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Risk Assessment: does it need redefinition?

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Risk assessment of chemicals in relation to human health

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

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)

BASIS OF TOXICITY

- interaction of chemical with biological system leading to adverse effects

- molecular target:
  “toxicological receptor”

BASIS OF TOXICITY

interactions are determined by:

- structural properties of compound

AND

- structural properties of target molecule

nature of physico-chemical reactions:

primary mechanism of toxic action

BASIS OF TOXICITY

following the primary interaction:

pathophysiological changes;

finally resulting in clinically manifest toxicity

complete process: ***mode of action***

PARADIGM:

toxicity is determined by:

the *critical*
concentration and time of exposure (dose metric) to

the *critical* compound (metabolite?) on

the *critical* site of action

in vitro toxicology

"Classical" *in vitro* toxicology:

finding concentrations (not dose)

need to extrapolate to intact organism

lack of biotransformation/ kinetics

concentrated on cytotoxicity, rather than on mechanisms of importance in vivo

basal cytotoxicity

disturbance of house-hold functions of cells:

* integrity of cellular membranes

* intactness of energy supplies

* maintenance of cellular compartments

cell-specific functional disturbance

differentiated functions of cells:

e.g.
neuronal activity
albumin production
barrier function / specific transport
etc.
etc.

in vitro toxicology

Mechanisms of action:

- may be studied in detail in *in vitro* sytems

- may give the intrinsic ability of a chemical to cause a certain toxic action

use of *in vitro*-derived data in hazard and risk assessments

e.g. reactivity + basal cytotoxicity:

answers towards: corrosivity, irritancy

reactivity towards DNA: genotoxicity

developments in cellular biology

general cellular biology

differentiation, de-differentiation, apoptosis

cell-cell interactions

receptor-mediated processes

signaling processes

metabolic changes

genomics, proteomics, metabonomics

adaptation processes

developments in cellular toxicology

biomarkers for cellular injury:

- oxidative stress
- disturbances in cellular membranes
- disturbances in intercellular communication
- cytokine profiles
- DNA damage
- etc.

cell- or tissue-specific effects

use *in vitro* assays in toxicodynamics

to determine (aspecific) cytotoxicity
to determine tissue-specific toxicity
to find relevant concentrations for functional cellular disorders (e.g. EC_{20} values):

'response surrogates'

developments in structure-activity relationships

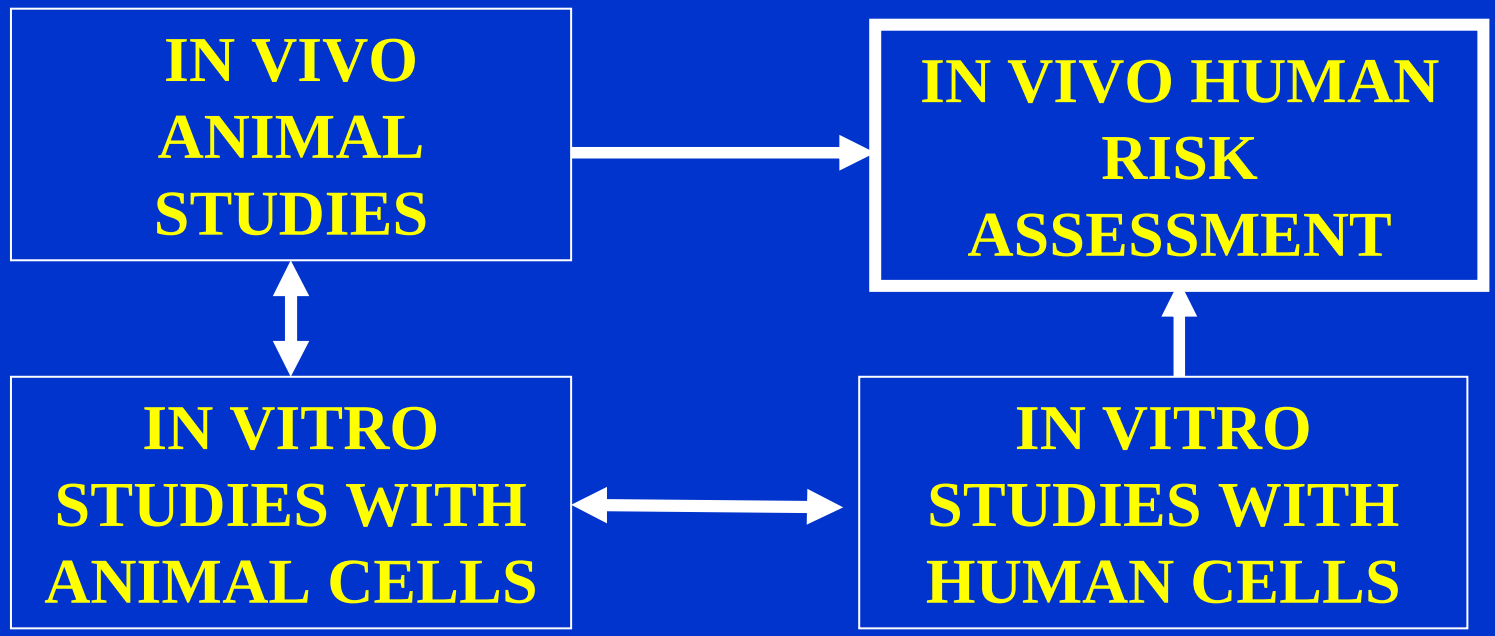
chemical-biological interactions:

- covalent binding
- receptor binding
- hydrophobic/hydrophilic interactions

knowledge-based computer systems

QSARs

THE PARALLELLOGRAM APPROACH



clinical effects

possibility of chemical to reach targets

- in appropriate amount or concentration
- in appropriate form (bound, metabolite?)

depends on:

- **exposure**
- **biokinetic behaviour:** absorption, distribution, metabolism, elimination

biokinetics

physicochemical properties

- of the chemical

as well as those

- of the tissues: **PARTITIONING**

AND

biokinetics

the physiology of the organism

e.g.

