Styrene Pharmacokinetics and Pharmacodynamics

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Establishing how a chemical is carcinogenic

- General questions:
  - mutagenic or non mutagenic?
  - Carcinogenic in one organ/one species?
  - Carcinogenic in multiple organs/multiple species?
  - Reactive metabolites?
  - Cytotoxic?
  - Relevant to humans?

Styrene

- Carcinogenic in Mouse Lung
- Hypothesized that carcinogenicity depends on:
  - Metabolism of styrene via CYP2F2 in the lung
  - More Clara cells in mouse lung than rat or human
  - Mice have a difference in metabolism of styrene via ring oxidation
  - Increased cell replication
  - Decreased CCSP

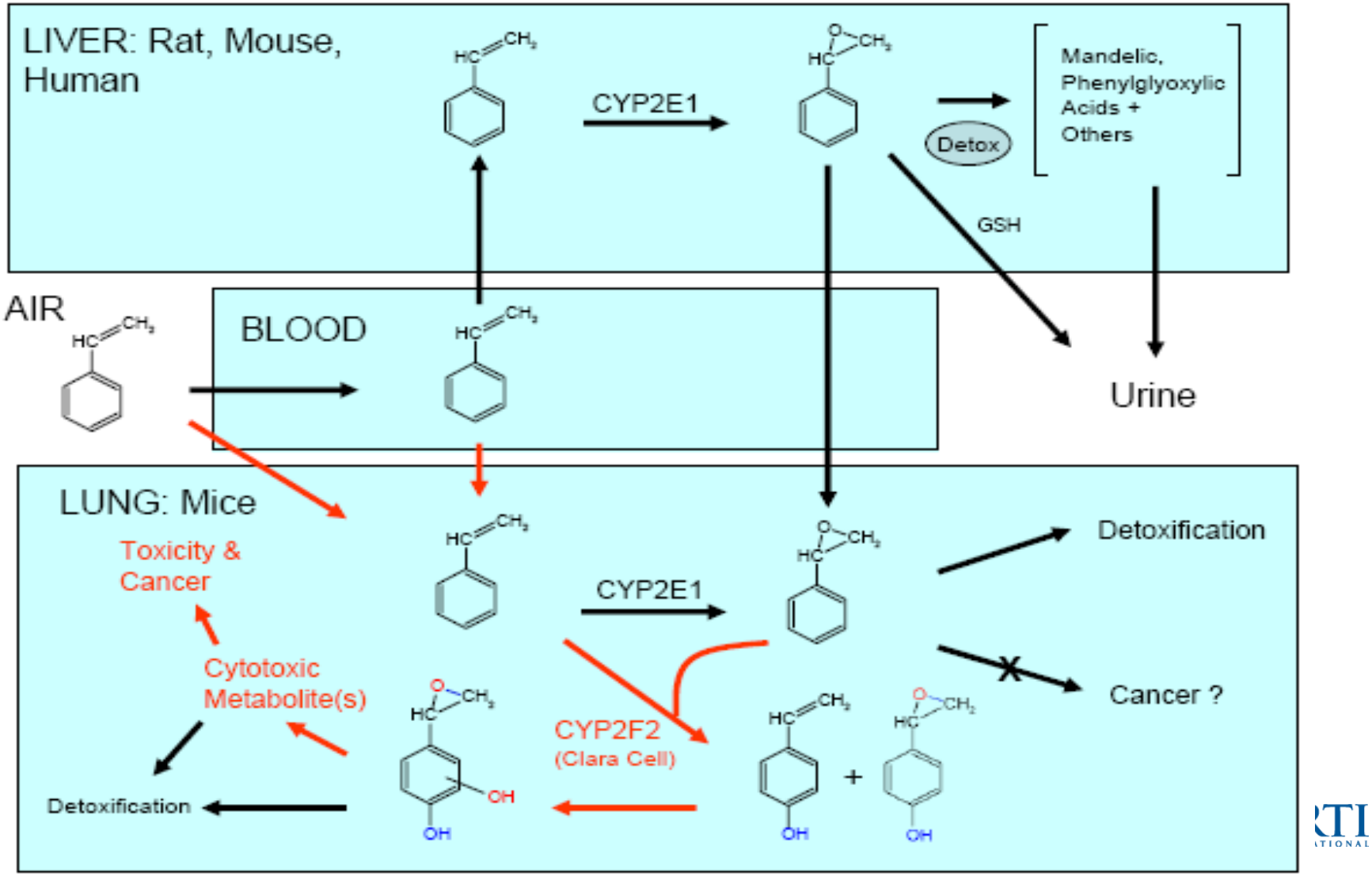
Support

- Comparison of activity in presence or absence of Cyp2F2.
  - Species differences
  - Cyp2F2 knockout mice
    - Toxicity
    - Cell proliferation
- Key question – is styrene carcinogenic in Cyp2F2 knockout mice?

Is the Mechanism Applicable to Humans?

- Single species/single organ?
- Reactive metabolite produced in target organ specific to that organ/species?
- Specific enzyme in activation? Is that enzyme present in humans? Is the activity present in humans?
- Pharmacodynamics applicable?
- Mutagenicity?
- Cell proliferation?
- Other?

Styrene MOA (from Cruzan)



Concerns about Metabolism

- Not clear what the metabolite of concern is.
  - Vinylphenol or a metabolite? Catechol? Ring opened? Epoxide?
  - Ring opened metabolite of styrene?
- Has the metabolite or its products been directly demonstrated in vitro?
- Has the metabolite or its products been directly demonstrated in vivo?
- Is there evidence for no metabolism via this pathway in man?

Metabolism in Humans

- ^{13}C labeled styrene exposure and NMR spectroscopy
  - Do see substantial differences in metabolism between rats, mice and humans.
- LC-MS analysis of styrene metabolites following workplace exposure to styrene including 4-vinylphenol glucuronide and sulfate

