

## Use of Kinetic and Mechanistic Data in Species Extrapolation of Bioactivation: Cytochrome P-450 Dependent Trichloroethylene Metabolism at Occupationally Relevant Concentrations

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**Abstract:** Use of Kinetic and Mechanistic Data in Species Extrapolation of Bioactivation: Cytochrome P-450 Dependent Trichloroethylene Metabolism at Occupationally Relevant Concentrations: John C. LIPSCOMB, *et al.* United States Air Force, Armstrong Laboratory, Toxicology Division—Trichloroethylene (TRI) is an industrial solvent and environmental contaminant; therefore exposure to TRI occurs in diverse human populations. TRI causes hepatocellular carcinoma in B6C3F1 mice, but not rats; this suggests that TRI may be metabolized differently in the two species. We investigated the metabolism of TRI and the effect of TRI on enzymatic activities indicative of specific cytochrome P450 (CYP) forms in hepatic microsomes from mice, rats and humans. Studies in microsomes estimated Michaelis-Menten kinetic parameters by saturation analysis.  $K_m$  values were 35.4, 55.5 and 24.6  $\mu\text{M}$  and  $V_{max}$  values were 5,425, 4,826 and 1,440 pmol/min/mg in pooled mouse, rat and human microsomes, respectively. TRI (1,000 ppm) inhibited CYP2E1 dependent activity in all three species and BROD activity in mice and rats; TRI (1,000 ppm) increased CYP1A1/1A2 activity, and had no effect on CYP2A activity. Inhibition studies with mouse hepatic microsomes demonstrated that TRI was a competitive inhibitor of CYP2E1, with  $K_i$  of 50 ppm. TRI noncompetitively inhibited CYP2B-dependent activities in the rat and mouse. Preincubation of microsomes with TRI and NADPH decreased the absorbance of CO-bound CYP in all three species, but the dose-dependence was most evident in mouse hepatic microsomes. These results have quantified the interspecies difference in CYP-dependent TRI bioactivation and indicate that under both equivalent and occupationally relevant (hepatic) exposure conditions the human is at less risk of forming toxic

CYP-derived TRI metabolites.  
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**Key words:** Trichloroethylene, Cytochrome P450, Metabolism, Microsomes, Risk assessment, Enzyme kinetics, Toxicity, Bioactivation, Chloral hydrate

Trichloroethylene (TRI) is an unsaturated, chlorinated hydrocarbon widely used as an industrial solvent and is now a relatively common ground water pollutant<sup>1</sup>. TRI produces liver and lung toxicity in mice<sup>2-6</sup> and kidney toxicity/tumors in rats<sup>6-8</sup>, but no current epidemiology studies indicate a cancer risk to humans. Studies in rats and mice revealed that metabolism of TRI by cytochrome P450 (CYP) enzymes accounts for its bioactivation<sup>9</sup> to compounds which mediate TRI's effects. This metabolism is saturated in rats, but not mice, receiving 2,000 mg TRI/kg<sup>10</sup>, and several CYP-dependent metabolites produce DNA strand breaks<sup>11</sup>. The formation of chloral hydrate (CH) by CYP<sup>12, 13</sup> is differentially increased by inducers (ethanol, phenobarbital and 3-methyl cholanthrene<sup>14, 15</sup>) and inhibited to different degrees by antibodies to CYP2E1 and CYP1A2 in mice and rats<sup>16</sup>. Such differences in metabolism across species<sup>17</sup> and among tissues<sup>18</sup> may be the basis for the apparent species-specific effect of TRI on hepatic DNA content and proliferation of peroxisomes<sup>19</sup>. Additional results<sup>20</sup> have demonstrated an association between peroxisome proliferation and species-specific hepatic, but not renal, tumors.

TRI concentrations as low as 10  $\mu\text{M}$ , *in vitro*, inhibit gap junction mediated intercellular communication in mouse but not rat hepatocytes, and effect which is blocked by CYP inhibition<sup>21</sup>. The production of CH, and DNA- and protein-adducts were more abundant in incubations containing mouse than rat and human microsomes exposed to much higher (1.0 mM) concentrations of TRI<sup>22</sup>. Finally, under equivalent exposure conditions *in vivo*, mice metabolize more TRI and exhibit more TRI-

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dependent hepatic macromolecular binding than rats<sup>23</sup>). Taken together, these reports indicate that species differences in susceptibility, in addition to differences in rates of formation of toxic metabolites, may mediate the observed species-dependent susceptibility to TRI's toxicity.

