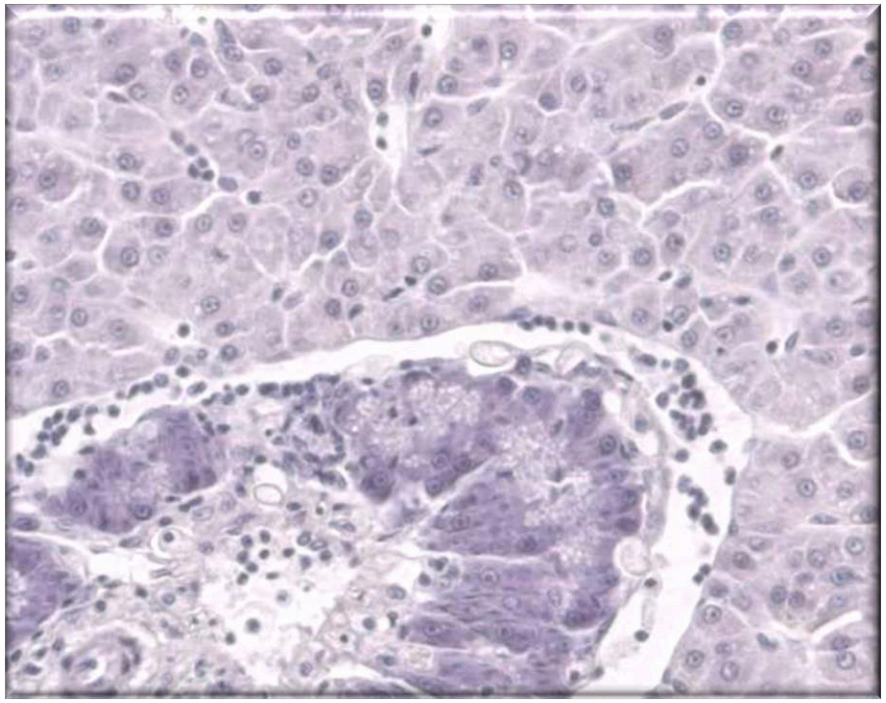
A stem cell based metabolic activation system: Potential for replacing rat liver S9 fraction

J. Müller, C. F. Lerche, T. Allner, J. Jeckel, B. Allner, P. Stahlschmidt-Allner

UNIKLINIK KÖLN

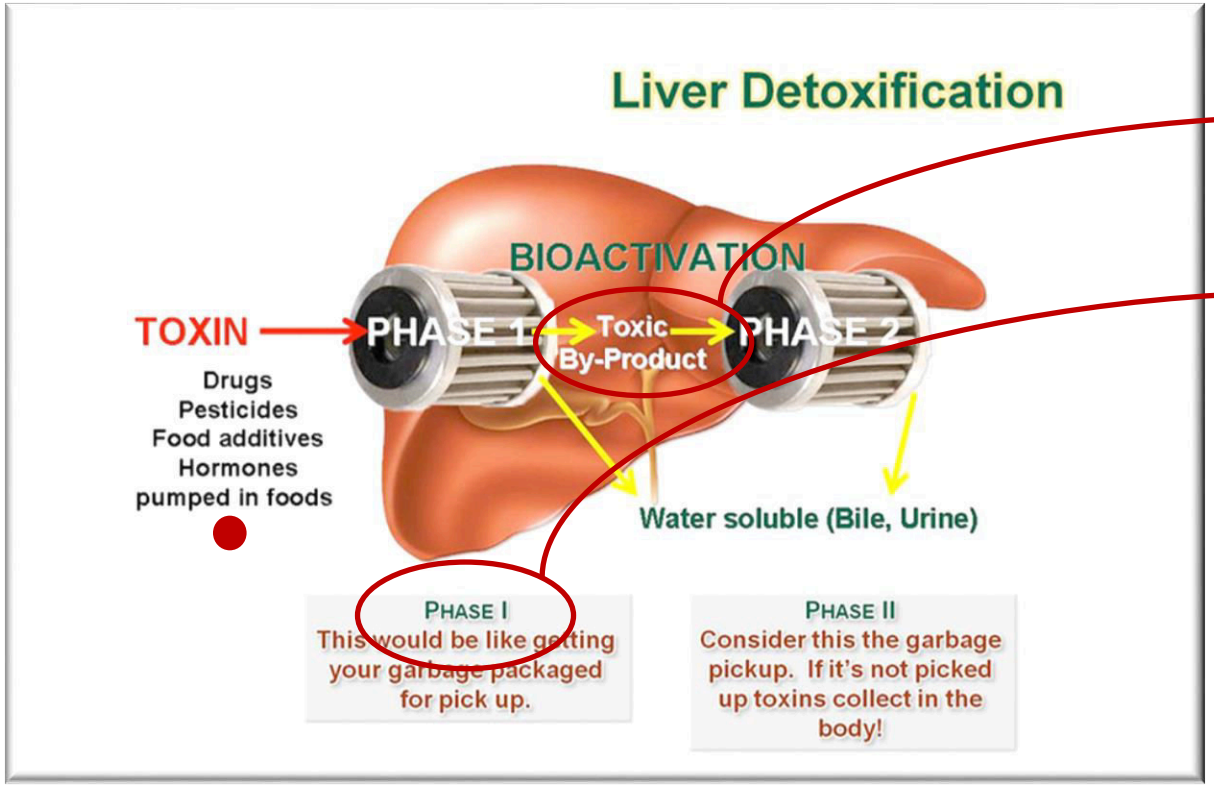


TECOmedical Group

GOBIO

Drug Metabolism

Xenobiotics → ✖ → *in vitro* systems



http://drjockers.com/complete-liver-health-analysis

Active metabolites
(e.g. activation of promutagens)

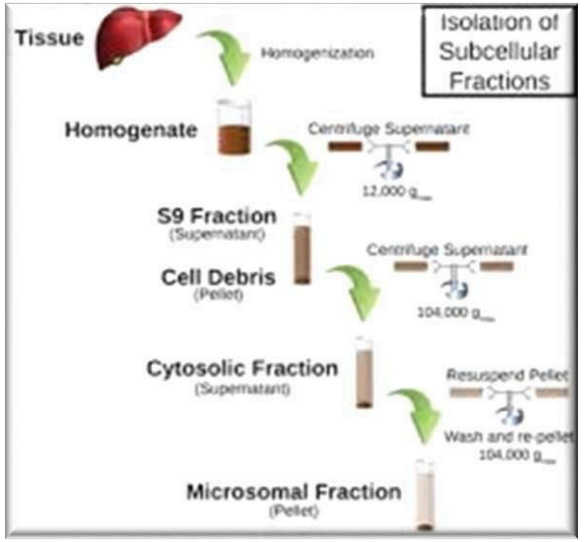
Phase I: Modification
(addition of polar / reactive groups)

- Oxidation / Reduction
- Hydrolysis
- (De) Cyclization

} Cytochrome P450 superfamily

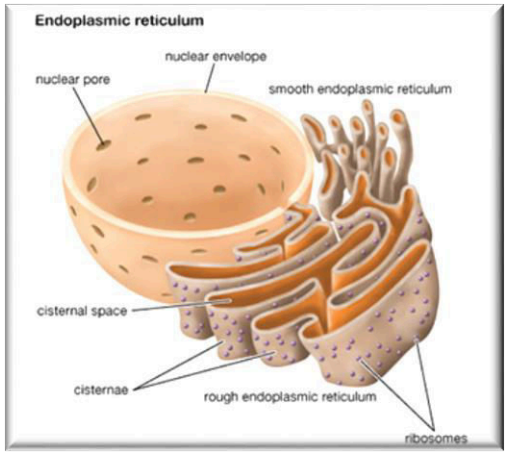
Phase II: Conjugation
e.g. glutathione S-transferase, N-acetyltransferases

Rat Liver S9 fraction



https://www.xenotech.com

Purified microsomes
(i.e. heterologous expression)



http://www.medicallibraryonline.com

Poisoning of animals

for performing *in vitro* tests!

