TRIP REPORT

Subject:	**37th Annual Meeting of the Society of Toxicology, Seattle, 1-5 March, 1998.**
Author:	Dr. A. R. Tricker
Date:	18th March, 1998

Organizer: Society of Toxicology

Participants: ca. 5,000

Other PM staff: 14 in total

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General Remarks

According to the organizers, 5,000 people registered to attend the meeting which had multiple parallel sessions for oral and poster presentations. In total the meeting contained 2,000 presentations and 200 trade stands. About 30 scheduled posters were not presented.
It was evident that considerable interest (and funding) remains in the U.S. for studies involving (i) PCB's – most of which were focused on environment/exposure/risk of female breast cancer, (ii) chlorinated solvents in drinking water, and (ii) exposure to heavy metals – particularly cadmium and lead.
Due to the large amount of work presented, this report is only a subjective selection of some of the presentations reporting product and smoking-related issues, inhalation studies, chemical and *in vitro* studies of tobacco smoke constituents, molecular and genetic susceptibility studies of different population groups to specific diseases. Copies of the abstracts (denoted #) are available from the author.

Smoking-related presentations

The meeting contained 4 posters from the Company on an electrically heated cigarette (#1449, #1450, #1451, #1452) and will not be discussed further.
Finch (Lovelace, Albuquerque, NM., #1728) reported a chronic inhalation study in which male and female F344/Crl rats were exposed to mainstream CS (100 or 250 mg TPM/m^3, 6 h/d, 5 d/wk, 30 m, whole-body) and received a single acute nasal exposure to $^{239}PuO_2$ during week 6. Crude tumor incidences at the end of exposure were as follows:

	Sham smoke exposure	Low smoke exposure (100 mg TPM/m^3)	High smoke exposure (250 mg TPM/m^3)
$^{239}PuO_2$ exposed	M: 33% F: 20%	M: 49% F: 61%	M: 72% F: 74%
No $^{239}PuO_2$ exposure	M: 2.5% F: 0%	M: 1.7% F: 2.8%	M: 8.0% F: 7.2%

Assuming an approximately linear dose-response relationship between radiation dose (Sham smoke: 3.8 Gy; low smoke exposure: 4.1 Gy; high smoke exposure: 7.6 Gy) and crude lung tumor incidence, no explanation could be given for the supperadditive increase in lung neoplasms in animals receiving both exposures.
Fung (Rutgers University, Piscataway, NJ., #470) reported induction of CYP1A1 (mRNA, protein and catalytic activity) in lung, liver and kidney of male SD rats exposed to diluted SS or MS of the 2R1 reference cigarette. Rats were exposed to smoke from 3 machine-smoked cigarettes using various experimental protocols (number of exposures, duration of exposure, and time of sacrifice after last exposure). MS was more effective than SS at inducing

CYP1A1 activity under most conditions. Induction by SS was observed in lung ≥ kidney, and to a lesser extent in liver.

Meckley (RJR, #1085) reported a standardized dermal tumor promotion assay protocol for CSC using female CD-1 mice initiated with a single dose of 75 μg DMBA followed by promotion 3 times/wk using 12, 24, 36, 48 and 60 mg 'tar' for 51 weeks. Using groups of 40 mice it was claimed that the protocol can differentiate between different doses of CSC. The 1R4F reference cigarette was used as a source of CSC. The assay protocol was suggested to replace existing protocols using the SENCAR mouse.

ETS/Smoking-related presentations

Pereg (CHUQ Research Center Ste-Foy, Qc., #1593) reported preliminary data for placental cadmium levels in nonsmokers (geometric mean: 0.026; range 0.024-0.028 μg/kg; n=15), smokers of 1-15 cpd (geometric mean: 0.058; range 0.053-0.064 μg/kg; n=14) and smokers of >15 cpd (geometric mean: 0.080; range 0.072-0.088 μg/kg; n=11). Significant correlation's were claimed between placental cadmium and cpd (Pearson's r=0.61; p<0.01), cotinine in meconium (r=0.87; p<0.001) and placental EROD activity (r=0.68; p<0.001). The study is being extended to 100 subjects and to determine total adducts using ^{32}P-postlabeling. Cadmium levels were claimed (verbally) to be higher in Canadian cigarettes compared to U.S. blend cigarettes. The ultimate study aim is to establish an association between self-reported exposure to ETS and placental cadmium. (Copy of poster available on request)

