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ORIGINAL ARTICLE

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Stereochemical metabolism of styrene in volunteers

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Abstract Objectives: To study the stereochemistry of styrene metabolism in volunteers, and its interindividual variability. **Methods:** Twenty healthy male volunteers (aged 18–37 years) were exposed to 360 mg/m³ styrene for 1 h while they performed 50 W physical exercise. Venous blood was drawn during and for up to 2 h after exposure. Urine was collected at time-intervals up to 24 h after exposure. The following parameters were determined: styrene, free and conjugated styrene glycol (SG) in blood, and conjugated SG, mandelic acid (MA) and phenylglyoxylic acid (PGA) in urine. **Results:** Average pulmonary retention of styrene was 62%. Excretion of the acidic metabolites MA and PGA accounted for 58% of the pulmonary uptake. The average maximum concentration ($C_{\max}$) and area under the curve (AUC) of free (*R*)-SG in blood were 1.3 and 1.7 times higher than those of (*S*)-SG respectively; the half-life of (*R*)-SG was longer (82 vs 62 min, $P < 0.005$). $C_{\max}$ and AUC of the conjugated SG enantiomers in blood did not differ, but again half-life for (*R*)-SG was longer (72 vs 64 min, $P < 0.05$). Cumulative excretion and renal clearance of conjugated (*S*)-SG in urine were three and four times higher, respectively, than that of (*R*)-SG. Cumulative excretion of (*S*)-MA was 1.6 times higher than (*R*)-MA. Interindividual differences in the kinetic parameters of the metabolites were two- to threefold. **Conclusions:** The enantiomeric excess found was different for each metabolite under study, implying different enantioselectivity and/or enantiospecificity of the enzymes and carrier-proteins involved in the biotransformation and excretion. The use of these metabolites as

biological indicators for prediction of the enantiomeric excess of the toxic metabolite styrene-7,8-oxide (SO) is therefore not justified. Interindividual differences in the stereochemical metabolism of styrene are moderate.

Key words Styrene · Stereoisomerism · Styrene glycol · Mandelic acid · metabolism

Introduction

Styrene is a solvent widely used in industry that can cause several adverse effects, including skin and pulmonary irritation at acute exposure, and central nervous system depression after acute as well as chronic exposure. The most extensive exposure to styrene occurs in plants manufacturing glass-reinforced plastics. Entry of styrene into the body occurs mainly via inhalation, and styrene metabolism takes place principally in the liver, although extrahepatic tissues may contribute. The first step in the major metabolic pathway is formation of styrene-7,8-oxide (SO) by cytochrome P450. SO is thereafter hydrolysed to styrene glycol (SG) by microsomal epoxide hydrolase (mEH); this metabolite is subsequently oxidised to mandelic acid (MA) and phenylglyoxylic (PGA) acid. These acids are the main urinary metabolites of styrene in humans, and account for 90% of the absorbed dose of styrene [1, 12].

Styrene is not chiral itself, but the first metabolite, SO, possesses an asymmetric centre and thus exists as (*R*)- and (*S*)-enantiomers. SG and MA also possess a chiral C-atom, but PGA is optically inactive. The chronic toxicity of styrene is mainly attributable to the reactive metabolite SO [4, 19, 20], and several studies have shown that a difference in toxicity between the enantiomers of SO exists. The (*R*)-enantiomer showed to be four times more mutagenic to *Salmonella typhimurium* TA 100 in the Ames test than the (*S*)-enantiomer [27, 29]. The (*R*)-enantiomer was also more toxic to lung and liver in mice in vivo [11]. However, another study showed that the (*S*)-enantiomer caused more sister

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chromatid exchanges in vivo in mice than did the (*R*)-enantiomer [30]. Hence, due to the different toxic potential of the SO enantiomers, determination of the racemic metabolites of styrene, which is the present practice, might not truly reflect the health risk associated with exposure to styrene. A number of studies have addressed the stereometabolism of styrene, but most of them have been performed in vitro [3, 5, 6, 31] or in vivo in laboratory animals, e.g. rats and mice [7, 8, 21, 22, 32]. The few studies performed in humans only measured enantiomers of MA [10, 13, 18]. The question arises, however, whether the enantiomeric composition of MA is representative of that of the ultimate toxic metabolite SO. The metabolism of SO to MA is mediated by a number of enzymes, some of which are known to be stereoselective, i.e. mEH.