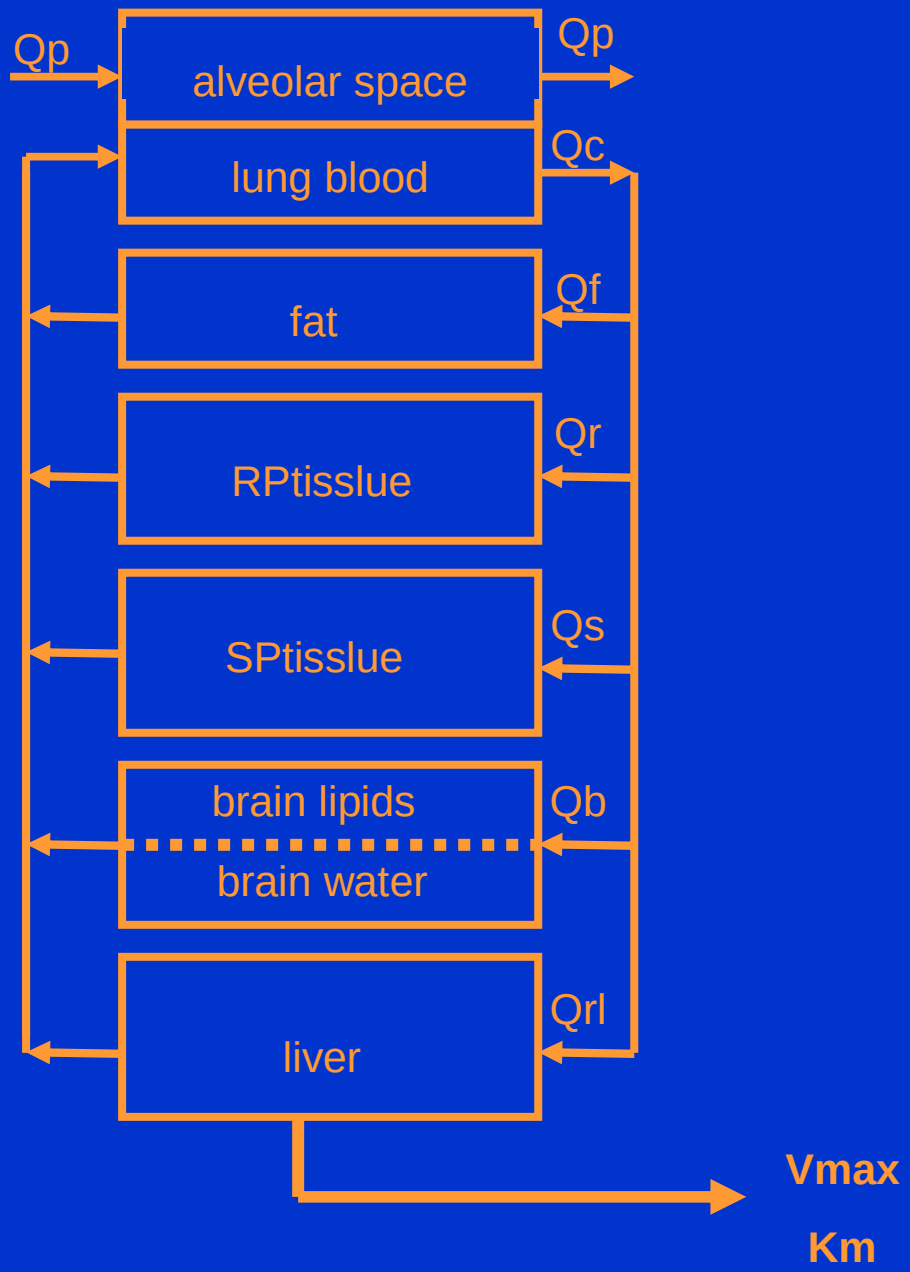
structure of the gut, nose, lungs, skin

blood flows

metabolising systems

kidney function

etc.


Qp
alveolar space
Qp
lung blood
Qc
fat
Qf
RPtisslue
Qr
SPtisslue
Qs
brain lipids
brain water
Qb
liver
Qrl
Vmax
Km

alternative methods

Many possibilities for studying mechanisms on the cellular and molecular level

However: use in risk assessment:

- very limited possibilities for a one-to-one replacement
- need for using information from different sources: **integration**

