Alternatives to Animal Testing in Toxicological Risk Assessment

Experimental Toxicology - now and in the future

Bas J. Blaauboer
Doerenkamp-Zbinden Chair
Institute for Risk Assessment Sciences (IRAS)
Utrecht University, the Netherlands

Risk assessment of chemicals

- hazard identification
- hazard characterisation (dose-response)
- exposure assessment
- risk characterisation

Objectives toxicity studies

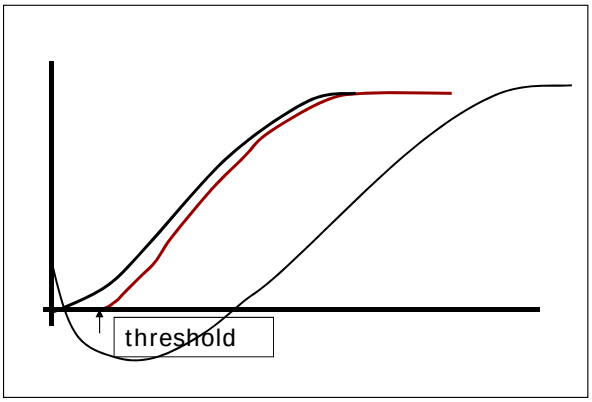
- type of adverse effect (classical protocol toxicology)
- (non) effective dose level (classical protocol toxicology)
- mechanisms
- significance for humans (risk assessment)

toxicological risk assessment

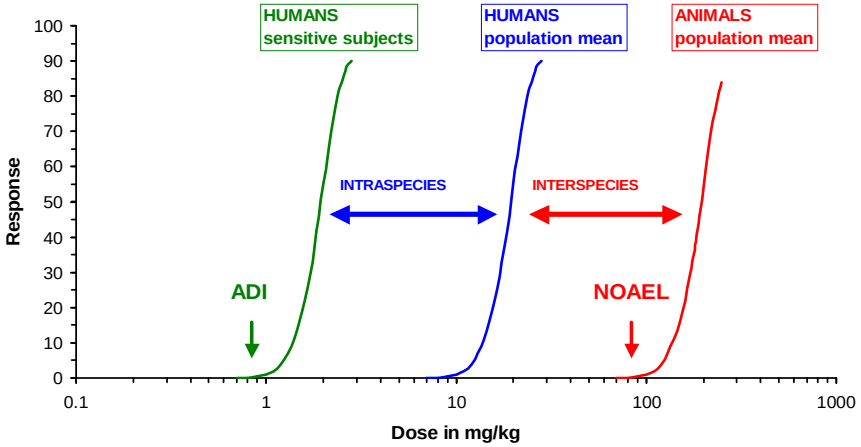
- "Classical:"
- animal experiments: quantification (LD50, NOEL)
- extrapolation to human situation (safety factors)
- threshold vs. non-threshold extrapolation approaches
- establish safety standards for human exposure

- uncertainties
- mechanisms of action (in vitro methods)

DOSE-EFFECT RELATIONSHIPS



Hypothetical dose response curves

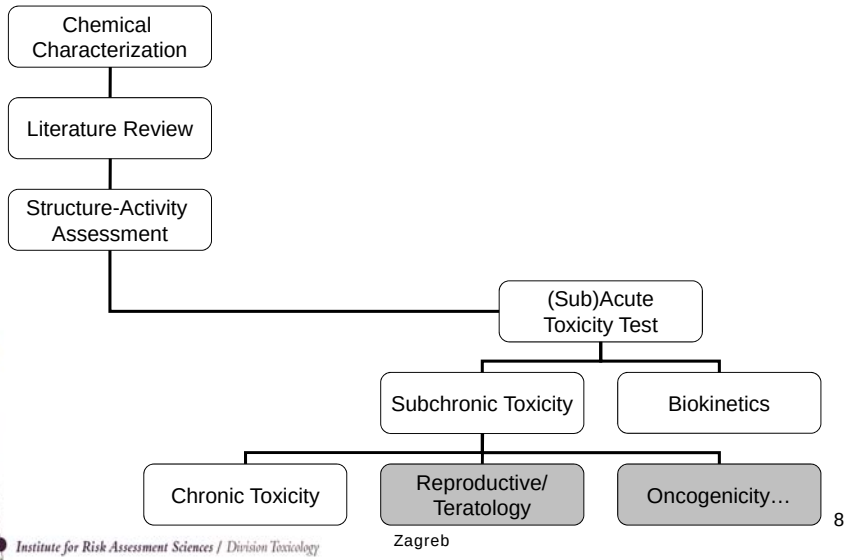


Animal studies

- acute toxicity: (LD_{50}, other acute tests)
- subchronic tests (28 or 90 days)
- chronic studies (two-year rat test)

- reproductive toxicity studies (fertility, developmental tox, teratology)
- genotoxicity (mutagenesis, carcinogenesis)
- specialised studies: biotransformation, organ toxicity, etc.

Tiered Testing Scheme



Risk Assessment: does it need redefinition?

Toxicity Testing: problem redefinition?

5.4 million animals + 950 million € extra / year
(Hartung and Rovida 2009 Nature 460: 1080-1081)

Ethics

- Toxicology 10% of animal tests
- Call for animal-rights and welfare
- Legal obligations (e.g. 7th Amendment to EU Cosmetics Act)

Costs

Science

- Species extrapolation
- Precision/variability
- Black-box models

From Casaretts and Doull's Toxicology (2008)

Cost concerns

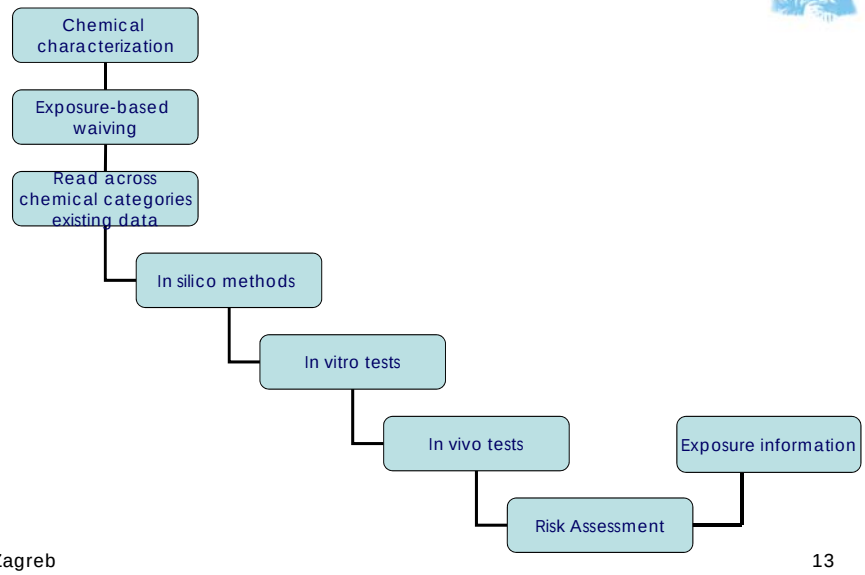
Type of Toxicity Study	Test System	Approximate cost/study (US$)
Acute oral	Rats	2000
Acute dermal	Rabbits	1500
Acute inhalation	Rats	5000
Ocular	Rabbits	1500
Skin irritation	Rabbits	100
Sensitization	Guinea pigs	3000
Neurotoxicity	Hens/rats	25,000
Mutagenicity	In vivo/in vitro	5000
Carcinogenicity/chronic toxicity	Rats Dogs	1,400,000
Reproduction & teratogenicity	Rats/rabbits	505,000
Toxicokinetics	Rats/mice	100,000