Since SO levels in blood are very low in humans, SG in blood has been suggested by Löf et al. [24] as a biomarker to reflect the levels of SO. In analogy, one could speculate whether the *R/S* ratio of SG in the blood reflects the enantiomeric ratio of SO. Others have suggested the use of *R/S* ratios of MA or conjugated SG in urine as indicators of the enantiomeric ratio of SO [18]. Direct measurement of SO in blood has been reported, although the enantiomeric composition was not measured [19, 20].

The enantiomeric ratio of styrene metabolites showed considerable interindividual variation [13]. Consequently, persons with a metabolic preference for one of the stereochemical forms could be at higher risk of the toxic effects of SO after styrene exposure. Interindividual variability in the stereochemical metabolism and kinetics of styrene has so far only been quantified in a limited number of styrene-exposed workers, not in volunteers under strictly controlled experimental conditions.

The aim of the present study was, therefore, to gain more insight into the stereochemical metabolism of styrene in humans, and its interindividual variability. For that purpose, we exposed 20 volunteers to styrene by inhalation, and monitored the stereochemistry of the blood and urinary metabolites (SG, MA) of styrene.

Methods

Subjects

The volunteers were 20 healthy men, ranging in age from 18–37 years (average 23.3). The body mass index (weight/height²) varied from 18 to 25 kg/m² (average 21.7). The subjects had not been occupationally exposed to solvents, and none had suffered from any respiratory or circulatory disease. Blood was taken for clinical chemistry analyses and haematological screening. All participants were non-smokers, two were alcohol-abstainers and 13 consumed more than 50 g ethanol/week. They were asked to abstain from alcohol during and also 48 h before the study. Each person was informed of the possible effects of styrene, and signed an informed consent prior to the beginning of the study. The study was evaluated and approved by the Medical Ethical Committee of the Amsterdam Medical Center and was performed according to the Declaration of Helsinki 1964.

Exposure to styrene

The volunteers were exposed to 360 ± 20 mg/m³ for 1 h. During exposure, they performed light physical exercise, 50 W, on a bicycle ergometer. The exposures were conducted in an open exposure chamber of 8 m³ volume, with an air-turnover rate of six times/h. Styrene vapour was generated by bubbling a constant flow of medical air through the solvent in two glass bottles connected in series. The solvent vapour was supplied at the fresh-air side of the ventilation system. The concentration of solvent in the chamber was continuously monitored with a Miran 1B infrared analyser. In order to determine the pulmonary ventilation and concentration of exhaled styrene, we collected exhaled air in a gas-sampling bag for 2 min at four time-periods during exposure. For the calculations the average value was used. Venous blood samples (two 7 ml-EDTA containers) were collected at several fixed time-points during exposure and up to 2 h after the end of exposure for determination of styrene, SO and SG. One tube was kept on ice until analysed, to prevent loss of styrene. Blood from the second tube was immediately extracted with hexane (2 × 3 ml), to prevent rapid decomposition of SO in blood [14]. Urine was collected before the exposure and at five time-intervals, 0–2, 2–4, 4–8, 8–12 and 12–24 h, after the end of exposure.

Analytical methods

For the determination of styrene in exhaled air, we withdrew 1 ml of air from the gas-sampling bag with a gas-tight syringe, and injected it into an HP5890 GC with flame-ionisation detection (FID) and a DB1701 (30 m, 0.53 mm, JW Scientific) column. To determine the styrene concentration in blood, we transferred 200 µl of blood into a 4-ml vial and heated it for at least 30 min at 50 °C. Then, 250 µl of headspace air was injected and analysed under the same conditions as styrene in air. *m*-Xylene was used as internal standard. For the determination of free SG, 1.5 ml of blood was extracted with 5 ml of ethyl acetate. Before extraction, phenyl-propylene glycol was added as internal standard. The conjugated SG was determined in the same sample of blood after removal of the ethyl acetate phase and subsequent incubation at 37 °C overnight with β -glucuronidase and sulfatase from *Helix pomatia* in 0.1 M acetate buffer, pH 4.7. The released SG was extracted with ethyl acetate. Ethyl acetate extracts, from both the free SG and conjugated SG samples, were evaporated to dryness and the residue derivatised with 20 µl of pentafluoropropionic anhydride (PFPA) and 0.5 ml of 1.5% pyridine in dichloromethane. Derivatives were analysed using gas chromatography (GC) with electron capture detection and a chiral column (CP Chirasil-Dex, 25 m, 0.25 mm ID, Chrompack, the Netherlands). Free and conjugated SG in urine were analysed similarly to those in blood. Free SG content in urine was less than 5% of the total SG content. MA and PGA in urine were determined by gas chromatography with FID according to Kežić et al. [15].

