

Hepatocyte Cultures as Model Systems for Trichloroethylene Hepatocarcinogenicity

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TCE Research Objectives

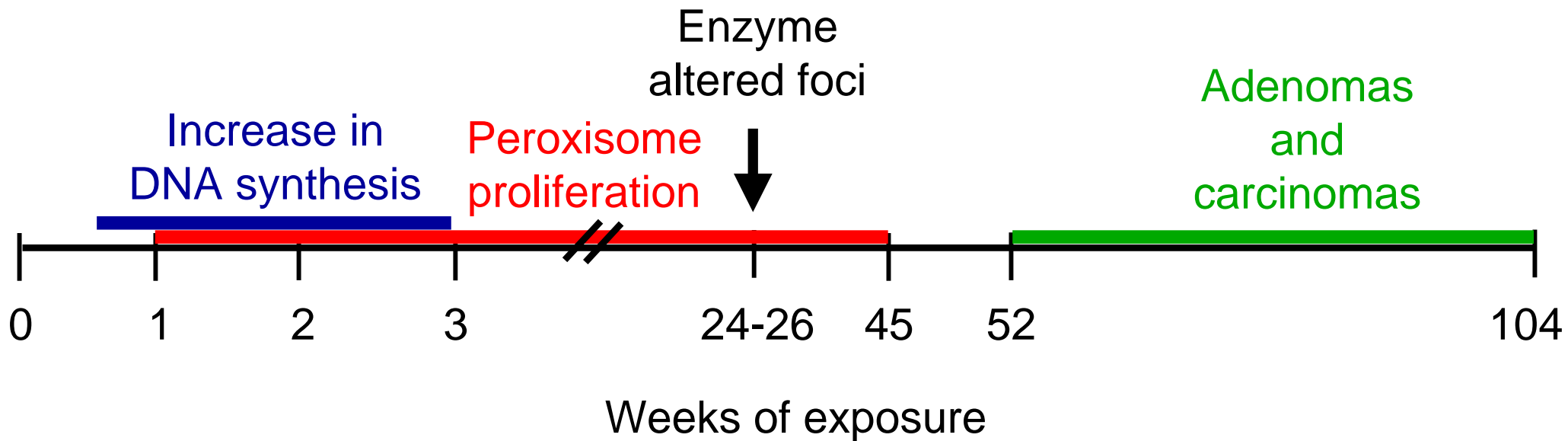
To determine:

- Relevance of peroxisome proliferation and increased hepatocyte mitogenesis to hepatocarcinogenicity of TCE and its metabolites
- Role of PPAR α in hepatocarcinogenicity of TCE and its metabolites
- Relevance of B6C3F₁ mouse liver events to human toxicity

TCE-Induced Hepatocarcinogenesis

- Response seen in B6C3F₁ 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 B6C3F₁ Mice



Overall Questions

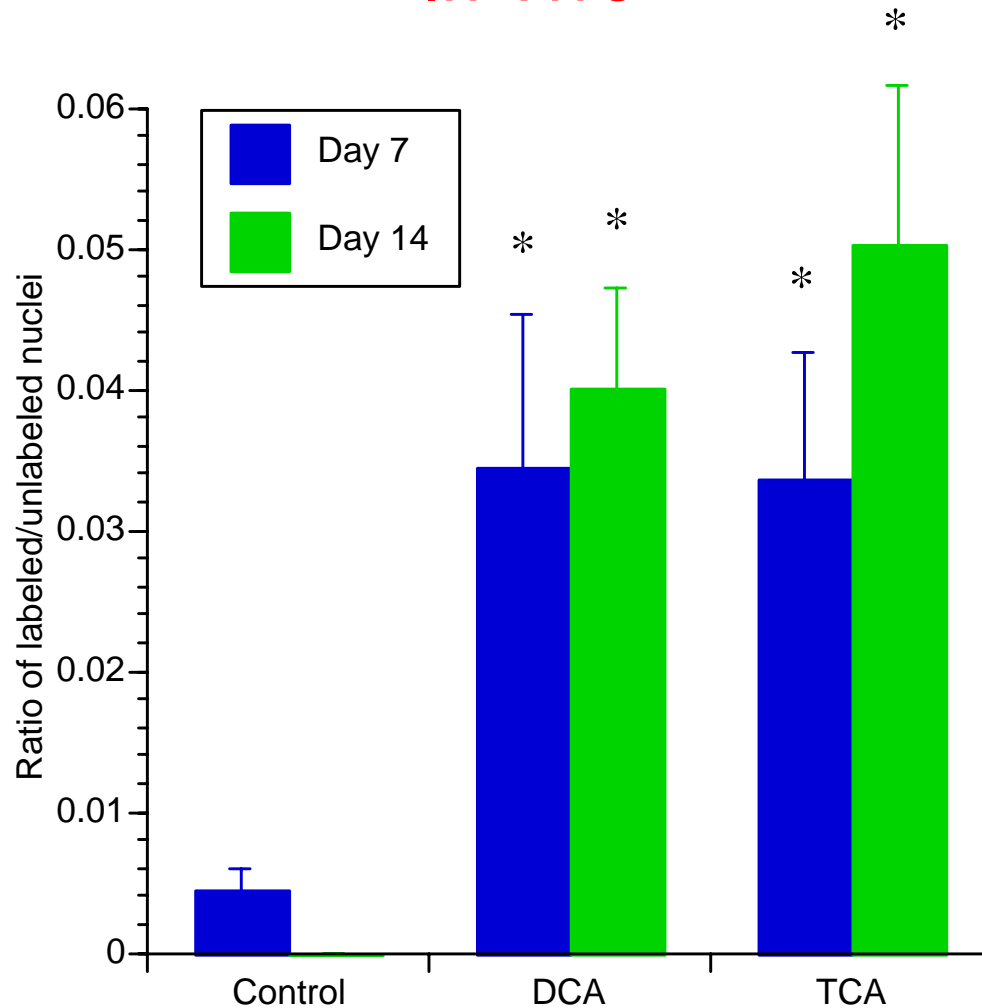
- Can rodent primary hepatocyte cultures be used to study the mechanism of:
 - hepatocyte mitogenesis?
 - peroxisome proliferation?
- Do human hepatocytes show responses?
 - qualitative
 - quantitative