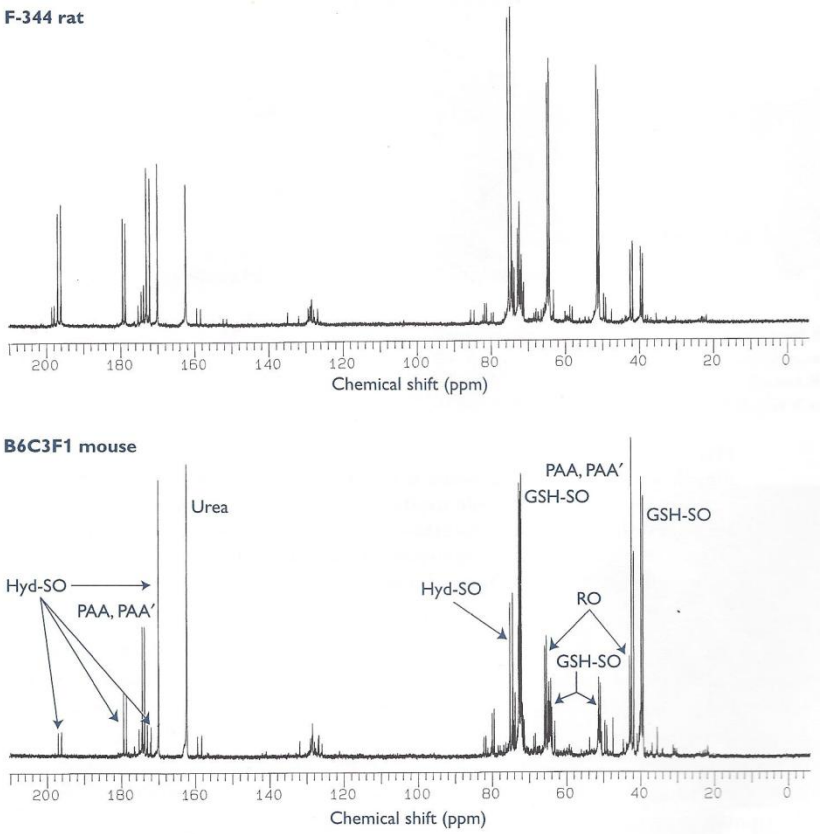


FIGURE 2
^{13}C-NMR spectra of urine from rats or mice administered [7,8-^{13}C]styrene had signals derived from the labeled carbons of styrene. These signals were assigned to metabolites based on chemical shifts expected for known styrene metabolites or calculated values of shift for proposed metabolites. Signals for ^{13}C-styrene-derived metabolites appeared as multiplet patterns due to spin-spin coupling between the labeled carbons. NMR spectra of urine from (top) the F344 rat and (bottom) the B6C3F1 mouse exposed to approximately 160 ppm [7,8-^{13}C]styrene indicated that mice have a greater production of metabolites from the phenylacetic acid and phenylaceturic acid (PAA, PAA′) pathway and signals consistent with the production of ring-open (RO) products. Abbreviations: GSH-SO, products formed following conjugation of SO with glutathione; Hyd-SO, products formed following hydrolysis of SO; SO, styrene 7,8-oxide.

Sumner et al., 2001; CIIT Activities, 21: no 3-4.

