The Importance of ADME/PK to Inform Human Safety Assessments Based on Animal Studies: Example with Furan

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Conflict of Interest

None

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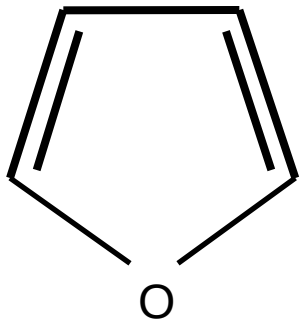
Furan



- Prototype of large group of chemicals, found in canned foods and coffee
- Used as solvent, in synthesis of pharmaceuticals
- Component of smog, cigarette smoke, spaceship air

Furan Hepatocarcinogenesis

- NTP Bioassay: hepatocellular carcinomas and cholangiocarcinomas in rats and mice (2, 4, 8, 15 mg/kg/d po in corn oil)

- Not mutagenic, no DNA excision-repair
  (Wilson *et al.* (1992) *Environ. Mol. Mutagen.* 19:209)

- No significant DNA binding, extensive protein binding
  (Burka *et al.* (1991) *J. Toxicol. Environ. Health* 34:245)

Furan Hepatotoxicity

- Frank hepatic necrosis above 8 mg/kg in rodents (bioassay dose)
- Midzonal hepatotoxicant (zone 2 →3)
- Hepatotoxicity requires cytochrome P450 bioactivation
- Sustained cell proliferation in response to cytolethality

Furan Hepatocarcinogenesis

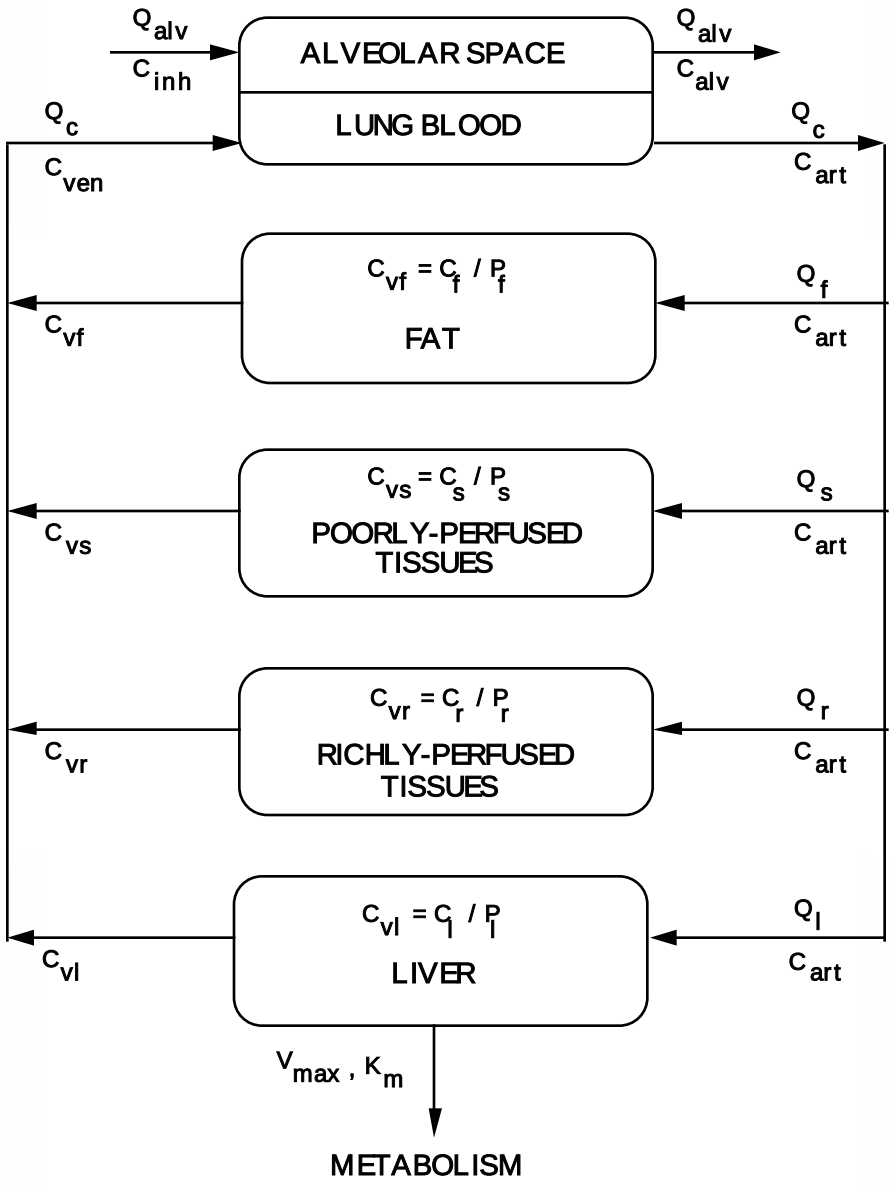
Hypothesis:

