Solvents are complex chemical mixtures containing many different hydrocarbon types, such as alkanes, alcohols, ketones, aldehydes, esters, ethers, and small aromatic molecules, that evaporate and become incorporated into environmental air as volatile organic compounds (VOCs). Although the percentage of each chemical in solvent formulations varies among commercial brands, toluene is the most abundant ingredient (Mercado-Calderón 1997). Products used in many industrial workshops and in the home contain potentially hazardous solvents. Therefore, people working in such industries or at home risk continuous exposure to VOCs released from solvents.

Toluene is a systemic toxicant that affects liver, kidney, and the central nervous system (Agency for Toxic Substances and Disease Registry (ATSDR) 2000), the last being considered the main target organ (Byrne et al. 1991). Toluene biotransformation takes place in the liver, kidney, and the central nervous system involved in mediating occupational chemical exposure effects, and to search for earlier and more sensitive biomarkers that will improve occupational safety (Mutti 1999). Consequently, exposure to toluene in an occupational setting could represent an increased risk of toxicity. CYP2E1 polymorphisms may also modulate this condition, although the extent to which 5′-flanking region RsaI/PstI polymorphisms alter induction is not clear (Wormhoudt et al. 1999).

Because of the significant health risks associated with high levels of solvent exposure, routine monitoring of exposed subjects and their work environment is necessary to ensure good occupational health and safety conditions. It is thus advisable to perform studies at multiple levels to elucidate the mechanisms involved in mediating occupational chemical exposure effects, and to search for earlier and more sensitive biomarkers that will improve occupational safety (Mutti 1999). Accordingly, several studies have been reported with the aim of assessing environmental and health conditions of VOC-exposed workers (Apostoli et al. 1982; Wallen 1986); CYP2E1 phenotype determination in such populations may provide additional information.

Chlorzoxazone (CHZ) is a centrally acting muscle relaxant drug that has been used extensively as a selective probe for CYP2E1 phenotyping; this isoform is the main enzyme involved in its 6-hydroxylation (6OH-CHZ) in humans (Lucas et al. 1999). However, because CHZ clinical prescription is no longer available in Europe and is under revision in the United States (Lucas et al. 2001), it should not be used as a routine monitoring probe (Tanaka et al. 2003). Therefore, alternative methods to assay CYP2E1 phenotype in vivo are being investigated. Raucy et al. (1997) reported a relationship between CYP2E1 activity in peripheral blood lymphocyte microsomes and plasma CHZ metabolic ratio (6OH-CHZ/CHZ quotient; CYP2E1 metabolism indicator) in alcoholic subjects. This association suggests that peripheral lymphocytes and liver are regulated similarly and could be advantageous for monitoring purposes (Gonzalez-Jasso et al. 2003). Little information, however, is available about the effects of environmental VOCs on CYP2E1 regulation in human peripheral lymphocytes. Consequently, we investigated the association of toluene exposure with both CYP2E1 mRNA content in peripheral lymphocytes and CHZ 6-hydroxylation in occupationally exposed subjects. We further assessed the contribution of the 5′-flanking region RsaI/PstI polymorphism in measured lymphocyte CYP2E1 mRNA content and CHZ 6-hydroxylation.

Materials and Methods

Study design. A cross-sectional study was performed with print industry workers in Mexico City. The study population consisted of

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OCCUPATIONAL TOLUENE EXPOSURE INDUCES CYTOCHROME P450 2E1 mRNA EXPRESSION IN PERIPHERAL LYMPHOCYTES

