SPECIES- AND SEX-RELATED DIFFERENCES IN METABOLISM OF TRICHLOROETHYLENE TO YIELD CHLORAL AND TRICHLOROETHANOL IN MOUSE, RAT, AND HUMAN LIVER MICROSOMES

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ABSTRACT:

Trichloroethylene (TRI) has been shown to cause a variety of tumors, particularly in mouse liver and lung and rat kidney. However, a clear association between exposure to TRI and cancer development in humans has not been established. Because TRI metabolism by cytochrome P450s has been implicated in the mechanisms of TRI-induced carcinogenicity in mice, the purpose of the present study was to characterize the kinetics of TRI oxidation in male and female mouse liver, rat, and human liver microsomes to possibly allow for a better assessment of human risk. Methods were developed to detect and quantitate chloral, trichloroethanol, trichloroacetic acid, dichloroacetic acid, chloroacetic acid, glyoxylic acid, and oxalic acid, known TRI metabolites in rodents or humans. However, only chloral and its further metabolite, trichloroethanol, were consistently detected in the various liver microsomes in the presence of NADPH. Chloral was the major metabolite detected, and its levels were species- and sex-dependent; the amounts of trichloroethanol detected were also species- and sex-dependent but never exceeded 15% of total metabolites. Double-reciprocal plots of metabolite formation with male and female rat and human liver microsomes indicated biphasic kinetics, but this trend was not observed with microsomes from male or female mouse liver. The V_{max} data are consistent, with male and female mice being more susceptible to TRI-induced liver carcinogenicity than male rats. However, the V_{max}/K_{m} ratios in male and female rat liver microsomes, in comparison with the male mouse liver microsomes, did not correlate with tumor incidences in these tissues. Furthermore, as only two out of six human liver samples examined exhibited V_{max}/K_{m} ratios similar or higher than the ratio obtained with male mouse liver, humans may vary in their toxic response after TRI exposure.

TRI (also known as trichloroethene) is a nonflammable liquid that is primarily used in industry as a metal degreasing agent. TRI was formerly used as a dry-cleaning solvent, an anesthetic drug, and as an extracting agent in the food and cosmetic industry, among its other uses (USEPA, 1985). Because of its widespread use, TRI is a common contaminant of ground and surface water and air around industrial sites (Davidson and Beliles, 1991). Although most epidemiological studies have been inconclusive regarding TRI carcinogenicity in humans (reviewed in Davidson and Beliles, 1991; Goepart et al., 1995; IARC, 1995), long-term exposure of mice and rats to TRI has been repeatedly associated with carcinogenicity; liver and lung tumors were clearly detected in male and female mice, whereas kidney and testicular tumors were detected only in male rats. Although these studies clearly showed that TRI is a rodent carcinogen, the molecular mechanisms of TRI-induced carcinogenicity and the biochemical basis for the observed tissue differences in susceptibility are not fully understood. Recently, mechanisms involving TRI oxidation by cytochrome P450s have been implicated in the hepatic and lung carcinogenicity of TRI in mice (Abbas and Fisher, 1997; Davidson and Beliles, 1991; Fahrig et al., 1995; Green et al., 1997), whereas conjugation of TRI with glutathione and further metabolism of the resulting glutathione conjugate by the mercapturic acid pathway have been implicated in the renal carcinogenicity in the male rat (Goepart et al., 1995; Lash et al., 1995, 1998). The relevance of these mechanisms to humans remains unclear.

The main site of TRI metabolism is the liver (Dekant et al., 1986; Green et al., 1995, 1998). Cytochrome P450 2E1 seems to have the highest affinity for TRI, whereas other cytochrome P450s, including 1A1, 2B1, and 2C11, are suggested to play a role at high TRI concentrations (Guengerich et al., 1991; Lipscomb et al., 1997; Nakajima et al., 1990, 1993). TRI metabolism by liver cytochrome P450 to yield the electrophilic metabolites trichloroethylene oxide, dichloroacetyl chloride, and chloral (fig. 1), which can bind to hepatocellular macromolecules, has been implicated in TRI-induced mouse liver carcinogenicity (Costa et al., 1980; Halmes et al., 1996; Miller and Guengerich, 1982, 1983). Chronic exposure to chloral hydrate via the drinking water significantly increased the prevalence of hepatocellular carcinomas and hepatocellular adenomas in male...
FIG. 1. Possible oxidative metabolism of TRI (1) in the liver to yield chloral (4), dichloroacetyl chloride (3), and trichloroethylene oxide (2). DCA (5), OXA (6), TCA (7), and TCE (8) are possible further metabolites and/or hydrolysis or decomposition products.

