

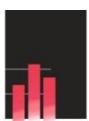
Workshop J: Is biologische monitoring overbodig geworden?

**Nieuw PBTK-model IndusChemFate voor
berekenen van bloed- en urine-concentratie na
inhalatie en/of huidblootstelling**

**Frans Jongeneelen, IndusTox Consult
Wil ten Berge, Santoxar**

Opzet workshop J

- 0-20 min: Korte introductie van model, instructie voor gebruik + voorbeeld
- 20-80 min: Laden programma en zelf oefeningen doen
- 10 min: Bespreking van oefeningen
- Wat is uw oordeel?



Overview of the PBTK-model IndusChemFate

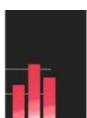
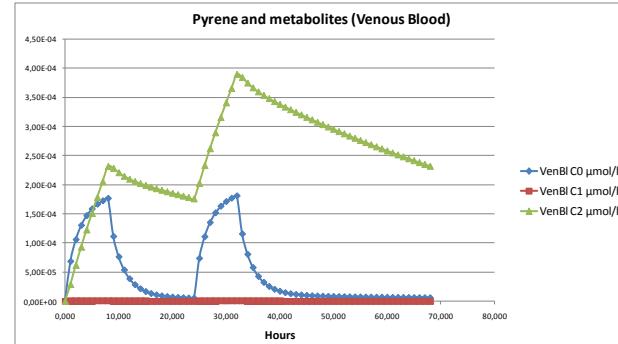
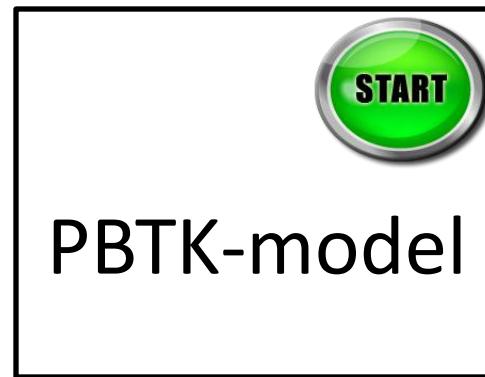
Exposure scenario

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



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

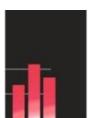
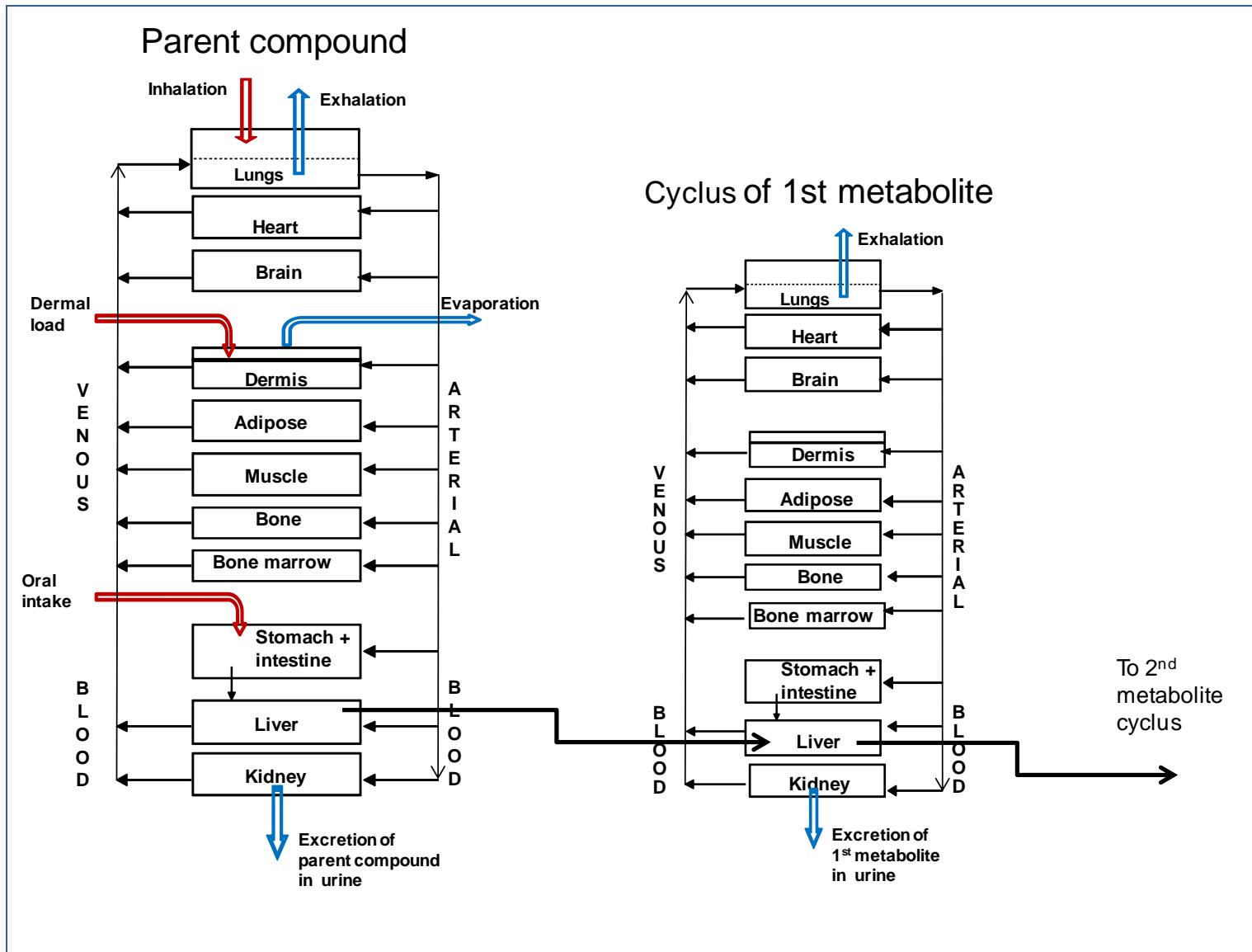


What is a PBTK-model?

- 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
- Compartments corresponds to predefined organs or tissues, with interconnections corresponding to blood
- Differential equations are 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

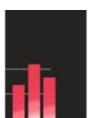
- QSPR algorithm for estimate of blood:air partitioning
- QSPR algorithm for estimate of tissue:blood partitioning