Technical expertise

State-of-the-art infrastructure

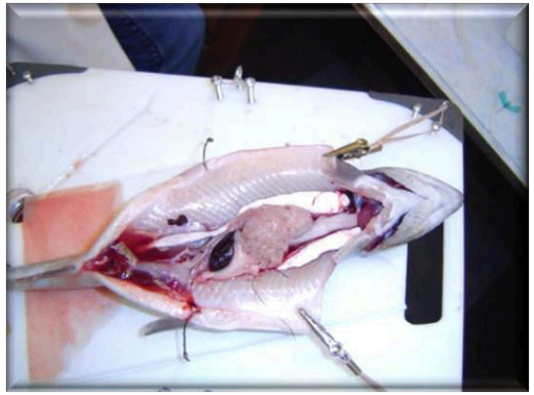
= $$$

Fish S9 – “Phylogenetic Refinement”

Johanning *et al.* 2012. Current Protocols in Toxicology, 53.

Assessing metabolic stability / estimating *in vivo* intrinsic clearance

Rainbow trout (*Oncorhynchus mykiss*)



http://onlinelibrary.wiley.com/doi/10.1002/0471140856.tx1410s53/pdf

No poisoning!

Fish S9 fractions
(e.g. rainbow trout TRS9PL subcellular fraction, ThermoFisher Scientific)

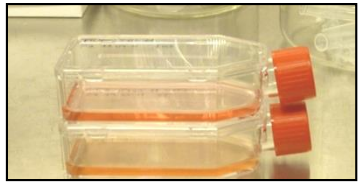
Not for diagnostics

KCB cell line

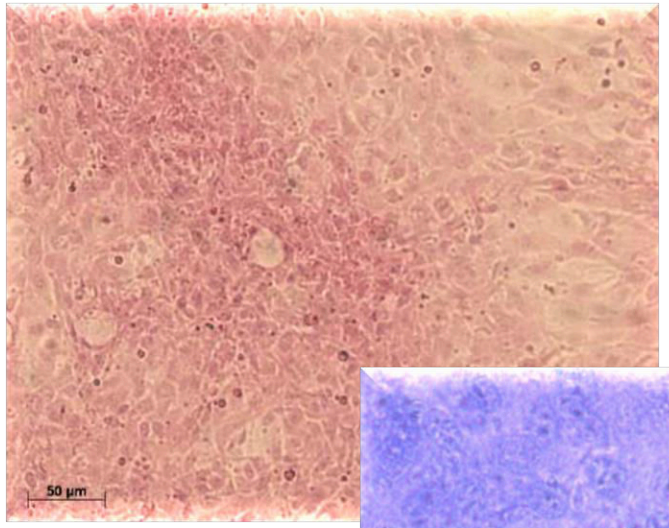
Adult gonadal reconstruction

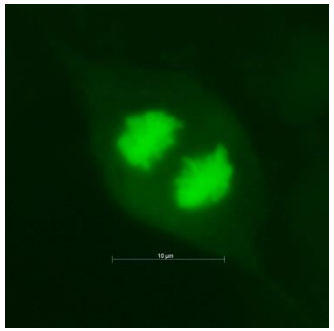
Livelong available PSCs

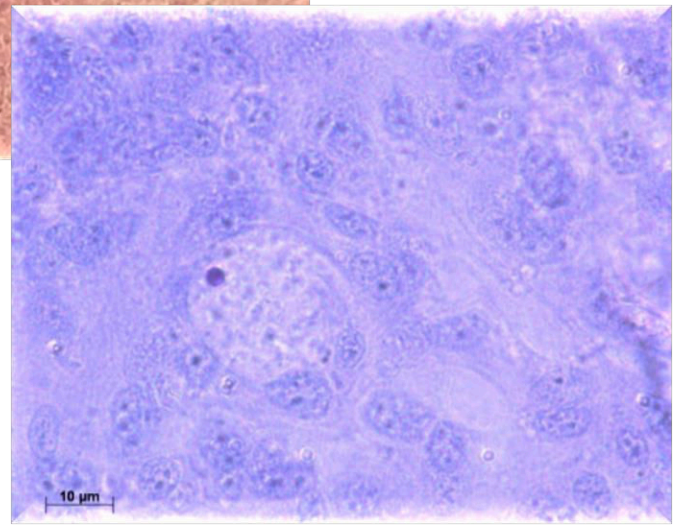
http://dnr.maryland.gov/fisheries/fishfacts/commoncarp.asp