B6C3F1 mice (Daniel et al., 1992). Because the further metabolites of chloral, namely, DCA and TCA (fig. 1) are also mouse liver carcinogens, a role for these metabolites in TRI-induced liver carcinogenicity has been proposed (Elinerbe et al., 1985; Larson and Bull, 1992).

The major metabolite detected in vitro is chloral, which can be reduced to TCE or oxidized to TCA (Costa et al., 1980; Miller and Guengerich, 1983). DCA, a minor metabolite of TRI in both rats and mice formed mostly by nonenzymatic hydrolysis of dichloroacetyl chloride, can also be formed by reductive dechlorination of TCA (Green and Prout, 1985; Templin et al., 1993). OXA and CAA are additional minor metabolites detected with rodents in vivo (Dekant et al., 1986).

Species and sex differences in susceptibility to TRI-induced carcinogenicity in rats and mice have been partially explained by differences in pharmacokinetics and rates of metabolism of TRI (Green and Prout, 1985; Templin et al., 1995). For example, a greater proportion of the TRI dose was metabolized in mice than in rats, and peak concentrations of chloral, TCE, and TCA were reached in the mouse within 2 hr after TRI administration, whereas 10–12 hr were required for peak metabolite concentrations in the rat (Prout et al., 1985).

Furthermore, chloral and TCE were rapidly eliminated from mouse blood, and the higher rate of TRI metabolism in the mouse resulted in overall TRI metabolism, species and sex differences may play a role in TRI-induced hepatocarcinogenicity. Furthermore, metabolites generated in the liver may also play a role in TRI-induced carcinogenicity in the extrahepatic tissues, as these metabolites may be translocated to these target tissues via the circulation. Thus, the purpose of the present investigation is to characterize the kinetics of TRI metabolism in male and female mouse, rat, and human liver microsomes over a wide range of TRI concentrations (0.01 to 2.0 mM) to help determine the more appropriate animal model and to improve assessment of human risk.
temperature of 250°C and a He carrier gas flow rate of 2 ml/min. For TCE analysis, the initial oven temperature was 60°C for 3.5 min. It was increased at 10°C/min to 240°C, where it was held for 2.5 min. The retention time of TCE was 6.6 min. Chloral was analyzed with an initial oven temperature of 32°C for 5 min followed by an increase to 35°C at a rate of 1°C/min. The temperature was then raised to 230°C at 70°C/min, where it was held for 2.5 min. Chloral had a retention time of 8.6 min. For analysis of CAA, DCA, TCA, GLY, and OXA, the injector temperature was 175°C. An initial oven temperature of 120°C was increased at a rate of 10°C/min to 155°C, where it was held for 7.5 min. The temperature was then increased to 175°C at 10°C/min and then to 240°C at a rate of 35°C/min, where it was held for 3.0 min. Retention times of the acids under these conditions were as follows: GLY, 5.0; CAA, 7.7; DCA, 9.8; TCA 12.3; and OXA, 18.3. Quantitations of all metabolites were done by comparing peak areas to standard curves generated in a similar manner and exhibiting correlation coefficients ≥0.99.