Question

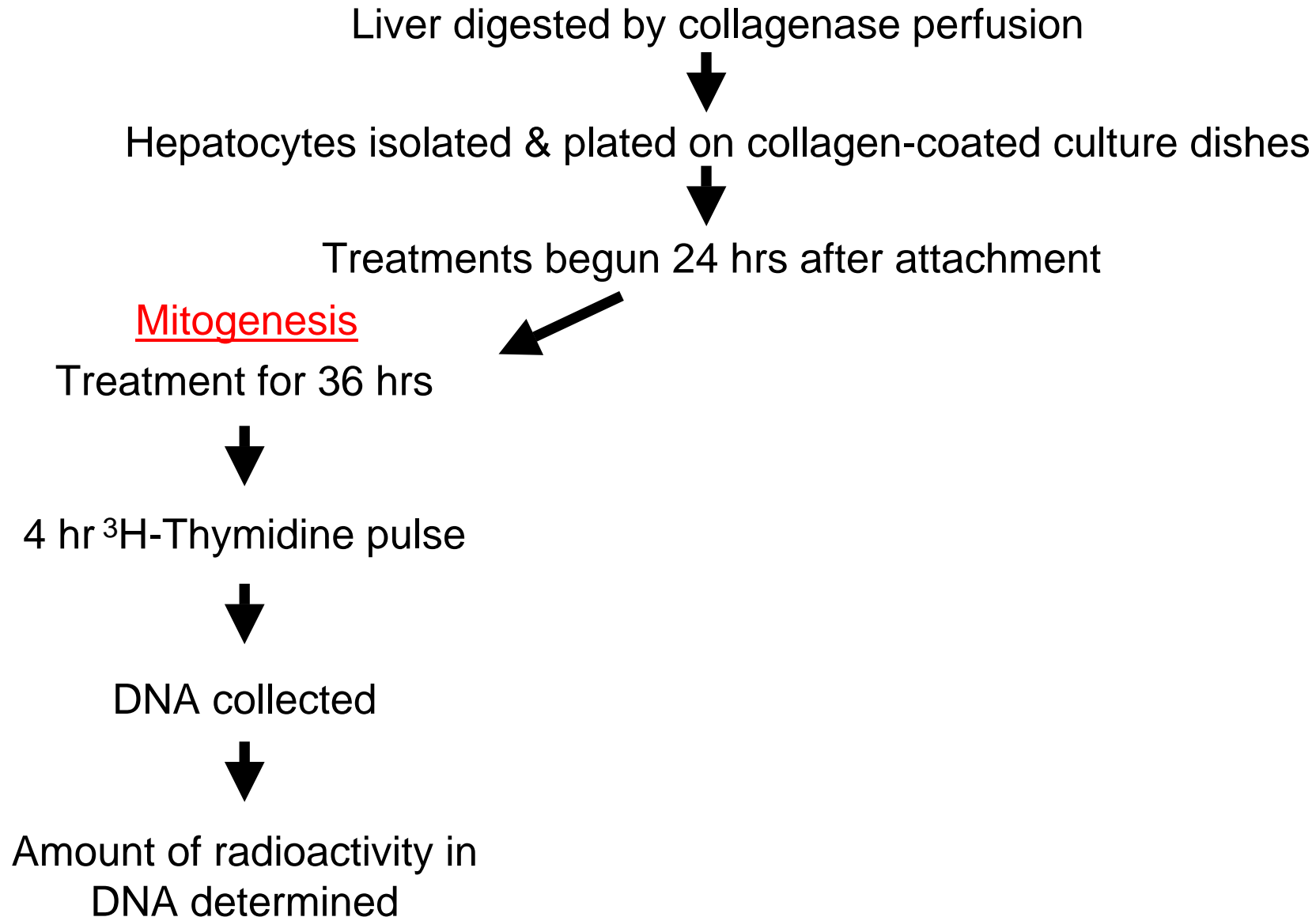
Are TCA and DCA mitogenic in rat and B6C3F₁ mouse hepatocyte cultures?