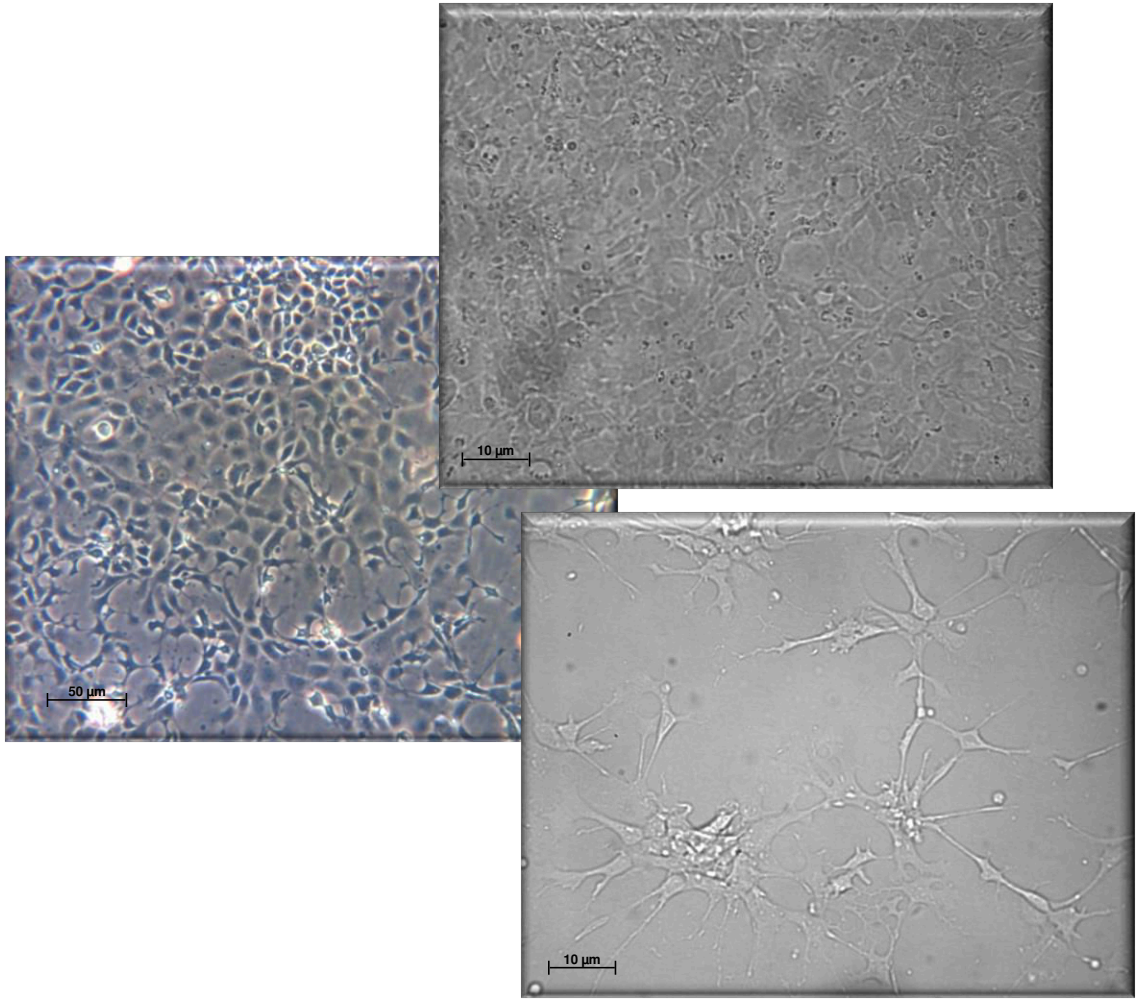


KCB cell line

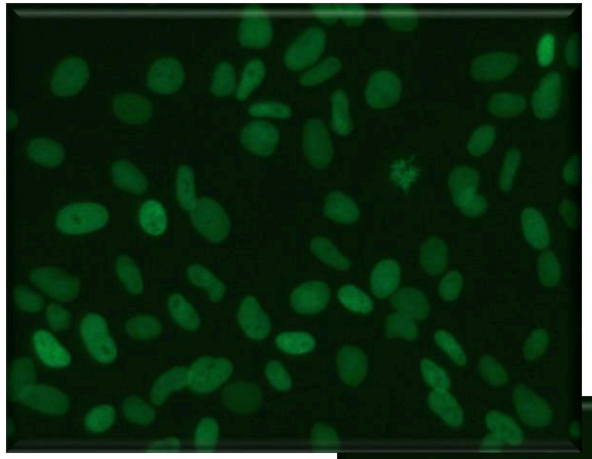


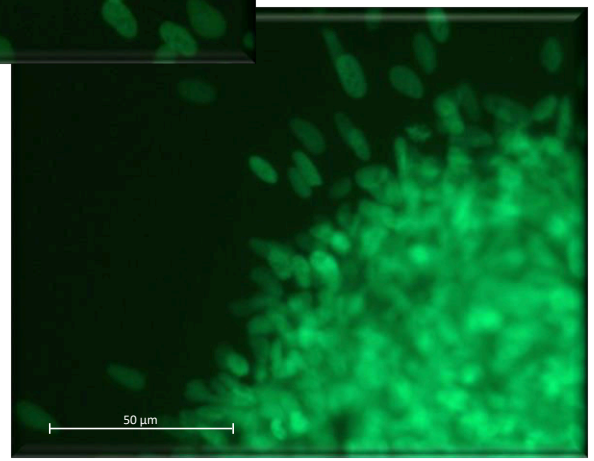


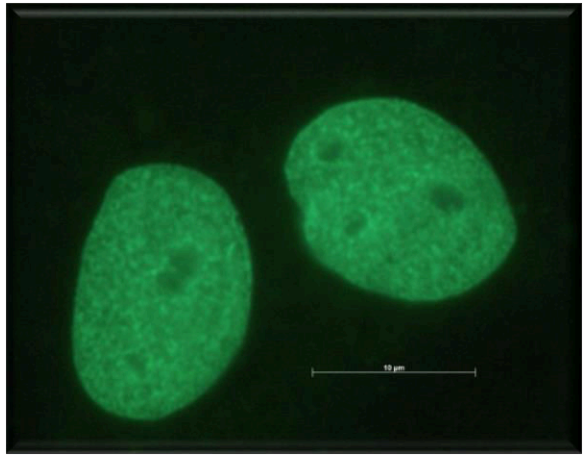


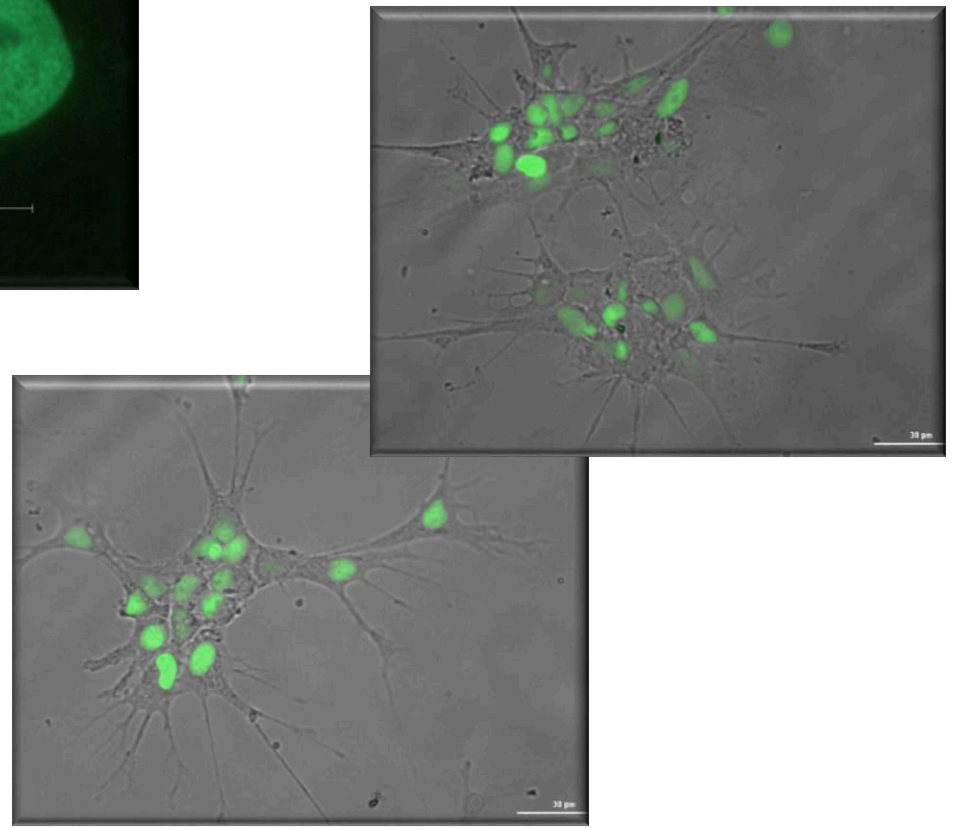


H2B-GFP labelled nuclei









Expression of genes in KCB cells

Genes for "undifferentiated" cells

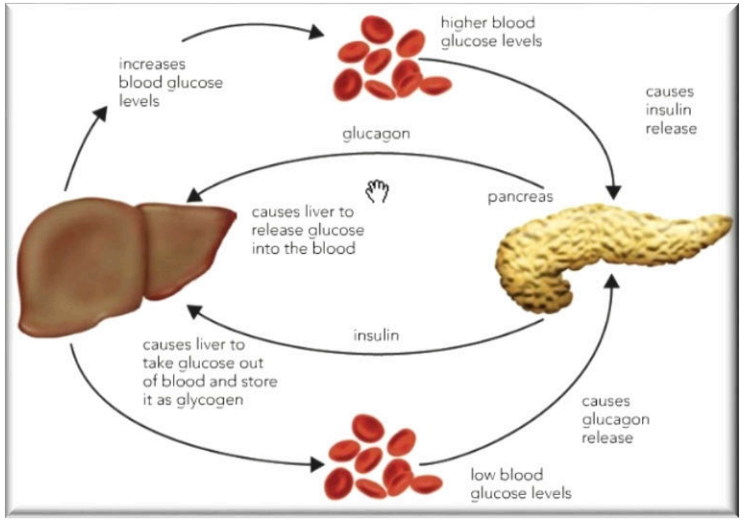
Confirmed expression: *pou2* (Oct3/4), *sox2* (Sox2), *c-myc* (c-Myc)
Under investigation: *prom1* (CD133), *nanog* (NANOG), *klf4* (KLF4),

Estrogen pathway

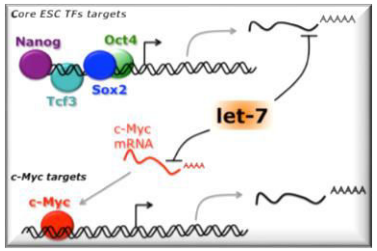
Spontaneous expression: *cyp19a1b* (AroB), *esr2* (ER-ß), *vtg* (VTG)
Induced expression: *esr1* (ER-α), *gper* (GPER),

Cytotoxicity

Spontaneous expression: *bax* (BAX)
Induced apoptosis / necrosis: *tnfa* (TNFα), *casp3* (CASP3), *casp8* (CASP8)



http://biology-igcse.weebly.com



Rosa & Brivanlou, 2013. Int. J. Mol. Sci., 14.