Hepatic cytolethality of furan drives cell proliferation and tumor development

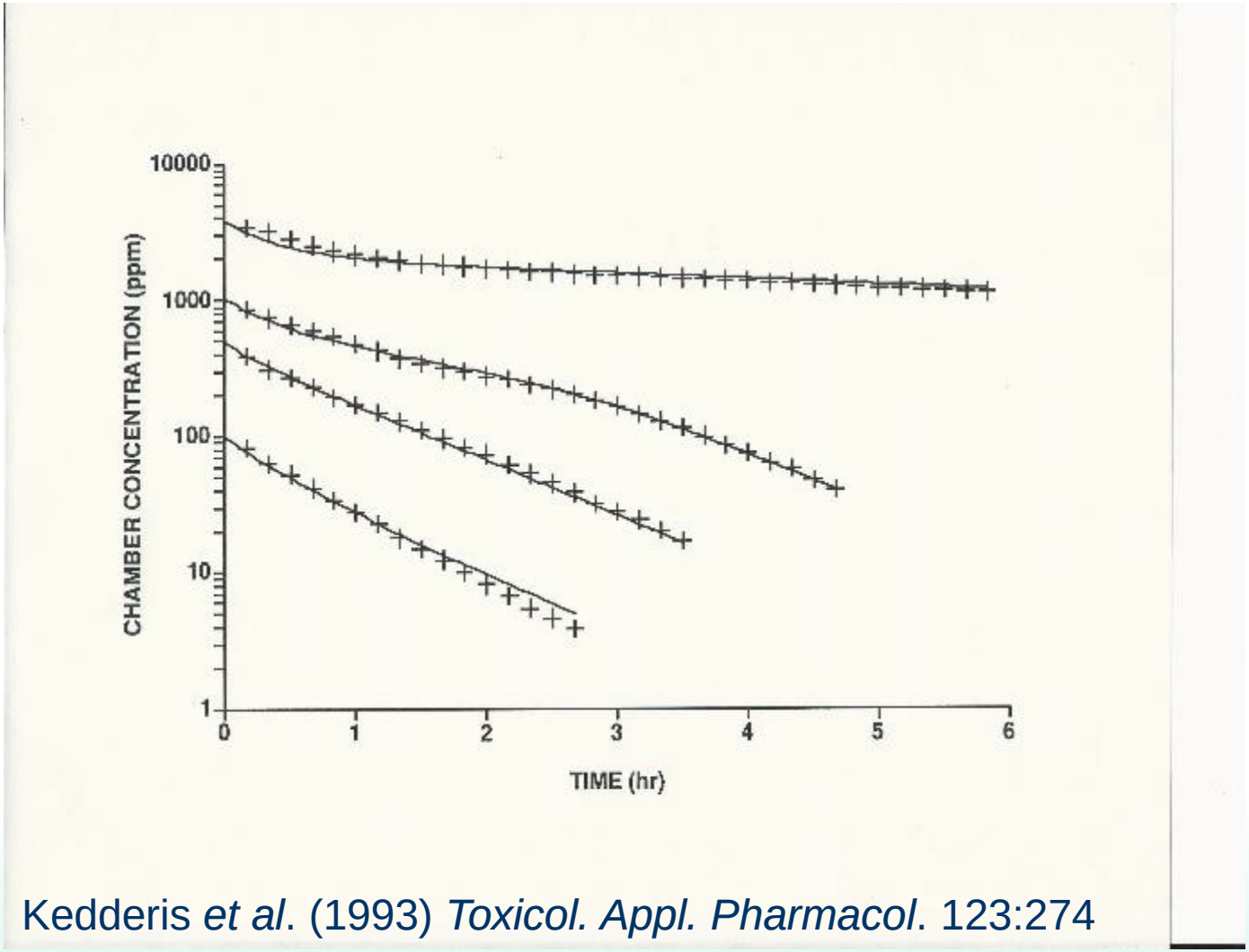
Research Objectives:

- Understand relationship between administered dose and target tissue (liver) concentration
- Determine biotransformation kinetics *in vivo* and *in vitro*
- Determine mechanisms of cytolethality in hepatocytes