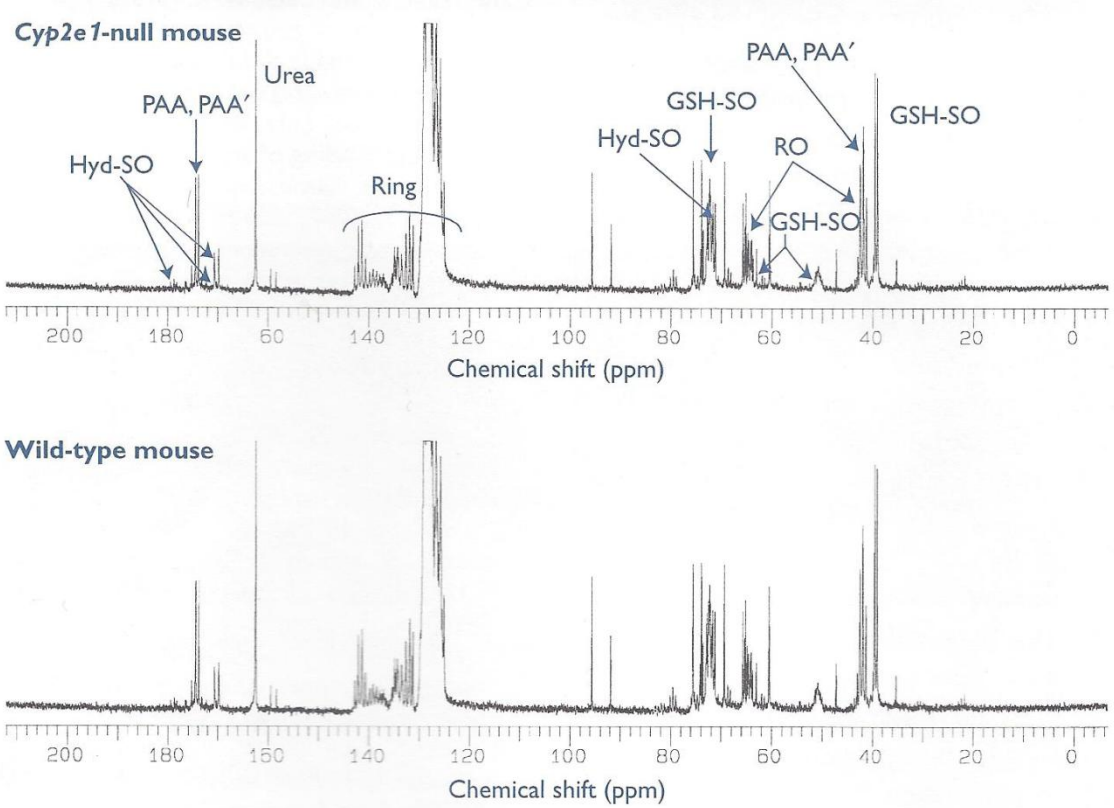


FIGURE 3
NMR spectra of urine from (top) the *Cyp2e1*-null mouse exposed to uniformly labeled ^{13}C-styrene were similar to those detected for (bottom) the exposed wild-type mouse, indicating that *Cyp2e1* is not essential for the in vivo conversion of styrene to styrene 7,8-oxide (SO). Abbreviations: GSH-SO, products formed following conjugation of SO with glutathione; Hyd-SO, products formed following hydrolysis of SO; PAA, PAA′, phenylacetic acid, phenylaceturic acid; RO, potential ring-open products.

Sumner et al., 2001; CIIT Activities, 21: no 3-4.