Toxicological risk assessment

"Classical" *in vitro* toxicology:

- finding concentrations (not dose)
- need to extrapolate to intact organism
- lack of biotransformation/ kinetics
- not focused on relevant biomarkers
- concentrated on cytotoxicity, rather than on mechanisms of importance in vivo

Integrated Testing Strategies (ITS)



THRESHOLD FOR TOXICOLOGICAL CONCERN

- **TTC principle is derived from FDA's Threshold of Regulation approach for food contact materials**
  - **Dietary concentration below 0.5 ppb (= 1.5 μg/person/day) is so negligible that it presents no public health concern**
  - **"Exempt from regulation"**

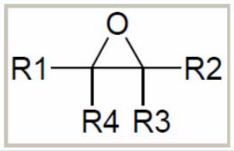
Structure Activity Relationships

Chemical Characterization

Literature Review

Structure-Activity Assessment

- Toxic effect related to molecular structure
- Similar toxicities within chemical category



Reprotoxic potential

(Sub)Acute Toxicity Test

Subchronic Toxicity

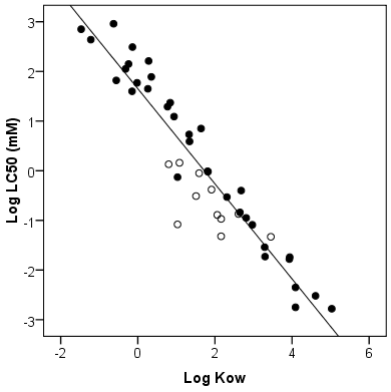
Biokinetics

Chronic Toxicity

Reproductive/ Teratology

Oncogenicity…

QSAR to Predict Baseline Fish Acute Toxicity

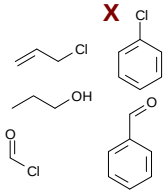


● Non-polar narcosis
○ Polar narcosis

Log LC_{50} (mM) = -0.94 Log *Kow* + 1.57 (R^2 0.89)

Quantitative Structure Activity Relationship (QSAR)

chemical structure or properties
X



fate or effect properties
Y

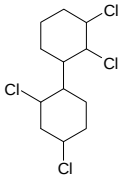
biodegradation rates
no-effect concentrations
soil sorption
bioaccumulation

quantitative model relating Y to X (QSAR)
Y = f (X)

prediction of fate or effect properties
from chemical structure for related chemicals

Activity of a chemical depends on:

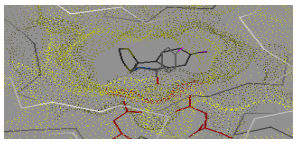
hydrophobicity / solubility in water



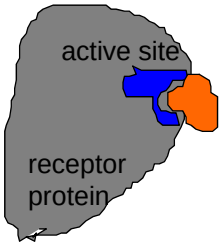
tetrachlorobiphenyl

methanol

size



electronic parameters: charge



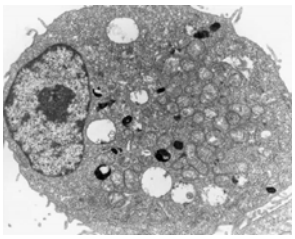
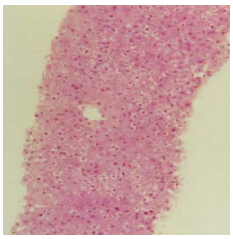
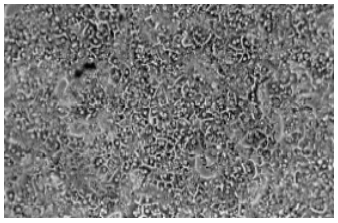
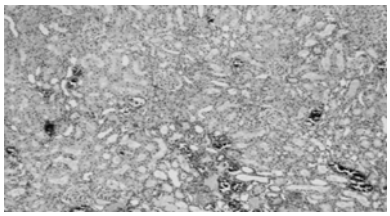
Quantitative structure-activity relationship: properties

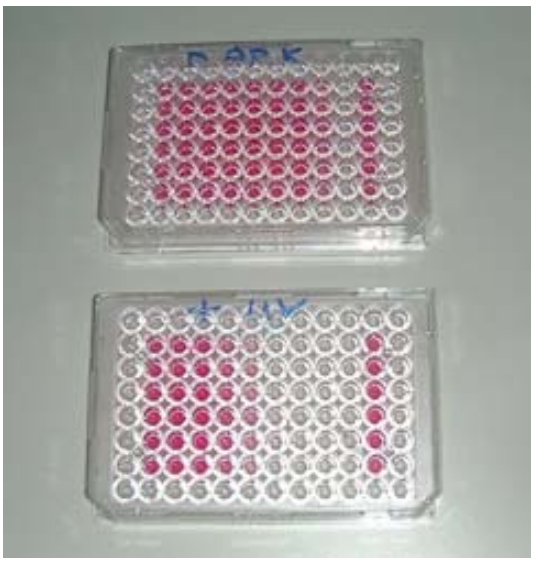
Hydrophobic parameters

Aqueous solubility
Octanol-water partition coefficient (K_{ow})
Total Surface Area (TSA)
Total Molecular Volume (TMV)

In Vitro Tests

In Vitro Test Systems



Paradigm shift:

- toxicity is determined by:
- the *critical* concentration and time of exposure (dose metric) to
- the *critical* compound (metabolite?) on
- the *critical* site of action

Toxicity testing: a more efficient approach.