Calculation of kinetic parameters

Alveolar ventilation was calculated from the pulmonary ventilation after correction for the anatomical dead-volume of the lungs. We calculated the respiratory intake rate of styrene by multiplying the styrene concentration in air by the alveolar ventilation [26]. We calculated the pulmonary uptake by multiplying the difference between the inhaled and the average exhaled concentration of styrene by the pulmonary ventilation. The kinetic parameters for styrene and SG in blood, i.e. half-life ($t_{1/2}$) and maximum concentration (C_{max}), were obtained from the blood concentration-time course. We calculated the apparent terminal elimination rate constant, k , using the least-squares analysis on the terminal portion of the semi-log blood concentration versus time curves. The $t_{1/2}$ was calculated by dividing $\ln 2$ by k . The area under the curve (AUC) from time zero to 180 min was calculated by a trapezoidal method (AUC_{0–180}). The extrapolation to time infinity (AUC_{0–∞}) was

determined by dividing the blood concentration at 180 min by k ($AUC_{180-\infty}$) and adding the result to the corresponding AUC_{0-180} .

We calculated the clearance (CL) of styrene by dividing the uptake by the $AUC_{0-\infty}$. The renal clearance (CL_r) of SG could only be calculated for the first time-period of urine sampling. We did this by dividing the amount of conjugated SG in urine at the first time-point of urine sampling by the AUC of conjugated SG in blood until that time point.

Cumulative excretion of conjugated SG in urine, (*R*)-MA, (*S*)-MA and PGA was calculated by the summation of the amounts of each metabolite excreted in each sampling period.

Statistical analysis

We checked normal distributions of all parameters with the Komogorov-Smirnov and Shapiro-Wilk test, and by graphically displaying the data in histograms and boxplots. Kinetic data of the (*R*)- and (*S*)-enantiomers of all metabolites were compared using Student's *t*-test for paired samples.

Results

Kinetic parameters of styrene are presented in Table 1. The pulmonary uptake of styrene during 1 h of exposure at 50 W was 3.0 mmol. Average pulmonary retention was 62%. The C_{max} of styrene in blood (C_{max} , STY) at the end of exposure was 26.7 ± 5.9 $\mu\text{mol/l}$. The elimination of styrene appeared to be biphasic. Half-lives were calculated by the method of residuals from semi-log plots, resulting in a linear initial phase with slope α and a linear terminal phase with slope β . The average $t_{1/2}$, β was 65 ± 12 min. In Fig. 1 the blood concentration profiles of free (*R*)-SG and (*S*)-SG are shown. The kinetic parameters derived from the blood concentration profiles are summarised in Table 2. The C_{max} , $AUC_{0-\infty}$ and the $t_{1/2}$ were significantly higher for the (*R*)-enantiomer ($P < 0.005$). Interindividual differences in *R/S* ratio were moderate, twofold for $t_{1/2}$ (range 1.0–2.0) and threefold for the C_{max} (range 0.8–2.2) and AUC (range 0.9–3.0). All volunteers, with the exception of one, had a higher C_{max} and AUC for the (*R*)-enantiomer.

In Fig. 2 the blood concentration profiles of the enantiomers of conjugated SG are shown. Calculated as $AUC_{0-\infty}$, conjugated SG amounted to 21.6% of the total blood SG. C_{max} and $AUC_{0-\infty}$ did not differ between the enantiomers. The difference in the half-lives of the enantiomers was small but statistically significant, with

Table 1 Kinetic parameters from 1 h of styrene exposure to 360 mg/m^3 in volunteers during physical exercise with a workload of 50 W ($n = 20$) (C_{max} , STY maximum concentration of styrene in blood, $t_{1/2}$, β terminal half-life of styrene, CL clearance)