HPLC Analysis for GLY. The ability to detect GLY as the 2,4-dinitrophenylhydrazone metabolite by HPLC (Wang et al., 1988) was also examined. The incubation was carried out as described above, and a 0.5-ml aliquot was removed. An equal volume of ice-cold ethanol (0.5 ml) was immediately added to quench the reaction and precipitate the protein followed by centrifugation at 3000 rpm for 15 min. To the supernatant, 0.5 ml of 2,4-dinitrophenyl hydrazine (6.3 mM in 6 N HCl) was added and incubated at 37°C for 24 hr. The derivatized product was pelleted by centrifugation and redissolved in 1 ml of acetonitrile. Samples were filtered through an Acrodisc LC 13 membrane filter (Gelman Sciences, Ann Arbor, MI) and analyzed by a slightly modified HPLC method previously described (Mentasi et al., 1987). Briefly, the sample (20 μl) was injected onto a Beckman Ultrasphere ODS 5 μm (4.6 mm × 25 cm) column. Separation was achieved with a Gilson 306 solvent delivery system with 1% (v/v) acetonitrile as solvent A and 100% acetonitrile as solvent B. The flow rate was 1 ml/min. The gradient began at 50% B, which was increased to 95% B over 12 min, where it was held for 5 min. The percentage of B then decreased to the initial concentration of 50% over 5 min for a total run time of 26 min. A Beckman 166 detector at 352 nm was used to monitor the derivative formation, which had a retention time of 7.7 min.

Analysis of Standards. TCE and chloral were quantitated by comparison of peak areas to standard curves prepared in their respective solvents and then

![FIG. 2. Typical electron-capture detector response chromatograms of chloral (A, B) and TCE (C, D) analyses. A and C, in the absence of NADPH; B and D, in the presence of NADPH. Peak I is TRI; peak II is chloral; peak III is TCE.](image)

![FIG. 3. Time dependence of chloral (•) and TCE (■) formation in male and female mouse, rat, and human liver microsomes. Typical results at 2 mM TRI are shown.](image)
analyzed by GC via their respective methods. Limits of detection were 6.7 pmol/ml for TCE and 0.68 pmol/ml for chloral. The reported values were corrected for recovery of the metabolites, which accounts for both extraction efficiency and/or protein binding. This was carried out by spiking the metabolite at the level formed in a typical incubation into either buffer alone or microsomal protein in the presence and absence of NADPH for the time of the incubation and then extracting with the appropriate organic solvent. When equivalent concentrations of chloral hydrate and chloral formed by treatment with sulfuric acid were used, similar GC peak areas were obtained. The acids CAA, DCA, TCA, and OXA were spiked into the incubation buffer at known concentrations and extracted and derivatized as described above. Limits of detection were as follows: CAA, 0.26 nmol/ml; DCA, 0.19 nmol/ml; TCA, 6.1 nmol/ml; and OXA, 55.5 nmol/ml. Limits of detection for GLY were determined by HPLC as described above and found to be 1.35 nmol/ml. The GC method used for detecting the other acids also detects GLY; however, the sensitivity is 200-fold better by HPLC.

**GC–Mass Spectral Characterization.** The pentafluorobenzyl derivatives of the acid metabolites were chemically synthesized and characterized by GC-MS. The instrument used was a Kratos MS25 with a Carlo Erba GC-mass spectrometer fitted with a DB-5 50 m capillary column. The ion source temperature was set at 300°C, and the injector temperature was 140°C. The initial oven temperature was 140°C and increased at a rate of 10°C/min to 240°C, where it was held for 2 min. Retention times of the acid derivatives were as follows: DCA, 3.95 min; GLY, 2.87 min; TCA, 4.43 min; CAA, 3.62 min; and OXA, 8.48 min. The major fragment of all the derivatives was 181, which corresponds to the pentafluorobenzyl moiety (Kassahun et al., 1990).

The samples also showed a small molecular ion peak. The spectra of CAA, DCA, and TCA gave the expected chlorine pattern appropriate for the number of chlorines present.

**Statistical Analyses.** Significant differences between means for the data were first assessed with a one-way analysis of variance using Minitab (State College, PA). When significant F values were obtained, the Tukey test for multiple comparisons was performed to determine which means were significantly different from each other using $p < 0.05$ as the criterion for significance.

