Hepatocyte Cultures as Model Systems for Trichloroethylene Hepatocarcinogenicity

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TCE Research Objectives

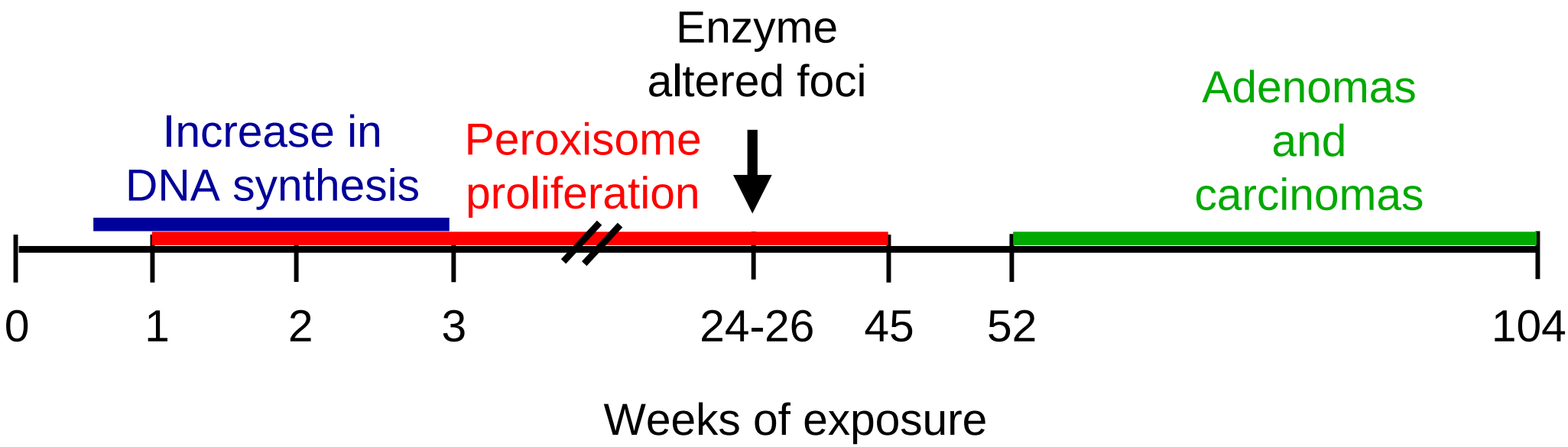
To determine:

- Relevance of peroxisome proliferation and increased hepatocyte mitogenesis to hepatocarcinogenicity of TCE and its metabolites
- Role of PPARα in hepatocarcinogenicity of TCE and its metabolites
- Relevance of B6C3F$_1$ mouse liver events to human toxicity

TCE-Induced Hepatocarcinogenesis

- Response seen in B6C3F_1 mice
- Metabolites: TCA &/or DCA
- Early responses *in vivo*
  - hepatocyte mitogenesis
  - peroxisome proliferation
- Response in humans is uncertain

Sequence of Hepatic Events in TCE-Treated $B6C3F_1$ Mice



Overall Questions

- Can rodent primary hepatocyte cultures be used to study the mechanism of:
  - hepatocyte mitogenesis?
  - peroxisome proliferation?

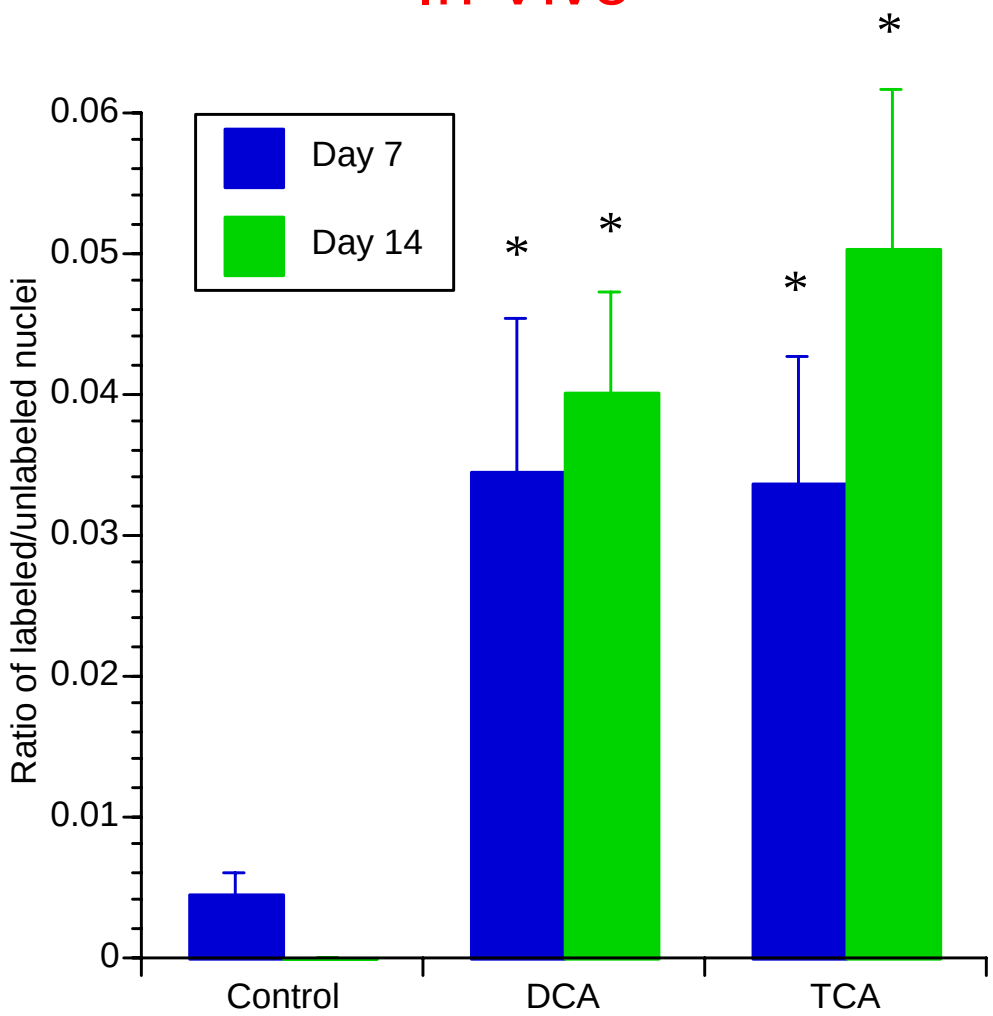
- Do human hepatocytes show responses?
  - qualitative
  - quantitative

