UNIVERSITÀ DEGLI STUDI DI MILANO
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Establishing toxicological end-points for human risk assessment: challenges and opportunities

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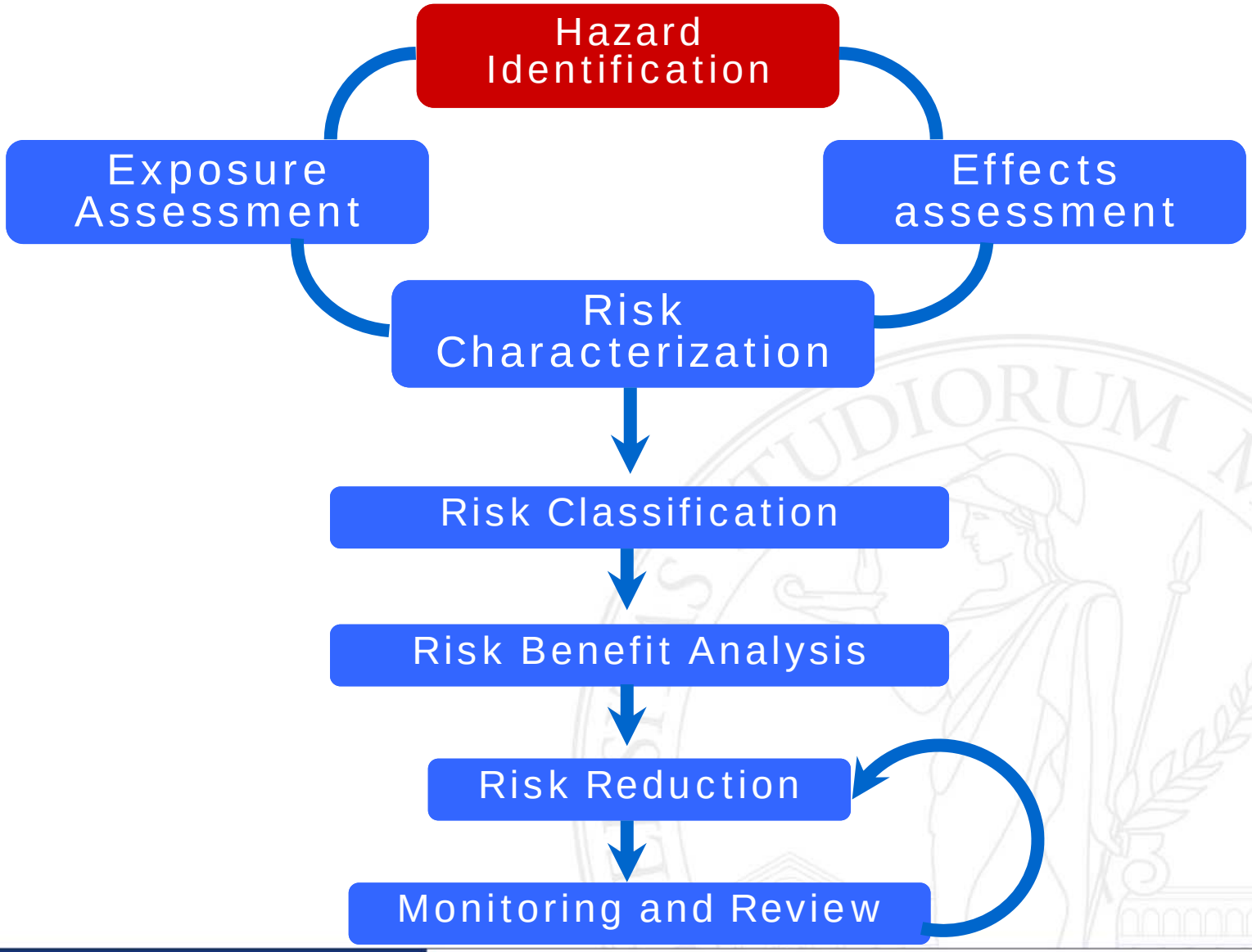
Step in the risk management process



Outline

- The current situation: the system is not efficient
- We need a change of paradigm (the RISK21 example)
- Some thoughts on future direction

Current situation

- Do all the toxicology
- Derive critical point-of-departure (e.g. NOAEL)
- Set exposure/intake limits
- Perform risk assessment

Anything less is second best or even unacceptable

Do all the toxicology

TOXICOKINETIC
Absorption
Distribution
Metabolism
Excretion

ACUTE TOXICITY
LD_{50} oral
LD_{50} dermal
LC_{50} inhalation
Skin irritation
Eye irritation
(Skin sensitization)/LLNA

GENOTOXICITY
Mutagenesis
Clastogenesis
Aneuploidy

DEVELOPMENTAL TOXICITY
Teratogenicity tests (Rat-Rabbit)

REPRODUCTIVE TOXICITY
Two generation reproductive toxicity

SHORT-TERM TOXICITY
Mouse	90 day toxicity
Rat	90 day toxicity
Dog	90 day toxicity
(Dog	1 year toxicity)

SPECIAL STUDIES
Acute/repeated neurotoxicity
Developmental neurotoxicity
Immunotoxicity
Others

LONG-TERM TOXICITY and/or CARCINOGENICITY
(Mouse 18 months)
Rat 104 weeks

Current situation

- Do all the toxicology
- **Derive critical point-of-departure (e.g. NOAEL)**
- **Set exposure/intake limits**
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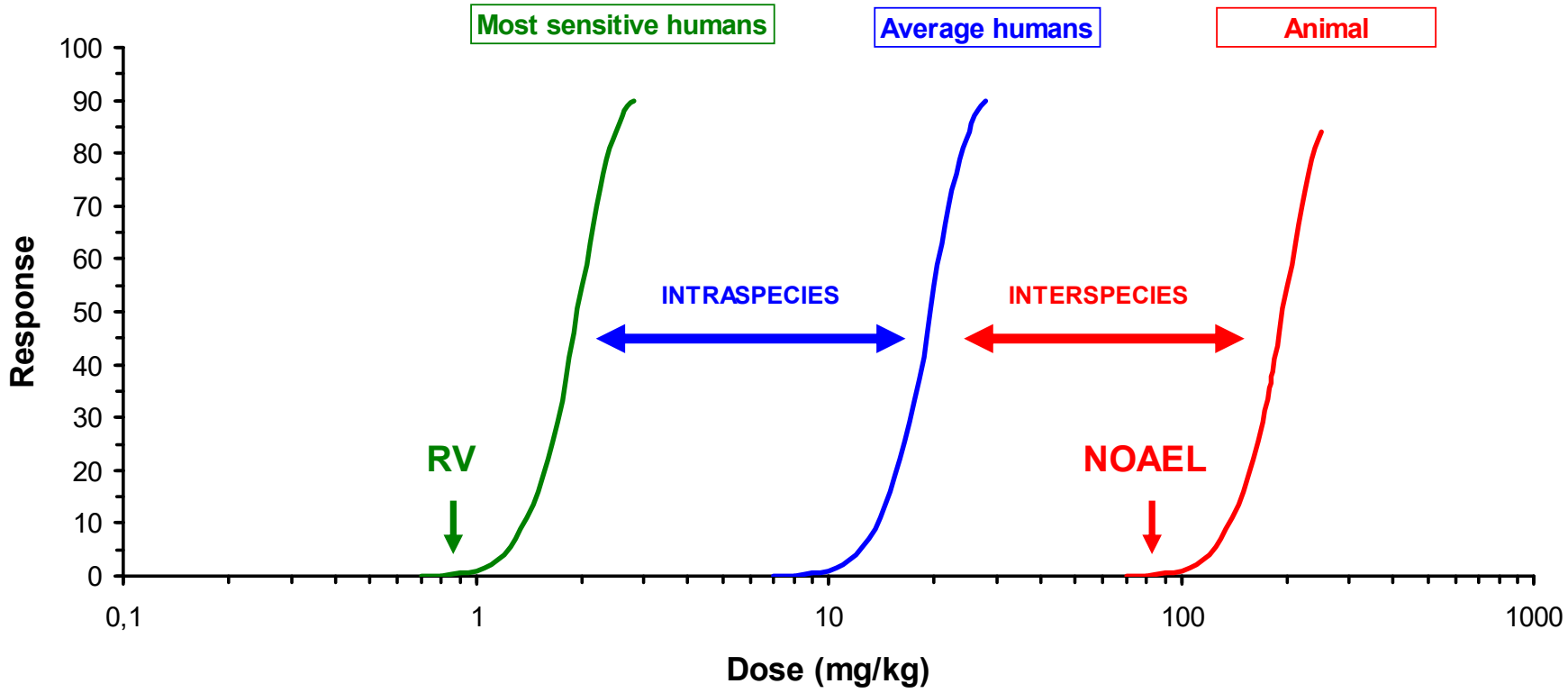
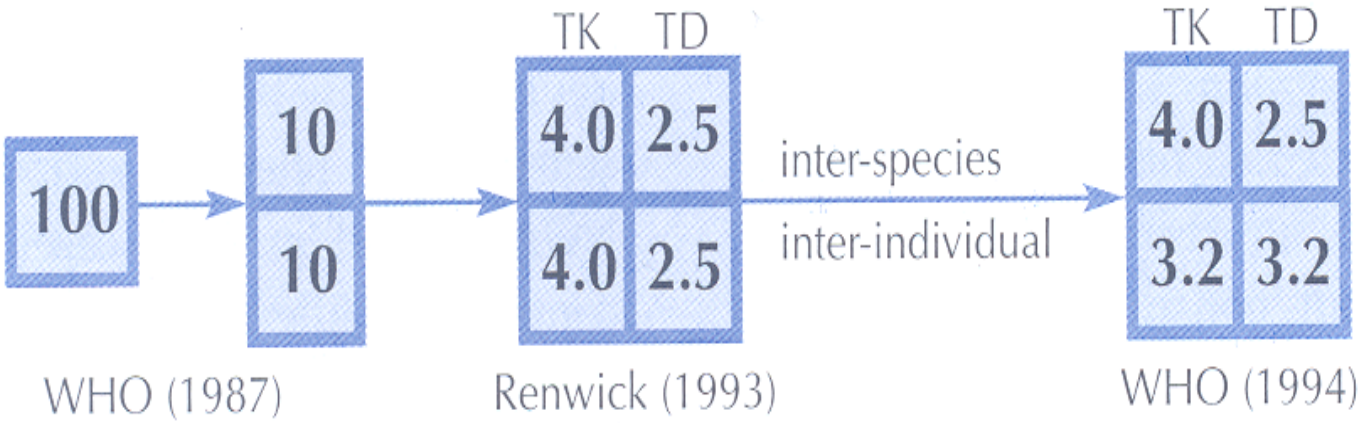
Dose-response curve: from animal to human



