Hepatocyte *in vitro* gene expression systems: what is the reason for differences between *in vivo* and *in vitro* systems?

Markus Schug, Dipl. Pharm.
Leibniz Research Centre for Working Environment and Human Factors (IfADo)
eSI workshop in Alicante (Spain)
October 17-19, 2008

Problems with *in vitro in vivo* correlation

- The problem: differences between RNA expression data *in vitro* and *in vivo*
- Published data: RNA expression of the following genes (*Abat*, *Sult1a1*, *Gsk3β*, *Myd116*) is altered *in vitro* (Beekman et al., 2006) but not *in vivo* (Ellinger-Ziegelbauer et al., 2005)
- Both *in vitro* and *in vivo* analyses have been done in rat (hepatocytes/liver)
- Are the gene expression alterations observed *in vitro* also relevant for the *in vivo* situation?

What is the most suitable culture system?

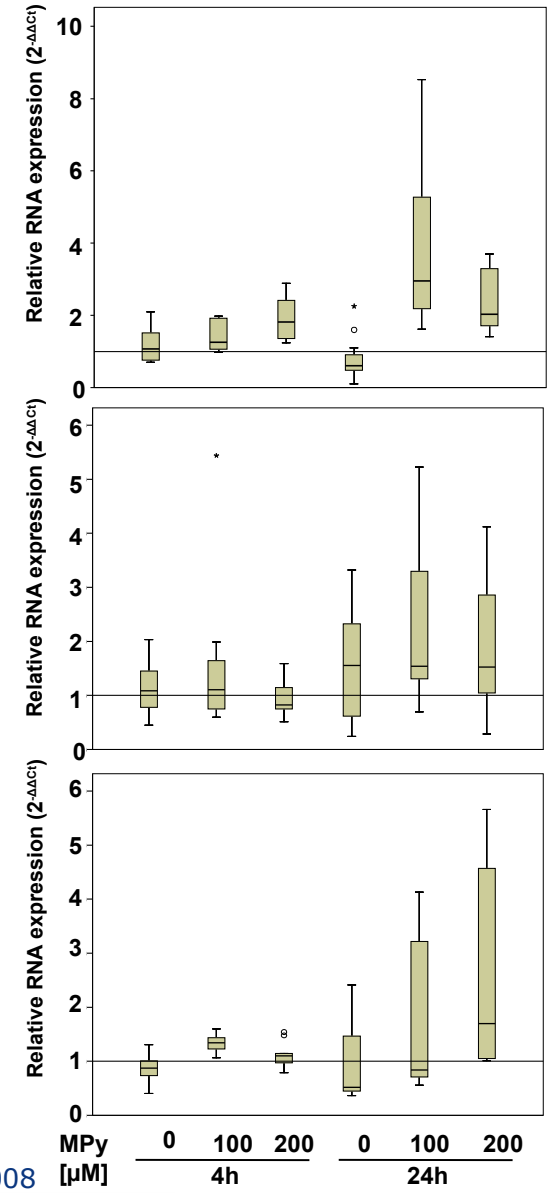
- Experimental design:
  - Culture system:
    - Collagen sandwich culture
    - Matrigel
    - 2D collagen culture
  - Substance concentrations:
    - 100 and 200 µM methapyrilene
  - Incubation time:
    - 4 and 24 h

What is the most suitable culture system?

Sandwich culture

Matrigel culture

Collagen 2D culture



- *Myd116*
  - Myeloid Differentiation Primary Response Gen 116
  - Levels upregulated with *in vitro* methapyrilene treatment (Beekman et al. 2006)