Question

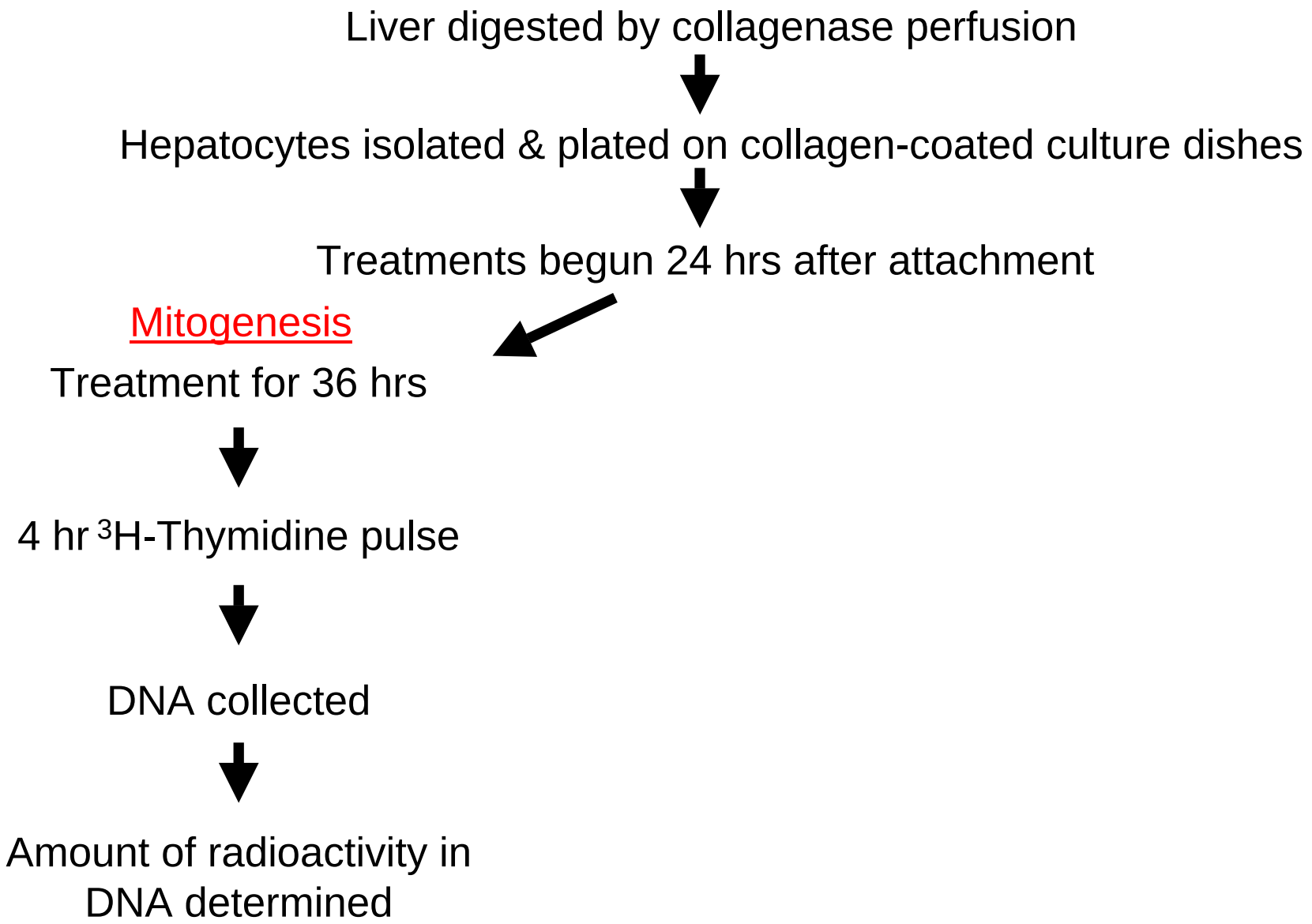
Are TCA and DCA mitogenic in rat and $B6C3F_1$ mouse hepatocyte cultures?