Kinetic parameter	Mean $\pm$ SD
Alveolar ventilation (l/min)	21.4 ± 3.5
Intake (mmol)	4.9 ± 0.8
Uptake (mmol)	3.0 ± 0.7
Pulmonary retention (%)	62 ± 7
C_{max} , STY ($\mu\text{mol/l}$)	26.7 ± 5.9
$t_{1/2}$, β (min)	65 ± 12
CL (l/min)	1.4 ± 0.4

Fig. 1 Blood concentration-time profiles of free styrene glycol enantiomers in volunteers during and after 1 h of exposure to 360 mg/m^3 styrene and 50 W physical exercise. ■ (*R*)-styrene glycol, □ (*S*)-styrene glycol. Average of 20 volunteers, errors bars indicate SEM

conjugated (*S*)-SG having the shorter $t_{1/2}$ ($P = 0.04$, Table 3). However, in urine, cumulative excretion of conjugated (*S*)-SG was much higher than (*R*)-SG (Fig. 3). The mean CL_r was 3.8 times higher for the (*S*)-enantiomer than for the (*R*)-enantiomer (Table 4).

Urine excretion profiles of the MA enantiomers and PGA are presented in Fig. 4 and kinetic parameters are shown in Table 5. No MA or PGA was found in urine samples taken before the onset of exposure. Cumulative excretion of (*S*)-MA was significantly higher than of (*R*)-MA ($P < 0.005$). However, half-lives were not significantly different (3.7 vs 3.9 h). The $t_{1/2}$ of PGA was considerably longer than that of MA, 9.6 ± 3.2 h. For three volunteers $t_{1/2}$ of PGA could not be calculated, due to extreme outliers. Total excretion of the acidic metabolites amounted to 58% of total uptake of which 39% was MA and 19% PGA. Interindividual differences in the *R/S* ratio of the cumulative excretion and the $t_{1/2}$ were twofold.

Discussion

To our knowledge, this is the first extensive study in volunteers on the stereochemical fate of styrene metabolites in blood and urine. The overall kinetics of styrene and its metabolites are in good agreement with previous studies [23, 28, 35]. We were not able to measure SO, because the hexane used for extraction of the blood samples was contaminated with small amounts of SO.

SG in blood was measured as free and conjugated fraction. The $AUC_{0-\infty}$ of conjugated SG in blood amounted to 22% of total SG, which is in close agreement with the data of Löf et al. [23]. To our knowledge, this is the first study to report on the stereochemical course of SG, which is the first and main metabolite of SO. The shorter $t_{1/2}$ of free (*S*)-SG in blood indicates that the further metabolism of this enantiomer to MA

Table 2 Kinetic parameters of the free styrene glycol (SG) enantiomers in blood and the *R/S* ratio after volunteers were exposed to 360 mg/m³ styrene during 1 h and 50 W of physical exercise (*n* = 20) (*C*_{max} maximum concentration of free styrene glycol in blood, *AUC* area under the curve, *t*_{1/2} half-life)

	(<i>R</i>)-SG Mean ± SD	(<i>S</i>)-SG Mean ± SD	<i>R/S</i>	
			Mean ± SD	Range
<i>C</i> _{max} (μmol/l)	1.6* ± 0.6	1.3 ± 0.7	1.3 ± 0.4	0.8–2.2
<i>AUC</i> _{0–∞} (μmol min/l)	223* ± 118	144 ± 88	1.7 ± 0.5	0.9–3.0
<i>t</i> _{1/2} (min)	82* ± 22	61 ± 13	1.4 ± 0.3	1.0–2.0

*Significantly different from (*S*)-enantiomer, determined by Student's paired *t*-test, *P* < 0.005

Fig. 2 Blood concentration-time profiles of conjugated styrene glycol enantiomers in volunteers during and after 1 h of exposure to 360 mg/m³ styrene and 50 W physical exercise. ■ (*R*)-styrene glycol, □ (*S*)-styrene glycol. Average of 20 volunteers, errors bars indicate SEM

