In vitro comparative metabolism studies to identify metabolites using microsomes: standards and criteria for acceptability and interpretation

Khaled Abass, Ph.D., ERT

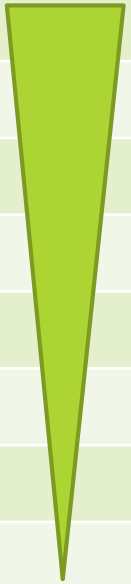
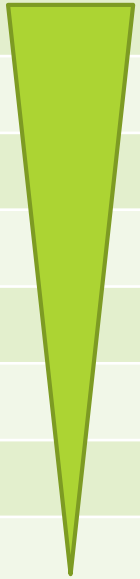
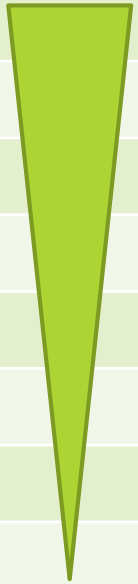
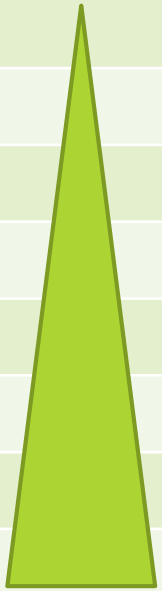
Faculty of Medicine, University of Oulu, Finland

Outlines

- Introduction
- Case studies (comparative qualitative and quantitative metabolism)
- Pesticide-CYP interactions
- Conclusion

Introduction

Models in order of *in vivo* resemblance.

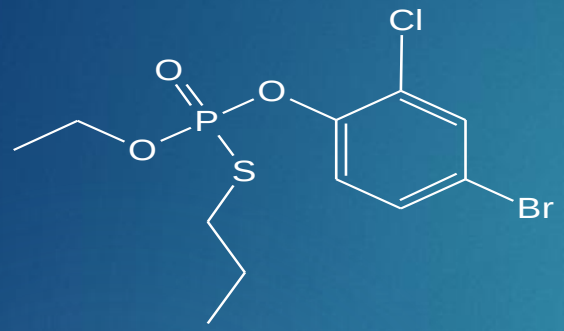
	Complexity	Easy applicable	Ethically acceptable	Resemblance of in vivo
Recombinant enzymes				
Microsomes				
S9 fraction				
Cell lines				
Primary hepatocytes				
Liver slices				
In vivo animal model				
Human				

Enzyme sources	Availability	Advantages	Disadvantages
Microsomes	Relatively good, from transplantations or commercial sources	Major Phase I enzymes. Inexpensive technique. Easy storage. Study of species-specific metabolic profile.	Cellular and organ architecture lost, Cofactor addition necessary, Lack of active uptake and transport

Case studies-qualitative and quantitative comparative metabolism

- Profenofos (Class: Organophosphorothiolate)

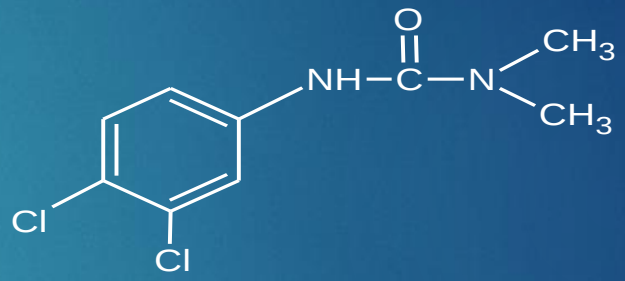
Abass et al. Pestic. Biochem. Physiol. 87 (2007)



- Diuron (Class: Phenyl urea)

Abass et al. Drug Metab. Dispos. 35 (2007)

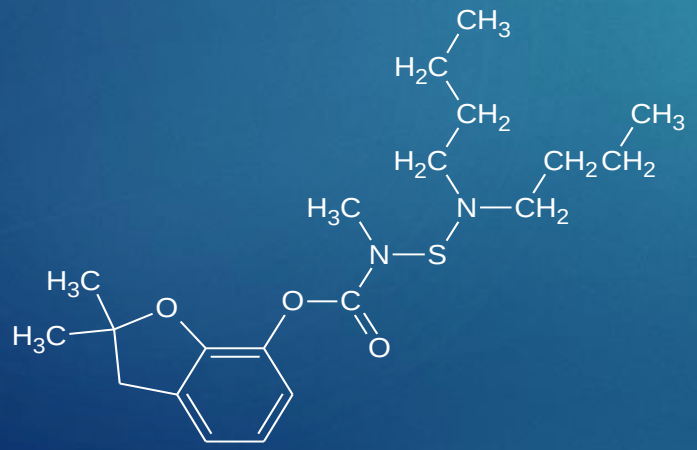
Abass Pestic. Biochem. Physiol. 107 (2013)



- Carbosulfan (Class: Carbamates)

Abass et al. Chem. Biol. Interact. 181 (2009)

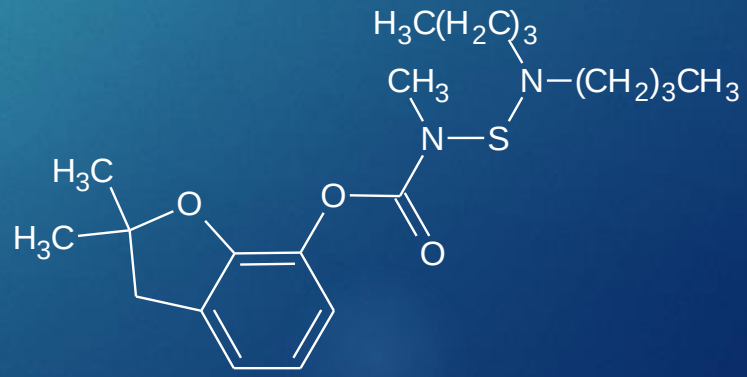
Abass et al. Chem. Biol. Interact. 185 (2010)