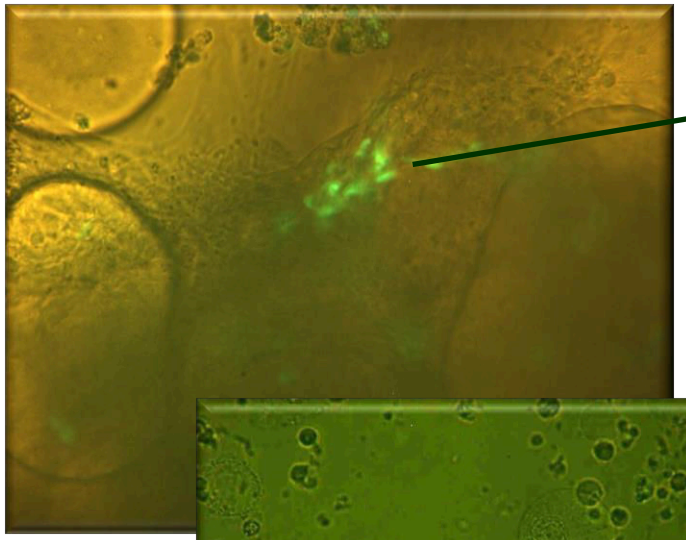
Expression of hepatic / pancreatic hormones and enzymes

Insulin-like growth factor 1

Insulin (Preproinsulin)

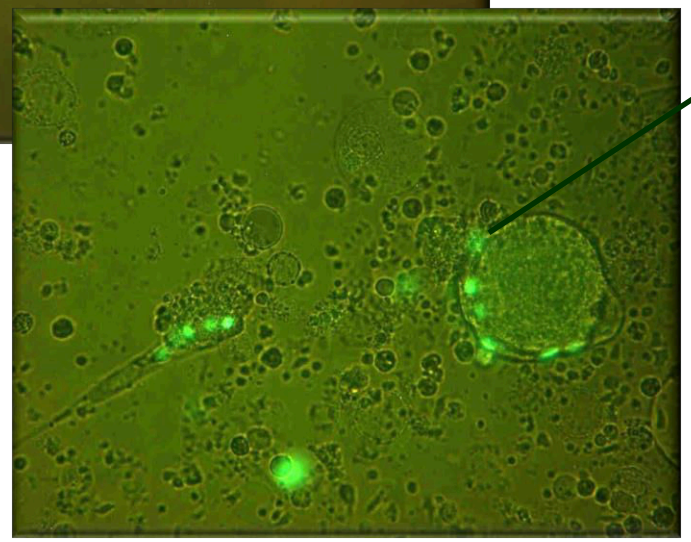
Cytochrome P450 3A

Co-cultivation

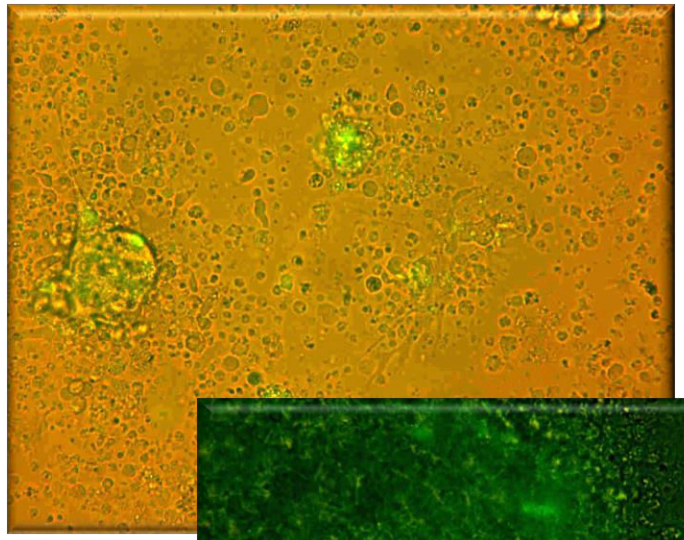


Stroma?

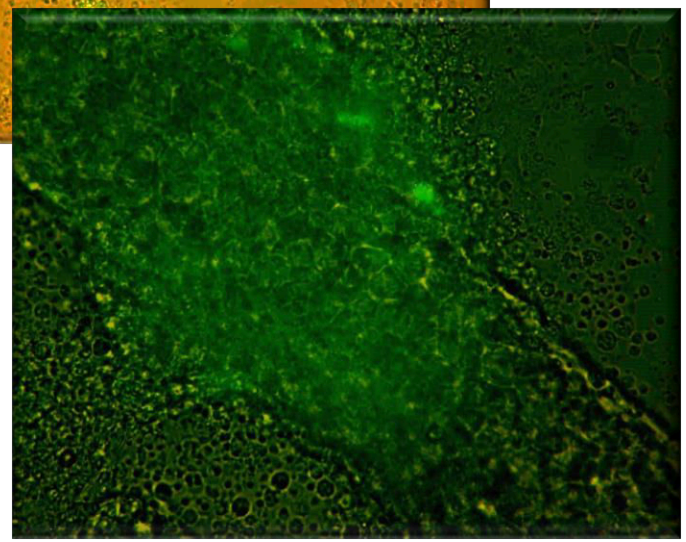
Follicle cells?