Fig. 3 Cumulative urinary excretion of conjugated styrene glycol enantiomers in volunteers after 1 h of exposure to 360 mg/m³ styrene and 50 W physical exercise. ■ (*R*)-styrene glycol, □ (*S*)-styrene glycol. Average of nine volunteers, errors bars indicate SEM

and/or conjugated SG is faster than that of (*R*)-SG. The higher *C*_{max} and *AUC* found for the (*R*)-enantiomer could therefore be explained by this faster metabolism of (*S*)-SG. However, several other explanations for the higher *C*_{max} and *AUC* of (*R*)-SG may be possible, i.e. (1) a higher rate of formation of (*R*)-SG by mEH than of (*S*)-SG, (2) product enantioselectivity of cytochrome P450, favouring (*R*)-SO, or (3) non-enantiospecific metabolism of SO to SG, i.e. (*S*)-SO is metabolised to (*R*)-SG. In vitro studies showed that cytochrome P450 has a small preference for the formation of (*S*)-SO [3, 31, 34], thereby making explanation (2) less likely. In addition, they showed that mEH-mediated hydrolysis of (*S*)-SO proceeds enantiospecifically [2, 33], making explanation (3) implausible. Although in the above mentioned studies hydrolysis of SO was shown to proceed enanti-

oselectively, favouring hydrolysis of (*R*)-SO, it is not very likely that differences in rate are reflected in the *AUC*. Therefore, enantioselectivity in mEH-mediated hydrolysis cannot be an explanation for the observed higher *AUC* of (*R*)-SG. It is possible, however, that differences in rate of hydrolysis influence *C*_{max}. Nevertheless, the *R/S* ratio of *C*_{max} and *AUC* of free SG in blood seems to be mainly a reflection of the elimination of the free SG enantiomers, and not that of the formation of the SO enantiomers.

In contrast to free SG, hardly any difference between the (*R*)- and (*S*)-enantiomers of conjugated SG in blood was observed. However, in urine, cumulative excretion of (*S*)-SG was 3.3 times higher than that of (*R*)-SG. Korn et al. [18] also reported a higher concentration of the (*S*)-enantiomer than of the (*R*)-enantiomer in urine

Table 3 Kinetic parameters of the conjugated styrene glycol (SG) enantiomers in blood and the *R/S* ratio after volunteers were exposed to 360 mg/m³ styrene during 1 h and 50 W of physical exercise (*n* = 20) (*C*_{max} maximum concentration of conjugated styrene glycol in blood, *AUC* area under the curve, *t*_{1/2} half-life)

	(<i>R</i>)-SG Mean ± SD	(<i>S</i>)-SG Mean ± SD	<i>R/S</i>	
			Mean ± SD	Range
<i>C</i> _{max} (μmol/l)	0.4 ± 0.2	0.4 ± 0.2	1.0 ± 0.2	0.6–1.3
<i>AUC</i> _{0–∞} (μmol min/l)	51 ± 25	50 ± 24	1.0 ± 0.2	0.6–1.5
<i>t</i> _{1/2} (min)	72* ± 29	64 ± 26	1.2 ± 0.2	0.6–1.6

*Significantly different from (*S*)-enantiomer, determined by Student's paired *t*-test, *P* = 0.04

Table 4 Kinetic parameters of the conjugated styrene glycol (SG) enantiomers in urine and the *S/R* ratio after volunteers were exposed to 360 mg/m³ styrene (*n* = 9) (*CL_r*, renal clearance)

	(S)-SG Mean ± SD	(R)-SG Mean ± SD	<i>S/R</i>	
			Mean ± SD	Range
Cumulative excretion (μmol)	11.7* ± 4.0	3.5 ± 1.1	3.3 ± 0.6	2.5–4.3
<i>CL_r</i> (ml/min)	196* ± 101	52 ± 26	3.8 ± 0.6	2.6–4.6

* Significantly different from (*S*)-enantiomer, determined by Student's paired *t*-test, *P* < 0.005

Fig. 4 Cumulative urinary excretion of mandelic acid (MA) enantiomers and phenylglyoxylic acid (PGA) in volunteers after 1 h of exposure to 360 mg/m³ styrene and 50 W physical exercise. ■ (*R*)-mandelic acid, □ (*S*)-mandelic acid, △ phenylglyoxylic acid. Average of 20 volunteers, error bars indicate SEM

collected at the end of workshifts of persons occupationally exposed to styrene. On the basis of this difference in ratio they concluded that the epoxide hydrolase system favours the (*S*)-enantiomer, consequently leading to a longer persistence of the (*R*)-enantiomer of SO in the organism. However, this would only be true if all other pathways, e.g. conjugation of SG and renal handling of the two enantiomers, showed no enantioselectivity. Our data show that *CL_r* of the two enantiomers differs almost fourfold, strongly indicating different renal handling of these two enantiomers. The differences in the *CL_r* between the enantiomers might be explained on the basis of their excretion mechanism. A *CL_r* between 125 and 625 ml/min, as found for the (*S*)-enantiomer, indicates tubular secretion in addition to glomerular filtration (and reabsorption). A *CL_r* below 125 ml/min, as found for the (*R*)-enantiomer, indicates only glomerular filtration and reabsorption and no