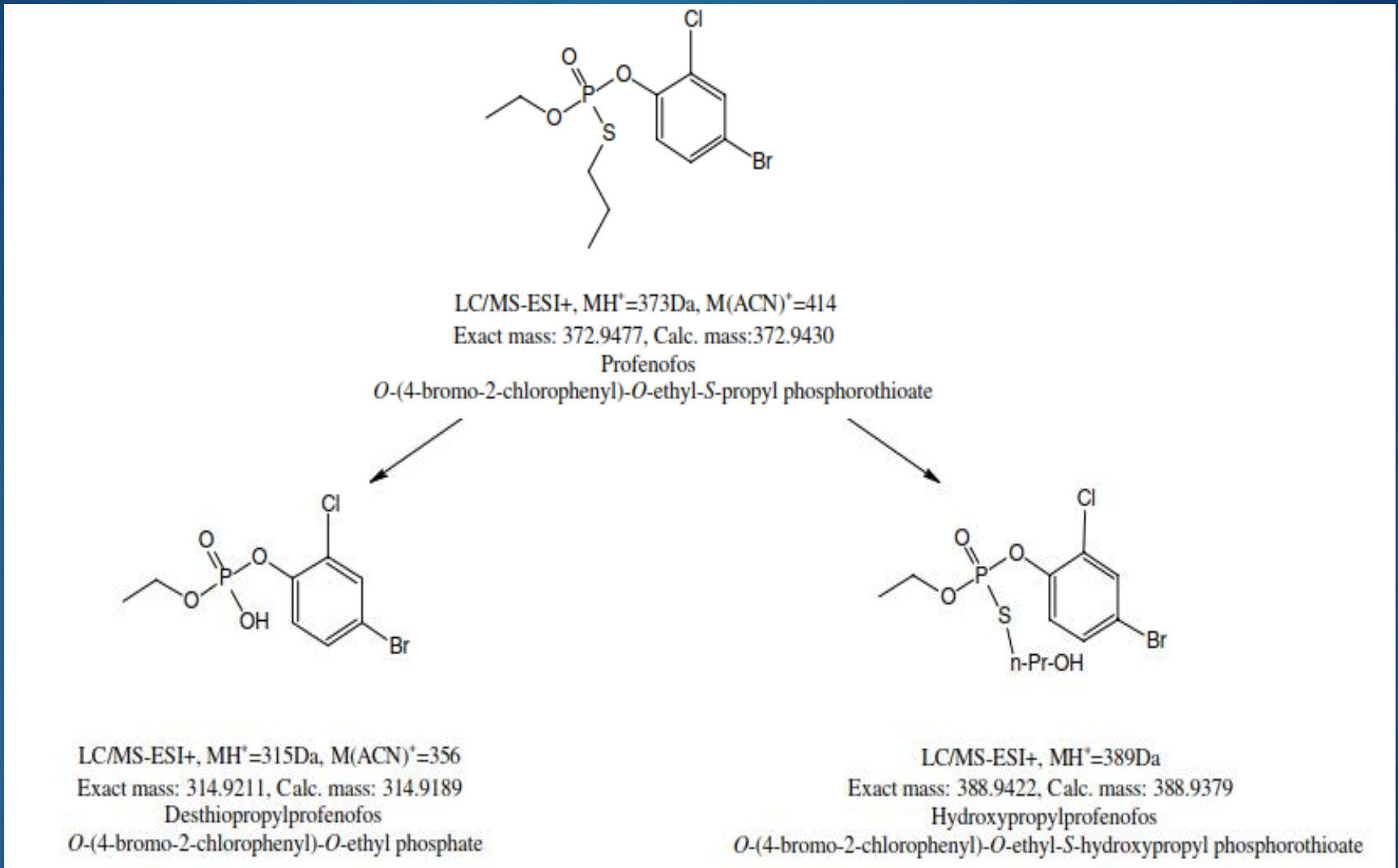
- Benfuracarb (Class: Carbamates)

Abass et al. Toxicology Letters 224 (2014), pp. 209-299

Abass et al. Toxicology Letters 224 (2014), pp. 300-310



1-Profenofos



Overall scheme of profenofos metabolism in human, mouse, and rat hepatic microsomes and exact masses of metabolites

Abass *et al.* Pestic. Biochem. Physiol. 87 (2007)

1-Profenofos

Kinetic parameters for the formation of profenofos metabolites by human, mouse and rat hepatic microsomes