- Metabolism

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

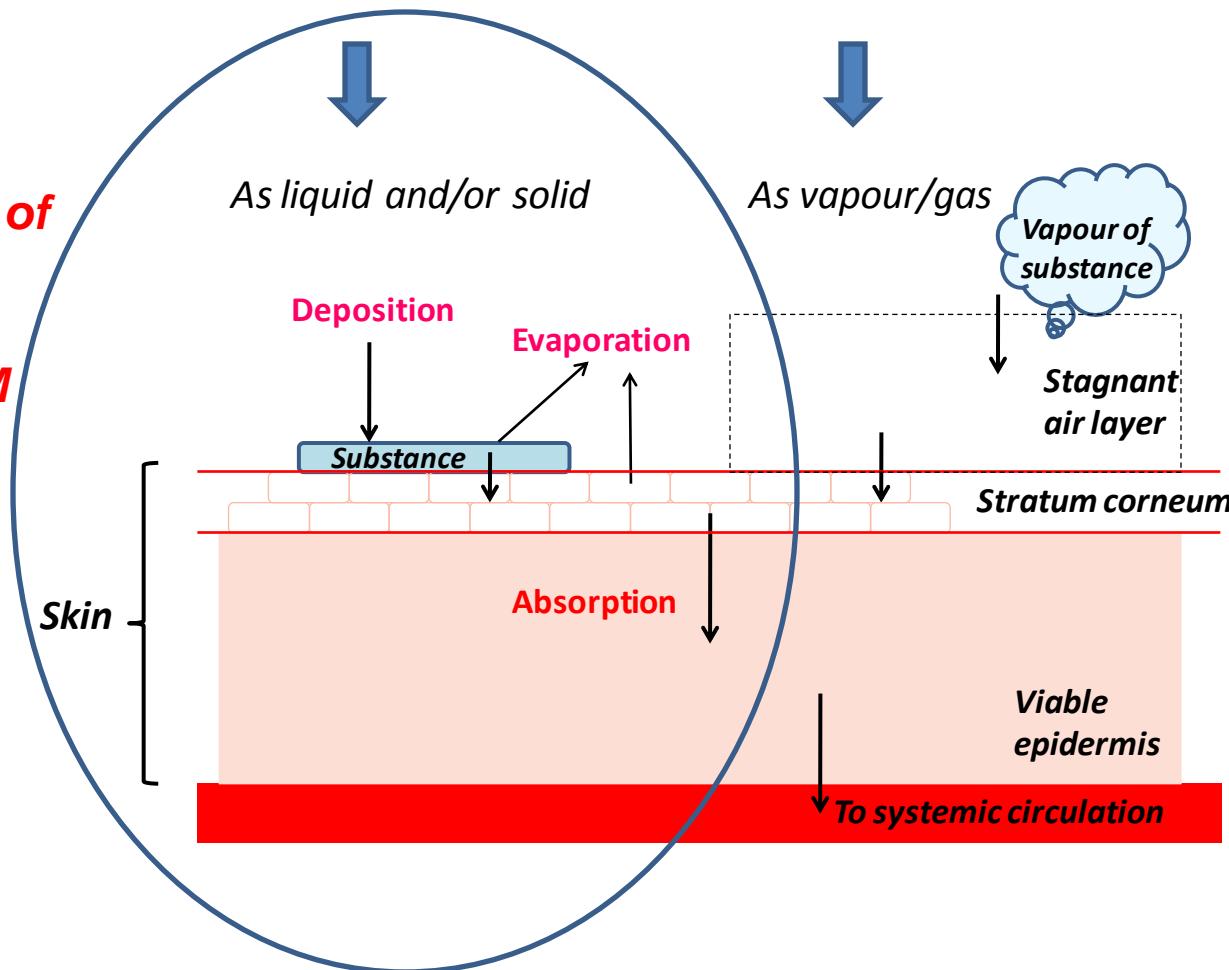
- Excretion

- Urine
- Exhaled air



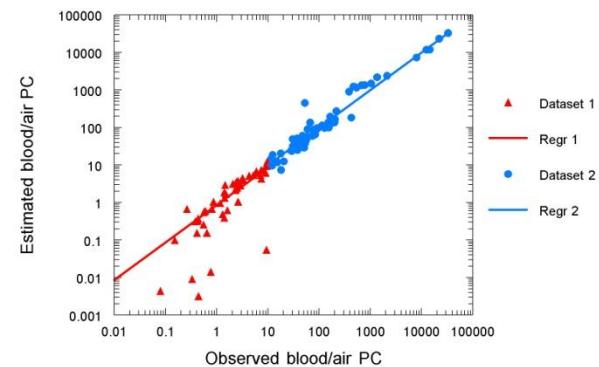
Dermal absorption module of the model

= New model of
AIHA-EASC
named
IH SKINPERM

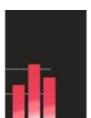


Distribution over compartments in the body

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



- Blood:tissue partition coefficient
 - QSAR 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.xls 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 example

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

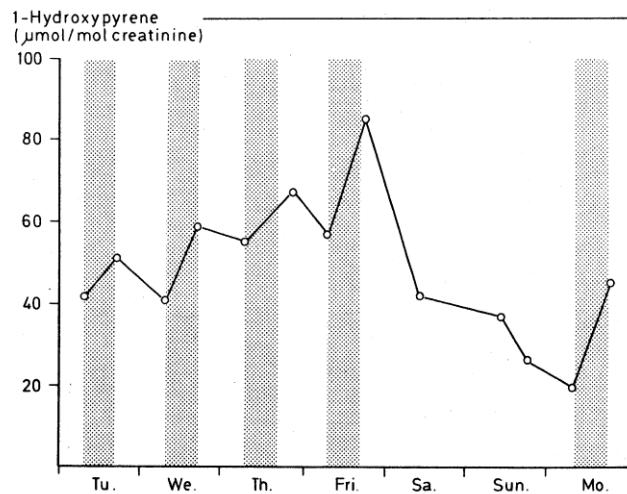
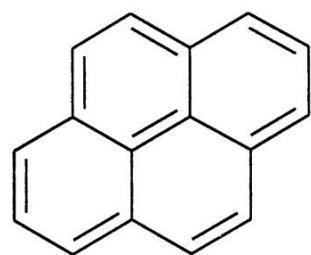


Figure 3-1A. Excretion of 1OHP in urine of a creosote impregnating worker (Jongeneelen et al, 1988)