Ovary



Kidney

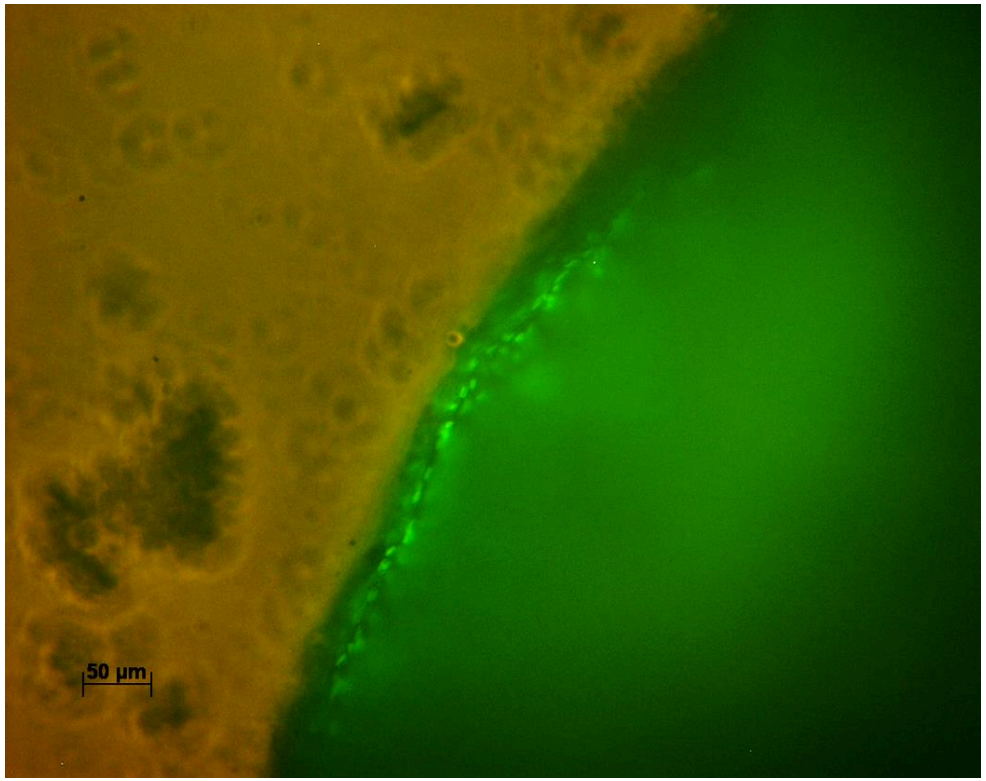


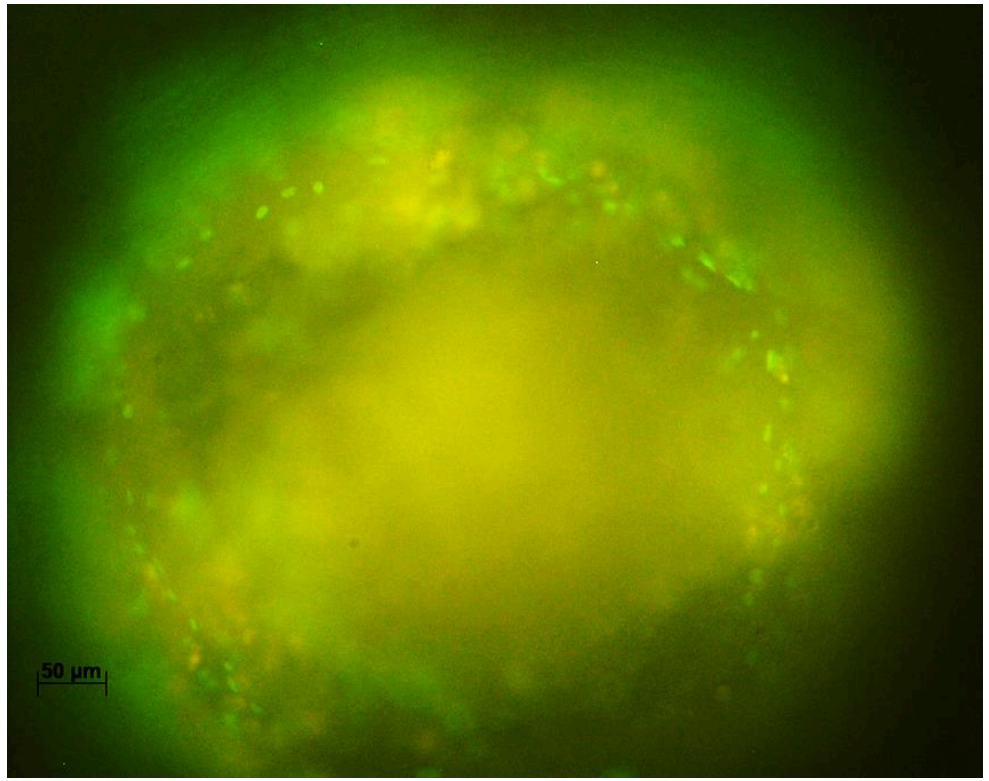
Gills

Integration in tissues

Embedding of primary cells

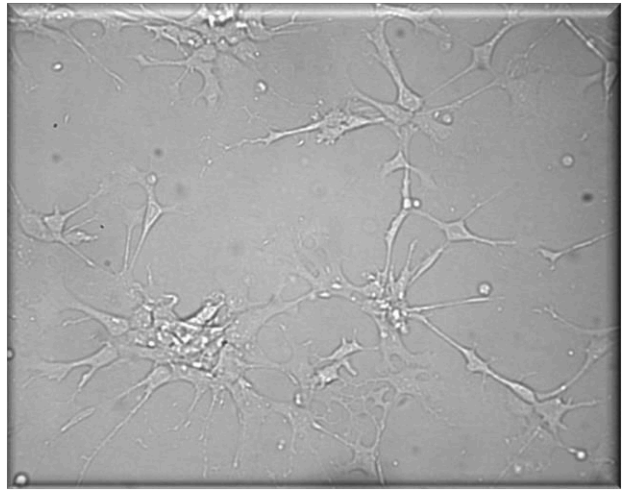
KCB / liver co-cultures

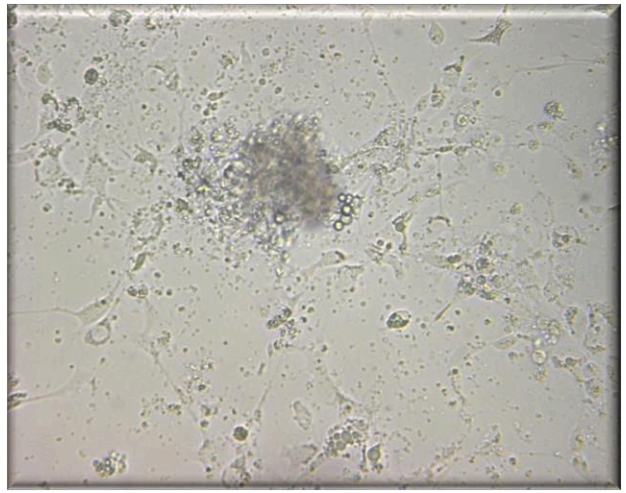


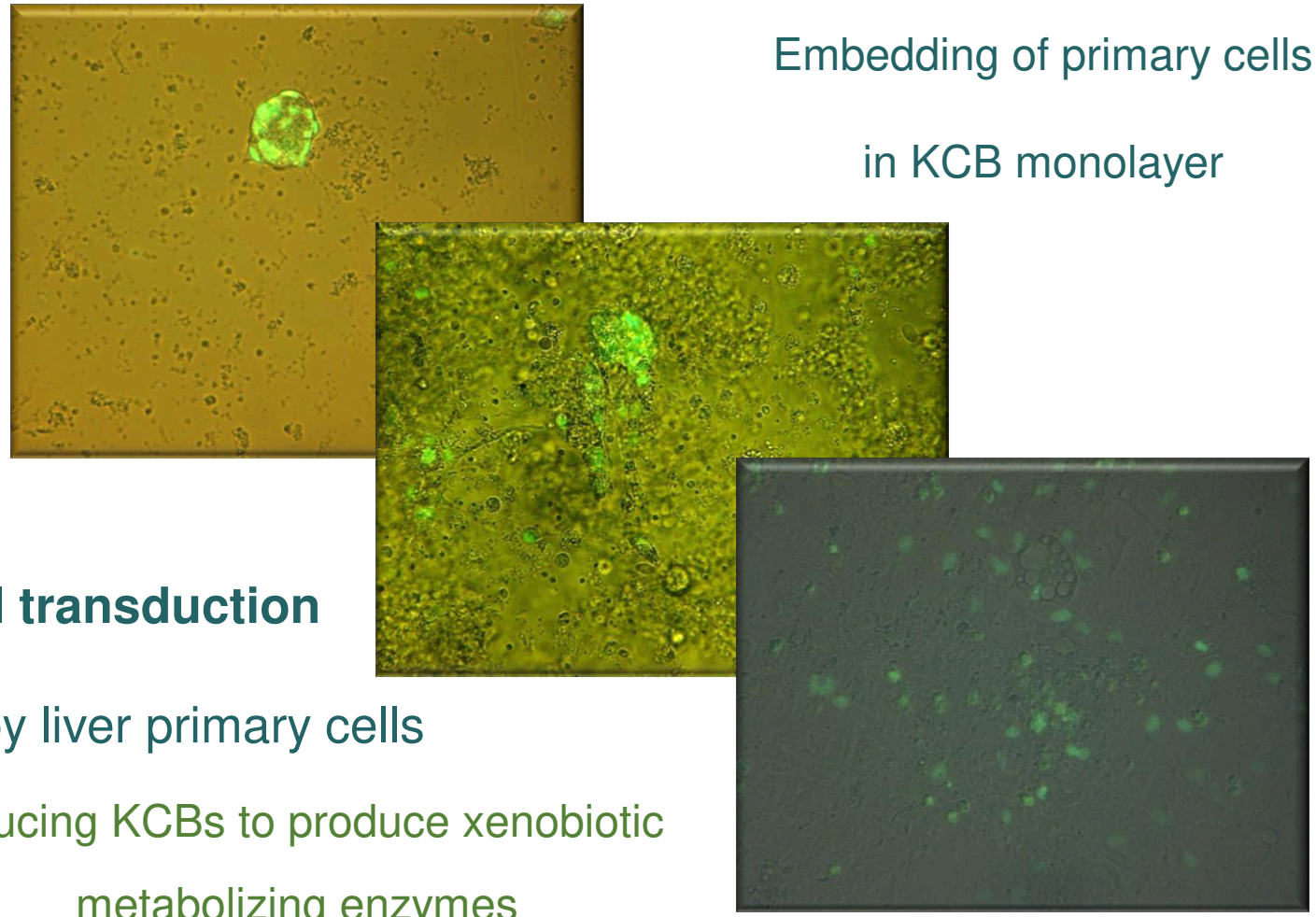


KCBs incorporated in liver fragments

KCB / liver co-cultures







Embedding of primary cells

in KCB monolayer

Signal transduction

triggered by liver primary cells

Inducing KCBs to produce xenobiotic

metabolizing enzymes

Metabolic induction assessment

Signal transduction in liver co-cultures:

Induction of CYP450 enzymes?

Paracrine?