TCA- and DCA-Induced BrdU Incorporation in $B6C3F_1$ Mouse Liver

In Vivo

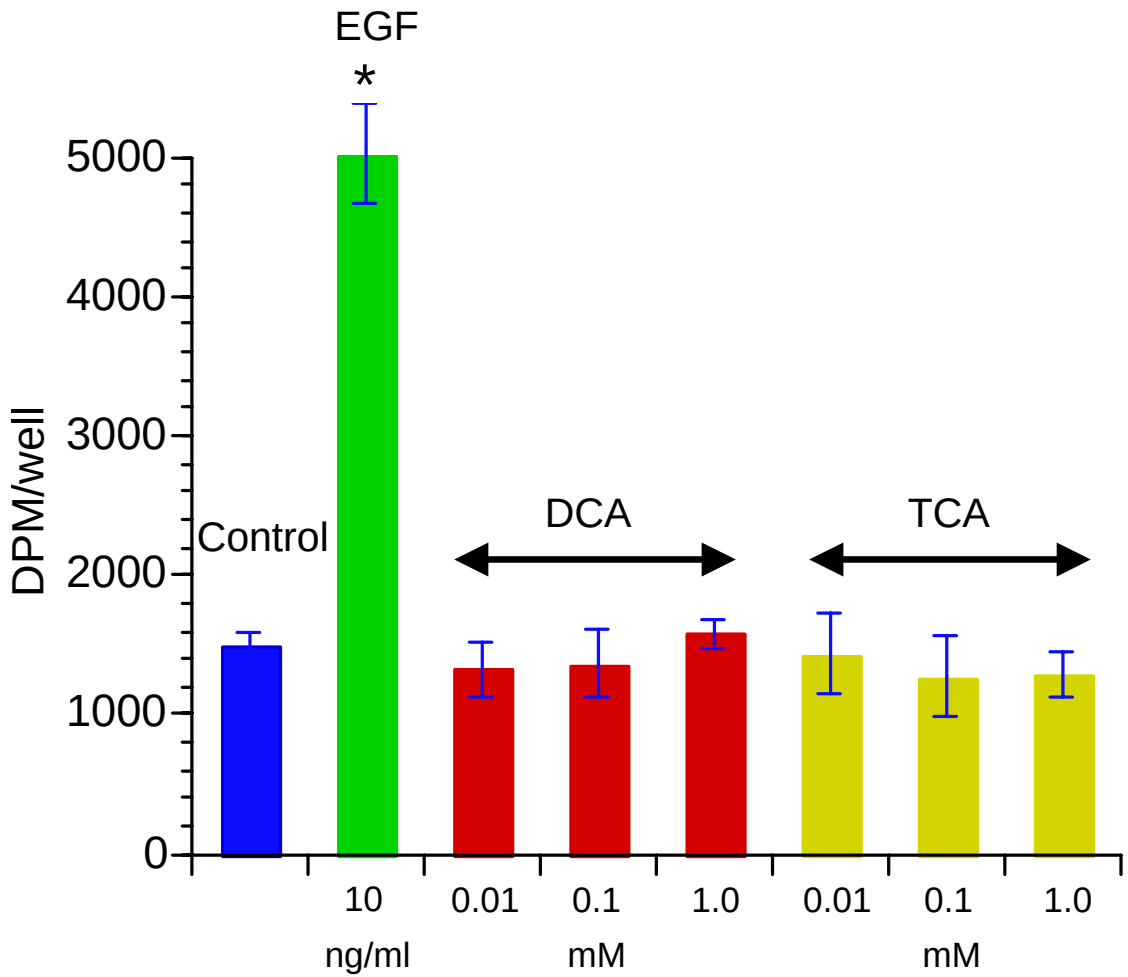


Rat & Mouse Hepatocyte Culture



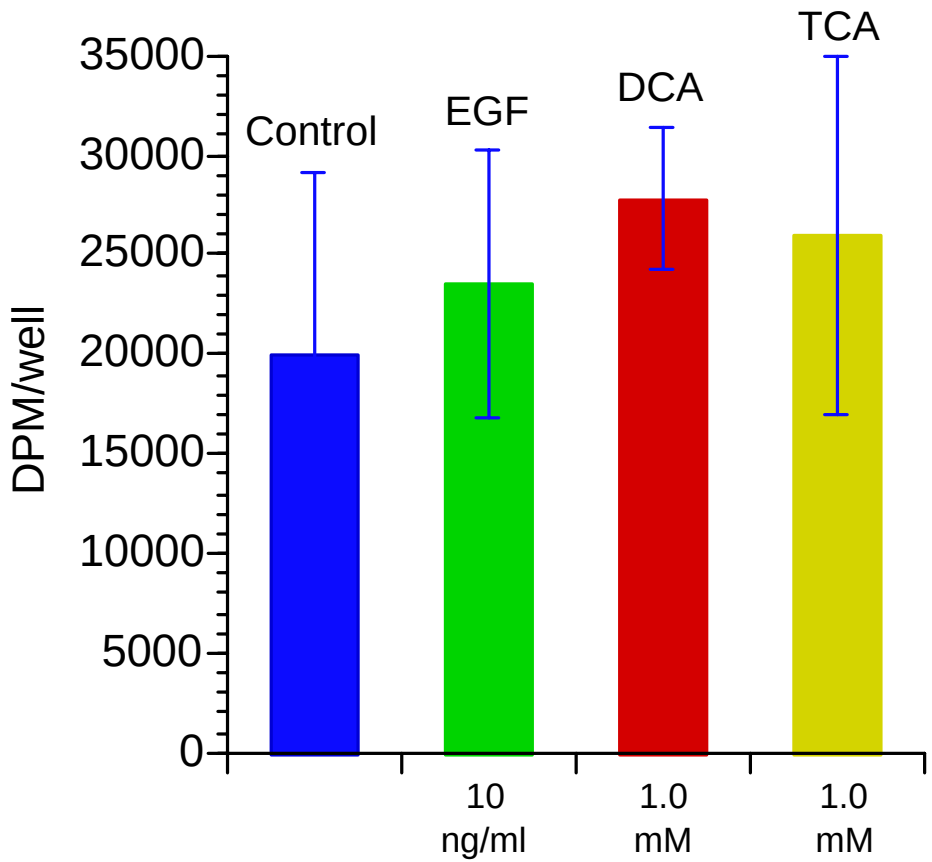
^{3}H-Thymidine Incorporation in Rat Hepatocytes

