



Simulation of urine levels of 1-hydroxypyrene with a generic PBTK-model in situations with inhalation and/or dermal exposure

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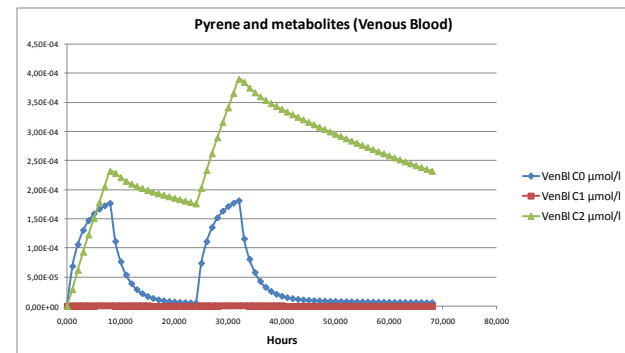
Overview of the PBTK-model IndusChemFate

Compound data

- Physical-chemical properties:
 - Density
 - Molecular weight
 - Vapour pressure
 - Log(K_{ow}) at pH 5.5 and 7.4
 - Water Solubility
- Biochemical parameters :
 - Metabolism (k_M and V_{max})
 - Renal tubular resorption
 - Enterohepatic circulation ratio

Exposure scenario

- Three routes of uptake:
 - Inhalation - concentration
 - Dermal – dose rate
 - Oral - dose
- Duration of exposure
- Personal Protective Equipment
- Physical activity level (rest/ light)

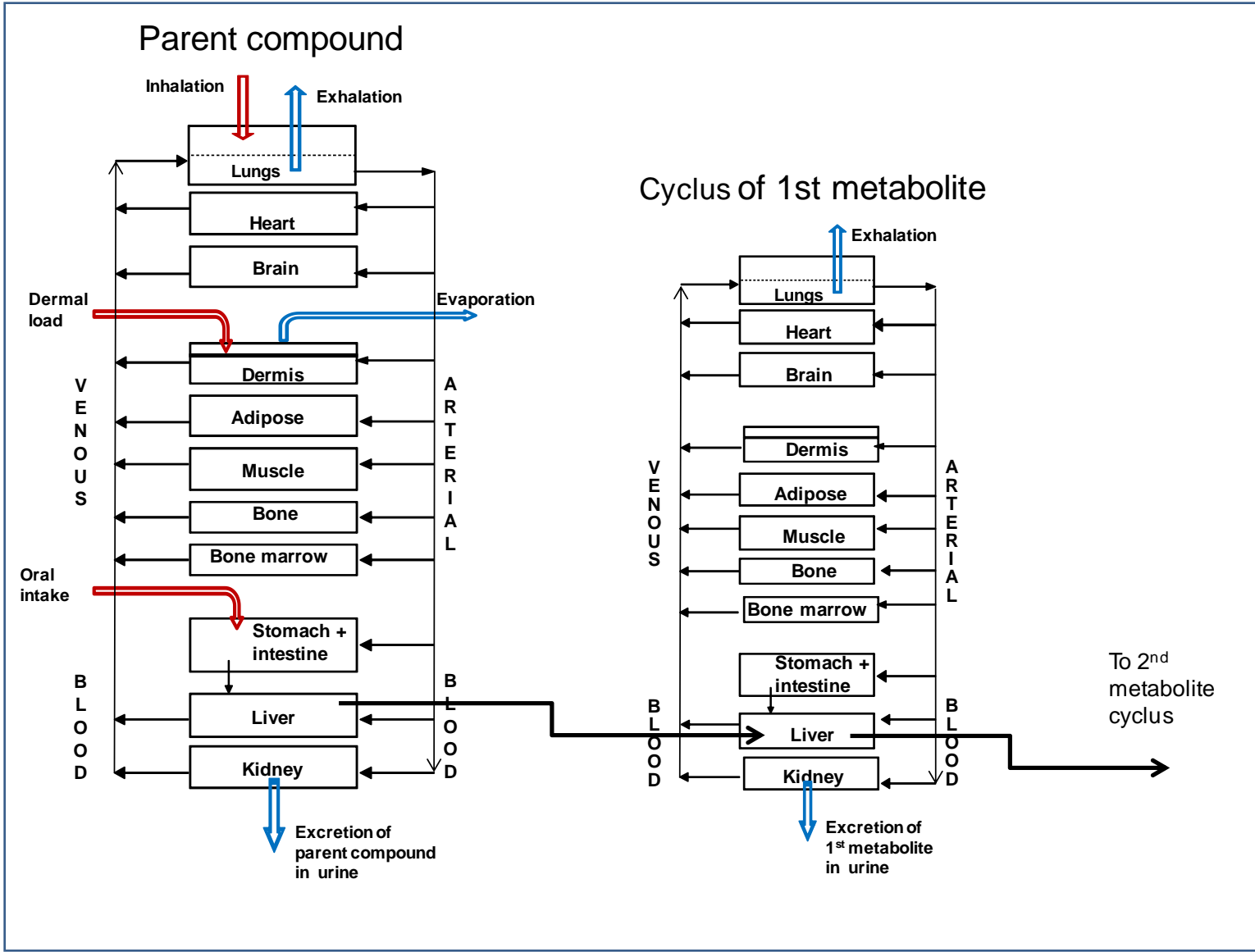


What is a PBTK-model?

- PBTK-model = Physiologically Based ToxicoKinetic model
- A PBTK-model is a mathematical description for absorption, distribution, metabolism and excretion (ADME) of a chemical in the body of experimental animals or humans
- Compartments corresponds to predefined organs or tissues, with interconnections corresponding to blood
- A system of differential equations is used to estimate the concentration of a chemical in each compartment
- Such a model can predict the time-course of concentrations in blood and/or urine after inhalation (or dermal exposure)



Scheme of the physiology of the PBTK-model



Routing of chemicals and metabolites in the PBTK-model

- Absorption

- Inhalation
- Oral uptake
- Dermal uptake

- Distribution over the body

- QSPR algorithm for estimate of blood:air partitioning
- QSPR algorithm for estimate of tissue:blood partitioning