Other induction mechanisms:

Liver homogenates

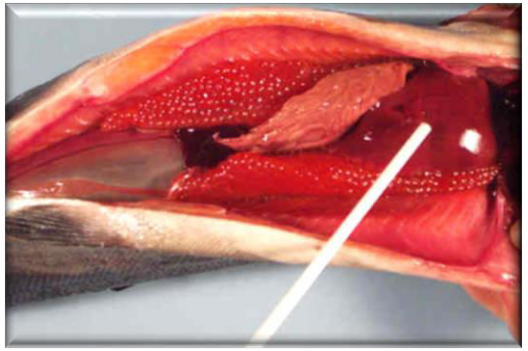
Specific transcription factors

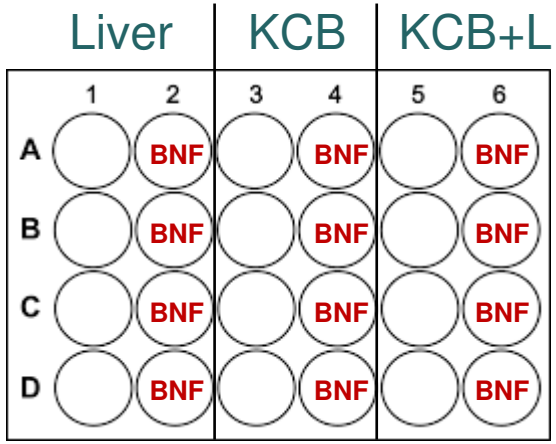
Cell differentiation protocols

Gene expression (RT-qPCR)

Enzymatic activity (EROD assay)

Biotransformation of 2 aminoanthracene (Umu Chromotest)



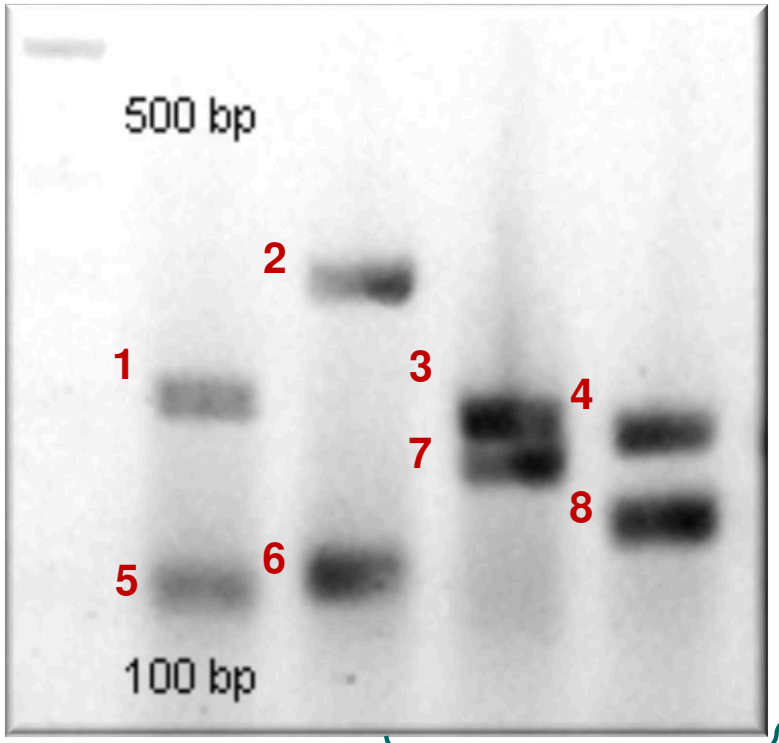


ß-naphtoflavone + Phenobarbital

Aroclor 1254

Endpoint PCR

Liver



1. CYP450 1b2 – 204 bp (AY437775.1)
2. CYP450 1c1 – 301 bp (AY437776.1)
3. Glutathione S-transferase α – 191 bp (DQ411310.1)
4. CYP450 1b – 178 bp (AB048942.2)
5. Microsomal glutathione S-transferase 1 – 98 bp (DQ411316.1)
6. CYP2K – 106 bp (GU046697.1)
7. CYP450 3a – 163 bp (GU046696.1)
8. CYP450 1a – 126 bp (AB048939.1)

- Only *cyp3a* spontaneously expressed in KCBs
- Expression of all genes in KCB / liver co-cultures

Semi-quantitative real time PCR

lifescience.roche.com

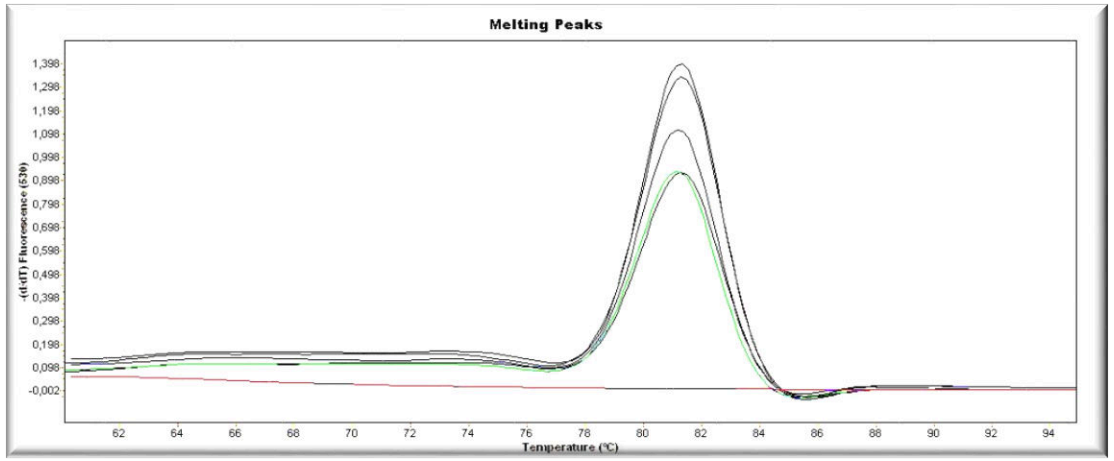
Normalization:

elf1a

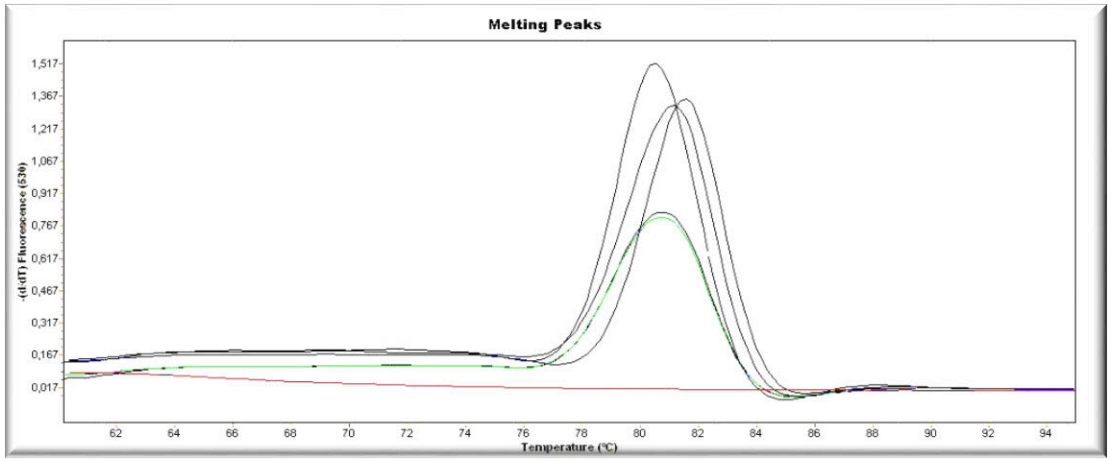
rps4

Gene	TmB (OligoCalc)	TmQ (qPCR)	ΔT%
cyp1a	80,7	81,34	0,79
cyp1b	81,6	81,88	0,34
cyp1b2	85,1	84,85	0,29
cyp1c1	83,4	83,5	0,12
cyp3a	81,9	80,81	1,33
cyp2k	78,7	78,51	0,24
mgst1	78,5	78,78	0,36
gsta	82,2	82,37	0,21