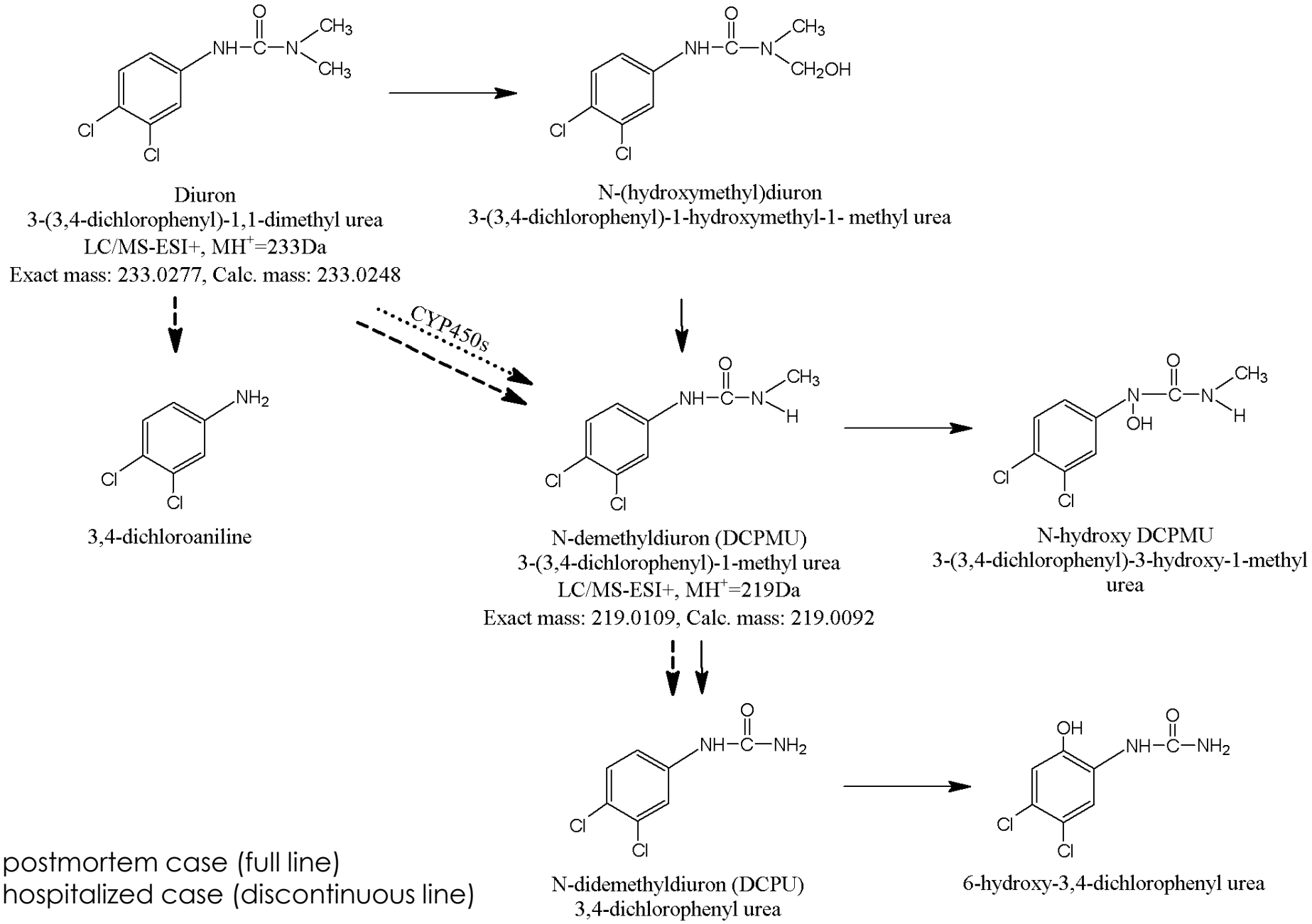
	Metabolites	CL_{int} µl / (mg protein * min)	Bioactivation/ hydroxylation	*In vitro* species differences
HumanLM				
	Desthiopropylation	27.9	82	
	Hydroxylation	0.3		
MouseLM				
	Desthiopropylation	37.5	14	1.3
	Hydroxylation	2.7		9.0
RatLM				
	Desthiopropylation	8.8	5	0.3
	Hydroxylation	1.9		6.3

Interspecies differences represents animal to human fold differences in *in vitro* toxicokinetics

Abass *et al.* Pestic. Biochem. Physiol. 87 (2007)

2-Diuron

The overall scheme of the diuron metabolites detected in postmortem and hospitalized cases. In addition to *in vitro* metabolism by seven mammalian LM.



postmortem case (full line)
hospitalized case (discontinuous line)

Abass *et al.* Drug Metab. Dispos. 35 (2007)
Abass Pestic. Biochem. Physiol. 107 (2013)

2-Diuron

Kinetic parameters of N-demethyldiuron formations obtained with different mammalian liver microsomes

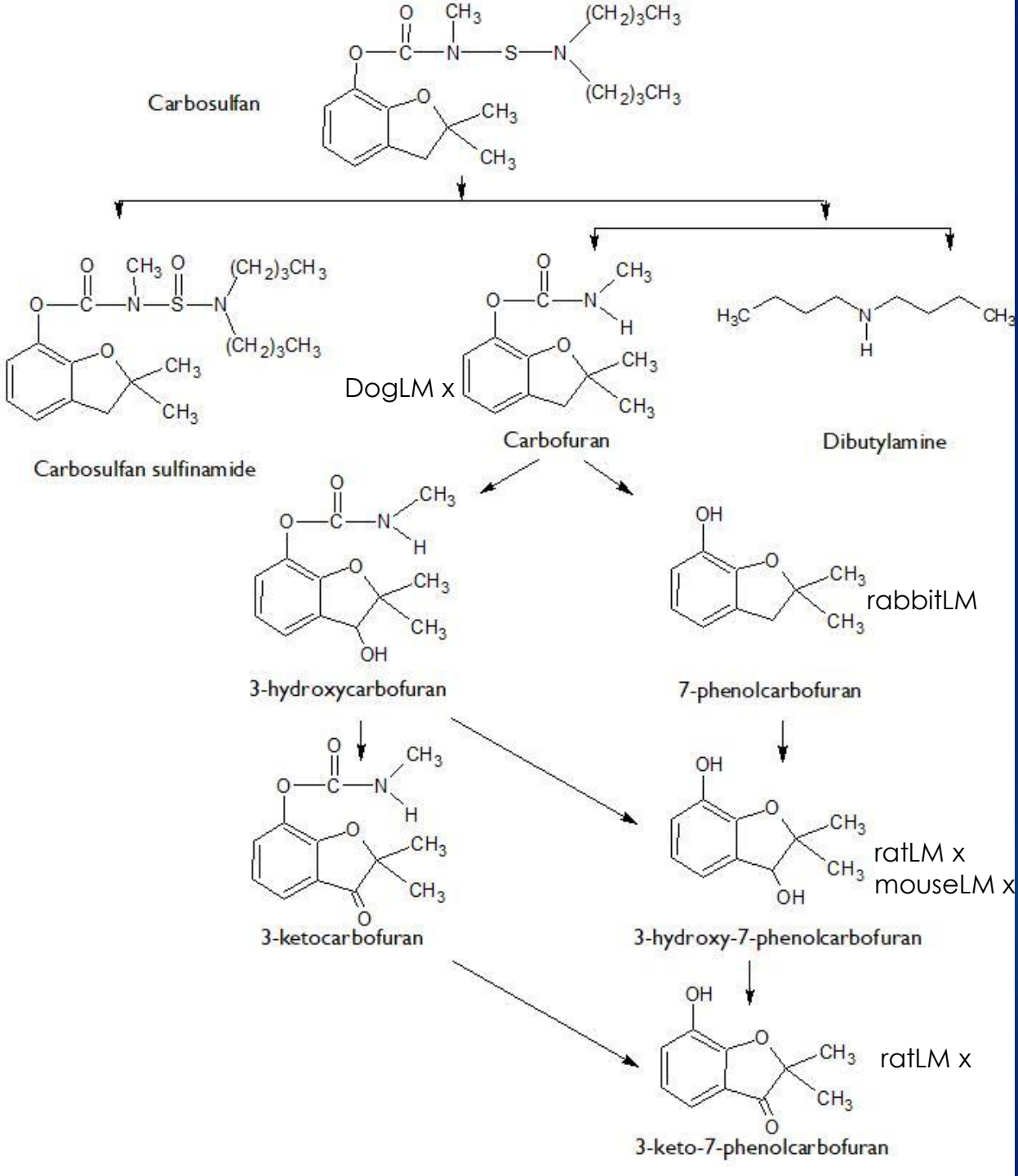
	Cl_{int} µl / (mg protein * min)	*In vitro* species differences
HumanLM	174.2	
RatLM	74.7	0.43
MouseLM	214.0	1.23
DogLM	401.3	2.30
MonkeyLM	327.2	1.88
MiniPigLM	159.7	0.92
RabbitLM	314.1	1.80