- Health Council of the Netherlands. The Hague: Health Council of the Netherlands, 2001; publication no. 2001/24E.
- ISBN: 90-5549-415-1
- http://www.gr.nl/ (go to reports, 20 November 2001)

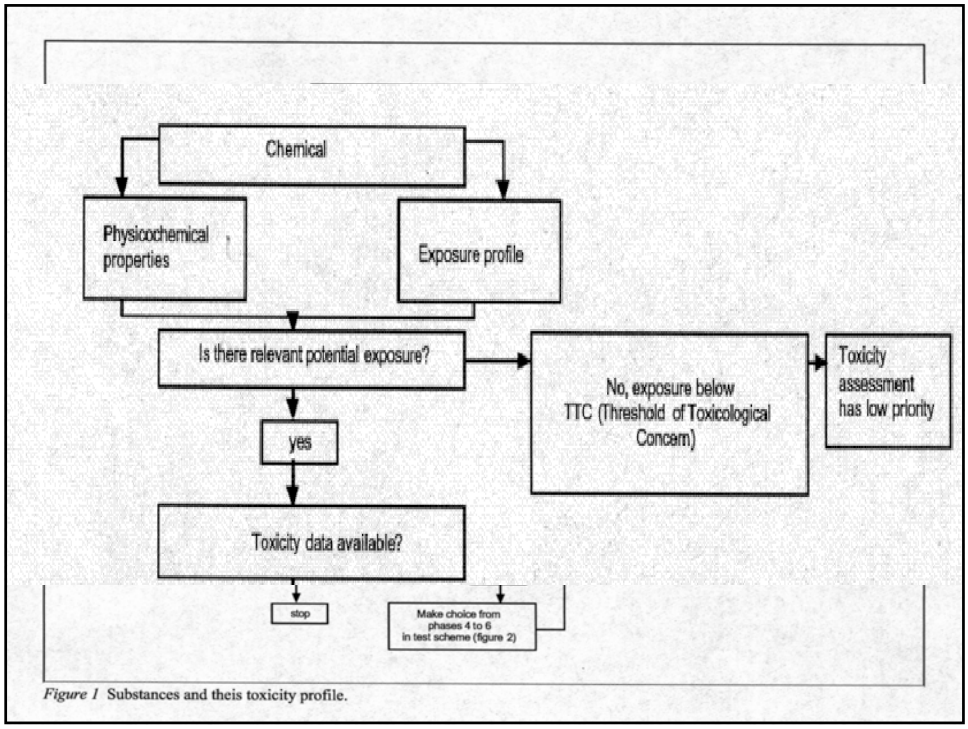


Figure 1 Substances and theis toxicity profile.

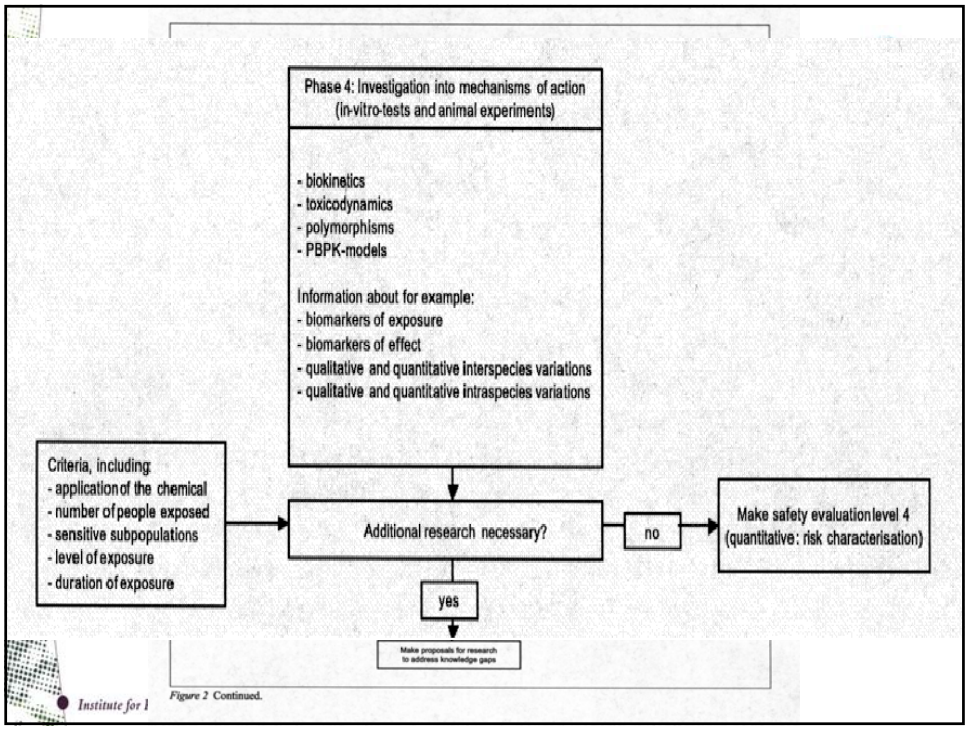


Figure 2 Continued.

Toxicity Testing in the 21st Century: A Vision and Strategy

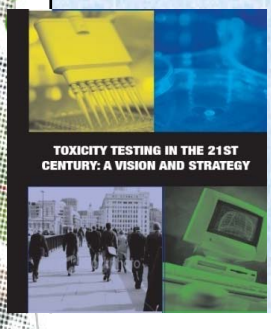
Committee on Toxicity Testing and Assessment of Environmental Agents

Board on Environmental Studies and Toxicology

Institute for Laboratory Animal Research

Division on Earth and Life Studies

National Research Council



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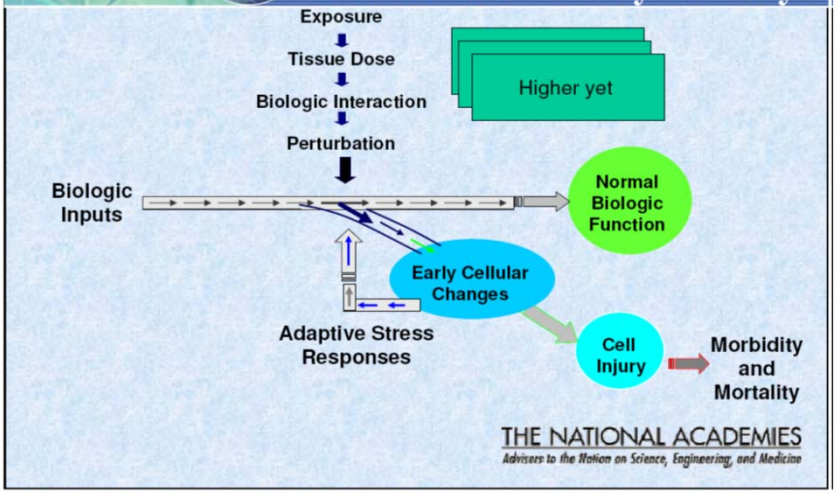
Advisers to the Nation on Science, Engineering, and Medicine