Howard (University of Nevada, Reno, NV., #744) – 'DNA damage from exposure to environmental tobacco smoke in the workplace'. Some discrepancies in the reported data in the poster and a handout publication at the poster boards were apparent (Howard *et al.*, Cancer Epidemiol. Biomarkers & Prev., 7, 141-146, 1998). Neglecting these discrepancies, the method used to determine 8-hydroxy-2'-deoxyguanosine as a marker of oxidative stress is known to be prone to artifact formation. The cotinine data was also suspect. (Publication available on request)

Costa-Mallen (University of Washington, Seattle, #1488) reported that polymorphism's of monoamine oxidase B (MAO-B) and the dopamine D2 receptor (DRD2) may influence risk of Parkinson's disease (PD). In 102 incident cases and 162 controls, smoking showed a significant negatively association with PD (OR=0.48; 95% CI 0.29-0.80). Increased risk of PD was evident among cases with the MAO-B intron 13 variant G allele (OR=1.57; 95% CI 1.03-2.39); significant in women (OR=3.57; 95% CI 1.59-8.04) but not in men (OR=1.21; 95% CI 0.64-2.26). Contrary to published studies, the DRD2 A1 allele was not associated with increased risk of PD (OR=1.23; 95% CI 0.77-2.08).

Nicotine-related presentations

Chang (RJR, #695) presented a PBPK nicotine model for estimating nicotine absorption from smoking different cigarette products.

Epps (Duke University Medical Center, Durham, NC., #1294A) investigated the affect of nicotine on neurotransmitter receptor development in rats. Following the diFranza and Lew study (J. Fam. Pract., 40, 385-394, 1996), the following working hypothesis was investigated: *Nicotine exposure during critical development period affects balance of stimulating M2-muscarinic receptors in the CNS, inhibits M2 receptors in the heart and excitory cardiac β-adrenergic receptors, leading to physiological abnormalities that could contribute to SIDS.* To test this hypothesis, either prenatal or postnatal exposure of SD rats to nicotine was studied:

Prenatal exposure to 6 mg/kg/day nicotine (ca. 40 ng/ml plasma) using osmotic minipumps (duration not stated) resulted in (i) decreased expression of excitory M2-muscarinic receptors in brain stem, (ii) increased expression of inhibitory M2-muscarinic cardiac receptors and decreased expression of excitory β-receptors, and (iii) receptor changes associated with greater inhibitory affects on cardiac function during hypoxia.

Postnatal exposure of pups to nicotine twice daily for 4 days (s.c. 0.3 or 3.0 mg/kg) and sacrifice 12 h after the last nicotine administration resulted in similar, but less evident effects. It was concluded that prenatal treatment of nicotine, and hence *in utero* exposure to nicotine, might contribute to cardiac arrest seen in SIDS. (When I asked about the physical condition of the rats I was told that if the doses are not high enough to make the animals 'shake' you cannot observe any effects!).

Mangipudy (University of Nebraska Medical Center, NE., #935) reported that smokeless tobacco extracts (0.5, 1.0, 2.5 and 5.0%) induced *in vitro* apoptosis of hamster cheek pouch cells in a dose-dependent manner. Addition of nicotine at 2.5 times the natural level in smokeless tobacco extracts inhibited apoptosis under identical conditions.

Marijuana smoke

Sarafian (UCLA, Los Angeles, CA., #1701) reported that marijuana smoke induces oxidative stress via reactive oxygen species (ROS) and depletes cellular GST levels *in vitro* in cultured human ECV304 endothelial cells. Oxidative stress was reduced in cells exposed to marijuana smoke containing no Δ^9-tetrahydrocannabinol.