Interspecies differences represents animal to human fold differences in *in vitro* toxicokinetics

Abass *et al.* Drug Metab. Dispos. 35 (2007)
Abass Pestic. Biochem. Physiol. 107 (2013)

3-Carbosulfan

The overall *in vitro* scheme of carbosulfan metabolism in mammalian liver microsomes



Abass *et al.* Chem. Biol. Interact. 181 (2009)
Abass *et al.* Chem. Biol. Interact. 185 (2010)

3-Carbosulfan

Kinetic parameters of the carbofuran- metabolic pathway obtained with different mammalian liver microsomes[a]

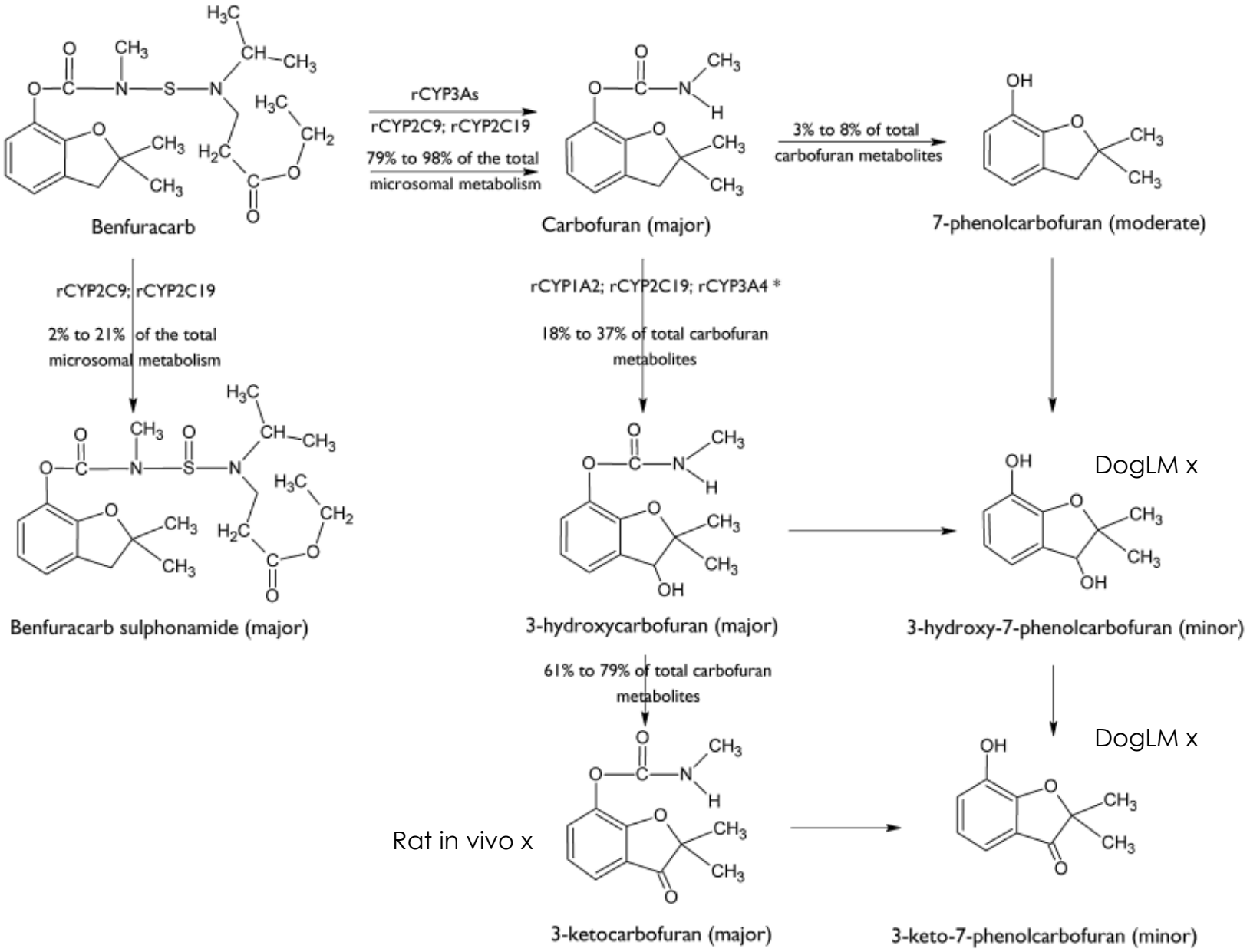
	Cl_{int} µl / (mg protein * min)	*In vitro* species differences
HumanLM	454.9	
RatLM	326.6	0.72
MouseLM	253.2	0.56
DogLM	223.9	0.49
RabbitLM	335.7	0.74
MinipigLM	471.4	1.03
MonkeyLM	450.8	0.99

[a] Sums of all the metabolites of the carbofuran pathway were used for the calculation of kinetic parameters
[b] Interspecies differences represents animal to human fold differences in toxicokinetics

Abass *et al.* Chem. Biol. Interact. 181 (2009)
Abass *et al.* Chem. Biol. Interact. 185 (2010)

4-Benfuracarb

The overall *in vitro* scheme of benfuracarb metabolites detected in mammalian liver microsomes. The percentage of metabolite formation in HLM and the contribution of hrCYP are shown.