- Metabolism

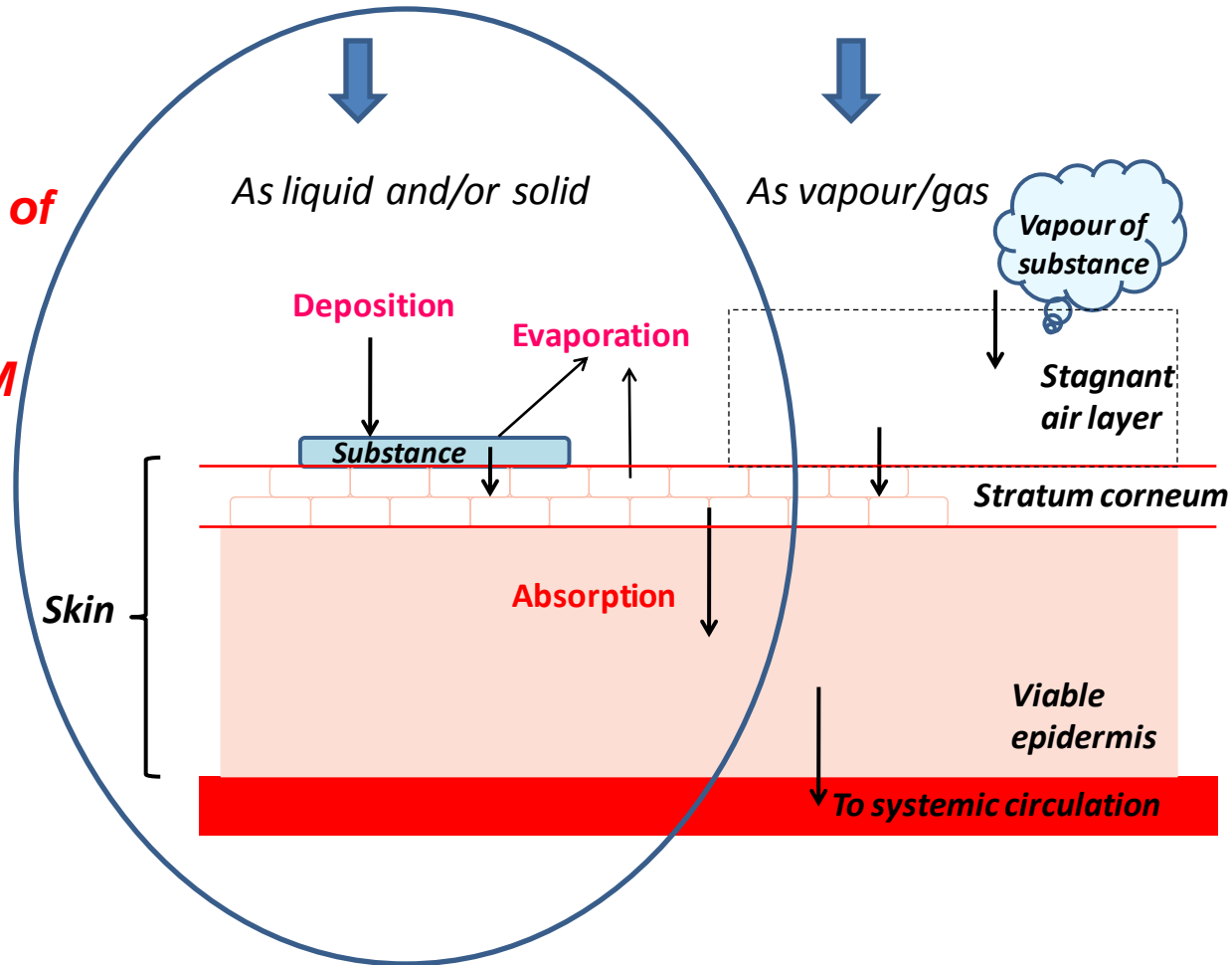
- Saturable metabolism according to Michaelis-Menten kinetics
- Metabolism in all tissues, only liver is default

- Excretion

- Urine
- Exhaled air

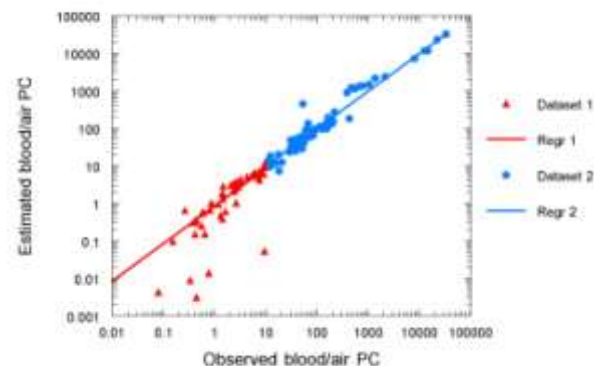
Dermal absorption module of the model

**= New model of
AIHA-EASC
named
IH SKINPERM**



Distribution over compartments in the body

- Blood:air partition coefficient
 - QSPR Algorithm for estimation of blood:air partitioning based on Henry coefficient and K_{oa}



- Blood:tissue partition coefficient
 - QSPR Algorithm for estimation of blood:tissue partitioning taken from De Jong et al (1997), based on lipid content and K_{ow}

The PBTK-model is build as application in MS-Excel, called IndusChemFate

- The differential equations of the PBTK-model are written in spreadsheet syntax (visual basic)
- The file IndusChemFate contains 4 sheets:
 1. Tutorial with instructions in short
 2. Worksheet
 - For data entry (exposure scenario, properties of chemical under study)
 - For numerical output
 3. Database of phys-chemical and biochemical properties of various chemicals
 4. Graphical output sheet

Simulation experiment 1

Operator creosote impregnating plant

- 1-hydroxypyrene was measured in urine of an operator of a creosote impregnating plant during 7-days
- Creosote oil = a timber protective agent that contains PAH
- Pyrene is metabolised to 1-hydroxypyrene