Although these effects have been examined in detail *in vitro*, most *in vitro* studies have been conducted at concentrations not predicted to occur *in vivo*. By employing the physiologically-based pharmacokinetic (PBPK) model for TRI in the human<sup>24</sup>) to simulate an occupational exposure (8 h at 50 ppm), we determined that human liver might contain 15–20  $\mu\text{M}$  TRI. Because of the similarity of the predicted liver concentration to the  $K_m$  value for CYP-mediated TRI metabolism in the human (28  $\mu\text{M}$ )<sup>25</sup>), we sought to produce data which might be used to compare the metabolism of occupationally relevant concentrations of TRI among three of the most toxicologically important species. To quantify species-related differences in TRI oxidation, we have prepared hepatic microsomes under identical conditions and determined the effect of TRI on specific CYP-dependent activities and the kinetic parameters governing TRI oxidation in hepatic microsomes of the Fischer 344 rat, the B6C3F1 mouse and the human.

## Methods

**Chemicals:** All chemicals were at least reagent grade and were obtained from Sigma (St. Louis, MO) or Aldrich (Milwaukee, WI) unless otherwise noted. Alkoxyresorufins were obtained from Molecular Probes (Eugene, OR). Glucose-6-phosphate and NADP<sup>+</sup> were obtained from Boehringer-Mannheim (Indianapolis, IN). University of Wisconsin solution was a generous gift from the Human Cell Culture Center (Silver Spring, MD).

**Animals:** Adult male B6C3F1 mice (25–28 g) and adult male Fischer 344 rats (180–220 g) were kept 5 or 3 per cage, respectively, in polycarbonate rodent cages with hardwood chip bedding with food (Ralston Purina, Bloomington, IN) and *Pseudomonas*-free water available *ad libitum*. Animals were not fasted prior to sacrifice by CO<sub>2</sub> asphyxiation. Livers were quickly perfused *in situ* with cold 0.1 M TRIS-HCl/0.05 M KCl, pH 7.4 (buffer), removed and placed in ice-cold saline. The animals used in the study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals* prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996, and the Animal Welfare Act of 1966, as amended.

**Microsome preparation:** Livers from mice and rats were homogenized on ice with a Teflon-coated tissue grinder using a 5:1 v/w ratio of buffer (above). Homogenates were centrifuged at 10,000 g for 10 min at 4°C and microsomes were prepared from the remaining

supernate by centrifugation at 105,000 g for 60 min at 4°C. Resulting microsomal pellets were resuspended in buffer at a density of 1.0 ml per gram tissue. Human microsomes were obtained from a commercial supplier (International Institute for the Advancement of Medicine, Exton, PA). Organ donors were identified prior to clinical death, at which time organ harvest teams perfused the liver *in situ* with University of Wisconsin medium, to maintain tissue viability for transplant. If no recipient had been identified, or if prior arrangements had been made with research suppliers, the organ was shipped to the processing facility where microsomes were prepared by the method of Guengerich<sup>26</sup>). The time which elapsed between *in situ* perfusion and freezing of the preparation did not exceed 24 h in 92+% of the cases. Microsomal protein content was determined by the BCA method with bovine serum albumin as a standard. Microsomes from nine individual humans (Table 1) were pooled for kinetic evaluation of TRI biotransformation.

**Effect of liver perfusion:** An experiment was conducted to examine the effect of time in cold storage prior to microsome preparation on CYP2E1 activity. We employed conditions which mimicked the times and treatments involved in harvesting human livers for *in vitro* experimental use. Rats were anesthetized with CO<sub>2</sub>, and, consistent with procedures for human liver harvesting, some had their livers immediately perfused with ice cold

**Table 1.** Human liver donor information

Donor	COD	Cigarettes (ppd)	Ethanol	CYP
36 C M	CHI	Yes	nr	0.31
38 C M*	SAH	2	nr	0.73
40 C M*	CHI	nr	Yes	0.45
52 C M*	SAH	2.5	Heavy	0.42
26 C F	CHI	1	Moderate	0.39
55 C F*	SAH	nr	nr	0.42
63 P F*	SAH	nr	nr	0.45
52 C F	SAH	1	Social	0.58
47 C F	OD	nr	nr	0.51

Samples are identified by age in years, ethnic background, sex, where C, Caucasian, P, Filipino; M, Male, F, Female. Cause of Death (COD) is identified by CHI, Closed Head Injury, SAH, Subarachnoid hemorrhage, OD, drug overdose. Cigarette smoking is reported in packs per day (ppd) when quantified. Ethanol consumption was subjectively reported; nr, a negative response was given for cigarettes and/or ethanol. Cytochrome P450 content of microsomes (CYP) is reported as nmoles CYP/mg microsomal protein. \*Kinetic parameters for TRI metabolism have been individually evaluated and reported<sup>20</sup>).

