Structural Basis of Species Differences between Rat and Human CYP1A1s in Metabolism of Polychlorinated Biphenyls

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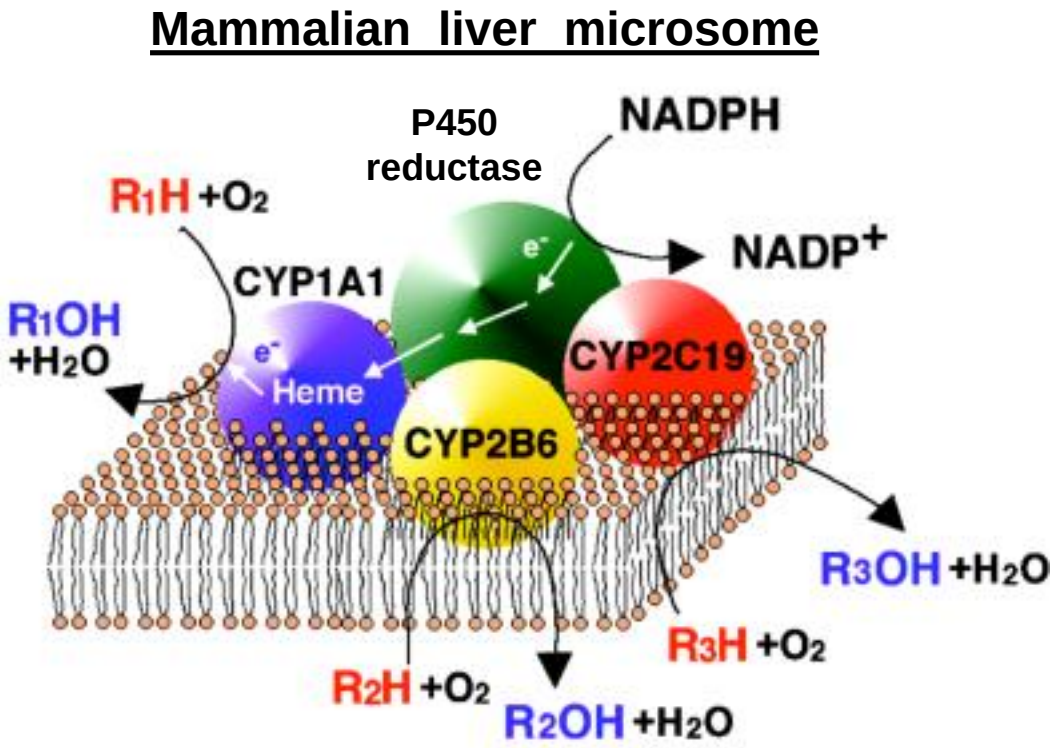
Cytochrome P450 (CYP) monooxygenases

- Monooxygenase reaction
- Endogenous and exogenous compounds as a substrate
- Broad and overlapping substrate specificity



$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

Critical roles in drug metabolism in mammals

Dioxin-like toxicity of PCBs

Persistent organic pollutants (POPs)
- **Persistency in the environment**
- **Wide distribution**
- **Bioaccumulation through the food chain**

Adverse effects to wildlife and human health by dioxin-like toxicity such as
- **Carcinogenicity**
- **Hepatotoxicity**
- **Teratogenicity**
- **Mutagenicity**

Congener	TEF
3,3',4,4'-TeCB (#77)	0.0001
3,4,4',5-TeCB (#81)	0.0003
3,3',4,4',5-PeCB (#126)	0.1
3,3',4,4',5,5'-HxCB (#169)	0.03
2',3,4,4',5-PeCB (#123)	0.00003
2,3',4,4',5-PeCB (#118)	0.00003
2,3,3',4,4'-PeCB (#105)	0.00003
2,3,4,4',5-PeCB (#114)	0.00003
2,3',4,4',5,5'-HxCB (#167)	0.00003
2,3,3',4,4',5-HxCB (#156)	0.00003
2,3,3',4,4',5'-HxCB (#157)	0.00003
2,3,3',4,4',5,5'-HpCB (#189)	0.00003

TEF; Toxic equivalency factor

WHO, 2005

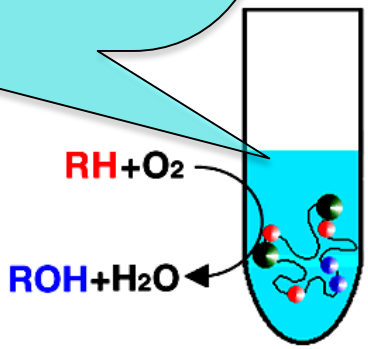
Metabolism of PCBs in microsomal fractions of rat and human CYP1A1s