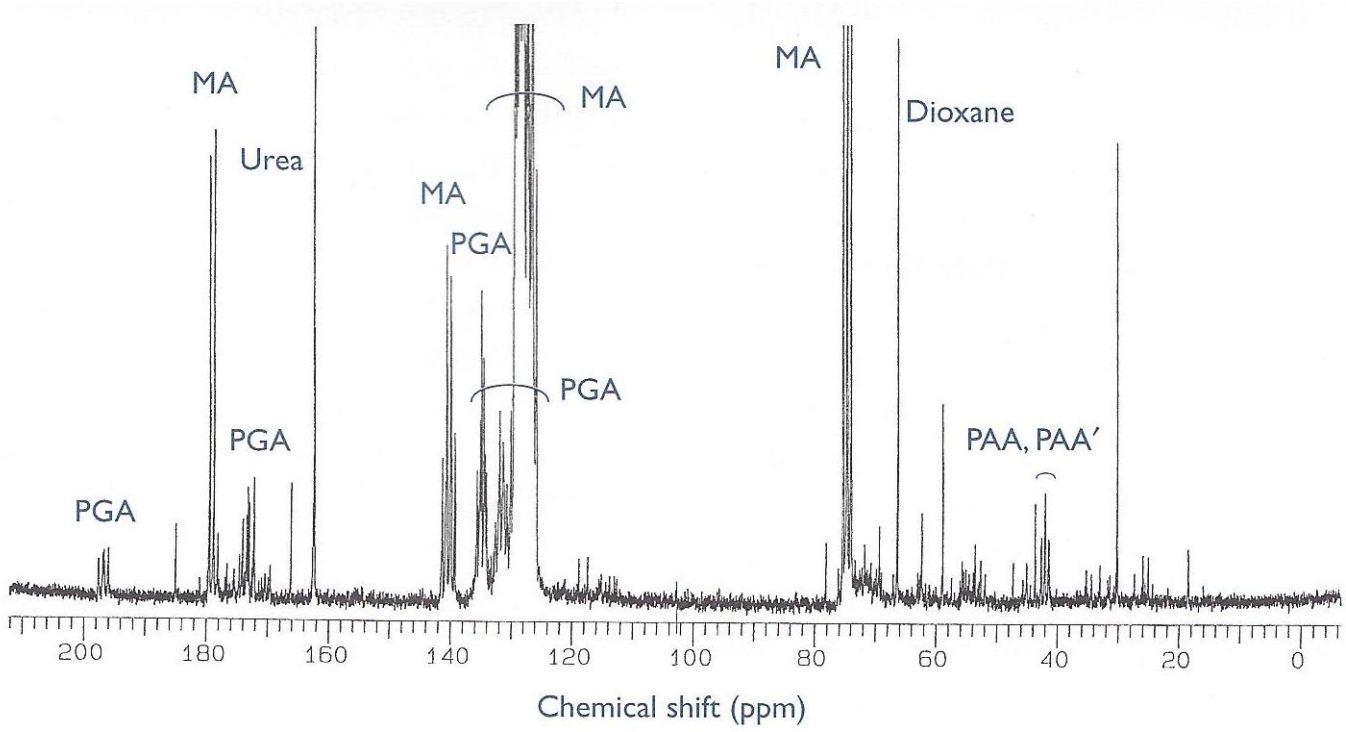


FIGURE 4
Human volunteers exposed to uniformly labeled ^{13}C-styrene vapor at 50 ppm for 2 hours had over 95% of assigned metabolites derived following hydrolysis of styrene 7,8-oxide (SO). Abbreviations: MA, mandelic acid; PAA, PAA′, phenylacetic acid, phenylaceturic acid; PGA, phenylglyoxylic acid.