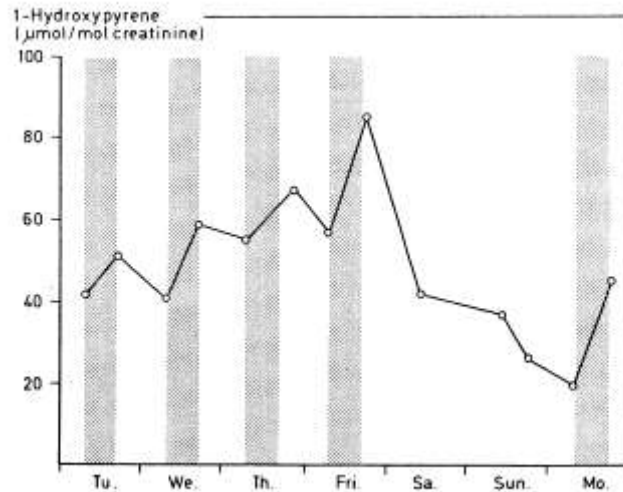
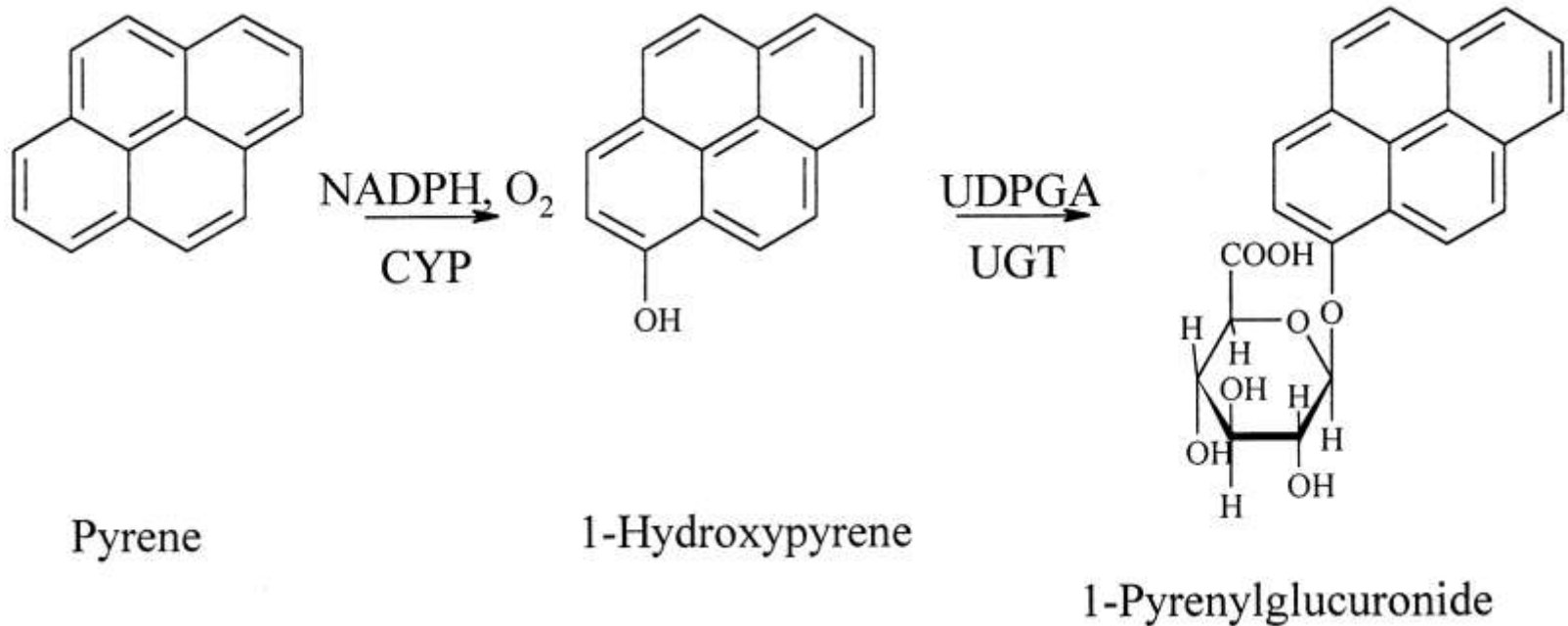


Figure 3-1A. Excretion of 1OHP in urine of a creosote impregnating worker (Jongeneelen et al, 1988)

Metabolism of pyrene



Human metabolism kinetics of pyrene

Step	Tissue	Parameter and value	ref
Pyrene to 1-OH-pyrene	Hepatic 9000*g fraction of 12 individuals	$V_{\max} = 180 \mu\text{mol/hr/kg}$ tissue $K_M = 4.4 \mu\text{M}$	Jongeneelen (1987)
1-OH-Pyrene to 1-OH-pyrene- gluc	Hepatic microsomal fraction of 3 individuals	$V_{\max} = 6,900 \mu\text{mol/hr/kg}$ tissue $K_M = 7.7 \mu\text{M}$	Luukkanen et al (2001)

Simulation experiment 1

Enter data

- ✓ Enter **phys-chemical properties** and biochemical parameters of parent compound and metabolites under study

- ✓ Enter **exposure scenario**
 - Inhalation: concentration and duration
 - Dermal: dose rate and duration
 - Oral: bolus dose

Simulation experiment 1 Entering properties of pyrene and metabolite

Pyrene

1-OH-Pyrene

1-OH-Pyrene-glucuronide

Parent Compound	Pyrene
CAS	129-00-0
Density (mg/cm ³ or grams/litre)	1270
Molecular weight	202,26
Vapour Pressure (Pa)	0,0106
Log(Kow) at skin pH 5.5	4,88
Log(Kow) at blood pH 7.4	4,88
Water solubility (mg/litre)	0,135
Resorption tubuli (y/n/?)	Y
Enterohepatic removal (relative to liver venous blood)	0
Vmax Liver (parent[total] µmol/kg tissue/hr)	360
Km Liver (parent[total] µmol/litre)	4,5
Vmax Liver (parent[specif] µmol/kg tissue/hr)	180
Km Liver (parent[specif] µmol/litre)	4,5
1st metabolite	Hydroxypyrene
CAS	5315-79-7
Density (mg/cm ³ or grams/litre)	1000
Molecular weight	218,28
Vapour Pressure (Pa)	0,000022
Log(Kow) at skin pH 5.5	
Log(Kow) at blood pH 7.4	4,45
Water solubility (mg/litre)	4
Resorption tubuli (y/n/?)	Y
Enterohepatic removal (relative to liver venous blood)	0
Vmax Liver (1st metab[total] µmol/kg tissue/hr)	6900
Km Liver (1st metab[total] µmol/litre)	7,7
Vmax Liver (1st metab[specif] µmol/kg tissue/hr)	6900
Km Liver (1st metab[specif] µmol/litre)	7,7
2nd metabolite	Hydroxypyrene Glucuronide
CAS	154717-05-2
Density (mg/cm ³ or grams/litre)	1000
Molecular weight	394
Vapour Pressure (Pa)	3,2E-17
Log(Kow) at skin pH 5.5	
Log(Kow) at blood pH 7.4	-2,12
Water solubility (mg/litre)	40000
Resorption tubuli (y/n/?)	n
Enterohepatic removal (relative to liver venous blood)	0,8
Vmax Liver (2nd metab[total] µmol/kg tissue/hr)	
Km Liver (2nd metab[total] µmol/litre)	
Vmax Liver (2nd metab[specif] µmol/kg tissue/hr)	
Km Liver (2nd metab[specif] µmol/litre)	