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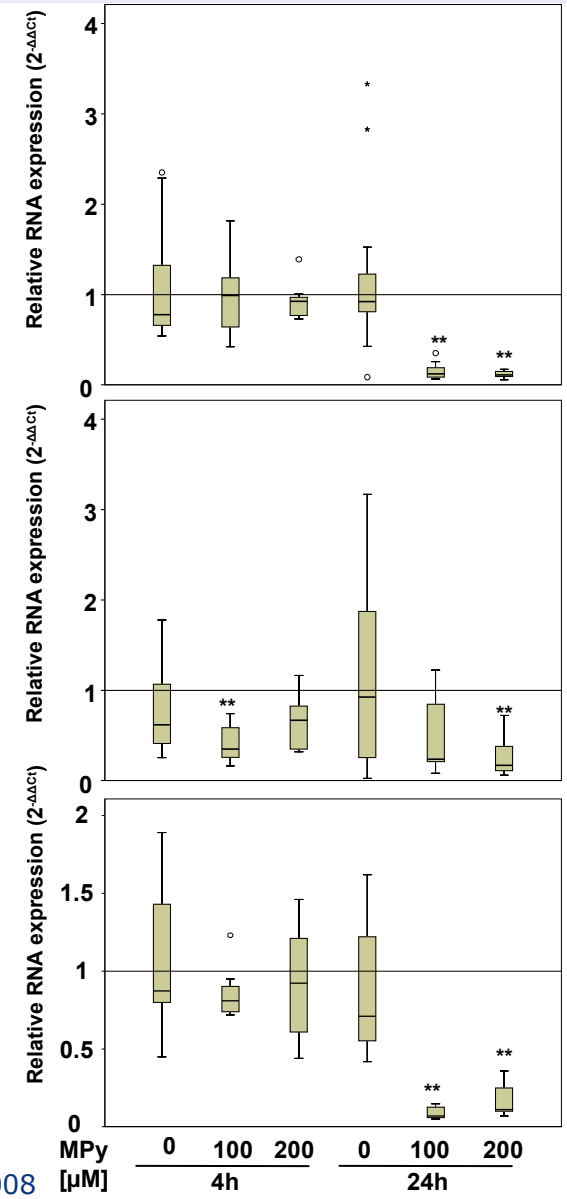

- *Sult1a1*
  - Sulfotransferase Family 1a, Phenol preferring, Member 1
  - Levels downregulated with *in vitro* methapyrilene treatment (Beekman et al. 2006)

Schug et al., 2008
Arch. Tox., accepted

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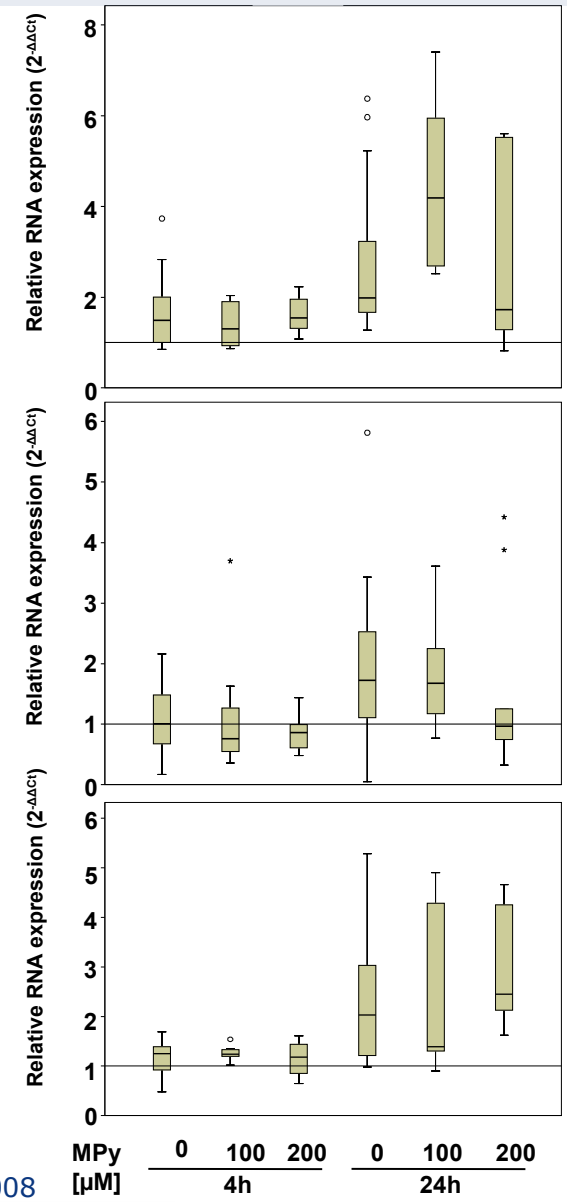

- *Gsk3β*
  - Glykogen Synthase Kinase 3-beta
  - Levels upregulated with *in vitro* methapyrilene treatment (Beekman et al. 2006)