Abass *et al.* Toxicology Letters 224 (2014), pp. 209-299

Abass *et al.* Toxicology Letters 224 (2014), pp. 300-310

4-Benfuracarb

Kinetic parameters of the carbofuran- metabolic pathway obtained with different mammalian liver microsomes[a]

	Cl_{int} µl / (mg protein * min)	*In vitro* species differences
HumanLM	99.95	
RatLM	253.7	2.5
MouseLM	255.2	2.5
DogLM	145.8	1.4
RabbitLM	134.7	1.3
MinipigLM	268.9	2.7
MonkeyLM	143.9	1.4

[a] Sums of all the metabolites of the carbofuran pathway were used for the calculation of kinetic parameters
[b] Interspecies differences represents animal to human fold differences in in vitro toxicokinetics

Abass *et al. Toxicology Letters 224 (2014), pp. 209-299*
Abass *et al. Toxicology Letters 224 (2014), pp. 300-310*

Qualitative Differences

	Profenofos	Diuron	Carbosulfan	Benfuracarb
HumanLM	-	- ***In vivo vs in vitro***	-	-
RatLM	-	-	*3-Keto-7-PhCF* ***ND*** *3-OH-7-PhCF* ***ND***	*3-Keto-CF* ***ND in vivo***
MouseLM	-	-	*3-OH-7-PhCF* ***ND***	-
DogLM		-	Carbofuran ***ND***	*3-Keto-7-PhCF* ***ND*** *3-OH-7-PhCF* ***ND***
RabbitLM		-	*7-PhCF* ***specific metab.***	-
MinipigLM		-	-	-
MonkeyLM		-	-	-

Quantitative Differences

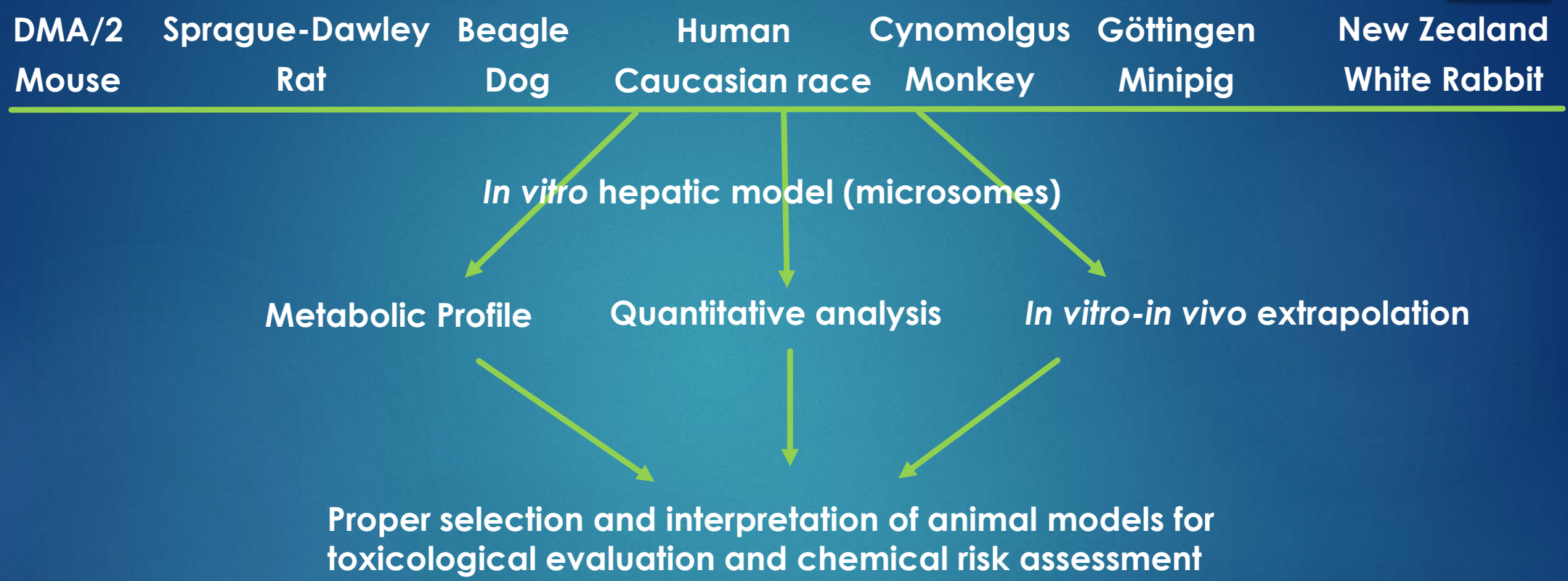
in vitro and *in vivo* extrapolated values for animal to human differences (fold) in toxicokinetic for the active chemical moieties

	Profenofos		Diuron		Carbosulfan		Benfuracarb	
	CL_{int}	CL_H	CL_{int}	CL_H	CL_{int}	CL_H	CL_{int}	CL_H
HumanLM								
RatLM	1.3	**3.3**	0.43	**2.3**	0.72	**3.1**	2.5	**4.1**
MouseLM	0.3	**4.1**	1.23	**3.9**	0.56	**3.5**	2.5	**4.8**
DogLM			2.30	**2.2**	0.49	**1.7**	1.4	**2.1**
RabbitLM			1.88	**2.3**	0.74	**2.0**	1.3	**2.3**
MinipigLM			0.92	**1.7**	1.03	**1.9**	2.7	**2.4**
MonkeyLM			1.80	**2.7**	0.99	**2.5**	1.4	**2.3**