University of Wisconsin (Belzer's) solution through the *vena cava*. Samples of non-perfused and perfused liver were immediately removed for light microscopy and an assessment of edema. Sections (5  $\mu\text{m}$ ) were prepared and stained with hematoxylin and eosin and evaluated by light microscopy. Four-millimeter sections of liver were weighed before and after drying at 80°C for 48 h and 72 h. Livers kept on ice in ten volumes of buffer were serially sampled by removing aliquots at either of two sets of times: 0, 6 and 12 h or 0, 12 and 24 h. Microsomes were prepared as above and CYP2E1-dependent demethylation of dimethylnitrosamine (DMN) was used as a marker for CYP2E1-dependent activity.

**Metabolism of TRI:** Incubations were carried out with microsomal protein, NADPH regenerating system and TRI in 0.1 M TRIS, 0.005 M  $\text{MgCl}_2$ , pH 7.4, the optimal pH for the reaction<sup>13,27</sup>. TRI was dissolved in acetone to final concentrations which allowed the introduction of acetone vehicle into microsomal incubations at concentrations of acetone up to 0.1% final volume. Previous experiments demonstrated that this concentration of acetone did not inhibit CYP2E1-dependent demethylation of dimethylnitrosamine. Analysis of metabolites was accomplished following previously published gas chromatographic methods<sup>25</sup>.

**Partition coefficient determination:** The partitioning of TRI into heat-inactivated rat liver microsomes was accomplished via the method of Sato and Nakajima<sup>28</sup>.

**Effect of TRI on CYP form-specific activities:** The effect of TRI on CYP activities was determined by adding TRI to the headspace of airtight vessels containing the reaction mixtures for each respective activity. TRI was diluted (to concentrations up to 100,000 ppm) into tedlar bags containing precisely metered volumes of air. TRI-containing air from bag samples was diluted into 10-ml serum vials sealed with Teflon-lined rubber septa. The activities of CYP1A1/1A2, CYP3A and CYP2B1/2B2 were estimated by determining the O-dealkylation of alkoxyresorufin substrates (ethoxy-EROD, benzoxy-BROD and pentoxy-resorufin, PROD, respectively) by the method of Burke *et al.*<sup>29</sup> Alkoxy resorufin assays were conducted at substrate concentrations of 2.0  $\mu\text{M}$ . Coumarin hydroxylase (COUM-OH) activity (CYP2A-dependent) was measured as described by Maenpaa *et al.*<sup>30</sup> using 10 mM substrate. CYP2E1-dependent, 4-Nitrophenol hydroxylase (PNP-OH, 100  $\mu\text{M}$ <sup>31</sup>) or N-dimethylnitrosamine N-demethylase (DMN, 1.0 mM<sup>32</sup>) activities, CYP1A2-dependent phenacetin O-deethylase (PAD, 1.0 mM) and CYP3A-dependent erythromycin N-demethylase (ERY, 1.0 mM) were assayed as previously described<sup>33</sup>. All reactions were initiated by the addition of a NADPH-regenerating system (in 0.1 M potassium phosphate buffer) which contained 14 mmol glucose-6-phosphate, 0.66 mmol  $\text{NADP}^+$ , and 3 units glucose-6-phosphate dehydrogenase.