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Print workers are exposed to organic solvents, of which the systemic toxicant toluene is a main component. Toluene induces expression of cytochrome P450 2E1 (CYP2E1), an enzyme involved in its own metabolism and that of other protocarcinogens, including some precarcinogens. Therefore, we investigated the association between toluene exposure and the CYP2E1 response, as assessed by mRNA content in peripheral lymphocytes or the 6-hydroxycortezoxazone (6OH-CHZ)/chlorzoxazone (CHZ) quotient (known as CHZ metabolic ratio) in plasma, and the role of genotype (5′-flanking region RsaI/PstI polymorphic sites) in 97 male print workers. The geometric mean (GM) of toluene concentration in the air was 52.80 ppm (10–760 ppm); 54% of the study participants were exposed to toluene concentrations that exceeded the maximum permissible exposure level (MPPE). The GM of urinary hippuric acid at the end of a work shift (0.041 g/g creatinine) was elevated relative to that before the shift (0.027 g/g creatinine; p < 0.05). The GM of the CHZ metabolic ratio was 0.33 (0–9.3), with 40% of the subjects having ratios below the GM. However, the average CYP2E1 mRNA level in peripheral lymphocytes was 1.07 (0.30–3.08), and CYP2E1 mRNA levels within subjects correlated with the toluene exposure ratio (environmental toluene concentration:urinary hippuric acid concentration) (ρ = 0.014). Genotype did not alter the association between the toluene exposure ratio and mRNA content. In summary, with further validation, CYP2E1 mRNA content in peripheral lymphocytes could be a sensitive and noninvasive biomarker for the continuous monitoring of toluene effects in exposed persons.

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103 unrelated male print workers employed as flat or rotary printing press operators (24%), operator assistants (33%), ink handlers (22%), machine maintenance personnel (mechanics and electricians) (13%), or supervisors (8%). Workers were exposed to a commercial non-benzene-containing organic solvent, composed of a complex mixture of VOCs, of which toluene and xylene were the main constituents. Inclusion criteria for participants were 2-fold: a) only clinically healthy males with normal hepatic function test values were included, and b) only those not taking drugs that alter CYP2E1 activity (e.g., acetaminophen, isoniazid, disulfiram, chlorothiazide, or chloroquine) were included (Frye and Branch 2002; Park et al. 1993; Simi and Ingelman-Sundberg 1999).

Workers were contacted through the National Coordination of Health at the Workplace (Instituto Mexicano del Seguro Social) and received a thorough explanation of the aims and procedures of this investigation; participants signed an informed consent form (response ratio, 85%). Subjects were directly interviewed by trained personnel in order to record clinical, occupational, and sociodemographic characteristics, tobacco and alcohol consumption, and use of personal protection equipment. In addition, the body mass index (BMI) was calculated for each subject as the quotient of body weight divided by the square of the height (Brown 1995). Subjects were instructed to avoid smoking and alcohol consumption for 5 days before the sample collection (Plee-Gautier et al. 2001).

The CINVESTAV-IPN Ethical Committee approved this investigation protocol.

**Quantitation of toluene levels in the environment.** Personal monitors measured toluene environmental levels in the indoor air of working areas. Each worker wore a Radiello passive diffusion monitor (code 130; Fondazione Salvatore Maugeri, Padua, Italy) placed within the respiratory area, for an 8-hr work shift. To extract absorbed solvent molecules, 2 mL carbon disulfide was applied to the workplace (Instituto Mexicano del Seguro Social) for 30 min and given an occasional shake. Extracts were analyzed by gas chromatography/mass spectrometry, using a Finnigan chromatograph (San Jose, CA, USA) equipped with a PONA capillary column (50 m × 0.25 mm inner diameter × 0.25 µm film thickness; stationary phase, 5% phenylmethyl silicone) and a flame ionization detector (FID). Run conditions were as follows: initial temperature was 40°C; then the temperature was increased at an initial ramp of 10°C/min to 150°C and a second ramp of 15°C/min to 180°C (2 min). The injector (splitless mode) temperature was 200°C, the FID temperature was 250°C, and the carrier gas (helium 99.998%; Praxair, Mexico City, Mexico) was sustained at a constant flow rate of 35 mL/min. We identified and quantified the chemicals present in the extract samples by comparison with standards (ChemService, West Chester, PA, USA) (Cocheo 1983).