TCA- and DCA-Induced BrdU Incorporation in B6C3F₁ Mouse Liver

In Vivo

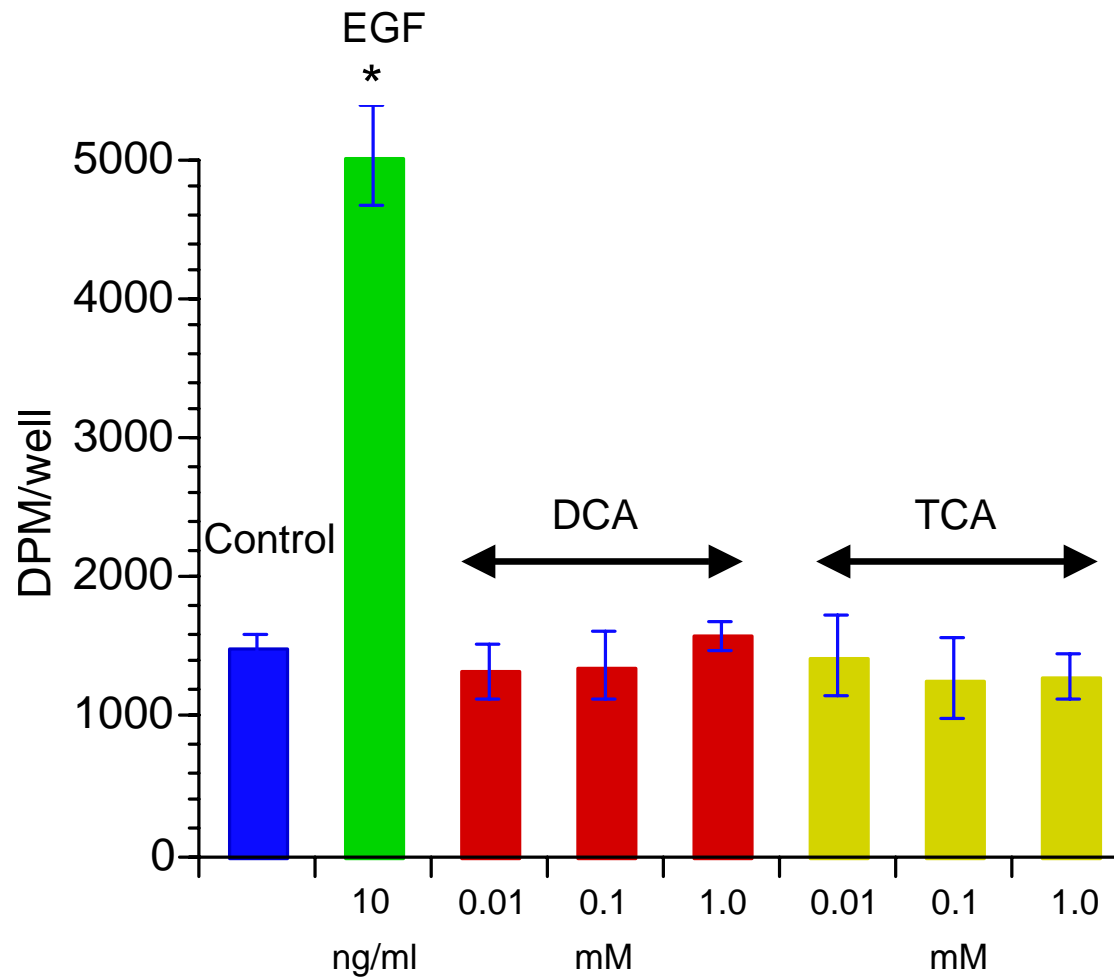


Rat & Mouse Hepatocyte Culture



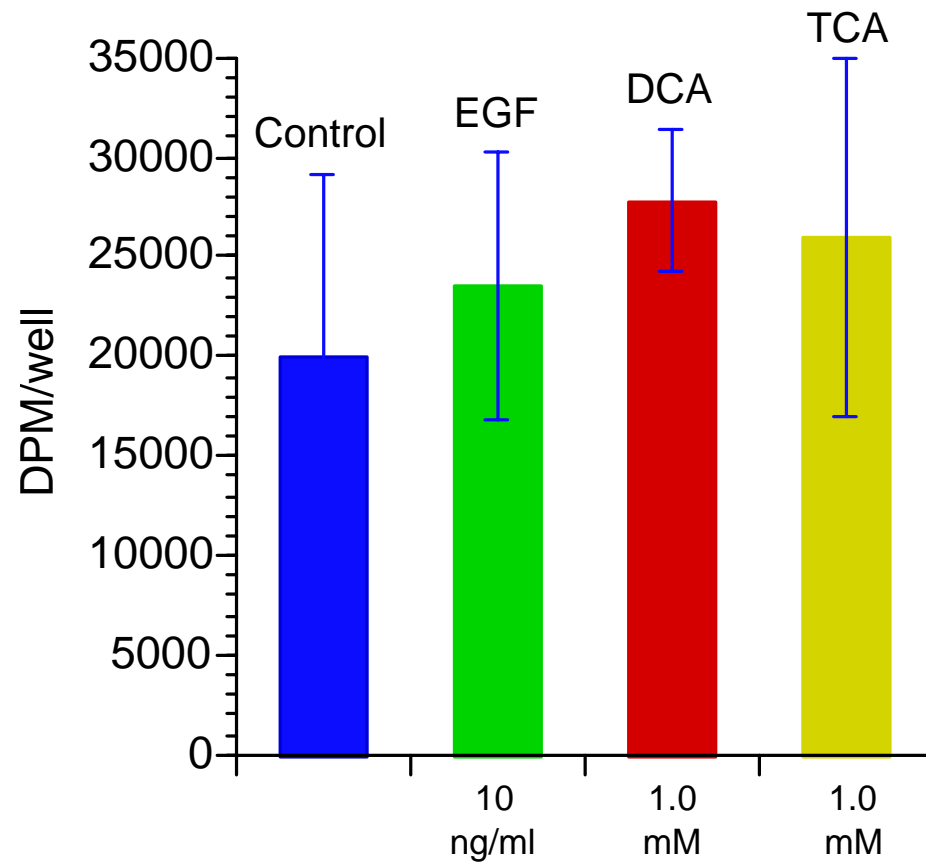
^3H -Thymidine Incorporation in Rat Hepatocytes