Integrated testing schemes:
elements

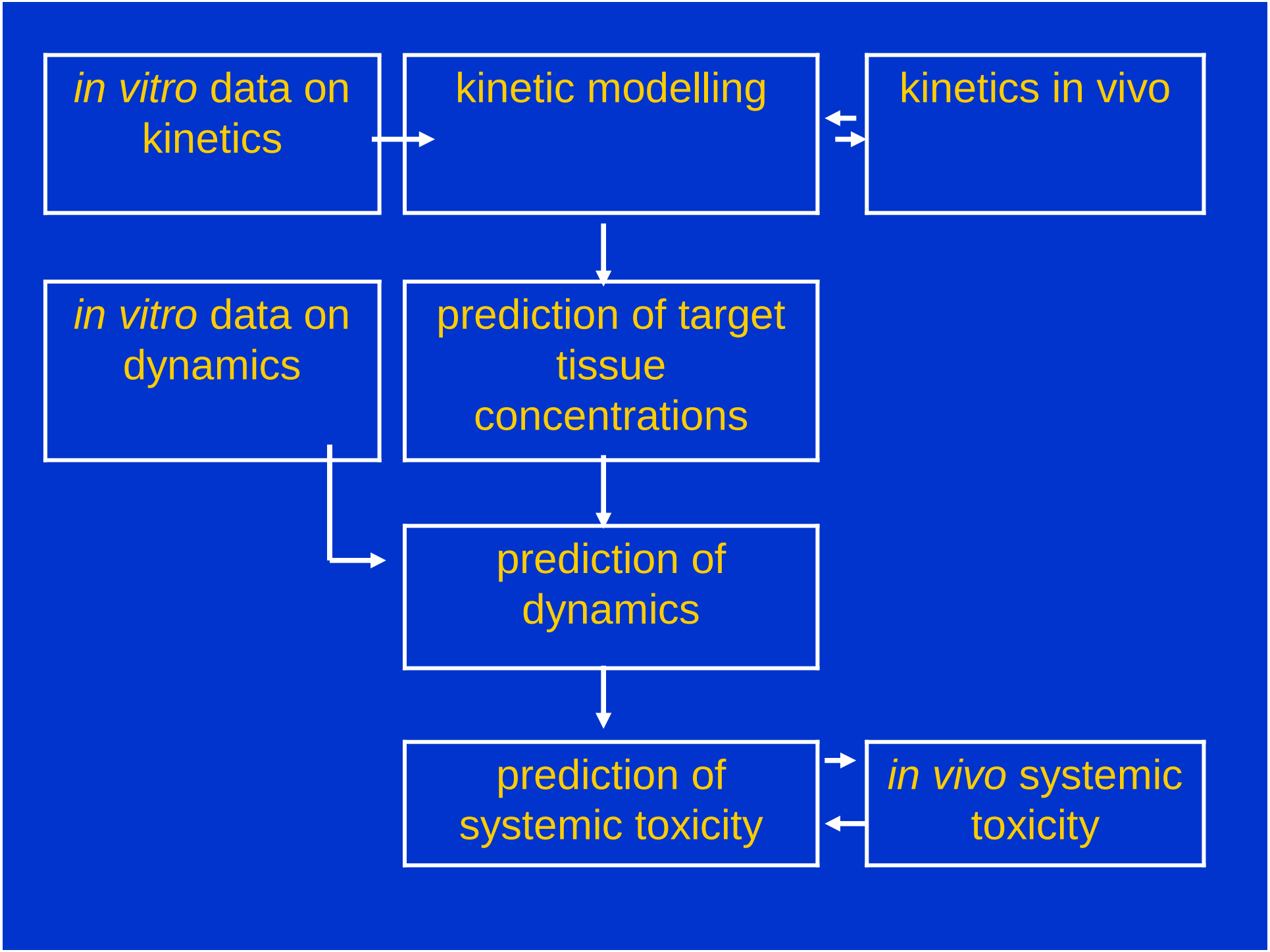
physico-chemical properties

QSARs

in vitro data

kinetic and dynamic modelling

evaluation against in vivo data


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 systemic toxicity

the case of acrylamide

In vitro data on toxicity

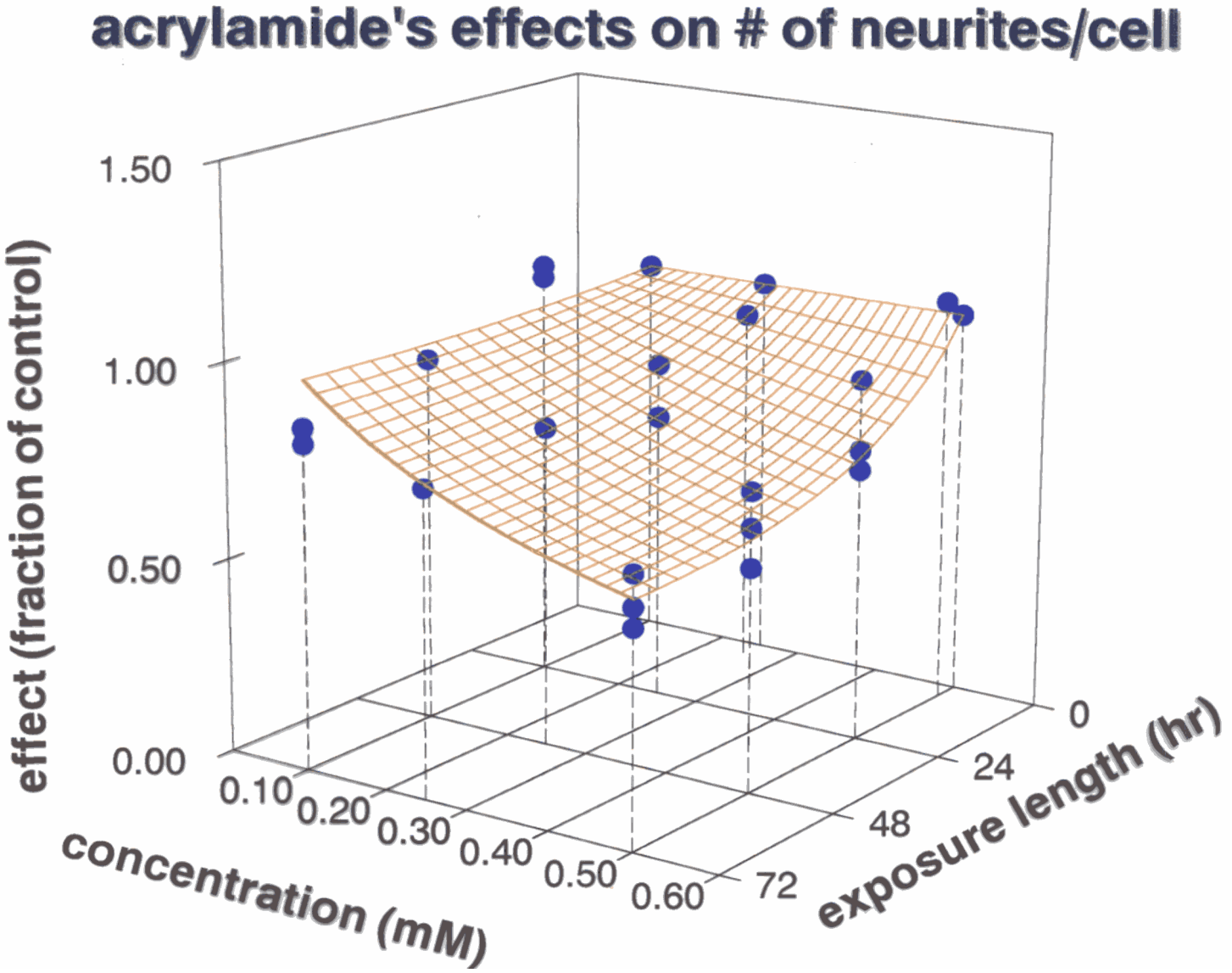
Kinetic model to predict dose causing toxic effects

determine basal cytotoxicity (e.g. Nutral Red test):

