (C_u) (y axis) of styrene. The C_i values ranged from 130 and $5156 \mu\text{mol}/\text{m}^3$ (median value $1138 \mu\text{mol}/\text{m}^3$; geometric standard deviation 2.23). The regression line is also drawn.

analytical procedures (3). Highly sensitive equipment is now available to solve the problem.

The slope of the regression lines was different in the four subgroups considered. It was lowest in subgroup 1 (1-h exposure at rest) (regression coefficient 0.11) and highest in subgroup 4 (2-h exposure spent partly at rest, partly performing light physical exercise of 50 W) (regression coefficient 0.26); 2 and 3 h of exposure at rest were characterized by intermediate slopes (regression coefficients 0.16 and 0.17, respectively) (figures 4 and 5).

The difference in the slope between the equations obtained after 1 and 2 h of exposure (0.11 against 0.16) was significantly higher than that between 2 and 3 h of exposure (0.16 against 0.17). This occurrence was probably due to a continuous increase in styrene concentration in arterial blood from the beginning of exposure up to 1 h or more later (6, 10, 37, 38).

The urinary concentrations should be in fact proportional to those in the arterial blood, with a proportional coefficient corresponding to the λ blood/urine value, on the assumption that the exchange of the solvent between the arterial blood and urine occurs after simple partition in accordance with Henry's law.

In this case, once a constant arterial concentration value has been reached, the slope of the regression lines should be independent of exposure time. The small slope difference of the regression lines corresponding to 2 and 3 h of exposure (figure 5) should mean that an increment in the blood concentration is still occurring throughout the third hour of exposure or that a stable level of urinary concentration is not rapidly reached, a phenomenon similar to what happens to the alveolar (constant) and arterial concentration (increasing up to over 75 min of exposure) (37, 38).

The greatest slope of the regression line in subgroup 4 (regression coefficient 0.26) was due to the work

Figure 4. Regression lines drawn on scatter diagrams relating the time-weighted average of the environmental concentration (in the breathing zone) (C_T) [x axis] and the urinary concentration (C_U) [y axis] of styrene (urine collected immediately after the exposure was over). [A = subgroup 1 (1-h exposure, at rest); B = subgroup 2 (2-h exposure, at rest); C = subgroup 3 (3-h exposure, at rest); D = subgroup 4 (2-h exposure during light physical exercise and alternately at rest)]

load. Under such conditions, R values being equal, the amounts of styrene taken up are clearly larger (depending on pulmonary ventilation), and greater solvent concentrations are therefore reached in arterial blood (10), and consequently greater urinary concentrations as well.

The four regression lines start practically from the origin of the Cartesian coordinates (figure 5) (intercepts of the y axis between -2.6 and 21.7 nmol/l) because the subjects under study had never been exposed to styrene previously.

A close relationship between the TWA environmental concentration in the breathing zone (C_T) and the urinary concentration of styrene in urine samples collected after 4 h of exposure also existed for the group of occupationally exposed subjects (correlation coefficient 0.88), the regression coefficient being 0.32.

The regression line does not start from the origin of the Cartesian coordinates but intercepts the y axis at 154 nmol/l (figure 3 and 6); this finding indicates that at the beginning of the exposure period there was some styrene in the blood of the subjects under study, as expected and previously documented (9, 10).

The urinary concentration (C_U) corresponding to the TLV-TWA ($2\,065 \mu\text{mol}/\text{m}^3$) is 815 nmol/l (figure 6). To propose a biological exposure limit — based on 4 h of occupational exposure and on conditions of a light work load — it is necessary to consider the corresponding 95 % lower confidence limit of the regression line (biological threshold 740 nmol/l). To compare the single urinary concentration values with the chosen threshold (for a decision of compliance or non-

compliance occupational exposure),³ it is advisable to consider the one-sided lower (LCL) and upper (UCL) confidence limits (at 95 %) [$\text{UCL (95 \%)} = C_U + 1.645 \cdot CV_T \cdot \text{TLV}$, $\text{LCL (95 \%)} = C_U - 1.645 \cdot CV_T \cdot \text{TLV}$, where CV_T is the coefficient of variation of the method and the factor 1.645 is the 95th percentile of the standardized normal distribution] of each single determination of urinary concentration starting from the total coefficient of variation of the method (26) (figure 6).

The biological indicators presently proposed to evaluate the occupational exposure to styrene are (1, 2, 5, 11, 18, 19): (i) the urinary concentration of mandelic and phenylglyoxylic acid, (ii) the styrene concentration in alveolar or mixed-expired air, and (iii) the styrene concentration of venous or arterial capillary blood. The proposed threshold values are 1 g (6.57 mol)/l for mandelic acid and/or 250 mg (1.66 mol)/l for phenylglyoxylic acid in urine (where the urine was collected at the end of the workshift), 40 ppb and 18 ppm of styrene in mixed-expired air (before and during the workshift, respectively), and 0.02 mg/l and 0.55 mg/l for styrene in venous blood (before and during the workshift, respectively) (2).