Animal to human quantitative differences (fold) in toxicokinetic for the active chemical moieties
The Extrapolated CL_H in animals were divided by the CL_H in humans

Abass Pestic. Biochem. Physiol. 107 (2013)

Comparative in vitro metabolism studies



Each pesticide is ''an individual'' with its own characteristics regarding toxicokinetics and metabolic pathway

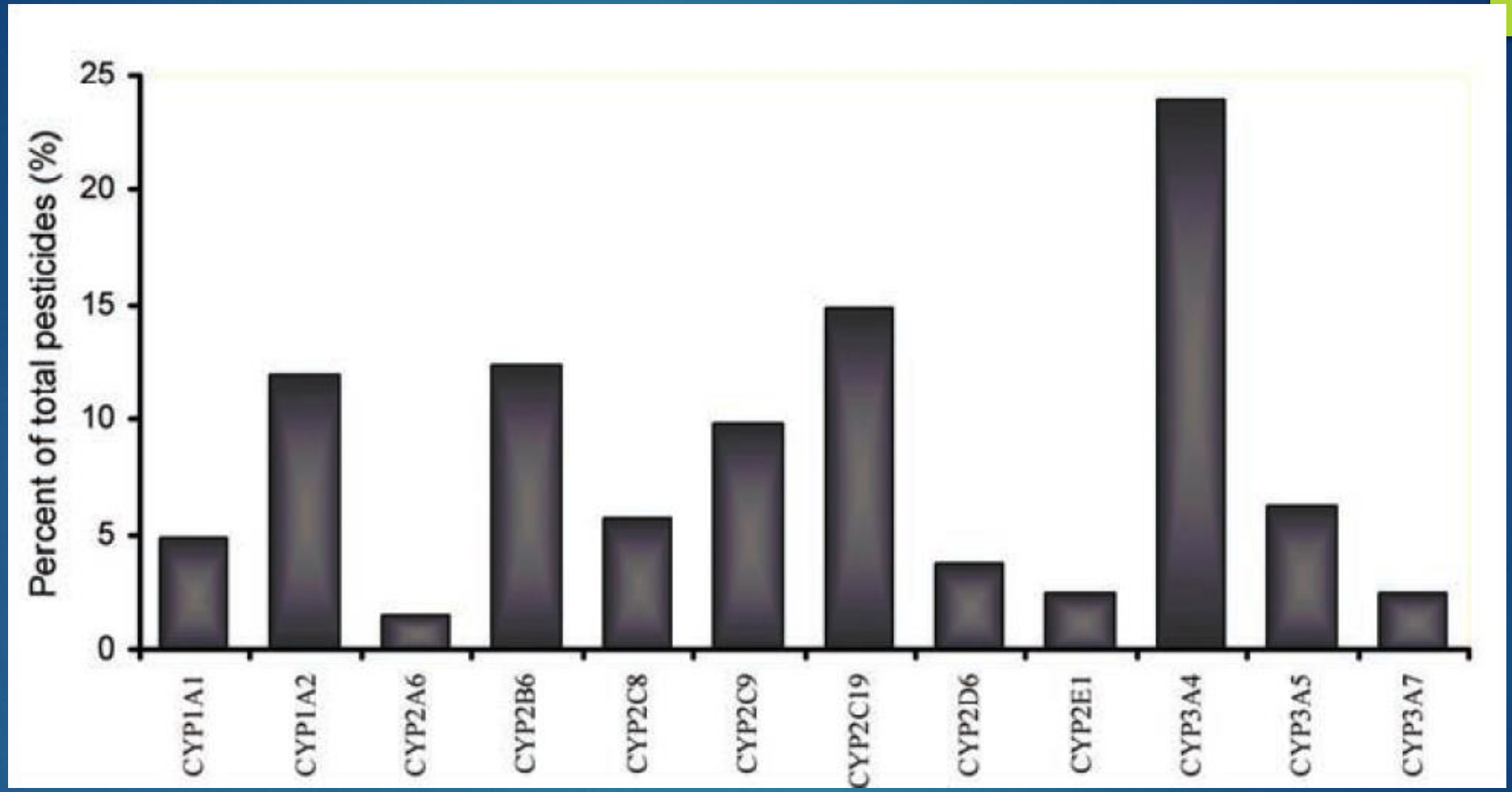
Abass *et al. Toxicology Letters 224 (2014), pp. 209-299*

Comparative in vitro metabolism studies

Limitations

- Results obtained from *in vitro* test systems are highly dependent on several technical factors
- The prediction of *in vivo* metabolic clearance is based on many scaling factors and physiological measures, which should either be assumed or measured.
- The best predictive value is usually obtained when the substrate concentration used is within the linear part of the time and protein concentration curves for substrate depletion or metabolite formation