**Biologic sampling.** Blood samples were collected from each subject’s radial vein and placed in tubes with or without EDTA for lymphocyte isolation and CHZ metabolism evaluation or liver function tests (serum), respectively. Urine samples were obtained at the beginning and end of an 8-hr work shift and used to measure hippuric acid levels. Liver function was assessed in serum by measuring alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transferase activities (Bergmeyer and Horder 1980). Creatinine was measured in urine by the Jaffe reaction method (Bartels and Bohmer 1972).

**Quantitation of urinary hippuric acid.** We measured urinary hippuric acid according to the recommendations of the National Institute for Occupational Safety and Health (NIOSH 1994). Briefly, we acidified a 1-mL urine sample by adding 40 µL hydrochloric acid, saturated the solution with 0.3 g sodium chloride, and then extracted hippuric acid by combining the solution with 4 mL ethyl acetate and shaking the mixture for 5 min. After centrifugation at 3,000 rpm for 10 min, the organic layer was evaporated under a nitrogen stream until dry and then redissolved in 1 mL distilled water. Recovery percentage was calculated by addition of a hippuric acid standard to urine samples; this control calculation with the standard revealed a 95% mean recovery rate.

Urine extracts were analyzed by high-performance liquid chromatography (HPLC) with a Perkin Elmer 235C chromatograph (PerkinElmer, Norwalk, CT) equipped with a diode array detector and a 20 µL loop. Samples were separated in a 250 mm × 5 mm Spherisorb Ps phaseb column packed with 5 µm C18 reversed phase (Phase Separation Inc., Norwalk, CT, USA). The mobile phase was a mixture of acetonitrile and 0.2% glacial acetic acid in water (10:90 vol/vol) and was maintained at a flow rate of 1 mL/min. The wavelength setting was 287 nm. This procedure achieved a 65% mean recovery rate for CHZ and 6OH-CHZ, and the CHZ metabolic ratios were reported.

**Evaluation of peripheral lymphocyte CYP2E1 mRNA content.** Isolation of total RNA. Lymphocytes were separated from 7 mL of whole blood as described by Boyum (1968). Briefly, blood diluted 1:1 with saline solution was layered over 5 mL Lymphoprep (Nycomed Pharma AS, Oslo, Norway). After centrifugation at 1,600 rpm for 25 min at 25°C, we transferred the cell interface to a new tube, washed it twice with saline solution, and then centrifuged it at 1,200 rpm for 10 min at room temperature. Finally, the supernatant was discharged, and cells were prepared for RNA extraction treatment. Total RNA was extracted using TRizol reagent (Gibco BRL, Life Technologies, Rockville, MD, USA) following the manufacturer’s instructions. Extracted RNA samples were suspended in diethylpyrocarbonate-treated water (20 µL). Total RNA was spectrophotometrically quantified, and its integrity was visualized by electrophoresis on 1.5% agarose gels stained with ethidium bromide.

**Preparation of standard RNA.** A set of primers was designed based on the human CYP2E1 cDNA sequence (GenBank accession number NM000773; National Center for Biotechnology Information, Bethesda, MD, USA) to amplify a 443-bp fragment of the CYP2E1 cDNA. The forward primer CYP2E1F (5'-ACAGGGACAGGGAATCAT-3') is located at the junction of exons 2 and 3, and the reverse primer CYP2E1R (5'-TGGGGTCCTCAGATTGATG-3') is located within exon 5. Using modified
versions of these primers, we constructed a standard RNA (recRNA) of the same length and sequence as endogenous CYP2E1 mRNA, except for a 71-bp deletion in the middle of the sequence. Construction of this competitor was accomplished in two steps. In the first step, primers T7CYP2E1 (5′-TGGGGTCCAGAGATTGATGCAGG-ACGACTCACTATAGGAAG-CAAGTAGTGTAGAAAGGAATCAT3′) and recRNA (5′-TGGGGTCCAGAGATTGATGCAGG-ACGACTCACTATAGGAAG-CAAGTAGTGTAGAAAGGAATCAT-3′) were used to amplify a cDNA from human peripheral blood lymphocytes. Primer T7CYP2E1 contains the T7 promoter sequence at the 5′-end of primer CYP2E1F (underlined sequence), and primer recRNA corresponds to CYP2E1R with the exception of a mismatching stretch of 20 bp located at the 3′-end (underlined sequence). In the human CYP2E1/cDNA sequence, the 20-bp stretch maps 98 bp upstream from the CYP2E1R sequence, but in the recRNA, primer both sequences were linked together to allow the further generation of the deleted polymerase chain reaction (PCR) fragment (Figure 1). The PCR product was purified with a column (Freeze ‘N Squeeze DNA gel extraction spin column; BioRad Laboratories, Hercules, CA, USA). In the second step, recRNA derived from the PCR fragment containing the T7′ promoter sequence was prepared by in vitro transcription with a T7 Transcription Kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer’s instructions. The recRNA was subsequently treated with RNase-free DNase (Boehringer Mannheim, Mannheim, Germany) to remove DNA template. recRNA was quantified by measuring its absorbance at 260 nm.