Entering exposure scenario of the creosote plant operator

Airborne exposure scenario

Parameters Airborne Exposure	
Concentration parent compound (mg/m ³)	0,003
Start of airborne exposure (hours)	0
Duration of airborne exposure (hours)	8
Respiratory protection factor (⇒ 1)	1
Dermal protection factor (air tight clothing ⇒ 1)	1

Dermal exposure scenario

Parameters Dermal exposure to parent compound	
Skin deposition pure substance (mg/cm ² /hour)	0,000006
Start of skin exposure (hours)	0
Duration of skin exposure (hours)	8
Skin temperature (centigrade)	25
Affected skin area (cm ²)	7500

Oral intake scenario

Parameters of oral absorption	
Bolus dose to stomach of parent compound (mg/kg bwt)	0
Time of application (time in hours)	0
Absorption rate into intestinal tissue (1/hour)	3

Selection of model parameters	
Select model (1=hum. rest, 2=hum. light act. 3=mouse 4=rat)	2
Repeating exposure for how many days?	5

Observation settings	
Start of observation (time in hours)	0
End of observation (time in hours)	168
Number of steps per hour	1000
Report times per hour	1

Simulation experiment 1

Run program - Results as table with levels and amounts in fluids and tissues

The screenshot displays a complex data table from a simulation program. The table is organized into several sections:

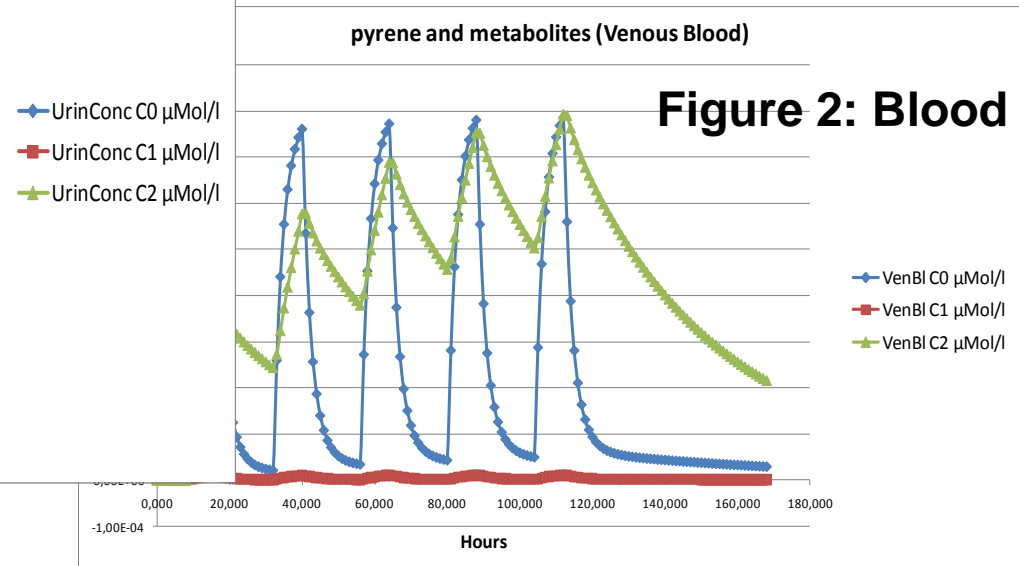
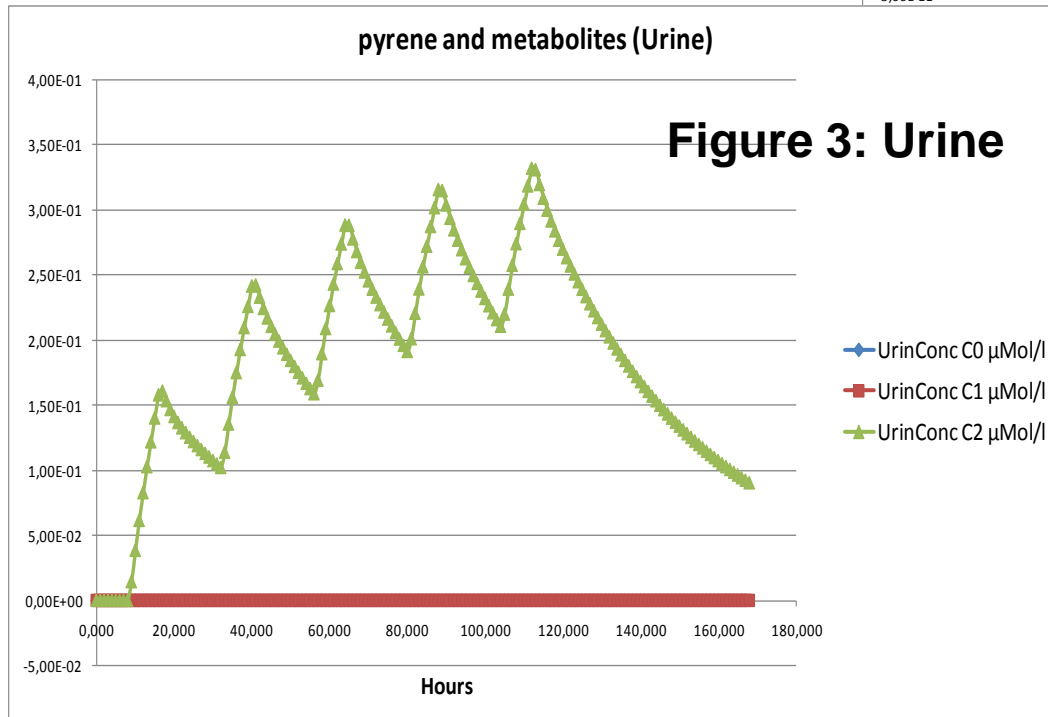
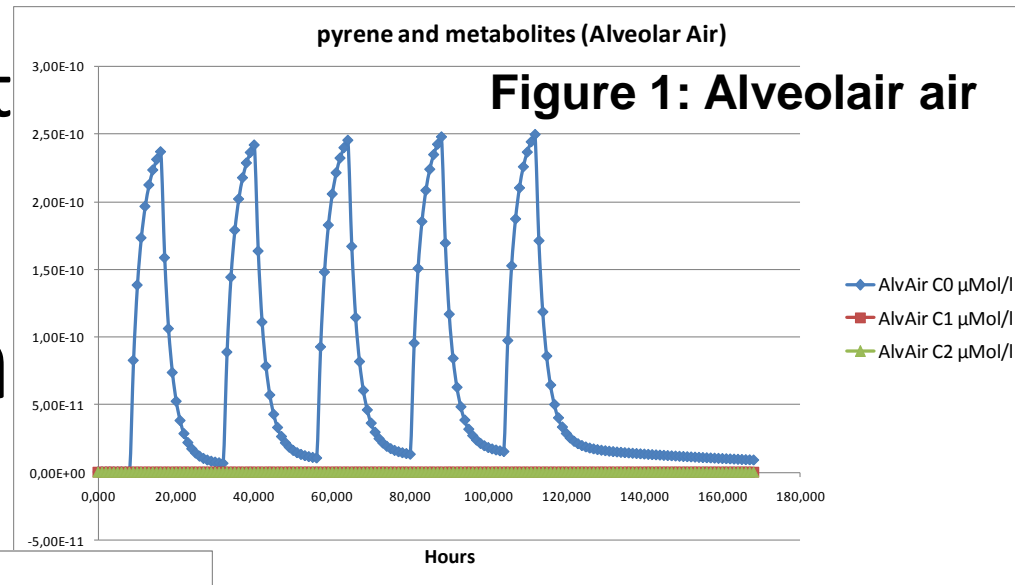
- Parent Compound:** Lists parameters for Butoxyethanol, such as 'Start of exposure (time in hours)', 'End of exposure (time in hours)', and 'Dose (mg/kg body weight)'. The results are numerical values in scientific notation.
- Metabolites:** Lists various metabolites like 'Sum Tissues', 'Sum Blood', and 'Sum Urine', with columns for 'Parent' and 'Absorbed' amounts.
- Absorption:** Lists different tissues and fluids, including 'Blood', 'Liver', 'Kidney', and 'Heart', with columns for 'Parent' and 'Absorbed' amounts.