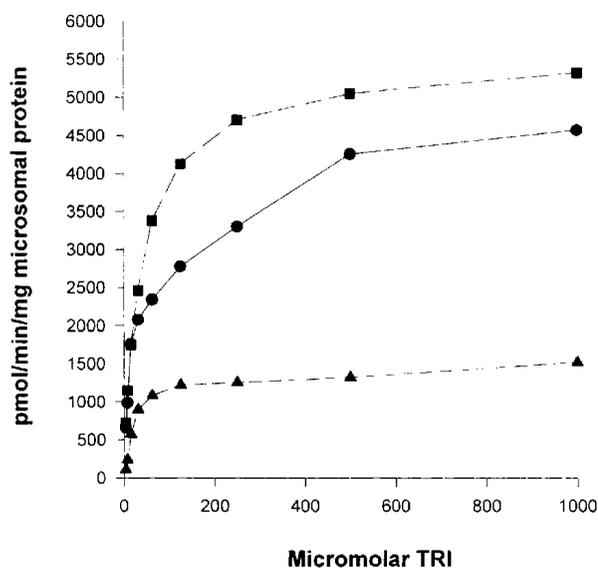
**Effect of TRI on total CYP:** The effect of TRI on total cytochrome CYP was determined by incubating microsomes with and without NADPH regenerating system and TRI (0, 250, 500 and 1,000 ppm present in vial headspace) for 15 min. Total CYP was then determined using the differential spectrophotometric method of Omura and Sato<sup>34</sup>.

**Statistical analysis:** Data from *in vitro* experiments were evaluated by analysis of variance with post-hoc evaluation of differences by Student-Newman-Keuls test ( $p \leq 0.05$ ). DMN activity data from microsomes derived from cold-stored liver were evaluated by paired t-test and analysis of variance (ANOVA). The tests were performed using a SAS General Linear Models program (SAS Institute, Cary, NC) on an IBM personal computer. Curve-fit mode for Michaelis-Menten kinetics (by saturation analysis) was performed using Enzfitter (Elsevier-Biosoft, Ferguson, MO) on an IBM personal computer. These analyses yielded values for  $K_m$  and  $V_{max}$  kinetic parameters.

## Results

**Effect of liver perfusion:** Light microscopic evaluation revealed no evidence of vascular damage; and, though not quantified, erythrocytes seemed absent from perfused liver. Increasing drying time from 48 to 72 h produced no additional change in sample weight (not shown). Perfusion produced a slight, but significant increase in the wet weight:dry weight ratios of perfused (3.86:1) versus non-perfused (3.54:1) rat liver. This indicates an increase in water content from 71.8% in control liver to 74.1% in perfused liver. Perfusion and cold-time (to 24 h) did not significantly alter CYP2E1-dependent activity towards DMN (data not shown).

**Metabolism of TRI:** Trichloroethylene metabolism by microsomes was dependent on NADPH and was highest in mice, slightly lesser in rats and much lower in the human (Fig. 1). Michaelis-Menten kinetics of microsomal TRI metabolism in the three species were used to calculate  $K_m$  and  $V_{max}$  values for each species. Michaelis-Menten analysis of saturation kinetics indicated  $K_m$  values of 35.4, 55.5 and 24.6  $\mu\text{M}$  and  $V_{max}$  values of 5,425, 4,826 and 1,440 pmol/min/mg microsomal protein for the mouse, rat and human, respectively. Because of previous reports<sup>14,16,35</sup> indicating that several CYP forms with different  $K_m$  values metabolize TRI in the rat, we examined TRI metabolism at low, intermediate and high TRI concentrations in microsomes of rats, mice and humans. Metabolic rates were examined at three substrate ranges: 0–250, 125–1,000 and 250–5,000  $\mu\text{M}$  (Table 2). We observed a pronounced shift in  $K_m$  value of the rat, but not the mouse or human. Indications of the activity of multiple forms in the rat was also confirmed using Eadie-Hoffstee analysis, but substrate-based inhibition complicated interpretation of results in the human (Fig. 2).



**Fig. 1.** Metabolism of TRI by microsomes. The metabolism of TRI by rat (circles), mouse (squares) and human (triangles) microsomes is presented as the mean from three experiments, nmol/min/mg microsomal protein. Microsomes were pooled from five rodents and seven humans.