Suddivision of the safety factor

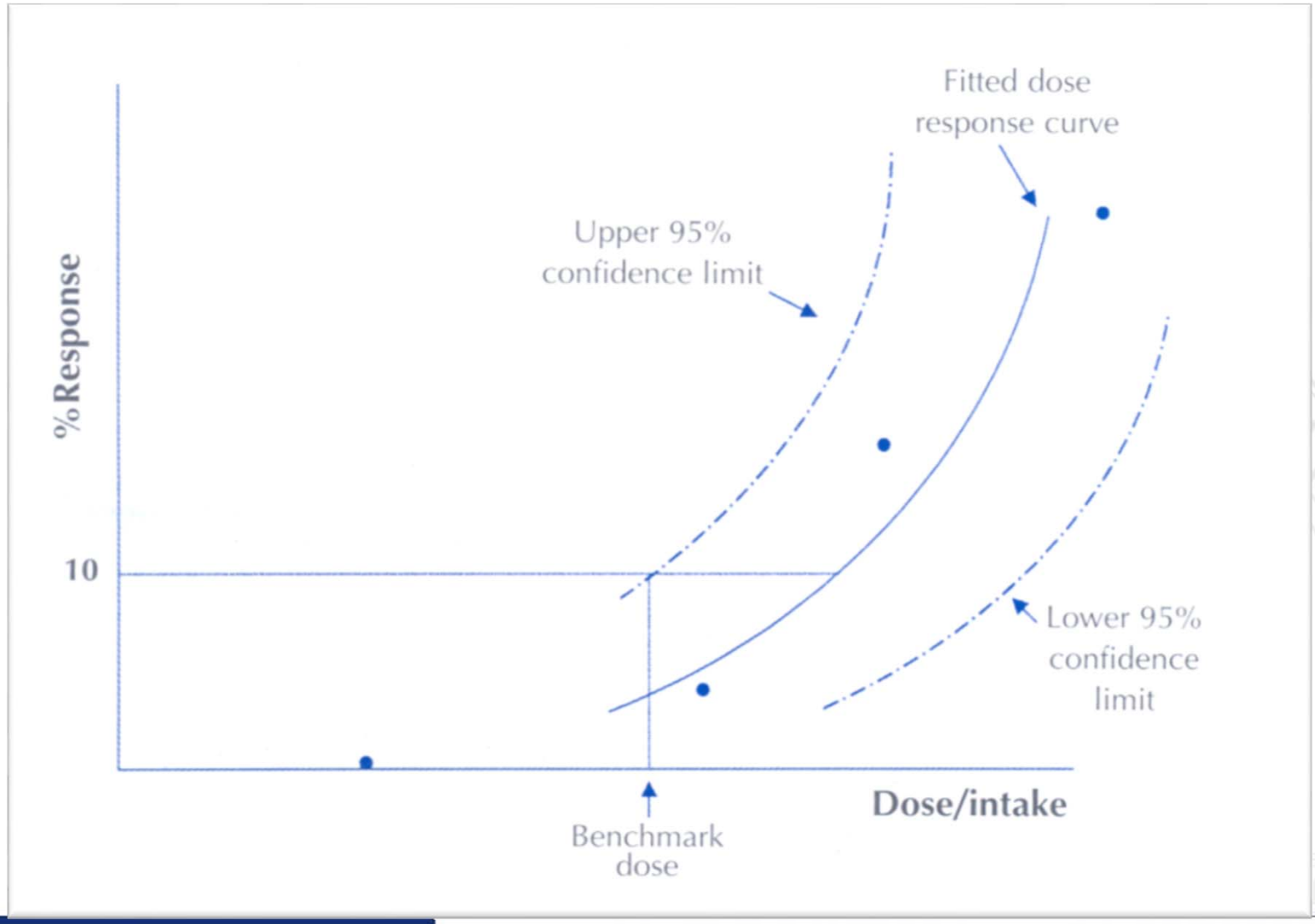


TK – toxicokinetics (fate of the chemical in the body)

TD – toxicodynamics (effetcs of the chemical on the body)

(from Renwick and Lazarus, 1998)

The Benchmark dose



Current situation

- Do all the toxicology
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- **Perform risk assessment**

Anything less is second best or even unacceptable

Outcome of the risk assessment of 84 a.i. performed by FAO/WHO JMPR (2013-2015)

International Estimated Daily Intake (IEDI)					
	% ADI				
	0-1	2-5	6-10	11-20	>20
% a.i.	35	29	19	4	13

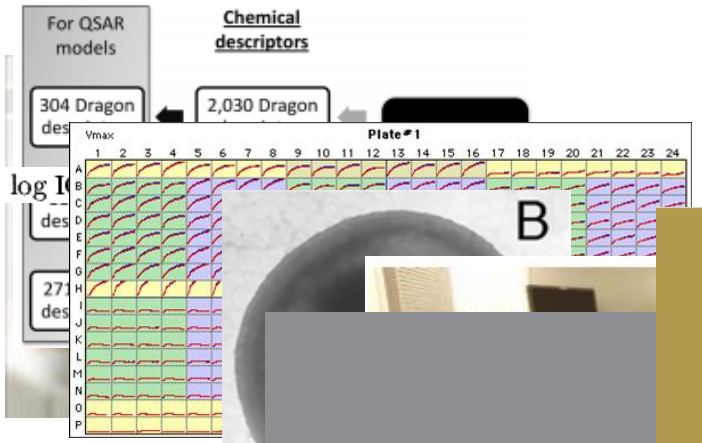
International Estimated Daily Intake (IEDI)