**TABLE 2**

<table>
<thead>
<tr>
<th>Species</th>
<th>$V_{\text{max}}$ (nmol/mg protein/min)</th>
<th>$K_m$ (mM)</th>
<th>$V_{\text{max}}/K_m$</th>
<th>$r$</th>
<th>$N$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>8.60 ± 4.50$^a$</td>
<td>0.378 ± 0.414</td>
<td>42.0 ± 28.5$^a$</td>
<td>0.95–0.99</td>
<td>5</td>
</tr>
<tr>
<td>Rat (high affinity)</td>
<td>0.96 ± 0.65$^{a,b}$</td>
<td>0.072 ± 0.082$^c$</td>
<td>23.8 ± 26.0$^{a,d}$</td>
<td>0.95–0.99</td>
<td>5</td>
</tr>
<tr>
<td>(low affinity)</td>
<td>2.48 ± 0.97$^{a,b}$</td>
<td>0.482 ± 0.104</td>
<td>5.3 ± 2.2$^a$</td>
<td>0.94–0.98</td>
<td>5</td>
</tr>
<tr>
<td>Human (high affinity)</td>
<td>0.52 ± 0.17$^{a,b}$</td>
<td>0.012 ± 0.003$^c$</td>
<td>48.0 ± 23.1$^b$</td>
<td>0.98–0.99</td>
<td>3</td>
</tr>
<tr>
<td>(low affinity)</td>
<td>0.93 ± 0.17$^{a,b}$</td>
<td>0.093 ± 0.026</td>
<td>10.7 ± 3.9$^{b,d}$</td>
<td>0.96–0.98</td>
<td>3</td>
</tr>
<tr>
<td>H1 (high affinity)</td>
<td>0.37</td>
<td>0.014</td>
<td>26.4</td>
<td>0.99</td>
<td>1</td>
</tr>
<tr>
<td>(low affinity)</td>
<td>0.88</td>
<td>0.123</td>
<td>7.2</td>
<td>0.98</td>
<td>1</td>
</tr>
<tr>
<td>H2 (high affinity)</td>
<td>0.61</td>
<td>0.009</td>
<td>67.8</td>
<td>0.98</td>
<td>1</td>
</tr>
<tr>
<td>(low affinity)</td>
<td>1.05</td>
<td>0.072</td>
<td>14.6</td>
<td>0.96</td>
<td>1</td>
</tr>
<tr>
<td>H3 (high affinity)</td>
<td>0.79</td>
<td>0.010</td>
<td>79.0</td>
<td>0.98</td>
<td>1</td>
</tr>
<tr>
<td>(low affinity)</td>
<td>1.24</td>
<td>0.075</td>
<td>16.5</td>
<td>0.97</td>
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</table>

<table>
<thead>
<tr>
<th>Female</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>26.06 ± 7.29</td>
<td>0.161 ± 0.029</td>
<td>162.8 ± 36.7</td>
<td>0.99</td>
<td>3</td>
</tr>
<tr>
<td>Rat (high affinity)</td>
<td>2.91 ± 0.71$^b$</td>
<td>0.042 ± 0.021</td>
<td>80.0 ± 33.9$^{b,c}$</td>
<td>0.96–0.98</td>
<td>3</td>
</tr>
<tr>
<td>(low affinity)</td>
<td>4.31 ± 0.31$^{a,b}$</td>
<td>0.111 ± 0.027</td>
<td>40.1 ± 7.1$^{a,b}$</td>
<td>0.92–0.99</td>
<td>3</td>
</tr>
<tr>
<td>Human (high affinity)</td>
<td>0.33 ± 0.15$^{a,b}$</td>
<td>0.026 ± 0.017$^c$</td>
<td>15.3 ± 10.1$^{a,d}$</td>
<td>0.97–0.99</td>
<td>3</td>
</tr>
<tr>
<td>(low affinity)</td>
<td>0.72 ± 0.60$^{a,b}$</td>
<td>0.160 ± 0.162</td>
<td>6.8 ± 5.6$^{b,d}$</td>
<td>0.96–0.99</td>
<td>3</td>
</tr>
<tr>
<td>H4 (high affinity)</td>
<td>0.19</td>
<td>0.014</td>
<td>13.6</td>
<td>0.99</td>
<td>1</td>
</tr>
<tr>
<td>(low affinity)</td>
<td>0.36</td>
<td>0.097</td>
<td>3.7</td>
<td>0.97</td>
<td>1</td>
</tr>
<tr>
<td>H5 (high affinity)</td>
<td>0.48</td>
<td>0.045</td>
<td>10.7</td>
<td>0.97</td>
<td>1</td>
</tr>
<tr>
<td>(low affinity)</td>
<td>1.42</td>
<td>0.333</td>
<td>4.3</td>
<td>0.99</td>
<td>1</td>
</tr>
<tr>
<td>H6 (high affinity)</td>
<td>0.38</td>
<td>0.013</td>
<td>29.2</td>
<td>0.99</td>
<td>1</td>
</tr>
<tr>
<td>(low affinity)</td>
<td>0.42</td>
<td>0.030</td>
<td>14.0</td>
<td>0.96</td>
<td>1</td>
</tr>
</tbody>
</table>