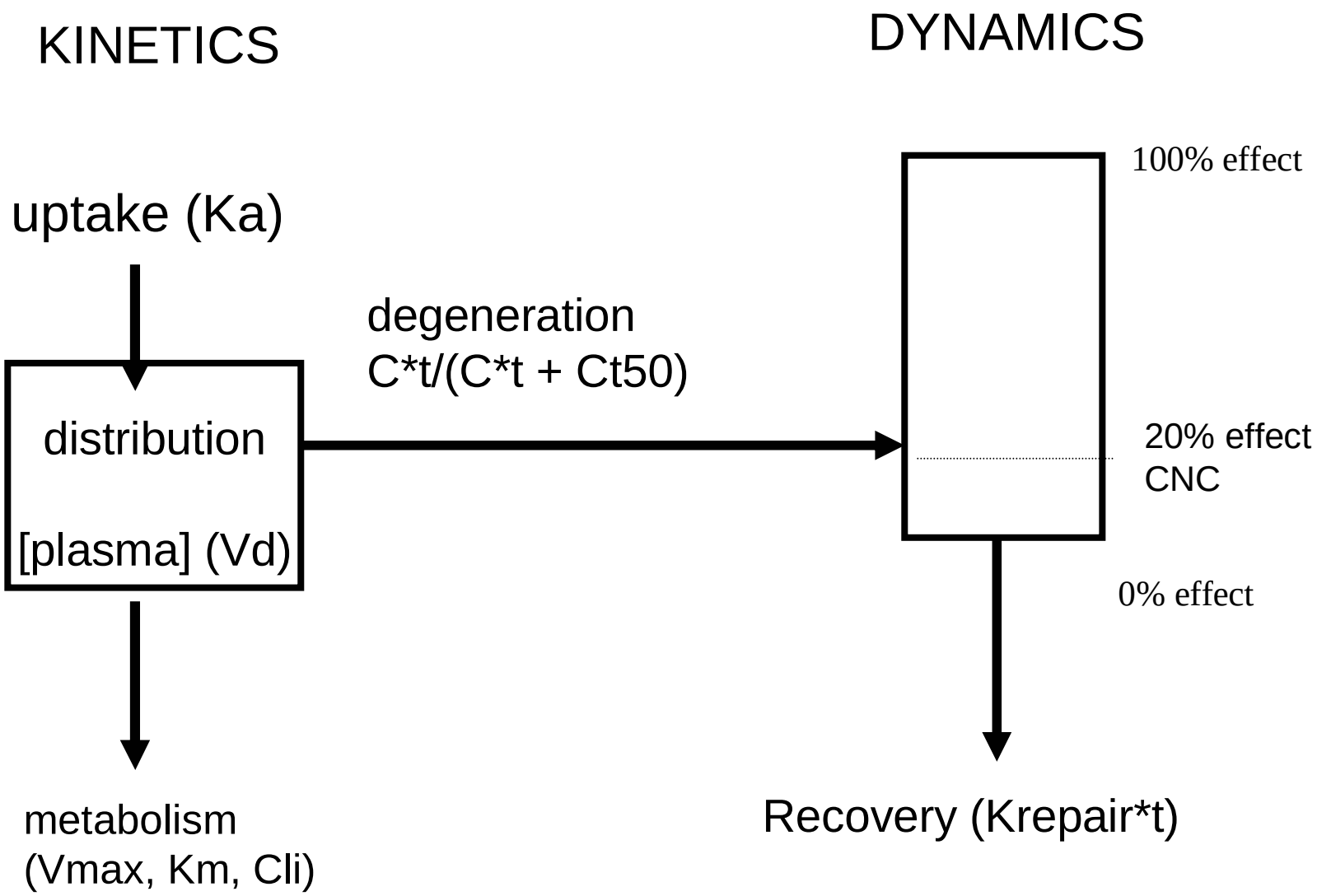
determine EC_{50} and EC_{20}

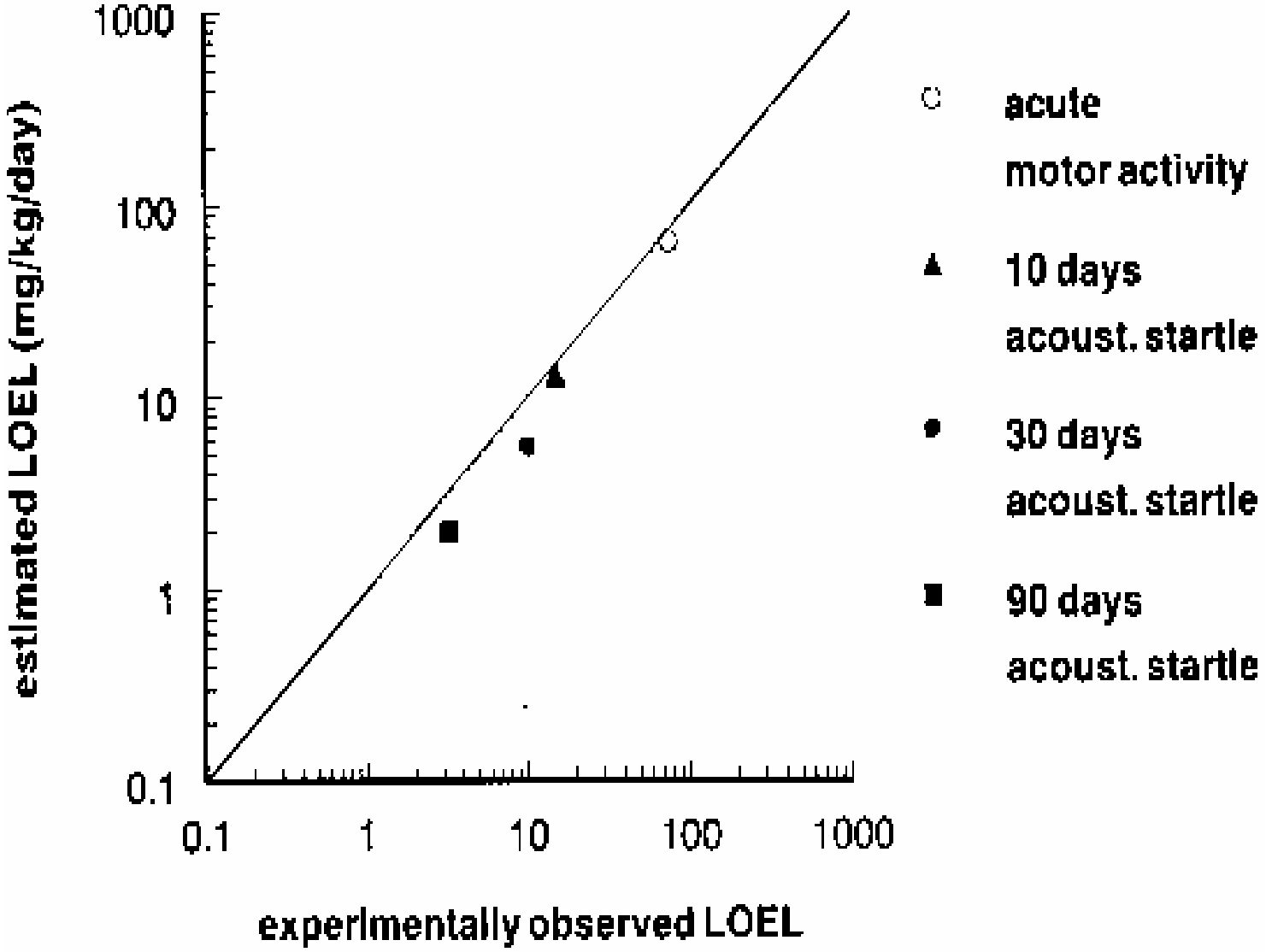
determine tissue-specific alterations, e.g. in neuronal cells:
-morphology
-changes in cell physiology
-neurochemical alterations
determine relevant EC_{50} and EC_{20}