1,3-Butadiene

Jackson (CIIT, #474) used a PBPK model to fit data obtained from exposing groups of B6C3F1 mice to 1,3-butadiene (1100 ppm), with or without, inhibition of CYP2E1 and CYP450 enzymes. It was concluded that CYP2A6 and CYP2E1 are involved in oxidation to

the monoepoxide, while CYP2E1 and CYP3A4 are involved in the formation of the diepoxide from the monoepoxide. (The high exposure conditions limit the usefulness of this study to predict enzymes involved in human metabolism).

Saranko (CIIT, #360) reported that the monoepoxide, and not the diepoxide, appeared to be responsible for the *lacI* mutational spectrum observed in Big Blue mice exposed to 1,3-butadiene.

Nieusma (University of Colorado, Denver, CO., #423) reported that stereochemical effects determine glutathione binding and depletion by epoxybutanediol.

Kemper (University of Wisconsin, Madison, WI., #1641) reported rapid metabolism of 3-butene-1,2-diol by B6C3F1 mice (i.p. 10-250 mg/kg) with less than 6% of administered dose recovered in 24 h urine as the unmetabolized parent compound. No significant formation and excretion of sulfate and glucuronide conjugates. Co-treatment of mice with 4-methylpyrazole, an inhibitor of alcohol dehydrogenase, decreased plasma clearance of 3-butene-1,2-diol from which the authors claimed that alcohol dehydrogenase mediates 3-butene-1,2-diol metabolism. (4-methylpyrazole also inhibits hepatic CYP2E1, which would be an equally good explanation for the reported results)

Shell Research in Amsterdam presented a series of four posters on 1,3-butadiene (**Boogaard**; #409, #428, #1060, #1795) based on a study in which male SD rats and B6C3F1 mice were exposed to [2,3-^{14}C]1,3-butadiene (200 ppm, 6 h nose-only inhalation) or treated with [2,3-^{14}C]butadiene monoepoxide (1-50 mg/kg, i.p.). Interspecies differences were reported for:

1. Urinary metabolite profiles for the glutathione conjugates 4-(N-acetylcystein-S-yl)-1,2-dihydroxybutane (metabolite M1) and N-acetylcystein-S-yl-hydroxybutene (metabolite M2).
2. Exhalation of CO_2 (ca. 40% of predicted uptake in rats compared to 5% in mice).
3. Hemoglobin adduct levels were 5 to 10-fold higher in rats than mice.
4. DNA adduct profiles in lung and liver were similar in rats and mice; however total adduct levels,were 4-fold higher in mice.

These results do not explain the 1000-fold greater susceptibility of mice to 1,3-butadiene. In discussion with Boogaard I heard that Shell's current research concludes that:

- Only 15% of total hemoglobin-bound material can be identified.
- C_4 lipid peroxidation contributes to background levels of adducts thought to be specific for 1,3-butadiene.
- Hemoglobin adducts cannot be used to determine occupational exposure to a TWA_8 of <2 ppm 1,3-butadiene.
- Biomonitoring of 1,3-butadiene excretion products (metabolites M1 and M2) cannot differentiate between nonsmokers and smokers of <2 packs/day.

Swenberg (University of North Carolina, Chapel Hill, NC., #890) reported data from a series of inhalation studies with SD rats and B6C3F1 mice (20-625 ppm, 6 h/d, 10 d, and single 6 h

exposure to 1250 ppm 1,3-butadiene). Detected levels of hepatic DNA adducts paralleled the increased sensitivity of mice compared to rats for liver cancer.

Henderson (Lovelace, Albuquerque, NM., #898) reported a higher frequency of *hprt* mutant T cells in the spleen of B6C3F1 mice compared to F344 rats exposed to 1,3-butadiene (0, 20, 62.5 and 625 ppm; 6 h/d, 5 d/wk for 4 wk), 1,3-butadiene monoepoxide (0, 2.5 and 25 ppm; 6 h/d, 5 d/wk for 4 wk), and 1,3-butadiene diepoxide (0, 2 and 4 ppm; 6 h/d, 5 d/wk for 4 wk). The diepoxide was concluded to induce *hprt* mutations, and mice were more sensitive than rats to 1,3-butadiene.