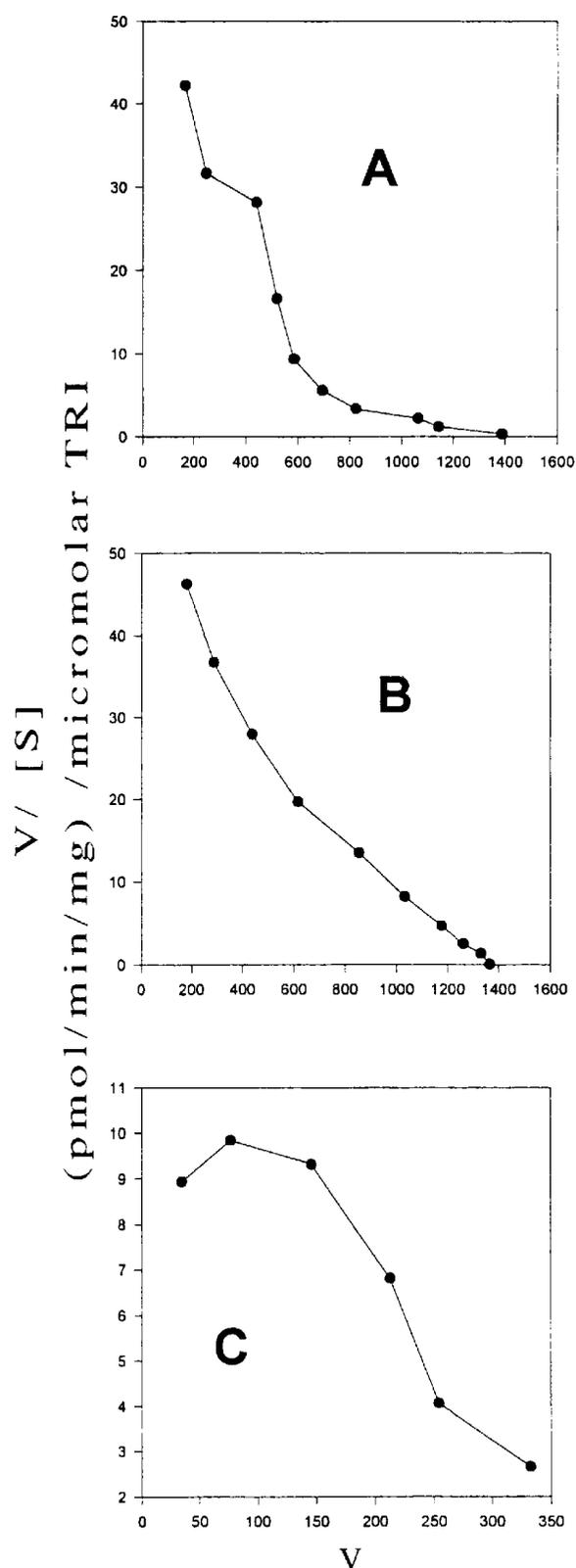
**Table 2.** Kinetic parameters of trichloroethylene metabolism by pooled<sup>a</sup> rat, mouse and human microsomes

Species	[ $\mu\text{M}$ TRI]	$K_m^b$	$V_{max}^c$	$CL_{int}^d$
Rat	0-5,000	55.5	1,206	21.7
	0-250	17.0	818	48.0
	125-1,000	114	1,272	11.2
	250-5,000	909	2729	3.0
Mouse	0-5,000	35.4	1,356	38.3
	0-250	31.9	1,301	40.0
	125-1,000	42.8	1,378	32.0
	250-5,000	43.0	1,379	32.0
Human	0-5,000	24.6	360	14.6

Data represent total metabolism of TRI to CH and TCOH. <sup>a</sup>n=5 rat and mouse, and n=7 human, <sup>b</sup> $K_m$  is presented as  $\mu\text{M}$  TRI in incubation medium, <sup>c</sup> $V_{max}$  is presented as nmol/min/mg microsomal protein, <sup>d</sup> $CL_{int}$  is presented as ml/min/mg microsomal protein.

**Partitioning of TRI:** Partitioning experiments carried out with heat-inactivated microsomes without NADPH regenerating system and with TRI in headspace at 1,000, 500 and 250 ppm indicated a partition coefficient of 1.78. This value predicts TRI concentrations in microsomal suspensions of 72, 36, and 18  $\mu\text{M}$ , respectively.

**Effect of TRI on P450 form-specific activities:** The effect of TRI (1,000 ppm) on P450-dependent, form-specific



**Fig. 2.** Eadie-Hoffstee depiction of microsomal TRI metabolism. Results from pooled rat (panel A) and mouse (panel B) microsomal samples and human microsomes from a single representative donor (panel C) are presented.