A New Paradigm: Activation of Toxicity Pathways



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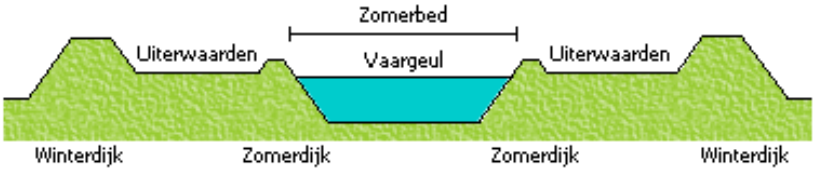
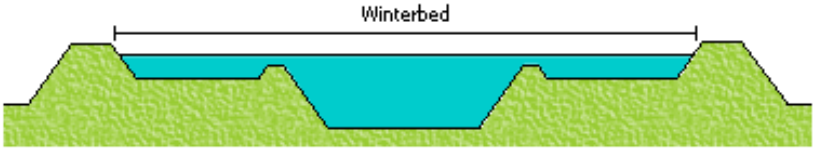
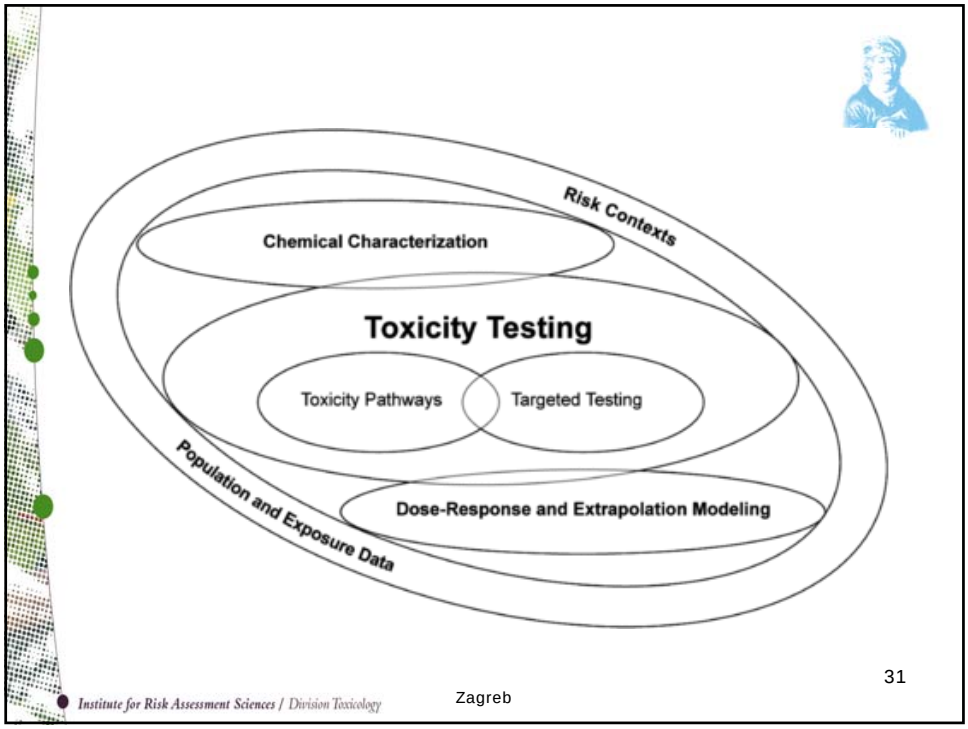
It's all about homeostasis:
keeping within the boundaries