Quantitative reverse transcriptase (RT)-PCR. Endogenous RNA and recRNA were co-reverse transcribed and amplified simultaneously. Reverse transcription reactions were performed in a final volume of 21 µL containing 16 mM Tris-HCl (pH 8.4), 40 mM KCl, 0.4 mM dithiothreitol, 4 mM MgCl₂, 0.4 mM of each deoxynucleotide triphosphate, 0.5 µg oligo(dT), 40 U recombinant ribonuclelease inhibitor RNaseOUT (Invitrogen, Carlsbad, CA, USA), and variable amounts of cellular RNA and recRNA. Sample solutions were incubated at 65°C for 10 min, 42°C for 50 min, and 70°C for 15 min. Fifty units of Superscript II reverse transcriptase was then added to each sample, and the samples were incubated at 42°C for 50 min and 70°C for 15 min. The reaction was terminated by rapid chilling on ice and by adding 4 U Escherichia coli RNase H (Invitrogen). The cDNA samples were amplified with primers CYP2E1F and CYP2E1R in 25 µL PCR buffer (100 ng of each primer, 1 U Taq polymerase, 200 mM Tris-HCl, pH 8.4, 500 mM KCl, 50 mM MgCl₂, and 0.2 mM dNTP mixture), using the following PCR conditions: an initial denaturing step at 95°C for 5 min followed by 37 cycles of amplification that consisted of a denaturing step at 95°C for 30 sec, an annealing step at 56°C for 30 sec, and an extension step at 72°C for 30 sec. The

**Table 1.** Anthropometric characteristics and habits of the study population.

| Variable                  | Frequency or value                  |
|---------------------------|-------------------------------------|
| No.                       | 97                                  |
| Age (years)a              | 34.2 ± 9.8 (18–63)                  |
| Years employedb           | 3.5 ± 1.6 (1–9)                     |
| BMI (kg/m²)c              | 26.98 ± 4.15 (20.38–40.03)          |
| Smoking status (%)d       | Nonsmoker 42 (43)                   |
| Smoker                    | 55 (57)                             |
| Drinking status (%)       | Never 8 (8)                         |
| Seldom                    | 79 (82)                             |
| Frequent                  | 9 (10)                              |

*Expressed as mean ± SD (range). **Expressed as number of individuals (percentage of population).
reaction was terminated with a final extension step at 72°C for 5 min. All reactions were carried out in a Thermocycler GeneAmp PCR System 2400 (Applied Biosystems, Foster City, CA, USA). The RT-PCR reaction yielded a 453 bp fragment from endogenous RNA and a 382 bp fragment from recRNA (Figure 2). The CYP2E1 mRNA ratio was obtained by dividing the intensity of the endogenous mRNA band by the intensity of recRNA band.

**DNA sequencing.** To verify the authenticity of cellular and standard PCR products, they were sequenced in an ABI PRISM 310 Genetic Analyzer using the ABI PRISM Big Dye DNA sequencing kit (Applied Biosystems).