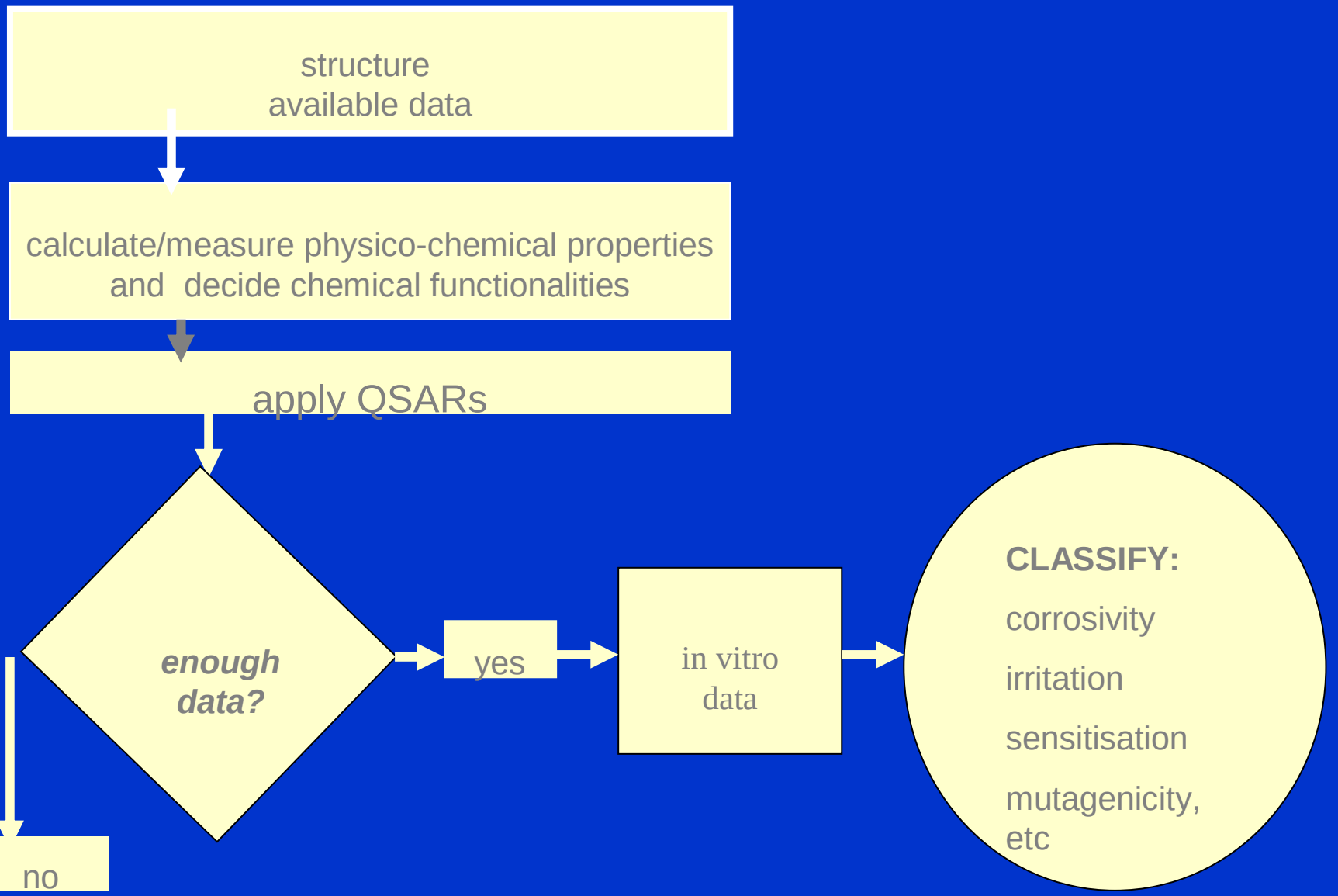
-determine ratios of EC_{50}s for basal/neurotoxic effects;
-decide on specific neurotoxicity action
-use EC_{20}-neurotoxicity as a basis for a LOAL calculation, making use of the integrated model described before.