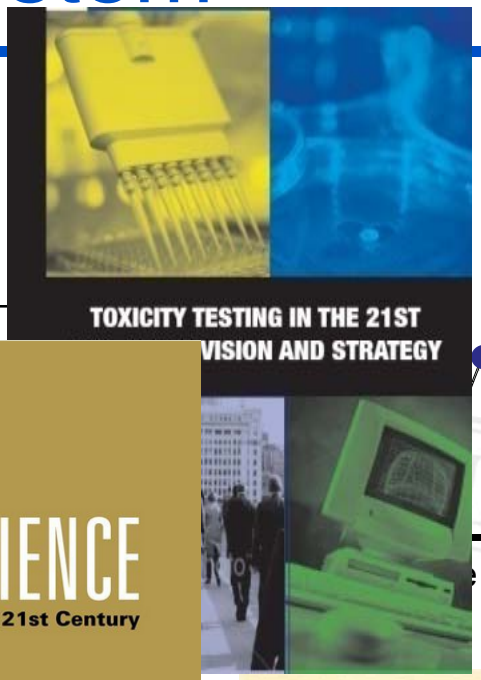
- Food balance sheets
- All crops treated with the compound
- No processing factors

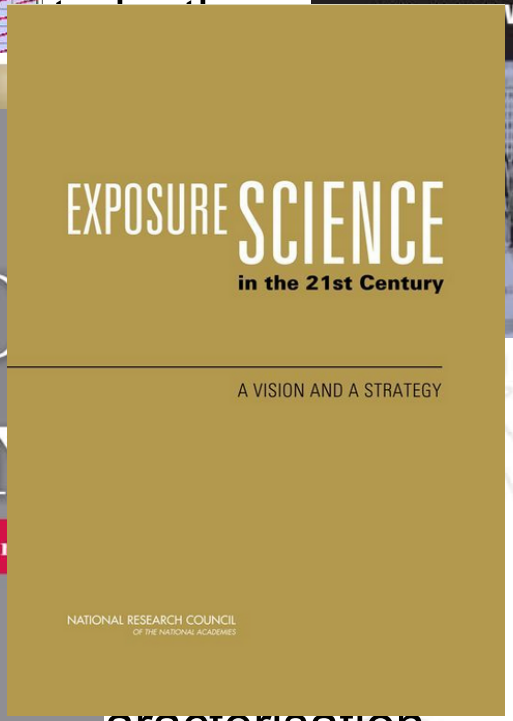
Outline

- The system is not efficient
- **We need a change of paradigm (the RISK21 example)**
- Some thoughts on future direction

Need to improve the system







Reference v
[RV] = POD/

aracterisation

MOE = POD/E

A change in philosophy

- From
  - Do all the toxicology then think about the risk assessment, anything less is second best or even unacceptable
- To
  - **Think** about the problem that needs to be addressed, then **select** sources of information which will have the most value

Problem Formulation: The Starting Point

www.hesiglobal.org

- **Sets out:**
  - Objectives
  - Scope
  - Hypotheses

- **Asks:**
  - what do you know?
  - what do you need to know?
  - How do you know when you're done?

Enough precision to make a decision

Mode of Action

In vivo

In vitro

QSAR/
TTC

3

Toxicity?

Biomonitoring

Probabilistic

Deterministic

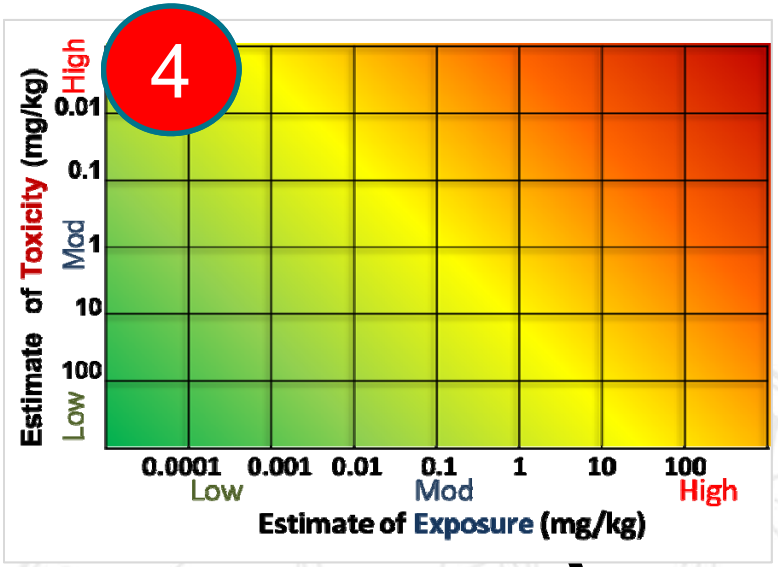
Minimal
Info

2

Exposure?

Risk? Safety?