$^a$Significantly different from the corresponding male mouse value.

$^b$Significantly different from the corresponding female mouse value.

$^c$Significantly different from the corresponding male rat (low affinity) value.

$^d$Significantly different from the corresponding female rat (high affinity) value.

![Fig. 4. Typical double-reciprocal plots of metabolite formation by liver microsomes from male and female mice and rats.](image-url)
Results

Metabolism of TRI by mouse, rat, and human liver microsomes in the presence of NADPH led to detection of two new peaks co-eluting with authentic chloral and TCE (fig. 2). The amounts of chloral and TCE detected exhibited time dependency (fig. 3), with TCE being detected at levels lower than that of chloral. Male mouse and male human liver microsomes produced TCE at levels higher than those produced by female mouse and female human or both sexes of rat liver microsomes. An apparent lag time in TCE formation was clearly observed in male mouse and male human liver microsomes (fig. 3), which is consistent with chloral being a precursor for TCE.

Over the full range of TRI concentrations (0.01–2.0 mM) used in the kinetic studies, chloral was the major metabolite detected with all microsomal samples. The amounts of TCE detected in male (N = 5) and female (N = 3) mouse liver microsomes represented 7.5 ± 5.5 and 0.4 ± 0.2% (means ± SD) of the total metabolites detected in these two tissues, respectively. TCE represented 1.9 ± 1.3 and 0.2 ± 0.1% of total metabolites detected in male (N = 3) and female (N = 3) rat liver microsomes, respectively, and 13.4 ± 2.3 and 7.0 ± 6.7% of total metabolites detected in male (N = 3) and female (N = 3) human liver microsomes, respectively.

Among the organic acids assayed for in the aqueous compartment, DCA was detectable only in mouse and rat liver microsomes at a high TRI concentration (2 mM); DCA was not detected in human liver microsomes. The DCA levels detected in mouse and rat liver microsomes were near the limits of detection of the assay. Attempts to detect dichloroacetyl chloride, presumably the main precursor of DCA (Green and Prout, 1985; Templin et al., 1993), in the organic extracts of the incubation mixtures were not successful. CAA, TCA, GLY, and OXA were also not detected in all microsomal assays, possibly because of the short incubation periods, the limits of detection of our assays, and/or the ineffectiveness of the microsomal proteins to carry out additional enzymatic steps, such as the oxidation of chloral to TCA (Lipscomb et al., 1996). In addition, the conditions used to investigate the oxidative metabolism of TRI may not be optimal for the metabolic conversion of chloral to TCE. Nonetheless, TCE formation under the oxidative metabolism conditions was determined to more accurately determine the total amounts of chloral formed in the incubation by combining the amounts of detectable chloral and TCE.

Kinetic constants for TRI metabolism to yield chloral and chloral and TCE combined were determined for male and female mouse, rat, and human liver microsomes by Michaelis-Menten plots; all plots gave good correlation coefficients (r > 0.92–0.99). Because inclusion of the relatively small amounts of TCE detected in the analyses did not affect the kinetic results, only the results obtained using total TRI metabolites are shown (table 2). Whereas male and female mouse liver kinetics are best described by single values for K_m and V_max, all of the male and female rat and human liver microsomes exhibited biphasic kinetics (figs. 4 and 5). This suggests the presence of both a high-affinity component and a low-affinity component for oxidative metabolism of TRI in rat and human liver microsomes.