Rat Primary Hepatocyte Culture



^{3}H-Thymidine Incorporation in B6C3F$_{1}$ Mouse Hepatocytes

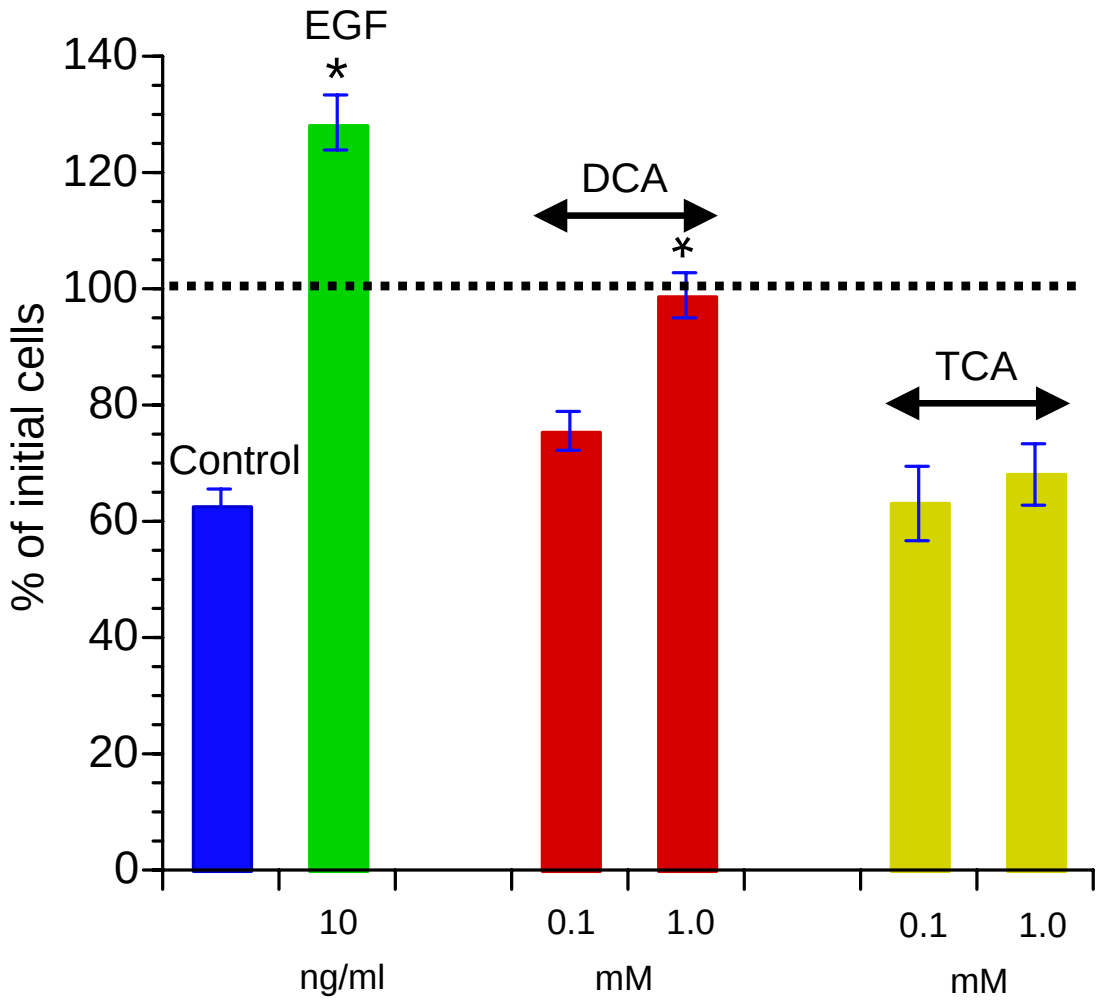
Mouse Primary Hepatocyte Culture



Cell Counts Following TCA and DCA Treatment

Rat Primary Hepatocyte Culture

40 hrs treatment



Question

Does TCA or DCA induce ^{3}H-thymidine incorporation in human hepatocyte cultures?

Isolation and Culture of Human Liver Cells

Donor tissue minced & digested by collagenase solution in Stomacher™ blender[1]