www.RISK21.org



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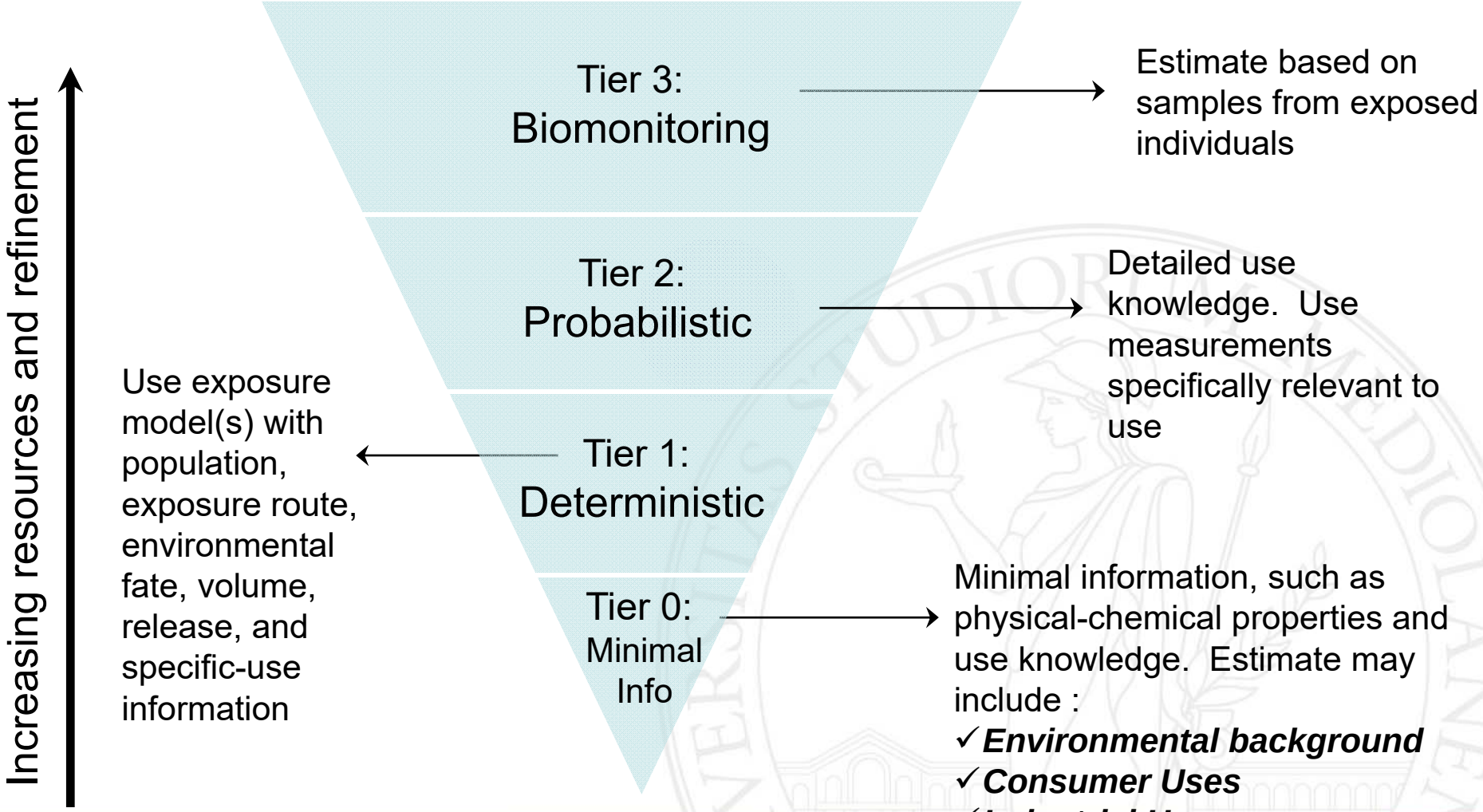
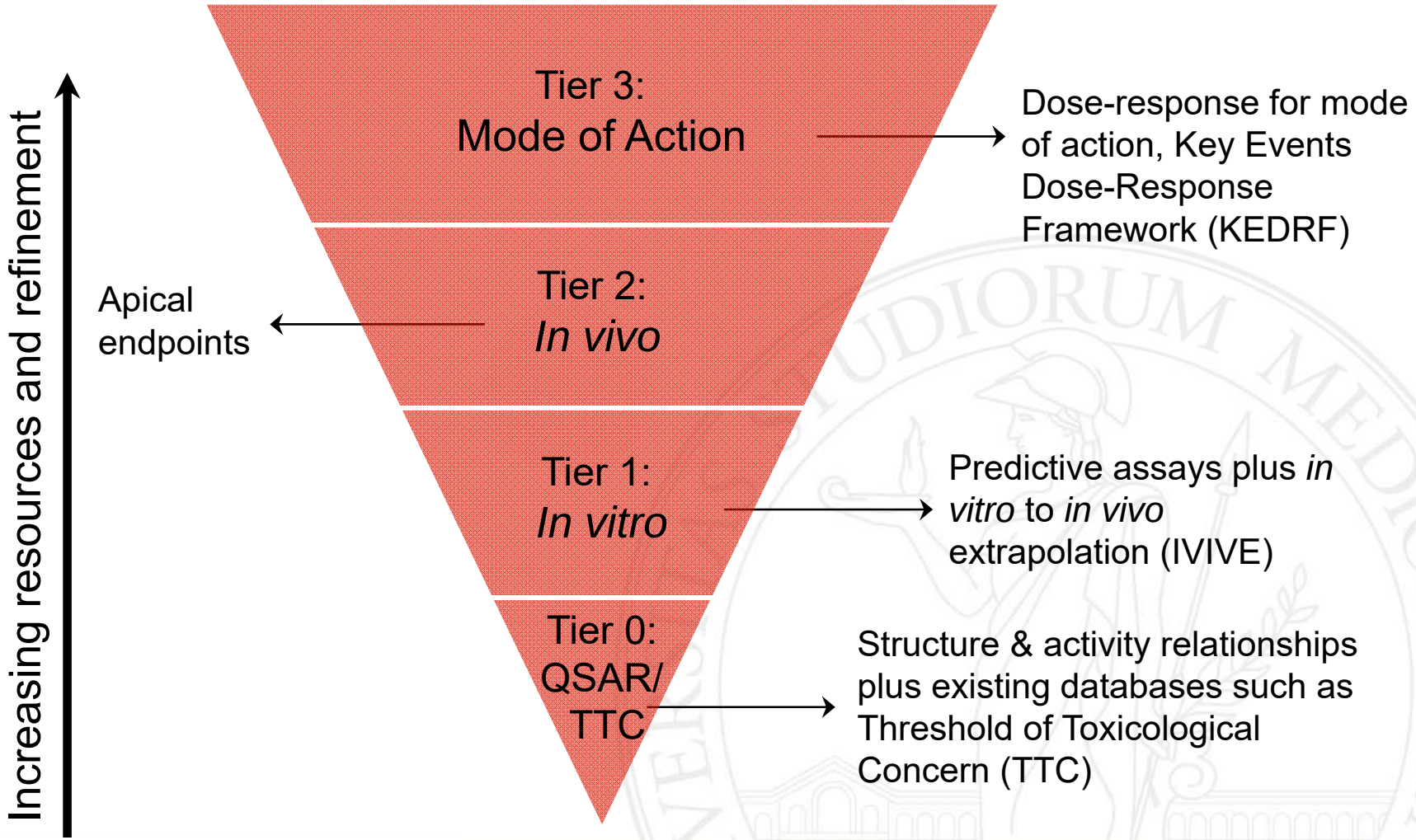
Problem Formulation

Conclude

Enough Precision for Exposure Estimate



Enough Precision for Toxicity Estimate



Case Study to Test the Approach: “Pseudomethrin”

Problem Formulation

- Can “Pseudomethrin” be used on bed nets to protect against mosquito bites?
- 11th pyrethroid
- Determine reasonable certainty of no harm for…
  - Bed-net dipping
  - Sleeping under treated net
- Use no more than 50 animals

Tier 0 Exposure

- Phys/Chem: Low volatility; therefore, inhalation negligible.
- Sub-chronic to chronic duration

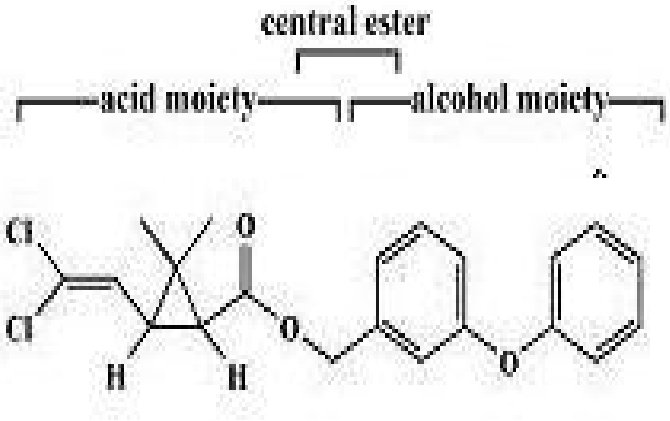
Use	Age	Dermal contact (mg/kg/d)	Hand to mouth (mg/kg/d)	Net mouthing (mg/kg/d)	Total / aggregate (mg/kg/d)
Net dipping (single exposure)	Adult	0.03 – 0.7	N/A	N/A	0.03 – 0.7
	Child	0.05 – 1.0	N/A	N/A	0.05 – 1.0
	Infant	N/A	N/A	N/A	N/A
Sleeping under net (chronic exposure)	Adult	0.0002 – 0.16	N/A	N/A	0.0002 – 0.16
	Child	0.0001 – 0.08	2e-6 – 0.006	N/A	0.0001 – 0.086
	Infant	0.0005 – 0.4	7e-6 – 0.003	0.01 – 0.04	**0.0106 – 0.443**

WHO (2004): A generic risk assessment model for insecticide treatment and subsequent use of mosquito nets"

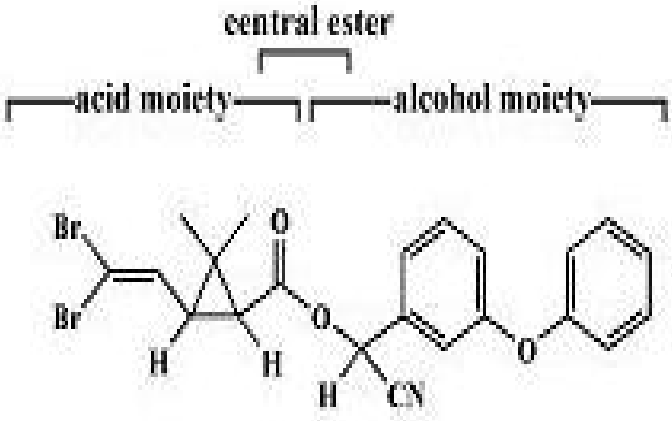
Pyrethroid Neurotoxicity

Administration to test animals and insects has identified two distinct poisoning syndromes:

- Type I: Aggressive sparring, increased sensitivity to external stimuli, fine tremors progressing to whole body tremors
- Type II: Pawing and burrowing, profuse salivation, course tremors progressing to seizures
- Mixed: some pyrethroids cause signs of both syndromes



Permethrin (1)