Mouse liver microsomes exhibited a sex-related difference in their rates of metabolism of TRI to chloral and TCE, yielding V_max values of 26.06 ± 7.29 (mean ± SD) and 8.60 ± 4.50 nmol/mg protein/min for females and males, respectively. The V_max values obtained with human and rat liver microsomes did not exhibit sex dependency and were nearly 2- to 77-fold lower than the values obtained with male and female mouse liver microsomes (table 2). Because of the variability of the human kinetic data, both individual sample data and means ± SD (N = 3) of data obtained with the male and female human liver samples are presented (table 2). Among the six human liver samples examined, differences in V_max values (up to 4-fold) were observed for both the high-affinity component and the low-affinity component; the human liver samples exhibited V_max values that ranged 0.19–0.79 and 0.36–1.42 nmol/mg protein/min for the high-affinity and the low-affinity components, respectively. Human liver microsomes exhibited K_m values ranging from 9 to 45 μM for their high-affinity components and from 30 to 333 μM for their low-affinity components. However, for the three species examined, there was no species or sex difference in K_m values. The V_max/K_m value for the female mouse liver microsomes was significantly higher than the corresponding values for male mouse liver microsomes and male and female rat and human liver microsomes.

Discussion

Although TRI has long been recognized as a rodent carcinogen affecting different tissues in mice and rats and extensive in vivo studies have indicated species differences in TRI pharmacokinetics and metabolism, there is limited in vitro data available to characterize TRI metabolism in these species and the corresponding human tissues. Using liver microsomes prepared from male Osborne-Mendel rats and male B6C3F_1 mice, Miller and Guengerich (1983) reported that mouse liver microsomes metabolized TRI (0.8 mM) to yield chloral at levels that were approximately 4-fold higher than that produced with rat liver microsomes. The amounts of chloral detected with four human liver microsomal samples under the same assay conditions were variable, with two samples exhibiting values similar to those
observed in the rat whereas the other two samples exhibited values similar to those observed in the mouse. Using liver microsomes from male Wistar rats and male B6C3F1 mice, Nakajima et al. (1993) reported that mouse liver microsomes metabolized TRI (0.2 mM) to chloral at a rate that was three times higher than the rate obtained with rat liver microsomes; when the TRI concentration was increased to 5.9 mM, the rate obtained with mouse liver microsomes was two times higher than the rate obtained with rat liver microsomes. Recently, Lipscomb et al. (1997) investigated the kinetics of TRI metabolism to chloral and TCE in male and female human liver microsomes; however, similar kinetic experiments with mouse or rat liver microsomes were not included. Thus, to our knowledge, this manuscript describes the first comprehensive kinetic study of oxidative metabolism of TRI in male and female mouse, rat, and human liver microsomes. The ten TRI concentrations (0.01 to 2.0 mM) used in our study were selected based on the American Conference of Governmental Industrial Hygienists (1990) recommendations for threshold limit values of 50 ppm as an 8-h time-weighted average and 200 ppm as a short-term exposure limit; the 50 ppm value will result in a blood concentration of 0.016 mM based on the human blood:air partition coefficient (Lipscomb et al., 1997).