PB-PK DOSIMETRY MODEL

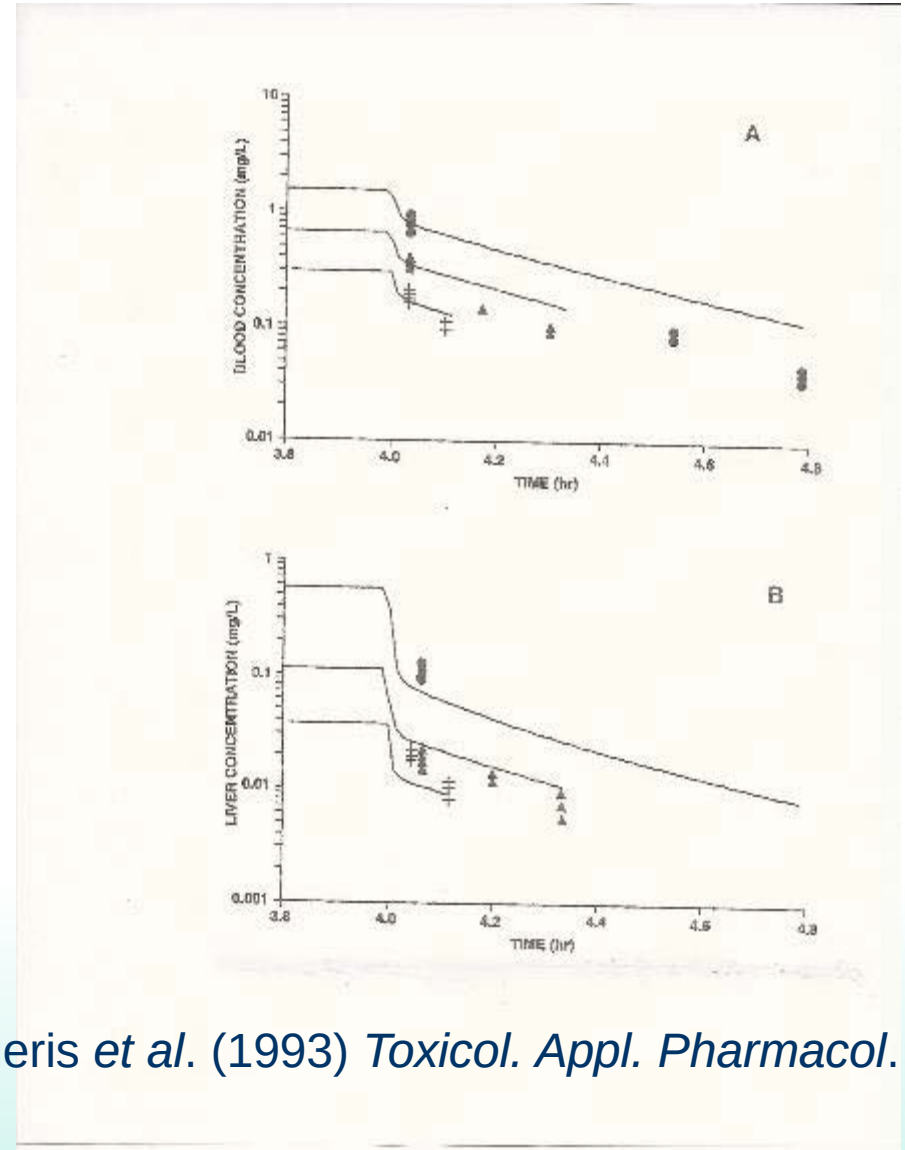


Furan Rats Closed Chamber



Kedderis *et al*. (1993) *Toxicol. Appl. Pharmacol*. 123:274

Furan in Rat Liver and Blood

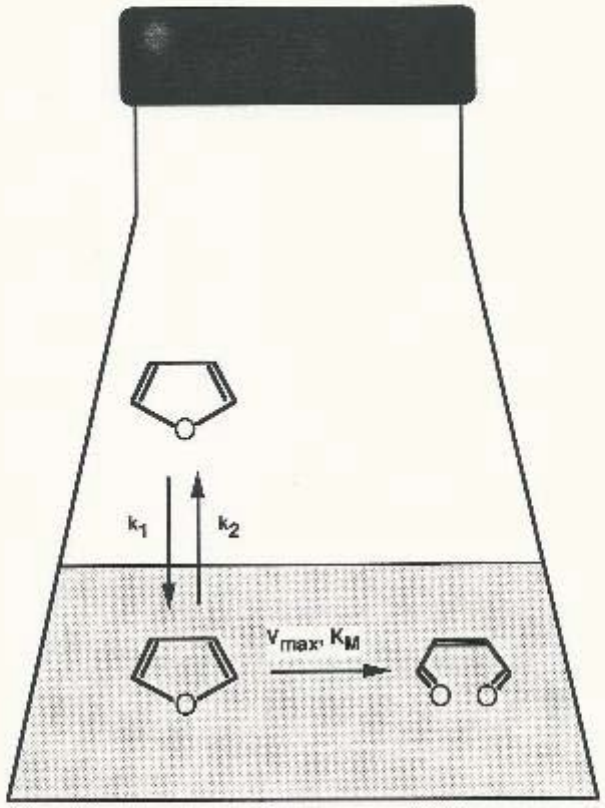


Kedderis *et al*. (1993) *Toxicol. Appl. Pharmacol*. 123:274

Hypothesis

Freshly isolated hepatocytes are a quantitative *in vitro* model for the biotransformation of chemicals which are metabolized primarily by the liver *in vivo*

2-COMPARTMENT VIAL MODEL



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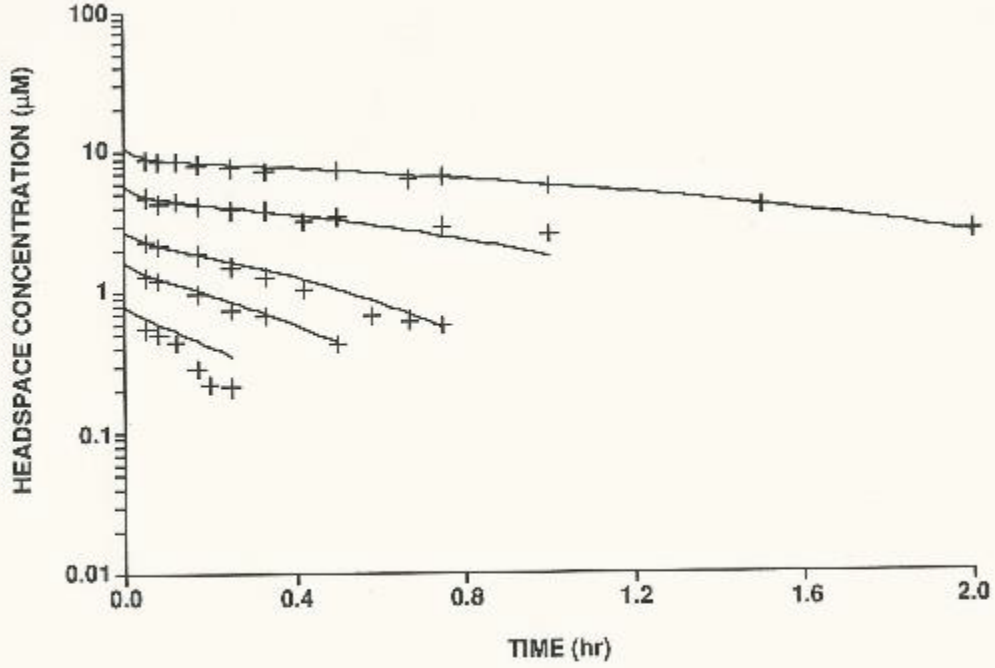
Furan Uptake by Rat Hepatocytes