tubular secretion. Glomerular filtration and reabsorption are passive processes, while tubular secretion is an active process involving carrier binding, which can be an enantioselective determinant [25]. Therefore, in our opinion, the enantiomeric ratio of SG in urine collected at the end of the workshift cannot be used as an indicator of the enantioselectivity of mEH.

The cumulative excretion of MA in urine was 1.6 times higher for the (*S*)-enantiomer than for the (*R*)-enantiomer. Similar values have been found in other studies, in humans [16] as well as in rats [21] and mice [22]. It has been suggested in those studies that this higher excretion of (*S*)-MA could be due to product-enantioselectivity of the oxidation of styrene to SO. However, it must be kept in mind that MA can only reflect CYP450 product-enantioselectivity if several assumptions hold true: the metabolism of SG to MA proceeds in an enantiospecific way, *CL_r* for both MA enantiomers is equal, and there is no stereochemical preference in the metabolism of MA to PGA. Little is known about the stereochemistry of these metabolic steps, certainly in humans. SG is assumed to be enantiospecifically metabolised to MA [17], but this assumption can be questioned on the basis of studies in rats [21] and mice [22]. Renal clearance of the MA enantiomers has not been investigated so far, but several organic acids are known to be excreted by tubular secretion [25], and on the basis of our *CL_r* data for conjugated SG, differences in tubular excretion between the MA enantiomers might be expected. To avoid differences in the enantiomeric ratio that are due to enantioselective formation or enantioselective *CL_r* of MA, the ratio of MA enantiomers could be determined in accumulated urine (24 h or longer). However, caution should still be taken in interpreting these ratios, since in rats an enantioselective oxidation of (*S*)-MA to PGA has been observed [9], which could seriously compromise findings. Interindividual differences in the enantiomeric ratios of

Table 5 Kinetic parameters of mandelic acid (MA) enantiomers and phenylglyoxylic acid (PGA) in urine, and the *S/R* ratio after volunteers were exposed to 360 mg/m³ styrene (*n* = 20)

	(S)-MA Mean ± SD	(R)-MA Mean ± SD	<i>S/R</i>		PGA Mean ± SD
			Mean ± SD	Range	
Cumulative excretion (μmol)	717* ± 336	453 ± 196	1.63 ± 0.3	1.2–2.3	586 ± 247
<i>t</i> _{1/2} ^a (hours)	3.7 ± 0.8	3.9 ± 0.8	1.0 ± 0.1	0.6–1.2	9.6 ± 3.2

* Significantly different from (*S*)-enantiomer, determined by Student's paired *t*-test, *P* < 0.005

^a *n* = 20 for MA; *n* = 17 for PGA

the metabolic parameters were never more than three-fold. For the urine data (SG and MA) these differences never exceeded a factor of 2 and the (*S*)-enantiomer was always higher. This means that the processes determining these ratios in blood and urine, i.e. metabolism and renal elimination, were relatively constant among the volunteers. Accordingly, the additional risk due to differences in stereometabolism might be relatively unimportant in humans.

In conclusion, we have studied the stereochemical pathway of styrene metabolism in order to gain insight into the stereochemical determinants of styrene metabolism, and to find a biomarker which would reflect the enantiomeric composition of SO in blood. As shown in this study, none of the determined parameters, in blood or urine, seems to be a good indicator of the enantiomeric ratio of SO. The enantiomeric ratios of the determined metabolites are dependent on a number of variables such as enantioselectivity of cytochrome P450, enantiospecificity of enzymes involved in the conversion of SG to MA, stereochemistry of phase II conjugating enzymes, stereochemistry of oxidation of MA to PGA, and renal excretion of the urinary metabolites. Much more information would be needed on the stereochemistry of the kinetic steps in humans, by the performance of either in vitro or in vivo studies, before a reliable biomarker for enantiomeric composition of SO can be established. It would also be important to improve the analytical methods for routine determination of SO enantiomers, to enable direct measurement of the SO enantiomers.

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