The table contains numerous rows of data, each representing a specific parameter or result. The values are presented in scientific notation, such as 1.00E+00, 1.23E-01, and 1.56E+01. The interface also shows a menu bar at the top with options like 'File', 'Edit', and 'View', and a status bar at the bottom indicating the current file and window state.

Simulation experiment

Run program-

Results as graph

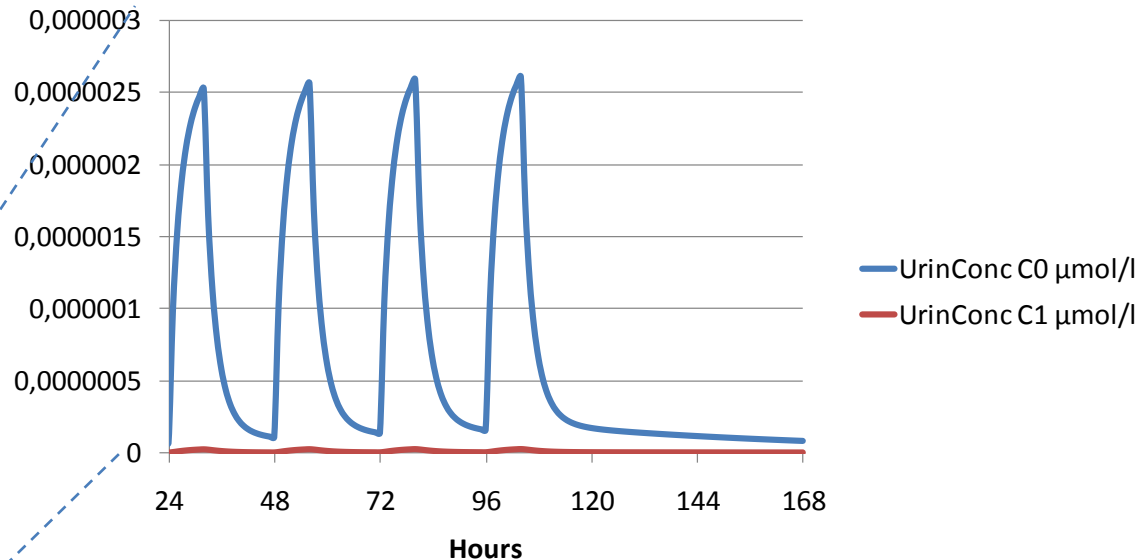


Simulation

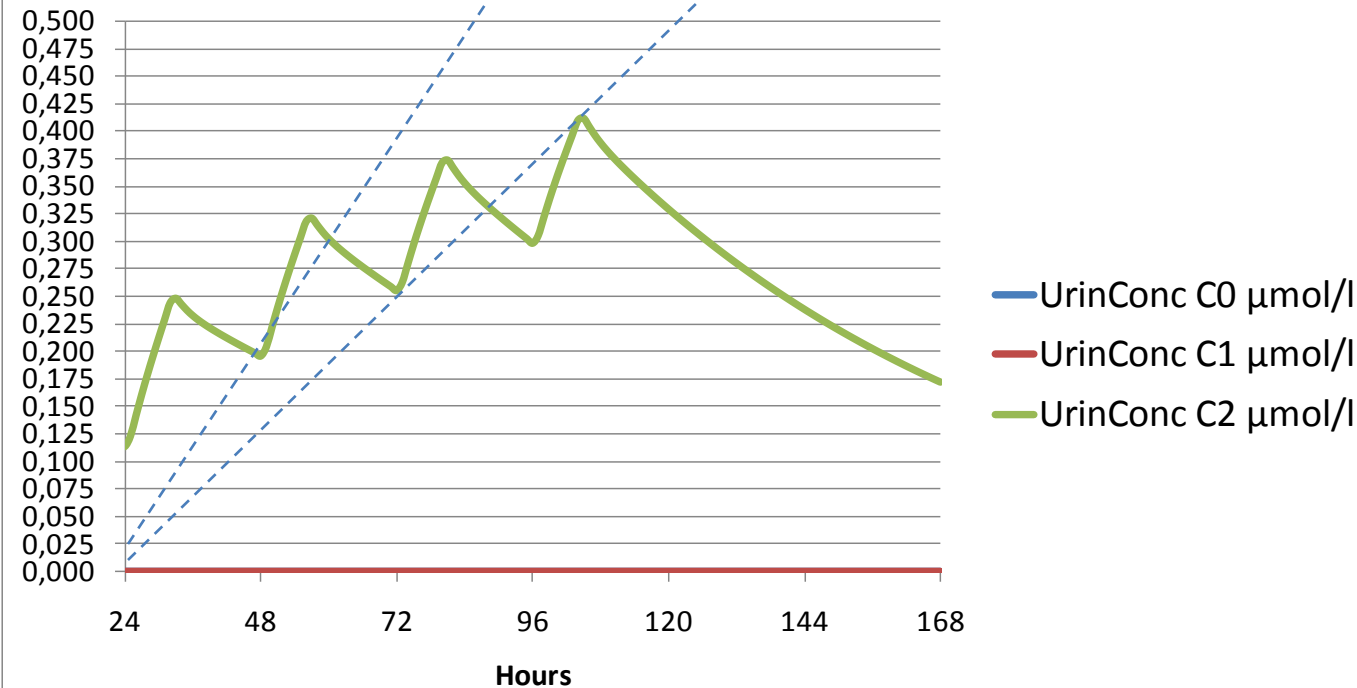
Experiment 1

**Results: levels
in urine**

Pyrene (C0) and free 1-OH-pyrene (C1) in urine

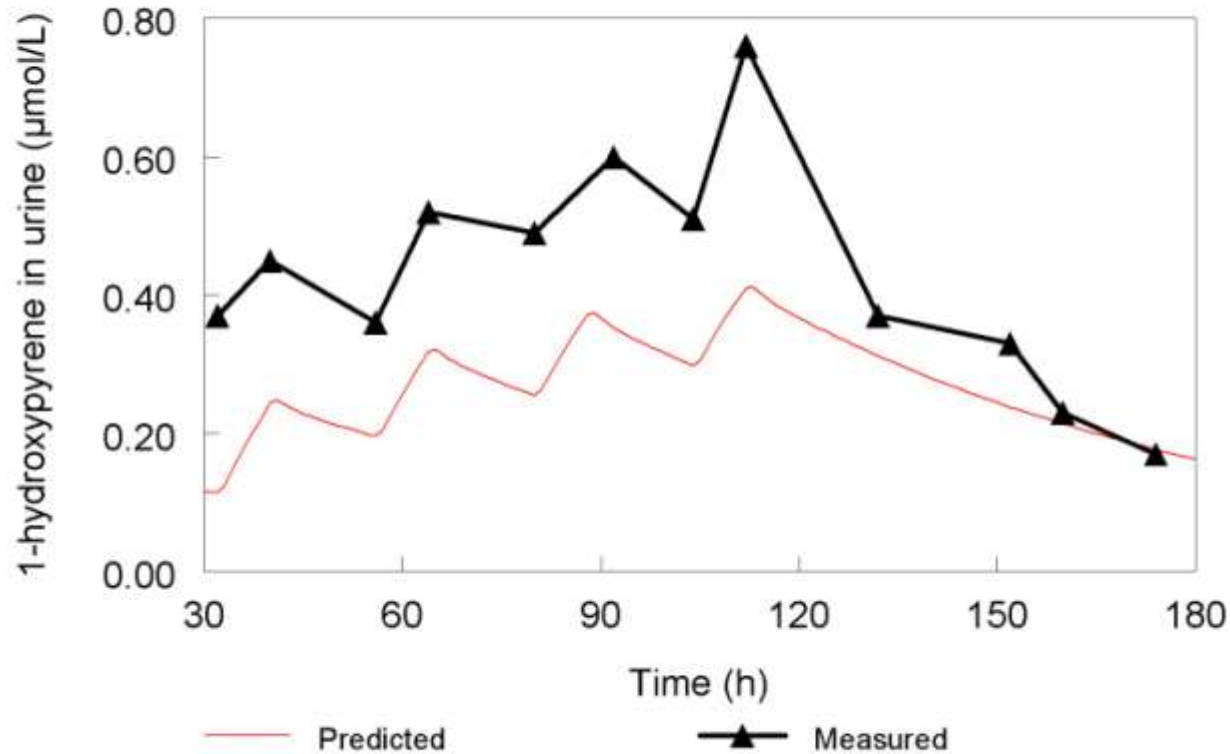


Pyrene and metabolites (Urine)



Simulation experiment 1

Comparison of measured and PBTK-model predicted level of 1-OH-pyrene in urine of operator