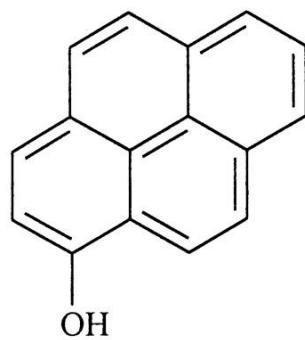
Simulation example

Metabolism of pyrene



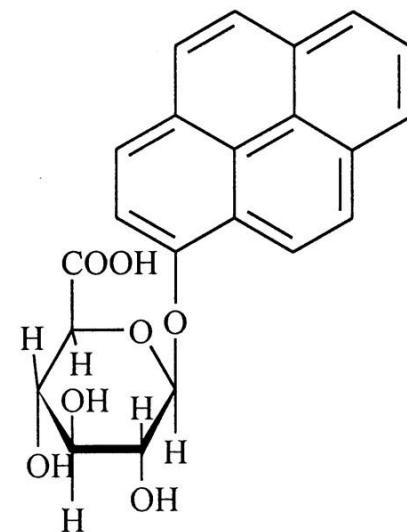
Pyrene

$\xrightarrow[\text{CYP}]{\text{NADPH, O}_2}$

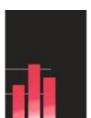


1-Hydroxypyrene

$\xrightarrow[\text{UGT}]{\text{UDPGA}}$



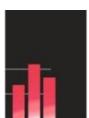
1-Pyrenylglucuronide



Simulation example

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 example

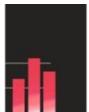
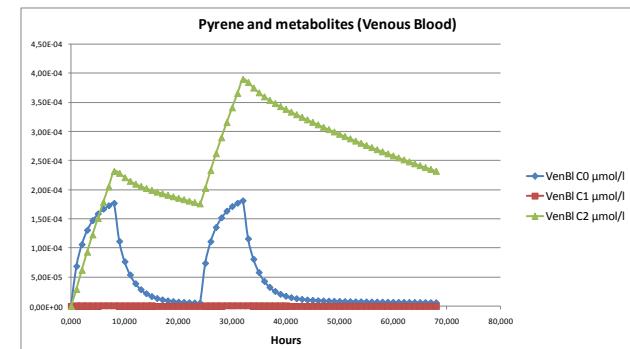
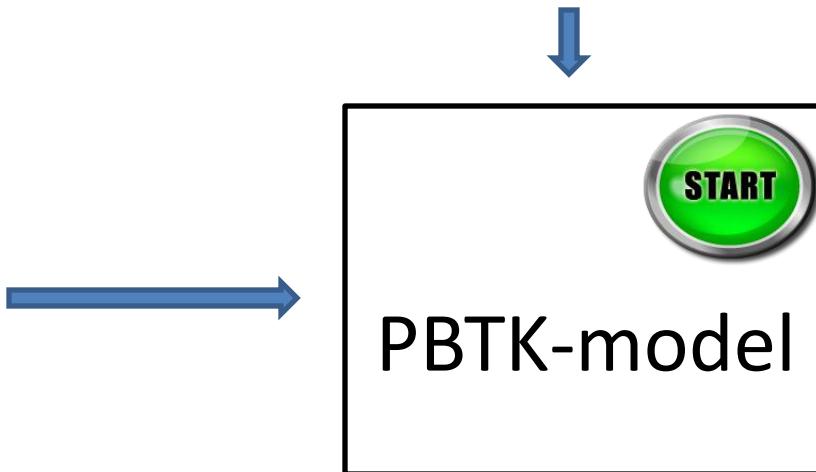
Enter compound data + Exposure scenario

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

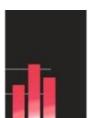
- Three routes of uptake:
 - Inhalation - concentration
 - Dermal – dose rate
 - Oral - dose
- Duration of exposure
- Personal Protective Equipment
- Physical activity level (rest/ light)



Simulation example

Data to be entered

- ✓ Phys-chemical properties and biochemical parameters
 - Parent compound: pyrene
 - Two metabolites: 1-OH-pyrene + 1-OH-pyrene-gluc
- ✓ Enter exposure scenario
 - Inhalation: concentration = 5 µg/m³, 8h/day (estimate)
 - Dermal: dose rate = 6 ng/cm², 8h/day (estimate)
 - Oral: 0 mg/kg bw



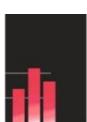
Simulation example

Pyrene

Properties of pyrene and 1-OH-Pyrene metabolites available from database-sheet

1-OH-Pyrene-glucuronide

Parent Compound	Pyrene
CAS	129-00-0
Density (mg/cm3 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
Enterhepatic 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/cm3 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
Enterhepatic 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/cm3 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
Enterhepatic 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)	



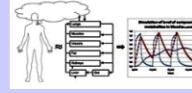
Simulation example

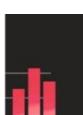
Entering exposure scenario of the creosote plant operator

Airborne
exposure
scenario

Dermal
exposure
scenario

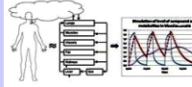
Oral intake
scenario

 Parameters Airborne Exposure	
Concentration parent compound (mg/m3)	0,003
Time of start of airborne exposure (hours)	0
Duration of airborne exposure per day (hours/day)	8
Respiratory protection factor (=> 1)	1
Dermal protection factor (air tight clothing => 1)	1
Parameters Dermal exposure to parent compound	
Skin deposition pure substance (mg/cm2/hour)	0,000006
Start of skin exposure (hours)	0
Duration of skin exposure per day (hours/day)	8
Skin temperature (centigrade)	25
Affected skin area (cm2)	7500
Parameters of oral absorption	
Bolusdose to stomach of parent compound (mg/kg bwt)	0
Time of application (time in hours)	0
Absorption rate into intestinal tissue (1/hour)	3
Setting of simulation parameters	
Select human or experimental animal subject (1-14)	3
Length of exposure period (in days)	8
Number of shifts/periods with exposure in a week (1 to 7)	5
Observation settings	
Time of start of observation (time in hours)	0
Time of end of observation (time in hours)	200
Number of steps per hour	1000
Report times per hour	1



Simulation example

Setting of species, simulation time and observation period

	
Parameters Airborne Exposure	
Concentration parent compound (mg/m3)	0,003
Time of start of airborne exposure (hours)	0
Duration of airborne exposure per day (hours/day)	8
Respiratory protection factor (=> 1)	1
Dermal protection factor (air tight clothing => 1)	1
Parameters Dermal exposure to parent compound	
Skin deposition pure substance (mg/cm2/hour)	0,000006
Start of skin exposure (hours)	0
Duration of skin exposure per day (hours/day)	8
Skin temperature (centigrade)	25
Affected skin area (cm2)	7500
Parameters of oral absorption	
Bolusdose to stomach of parent compound (mg/kg bwt)	0
Time of application (time in hours)	0
Absorption rate into intestinal tissue (1/hour)	3
Setting of simulation parameters	
Select human or experimental animal subject (1-14)	3
Length of exposure period (in days)	8
Number of shifts/periods with exposure in a week (1 to 7)	5
Observation settings	
Time of start of observation (time in hours)	0
Time of end of observation (time in hours)	200
Number of steps per hour	1000
Report times per hour	1