Zomersituatie



Wintersituatie


Risk Contexts
Chemical Characterization
Toxicity Testing
Toxicity Pathways
Targeted Testing
Dose-Response and Extrapolation Modeling
Population and Exposure Data
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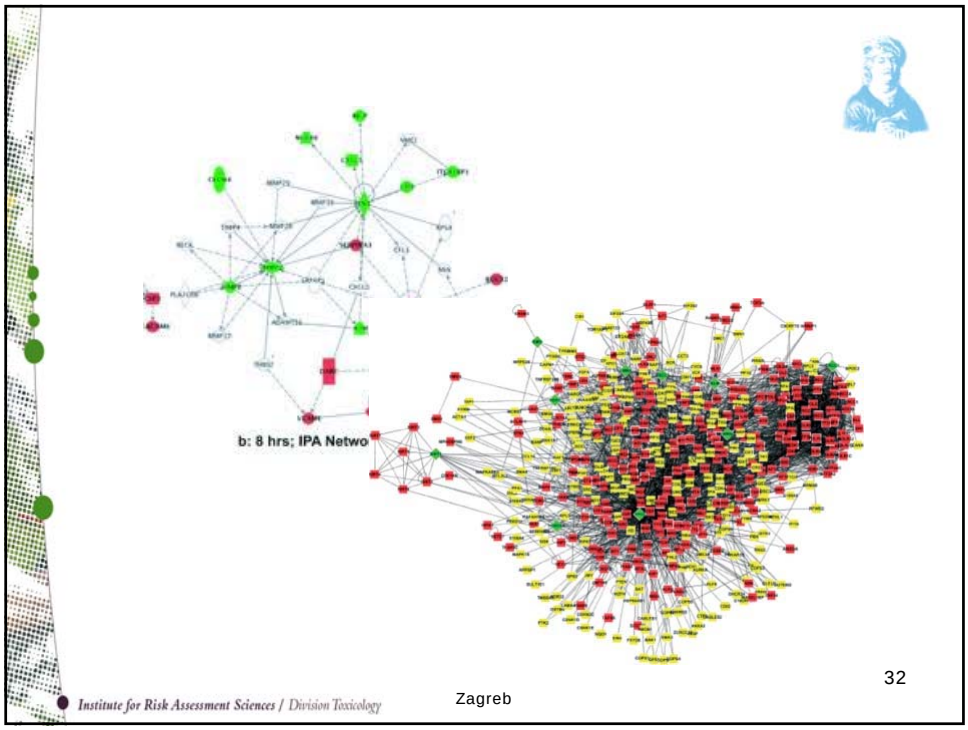
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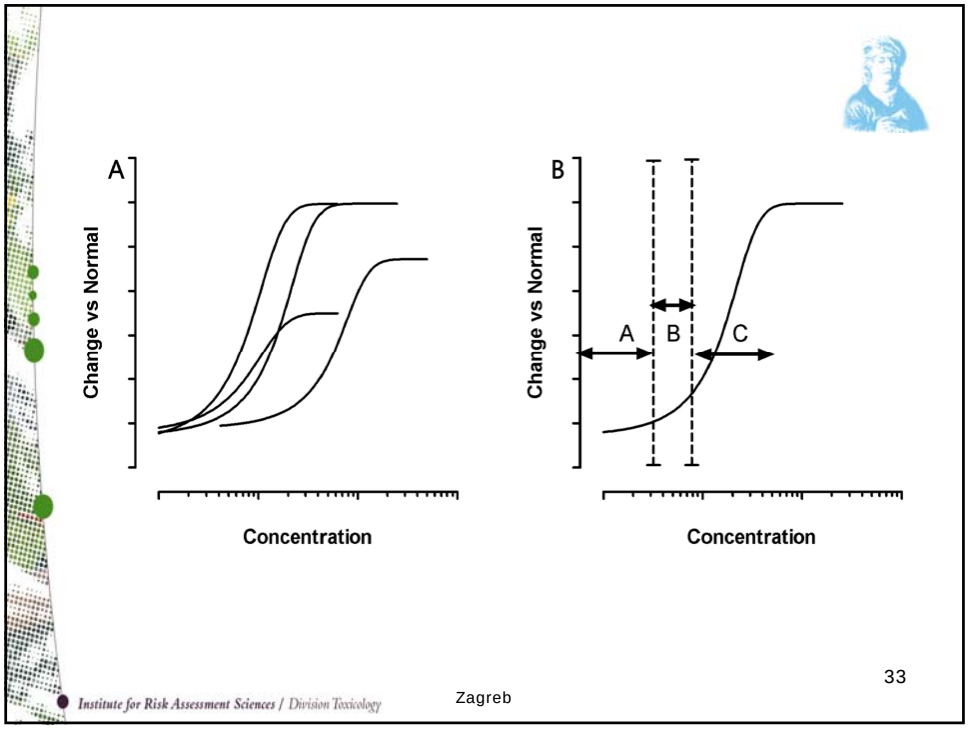
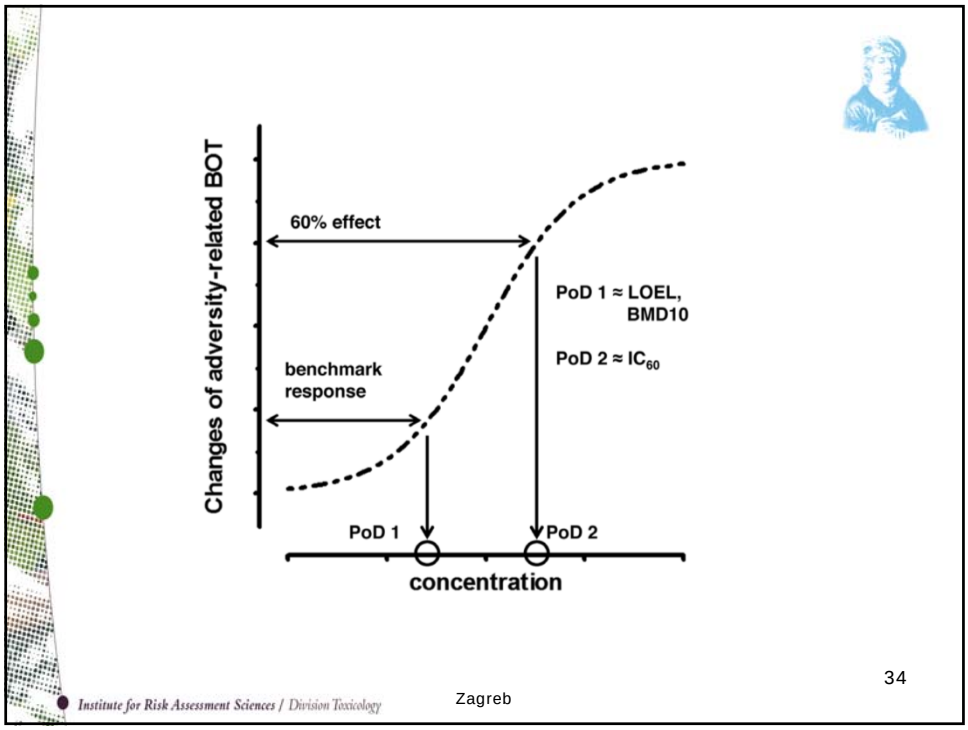
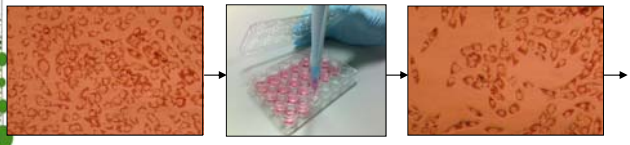
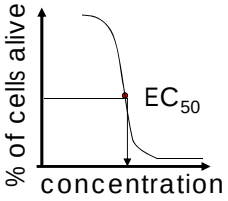

A
Change vs Normal
Concentration
B
Change vs Normal
A
B
C
Concentration


Changes of adversity-related BOT
60% effect
PoD 1 ≈ LOEL, BMD10
PoD 2 ≈ IC60
benchmark response
PoD 1
PoD 2
concentration