Level is expressed as sum of free 1-OHP and 1-OHP-glucuronide

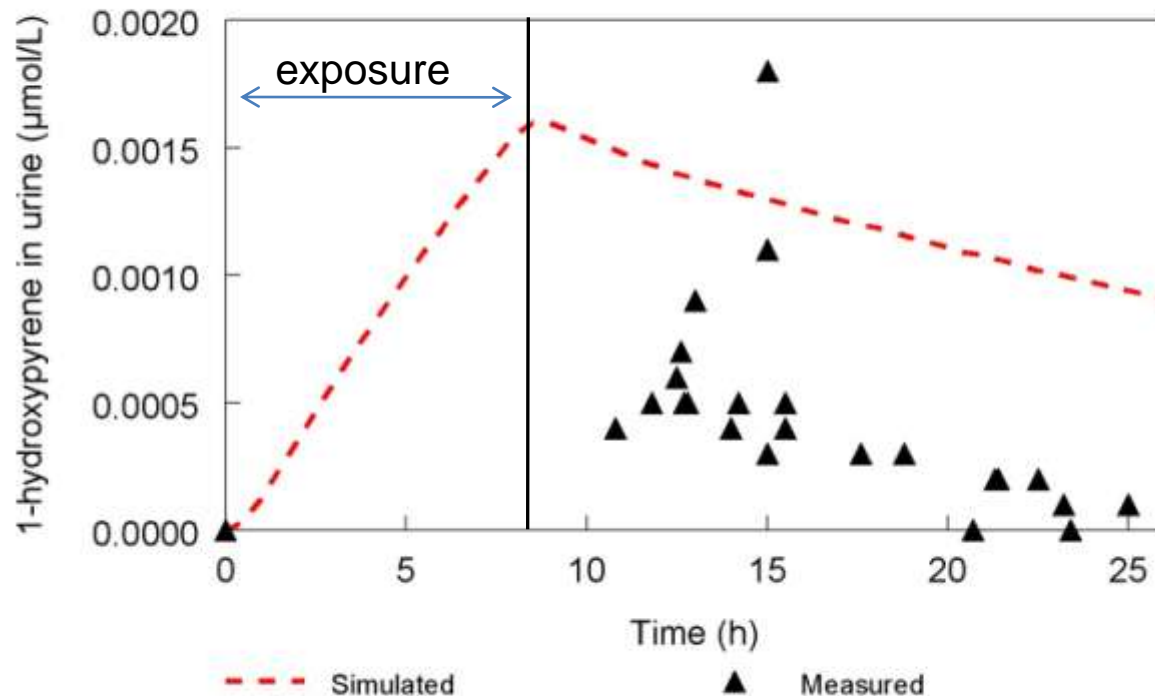
Other comparisons of experiments/field measurements with simulations

Nr.	Type of study	Exposure route	Exposure scenario	Reference
2	Bitumen fume exposed volunteers with RPE (n=10)	Dermal	8h exposure to 20 mg/m ³ of bitumen fume = 0.65 µg/m ³ pyrene	Walter & Knecht 2007
3	Intervention study with RPE of electrode paste plant workers (n=18)	Inhalation	Two weeks 5 shifts*8h exposure to 2.75 µg/m ³ pyrene	Bentsen et al, 1998
4	Individual differences among coal liquefaction workers (n=5)	Inhalation and dermal	4 shift*12h at work with 1.3 µg/m ³ pyrene. + 96h off work.	Quinlan et al, 1995



Results experiment 2: Dermal uptake of bitumen fume among volunteers (Walter & Knecht, 2007)

- Non-smoking volunteers with only shorts
- Volunteers used RPE to prevent inhalation
- 8h exposure to 20 mg/m³ bitumen fume = 0.65 µg/m³ pyrene



--- = sum of free 1-OHP and 1-OHP-glucuronide

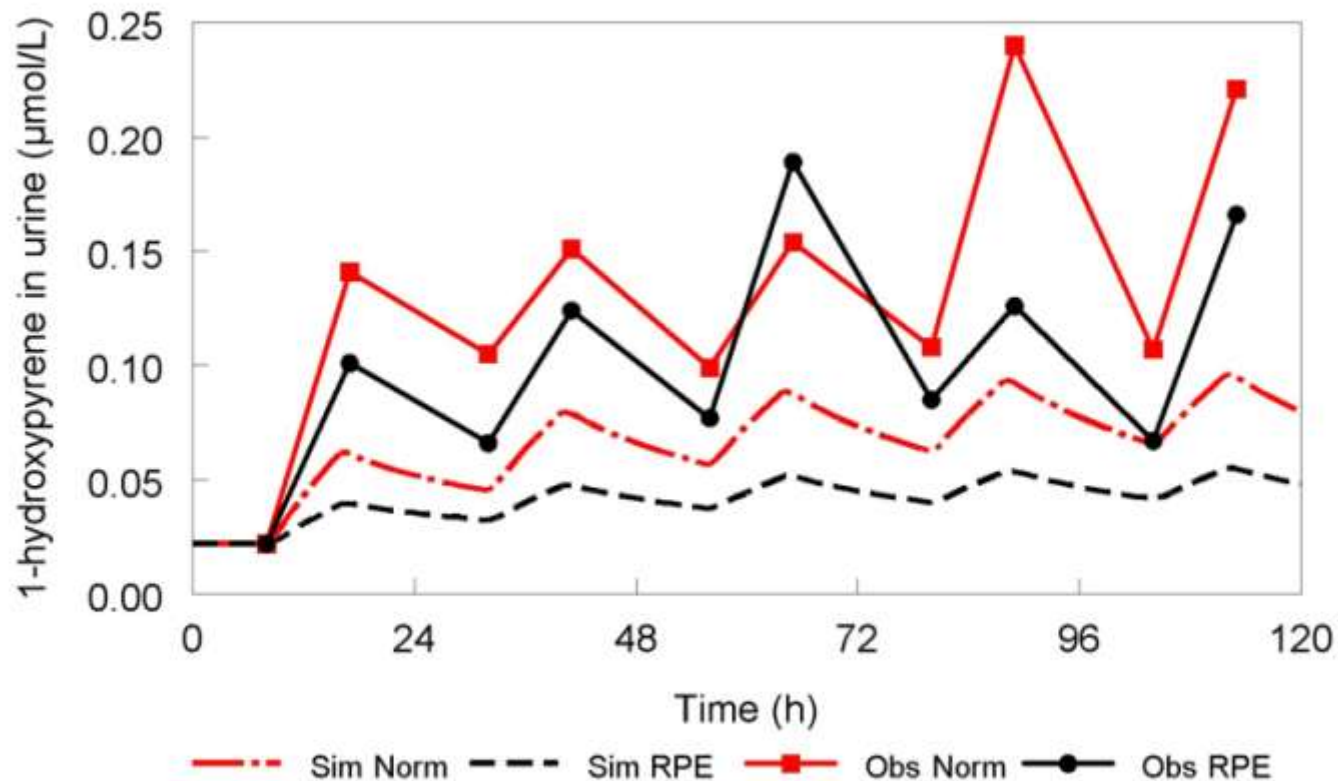
Experiment 3: Reduction of exposure after extra respiratory protection

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Results experiment 3: Reduction of exposure after extra respiratory protection (Bentsen et al, 1998)

- Pre- and postshift urine samples during 5-days working week
- **Regular RPE (red lines)** and week with **extra RPE (black lines)**
- Measured (contineous lines) and predicted (broken lines)



Dermal exposure was not measured and set at zero in simulation !