Sumner et al., 2001; CIIT Activities, 21: no 3-4.

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RCM

Liquid chromatography/electrospray tandem mass spectrometry characterization of styrene metabolism in man and in rat

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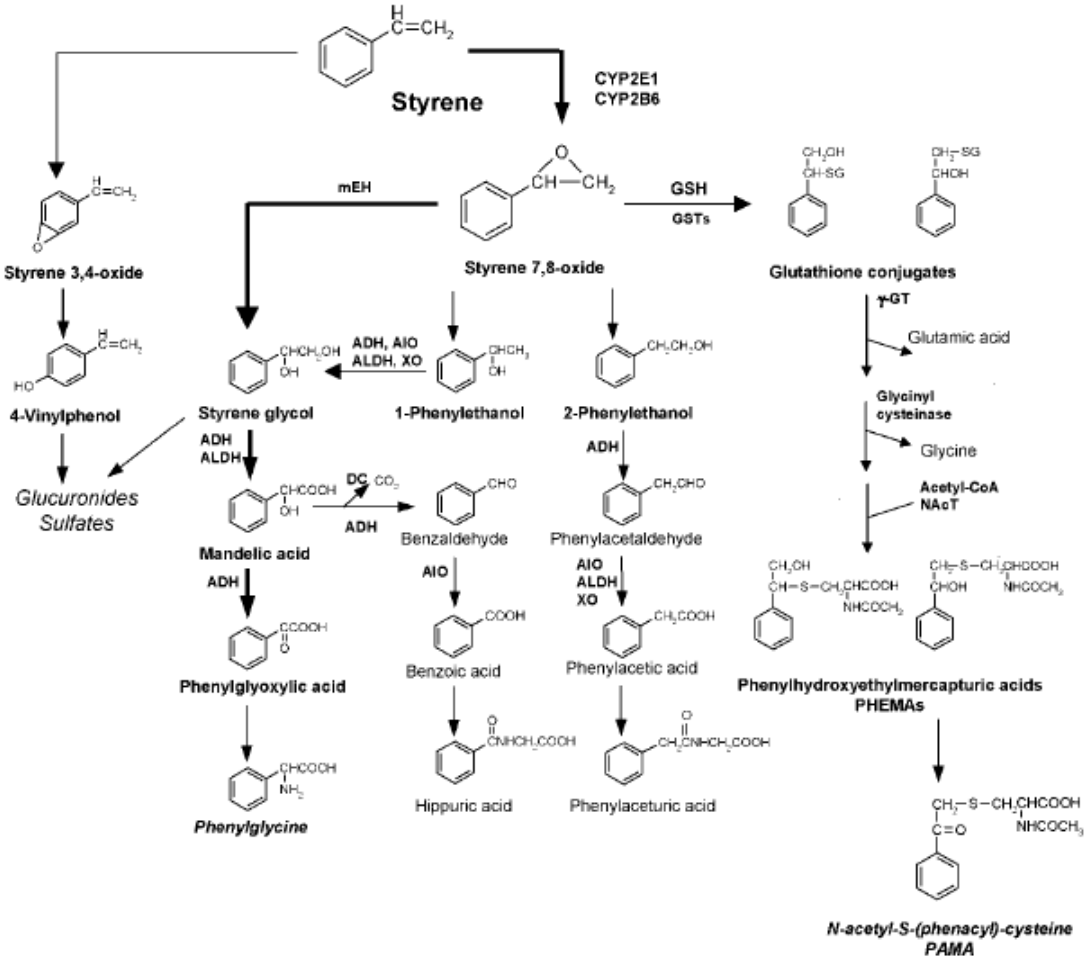