**CYP2E1 genotype.** CYP2E1 genotype was assessed by restriction fragment length polymorphism analysis for the 5’-flanking region RsaI/PstI polymorphic sites (Figure 1), according to the method described by Hayashi et al. (1991).

**Statistical analysis.** Differences between initial and final hippuric acid concentrations were compared by Student’s t-test. Log transformations improved normalcy for CYP2E1 activity and urinary hippuric acid concentrations. Simple and multiple linear regression analyses were carried out to determine whether there were any associations between toluene exposure and CYP2E1 phenotype (enzymatic activity or mRNA content). These relationships were stratified by RsaI/PstI polymorphic site alleles. Multiple regression models were adjusted by age, BMI (classified as normal, overweight, and obesity), current drug consumption (categorized as yes or no), smoking habit (smokers or nonsmokers), and alcohol consumption (ranked as never, <1 serving/week; seldom, 1 to 2 servings/week; frequent, >2 servings/week). All statistical analyses were conducted using the STATA 8.0 software package (Stata Corporation, College Station, TX, USA).

**Results**

**Demographic and health characteristics of the study population.** Among 103 male workers, 6 subjects were excluded because of abnormal liver function. The remaining 97 subjects had an age range of 34.2 years (range, 18–63 years) and a mean employment time of 3.5 years (range, 1–9 years). The BMI data indicated that 70% of the subjects exhibited some degree of obesity. In addition, 55% of the study subjects were smokers, 92% of them declared some degree of alcohol consumption, and 10% considered themselves to be frequent drinkers. Table 1 summarizes the anthropometric characteristics and habits of the study population.

**Environmental toluene and urinary hippuric acid levels.** Table 2 summarizes the indoor air toluene concentrations to which the workers were exposed in the print workshop and the levels of toluene exposure biologic markers. Air toluene concentrations monitored individually for a work shift had a geometric mean (GM) of 52.80 ppm (range, 10–760 ppm). Thus, 54% of the subjects studied were exposed to toluene levels that exceeded the 50 ppm maximal permissible exposure level (MPEL) (NOM-010-STS-1999-2000). The GM of the urinary hippuric acid at the beginning and the end of a work shift were 0.027 and 0.041 g/g creatinine, respectively. Both values were below the maximal biologic permissible level (MBPL) of 2.5 g/g creatinine (NOM-047-SSA1-1993 1996). However, these data represent a 48% increase in urinary hippuric acid GM from the beginning to the end of the shift (p < 0.05) (Figure 3). This increase is consistent with the occurrence of active toluene absorption and biotransformation during the work shift.

**CYP2E1 mRNA associations with toluene, hippuric acid, and the toluene exposure ratio.** In the present study, we assessed toluene exposure in two steps. First, toluene levels in work area air and hippuric acid levels in urine were measured for each subject, and these data were individually associated with the subject’s CYP2E1 mRNA data. These comparisons yielded statistically significant but opposite correlations. That is, environmental toluene correlated positively with CYP2E1 mRNA levels ($\beta = 0.062$, $\sigma = 0.011$, $p = 0.029$; $n = 76$), whereas urinary hippuric acid levels correlated negatively with CYP2E1 mRNA levels ($\beta = 0.065$, $\sigma = -4.120$, $p = 0.027$; $n = 71$). This dissociation may be due to the capacity of toluene to induce CYP2E1 mRNA expression, whereas hippuric acid reflects toluene disposition. Therefore, we conducted further analysis with the toluene exposure ratio, which was calculated as the quotient of each individual’s environmental toluene concentration exposure divided by his measured urine hippuric acid concentration. CYP2E1 mRNA level and the toluene exposure ratio showed a statistically significant relationship ($p < 0.05$) (Figure 4). Using this ratio enabled each subject’s exposure to be more accurately described, bearing in mind the variability between internal and external conditions among workers and allowing us to normalize the individual differences observed in toluene metabolism. Such interindividual differences are likely to result from several factors: a) differing toluene exposures that lead to different hippuric acid production; b) a competitive inhibition of toluene metabolism by other VOCs, which was greater in workers exposed to high toluene concentrations; c) differing metabolic statuses, depending on individual genetic characteristics and the extent of CYP2E1 induction; and d) possible development of a tolerance to toluene, as has been described for other CYP2E1 substrates (Shyag et al. 1999). The GM for the toluene exposure ratio was 0.122, ranging between 0.007 and 3.366 (Table 2).