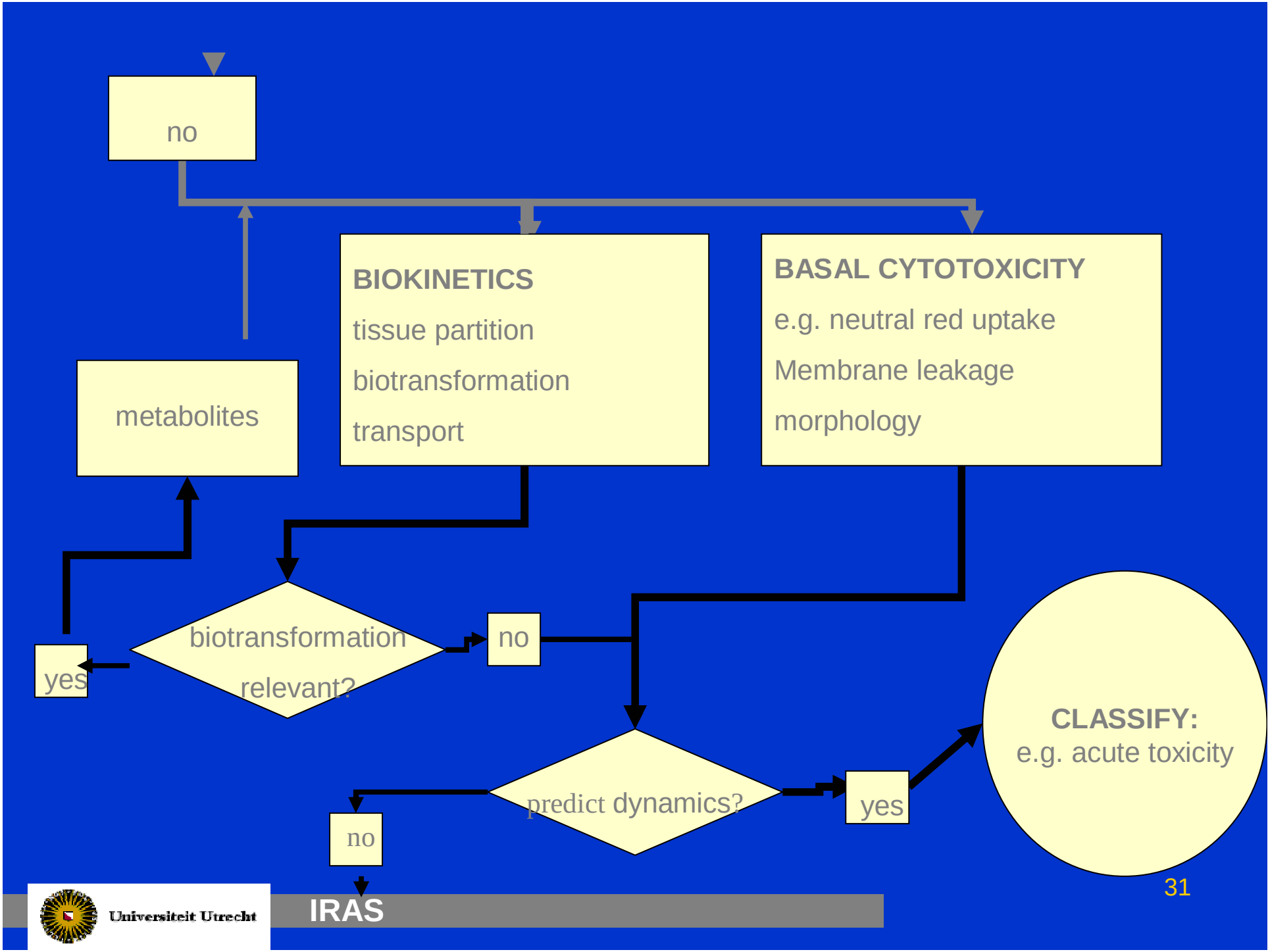

acrylamide's effects on # of neurites/cell
effect (fraction of control)
1.50
1.00
0.50
0.00
0.10
0.20
0.30
0.40
0.50
0.60
concentration (mM)
0
24
48
72
exposure length (hr)