Figure 1. Scheme of styrene metabolism in man. Abbreviations: CYP2E1 and CYP2B6 cytochrome P-450 monooxygenase, mEH microsomal epoxide hydrolase, ADH alcohol dehydrogenase, AIO aldehyde oxidase, ALDH aldehyde dehydrogenase, XO xanthine oxidase, DC decarboxylase, GSH glutathione, GSTs glutathione S-transferases, γ-GT gammaglutamyl transpeptidase, NAcT N-acetyltransferase.

Table 2. Metabolite excretion in rats exposed to styrene and co-exposed to styrene and styrene-d_8

		Control		Styrene-d_8		Styrene + styrene-d_8	
Compound	SMR	24 h	48 h	24 h	48 h	24 h	48 h
Main pathway							
MA	151 → 107	–	–	–	–	+	+
d_6-MA	157 → 113	–	–	+	+	+	+
PGA	149 → 105	–	–	–	–	+	+
d_5-PGA	154 → 110	–	–	+	+	+	+
PGLY	152 → 135	–	–	–	–	+	+
d_5-PGLY	157 → 140	–	–	+	+	+	+
Mercapturic acids							
PHEMAs	282 → 153	–	–	–	–	+	+
d_8-PHEMAs	290 → 161	–	–	+	+	+	+
PAMA	280 → 151	–	–	–	–	+	+
d_7-PAMA	287 → 158	–	–	+	+	+	+
4-VPMA a	282 → 153	–	–	–	–	–	–
d_7-4-VPMA a	290 → 161	–	–	–	–	–	–
4-VPMA b	264 → 135	–	–	–	–	–	–
d_7-4-VPMA b	271 → 142	–	–	–	–	–	–
Sulfates							
PE-S	201 → 121	+	+	+	+	+	+
d_8-PE-S	209 → 129	–	–	–	–	–	–
SG-S	217 → 137	–	–	–	–	(trace)	(trace)
d_8-SG-S	225 → 145	–	–	(trace)	(trace)	(trace)	(trace)
4-VP-S	199 → 119	–	–	–	–	+	+
d_7-4-VP-S	206 → 126	–	–	+	+	+	+
Glucuronides							
PE-G	297 → 175,113	+	+	+	+	+	+
d_8-PE-G	305 → 175,113	–	–	–	–	–	–
SG-G	313 → 175,113	–	–	–	–	+	+
d_8-SG-G	321 → 175,113	–	–	+	+	+	+
4-VP-G	295 → 175,113	–	–	–	–	(trace)	(trace)
d_7-4-VP-G	302 → 175,113	–	–	(trace)	(trace)	(trace)	(trace)

Manini et al., 2002

Table 5. LC/MS/MS analysis of styrene metabolites in 'end-of-shift' and 'next morning' samples from 10 workers exposed to styrene. Concentrations are expressed in mg/g creatinine. Relative percentages (%) of metabolites were calculated assuming the sum of MA, PGA, PHEMAs, 4-VP-G and 4-VP-S as the 100%