The three experimental models examined in this study exhibited distinct species- and sex-related differences in both the kinetics of TRI metabolism and the relative amounts of chloral and TCE detected (table 2; fig. 3). Female mouse liver microsomes exhibited \( V_{\text{max}} \) and \( V_{\text{max}}/K_{\text{m}} \) values that were much higher than the corresponding male mouse liver microsomes or male and female rat and human liver microsomes. Female mouse liver microsomes were also less efficient than male mouse liver microsomes in converting chloral to TCE, which is considered a detoxication reaction (Dekant et al., 1986; Goepart et al., 1995). Thus, if the rates of TRI metabolism to chloral and the subsequent rates of chloral metabolism to TCE are important determinants of TRI carcinogenicity, our data would suggest that the female mouse may be at higher risk than the male mouse or either sex of rats and humans. However, a clear correlation between the higher rates of oxidative metabolism of TRI in female B6C3F1 mice and liver tumor incidence is difficult to make. Although male B6C3F1 mice generally exhibit higher incidences of liver tumors than females, the males have a markedly higher frequency of background liver tumors, and the various bioassays have often used dosing regimens that are difficult to compare (Maltoni et al., 1986; National Toxicology Program, 1990). Hence, a clear conclusion about which sex of B6C3F1 mice is more susceptible to hepatocarcinogenesis is not possible at present. Similarly, an explanation for the lack of correlation between the high \( V_{\text{max}}/K_{\text{m}} \) ratio for oxidative metabolism of TRI in male and female Fischer 344 rat liver microsomes, in comparison with the male mouse liver microsomes, and liver tumor incidences is not possible at present. The \( V_{\text{max}} \) value obtained with male mouse liver microsomes was statistically different than the \( V_{\text{max}} \) values obtained for the high-affinity components of male and female rat and human liver microsomes (table 2). These results suggest that at low TRI concentrations, male mouse liver microsomes are likely to metabolize TRI at rates higher than rat or human liver microsomes.

The kinetic constants \( (V_{\text{max}} \text{ and } V_{\text{max}}/K_{\text{m}}) \) obtained in this study for TRI oxidation in male and female mouse liver microsomes (table 2) do not correlate with the kinetic constants derived from gas uptake studies in male and female B6C3F1 mice (Fisher et al., 1991), which showed that female mice metabolized TRI at a rate \( (V_{\text{max}} = 23.2 \pm 0.1 \text{ mg/kg/hr}) \) lower than the rate obtained with male mice \( (V_{\text{max}} = 32.7 \pm 0.06 \text{ mg/kg/hr}) \). The reason for this difference between the two studies is presently unclear.

Because of the wide range of TRI concentrations (0.01 to 2.0 mM) used in our study in comparison with that (0.039 to 0.125 mM) used by Lipscomb et al. (1997), we were able to detect biphasic kinetics for TRI metabolism to chloral and TCE in all human liver microsomal samples (table 2; figs. 4 and 5). In addition, our results (table 2) indicated no sex-related differences in \( K_{\text{m}} \) values for TRI in human liver microsomes. Whereas the latter difference between our results and those of Lipscomb et al. could also be explained by the different TRI concentrations used in the two studies, the small number of human liver samples used in our study may have also contributed to this difference. Because human liver microsomes metabolized TRI to chloral and TCE in a manner similar to that in mouse liver microsomes, the high-affinity component for TRI metabolism in human liver microsomes may be of toxicological significance. TRI concentrations attainable in current exposure situations may saturate this component, leading to the formation of toxic metabolites.

Among the six human liver microsomes examined, two samples exhibited \( V_{\text{max}}/K_{\text{m}} \) values similar to or higher than the \( V_{\text{max}}/K_{\text{m}} \) values obtained with male mouse liver microsomes. These results, and the previous report that humans exhibited dose-response relationships for TRI metabolism to TCA that were more similar to those exhibited with mice than with rats (Goepart et al., 1995), suggest that human exposure to TRI may be associated with risk for cancer development. Our data along with those of others (Ikeda, 1977; Kimmel and Eben, 1973; Lipscomb et al., 1997; Monster et al., 1976) also suggest that risk to humans can vary depending on the individual. Because the biphasic kinetic data observed in rat and human liver microsomes are consistent with the involvement of multiple cytochrome P450 enzymes in TRI metabolism (Lipscomb et al., 1997), it is important to characterize human variability in TRI metabolism to chloral. Human variability in further metabolism of chloral to TCE should also be characterized further because this metabolic reaction may be an important detoxication pathway, and our data and that obtained by others (Ikeda, 1977; Kimmel and Eben, 1973; Monster et al., 1976) showed considerable variability between sexes and among individuals. These studies should improve human risk assessment.

References