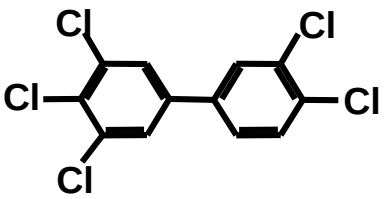
Reaction condition

40pmol	Microsomal fraction containing rat or human CYP1A1
0.5mM	NADPH
5mM	G6P
1U	G6PDH
3.3mM	$MgCl_2$
100mM	Potassium phosphate buffer
~2ppm	CB126, CB77
0.5ml	

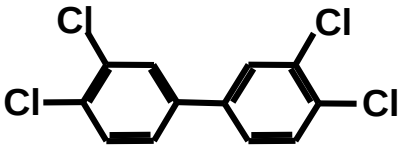
37℃, 2-hour incubation

1. Addition of NADPH to start reaction
2. A reaction mixture without NADPH is used as a control.
3. Addition of ^{13}C-OH-PCBs after stopping a reaction
4. Extraction of metabolites with hexane
5. Dryness and methylation
6. Quantification and identification of metabolites with high resolution GC/MS



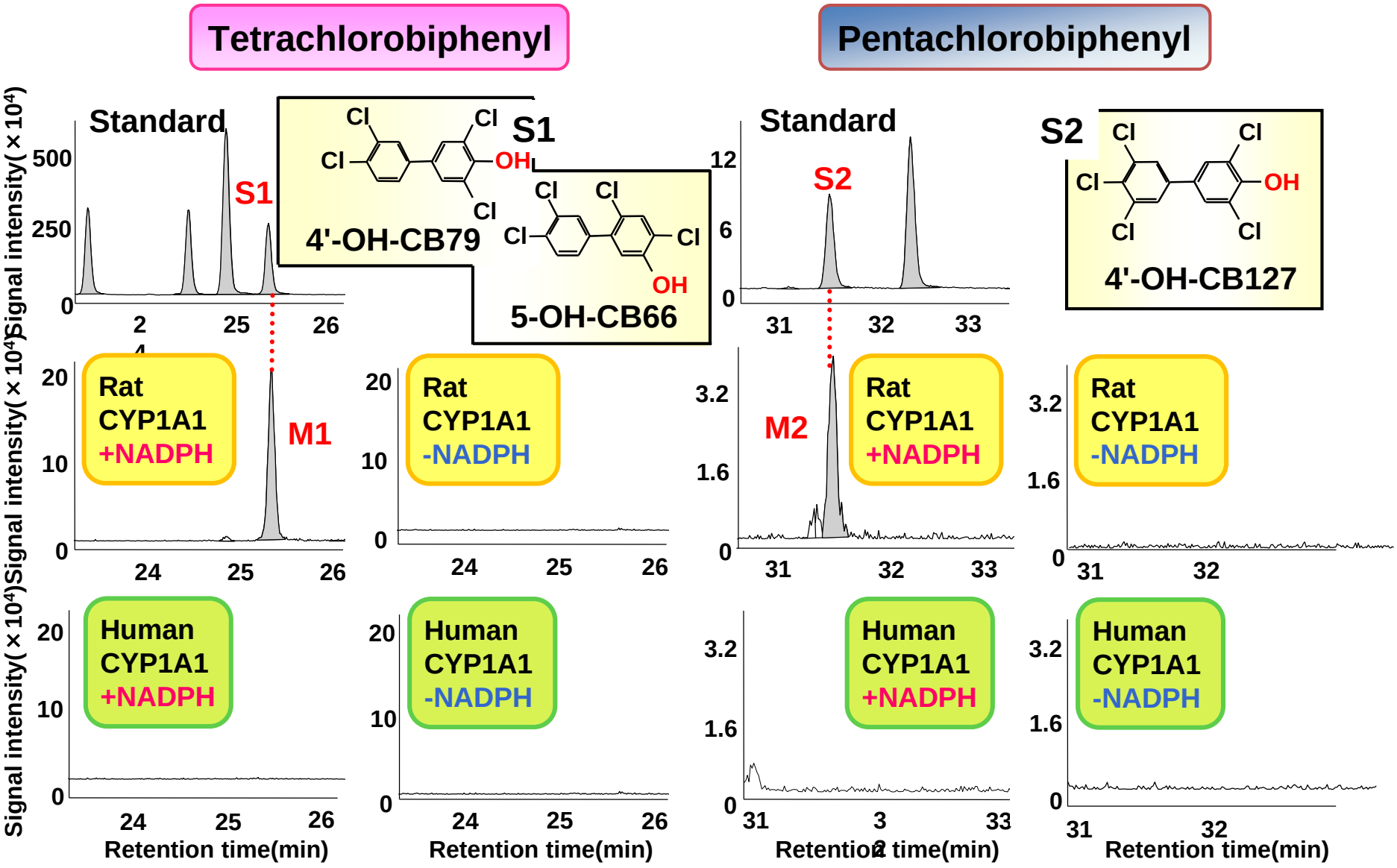


CB126



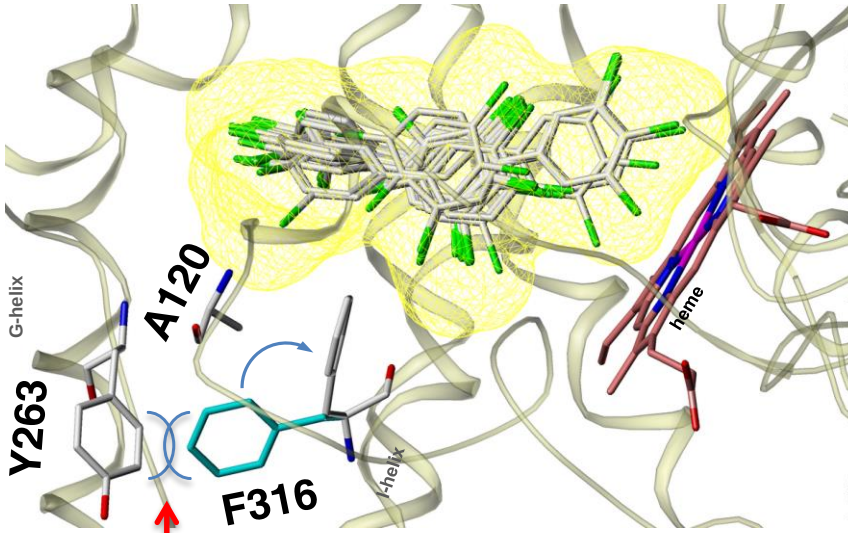
CB77

Metabolism of CB126 by rat and human CYP1A1s



Homology modeling of rat and human CYP1A1s

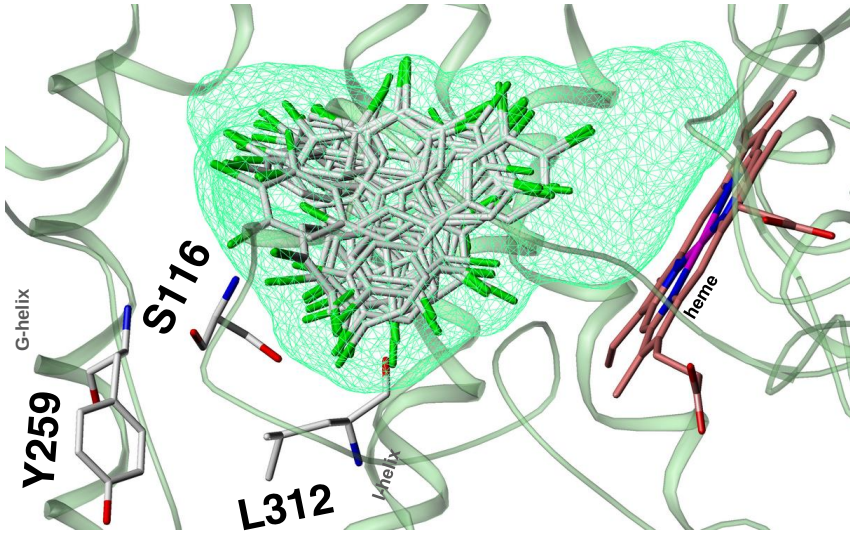
Rat CYP1A1



510Å^3

Conflict !