* http://biotools.nubic.northwestern.edu/OligoCalc.html



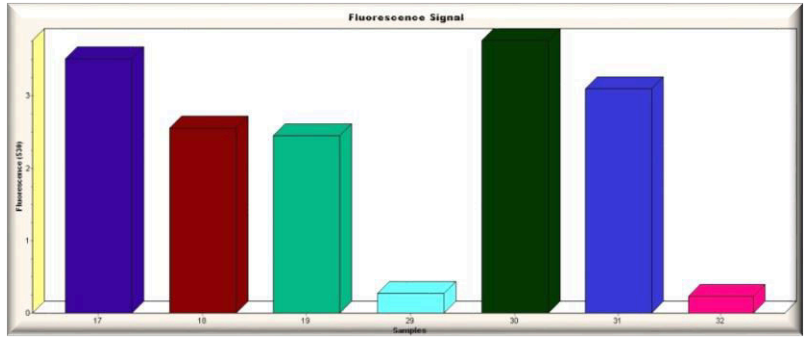
CYP450 1a



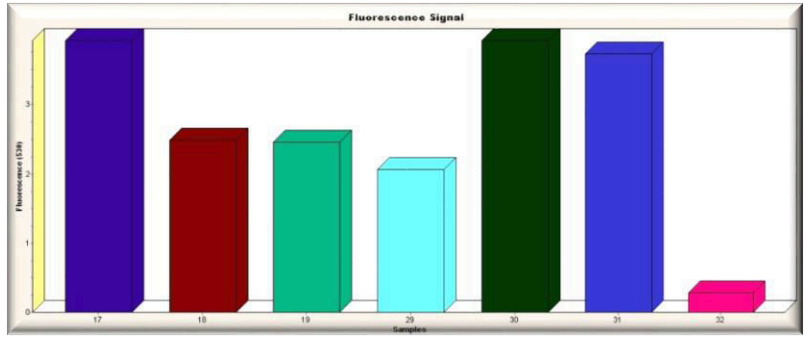
CYP450 3a

Semi-quantitative real time PCR

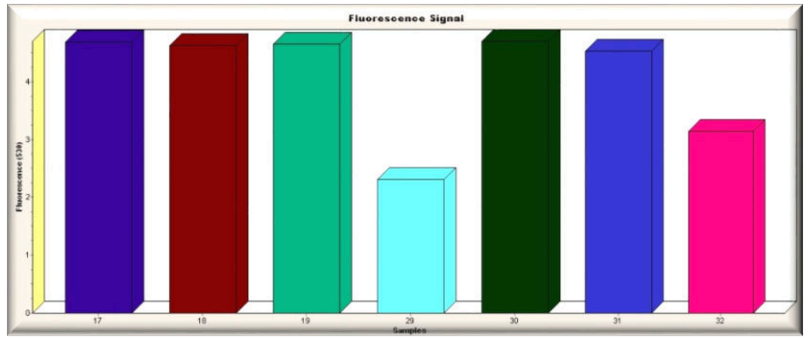
CYP450 1a



CYP450 3a

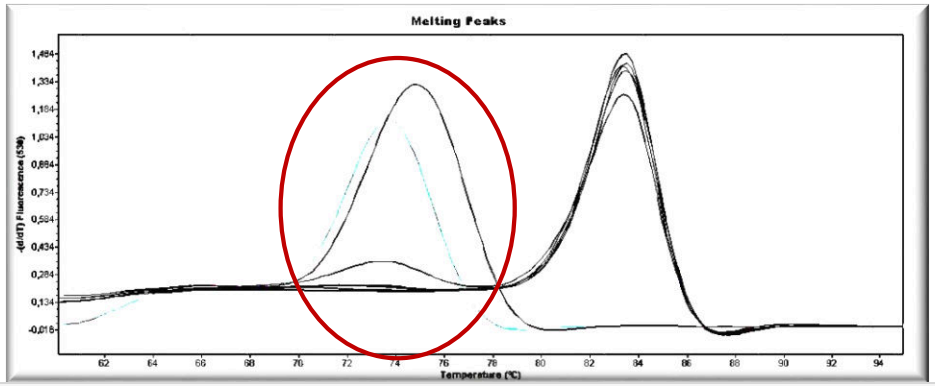


CYP450 1c1

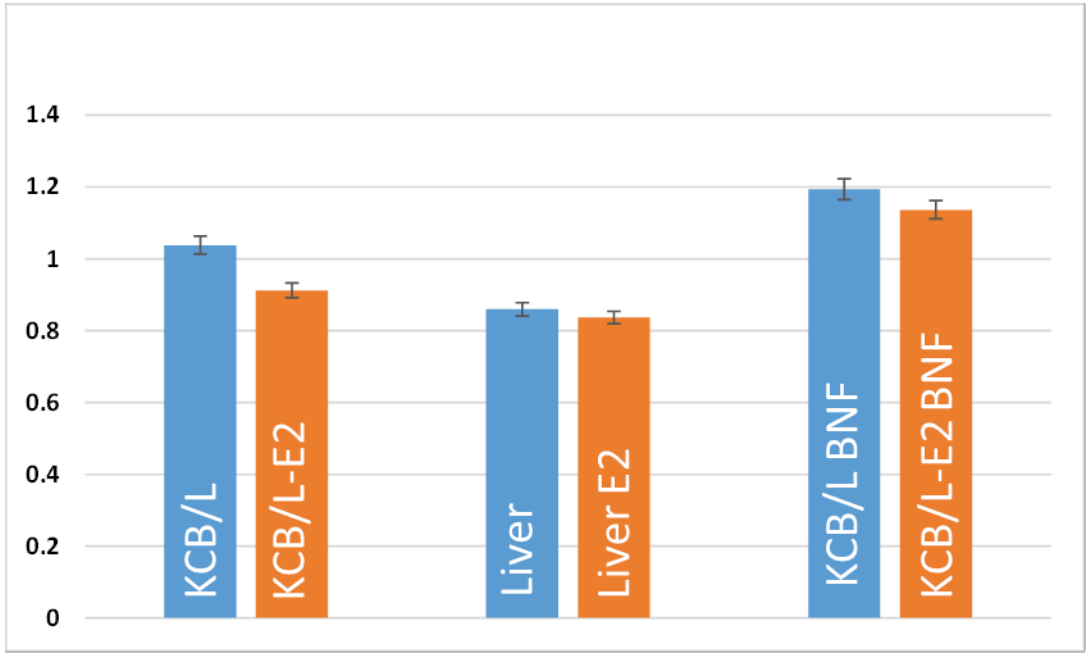


- Expression of *cyp3a* in the KCBs
- Expression of all investigated genes in the KCB/liver co-culture
- Enhanced expression of *mgst1, cyp1c1* and *cyp2k* in co-cultures exposed to BNF