Simulation example

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

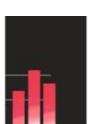
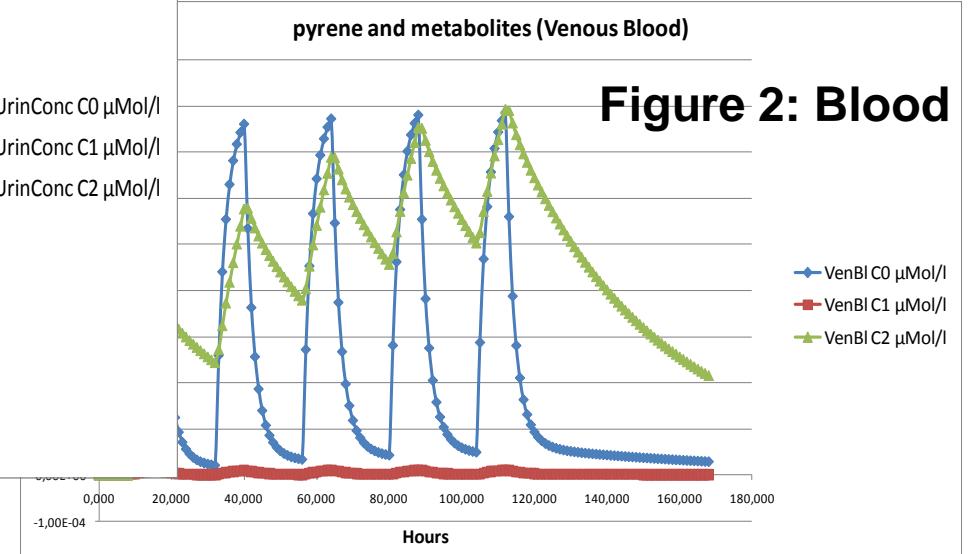
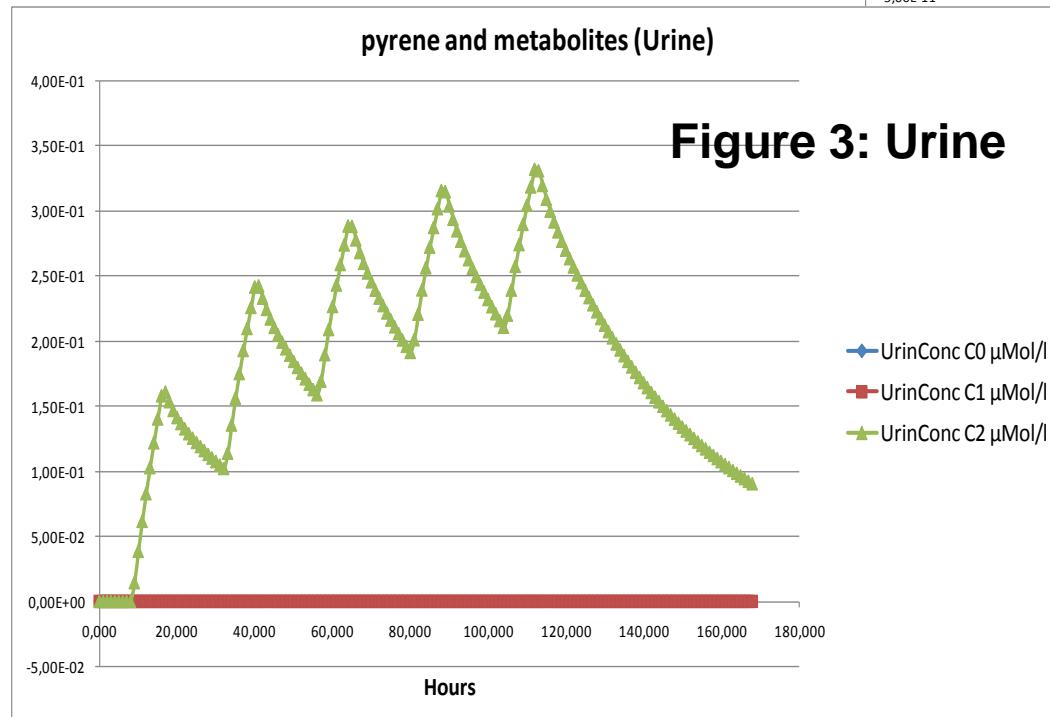
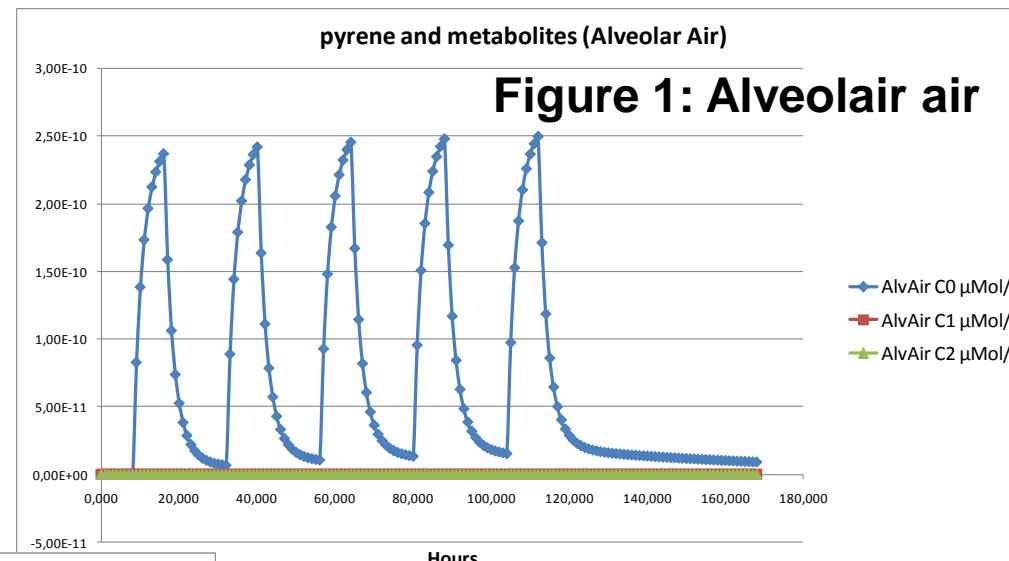
The screenshot shows a Microsoft Excel spreadsheet titled "IndusChemFile_vs1_6_20101022 [Compatibility mode] - Microsoft Excel". The spreadsheet is organized into several sections:

- Section 1: Parameters**
 - Airborne Exposure (e.g., Concentration parent compound: 246 μg/m³)
 - Start of airborne exposure (hours): 0
 - Duration of airborne exposure (hours): 2
 - Respiratory protection factor (>= 1): 1
 - Dermal protection factor (air/light clothing >= 3): 1
- Section 2: Dermal exposure to parent compound**
 - Skin deposition pure substance (mg/cm²/hour): 0
 - Start of skin exposure (hours): 0
 - Duration of skin exposure (hours): 8
 - Skin temperature (centigrade): 25
 - Affected skin area (cm²): 7500
- Section 3: Parameters of oral absorption**
 - Bolusdose to stomach of parent compound (mg/kg bwt): 0
 - Time of application (time in hours): 0
 - Absorption rate into intestinal tissue (1/hour): 3
- Section 4: Selection of model parameters**
 - Select model (1=hum, rest, 2=hum, light etc, 3=mouse 4=rat): 2
 - Repeating exposure for how many days?: 1
- Section 5: Observation settings**
 - Start of observation (time in hours): 0
 - End of observation (time in hours): 16
 - Number of steps per hour: 1000
 - Report times per hour: 2
- Section 6: Parent Compound**
 - Butoxyethanol
- Section 7: CAS**
 - 111-76-2
- Section 8: Density (mg/cm³ or grams/litre)**
 - 901.5
- Section 9: Molecular weight**
 - 118.18
- Section 10: Vapour Pressure (Pa)**
 - 117
- Section 11: Log(Kow) at skin pH 5.5**
 - 0.86
- Section 12: Log(Kow) at blood pH 7.4**
 - 0.86
- Section 13: Water solubility (mg/litre)**
 - 90100
- Section 14: Resorption tubuli (v/n?)**
 - v
- Section 15: Enterohepatic removal (relative to liver venous blood)**
 - 0
- Section 16: Vmax Adipose tissue (parent/total) μmol/kg tissue/hr**
 - 4.1
- Section 17: Km Adipose tissue (parent/total) μmol/litre**
 - 1.53E-01
- Section 18: Vmax Bone (parent/total) μmol/kg tissue/hr**
 - 4.1
- Section 19: Km Bone (parent/total) μmol/litre**
 - 1.53E-01
- Section 20: Vmax Brain (parent/total) μmol/kg tissue/hr**
 - 4.1
- Section 21: Km Brain (parent/total) μmol/litre**
 - 1.53E-01
- Section 22: Vmax Heart (parent/total) μmol/kg tissue/hr**
 - 4.1
- Section 23: Km Heart (parent/total) μmol/litre**
 - 1.53E-01
- Section 24: Vmax Kidney (parent/total) μmol/kg tissue/hr**
 - 4.1
- Section 25: Km Kidney (parent/total) μmol/litre**
 - 1.53E-01
- Section 26: Vmax Intestines (parent/total) μmol/kg tissue/hr**
 - 4.1
- Section 27: Km Intestines (parent/total) μmol/litre**
 - 1.53E-01
- Section 28: Vmax Liver (parent/total) μmol/kg tissue/hr**
 - 5680
- Section 29: Km Liver (parent/total) μmol/litre**
 - 200
- Section 30: Vmax Lung (parent/total) μmol/kg tissue/hr**
 - 4.1
- Section 31: Km Lung (parent/total) μmol/litre**
 - 1.53E-01
- Section 32: Model (parent/total) μmol/kg tissue/hr**
 - 4.1
- Section 33: Exposure Conditions+Calculation**
- Section 34: Database**
- Section 35: Graphical Output**