**Table 3.** The effect of TRI on cytochrome P450 form-specific activities in microsomes from mouse, rat and human

Species	TRI <sup>a</sup>	DMN <sup>b</sup>	PAD	ERY	EROD	COUM-OH	PROD	BROD
Rat	-	1.40 ± 0.26	0.37 ± 0.09	1.16 ± 0.06	83.00 ± 4.36	44.60 ± 0.66	35.00 ± 8.89	152.33 ± 18.56
Rat	+	0.99 ± 0.09*	0.52 ± 0.24	1.10 ± 0.17	157.33 ± 10.97*	46.77 ± 1.28	36.66 ± 9.24	46.00 ± 8.66*
Mouse	-	0.98 ± 0.09	0.16 ± 0.05	1.93 ± 0.30	169.33 ± 4.51	134.97 ± 5.34	14.00 ± 0.10	76.80 ± 1.85
Mouse	+	0.30 ± 0.09*	0.25 ± 0.09	1.07 ± 0.07*	326.43 ± 27.03*	112.37 ± 1.85*	18.00 ± 1.73	23.70 ± 2.42*
Human	-	1.73 ± 0.58	N.T. <sup>c</sup>	5.51 ± 2.25	80.05 ± 59.67	N.T.	4.25 ± 1.39	15.13 ± 9.85
Human	+	0.76 ± 0.18*	N.T.	6.81 ± 2.47	149.67 ± 30.98*	N.T.	4.02 ± 2.00	9.02 ± 4.22

Assay used were Dimethylnitrosamine N-demethylase, DMN (CYP2E1); Phenacetin O-deethylase, PAD (CYP1A2), Erythromycin O-deethylase, ERY (CYP3A); ethoxy- (EROD), benzoxy- (BROD) and pentoxy-resorufin O-dealkylase (PROD), were used to measure CYP1A-, CYP3A- and CYP2B-dependent activities, respectively. Coumarin hydroxylase (COUM-OH) was used to estimate CYP2A. \*Significantly different from control activity,  $p \leq 0.05$ . <sup>a</sup>Trichloroethylene, 1,000 ppm in headspace. <sup>b</sup>Units of activity: DMN, PAD, ERY=nmol/min/mg; EROD, COUM-OH, PROD, BROD=pmol/min/mg; PNP=nmol/min/mg. <sup>c</sup>Not tested in this species.

**Table 4.** The effect of Various concentrations of TRI on PNP-OH (CYP2E1) activity

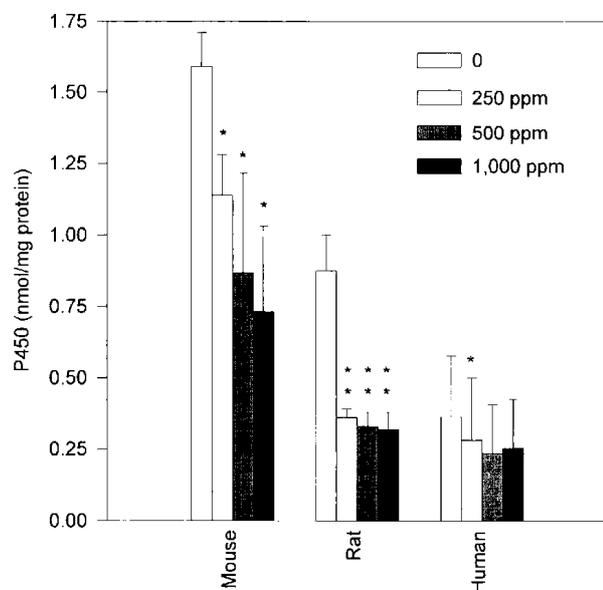
PNP ( $\mu$ M)	TRI (ppm)	Activity (nmol/min/mg Prot)		
		Human: 38	36	43
100	0	1.11	2.41	3.29
100	50	1.10	1.77	3.16
100	100	1.00	1.37	2.56
100	500	0.97	1.01	1.98
20	0	1.08	2.01	3.12
20	50	1.16	1.61	3.08
20	100	0.99	1.09	2.37
20	500	0.95	0.93	1.61
10	0	1.08	1.70	2.98
10	50	1.03	1.24	1.95
10	100	0.97	1.02	1.11
10	500	0.58	0.96	0.85

Microsomes from three humans displaying either low (#38), medium (#36) or high (#43) activity of CYP 2E1 were incubated with 0, 50, 100 and 500 ppm TRI in head space. Results indicate that PNP-OH activity is decreased by TRI in a dose-dependent manner and that the extent of inhibition is correlated with basal levels (0 ppm TRI) of CYP2E1-dependent activity.