Nitrosamines

Data was presented by **Tricker** and **Richter** (#900) and Smith (#1399) for metabolism of NNK in human lung tissue and isolated lung cell fractions (cell digest, Type II cells and macrophages), respectively. Tricker and Richter compared metabolism in lung and liver of the A/J mouse, F344 rat and man, and concluded that NNK to NNAL reduction accounted for >97% of NNK metabolism in human lung and liver, while significant α-hydroxylation occurred in tissues from experimental animals with reduced formation of NNAL. **Smith** (Queen's University, Kingston, ON., #1399) reported human lung cell fractions primarily metabolize NNK by reduction to NNAL (>95% of total metabolism). Smith used a single concentration of 4.2 μM NNK with 24-h incubation, separation of metabolite peaks by HPLC fractionation and solid-phase radioactivity monitoring. Neglecting differences in experimental and analytical protocols, both studies indicated <1.5% α-hydroxylation of NNK in human lung preparations. **Gerde** (Lovelace, NM., #901) reported absorption of NNK and B(a)P over canine tracheal epithelium following instillation in the distal trachea. NNK, but not B(a)P, was rapidly absorption over the trachea. Two to 3-fold higher binding of NNK occurred in trachea than observed in distal tissues including lung. No NNK was detected in systemic circulation 18 min after administration. It was concluded that (I) absorption of B(a)P was considerably slower than that of NNK, (ii) NNK absorption over lung epithelial is likely to be so rapid that significant metabolism does not occur in this organ, and (iii) the slower absorption of B(a)P probably induces biological effects at the site-of-entry. **Chang** (RJR, #899) reported an old study in which NNK-induced O^6-methylguanine formation in lung and liver of the A/J mouse was significantly suppressed by inhalation of cigarette smoke. (Copy available on request)

Iatropoulos (AHF, #63) presented an extension of a previously published study (Williams *et al.*, Carcinogenesis, 14, 2149-2156, 1993 and 17, 2253-2258, 1996) showing non-linearity of NDEA liver neoplasms in male F344 rats. The NOEL for initiation was 0.5 mmol/kg cumulative dose. (Copy available on request)

Smith (MRC Toxicology Unit, Leicestershire, UK, #64) reported that iron overload (500 mg/kg bw iron dextran) promoted NDEA-initiated GST-P foci in the rat liver. It was suggested that iron might also act as a promotor of human liver cancer. (Study published by Carthew *et al.*, Carcinogenesis, 18, 599-603, 1997, and available on request)

Bichet (SANOFI Research, Montpellier, France, #390) reported that N-nitroso-N-methylpiperazine might be a suspect hepatocarcinogen in the rat. The biological activity of this compound in the rat liver was actually reported by Druckrey et al., Z. Krebsforsch., 69, 103-201, 1967 – probably before the presenter was born!

PAHs

Harms (Purdue University, Lafayette, IN., #399) reported that B(a)P induced DNA adducts in spermatozoa of mice treated with B(a)P (500 mg/kg, 4 days, i.p.). *In vitro* incubation of trout sperm with BPDE (10 µg/ml) also induced adducts. (Both results are hardly surprising considering the experimental conditions)

Bouchard (University of Montreal, #140, #146) provide further experimental data to support the use of 1-hydroxypyrene (1-HOP) as a urinary biomarker for pyrene exposure. Treatment of F344 rats with [^{14}C]pyrene (50 µmol/kg, i.v.) resulted in 57.2% recovery in urine and 18.3% in feces within 24 h. Only 4.6% of the dose was recovered as 1-HOP in urine. Administration of [^{14}C]pyrene (1.5-100 µmol/kg, p.o.) resulted in a lower recovery of 2.6-3.3% 1-HOP in urine. Despite these low recoveries, 1-HOP is now an accepted biomarker for occupational biomonitoring of PAH exposure.