**CYP2E1 phenotype and genotype in the study population.** CHZ biotransformation is related to CYP2E1 activity (Lucas et al. 1999), which is induced by toluene (Uaki et al. 1995). However, CHZ metabolism can be competitively inhibited by other contaminants in an occupational setting (Lucas et al. 1999); such effects will be reflected in the CHZ metabolic ratio. The GM of the CHZ metabolic ratio was 0.33 (0–9.3); however, 40% of the study population values were <0.3. On the other hand, blood peripheral lymphocyte CYP2E1 mRNA content was 1.07 with a range of 0.03–3.08 (Table 2).
Genotype frequencies for the *RsaI/PstI* polymorphisms were reported previously (Mendoza-Cantú et al. 2003). Briefly, 60 native homozygous (c1/c1), 34 heterozygous (c1/c2), and 3 homozygous (c2/c2) subjects were found. These genotype frequencies are in accordance with the Hardy-Weinberg equilibrium model (Klug and Cummings 2000).

The stratification of CYP2E1 phenotype parameters per genotype showed the following values: mRNA content c1/c1: GM = 1.0645; range = 2.069–0.304; c1/c2, GM = 1.056; range = 2.123–0.477; c2/c2, GM = 1.319; range = 1.708–1.153. The CHZ metabolic ratios for each genotype were as follows: c1/c1, GM = 0.327, range = 2.362–0.0023; c1/c2, GM = 0.367, range = 1.597–0.0310; c2/c2, GM = 0.430, range = 2.287–0.117. There was no apparent influence of genotype on the measured mRNA content levels and CHZ metabolic ratios.

**Relationships between toluene exposure and CYP2E1 phenotype.** We found a correlation between the toluene exposure ratio and CYP2E1 mRNA content ($r^2 = 0.0854$, $\beta = 0.0929$, $p = 0.0116$; $n = 67$) (Table 4). However, the CHZ metabolic ratio did not correlate with either the toluene exposure ratio ($r^2 = 0.237$, $\beta = -0.007$, $p = 0.744$; $n = 84$) or CYP2E1 mRNA content ($r^2 = 0.215$, $\beta = -0.026$, $p = 0.686$; $n = 78$) (Table 3).

**Discussion**

We investigated the effects of toluene exposure on expression of the CYP2E1 phenotype by measuring CYP2E1 mRNA induction in peripheral lymphocytes and CHZ metabolism in workers exposed to organic solvents and then determining whether these mRNA content and CHZ metabolic data were associated with genotype.

**Toluene in the work environment.** Toluene exposure in the present group of subjects (GM = 52.80 ppm) was similar to the lower limit reported for toluene exposure in a previous study of workers from 25 Mexican companies (52.5–931 ppm) in which the workers routinely handled organic solvents, paints, and varnishes (Madrigal-Bujaidar et al. 1996). Regrettably, both studies revealed environmental toluene concentrations that exceeded the limits set by Mexican regulations (NOM-010-STPS-1999 2000). Indeed in the present study, 54% of our study population was above the toluene MEP. Therefore, stricter regulations should be implemented to ensure safe conditions in the occupational environment.