Primer dimers

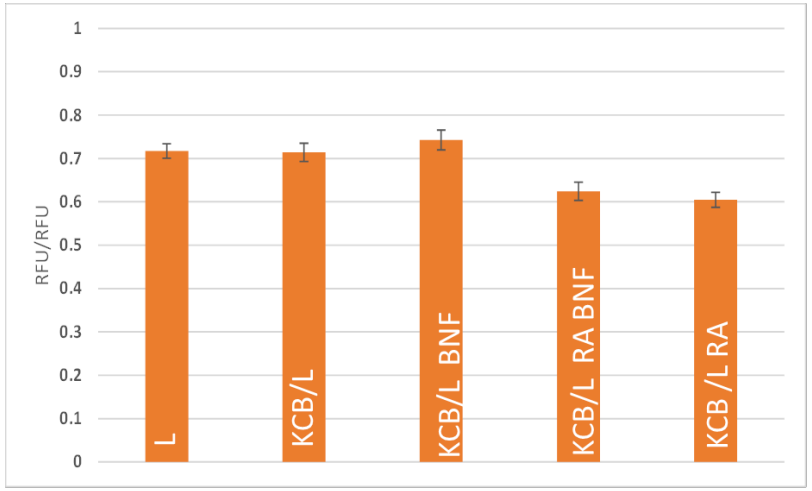


Semi-quantitative real time PCR



- *cyp2k* gene expression

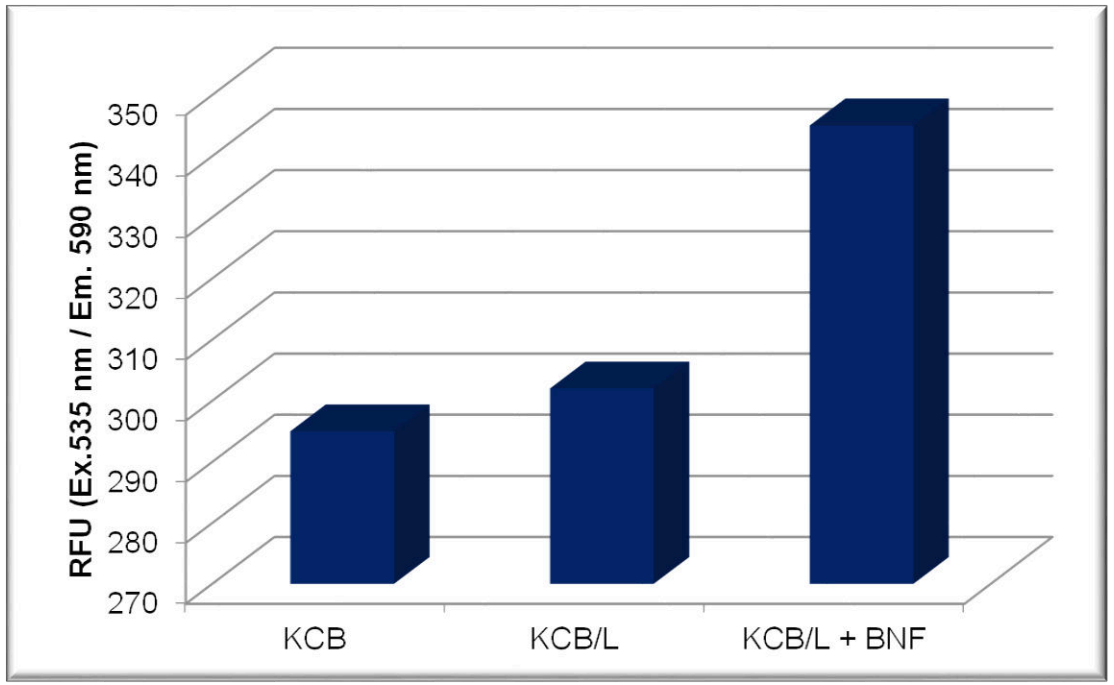
- Enhanced gene expression for KCB/L –E2 for *cyp1a, cyp1b, cyp3a*
- Induction of mgst1, cyp1c1 and cyp2k by BNF



- *cyp1a* gene expression

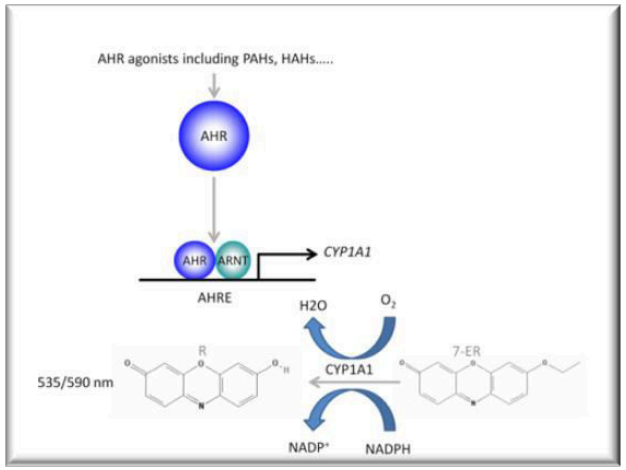
EROD assay

7-ethoxyresorufin-O-deethylase (EROD) activity



CYP1A – hydroxylation of PAHs (aryl hydrocarbon hydroxylase)

Mohammadi-Bardbori, A., 2014.



http://www.nature.com

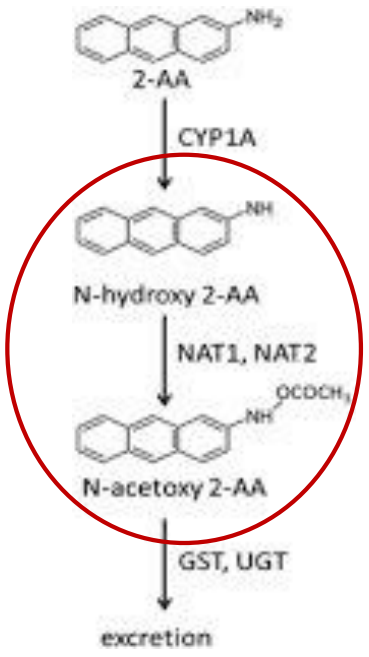
Any enzyme activity involved in generating NADH or NADPH

Vehniäinen et al. 2012. "Decrease of the fluorescence (in the EROD assay) due to enzymes that reduce resorufin to a non-fluorescent form."

Umu Chromotest

	Replicate 1	Replicate 2	Replicate 3	Mean	SD
KCB	1,26	1,31	1,35	1,31	0,04
KCB + BNF	1,06	1,38	1,35	1,21	0,14
	1,23	1,09	1,15		
KCB/L	1,18	1,29	1,06	1,20	0,11
	1,08	1,32	1,27		
KCB/L + BNF	1,25	1,45	1,26	1,34	0,08
	1,37	1,35	1,38		

Average growth factor=0.51



CYP450 1A subfamily

N-Acetyltransferases

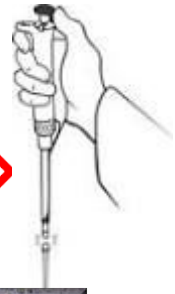
Darwish et al 2015.
J. Food Sci., 80.

- Increase in metabolized (active) 2-AA in co-cultures due to exposure to BNF

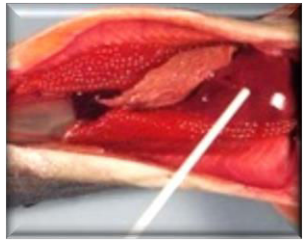
3 R´s

Refinement

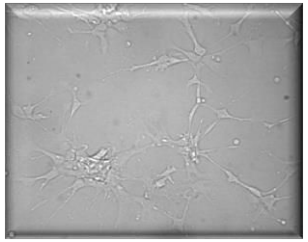
http://www.criver.com



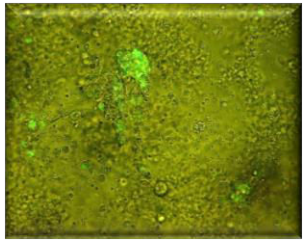
Reduction

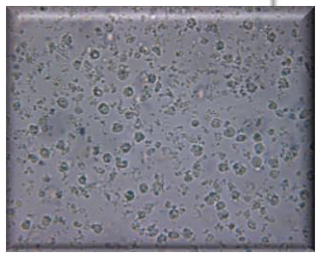


+



=



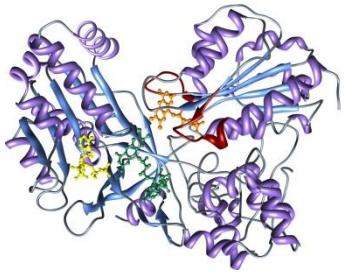


Replacement

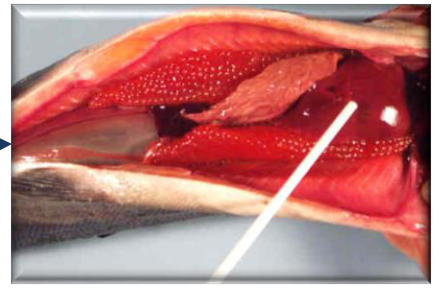
+

=

http://www.p450.kvl.dk



Outlook



Aroclor 1254

EROD assay

Umu Chromotest

Gene expression

Enzymatic activity

www.the-scientist.com

Acknowledgements

Supported by:

Federal Ministry for Economic Affairs and Energy

on the basis of a decision by the German Bundestag

GOBIO

Institute for Ecology of Waters and Applied Biology

TECOmedical Group

always your partner

Thank you!

UNIKLINIK KÖLN

EUSAAT

European Society for Alternatives to Animal Testing