IfADo Schug et al., 2008 Arch. Tox., accepted

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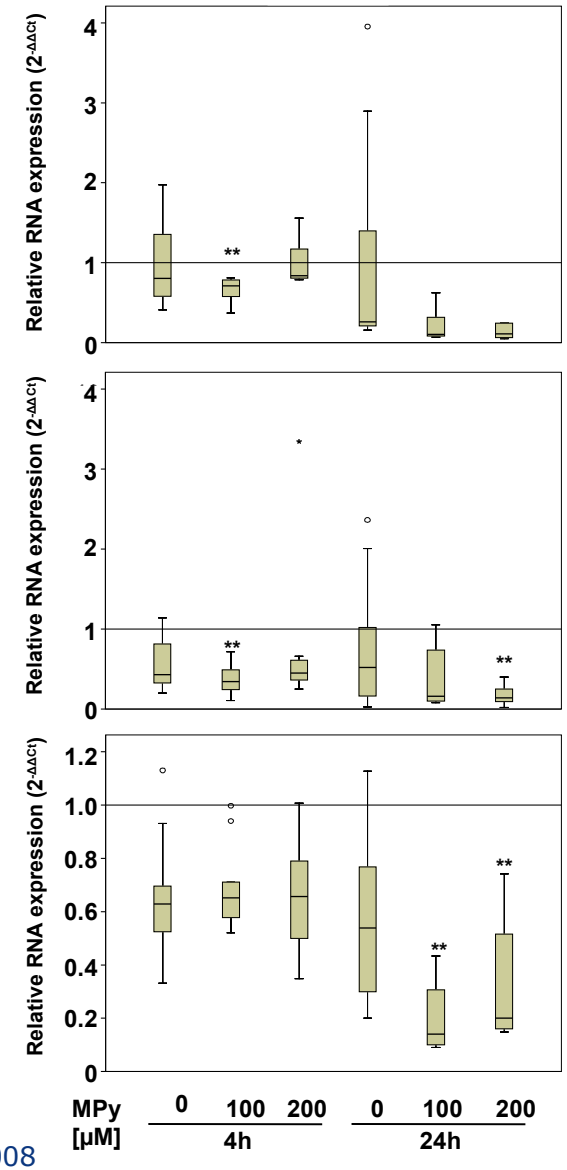

- *Abat*
  - Aminobutyrate aminotransferase
  - Levels downregulated with *in vitro* methapyrilene treatment (Beekman et al. 2006)

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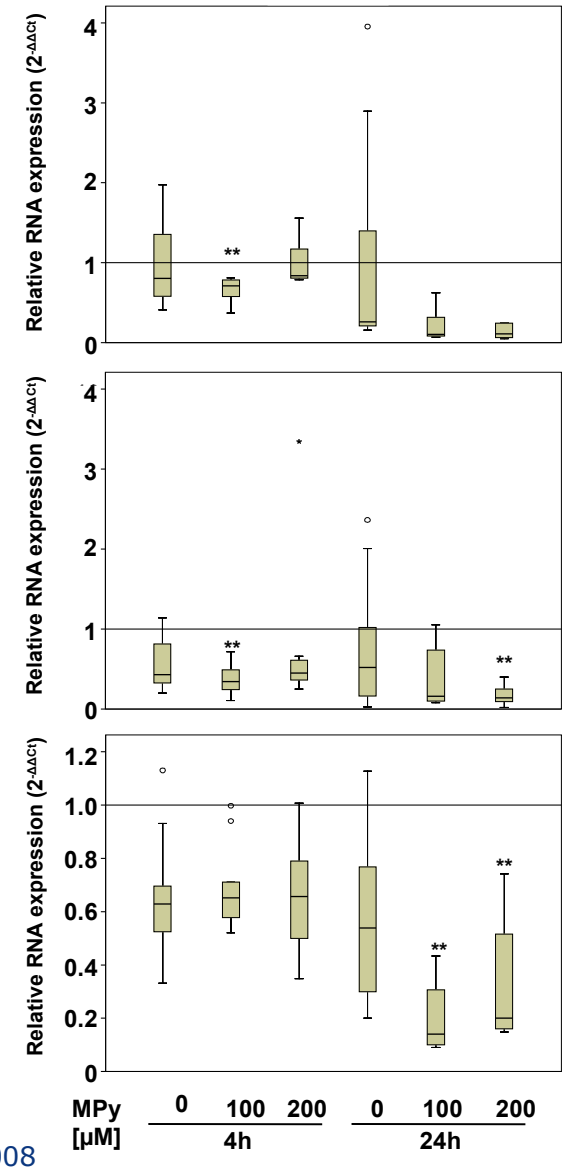

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Same results for all genes after a cell maintenance time of 3 weeks in sandwich culture before starting the incubation