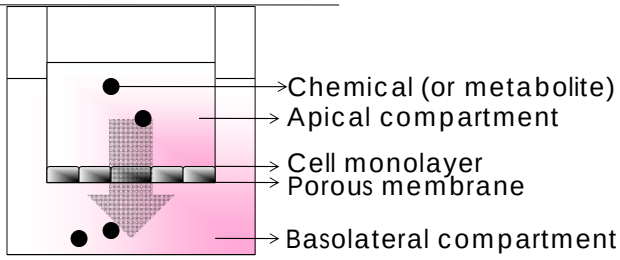
In Vitro Tests

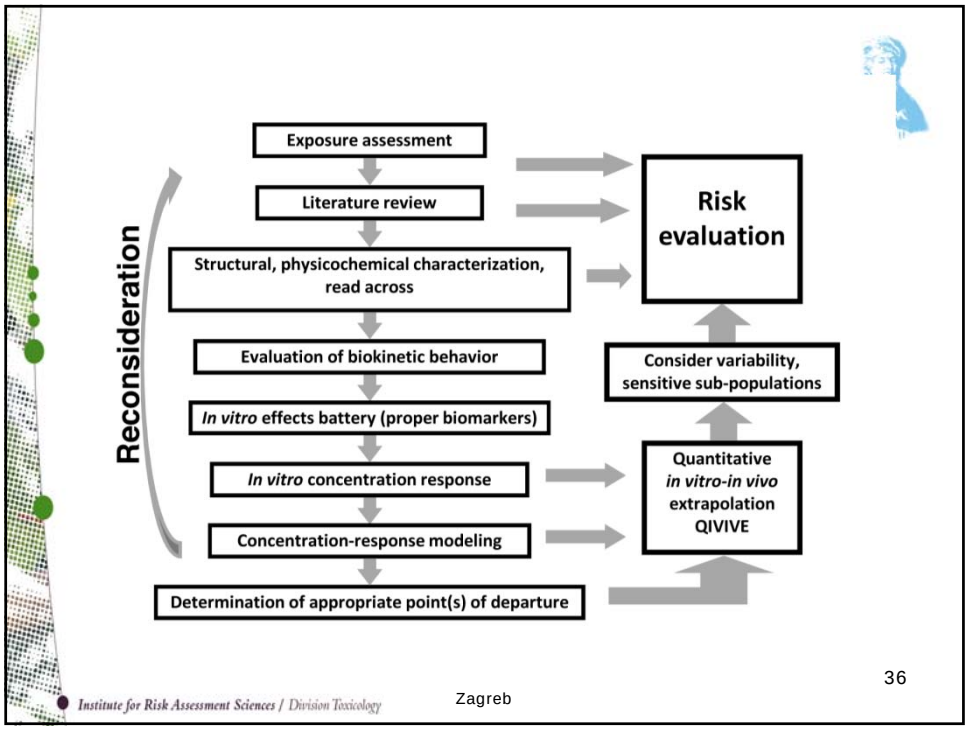
- In toxicity testing…



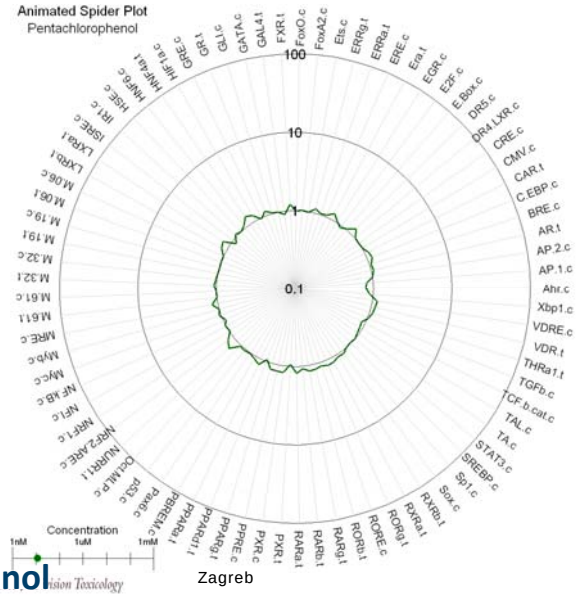


- In ADME studies…





Attagene Transcription Factor Profiling



Pentachlorophenol

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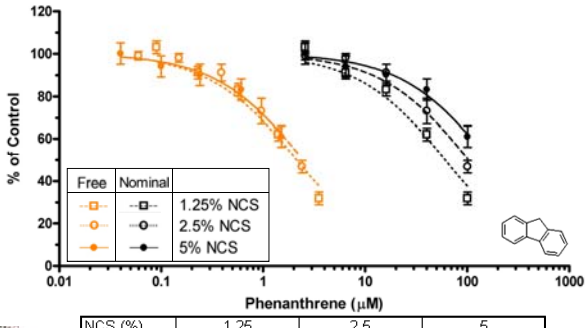
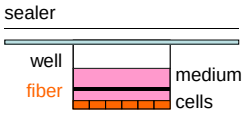
Cytotoxicity vs Serum Level



NCS (%)	1.25		2.5		5	
Log EC_{50}	1.79 ± 0.06	0.33	2.02 ± 0.04	0.40	2.22 ± 0.03	0.40
EC_{50} (µM)	61.3	2.15	104.1	2.49	165.5	2.48



% NCS	% Free	
	Measured	
1.25	6.85 ± 0.75	
2.5	4.55 ± 1.01	
5	2.70 ± 0.20	

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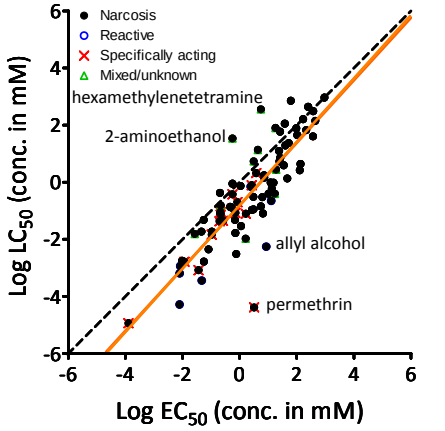
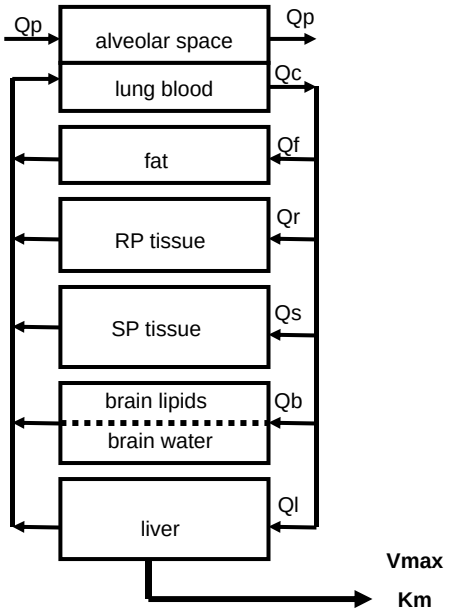
In Vitro – In Vivo Extrapolation

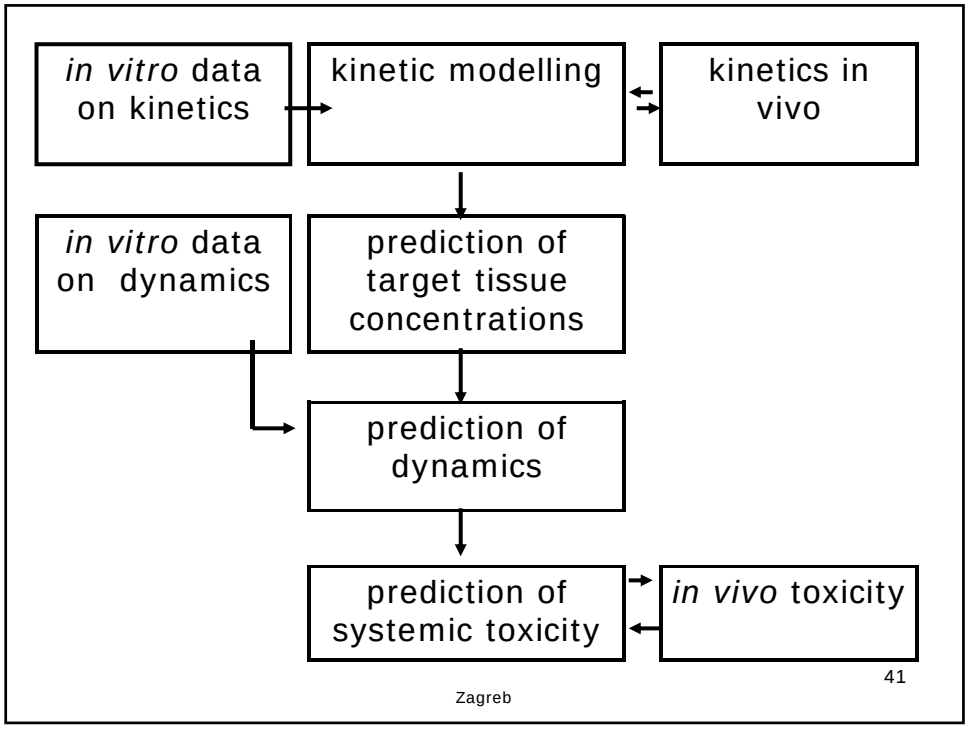
Halle Registry of Cytotoxicity
vs.
U.S. EPA Duluth Fathead
Minnow Database