Kedderis *et al*. (1993) *Toxicol. Appl. Pharmacol*. 123:274

Extrapolation of *In Vitro* Metabolic Rates to Intact Animals *In Vivo*

- Rate of enzyme-catalyzed reactions directly proportional to total enzyme $[E]_T$

$$v_{app} = k_{app}[E]_T$$

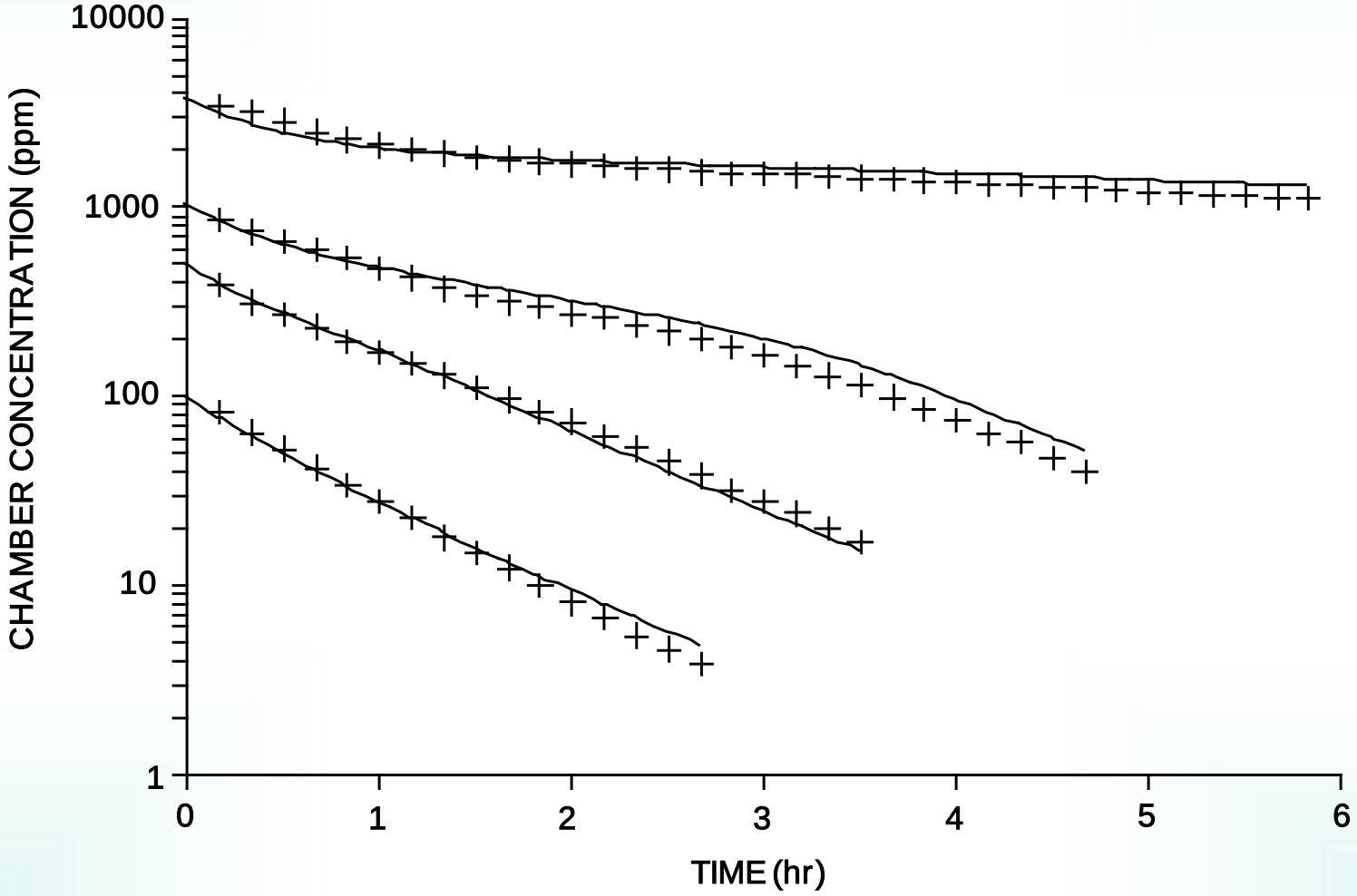
- Extrapolation based on cellularity of liver: ~130 million hepatocytes/g mammalian liver
- Need to know overall enzyme mechanism: most enzymes follow Michaelis-Menten kinetics
- Need model of animal: PBPK model

Furan Biotransformation by Rats *In Vivo* and Hepatocytes *In Vitro*

	K_M (μM)	V_{max} (μmoles/hr/250 g rat)
In Vivo	3.3	27.7
In Vitro	0.74	24.1

Kedderis *et al*. (1993) *Toxicol. Appl. Pharmacol*. 123:274

The Hepatocyte Biotransformation Kinetics of Furan *In Vitro* Accurately Predict Pharmacokinetics *In Vivo*