Schug et al., 2008
Arch. Tox., accepted

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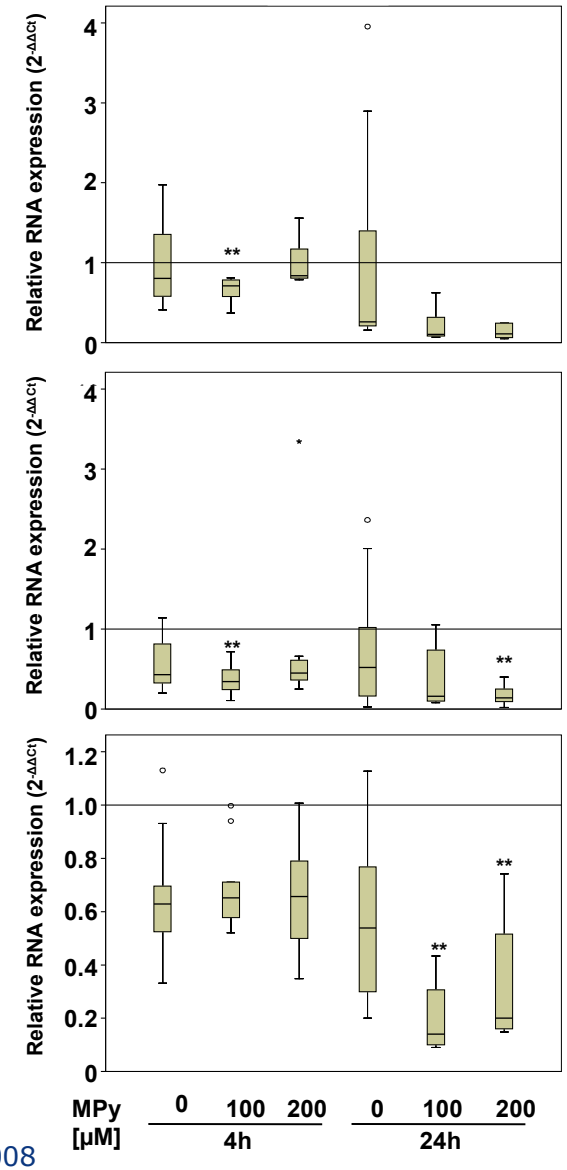

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Collagen sandwich culture is the most suitable culture system

Schug et al., 2008
Arch. Tox., accepted

Do *in vivo* relevant concentrations cause effects *in vitro*?

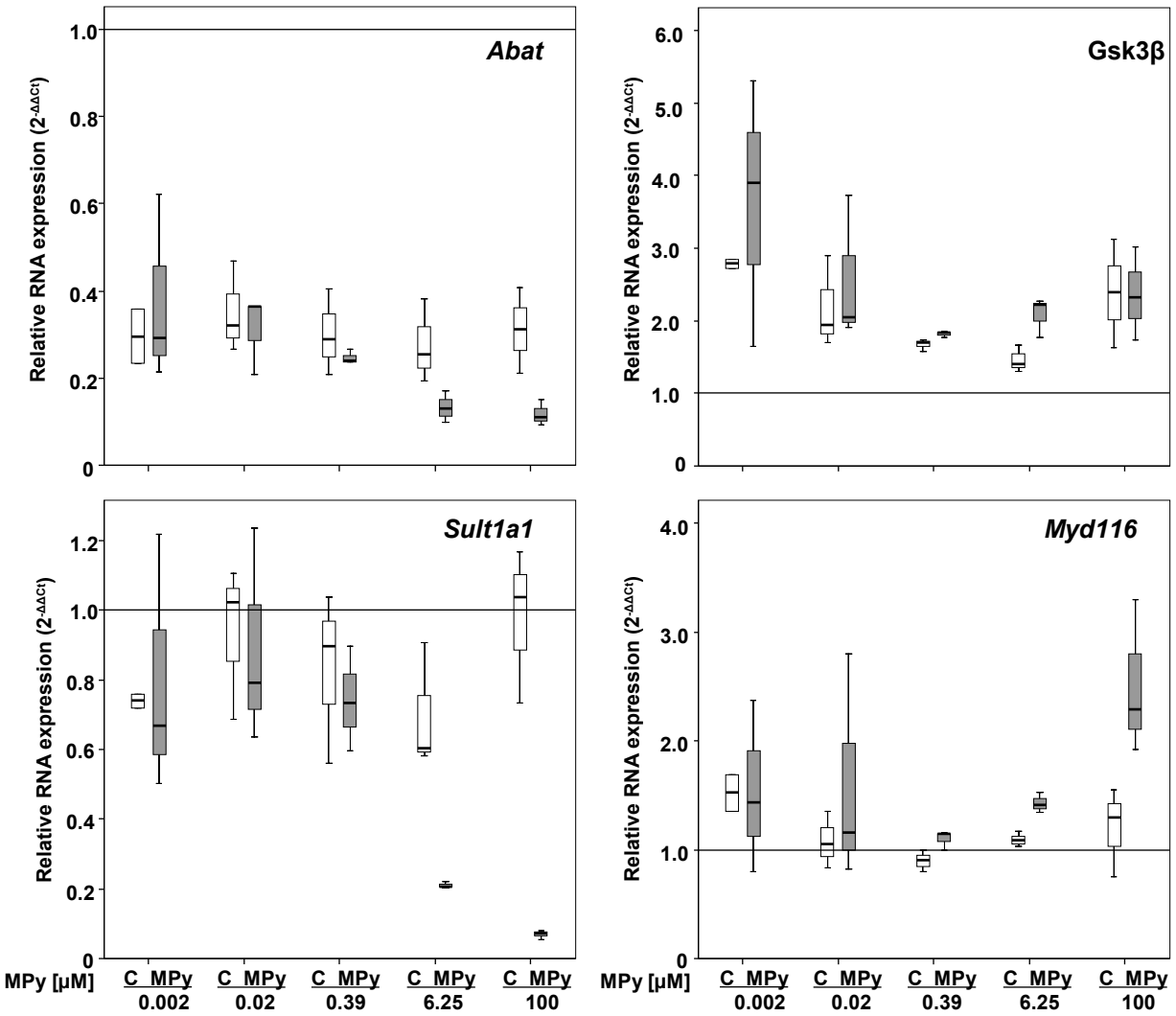
- Experimental design
  - Culture matrix:
    - Collagen sandwich culture
  - Substance concentrations:
    - 0.002, 0.02, 0.39, 6.25, 100 µM methapyrilene
  - Incubation time:
    - 24 h