↓

Digested tissue filtered & hepatocytes pelleted **(50 x g)**

↓

Viable hepatocytes counted & plated

↓

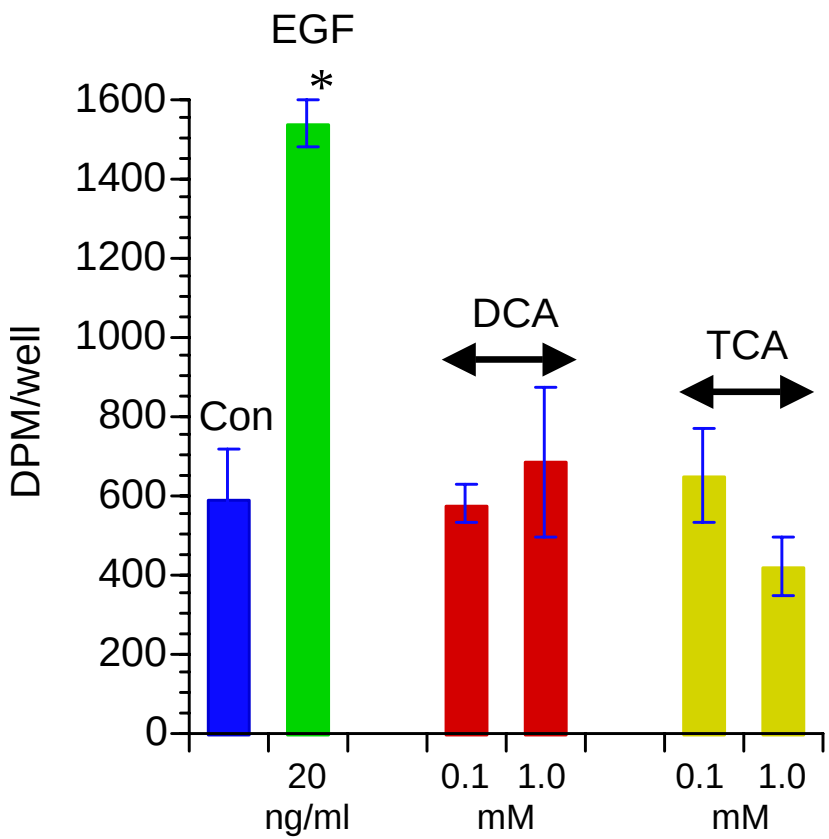
Cells grown to desired cell density

Primary cells	Subpassaged cells
Cells used	Cells passaged and used subsequently

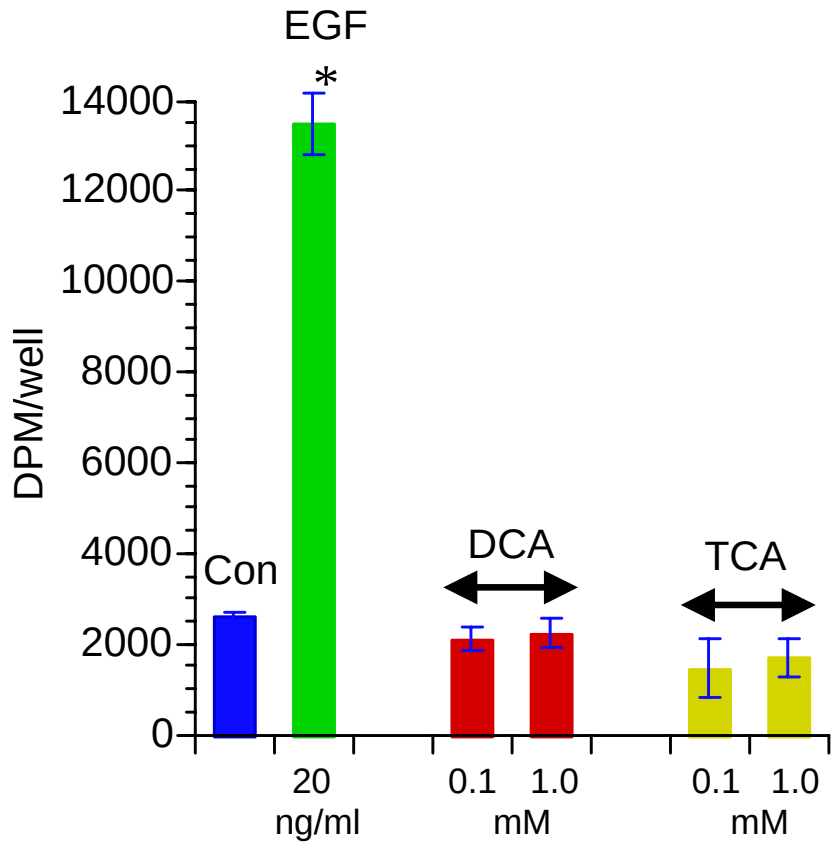
[1]Gibson, D. *et al.*, 1993. *Cell Biol. Toxicol.* **9**: 385-403.

^{3}H-Thymidine Incorporation in Human Cells

HL6 primary



HL6.5 subculture



Walgren, *et al.,* 2000 *Cell Biol. Toxicol*. 16: 257-273.

Summary

- Rodent hepatocytes
  - Mitogenic activity of DCA or TCA was not detected
- Human hepatocytes
  - No mitogenic effect with DCA or TCA

Question

Do TCA &/or DCA induce peroxisome proliferation in rat or mouse hepatocyte cultures?

Palmitoyl-CoA Oxidation in Intact Liver

In Vivo

	Palmitoyl-CoA oxidation			
	Control (nmol/min/mg prot)	TCA (fold increase)	DCA (fold increase)	Wy-14,643 (fold increase)
Rat	4-10	4	2	18
$B6C3F_1$ mouse	2-10	2.5	2	13
Human	2.8 $\pm$ 0.4*	—	—	—