Human CYP1A1

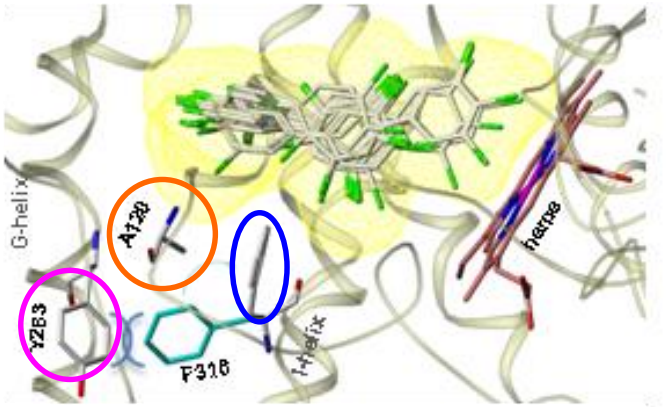


600Å^3

F316 is conflicted with Y263, and then flipped to the side of substrate binding pocket. It results in a small volume of substrate binding pocket in rat CYP1A1.

Structures of amino acid residues consisting of a substrate binding pocket in mammalian CYP1A1s

Number (rat)	Human	Rat	Dog	Golden hamster	Guinea pig	Monkey	Mouse	Rabbit
A120	Ser	Ala	Thr	Thr	Ser	Ser	Thr	Thr
Y263	Tyr	Tyr	Tyr	Tyr	Ser	His	Tyr	Tyr
F316	Leu	Phe	Leu	Leu	Leu	Leu	Leu	Leu

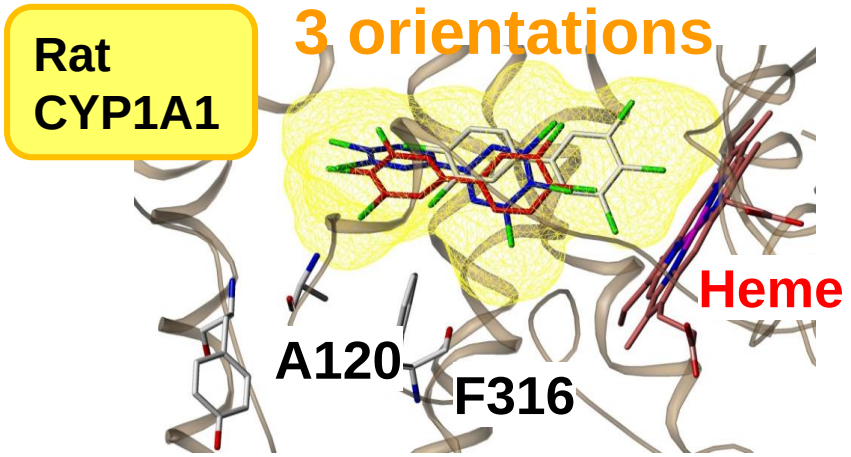


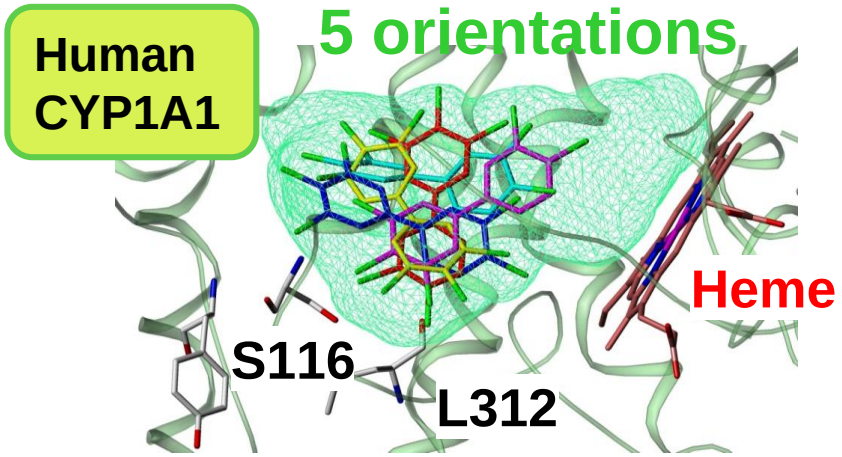
The Y263 causing the conflict is well conserved. Both residues A120 and F316, seen in rat CYP1A1, are rare in other well-studied mammalian CYP1A1s.

These findings suggest that the cavity shape and catalytic activity of rat CYP1A1 may be unique among experimental animals.