In vivo observed plasma concentrations after i.p. administration of methapyrilene: 3.5 mg/kg BW (Kelly et al. 1990)

time after i.p. Injection	animal no.	MPy in plasma [µM]
5 min	1	1.35
	2	1.72
	3	0.91
20 min	1	1.22
	2	0.62
	3	0.7
80 min	1	0.32
	2	nd*
	3	0.35
320 min	1	nd*
	2	nd*
	3	nd*

Schug et al., 2008 Arch. Tox, accepted

Do *in vivo* relevant concentrations cause effects *in vitro*?



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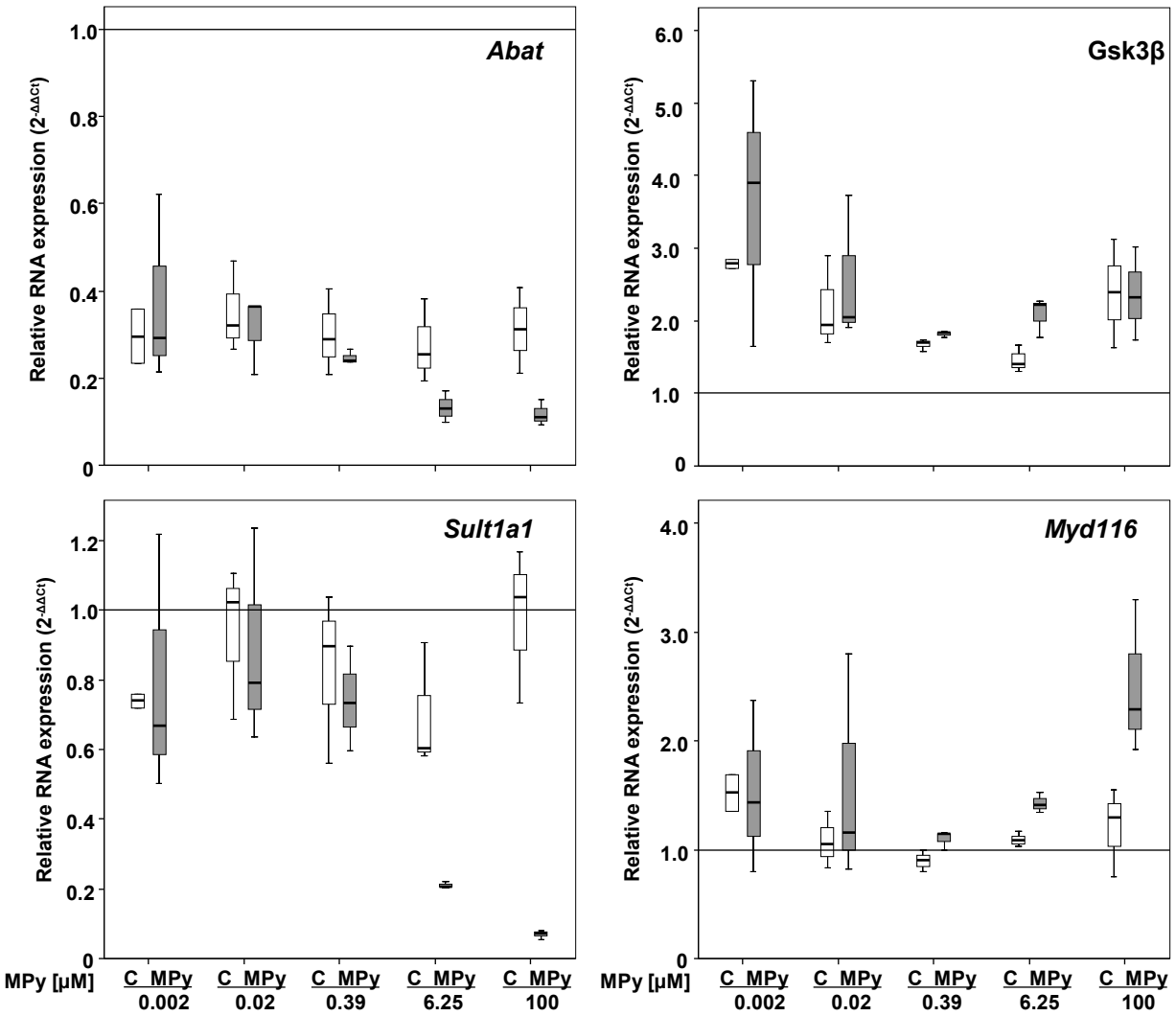
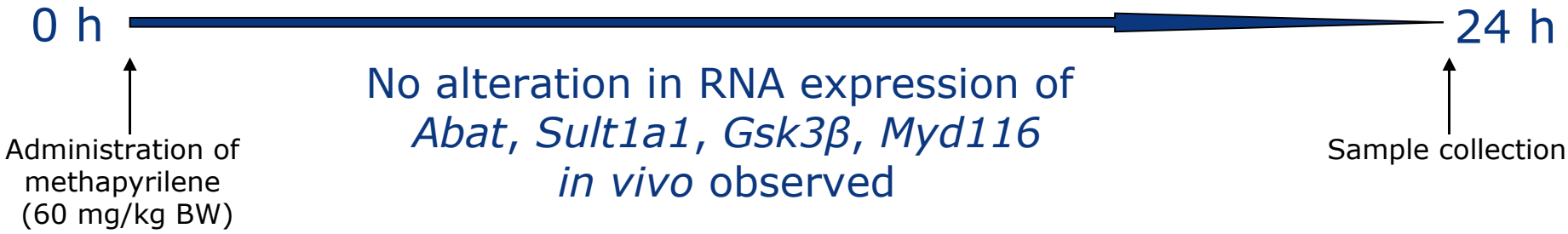


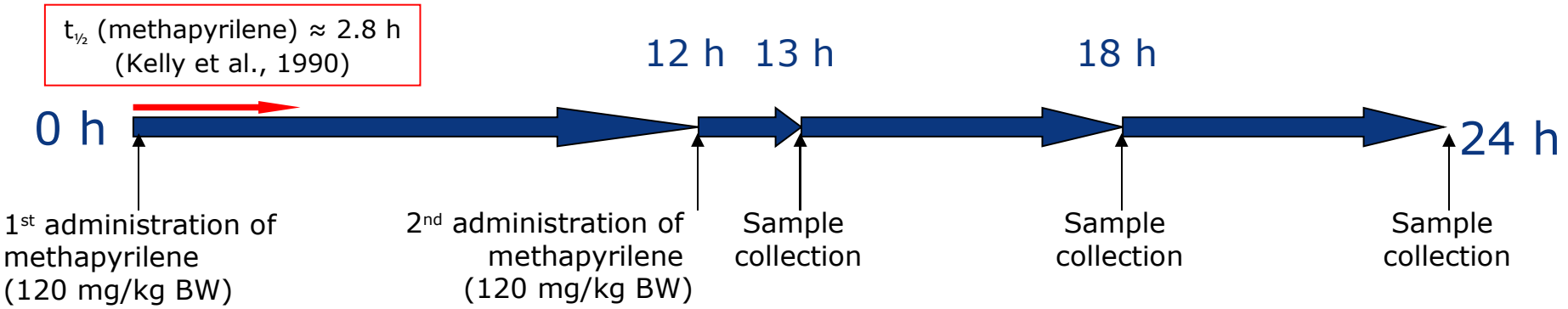
Alterations in gene expression is also observed at *in vivo* relevant concentrations

Are the gene expression alterations observed *in vitro* not relevant to the *in vivo* situation?