The results obtained both experimentally in volunteers exposed in an exposure chamber and in groups

³ Compliance exposure: 95 % confidence based on measurements that a worker's exposure is below the standard; non-compliance exposure: 95 % confidence that a worker's exposure is above the standard; possible overexposure: any exposure which cannot be classified as compliance or non-compliance exposure.

Figure 5. Regression lines relating the time-weighted average of the environmental concentration (in the breathing zone) (C_i) and the urinary concentration of styrene (C_u); A B C D refer to the four subgroups considered. See figure 4 for an explanation of the groups. Arrows indicate the C_u extrapolated values at the time-weighted average of the threshold limit value for styrene ($2065 \mu\text{mol}/\text{m}^3 = 215 \text{ mg}/\text{m}^3$) (C_u biological exposure limit: A = $242 \text{ nmol}/\text{l}$, B = $328 \text{ nmol}/\text{l}$, C = $371 \text{ nmol}/\text{l}$, D = $559 \text{ nmol}/\text{l}$)

of subjects occupationally exposed enable us to include also the styrene urinary concentration in the already proposed biological exposure indice. With regard to the meaning and the value used to ascribe to the various biological indicators proposed, it should be pointed out that the blood (C_a , C_a) and air (C_a , C_a) measurements are instantaneous values, while the unchanged urinary styrene (C_u) and styrene urinary metabolites are weighted values. However they are not indicators of the same exposure time. In fact the metabolic indicator phase depends on the rate of biotransformation. Its metabolites can also be classified as effect indicators. On the contrary the unchanged styrene urinary elimination during exposure indicates a natural integration over time of the rather fast partition between air and arterial blood and between arterial blood and urine (the bladder serving as a collecting and mixing vessel).

Besides, the situations of highest uptake of styrene (environmental concentration being equal) by subjects working under different work loads are clearly indicated by the urinary concentration values. On the contrary it is well known that, mainly with highly soluble solvents (like styrene), increases in pulmonary ventilation do not bring about proportional increases in alveolar concentration (1, 37). Thus C_a values correlate

Figure 6. Regression line relating the time-weighted average (TWA) of the environmental concentration (in the breathing zone) (C_i) and the urinary concentration of styrene (C_u) for occupationally exposed subjects (urine collected every 4 h of exposure); the corresponding regression equation is the following: $C_u = 0.32 \cdot C_i + 154.2$ (correlation coefficient = 0.88; $N = 101$). [TLV: threshold limit value; a: C_u value at $C_i = 2065 \mu\text{mol}/\text{m}^3$ (TLV-TWA); b: 95 % lower confidence limit = biological exposure limit; c: hypothetical value of C_u in an occupationally exposed subject; d: one-sided upper confidence limit (at 95 %) of C_u ; e: one-sided lower confidence limit (at 95 %) of C_u ; classification system: (i) $d < b$ (or $d/b < 1$) = compliance exposure, (ii) $e > b$ (or $e/b > 1$) = noncompliance exposure, and (iii) any individual who cannot be classified in i or ii = possible overexposure]

well with C_i values (5) but not with the amounts of styrene taken up.

Finally it is to be pointed out that the unchanged styrene urinary concentration should not be affected either by other solvents interfering with each other in the biotransformation rate or by the presence of common metabolites (38). Last, the method is simple, quickly performed, and noninvasive.

The exchange of styrene between renal arterial blood and urine could occur (similar to what is believed to take place at the alveolocapillary level) by means of a simple partition mechanism between these two fluids regulated by the λ blood/urine value; in this case a pressure equilibrium should be reached with $P_a = P_u$ (20) (and then $C_a/C_a = \lambda$ blood/air; $C_u/C_u = \lambda$ blood/urine). Such a hypothesis has already been advanced (existence of an alveolourinary inert gas partial pressure equilibrium) and employed in the field of pulmonary physiology (4, 24, 30).

The mean value of C_u in the group of subjects exposed for 3 h at rest (subgroup 3) was $145 \text{ nmol}/\text{l}$, and the mean value of C_a in the same group was $103 \mu\text{mol}/\text{m}^3$.

The experimental value of the $C_a:C_a$ ratio corresponded to the in vitro λ blood/urine value (37). Therefore the styrene arterial concentration (C_a) (at

equilibrium) is likely to be about $C_A \cdot \lambda$, ie, in this case, 5 350 nmol/l. [No direct measurement of the styrene concentration in arterial blood was made because the introduction of a catheter into an artery was not included in the acceptance protocol of the volunteers and because reference data are already available (1, 12, 37, 38).]

On the assumption that the $C_a:C_u$ ratio corresponds to the in vitro λ blood/urine value ($\lambda = 7.29$) (17), the value of C_u in the last parts of the urine, filtered towards the third hour of exposure, should be 735 nmol/l; the time-weighted value of C_u , of the entire urine sample filtered throughout the 3 h of exposure, was 145 nmol/l. Such a value is not in contrast with the hypothesis advanced. Moreover none of the C_u values suggested an active mechanism of renal styrene elimination.

In conclusion, analogous to what has been observed for other substances [n-hexane (23), toluene (29), p-dichlorobenzene (17), methyl ethyl ketone (27), nitrous oxide (34, 35), and methanol (14,33)], we believe that also for styrene the measurement of urinary concentration is a useful biological indicator of exposure.

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