Kedderis *et al*. (1993) *Toxicol. Appl. Pharmacol*. 123:274

Kinetic Parameters for Furan Biotransformation by Isolated Human and Rodent Hepatocytes

Sample	Age	Sex	Vmax (nmol/hr/million cells)	K_M (µM)
Human 1	53	M	19.0	2.1
Human 2	14	F	38.5	3.3
Human 3	34	M	43.9	3.3
B6C3F1 Mouse		M	48.1	1.0
F344 Rat		M	18.0	0.4

Kedderis and Held (1996) Toxicol. Appl. Pharmacol. 140:124

Furan Dosimetry after a 4-Hr Exposure to 10 ppm

Species	Absorbed Dose (mg/kg)	Integrated Liver Exposure to Furan Metabolites (μM)
Rat	1.4	480
Mouse	4.1	1075
Human	0.4	168

Absorbed Dose = (Inhaled – Exhaled)/BW

Kedderis and Held (1996) Toxicol. Appl. Pharmacol. 140:124

What is Dose?

- Exposure concentration is not dose
- Administered Dose vs Target Tissue Dose
- Species differences in size, breathing, metabolic rates
- Allometry: smaller mammals breath faster
- Nonlinearity of metabolism, saturation

Blood Flow Limitation of Furan Biotransformation [a]

Species	Blood Furan (µM)	Liver Perfusion [b] (µmol/min)	Furan Oxidation [c] (µmol/min)
Rat	0.80	0.017	0.213
Mouse	0.80	0.005	0.122
Human	0.68	1.02	25.6 - 37.7

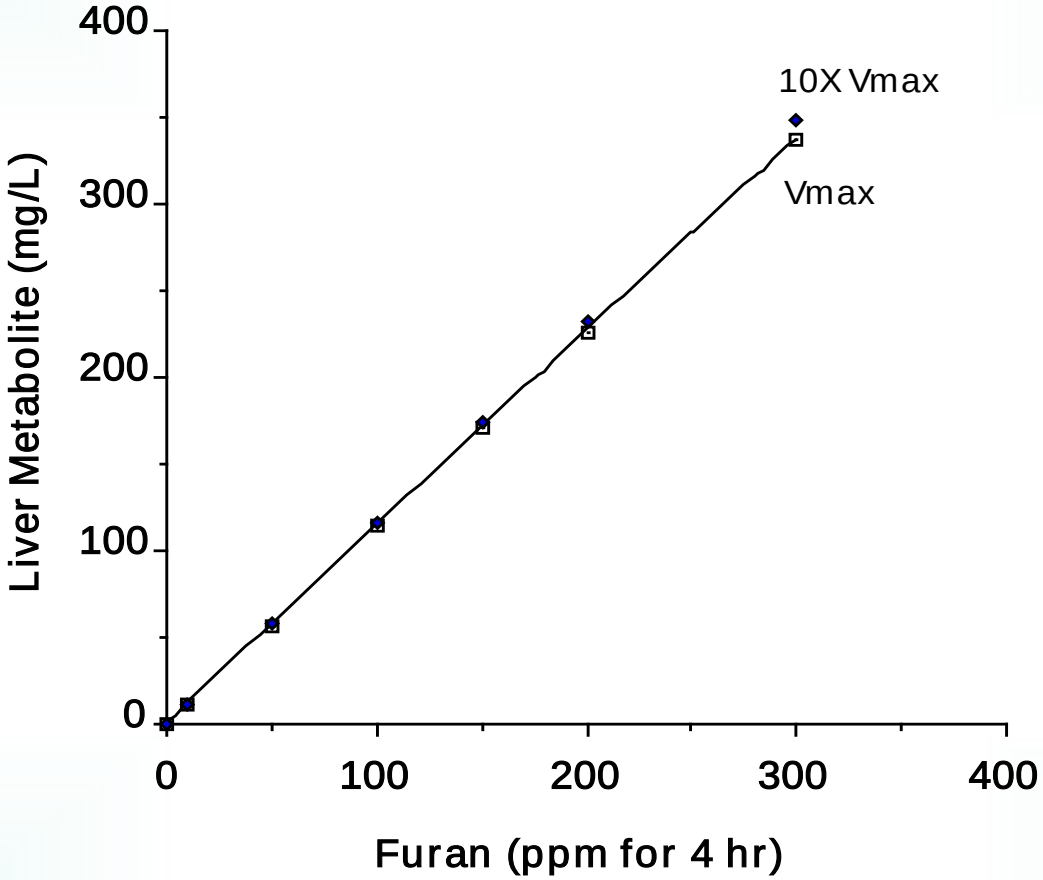
[a] Exposure to 10 ppm furan for 4 hr.

[b] Blood concentration * liver blood flow.

[c] Initial rate of metabolism (V/K) * blood concentration.