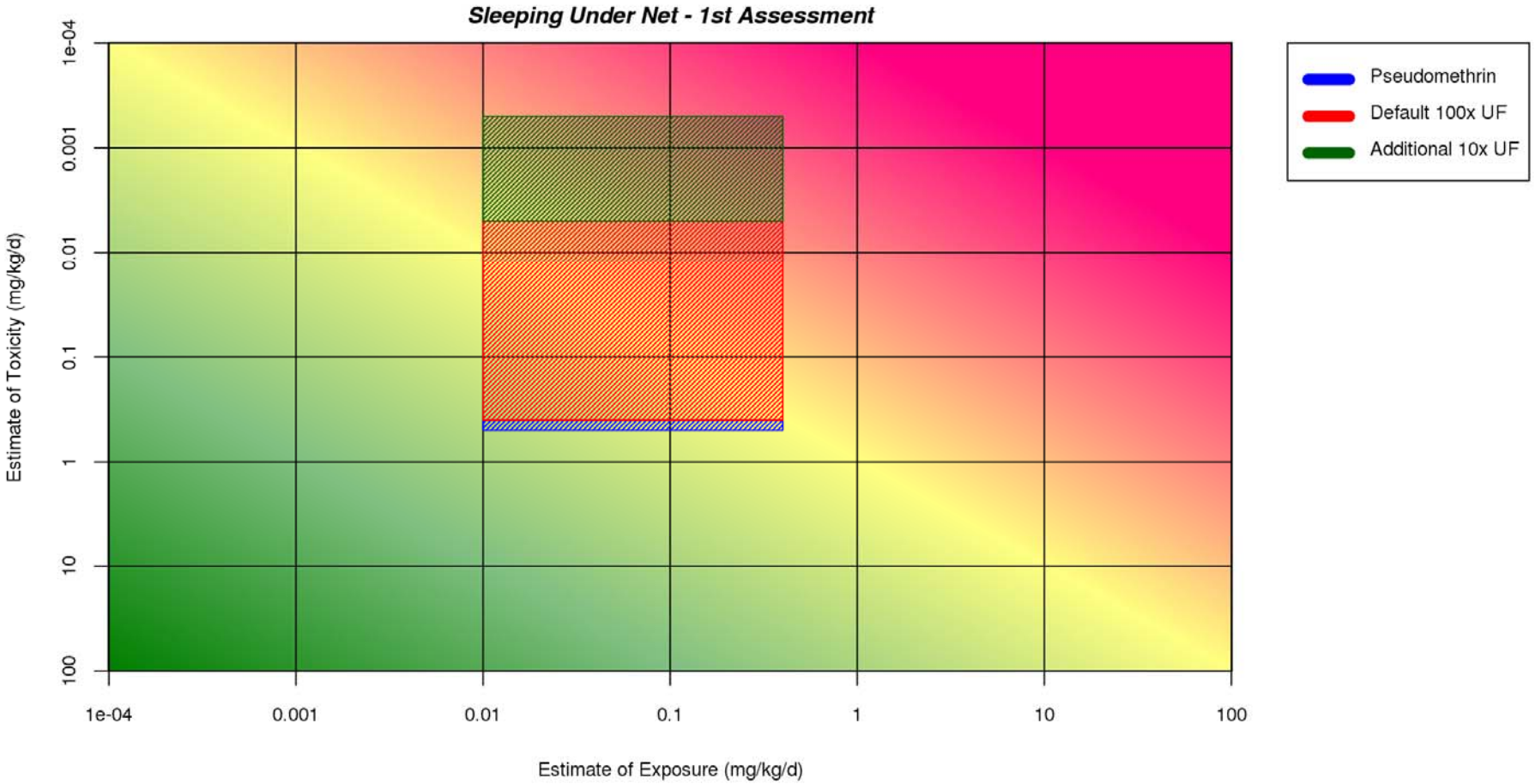
Deltamethrin (9)

Toxicity Values for Pyrethroids

		Type I non-cyano			Type II alpha-cyano						
		Permethrin	*Bifenthrin*	*Resmethrin*	*S-Bioallethrin*	*Cyfluthrin*	*Cypermethrin*	*Pseudomethrin*	*Esfenvalerate*	*Fenpropathrin*	*lambda-Cyhalothrin*
Short-term/ Acute	BMD20 (Single Dose)	156	14.3	**291**	135	12.6	76	14.5	40.5	35	**8.9**
Intermed.	Ref 90d NOEL	5	2.5	**80**	20	1.3	12.5	1	7.5	7	**0.5**
Long-Term/ Chronic	Ref Chron NOEL	5	1.5	3	**14**	6.2	7.5	1	2	3	**0.5**

Highest and lowest values for each row are **bolded**

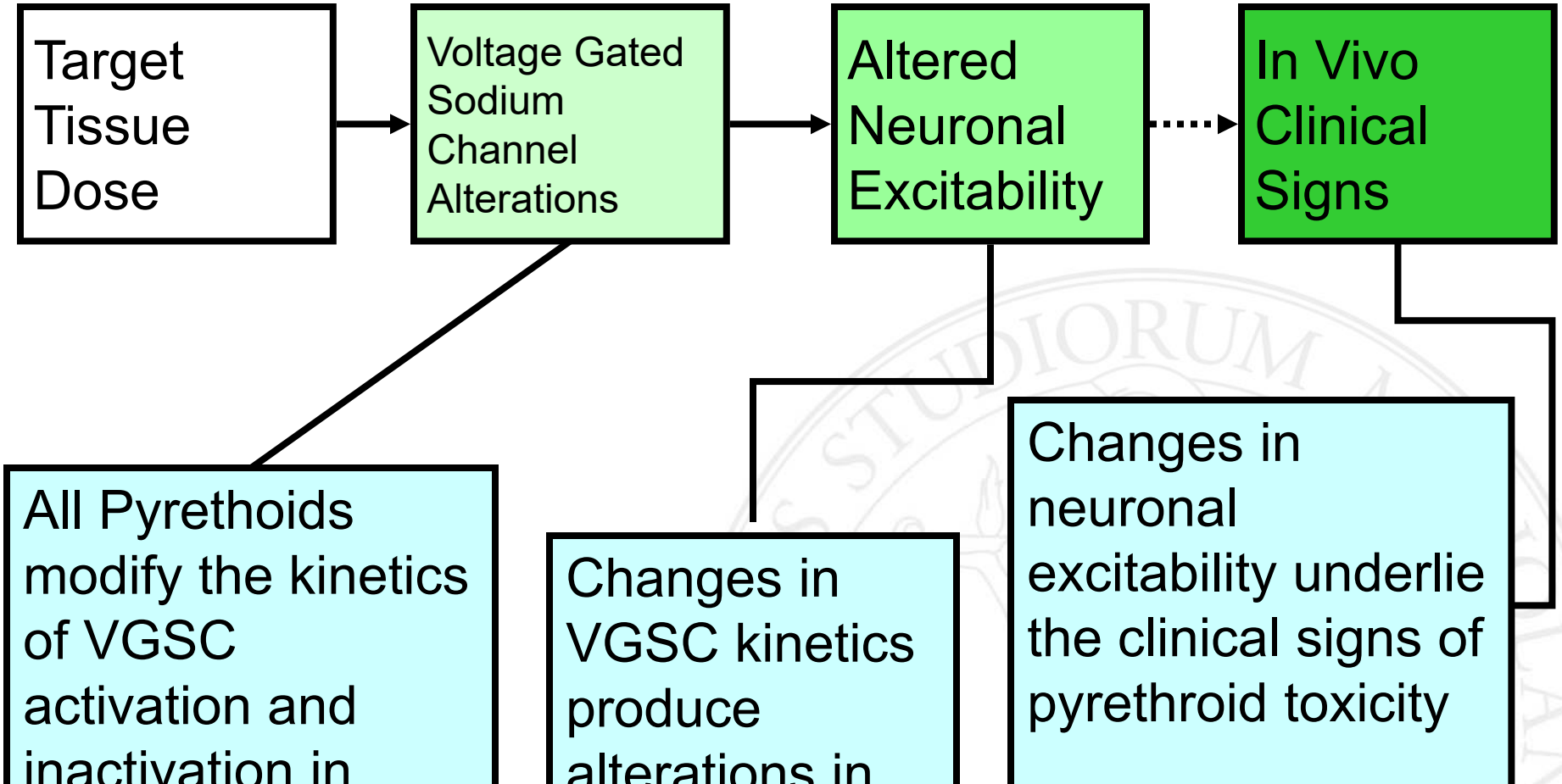
Sleeping under net: Tier 0



Exposure range: 0.1 – 0.443 mg/kg/d (infant, aggregate, sleeping)