- *In vivo* study from Ellinger-Ziegelbauer et al. 2005



- Hypothesis: *in vivo* observable after a shorter period?



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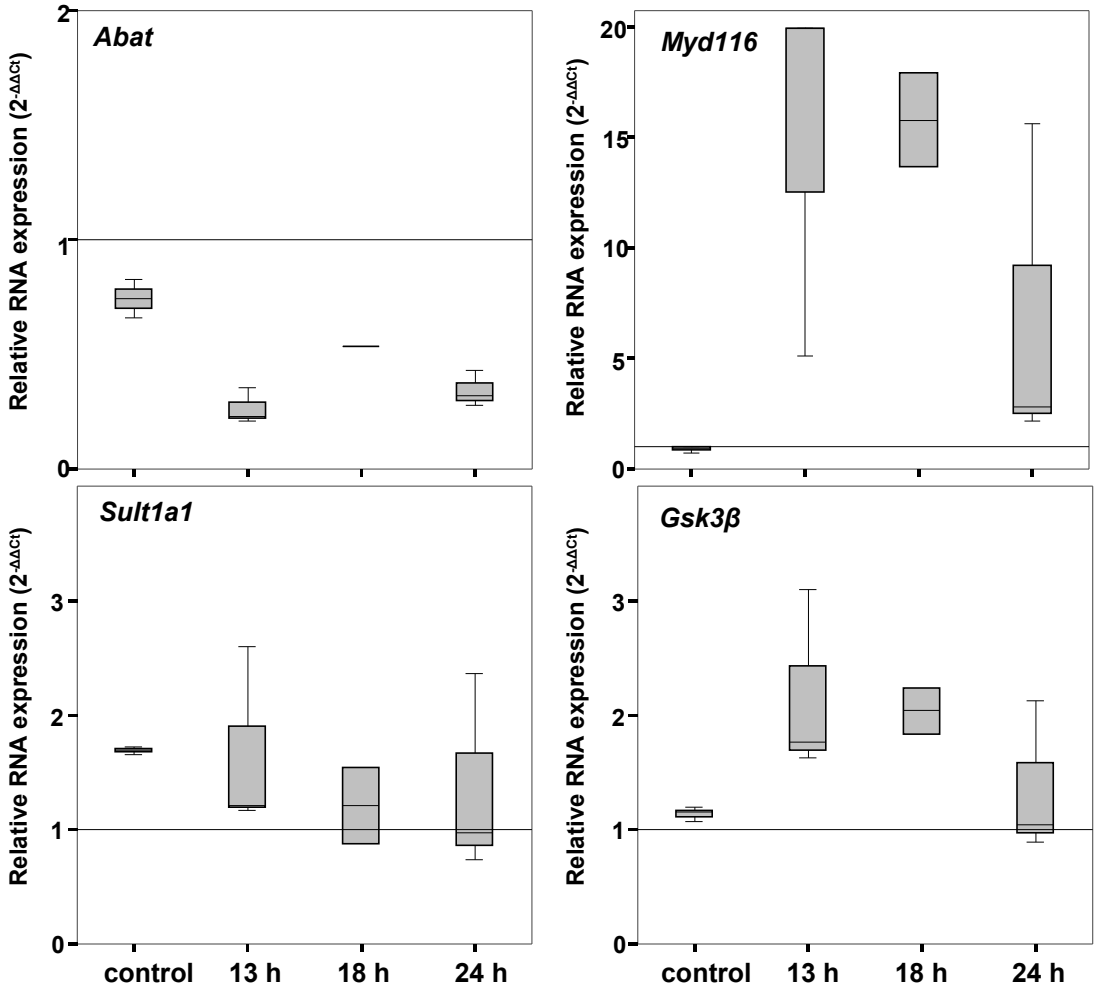