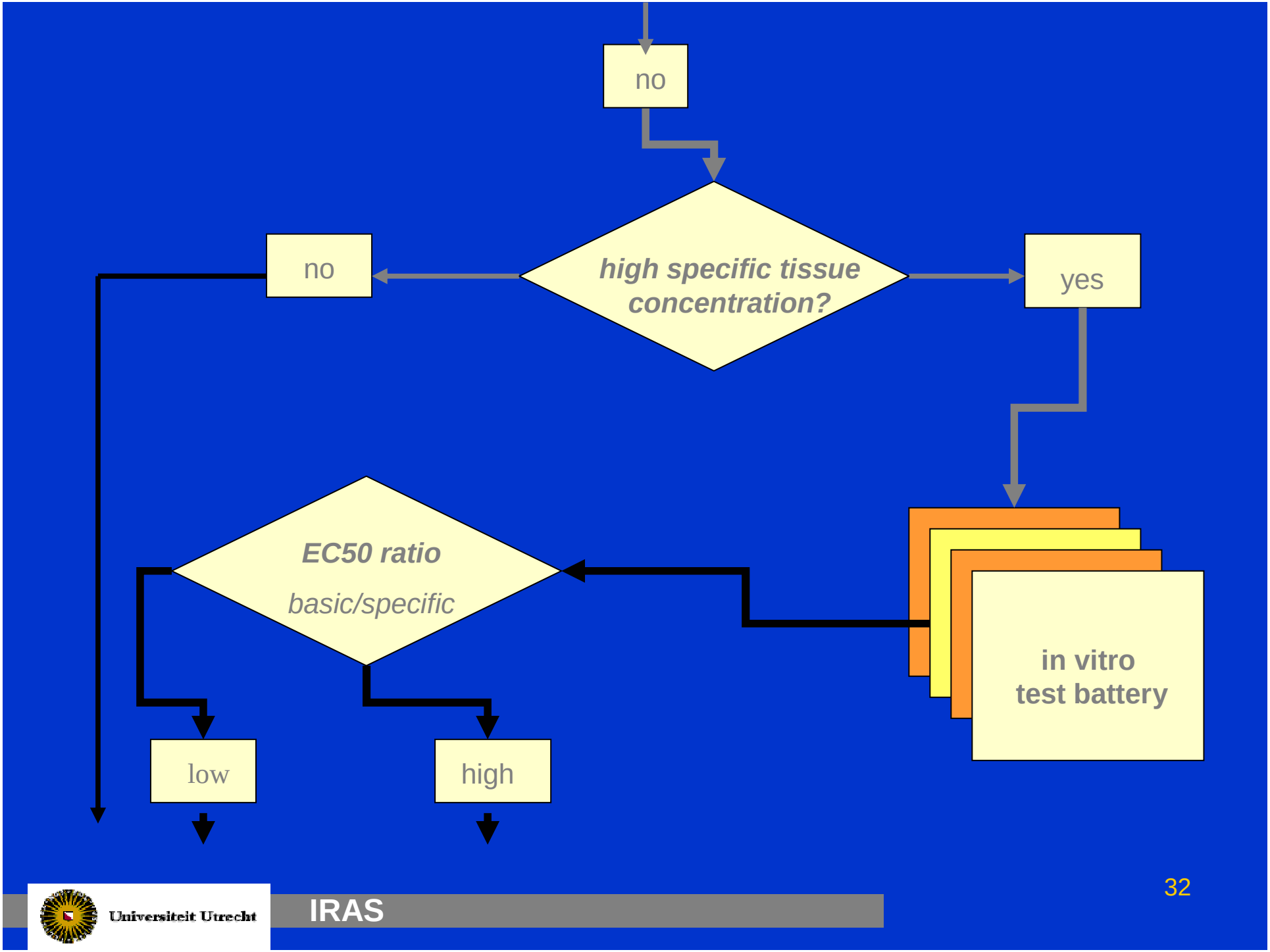
KINETIC-DYNAMIC MODELLING

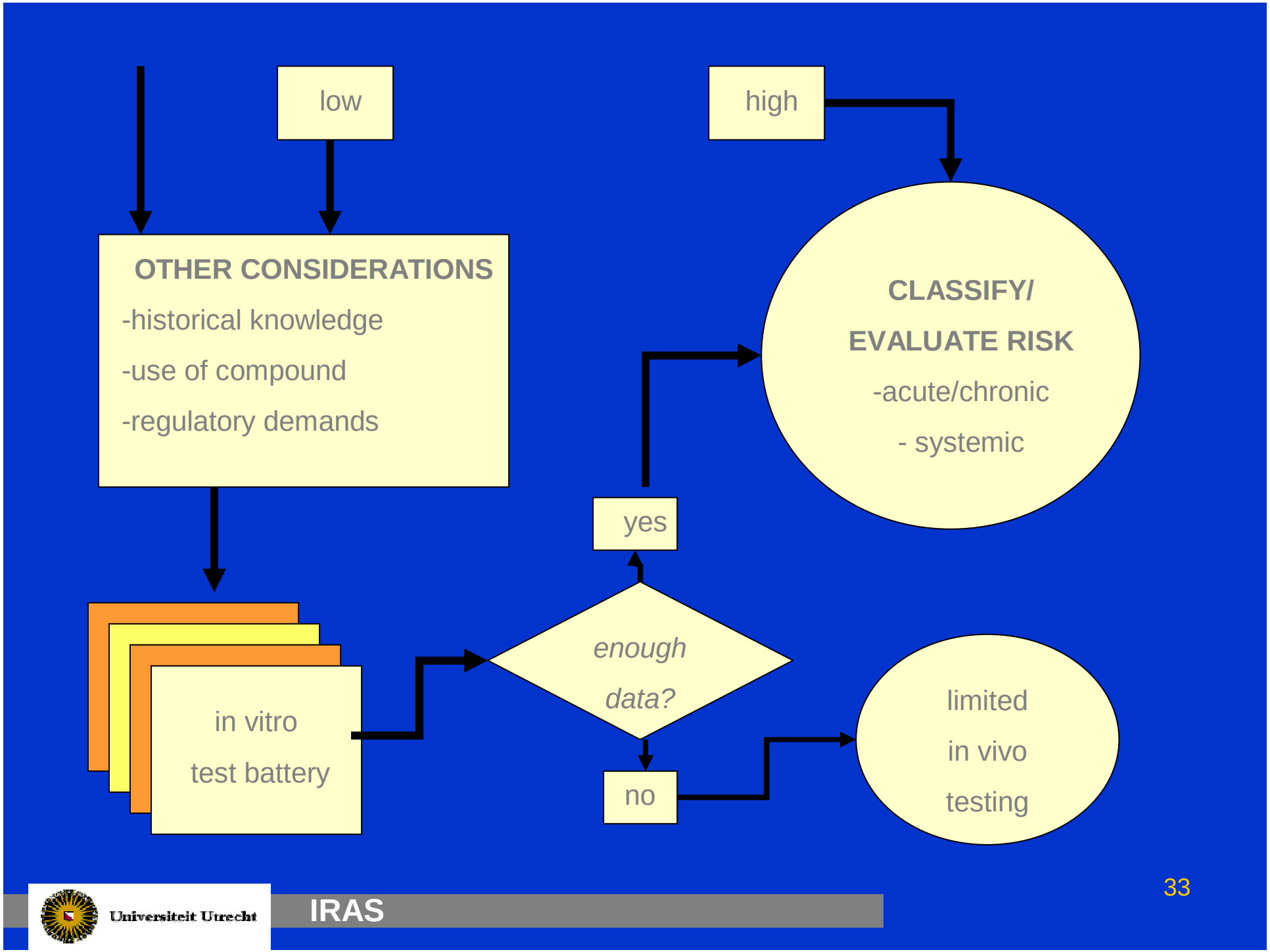


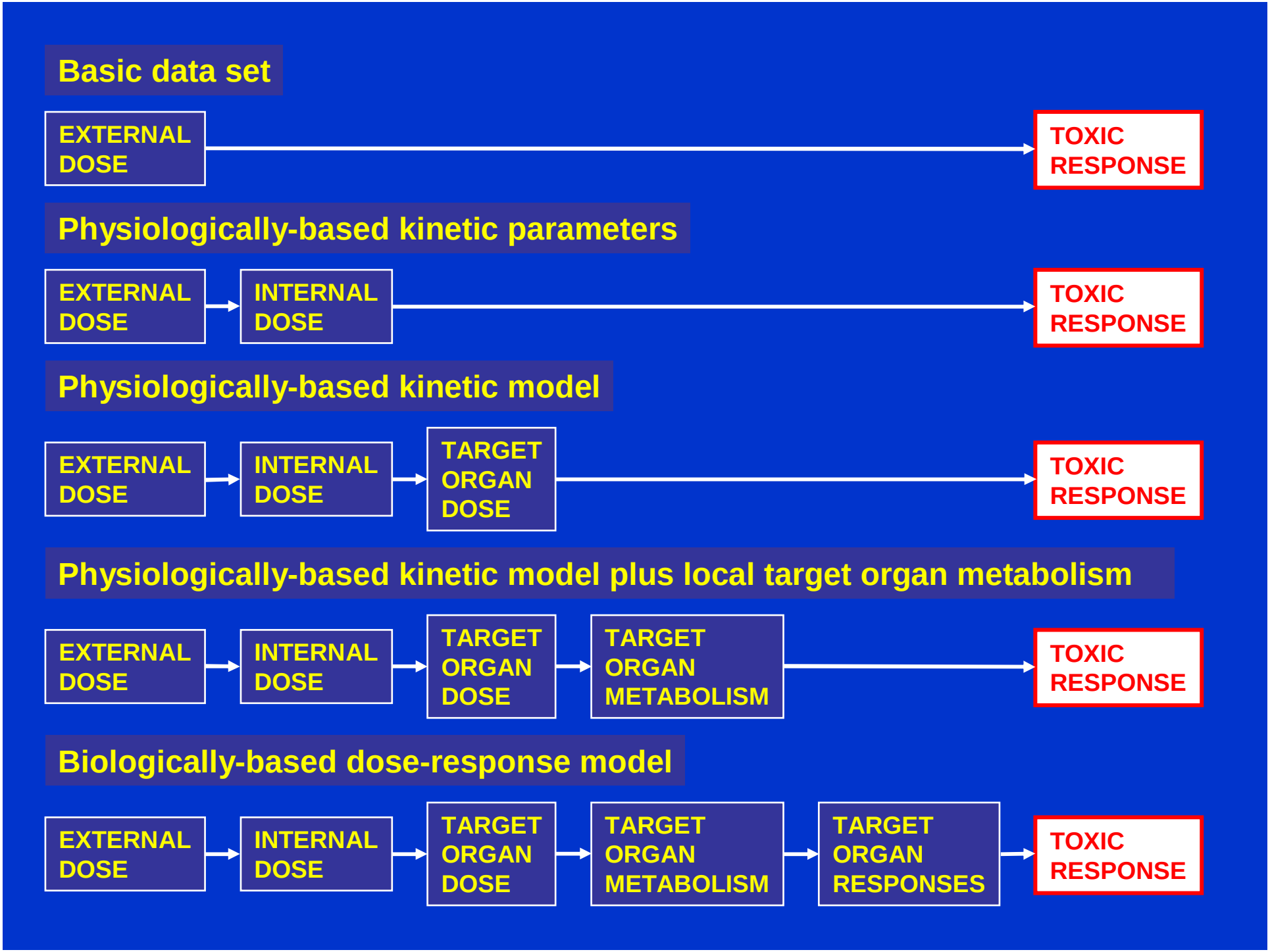

1000
100
10
1
0.1
estimated LOEL (mg/kg/day)
0.1
1
10
100
1000
experimentally observed LOEL
acute
motor activity
10 days
acoust. startle
30 days
acoust. startle
90 days
acoust. startle


structure
available data
calculate/measure physico-chemical properties
and decide chemical functionalities
apply QSARs
enough
data?
yes
no
in vitro
data
CLASSIFY:
corrosivity
irritation
sensitisation
mutagenicity,
etc


no
BIOKINETICS
tissue partition
biotransformation
transport
BASAL CYTOTOXICITY
e.g. neutral red uptake
Membrane leakage
morphology
metabolites
biotransformation relevant?
yes
no
predict dynamics?
yes
no
CLASSIFY:
e.g. acute toxicity


no
high specific tissue concentration?
no
yes
EC50 ratio
basic/specific
in vitro
test battery
low
high


low
high
OTHER CONSIDERATIONS
-historical knowledge
-use of compound
-regulatory demands
CLASSIFY/
EVALUATE RISK
-acute/chronic
- systemic
yes
in vitro
test battery
enough
data?
no
limited
in vivo
testing


Basic data set
EXTERNAL DOSE
TOXIC RESPONSE
Physiologically-based kinetic parameters
EXTERNAL DOSE
INTERNAL DOSE
TOXIC RESPONSE
Physiologically-based kinetic model
EXTERNAL DOSE
INTERNAL DOSE
TARGET ORGAN DOSE
TOXIC RESPONSE
Physiologically-based kinetic model plus local target organ metabolism
EXTERNAL DOSE
INTERNAL DOSE
TARGET ORGAN DOSE
TARGET ORGAN METABOLISM
TOXIC RESPONSE
Biologically-based dose-response model
EXTERNAL DOSE
INTERNAL DOSE
TARGET ORGAN DOSE
TARGET ORGAN METABOLISM
TARGET ORGAN RESPONSES
TOXIC RESPONSE

in vitro data in risk assessment: perspectives

use as stand-alone methods

use of in vitro data in integrated schemes

challenges: choice of the most relevant battery of tests

can we predict mode of action/target organ on the basis of structure

CONCLUSIONS

- integration of *in vitro* data in risk evaluation is possible, provided that:
  biokinetics are taken into account (absolute necessity)
- integration of all 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