Toxicity value: most potent chronic NOAEL (lambda-cyhalothrin): 0.5 + UFs

Common Mechanism of Toxicity

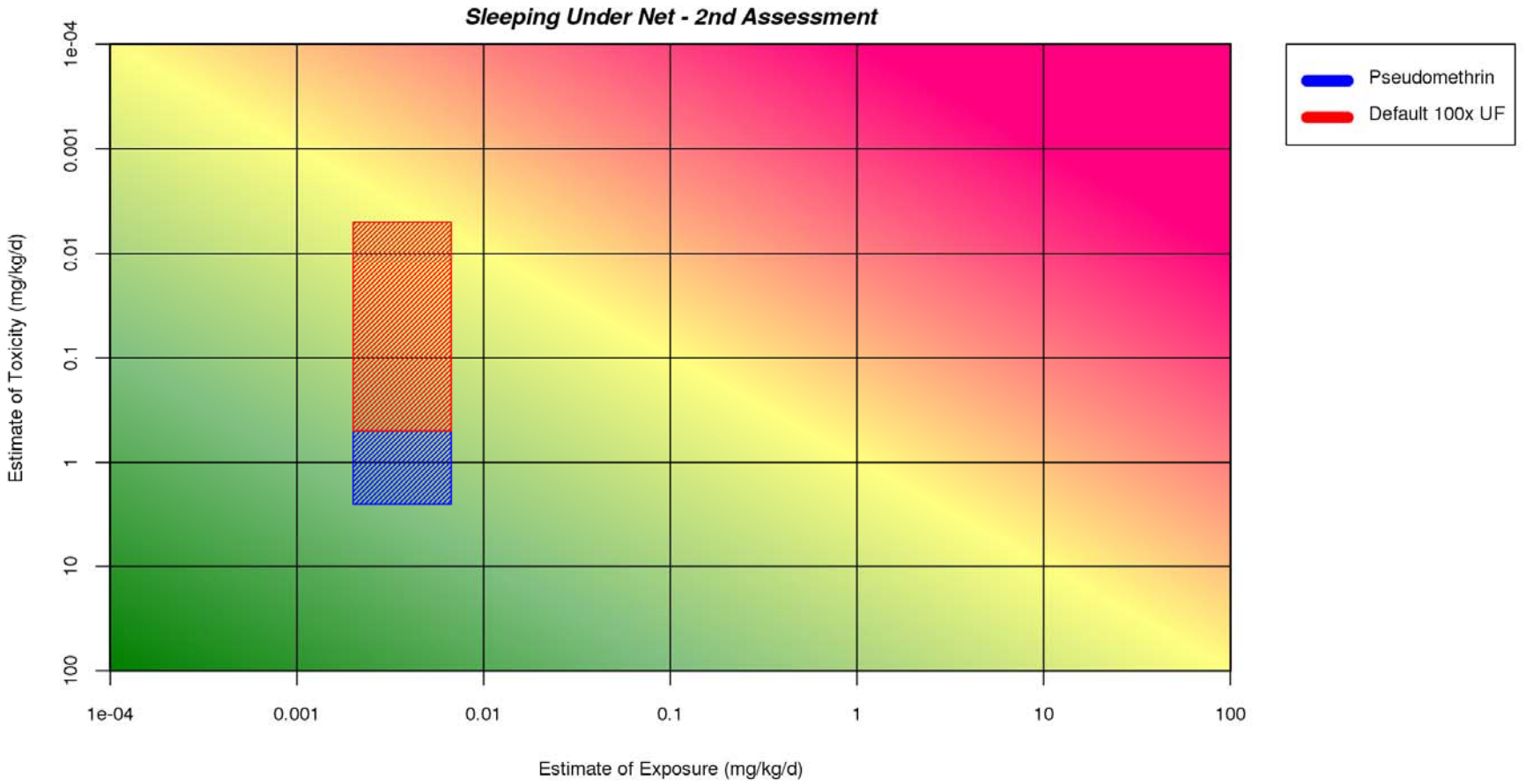


Toxicity Values for Pyrethroids

	Type I non-cyano				Type II alpha-cyano					
	Permethrin	*Bifenthrin*	*Resmethrin*	*S-Bioallethrin*	*Cyfluthrin*	*Cypermethrin*	*Pseudomethrin*	*Esfenvalerate*	*Fenpropathrin*	*lambda-Cyhalothrin*
BMD20	156	14.3	291	135	12.6	76	14.5	40.5	35	**8.9**
Ref 90d NOEL	5	2.5	80	20	1.3	12.5	1	7.5	7	**0.5**
Ref Chron NOEL	5	1.5	3	14	6.2	7.5	1	2	3	**0.5**
MEA IC50	719	439	1685	1525	305	181	**175**	809	1518	**25**

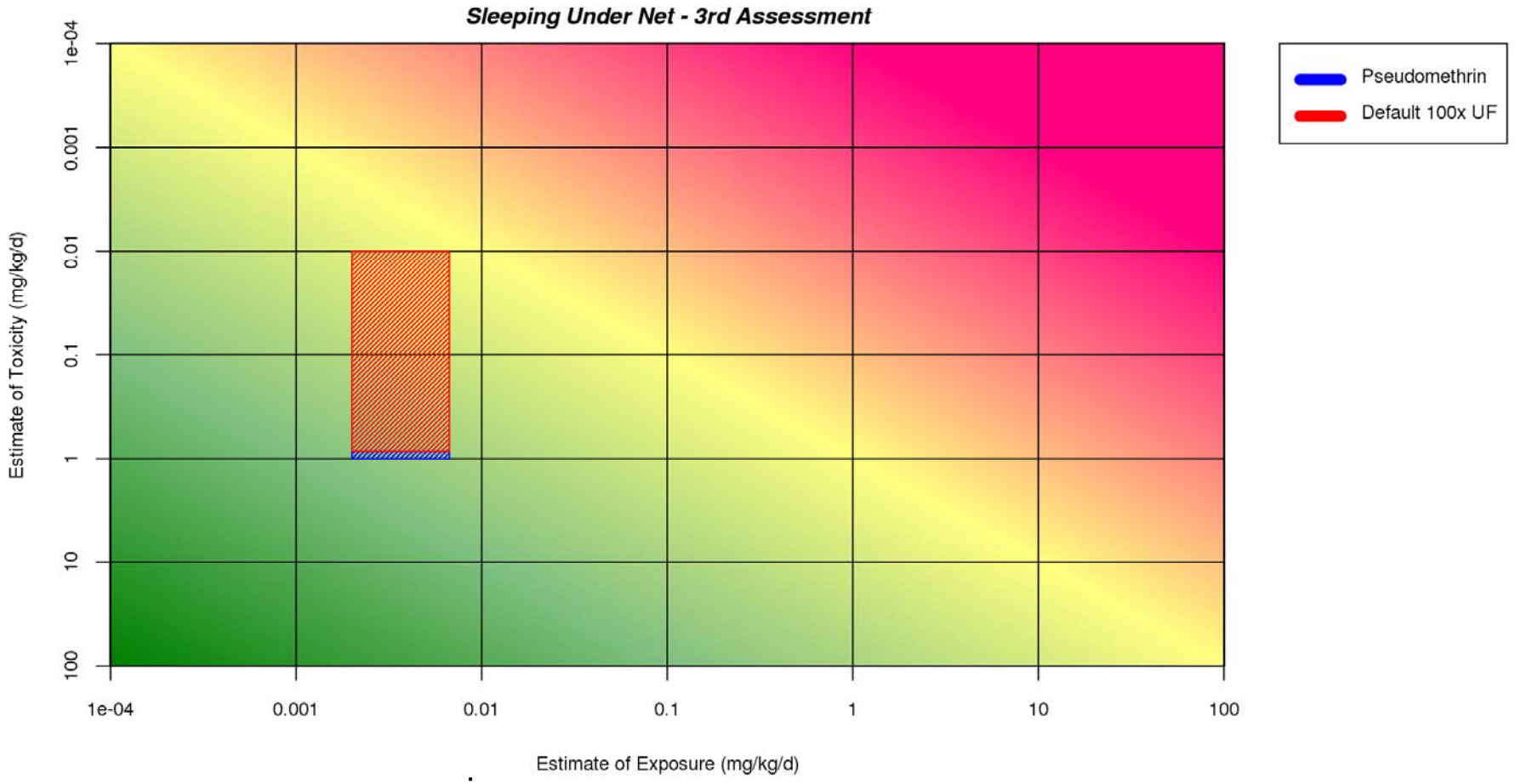
5-fold difference in potency between pseudomethrin and most-potent