Docking model of CB126 with rat and human CYP1A1s

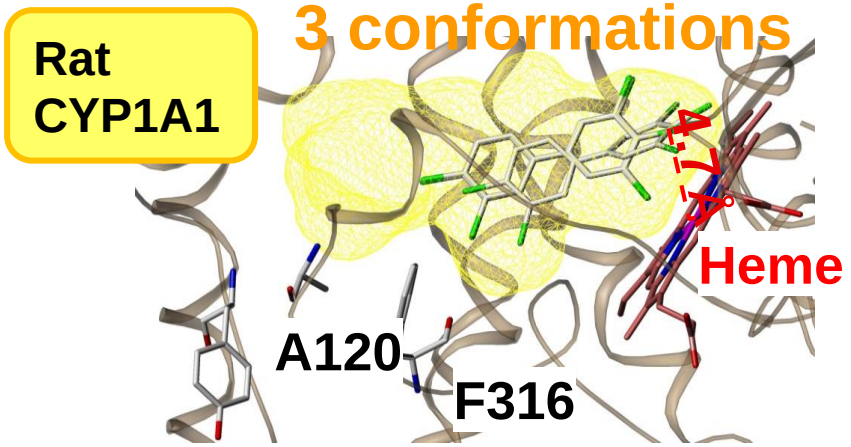
Orientation

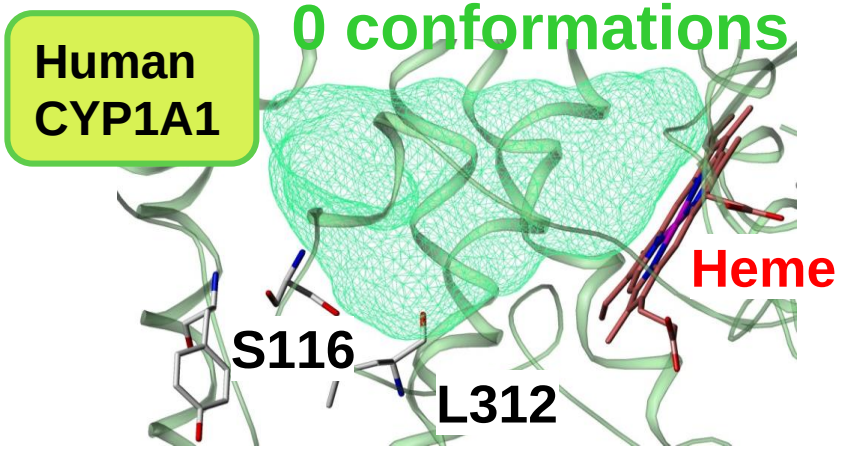




CB126 is more stable in the cavity of rat CYP1A1 than that of human CYP1A1.

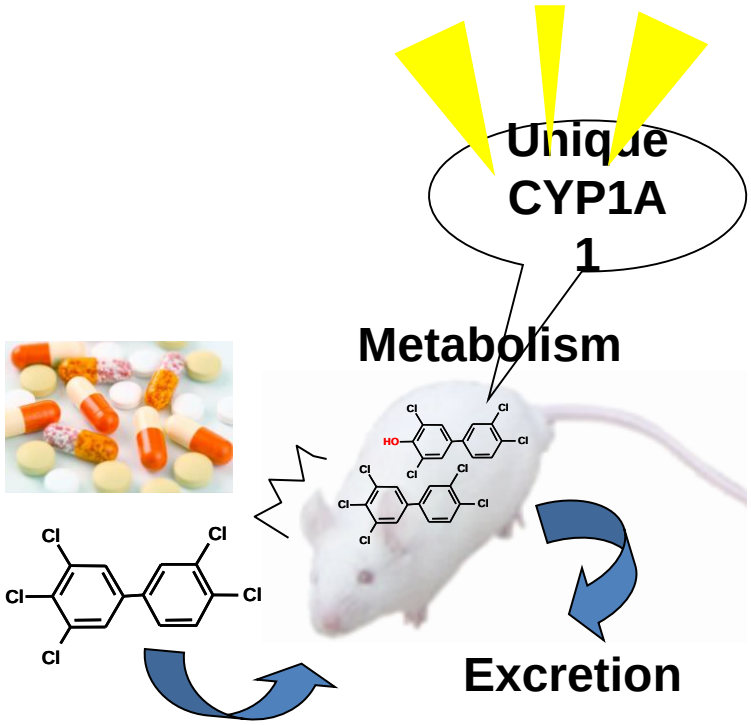
Distance from the iron of the heme （Fe-C4≦5Å）





CB126 is more accessible to the heme of rat CYP1A1 than that of human CYP1A1.

Species differences on metabolism of PCBs between human and rat CYP1A1s



Lower AhR-mediated dioxin toxicity than human

Higher endocrine disruption than human

Extrapolation

Detoxified? Not detoxified?
Safe? or not?

Difficult, or even wrong

The rat is not always a suitable animal for estimating effects in humans, such as dioxin toxicity and endocrine disruption, if they are metabolized by CYP1A1.