Log LC_{50} (mM) = 1.110 + 0.881 Log EC_{50} (mM) – 0.811 + 0.121, R^2 = 0.870.

biokinetic model




in vitro data on kinetics
kinetic modelling
kinetics in vivo
in vitro data on dynamics
prediction of target tissue concentrations
prediction of dynamics
prediction of systemic toxicity
in vivo toxicity
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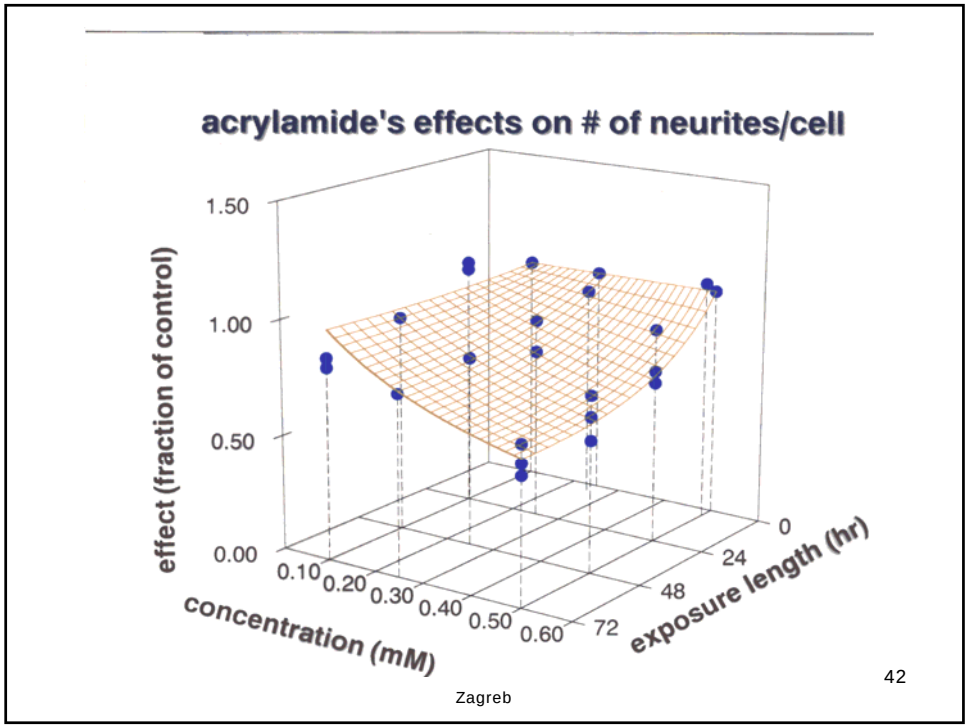
acrylamide's effects on # of neurites/cell
effect (fraction of control)
1.50
1.00
0.50
0.00
0.10
0.20
0.30
0.40
0.50
0.60
concentration (mM)
0
24
48
72
exposure length (hr)
42
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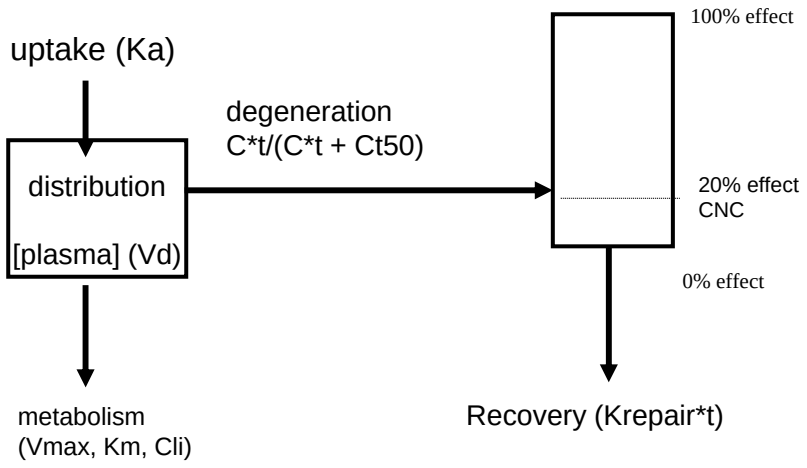
KINETIC-DYNAMIC MODELLING

KINETICS

DYNAMICS



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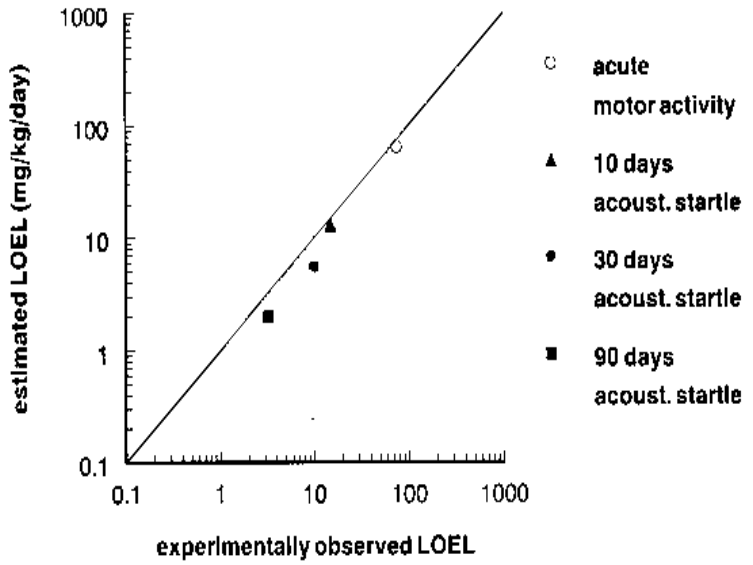