Sleeping under net: 2nd Assessment



Exposure range: 0.002 – 0.0067 (infant, aggregate, sleeping) –dermal absorption estimates
Toxicity range: 0.5 – 2.5 [derived from most potent chronic NOAEL (lambda-cyhalothrin) and 5-fold lower potency of pseudomethrin based on MEA IC50] + UFs

Sleeping under net: 3rd Assessment



Exposure range: same as previous

Toxicity range: 5-day dog study (neurological NOAEL of 1 mg/kg/d) with UF and in vitro screens

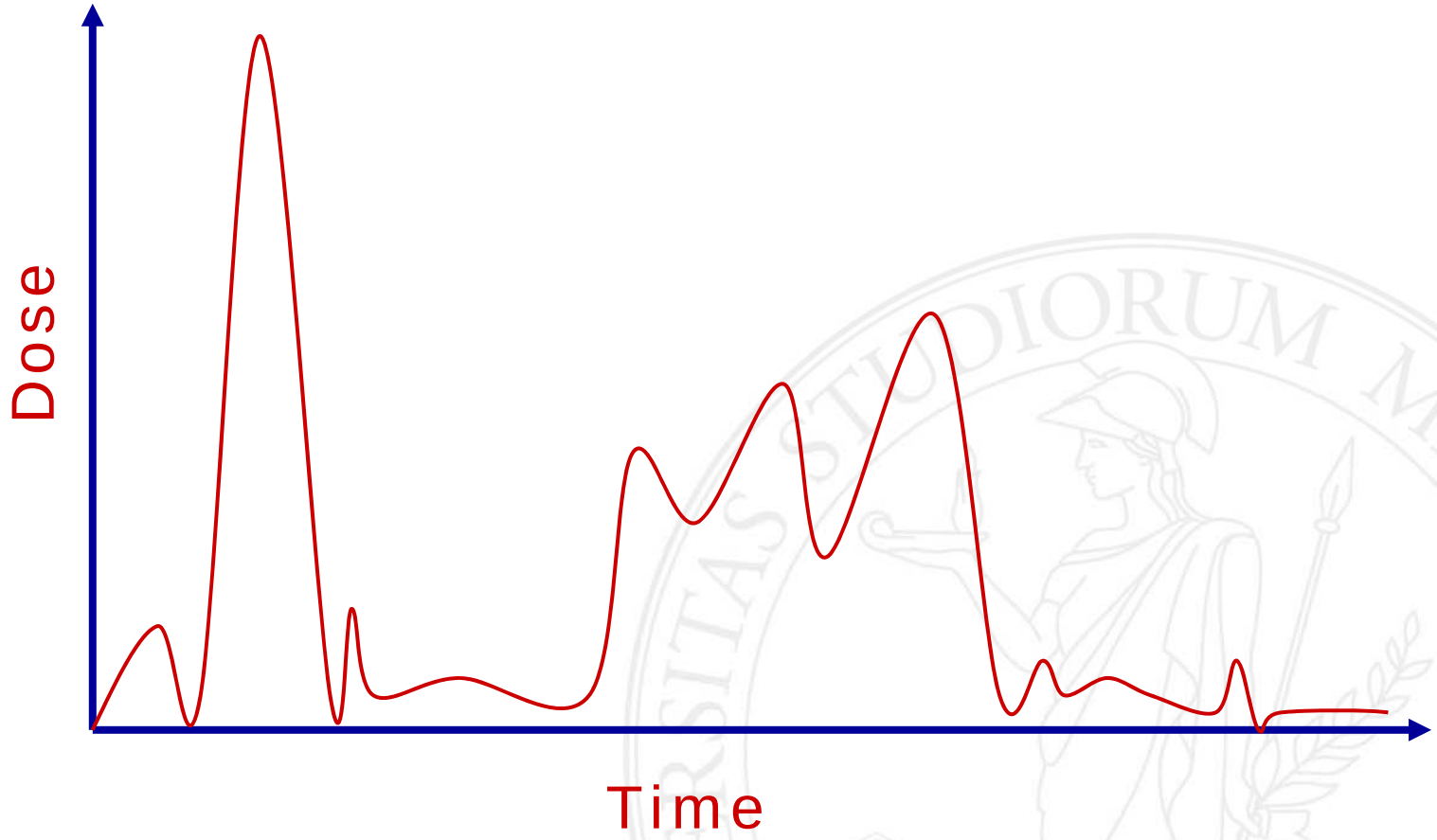
Outline

- The system is not efficient
- We need a change of paradigm (the RISK21 example)
- **Some thoughts on future direction**

Some thoughts on future direction(s)

- **We need to improve our exposure assessment**
- We need to understand the meaning and how to use the new (and old) *in silico* and *in vitro* tools

How do we deal with varying or intermittent exposures?



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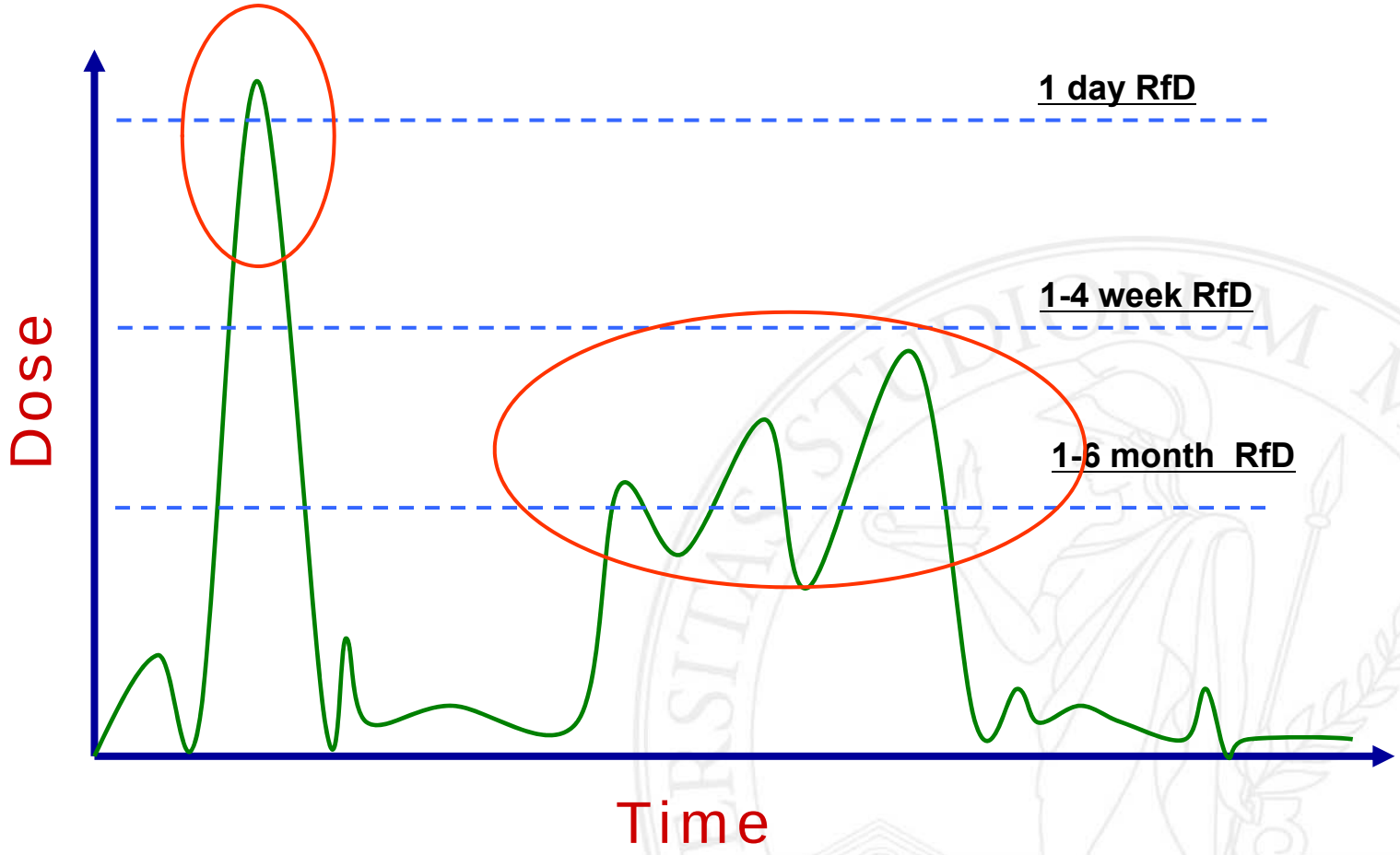