Metals

Evans (PTI Environmental Services, Bellevue, WA., #205) used various published data to predicted daily intake for cadmium in adults:

Food	0.18 µg/kg/day	2.5% absorption → 0.0045 µg/kg
Drinking water	1 µg/l	5% absorption → 0.0018 µg/kg
Air	0.02 µg/m^3	50% respirable fraction 25% deposition and absorption → 0.0009 µg/kg
Smoking	1 µg/pack	→ 0.0067 µg/kg/pack

Salnikow (New York University Medical Center, #1575) reported that Ni^{2+} induces the CAP43 gene in human bronchoalveolar A549 cells. The function of the CAP43 gene in cellular growth is under current investigation. Normal ambient air was claimed to result in an exposure to 0.2-0.4 µg Ni.

DNA and protein adducts

DeBord (NIOSH, Cincinnati, OH., #892) reported the development of a simple electrochemical detection method to measure O^6-methylguanine (O^6mG) in peripheral lymphocytes of workers occupationally exposed to N-nitrosamines. N^7-methyl-deoxyguanosine (7mG) was detected using conventional ^{32}P-postlabeling assay. The poster provided no data for N-nitrosamine exposure, which was assumed to occur according to the NIOSH HETA 9'-0072 category of workplaces. Neither adduct correlated with assumed workplace exposure to N-nitrosamines; most subjects had no detectable O^6mG adduct levels. (Most of the cited references did not fit the poster text)

Myers (University of Louisville, KY., #213) reported 4-aminobiphenyl (4-ABP) and B(a)P tetrol hemoglobin adducts in maternal-fetal pairs. The 4-ABP data has already been published (J. Toxicol. Environ. Health, 47, 553-557, 1996). The abstract, but not the poster, claimed several associations with GSTM1 and GSTT1 polymorphisms: '*The data failed to demonstrate a statistically significant effect of the glutathione S-transferase Theta null genotype on the amount of hemoglobin-tobacco smoke carcinogen adducts. However, at the low exposure range (<1,000 ng cotinine/mL), the data do suggest that individuals with the GST T1 null genotype have increased benzo(a)pyrene hemoglobin adducts.*' Wishful thinking since genotyping has not yet been performed!

	4-Aminobiphenyl (pg/g globin)		B(a)P tetrol (pg/g globin)	
	Maternal	Fetal	Maternal	Fetal
Nonsmoker, n=74	18.3±12	9±6	6.8±4	3.4±1.8
ETS exposed, n=20	118±38	61±28	10±2.5	5.6±2.5
Smoker <1 p/d, n=16	144±22	74±18	18.9±5	10.3±3.1
Smoker 1, p/d, n=19	250±33	123±27	33±7	19.9±4.4
Smoker 1-2 p/d, n=19	394±64	196±40	48±15	23.2±6.9
Smoker 2< p/d, n=20	633±87	319±50	97±19	52.5±14.3

Swenberg (University of North Carolina, Chapel Hill, NC., #888) reported GC-MS methods for determining N7-(2-hydroxypropyl)guanine DNA and N-(2-hydroxypropyl)valine (HOPVal) hemoglobin adducts in nasal respiratory mucosa, nasal olfactory mucosa, lung, spleen, liver and testis of F344 rats exposed by inhalation to propylene oxide. HOPVal was concluded to be a sensitive biomarker of exposure to propylene oxide, but not a good surrogate marker for DNA adduct levels in different target tissues.

Fretland (University of North Dakota, Grand Forks, ND., #1381) reported PhIP-induced ^{32}P-postlabeled DNA adducts in prostrate, urinary bladder, heart, colon and liver in hamsters treated with a total dose of 100 mg/kg PhIP. The prostate was concluded to be a possible

target organ for PhIP carcinogenesis in the hamster. Rapid acetylator congenic hamsters had significantly higher adducts in the prostrate than slow acetylator hamsters; acetylator status did not affect adduct levels in other organs.