Simulation example

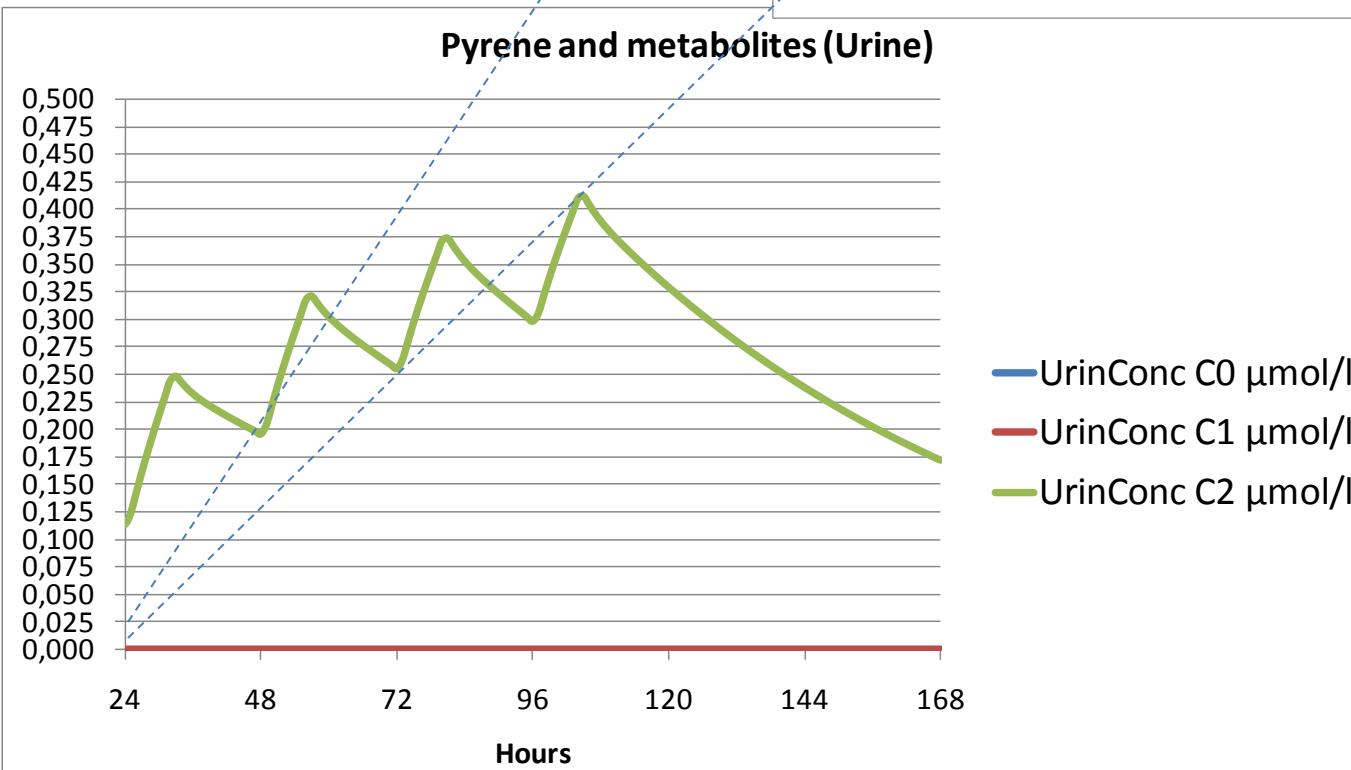
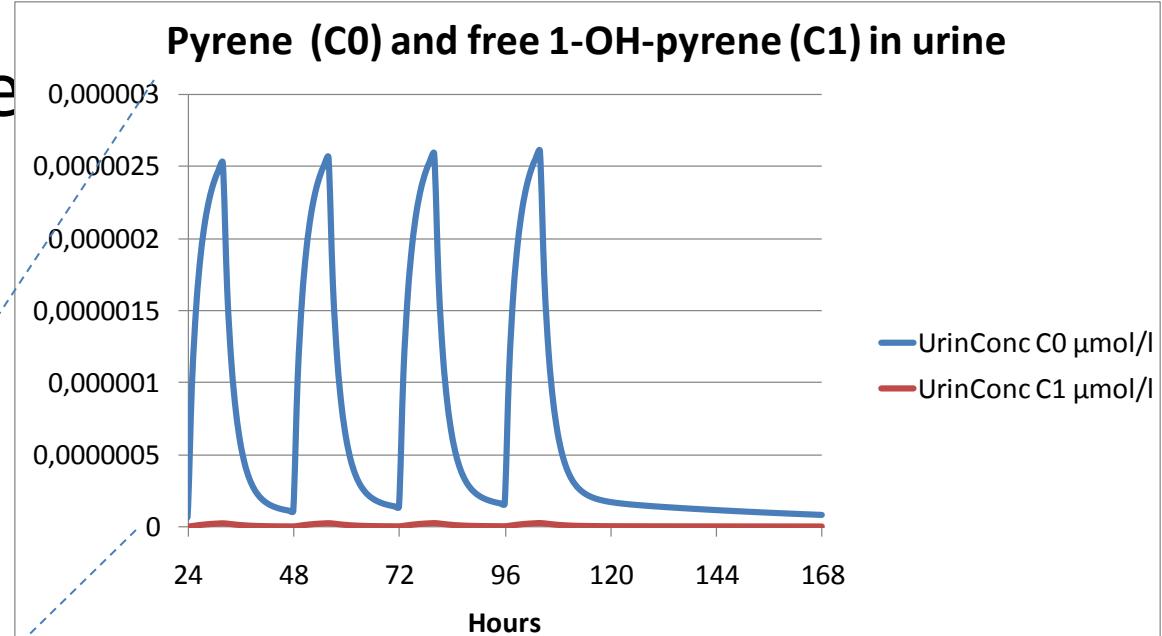
Run program -