**Hippuric acid in the urine.** Hippuric acid has been used extensively as a toluene exposure biomarker, but this metabolite may occur in the diet, that is, via some fruits and food preservatives (Kawamoto et al. 1996). In this study, a statistically significant increase in hippuric acid concentrations during the work shift (48%) demonstrated active absorption and biotransformation of toluene by exposed workers. Despite the high toluene levels in air, the hippuric acid concentrations remained below the MBPL. This apparent inconsistency between the environmental and the biological data leads to a disagreement between the MEP and MBPL. Thus, an environmental exposure to toluene of 50 ppm for an 8 hr work shift resulted in a urinary hippuric acid concentration of < 2.5 g/g creatinine. De Bruin (1980) studied a group of volunteers exposed to 100 ppm toluene for 8 hr (twice the MEP), but this high level of exposure produced a relatively low urinary hippuric acid excretion of 1.2 g/g creatinine. Although toluene is one of the main constituents of the organic solvent used in the print industry, these mixtures also contain many other VOCs that may be potential CYP2E1 substrates that may competitively inhibit its activity, as has been described for other chemicals (Campo et al. 1998; Tardif et al. 1993; Uaki et al. 1995).

**Toluene effects on CYP2E1 phenotype.** Various factors, including exposure to certain xenobiotics, can induce CYP2E1 expression and thereby result in an increase in its metabolic activity. Such induction could be a risk to the health of persons exposed to CYP2E1 protonic substrates; thus, CYP2E1 expression demands close monitoring in those individuals.

**The CHZ metabolic ratio.** Most of our subjects showed small CHZ metabolic ratios (40% had ratios < 0.3), indicating a very low CHZ biotransformation rate, a finding that may be attributable to competitive inhibition of CHZ metabolism by VOCs. We did not detect significant differences between the toluene exposure ratio and CHZ metabolic ratio, or between CHZ metabolism and mRNA content. Lucas et al. (1999) reported a decreased CHZ metabolic ratio in shoemakers exposed to glue and suggested that there may be metabolic competition between CHZ and the organic chemicals present in the solvent’s emissions. Furthermore, Ernstgård et al. (1999) found evidence that coexposure to acetone and CHZ or to toluene and CHZ increased plasma concentrations of both the organic chemicals and CHZ in human males, indicating that there was metabolic competition as a result of these interactions. In addition, Powell et al. (1998) found that human livers showed no correlation between mRNA levels and CYP2E1 protein content and *p*-nitrophenol and CHZ hydroxylation activity. Likewise, Sumida et al. (1999) reported no correlation between CYP2E1 mRNA level and CHZ hydroxylation in human liver biopsies. Thus, these lack of associations suggests that the CHZ metabolic ratio may not be an appropriate biomarker for toluene exposure when evaluated directly at the occupational site.
CYP2E1 mRNA induction. The associations of peripheral lymphocyte CYP2E1 mRNA content with environmental toluene or urinary hippuric acid were statistically significant. Furthermore, the peripheral lymphocyte CYP2E1 mRNA with the toluene metabolic ratio suggests that CYP2E1 mRNA content is a more significant variable than is the CHZ metabolic ratio. Moreover, CYP2E1 mRNA content is sensitive, noninvasive test, for which samples can be obtained on site, and its expression in human peripheral lymphocytes appears to be regulated by the same factors as those affecting the liver (Raayt et al. 1997).

The CYP2E1 response we observed in this study demonstrated that toluene exposure affects CYP2E1 mRNA content, but further studies on tolulene toxicity are needed to fully characterize the mechanism of induction. Other chemicals, such as ethanol, can regulate CYP2E1 activity by altering protein stabilization without affecting transcriptional activity (Roberts et al. 1995). Ethanol has also been shown to increase CYP2E1 gene expression at high doses (>300 mg/dL blood) (Badger et al. 1993). Finally, CYP2E1 genotype stratification did not modify the association between toluene exposure and mRNA content in peripheral lymphocytes.

In conclusion, our results suggest that CYP2E1 mRNA content may be a useful biomarker of physiologic effects in humans exposed to toluene. The mRNA response is the first step after its modulation and may not be affected by other factors that modify CYP2E1 enzyme activity, as has been suggested to be a complicating factor for CHZ metabolism. However, further studies are needed to fully characterize and validate the use of CYP2E1 mRNA content for health surveillance purposes.