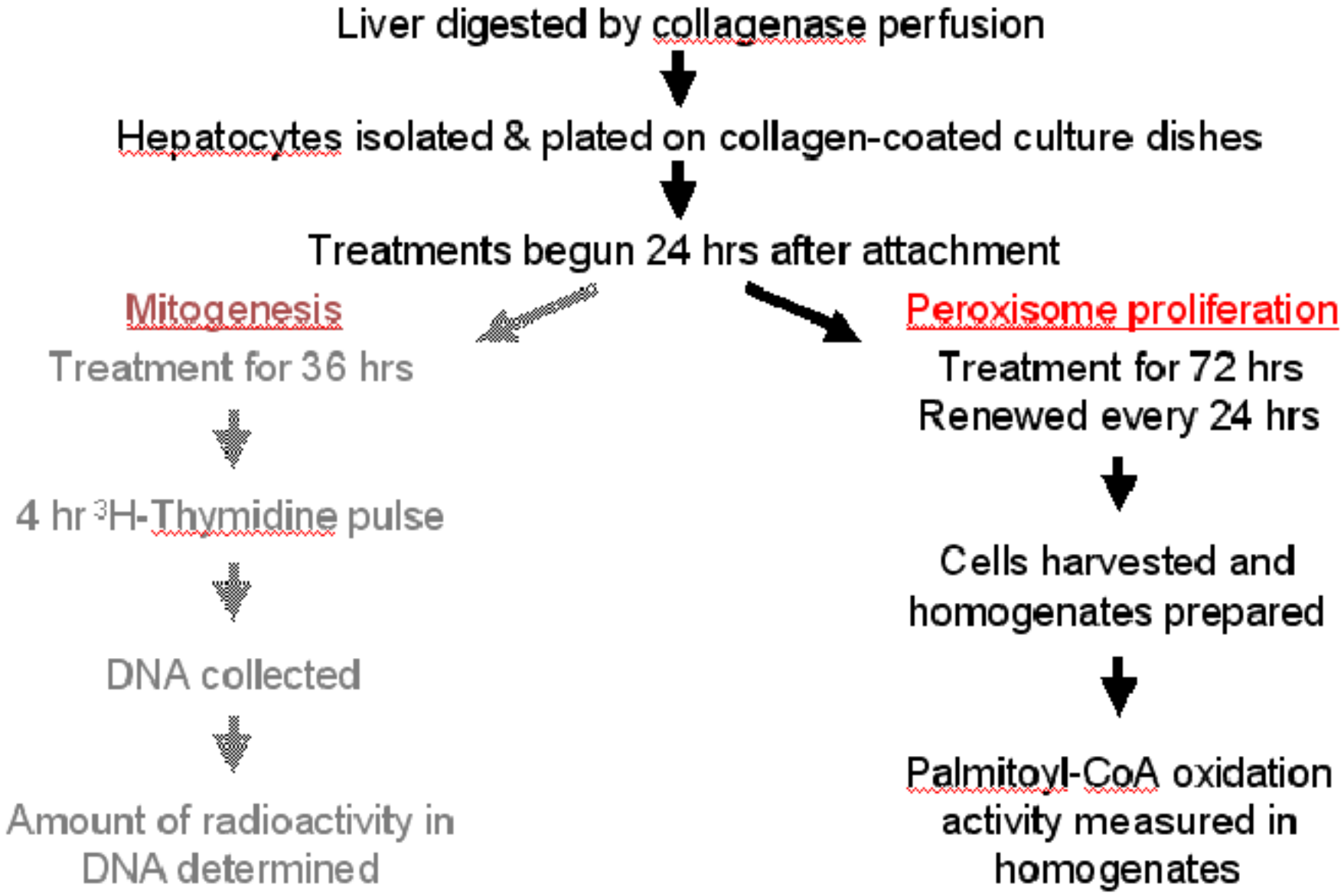
Elcombe, CR. 1985. *Arch. Toxicol.* Suppl. 8, 6-17.

DeAngelo et al. 1989. *Toxicol. Appl. Pharmacol.* 101, 285-298.

Bentley et al. 1993. *Fd. Chem. Toxic.* 31, 857-907.

*Walgren, *et al.,* 2000. *Cell Biol. Toxicol*. 16: 257-273

Rat & Mouse Hepatocyte Culture



Palmitoyl-CoA Oxidation in Cultured Hepatocytes

	Palmitoyl-CoA oxidation (nmoles NADH/mg protein/min)			
	Control	TCA	DCA	Wy-14,643
		[2 mM]	[2 mM]	[0.005 mM]
Rat	1.4 ± 0.1	3.2 ± 0.1 (2.5)	7.0 ± 0.2 (5)	12.1 ± 2.4 (8.5)
B6C3F1 mouse	0.2 ± 0.03	0.7 ± 0.1 (3.5)	1.1 ± 0.2 (5.5)	--
		[4 mM]	[4 mM]	[0.1mM]
Human	BLD (<20 pmol/min/mg)	BLD	BLD	BLD

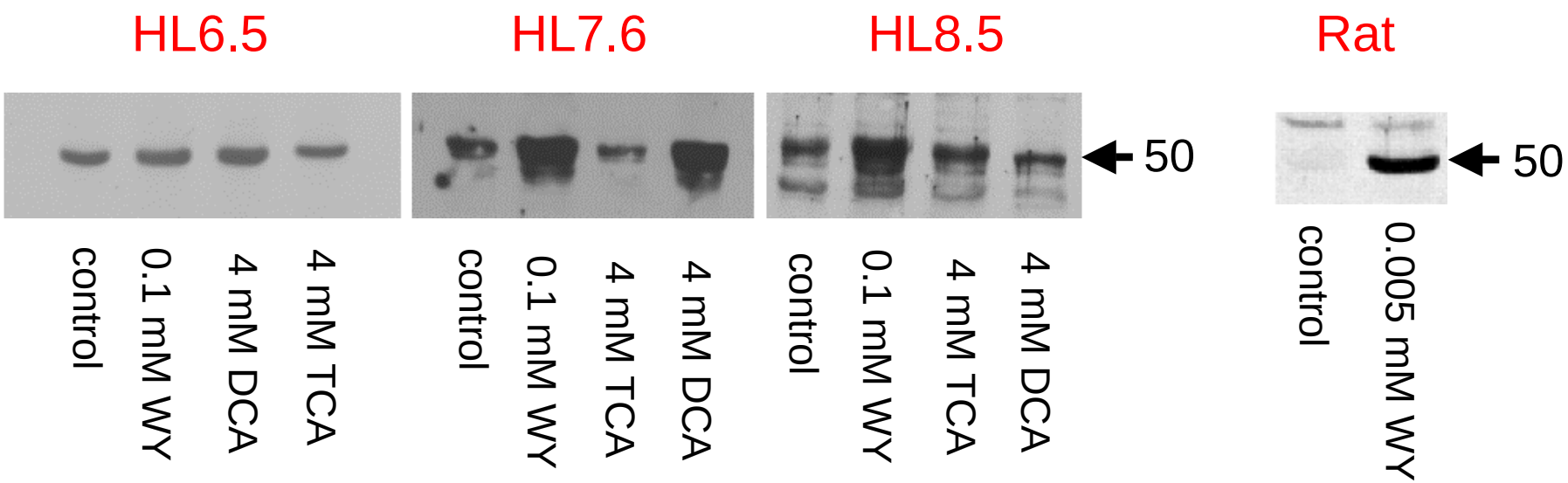
Walgren, *et al.,* 2000. *Cell Biol. Toxicol*. 16: 257-273

Questions

- Can cytochrome P450 4A be detected in human hepatocyte cultures?