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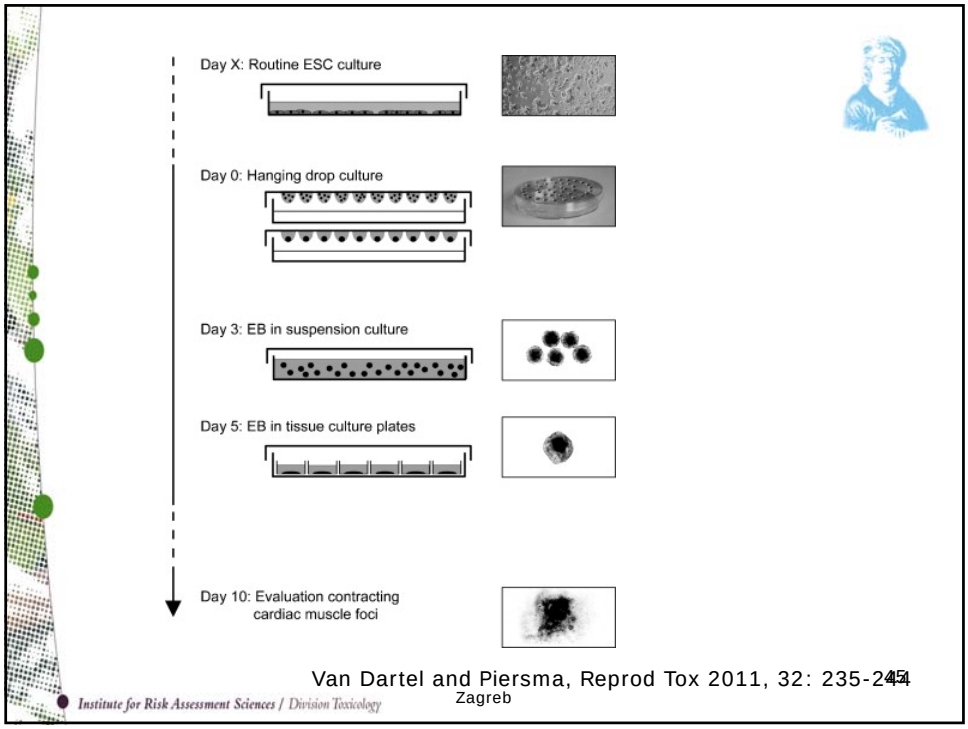
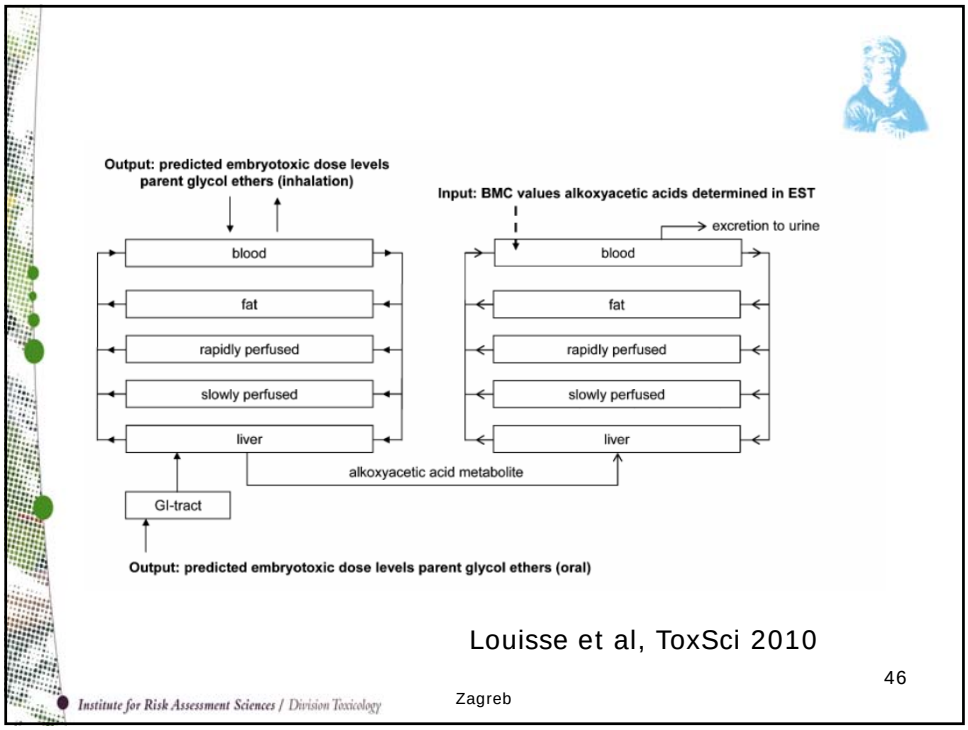
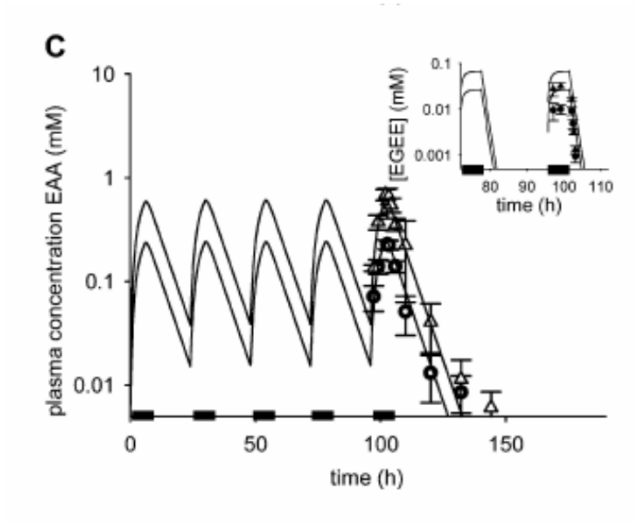


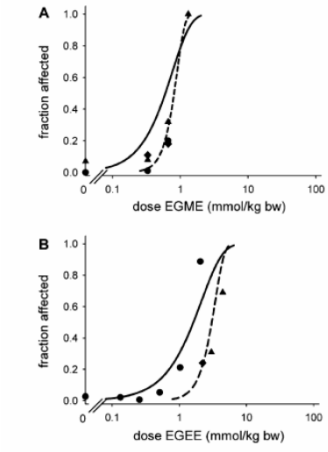
Van Dartel and Piersma, Reprod Tox 2011, 32: 235-241



Louisse et al, ToxSci 2010



Louisse et al, ToxSci 2010



Louisse et al, ToxSci 2010

Conclusions 1

- Risk assessment = hazard characterization + exposure assessment
- Traditional toxicology tests are performed on animals and in a tiered manner: acute – subchronic – chronic and specific toxicity tests
- Animal welfare and cost concerns as well as legal obligations and questionable scientific results = major impetus for research in alternatives testing methods and strategies in toxicology

Conclusions 2

- integration of *in vitro* data in risk evaluation is possible, provided that:
  biokinetics are taken into account (absolute necessity)
- integration of <u>all</u> available data in a stepwise (hierarchical) approach will improve the transparency and efficacy of the risk assessment process
- there are many possibilities for the use of non-animal data in this process

Conclusions 3

- New toxicological paradigm: tiered integrated testing using
  - Exposure-based waiving (TTC approach), read across (SAR) and chemical categories
  - In silico methods (QSARs, systems biology, pathway modeling, PBPK and PKPD modeling)
  - In vitro methods (testing battery for both toxicological endpoints and kinetic parameters)
- Read 'Toxicity Testing in the 21st Century'- Report by NRC

Conclusions 4

- So….

more science, less animals used