Kedderis and Held (1996) Toxicol. Appl. Pharmacol. 140:124

Human Liver Bioactivation of Furan

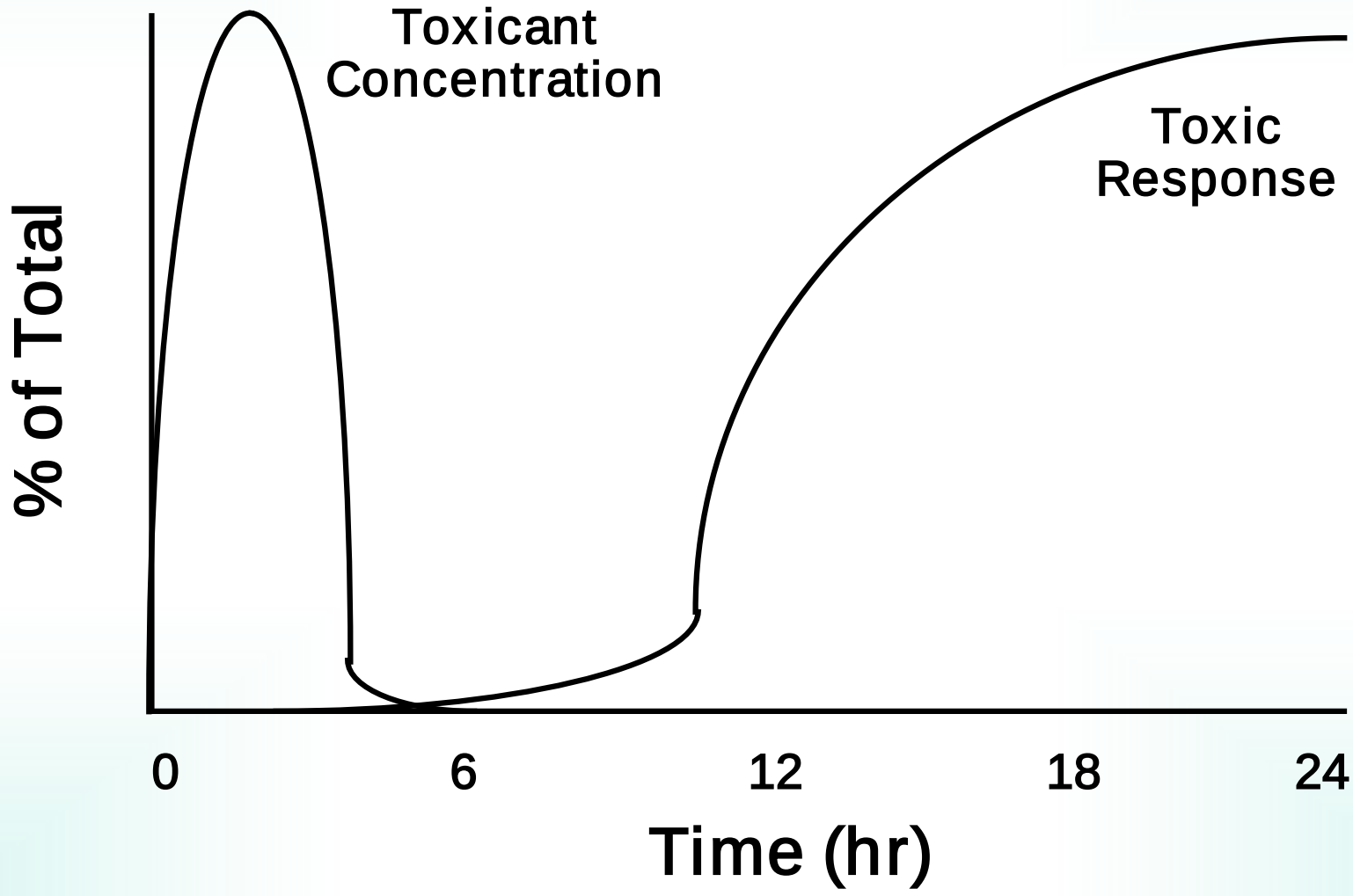


Kedderis and Held (1996) Toxicol. Appl. Pharmacol. 140:124

Furan Cytolethality Objective

Develop an *in vitro* hepatocyte system to study the mechanisms of furan-induced cytolethality which accurately reflects the exposure of the liver to furan *in vivo*.

Relationship between Exposure and Toxicity



Comparison of Furan Dosimetry in Rats *In Vivo* and Hepatocytes *In Vitro*

	Peak Conc. in Liver or Media (μM)	Conc. in Liver or Media after 4 hr (μM)	Amount Metabolized (μmoles in 4 hr)
In Vivo			
8 mg/kg†	36	0.006	24.5
30 mg/kg‡	226	0.07	50.6
In Vitro			
2 μM	2	<0.001	11.6
10 μM	14	<0.001	58.1
100 μM	160	136	92.3