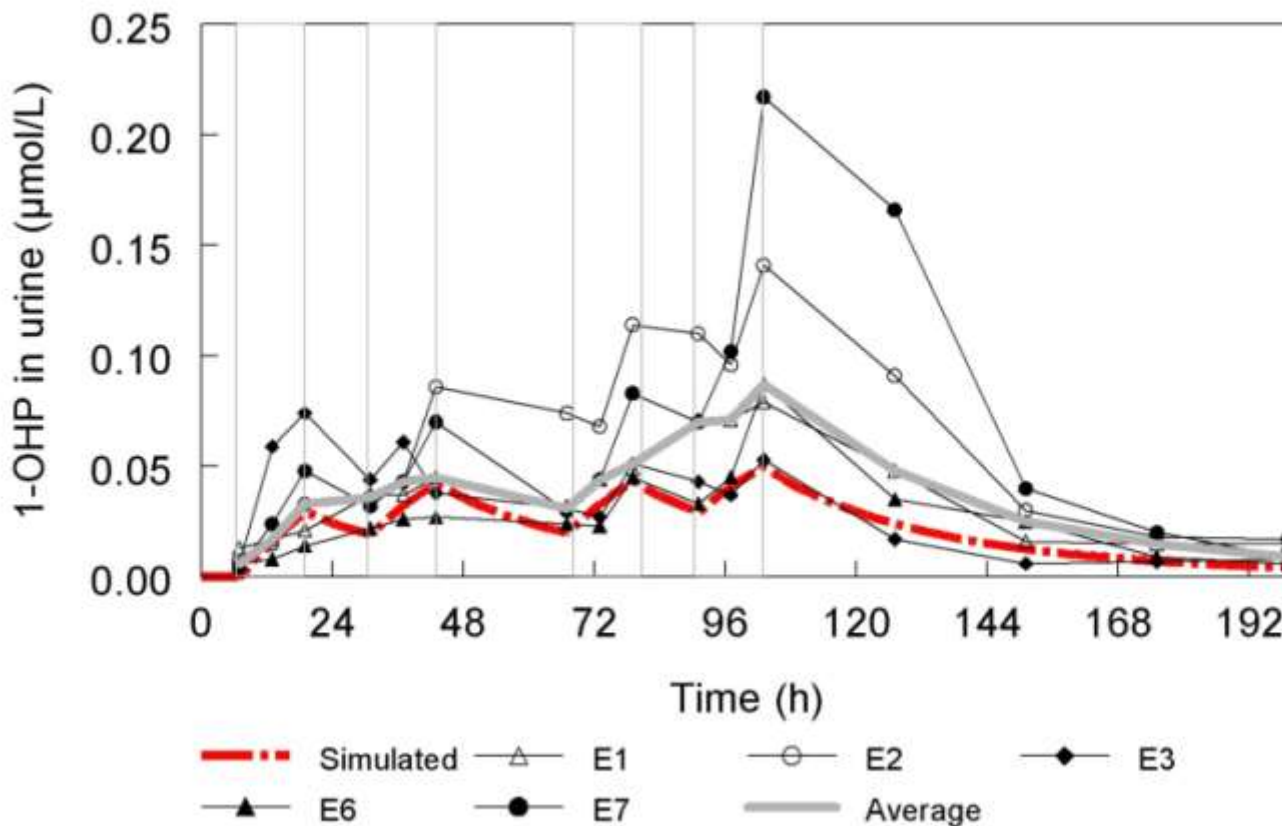
Experiment 4: Average level versus boundaries of interindividual differences

Nr.	Type of study	Exposure route	Exposure scenario	Reference
2	Bitumen fume exposed volunteers with RPE (n=10)	Dermal	8h to 20 mg/m ³ bitumen fume = 0.65 µg/m ³ pyrene	Walter & Knecht 2007
3	Intervention study with RPE of electrode paste plant workers (n=18)	Inhalation	Two weeks 5 shifts*8h exposure to 2.75 µg/m ³ pyrene	Bentsen et al, 1998
4	Individual differences among coal liquefaction workers (n=5)	Inhalation and dermal	4 shift*12h at work with 1.3 µg/m³ pyrene. + 96h off work.	Quinlan et al, 1995



Results experiment 4: Average versus interindividual differences (Quinlan et al, 2005)

- Week with 4 shifts of 12 h on work and 96 h off work
- Airborne concentrations were measured
- Black lines are experimental data, **red broken line is predicted level**



Dermal exposure was not measured and set at zero in simulation !⁴

Conclusions on quality/accuracy of PBTK- prediction of levels of pyrene metabolites in urine

- Accuracy
 - Estimated level is within the boundaries of interindividual differences
- Limitations
 - Simplified physiological structure
 - Metabolism in liver only
 - Sensitivity tests shows strong dependancy of the parameters of hepatic *in vitro* metabolism kinetics

What are the differences between this PBTK-model and other PBTK-models?

- **GENERIC MODEL**

- Partitioning in the body of the chemical/metabolite is estimated by algorithms, thus model can be used for multiple volatile and semi-volatile chemicals

- **WIDELY AVAILABLE SOFTWARE**

- The software application is running in EXCEL, a software platform that is widely available

Suggested application domain of this PBTK-model IndusChemFate

■ Pyrene/PAH

- ✓ Fine-tuning of urine sampling program
- ✓ Assessment of blood and urine levels when air concentrations are known
- ✓ Assessment of contribution of dermal uptake to body burden

■ Other volatile and semi-volatile chemicals

- ✓ *A priori* (= 1st tier) estimation of concentration in blood and/or in urine and/or in exhaled air concentrations after exposure
- ✓ Screening of absorption and fate of data-poor substances in human body
- ✓ Education of students to understand toxicokinetics of chemicals in human body

Where to get more info?

- Download **EXCEL-application file** and **user manual** from:
 - Website CEFIC LRI, on page IndusChemFate
<http://www.cefic-lri.org/lri-toolbox/induschemfate>
 - The software application is free of charge
- Info on **www.industox.nl**
- **Two papers** are submitted
- Ask for a **live-demonstration** in lobby of AIRMON

Acknowledgement

Funding from CEFIC-LRI



Thank you

Any questions?