Rat Primary Hepatocyte Culture



^3H -Thymidine Incorporation in B6C3F₁ Mouse Hepatocytes

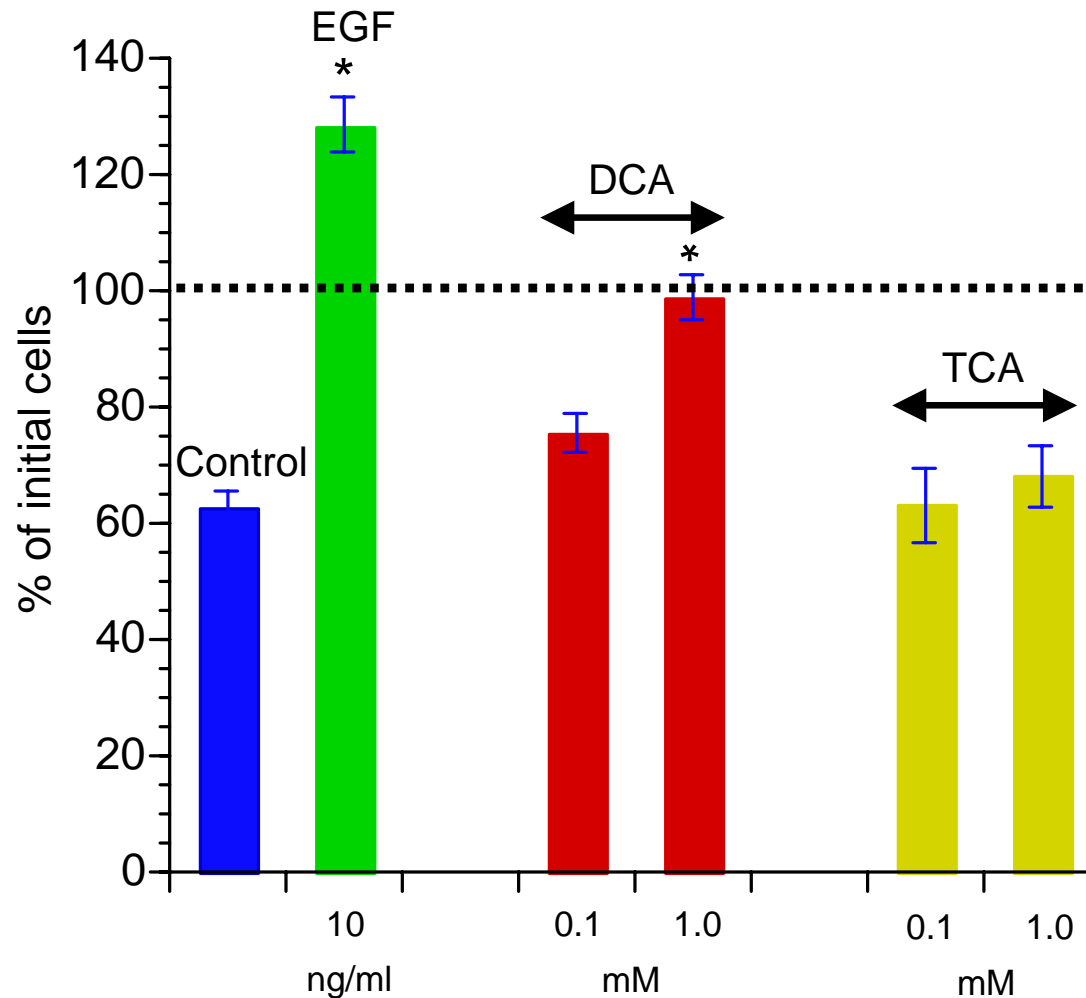
Mouse Primary Hepatocyte Culture



Cell Counts Following TCA and DCA Treatment

Rat Primary Hepatocyte Culture

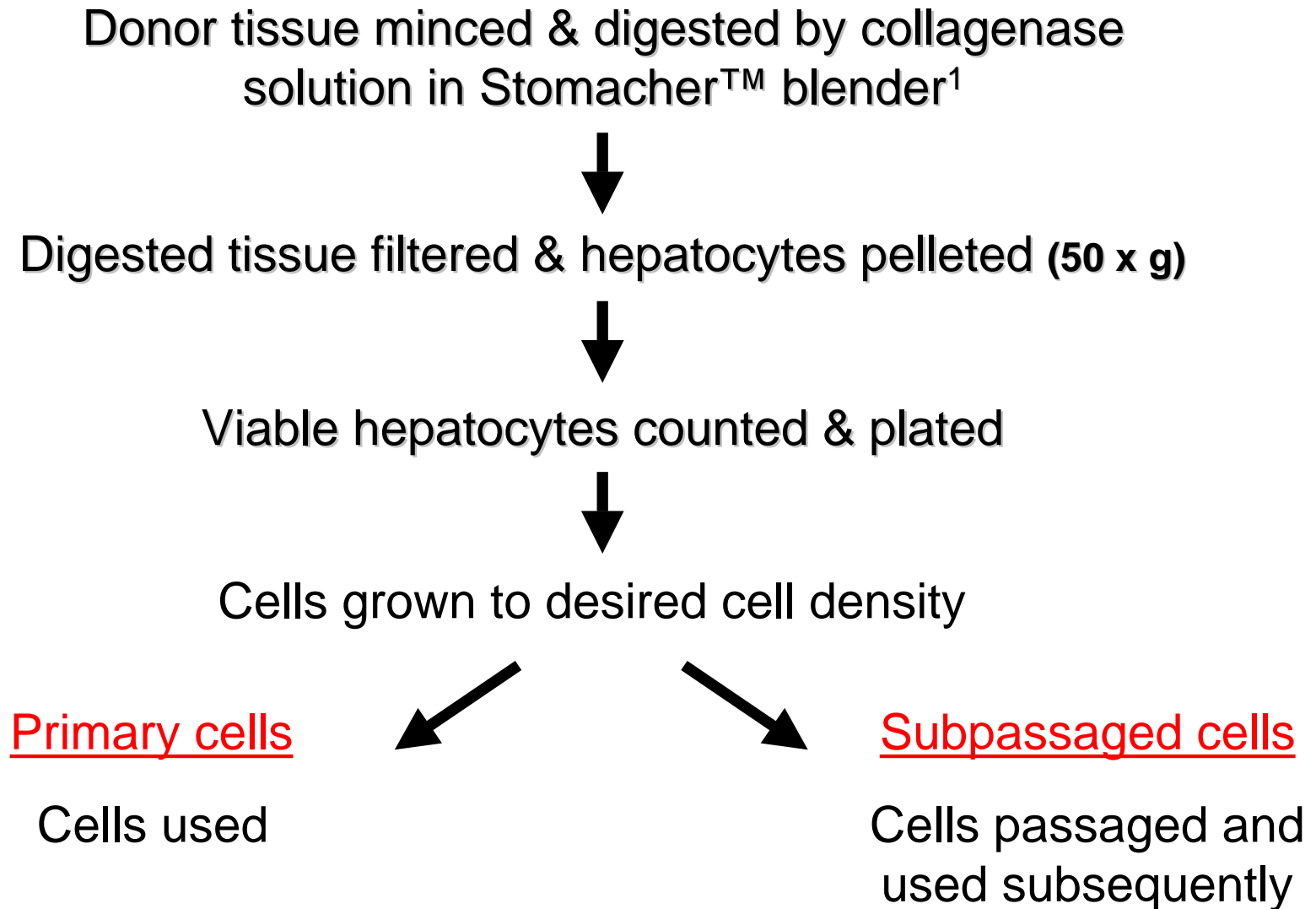
40 hrs treatment



Question

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

Isolation and Culture of Human Liver Cells

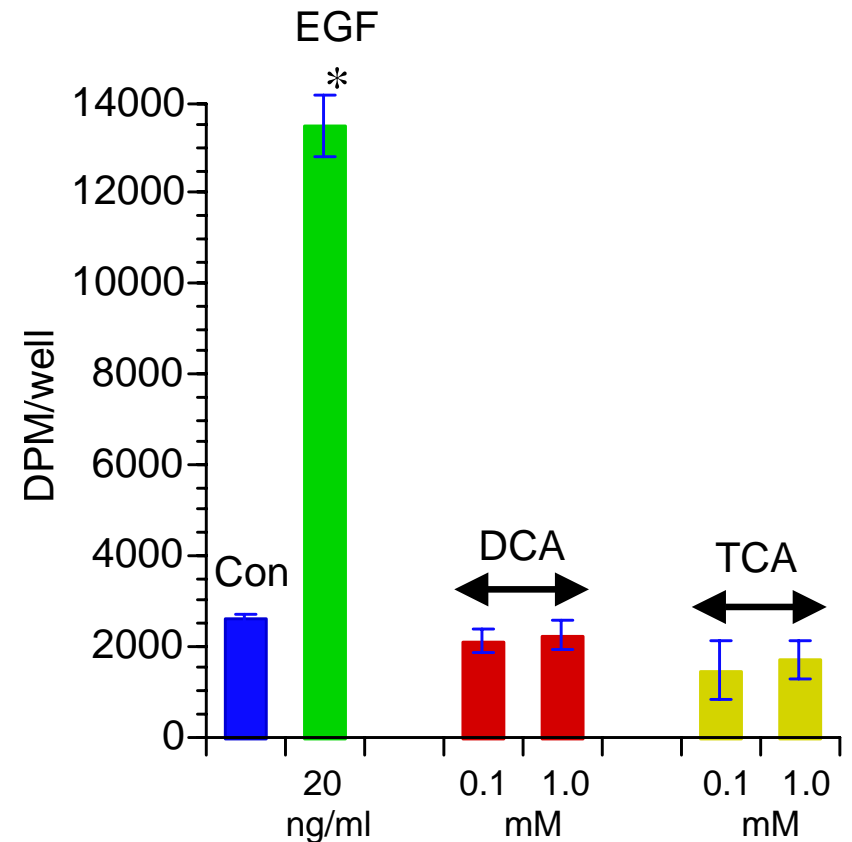
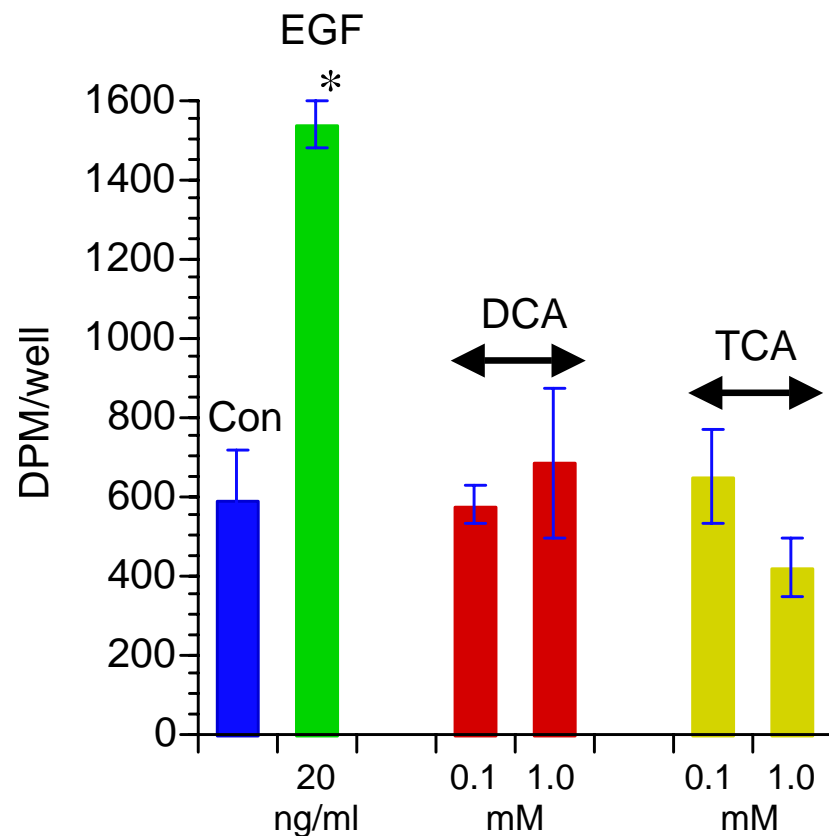