Drug metabolism polymorphisms and genetic susceptibility

Taylor (NIEHS, #564) in an oral presentation concluded that there is an interaction between smoking, polymorphisms of NAT1 and NAT2 and risk of bladder cancer. GSTM1 null subjects being at considerably higher risk. Low urinary pH also appeared to increase risk significantly. However, attributable risk is difficult to calculate for smokers, and is negligible in nonsmokers. True polymorphisms of NAT1 and NAT2 are difficult to define and it was suggested that a DNA sequence change occurring with a frequency of >1% should constitute a polymorphism (allele), while a DNA sequence change occurring with a frequency of <1% should be regarded as a mutation.

Hein (University of Louisville, KY., #565) reported that the functional significance of 19 different alleles of NAT1 and 24 alleles of NAT2 are now well known from recombinant expression systems. Despite this knowledge, there is a lack of a consistent association between NAT2 polymorphisms and risk of colon cancer, presumable due to misclassification of acetylator status in early studies in which not all alleles had been identified or characterized. It was assumed that heterocyclic aromatic amines are an etiological risk factor.

Furlong (University of Washington, Seattle, #566) reported that newborn rats and mice have very low paraoxonase (PON1) levels and require about 3 weeks before PON1 expression develops. PON1, also known as arylesterase, is located in HDL particles and may be a genetic susceptibility factor for vascular disease and Gulf War syndrome. The $Gln^{192}\rightarrow Arg$ amino acid change in PON1 is associated with reduced lipid oxidation. A second silent polymorphism in codon 55 (not fully characterized) does not appear to have a functional effect.

Loo (University of Washington, Seattle, #1445) reported a rapid ELISA-based oligonucleotide ligation assay to detect $Ile^{104}\rightarrow Val$ and $Ala^{113}\rightarrow Val$ polymorphisms in GSTP1. Variant allele frequencies of 33.6% and 10.8% were reported for Val^{104} and Val^{113}, respectively, among controls. No increase in risk of Parkinson's disease was found for the Val^{104} allele (OR=0.83; 95% CI 0.35-1.99) or the Val^{113} allele (OR=0.93; 95% CI 0.27-3.22). The same polymorphisms are being investigated for lung and esophageal cancer.

Shinde (University of Arkansas for Medical Sciences, AR., #1567) reported a bp-34 (T→C) substitution in the 5' promotor region of CYP17 which appears to increase gene expression. Since CYP17 is involved in testosterone biosynthesis, this point mutation may increase androgen levels. Preliminary results indicate an increased risk of prostate cancer in combined heterozygotes and homozygotes men for the variant allele. (This is consistent with the increased androgen level hypothesis)

Omiecinsky (University of Washington, Seattle, #567) reported that exon 3 Tyr^{113}→His and exon 4 His^{139}→Arg polymorphisms in microsomal epoxide hydroxylase (mEH) have no significant effect on enzyme function since genotype and function are only weakly correlated. In addition to these two polymorphisms, a further 7 polymorphic sites have been identified, 3 of which impact post-transcription regulation. Failure to consider these new polymorphisms questions the validity of previous associations reported for mEH polymorphisms:

His^{139}→Arg increases the risk of hepatocellular cancer (McGlynn *et al.*, PNAS 92, 2384, 1995)

Tyr^{113}→His increases the risk of ovarian cancer (Lancaster *et al.*, Mol. Car., 17, 160, 1996)

Neither polymorphism related to bladder cancer (Brockmöller *et al.*, Cancer Res., 56, 3915, 1996).

Polymorphisms of mEH are currently being studied at the University of Washington as genetic risk factors for lung cancer and Parkinson's disease.

Maier (University of Cincinnati Medical Center, OH., #1222) presented initial data indicating 10 polymorphic sites in the coding region of the mouse Ah receptor. A further polymorphic site was identified in a non-coding region.

Henry (University of Pittsburgh, PA., #91) presented initial data for phenotyping using the 'Pittsburgh cocktail', a mixture of 5 test substrates to determine phenotype for CYP1A1, CYP2C9, CYP2E1, CYP2D6 and NAT2 acetylator status. Although the 'cocktail' was claimed not to result in cross-inhibition of CYP activity, the presented results suggest that significant inhibition of CYP2D6 metabolism occurred since 4 of 18 subjects (22%) were classified as CYP2D6 PM phenotypes compared to an expected 6-8%.