	End-of-shift			Next morning		
Metabolite	Median	range	%	Median	range	%
MA	981	101–3196	73.2	160	29.9–511	56.0
PGA	323	34.7–813	24.9	111	23.0–276	38.9
MA + PGA	1337	136–4009		283	52.9–787	
PGLY	5.4	1.1–9.1	0.40	6.0	1.3–11.6	2.12
(R,R)-M1	4.0	0.04–19.5		1.4	0.16–5.7	
(S,R)-M1	0.23	0.01–0.98		0.02	0.01–0.09	
(S,R)-M2	2.5	0.05–9.8		0.48	0.08–1.96	
(R,R)-M2	0.37	0.02–0.97		0.09	0.01–0.23	
Total PHEMAs	7.1	0.12–31.2	0.53	1.9	0.31–7.9	0.69
4-VP-G	7.5	0.91–55.1	0.56	2.6	0.40–9.0	0.91
4-VP-S	6.5	0.73–21.2	0.49	3.9	0.83–11.2	1.40

Manini et al., 2002

Role of CYPs in the Metabolism of Styrene

- Types of studies:
  - Inhibition of metabolism
  - Antibody inhibition
  - Recombinant cyps
  - Knockout mice
- Styrene has been identified as one of the substrates for Cyp2E1 and CYP2F2.
- However, it is also a substrate for many other CYPs.
- Most of the analyses of activity have focused on the oxidation of styrene to styrene oxide or to styrene glycol.

Lack of Styrene Metabolism by Human CYP2F1?

- "The human lung CYP2F1 is expressed at levels well below those found in the non-responsive rat (reviewed in Cruzan et al., 2009), and in vitro studies in human BEAS-2B cells overexpressing CYP2F1 also did not detect any styrene metabolism (Carlson, 2008)." Cruzan et al., (2013) Regulatory Toxicology and Pharmacology 66: 24-29.

- Carlson (2008). Critical appraisal of the expression of cytochrome P450 enzymes in human lung and evaluation of the possibility that such expression provides evidence of potential styrene tumorigenicity in humans. Toxicology 254 (2008) 1–10.

- What it says: "Sheets et al. (2004) examined the metabolism of benzene in human lung cells (BEAS-2B) overexpressing CYP2F1……..However, unpublished studies have not been able to demonstrate the metabolism of styrene by this CYP2F1 containing system."

However: cDNA-expressed Cyp Activities

Table 3. Formation of Styrene Glycol from Styrene in Vaccinia Virus-Expressed Human, Mouse, and Rat Cytochrome P450s

recombinant virus	cell[a] (nmol/(dish·2 h))	lysate[b] (nmol/(mg of protein·min))
	Human	
wild type	ND[c]	ND
CYP1A2	63.2 ± 8.2	0.096
CYP2A6	2.7 ± 0.4	ND
CYP2B6	119.6 ± 13.4	0.147
CYP2C8	47.6 ± 6.9	0.050
CYP2C9	5.7 ± 0.9	ND
CYP2D6	2.7 ± 0.4	ND
CYP2E1	63.4 ± 2.0	0.161
CYP2F1	103.9 ± 4.6	0.105
CYP3A3	23.6 ± 3.8	0.035
CYP3A4	24.0 ± 2.5	0.034
CYP3A5	11.8 ± 1.7	0.025
CYP4B1	13.3 ± 1.0	0.025
	Mouse	
CYP1A1	85.0 ± 2.7	0.099
CYP1A2	17.2 ± 5.3	0.031
	Rat	
CYP2B1	198.8 ± 19.0	0.358
CYP2B2	81.9 ± 2.6	0.092

[a] Hep G2 cells, cultured in dishes containing DMEM and 10% fetal calf serum, were infected with vaccinia virus encoding one of P450 isozymes, and styrene was directly added to the dishes. Each value represents the mean ± SD of triplicate determinations. [b] Hep G2 cells expressing P450 isozyme were destroyed by sonication, and the microsomal fraction was used as a source of enzyme. Each value represents mean of duplicate determinations. [c] ND, not detected.