¹Gibson, D. *et al.*, 1993. *Cell Biol. Toxicol.* **9**: 385-403.

^3H -Thymidine Incorporation in Human Cells

HL6 primary

HL6.5 subculture



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 ± 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

Liver digested by collagenase perfusion



Hepatocytes isolated & plated on collagen-coated culture dishes



Treatments begun 24 hrs after attachment

Mitogenesis



Treatment for 36 hrs



4 hr ³H-Thymidine pulse



DNA collected



Amount of radioactivity in DNA determined

Peroxisome proliferation



Treatment for 72 hrs
Renewed every 24 hrs



Cells harvested and homogenates prepared



Palmitoyl-CoA oxidation activity measured in homogenates

Palmitoyl-CoA Oxidation in Cultured Hepatocytes

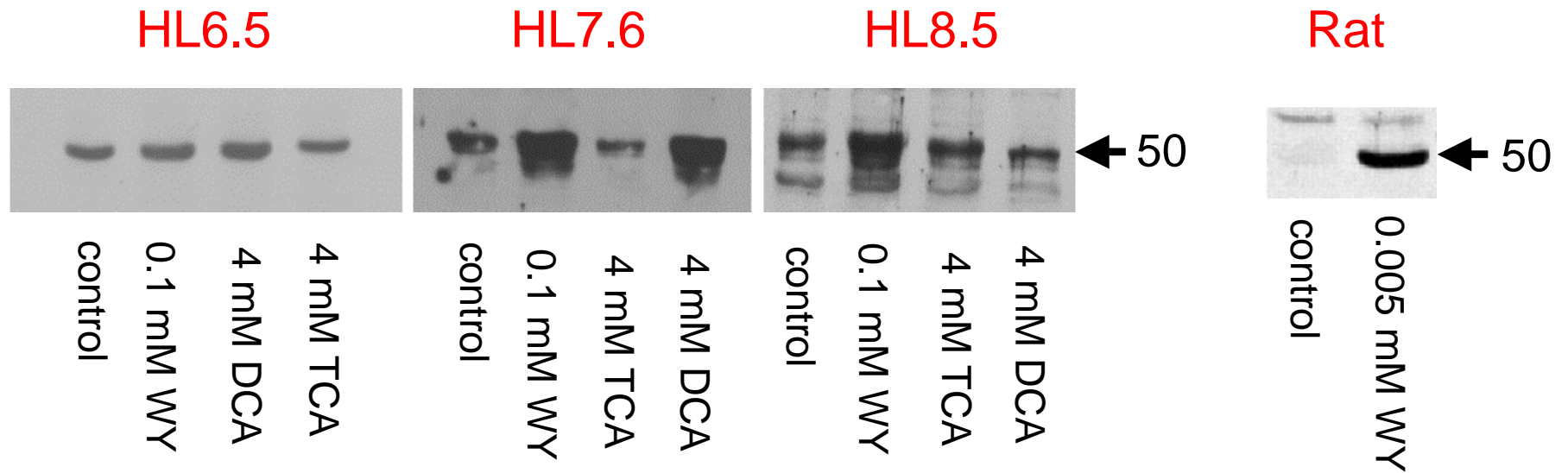
	Palmitoyl-CoA oxidation (nmoles NADH/mg protein/min)			
	Control	TCA [2 mM]	DCA [2 mM]	Wy-14,643 [0.005 mM]
Rat	1.4 ± 0.1	3.2 ± 0.1 (2.5)	7.0 ± 0.2 (5)	12.1 ± 2.4 (8.5)
B6C3F1 mouse	0.2 ± 0.03	0.7 ± 0.1 (3.5)	1.1 ± 0.2 (5.5)	--
		[4 mM]	[4 mM]	[0.1mM]
Human	BLD (<20 pmol/min/mg)	BLD	BLD	BLD