activities varied with the assay performed. Those activities dependent on CYP2E1 (DMN, PNP-OH), CYP3A (BROD, ERY) and CYP2B (PROD) were decreased by the presence of TRI in the reaction vessel headspace. Evaluation of the interactions revealed a competitive inhibition with respect to CYP2E1-dependent activity ( $K_i=50$  ppm TRI), and a non-competitive inhibition of CYP2B-dependent activity (not shown). CYP1A1/1A2-dependent EROD activity was

increased by TRI as was CYP1A2-dependent phenacetin O-deethylase activity. Coumarin hydroxylase (CYP2A) was not affected by TRI (Table 3). To examine the effect of TRI on human CYP2E1 activity, microsomes were examined for PNP-OH activity. Microsomal samples possessing low, medium and high PNP-OH activity were incubated with increasing concentrations of TRI. Results presented in Table 4 indicate that TRI produced a concentration-dependent decrease in PNP-OH activity in all three samples, but the greatest inhibition (71%) was observed in the microsomal sample expressing the highest PNP-OH activity. This may indicate that the formation of a reactive metabolite is responsible for inhibition. To determine the sensitivity of microsomal PNP-OH activity to inhibition by TRI, we determined the concentration of TRI necessary to inhibit PNP-OH activity to 50% the original (uninhibited) value ( $I_{50}$ ). We determined that the  $I_{50}$  was 26 ppm in mouse microsomes and varied in the human samples evaluated: the sample showing the highest basal PNP-OH activity was the most sensitive ( $I_{50}=79$  ppm), the sample with intermediate PNP-OH activity demonstrated an  $I_{50}$  of 284 ppm TRI, and the sample with the lowest PNP-OH activity was the most resistant to inhibition by TRI ( $I_{50}=587$  ppm). These results may also suggest that samples (either from individuals within a species or among species) with higher intrinsic CYP2E1 activity may more susceptible to CYP2E1 inhibition and CYP destruction from reactive TRI metabolites.

**Effect of TRI on total CYP:** To examine the effect of TRI on total CYP, samples of microsomes from mice, rats and humans were incubated for 15 min with increasing concentrations of TRI and an NADPH-regenerating system. Control levels of CYP were highest for mouse>rat>human. Results presented in Fig. 3 indicate that TRI produced a dose-dependent increase in the destruction of P450 in mouse microsomes, as



**Fig. 3.** The effect of TRI on total cytochrome P450. Results are presented as mean  $\pm$  S.D. TRI was incubated with mouse microsomes and a source of NADPH for 15 min. \*denotes  $p \leq 0.05$ , \*\*denotes  $p \leq 0.0001$ .

determined by CO-binding spectrum. The rat demonstrated the highest loss of CYP, and microsomes of the human demonstrated the lowest loss (<20%). Further experiments with mouse microsomes determined that the decrease by TRI was dependent on both time and NADPH (data not shown).

## Discussion

Because of the low level of TRI to which humans are occupationally exposed, and the further diminution of the absorbed dose prior to distribution to the liver, we sought to compare metabolism of physiologically-relevant doses of TRI. Previous studies<sup>22, 36</sup> have used concentrations of TRI which are saturating in the human to demonstrate species rank order of metabolism. By defining the kinetic constants for CYP mediated TRI metabolism, it is possible to compare these rats among species at concentrations more relevant to human exposures. While metabolic saturation may be evident in data from studies with experimental animals or *in vitro*, kinetic data from human microsomes combined with the available human PBPK model for TRI predict that occupationally relevant exposures to TRI will not produce saturation in the human *in vivo*.

Our comparison of metabolic rates *in vitro* assumes that enzymes in subcellular preparations from human liver are not compromised. We have demonstrated that CYP2E1 is the primary form responsible for metabolism of TRI, that activity characteristic for CYP2E1 is not compromised in our human hepatic microsomes, and that the