- Can cytochrome P450 4A be induced in human hepatocyte cultures?

CYP4A Levels in Human Hepatocytes



Walgren, *et al.,* 2000 *Cell Biol. Toxicol*. 16: 257-273

Summary

- Rat and mouse hepatocytes:
  - Palmitoyl-CoA oxidation is detectable
  - Palmitoyl-CoA oxidation is inducible by TCA and DCA
- Human hepatocytes:
  - Palmitoyl-CoA oxidation was not detectable
  - Induction of palmitoyl-CoA oxidation could not be detected
  - CYP 4A was detectable and maintained in the passaged cells
  - Induction of CYP 4A was observed
  - CYP 4A induction and the extent of induction were variable in different hepatocyte preparations

Question

Why are rodent and human responses different?

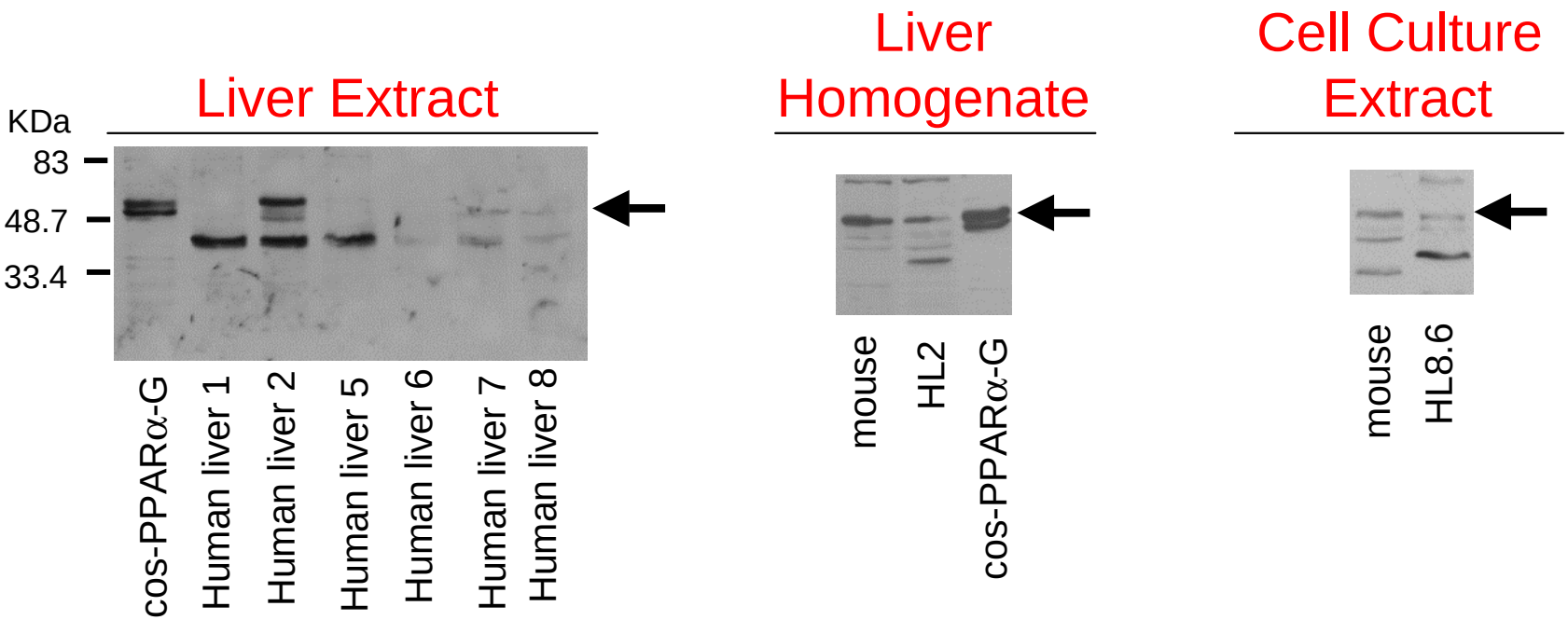
Human *vs.* Rodent Peroxisomal Responses

- PPARα mRNA levels are 10-fold greater in mouse *vs.* human liver
- Evidence for 2 or more variants of human PPARα
- No evidence for increased peroxisomal enzyme activities in humans taking fibric acid drugs

Question

- Are the differences in response due to differences in
  - receptor?
  - response element activation?