† bioassay dose
‡ cytotoxic dose

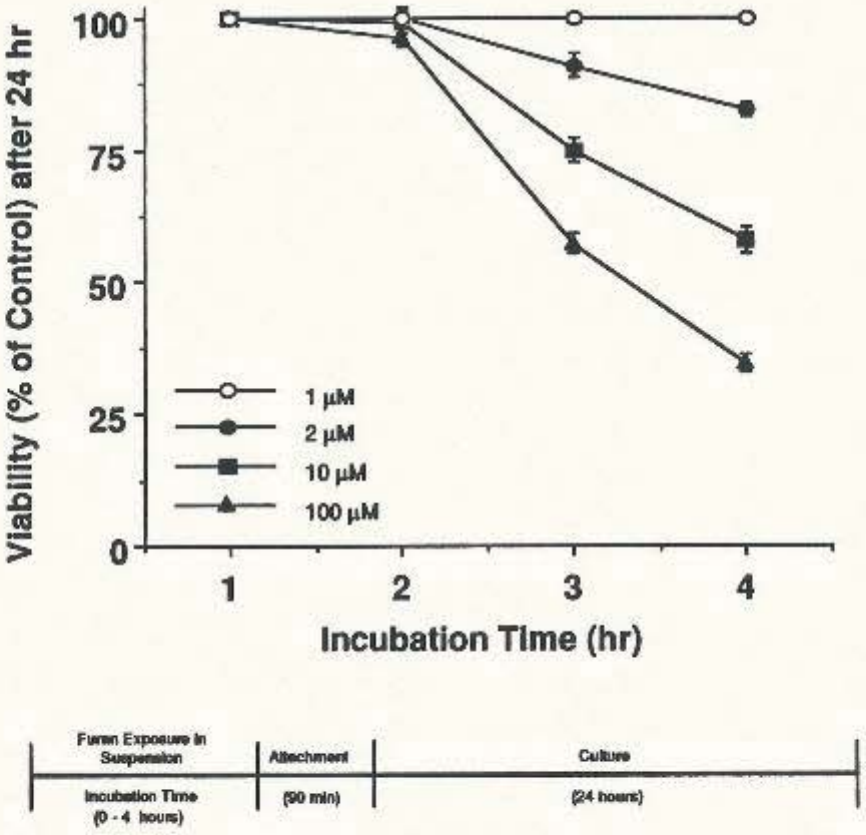
Carfagna *et al*. (1993) *Toxicol. Appl. Pharmacol*. 123:265

Hepatocyte Suspension-Culture Approach

- Exploit metabolic competency of freshly isolated hepatocytes and longevity of monolayer cultures
- Incubate hepatocyte suspensions with toxicant
- Toxicant concentrations and incubation times from PBPK dosimetry model
- After incubation, cells placed in monolayer culture to express toxicity at 24 hr

Carfagna et al. (1993) Toxicol. Appl. Pharmacol. 123:265

Cytolethality of Furan



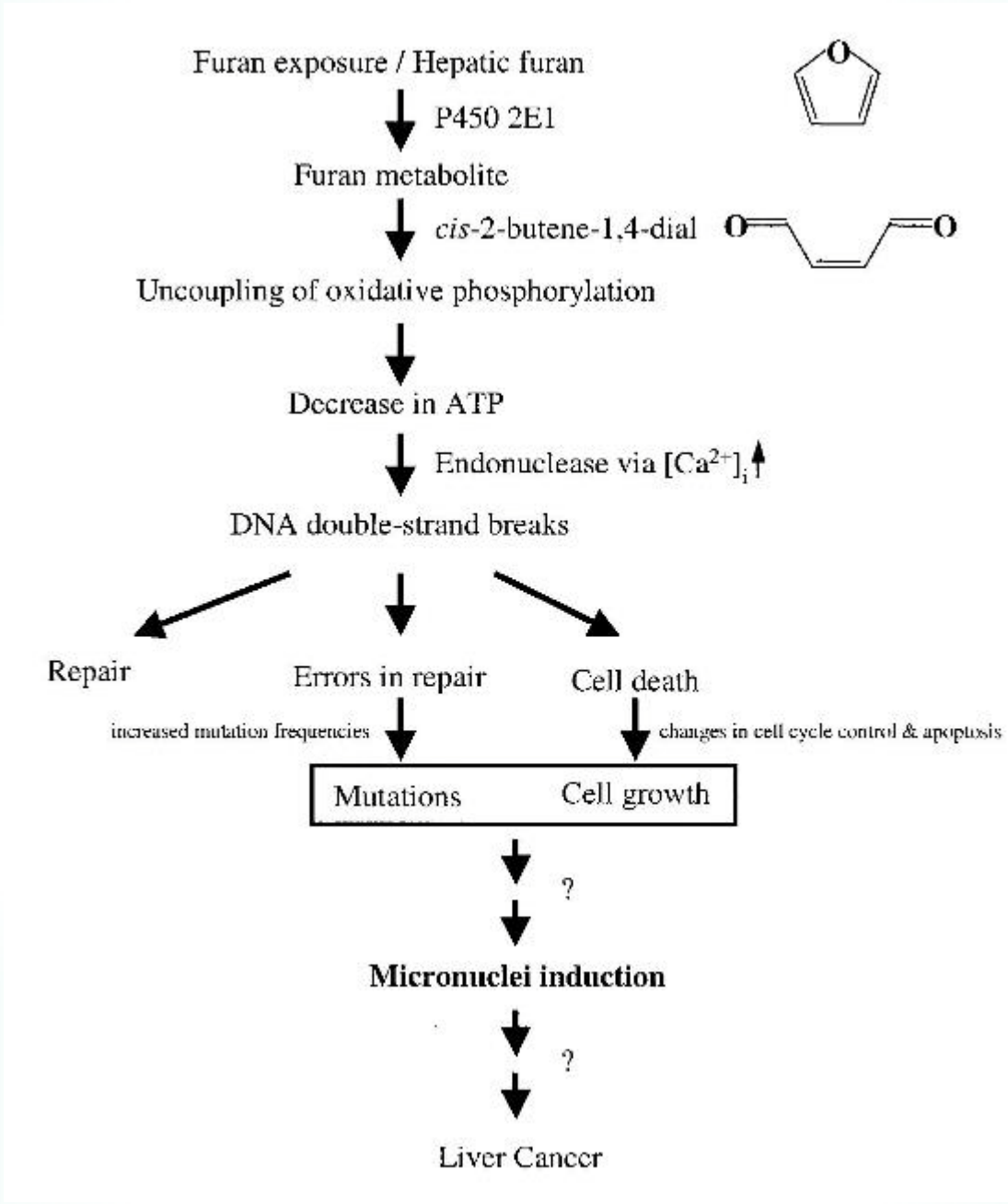
Carfagna *et al*. (1993) *Toxicol. Appl. Pharmacol*. 123:265