maximal rate of TRI metabolism ( $V_{max}$ ) in human microsomes is approximately one-third that in rat microsomes and approximately one-fourth that in mouse microsomes. Also, clearance values ( $V_{max}/K_m$ ) are 0.153, 0.087, and 0.058 ml/min/mg microsomal protein for the mouse, rat and human, respectively, which indicate that the human is least able to metabolically dispose of TRI. Michaelis-Menten enzyme kinetic analysis determined that while mouse and human microsomes have a similar  $K_m$  value (35.4 versus 24.6  $\mu$ M TRI, respectively), rats have an apparent high, medium and low affinity form-specific metabolism of TRI (with  $K_m$  values of 17, 114 and 909  $\mu$ M TRI, respectively). This observation extends original reports<sup>14, 16</sup> that rat microsomes possessed multiple  $K_m$  values for TRI metabolism. In the Wistar rat, phenobarbital treatment induced TRI metabolic rate at high, but not low, TRI concentrations<sup>14</sup> indicative of a contribution of CYP2B<sup>37</sup>; although inhibition of TRI metabolism by ketoconazole<sup>25</sup> may indicate some contribution by CYP3A forms. Because of the high distribution of CYP3A forms in human liver<sup>38</sup>, the  $K_m$  value for the high- $K_m$  enzyme in rat liver microsomes (relative to concentrations of TRI in human liver) may indicate that hepatic CYP3A forms are not well suited for TRI metabolism, given the constraints of occupational exposures. Further, Miller and Guengerich<sup>22</sup> have demonstrated that rat hepatic CYP3A (P-450<sub>PB/PCN-E</sub>) does not contribute to TRI metabolism *in vitro*. However, the extreme lipophilicity of TRI may produce concentrations in fat which may stimulate metabolism by CYP3A and CYP2E1 enzymes expressed therein<sup>39, 40</sup>. Because of the potential metabolism of TRI by these CYP forms, the pharmacokinetic contribution, beyond a depot, of fat may be reconsidered.

Additional species-related differences suggest that the human might be at lower risk of forming toxic CYP-derived metabolites; mouse and rat microsomes are capable of metabolizing TRI across a wide range of concentrations while microsomes from humans display substrate based inhibition of TRI metabolism at higher concentrations (>150  $\mu$ M TRI). By using the Michaelis-Menten equation, the metabolic rate in human microsomes exposed to the occupationally relevant concentration of 15  $\mu$ M is 545 pmol/min/mg protein, while that concentration produces rates of 1,026 and 1,614 pmol/min/mg in rat and mouse microsomes, respectively. To further demonstrate the reduced capacity of human microsomes to biotransform TRI, 545 pmol/min/mg would be produced in rat and mouse microsomes exposed to 7.1 and 4.0  $\mu$ M TRI, respectively.

The interaction between TRI and several form-selective substrates metabolized by cytochrome P450 forms was also investigated. TRI is a competitive inhibitor of mouse CYP2E1-dependent activity (PNP-OH), which supports the contention that this same highly-conserved CYP is responsible for TRI metabolism in rodents and humans.

This metabolic similarity may assist in the extrapolation of rodent metabolism data to the human. However, we found several other differences in metabolic effects of TRI between rodent and human.

A finding which is not so easily interpreted is the increased activity of CYP1A forms in the presence of TRI. Our data (PAD and EROD) in the rat and mouse indicate substantial increases in the activity of these forms in the presence of TRI. The lipophilicity of TRI may contribute to a detergent-like effect, solubilizing the membrane and releasing the enzyme to allow more direct interaction between substrate and enzyme (e.g. bilirubin glucuronyl transferase assays), but this would have produced a non-selective increase in CYP activity, an effect which was not observed. It is also possible that TRI occupied additional non-metabolically active, or slow turn-over binding sites normally occupied by phenacetin or ethoxy resorufin, thereby increasing substrate availability at higher turnover sites on CYP1A. Other authors have attributed similar *in vitro* effects of CYP2E1 specific substrates (halothane and acetone) to conformational change produced in the affected CYP form<sup>41, 42</sup>.

The TRI-induced decrease in total P450 as measured by spectrophotometry was higher in rats and mice than in the human samples evaluated. Because of the higher total CYP content of rodent microsomes@1the higher extent of CYP destruction in the rodent may be the basis for the higher degree of PNP-OH inhibition observed. The difference in inhibition of PNP-OH activity in the three human samples may also reflect the contribution of a P450 form other than CYP2E1 towards PNP. These results will require further experimentation to fully and accurately characterize the effect of TRI on this activity.

These data indicate that, like many other industrial chemicals<sup>43, 44</sup>, the mouse is best able to metabolize TRI. This higher extent of CYP-dependent TRI metabolism in the mouse is one factor which may predispose this species to the demonstrated hepatotoxic effects of TRI. The results presented in this report represent the first time that microsomal TRI metabolism of the rat, mouse and human have been fully characterized and precisely compared *in vitro*. This report has demonstrated that CYP2E1 is the CYP form most responsible for TRI metabolism in these species, indicating that factors which modify CYP2E1 expression in the human, as in the rat, should be considered as modifiers of TRI toxicity. Our data on CYP3A4 warrant further investigation: the potential for TRI metabolism by enzymes distributed within the fat compartment is being investigated.

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