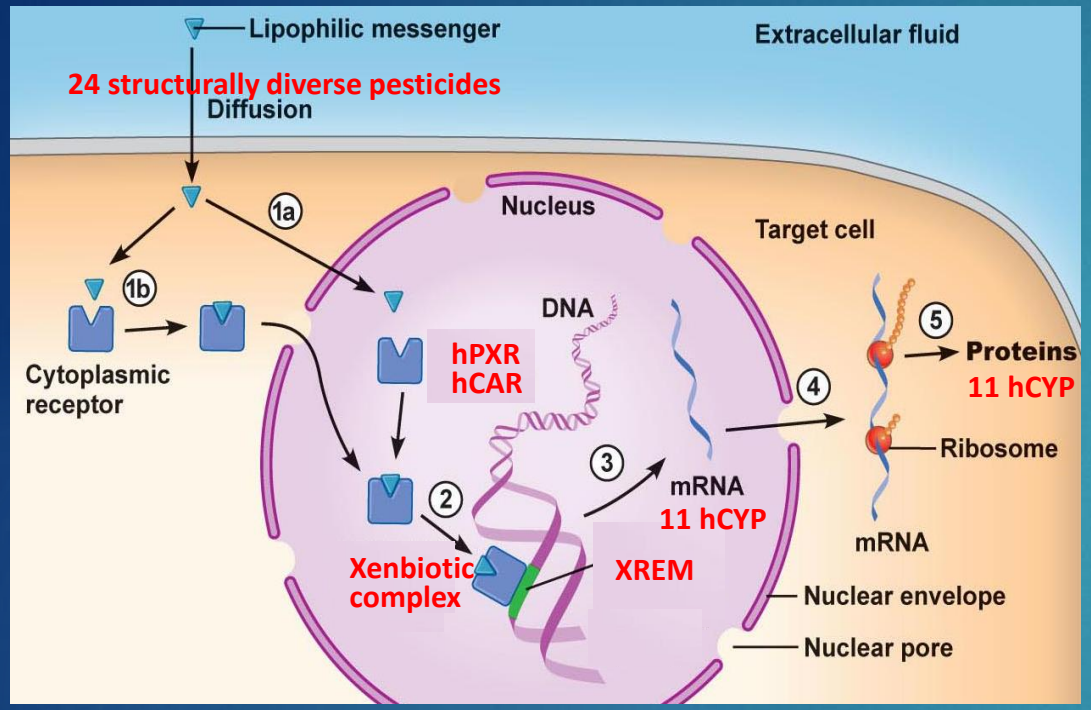
Pesticides-CYP Intercations



The percentage of hrCYPs involved in pesticides metabolism. 63 compounds (36 insecticides; 14 fungicides; 10 herbicides; 2 plant growth regulators and a biocide agent) were metabolized at least in part by one or more hrCYP yielded 495 metabolic reactions (restricted to 2011 survey).

Abass et al. 2012, Insecticides, ISBN 979-953-307-667-5, pp. 165-194.

Pesticide-CYP Intercations

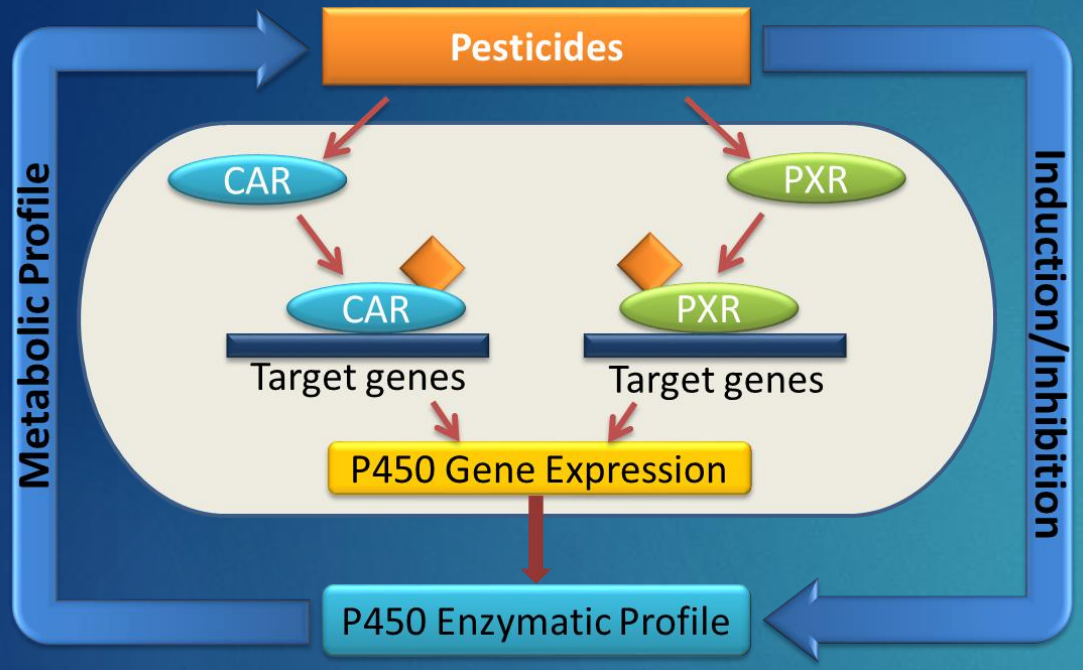


CYP mRNA levels in human HepaRG after 24 h exposure to tested pesticides.

mRNA		10 µM /fold induction	50 µM /fold induction
CYP1A2	**TCDD**	218 (10 nM)	
	Diuron	9	100
CYP2B6	**Phenobarbital**	6 (500 µM)	
	Isoproturon	20	22
	Atrazine	11	13
CYP3A4	**Rifampicin**	20	19
	Cypermethrin	6	35
	Fenvalerate	17	22
	Cyhalothrin	13	28

Abass *et al.* 2012, Toxicology. 294: 17–26
Abass *et al.* 2013 Toxicol In Vitro. 27(5) 1584-8

Pesticide-CYP Intercations



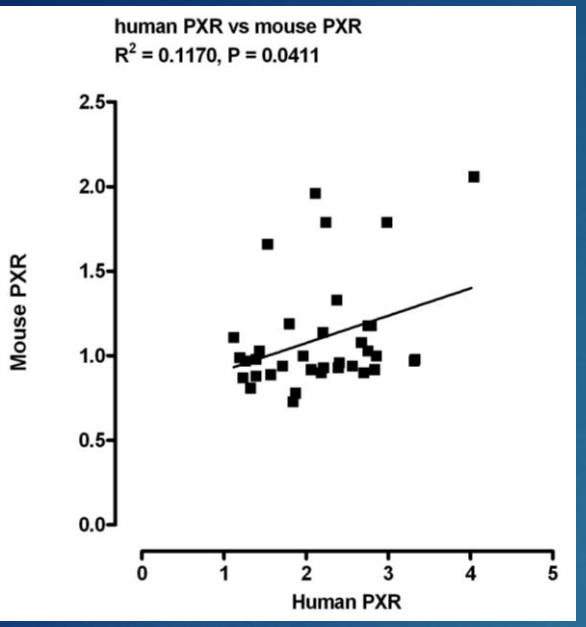
Pesticides can induce/inhibit the CYPs involved in their own metabolism