Furan Cytolethality Toward Hepatocytes

- NOAEL ~1 μM *in vitro*
- P450 inhibitors prevent cytolethality, GSH depletion
- ATP depletion due to irreversible uncoupling of oxidative phosphorylation *in vitro* and *in vivo*
- ATP depletion/uncoupling prevented by P450 inhibitors
- Activation of endonucleases: DNA double-strand breaks

Carfagna *et al*. (1993) *Toxicol. Appl. Pharmacol*. 123:265

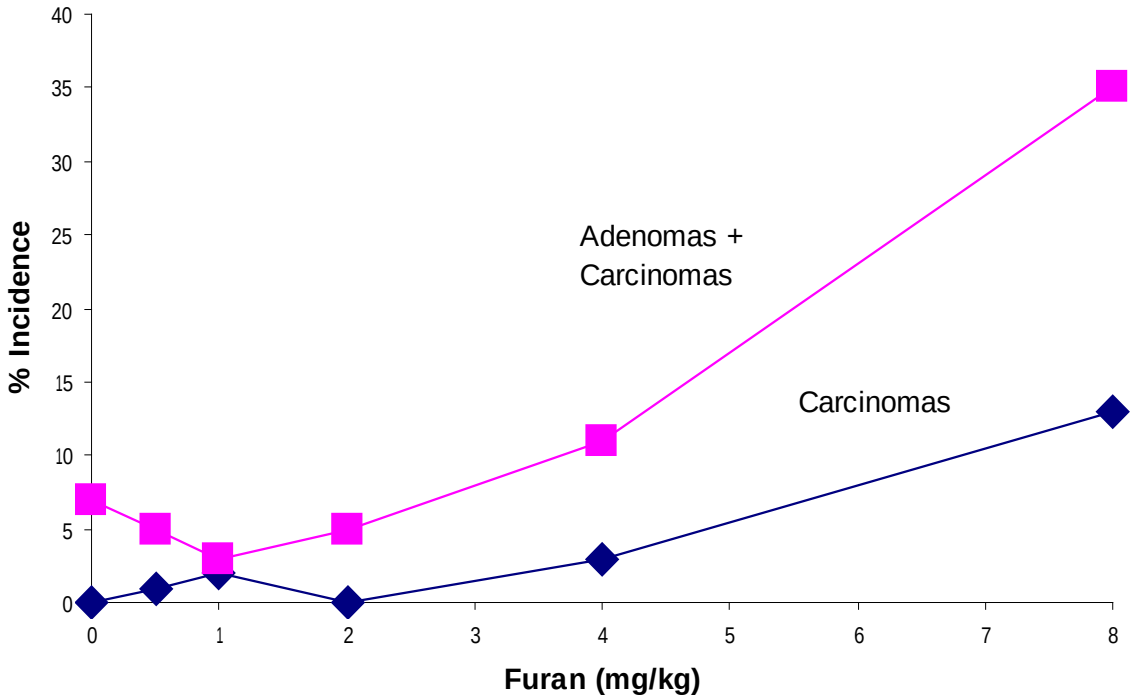
Mugford *et al*. (1997) *Toxicol. Appl. Pharmacol*. 144:1


Furan exposure / Hepatic furan
P450 2E1
Furan metabolite
cis-2-butene-1,4-dial
Uncoupling of oxidative phosphorylation
Decrease in ATP
Endonuclease via [Ca2+]i
DNA double-strand breaks
Repair
Errors in repair
Cell death
increased mutation frequencies
changes in cell cycle control & apoptosis
Mutations
Cell growth
?
Micronuclei induction
?
Liver Cancer

Furan Risk Assessment Considerations

- Toxicity mechanism exhibits NOAEL
- Health Canada oral gavage 90-day studies: NOAEL 0.03 mg/kg rats, 0.12 mg/kg mice
  Gill *et al*. (2010) *Toxicol. Pathol*. 38:619; (2011) 39:787
- NTP mouse 2-year study: nonlinear increase in toxicity and hepatic tumors, NOAEL 0.5 mg/kg
  Moser *et al*. (2009) *Exp. Toxicol. Pathol*. 61:101
- Changes in gene expression (NFR2), long non-coding RNAs in mouse liver: epigenetic markers?
  Recio *et al*. (2013) *Toxicol. Sci*. 135:369
  Jackson *et al*. (2014) *Toxicol. Appl. Pharmacol*. 274:63

Dose-Dependence of Furan Hepato-carcinogenesis in Female B6C3F1 Mice



Moser et al. (2009) Exp. Toxicol. Pathol. 61:101

Problematic Dosimetry of Furan in Foods

- Formed in canned foods by heat, irradiation from sugars, ascorbic acid, fatty acids

  Fan (2005) *J. Agric. Food Chem*. 53:7826; (2008) *J. Agric. Food Chem*. 56:9490; (2015) *Food Chem*. 175:439
- Not found in fresh-cut fruits and vegetables

  Fan and Sokorai (2008) J. Food Sci. 73:C79
- Exposure from eating prepared foods, volatilization
- But how much furan is really there?
- Baby food: infants do not have P450 capacity of adults

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