Some thoughts on future direction

- We need to improve our exposure assessment
- **We need to understand the meaning and how to use the new (and old) *in silico* and *in vitro* tools**

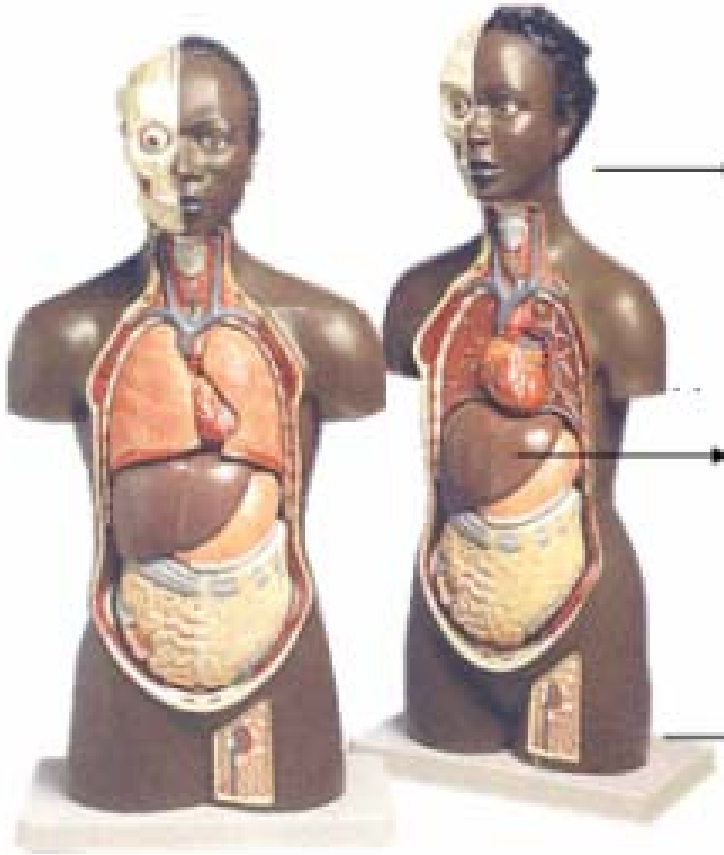
in vitro tools

- “omics”
- High-throughputs
- Receptor assays
- …..

In silico tools

- (Q)SAR
- Receptor/protein docking
- TTC
- Read-across
- PBPK-PD
- ….

PB-PK modeling internal dose



Inhalation

$$V_{\dots}\frac{dC}{dt} = Q_{\dots}(C_{\dots} - C_{\dots}) + Q_{\dots}(C_{\dots} - C_{\dots})$$

Concentration in Liver

$$V_{\dots}\frac{dC}{dt} = Q_{\dots}\left(C_{\dots} - \frac{C_{\dots}}{P_{\dots}}\right) - \dots - Q_{\dots}\left(C_{\dots} - \frac{C_{\dots}}{P_{\dots}}\right) - V_{\dots}\frac{C_{\dots}}{K_{\dots} + C_{\dots}}$$

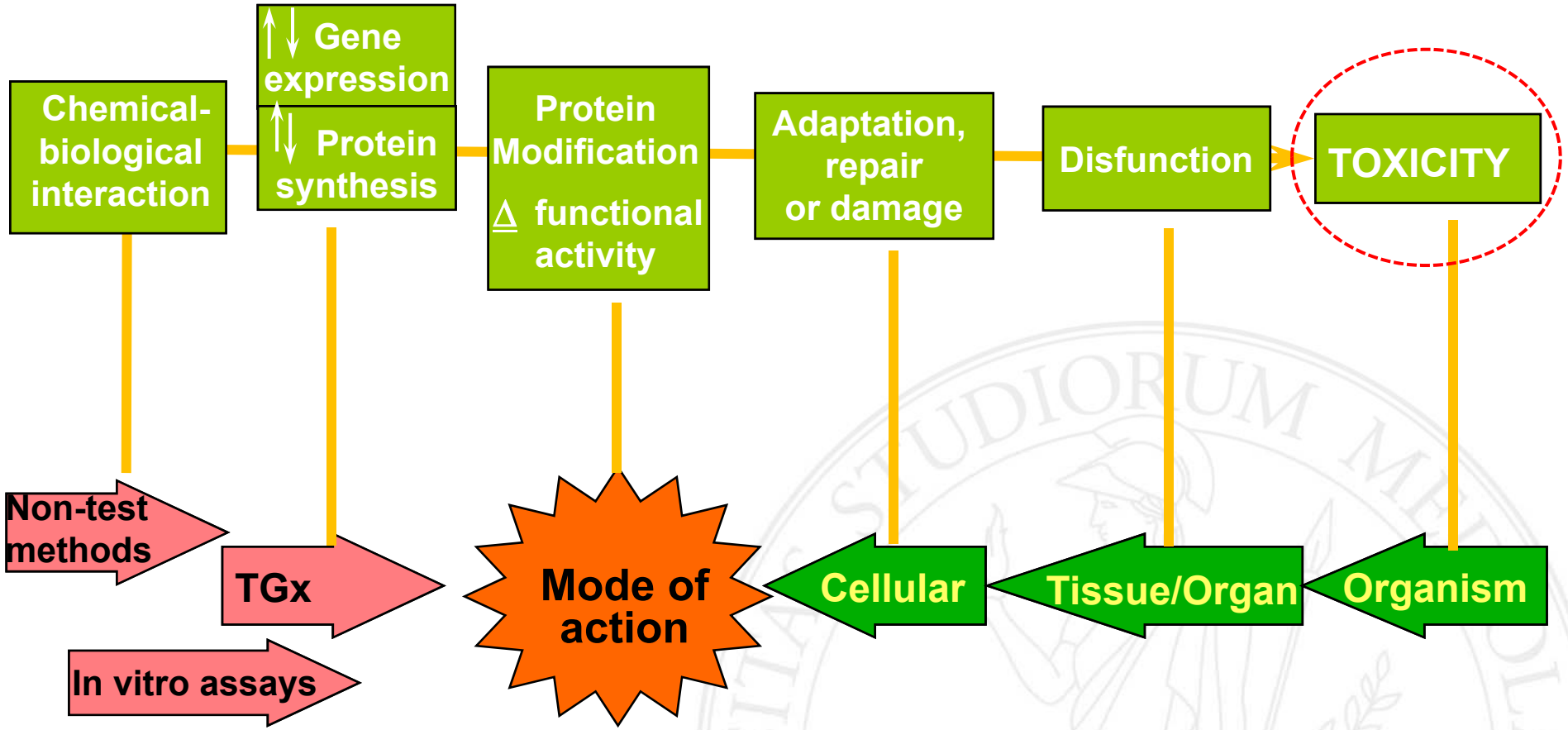
Skin Penetration

$$V_{\dots}\frac{dC_{\dots}}{dt} = Q_{\dots}\left(C_{\dots} - \frac{C_{\dots}}{P_{\dots}}\right) + C_{\dots}K_{\dots}A$$

We need to understand the meaning and how to use the new (and old) *in silico* and *in vitro* tools **because…..**

we need a shift in the approach to toxicology

Use of the MoA (AOP) concept



Conventional toxicity testing = "Top down"

21st century toxicity evaluation = "Bottom up"

Thank you
for your attention
and patience