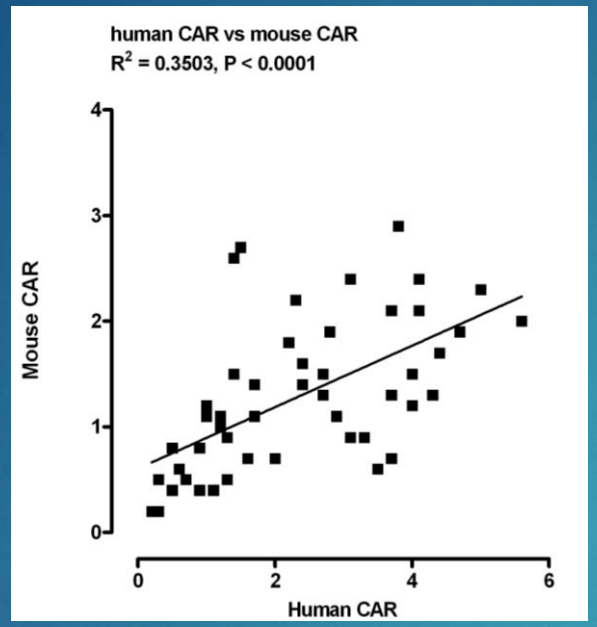
CYP mRNA levels in human HepaRG after 24 h exposure to tested pesticides.

mRNA		10 µM /fold induction	50 µM /fold induction
CYP1A2	**TCDD**	218 (10 nM) (20-fold Mela-OH)	
	Diuron	9	100-fold (IC50 =3 µM)
CYP2B6	**Phenobarbital**	6 (500 µM) (3-fold Bup-OH)	
	Isoproturon	20 (7-fold Bup-OH)	22 (7-fold Bup-OH)
	Atrazine	11 (3-fold Bup-OH)	13 (4-fold Bup-OH)
CYP3A4	**Rifampicin**	20 (10-fold Tes-6OH)	19 (10-fold Tes-6OH)
	Cypermethrin	6	35
	Fenvalerate	17 (3-fold Tes-6OH)	22 (10-fold Tes-6OH)
	Cyhalothrin	13 (5-fold Tes-6OH)	28 (8-fold Tes-6OH)

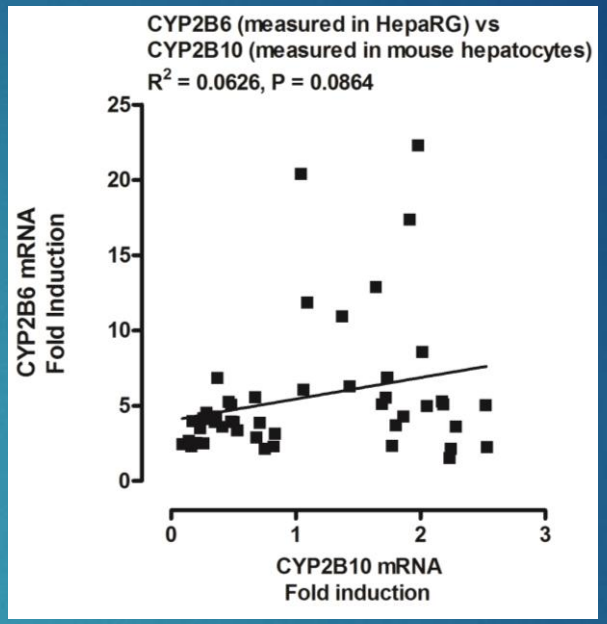
Abass *et al.* 2012, Toxicology. 294: 17–26
Abass *et al.* 2013 Toxicol In Vitro. 27(5) 1584-8

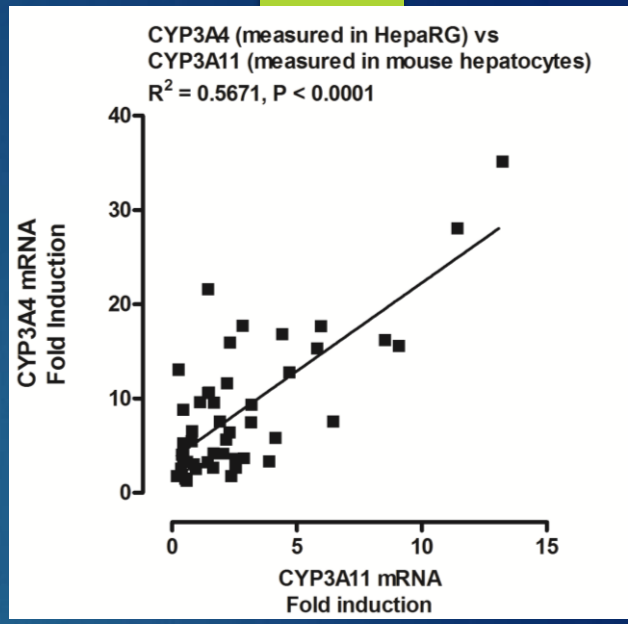
Pesticide-CYP Intercations-Species differences





Nuclear receptor activity was measured by luciferase reporter gene





CYP-mRNA induction in HepaRG and in mouse primary hepatocytes

Comparison of the mouse and human CAR/PXR and CYP2B/CYP3A mRNA induction by 24 structurally diverse pesticides

The observed differences emphasize the importance of using human-based cellular screening models in comparative metabolism studies

Abass *et al.* 2012, Toxicology. 294: 17–26

Conclusion

- *in vitro* screening of metabolite profiles and toxicokinetics are desirable for the proper selection of animal models for toxicological evaluation
  - Rate of metabolism
  - Spectrum of metabolites produced
  - Intrinsic clearance

- to include interactions at different biological levels and to maximize the chance of having all possible metabolites, Living cells, if metabolically competent, would give metabolite patterns closer to *in vivo* situation than tissue fractions.

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Thank you

Arctic Health; University of Oulu, Finland