Andersen (University of Washington, Seattle, #83) genotyped expression of CYP2D6 in a human liver bank. Although not mentioned in the abstract, data was presented in the poster to show expression of CYP2D6, CYP2D7 and CYP2D8P in human lung.

Paine (University of Michigan, Ann Arbor, MI., #1644) presented data to show that ketoconazole, originally thought to be a mechanism-based inhibitor of CYP3A4, potentially inhibits metabolism by CYP1A1.

Moron (University of Colorado, Denver, CO., #1393) reported that the bp609 (C→T) point mutation resulting in a Pro^{187}→Ser amino acid exchange in NAD(P)H:quinone oxidoreductase (NQO1) increases *in vitro* benzene toxicity in human bone marrow cells.

Studies using precision-cut tissue slice

This model is currently being used in a Philip Morris funded project (Richter and Tricker, #900). Evident from the meeting was a renewed interest in using this model for studying the metabolism and toxicity of various xenobiotics:

Tissue	Substrate/conditions	Authors
Lung and kidney (man)	TCDD, 0.1-10 nM, <96 h (CYP1A1 and 1B1 induction)	**Slezak** (SUNY at Buffalo, Madison, WI., #1906)
Liver (man)	2AAF, AFB_1, PhIP, various (induction of UDS)	**Beamand** (BIBRA, UK, #1951)
Liver (man)	Rifampicin, 200-300 µM, 72h (CYP1A2 and 3A4 induction)	**Lake** (BIBRA, UK, #1953)
Liver (man)	Various, <96 h (induction of CYP isoforms)	**Yager** (John Hopkins School of PH, Baltimore, MD., #318)
Liver (man)	Furfural, <25 mM, 24 h (induction of UDS)	**Adams** (BIBRA, UK, #389)
Lung (rat)	(Validation of model system for hypoxia)	**Monteil** (Inserm, Rouen, France, #1699)
Liver and kidney (F344 rat)	Chlorotrifluoroethylene, 10-100 µM, 6-24 h	**Hasal** (University of Arizona, Tucson, AZ., #420)
Kidney (F344 rat)	3,4-dichloroaniline, *ex vivo* (toxicity study)	**Valentovic** (Marshall Univ., Huntington, WV., #1854)
Liver (SD rat)	Galactosamine, 1-20 mM, 24h (toxicity study)	**Masutomi** (Mitsubishi Chemical Co., Japan, #1950)
Liver (F344 rat)	Fumonisins, 250 µM, 20-48 h (toxicity study)	**Norred** (USDA, Athens GA., #1420
Liver (F344 rat)	(Validation of model system)	**Jensen** (Eli Lilly and Company, #482)
Liver (F344 rat)	(Validation of model system)	**Price** (BIBRA, UK, #1952)
Liver (F344 rat and B6C3F1 mouse)	2-Butoxyethanol, various	**Grant** (Battelle, Richland WA., #1624)
Liver (F344 rat, B6C3F1 mouse, man)	*Trans*-Methylstyryl ketone, 100-1000 µM, 18 h	**Sipes** (University of Arizona, Tucson, AZ., 1390)
Kidney (rabbit)	Arsenite, 0.010-10 µM, 2-8 h (toxicity study)	**Zheng** (University of Arizona, Tucson, AZ., #1588)
Kidney (rabbit)	Hg(II), 0.01-10.0 µM, 2-8 h (toxicity study)	**Turney** (University of Arizona, Tucson, AZ., #1872)

The above studies used various culture media, incubation times and protocols. It was evident from the combined presentations that each group has their own preferred method and conditions. Comparative studies showed interspecies differences for *trans*-methylstyryl ketone metabolism by rat, mouse and human liver (**Sipes**, #1390) and for 2-butoxyethanol metabolism by F344 rat and B6C3F1 mouse liver (**Grant**, #1624). Grant also reported age-related differences in hepatic metabolism of 2-butoxyethanol. Most studies provided data to confirm vitality of fresh liver slices for 36-96 h.

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