Results appear also as graphs



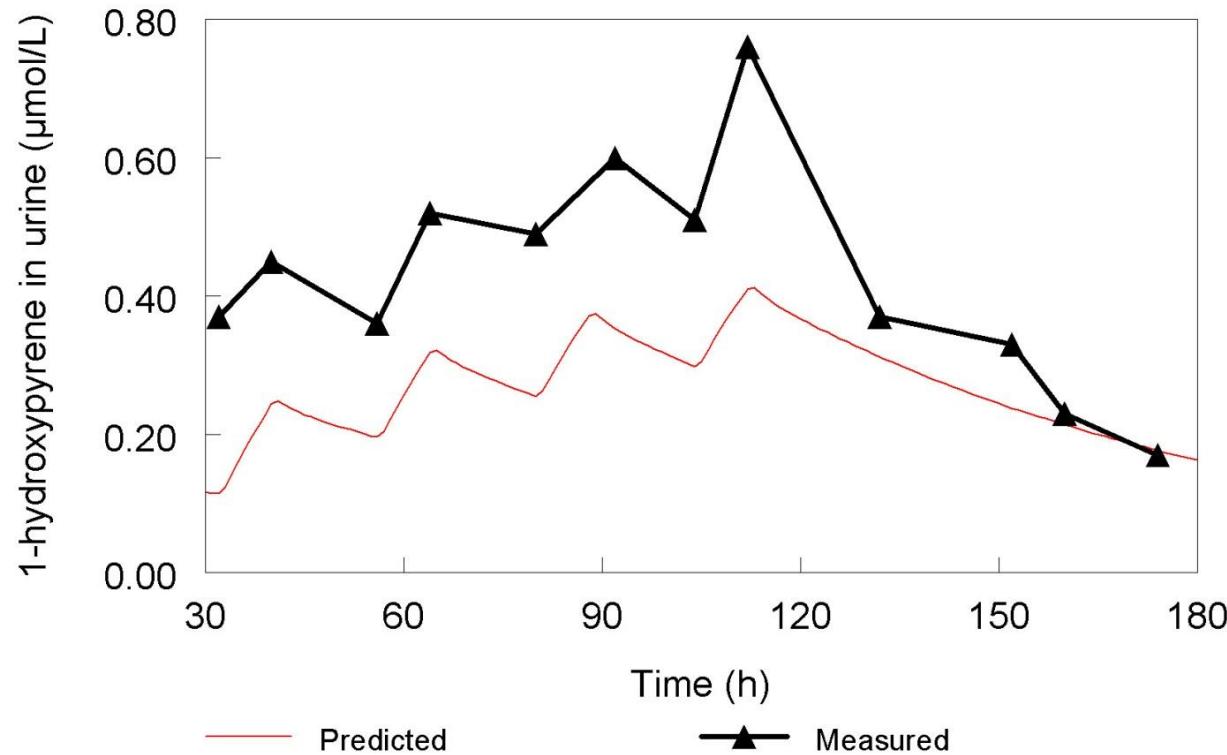
Simulation Example

Results: level 1-OH-pyrene in urine



Simulation example

Comparison of measured and predicted level of 1-OH-pyrene in urine of creosote plant operator

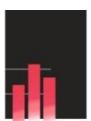


Level is expressed as sum of free 1-OHP and 1-OHP-glucuronide

Oefeningen - zelf simulaties doen

Nabootsen van metingen van 1-OH-pyreen in urine bij bekende blootstelling

- ✓ Excel-file IndusChemFate.xls laden
- ✓ Samen 1^e oefening doen
- ✓ Dan zelf oefening 2 t/m 4



Laden Excel-file Induschemfate

1. Laad de Excel applicatie:

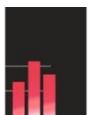
IndusChemFate model 2.00

vanaf website CEFIC LRI, page IndusChemFate

<http://www.cefic-lri.org/lri-toolbox/induschemfate>

Let op: na openen Excel-file induschemfate.xls

- ✓ Macro's aanzetten
- ✓ Check of de stof pyrene is geselecteerd



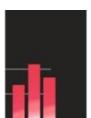
Samen 1^e oefening doen

Oefening 1: Bereken het verloop van de urineconcentratie 1-hydroxypyreen van de creosoteerder over een werkweek + weekend

Op werkdagen is blootstellingsscenario:

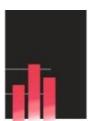
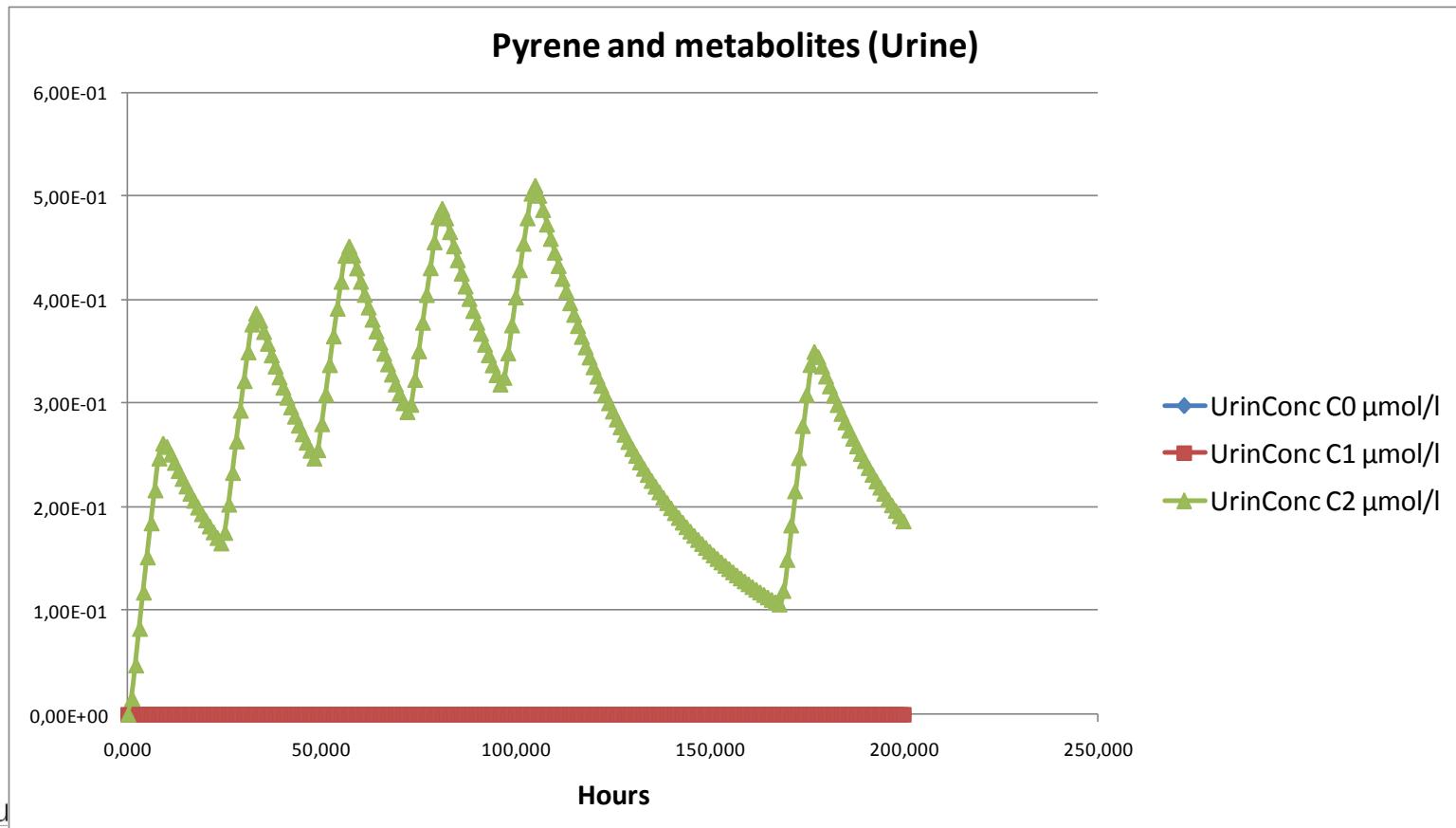
- Inhalatie: 3 µg pyreen/m³, 8h
- Huidblootstelling: depositie snelheid = 6 ng pyreen/cm²/uur op 7500 cm² huid