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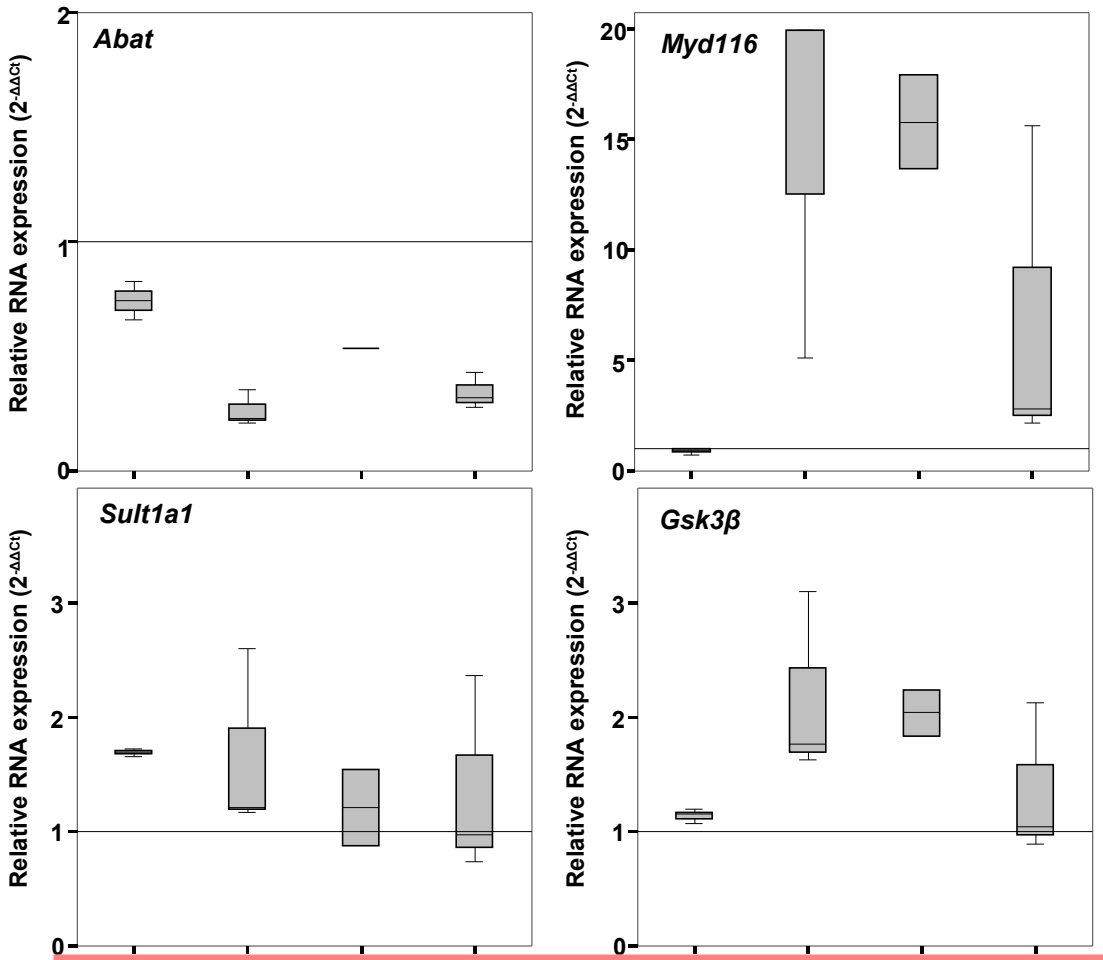

The *in vitro* observed alterations in gene expressions are indeed relevant for the *in vivo* situation. The reason for previous observed discrepancies could be due to different pharmacokinetics *in vitro* and *in vivo*

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Summary

- Collagen Sandwich culture is the most suitable culture system
- Alterations in gene expression is also observed at *in vivo* relevant concentrations
- The *in vitro* observed alterations in gene expression of *Abat*, *Sult1a1*, *Gsk3β*, *Myd116* are indeed relevant for the *in vivo* situation. The reason for previous observed discrepancies could be due to different pharmacokinetics *in vitro* and *in vivo.*

Acknowledgments

- IfADo Dortmund
  - Prof. Jan G. Hengstler
  - Alexander Bauer
  - Georgia Günther
  - Dr. Meinolf Blasczkewicz
  - Gabriele Baumhoer
  - Dr. Matthias Hermes
  - Dr. Rosemarie Marchan
  - And many others
- BfR Berlin
  - Dr. Tanja Heise
  - Dorothe Storm
  - Dr. Axel Oberemm
  - Prof. Ursula Gundert-Remy
  - Dr. Klaus E. Appel
- Bayer Health Care
  - Dr. Heidrun Ellinger-Ziegelbauer
- BMBF (grant-no. 0313854C)

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