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?

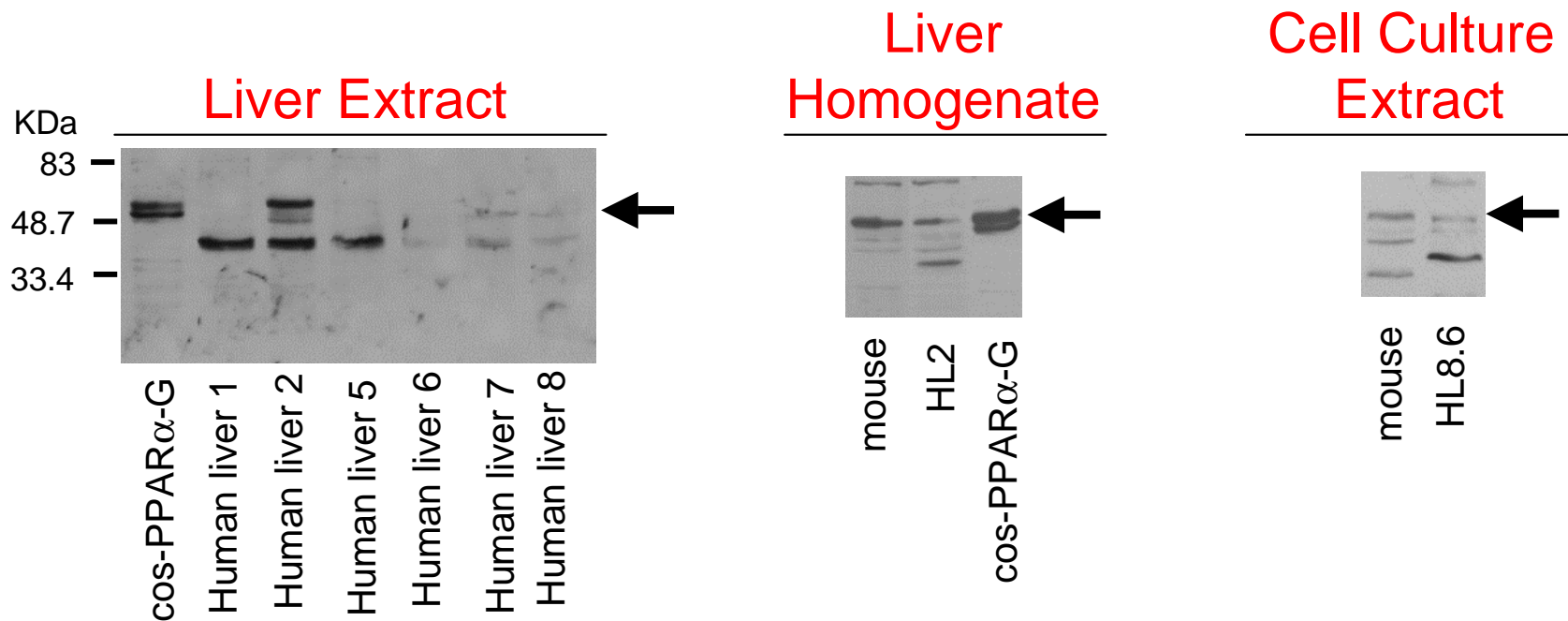
Human vs. Rodent Peroxisomal Responses

- PPAR α mRNA levels are 10-fold greater in mouse vs. human liver
- Evidence for 2 or more variants of human PPAR α
- 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?

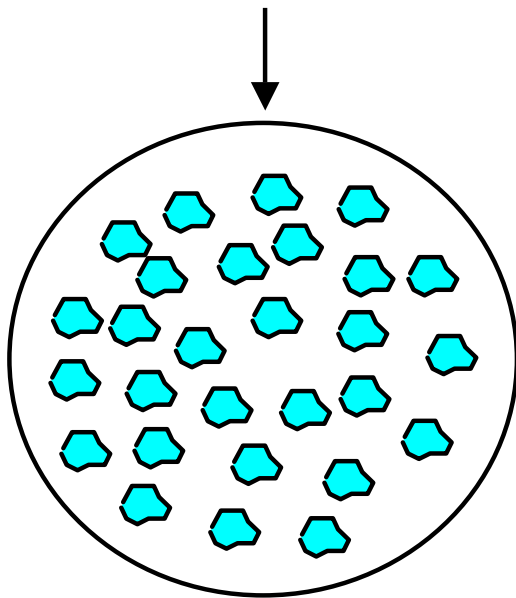
PPAR α Protein Levels in Human Liver and Cultured Hepatocytes