PPARα Protein Levels in Human Liver and Cultured Hepatocytes

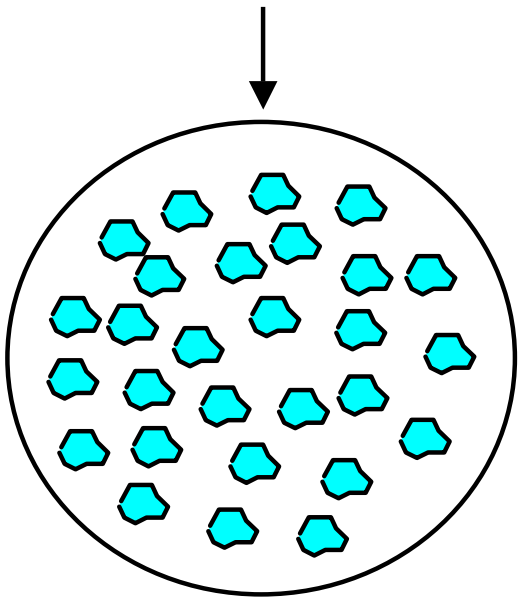


Walgren, *et al.,* 2000. *Res. Commun. Mol. Pathol. Pharmacol*. 108: 116-132

Transfection of Human Hepatocytes

Endogenous

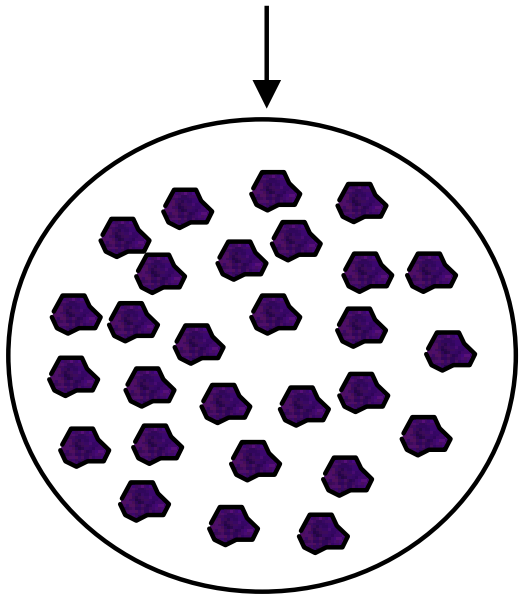
PPRE–CAT
pcDNA3



-CAT assay:
endogenous hPPAR activity

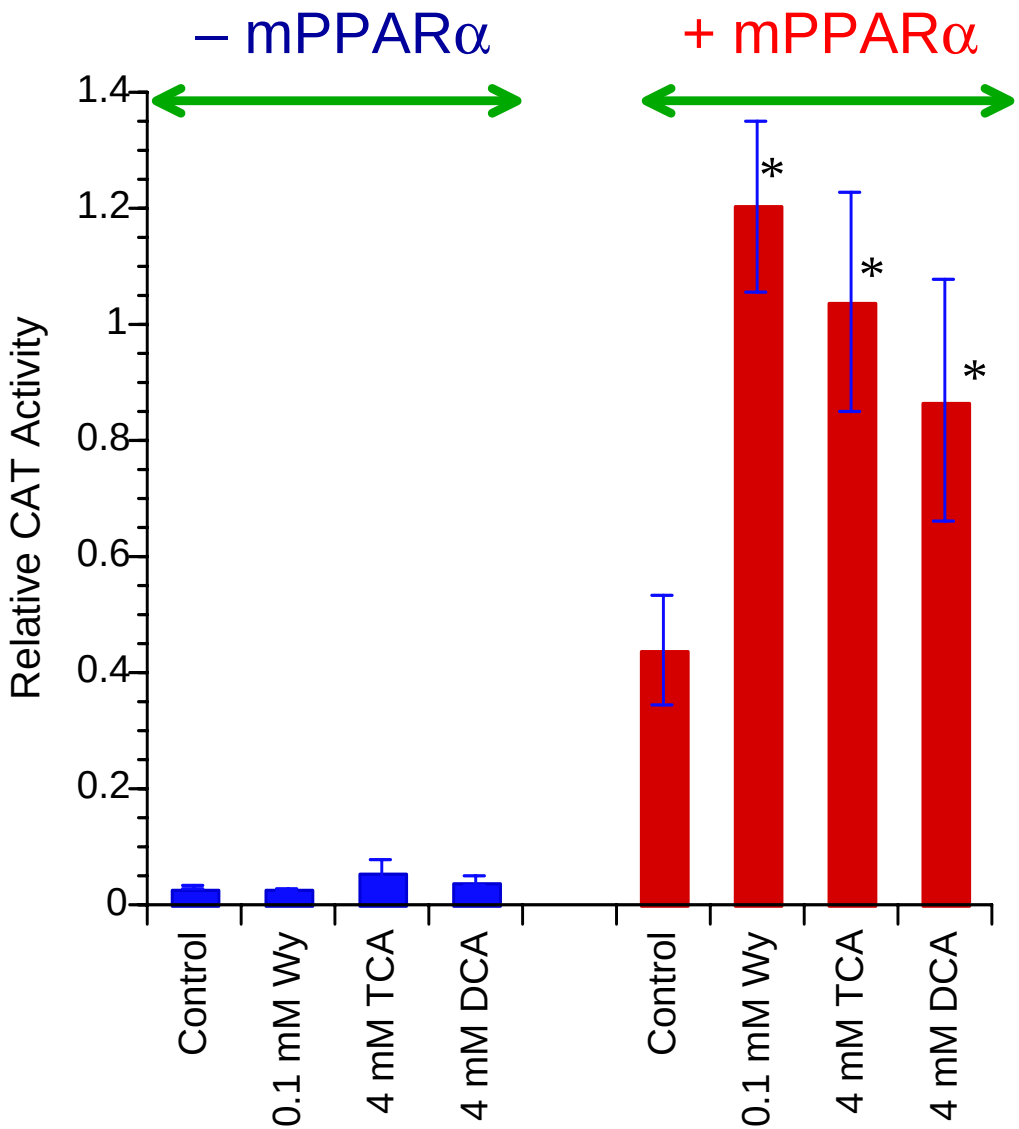
Transfected

PPRE–CAT
mPPARα
mRXRα



-CAT assay:
transfected mPPAR activity

PPRE Activation in Human Cells Transfected with Mouse PPARα



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Conclusions

Reproduction of early *in vivo* responses

	Rodent	Human
Direct mitogenesis	–	–
Palmitoyl-CoA oxidation	+	–
P450 4A induction	+	+

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