Uitvoering van de oefening wordt geprojecteerd op het scherm



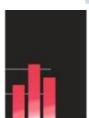
Resultaat 1^e oefening

1. Figuur met verloop van pyreen en metabolieten in urine
2. C0 = pyrene, C1 = vrij hydroxypyrene, C2 = 1-hydroxypyrene-glucuronide



Volgende oefeningen

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

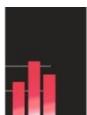
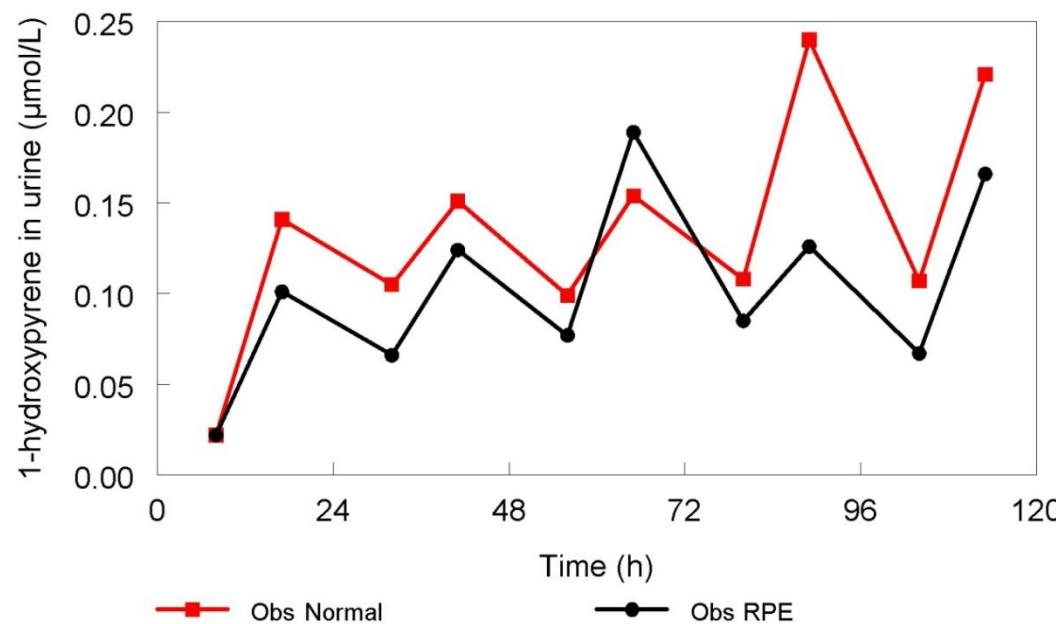


Oefening 2. Simulate results of intervention study with RPE of electrode paste plant workers

Working week = 5 shifts * 8h exposure. Average pyrene level $2.75 \mu\text{g}/\text{m}^3$.

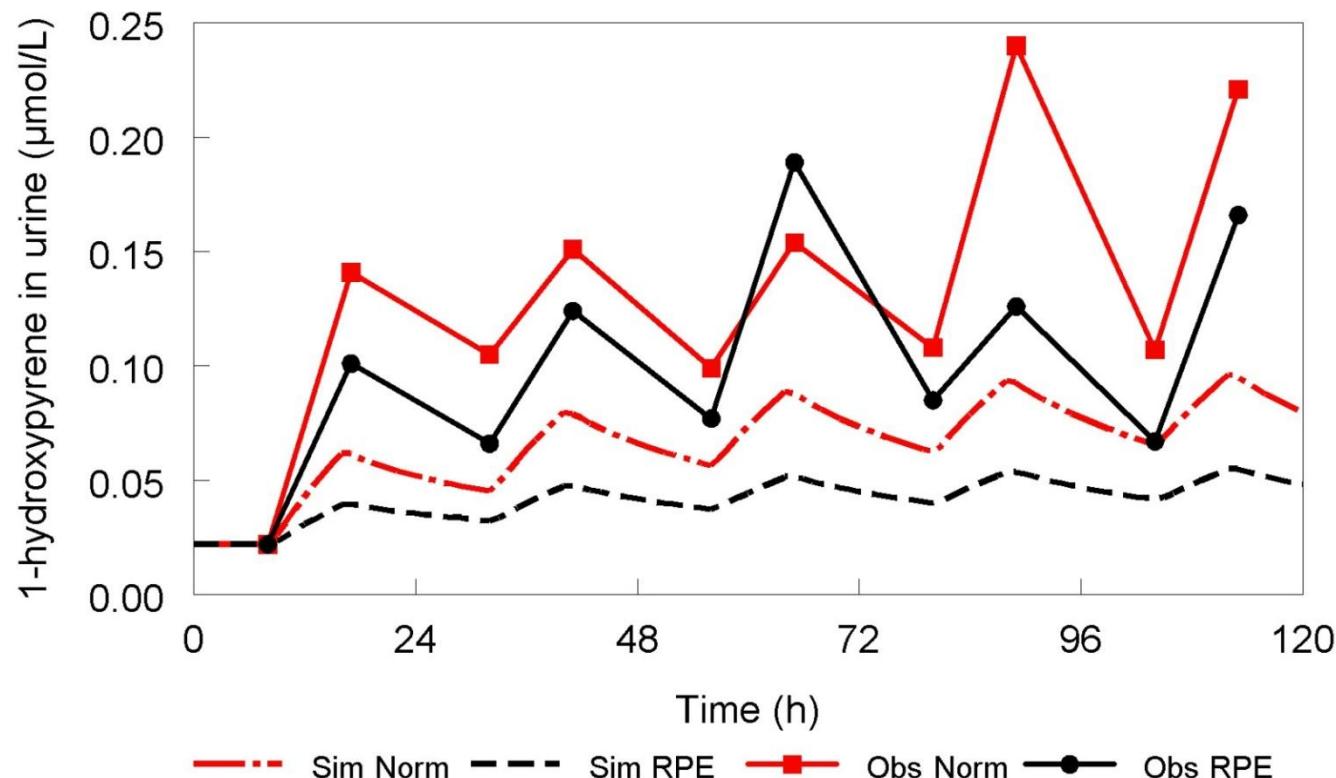
One week normal, one week extra RPE. Reduction factor extra RPE unknown

Figure. Measured time course of 1-hydroxypyrene in urine



Resultaat oefening 2

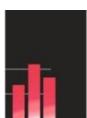
- Pre- and postshift urine samples during 5-days working week
- **Regular RPE (red lines)** and week with **extra RPE (black lines)**
- Measured (continuous lines) and predicted (broken lines)