From: Nakajima et al., (1994) Chemical Research in Toxicology, 7: 891-896

In Vitro Metabolism of Styrene in Mouse Lung Microsomes

Table 2. Velocity of the Production of Vinyl Phenols and Styrene Glycol in *Cyp2e1*-null and Wild-Type Mouse Liver and Lung Microsomal Incubations with Styrene (500 μM)[a]

	liver microsomes				lung microsomes			
	2-VP	3-VP	4-VP	SG	2-VP	3-VP	4-VP	SG
129S1/SvImJ	1.85 ± 0.15	0.089 ± 0.017	0.877 ± 0.026	2.09 ± 0.04	6.47 ± 0.96	0.155 ± 0.020	0.234 ± 0.047	1.31 ± 0.13
Cyp2e1-null	0.717 ± 0.145[b]	0.063 ± 0.011	0.278 ± 0.015[b]	1.17 ± 0.126[b]	4.35 ± 0.623[c]	0.163 ± 0.020	0.232 ± 0.015	1.37 ± 0.02

[a]Mean ± SD, $n = 3$. VPs, pmol/min/mg of protein; SG, nmol/min/mg of protein. [b]$p < 0.01$. [c]$p < 0.05$ compared with the wild-type mice.

Table 3. Velocity of the Production of Vinyl Phenols and Styrene Glycol in *Cyp2f2*-null and Wild-Type Mouse Liver and Lung Microsomal Incubations with Styrene (500 μM)[a]

	liver microsomes				lung microsomes			
	2-VP	3-VP	4-VP	SG	2-VP	3-VP	4-VP	SG
C57BL/6J	2.93 ± 0.15	0.370 ± 0.035	0.925 ± 0.082	2.44 ± 0.08	3.72 ± 0.54	0.181 ± 0.007	0.334 ± 0.019	1.13 ± 0.17
Cyp2f2-null	1.90 ± 0.58[c]	N.D.	N.D.	1.83 ± 0.02[b]	N.D.	N.D.	N.D.	0.410 ± 0.007[b]

[a]Mean ± SD, $n = 3$. VPs, pmol/min/mg of protein; SG, nmol/min/mg of protein. [b]$p < 0.01$. [c]$p < 0.05$ compared with the wild-type mice. N.D.: not detected.

Shen et al., Metabolism of Styrene to Styrene Oxide and Vinylphenols in Cytochrome P450 2F2- and P450 2E1-Knockout Mouse Liver and Lung Microsomes. Chemical Research in Toxicology, in press.

RTI INTERNATIONAL

Cyp2F1 Humanized Mouse

- CYP2F2(-/-)/CYP2F1/2A13/2B6-transgenic (TG) mice and CYP2F2(-/-) mice vs. WT.
- No cytotoxicity or no increase in BrDU labeling with S or SO in the transgenic mice compared with the wild type (200 mg/kg/day ip for 5 days).
- Decreased BrDU labeling with KO and TG mice vs WT when administered 4-vinylphenol.
- Could interpret as a lack of metabolism via human CYP2F1 in mice. However, it is ambiguous, and changes could also result from alterations in metabolism resulting from the CYP2A13 or 2B6 isoforms, both of which can oxidize styrene (Fukami et al., 2008, and Nakajima et al., 1994).

Cruzan et al., Studies of styrene, styrene oxide and 4-hydroxystyrene toxicity in CYP2F2 knockout and CYP2F1 humanized mice support lack of human relevance for mouse lung tumors. Regulatory Toxicology and Pharmacology, (2013), http://dx.doi.org/10.1016/j.yrtph.2013.02.008

Questions?

- Is there a species difference in lung metabolism?
- Are the toxic metabolites so reactive that they have to be produced in situ?
- Are vinylphenols mutagenic with activation with lung microsomes?
- Are the toxic metabolites so reactive that they can not be detected directly?
  - Can they be detected indirectly?
- Can a marker be developed that indicates they were formed? Protein or DNA adduct?

Potential Ways to Elaborate on Activity

- Metabolomics - Changes in endogenous metabolites
- Gene expression
- Protein expression
- Protein modification
  - Blood protein adducts
  - Tissue adducts
- Dose and time response. Is there a correlation with covalent binding and GSH depletion?