Transfection of Human Hepatocytes

Endogenous

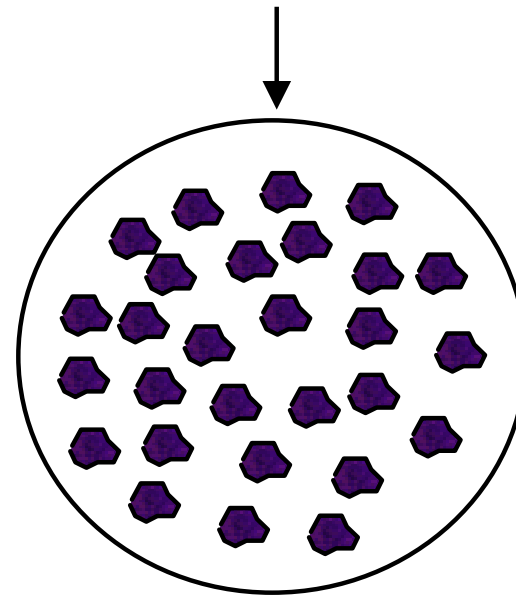
PPRE-CAT
pcDNA3



-CAT assay:
endogenous hPPAR activity

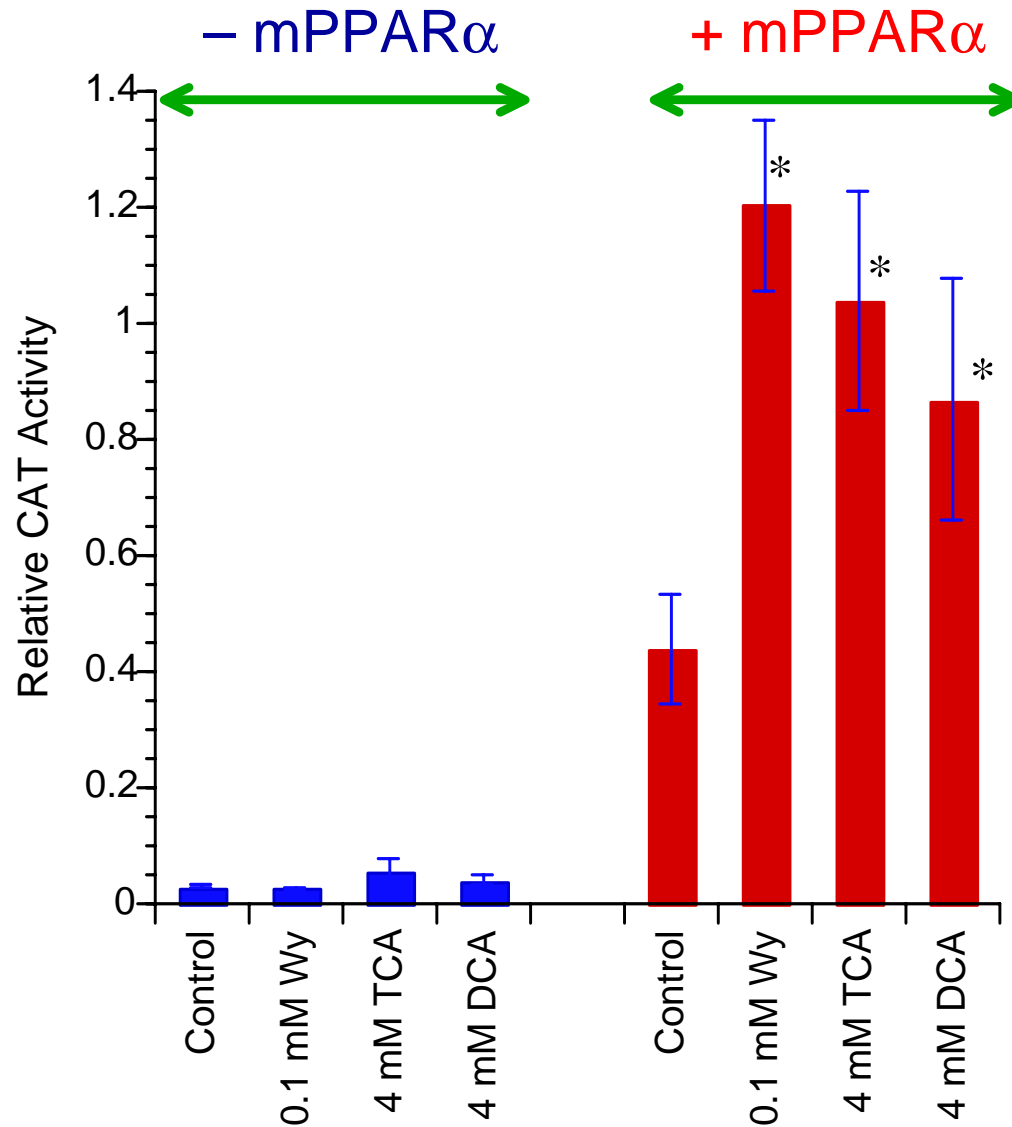
Transfected

PPRE-CAT
mPPAR α
mRXR α



-CAT assay:
transfected mPPAR activity

PPRE Activation in Human Cells Transfected with Mouse PPAR α



Conclusions

Reproduction of early *in vivo* responses

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

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