Dermal exposure was not measured and set at zero in simulation !²⁸

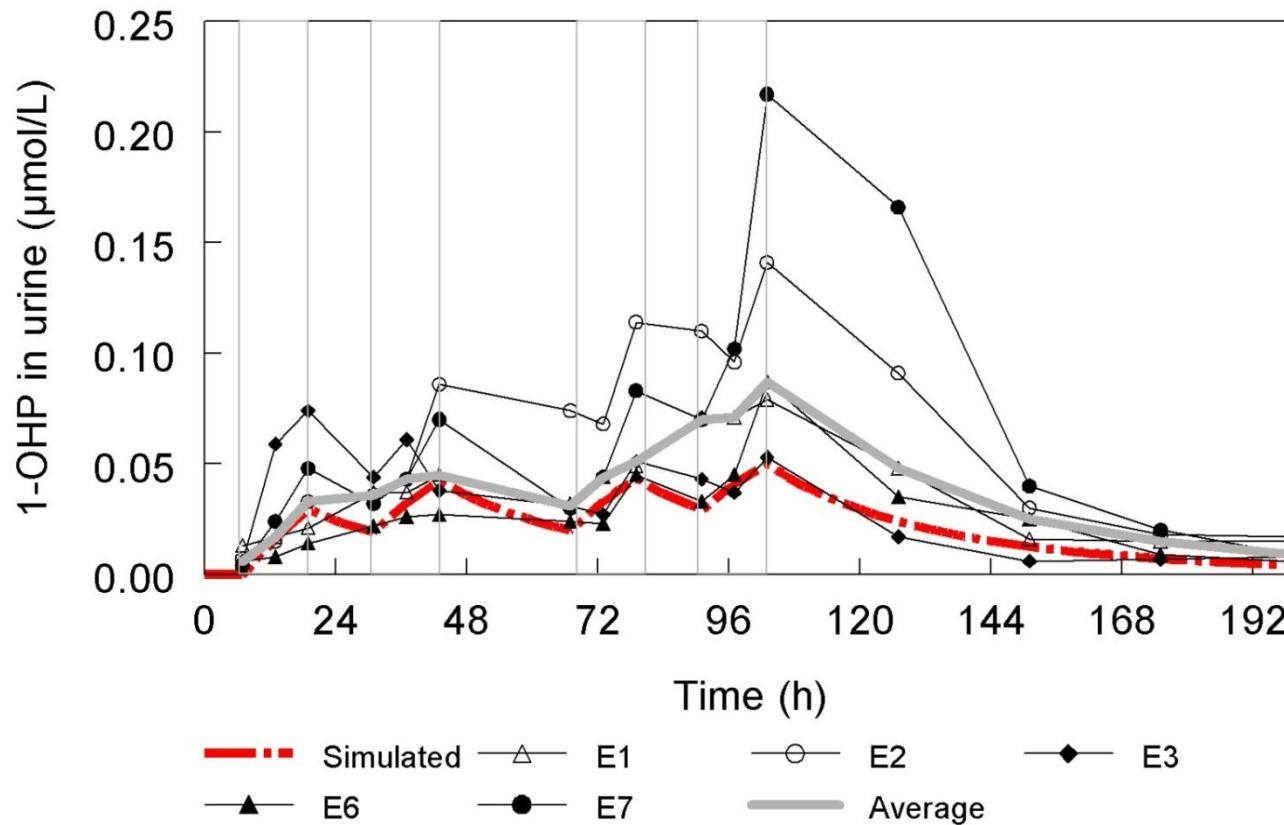
Oefening 3: coal liquefaction workers

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



Results oefening 3: Average versus interindividual differences (Quinlan et al, 2005)

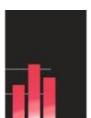
- Week with 4 shifts of 12 h on work and 96 h off work
- Airborne concentrations were measured
- Black lines are experimental data, red broken line is predicted level



Dermal exposure was not measured and set at zero in simulation !⁰

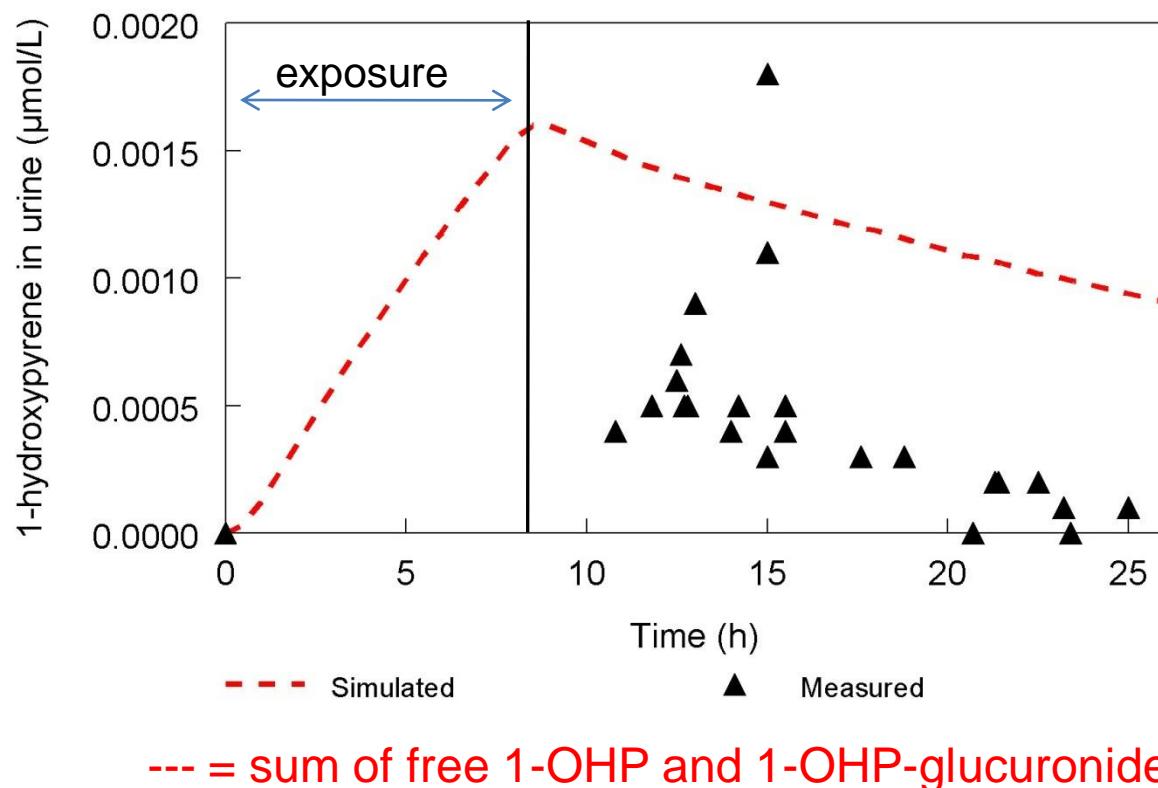
Oefening 4 : Bitumen fume exposed volunteers

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



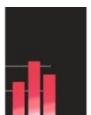
Resultaat oefening 4: Dermal uptake of bitumen fume among volunteers (Walter & Knecht, 2007)

- 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



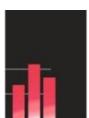
Conclusions on quality/accuracy of PBTK-prediction of levels of pyrene metabolites in urine

- 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



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 **user manual** from:
 - Website CEFIC LRI, on page IndusChemFate
<http://www.cefic-lri.org/lri-toolbox/induschemfate>
- **Two recent papers** are available:
 - Jongeneelen & ten Berge. Annals Occupational Hygiene, 2011
 - Jongeneelen & ten Berge. Int Arch Occup Environ Health , first online 2011

