Data from alternative methodologies for cumulative risk assessment

– An industry perspective

Robert Landsiedel

at the Workshop

'What does the future hold for harmonised human health risk assessment of plant protection products?'

Berlin, 24th of November 2017

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Key Issues

- key issue #1: For which toxicological endpoints do alternative test methods already exist?
- key issue #2: How to improve the reliability of in-silico methods?
- key issue #3: How to use data from in-silico tools and alternative test methods to perform cumulative risk assessment?

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For which toxicological endpoints do alternative test methods already exist?

- **Skin irritation and corrosion** (OECD t.g no. 430, 431, 435, 439)
- **Photocytotoxicity** (OECD 432)
- **Eye irritation** (OECD 437, 438, 460, 491, 492)
- **Skin sensitization** (OECD 442C, D, E)
- **Genotoxicity and Mutagenicity**
  (OECD 471, 473, 476, 480, 481, 482, 487, 490)
- **Steroid hormone receptor binding and synthesis**
  (OECD 455, 456, 457, 458, 493)

For which toxicokinetic data do alternative test methods already exist?

- **Dermal penetration** (OECD t.g. no. 428)

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- **key issue #3:**
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Substances in complex mixtures ...

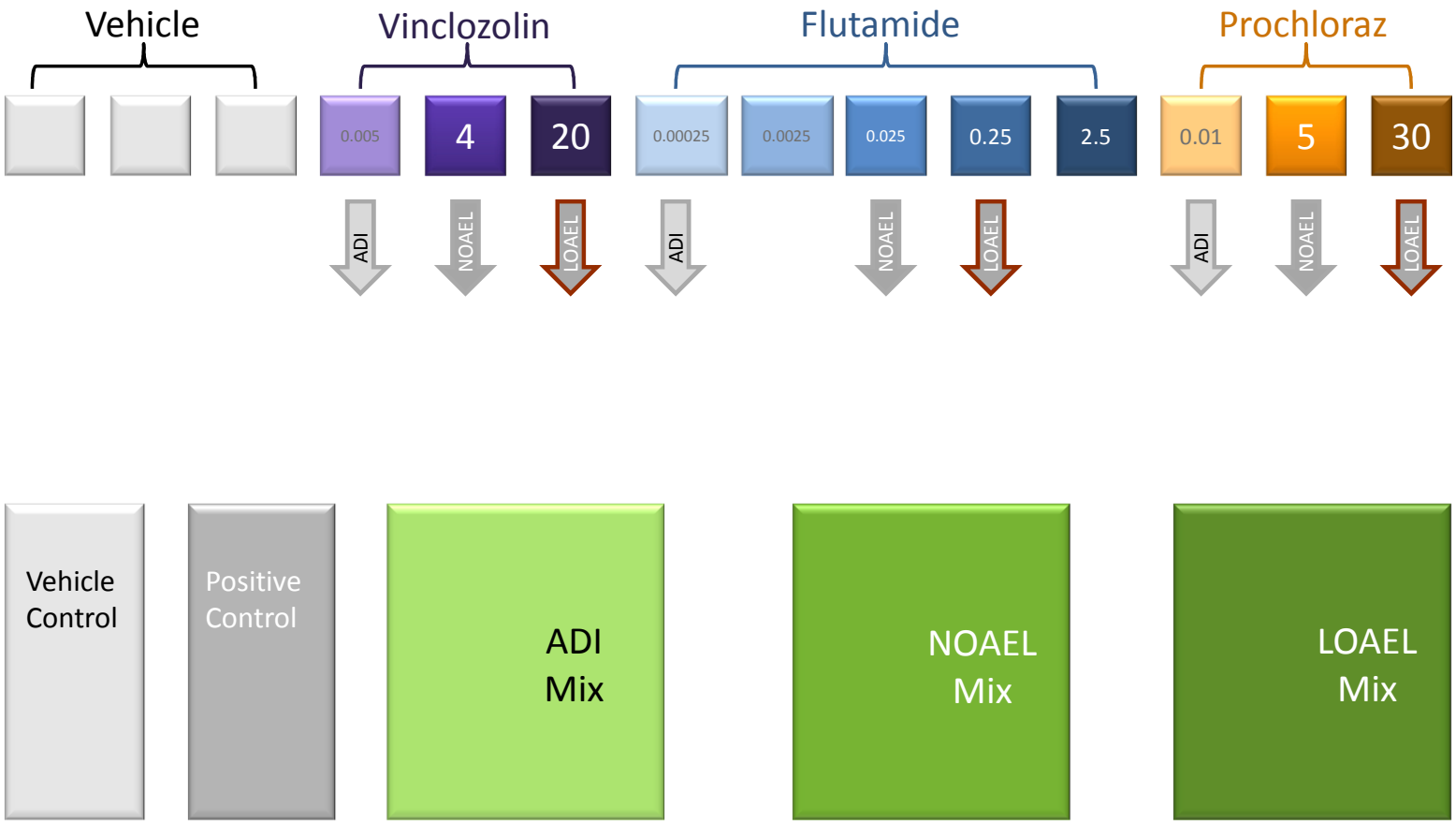
- **Substances in**
  - formulations
  - multiple residues
- **Substances acting**
  - independent
  - additive
  - inter-acting
    - over-additive
    - under-additive

1 + 1 = 1
1 + 1 = 2
1 + 1 = 1.6

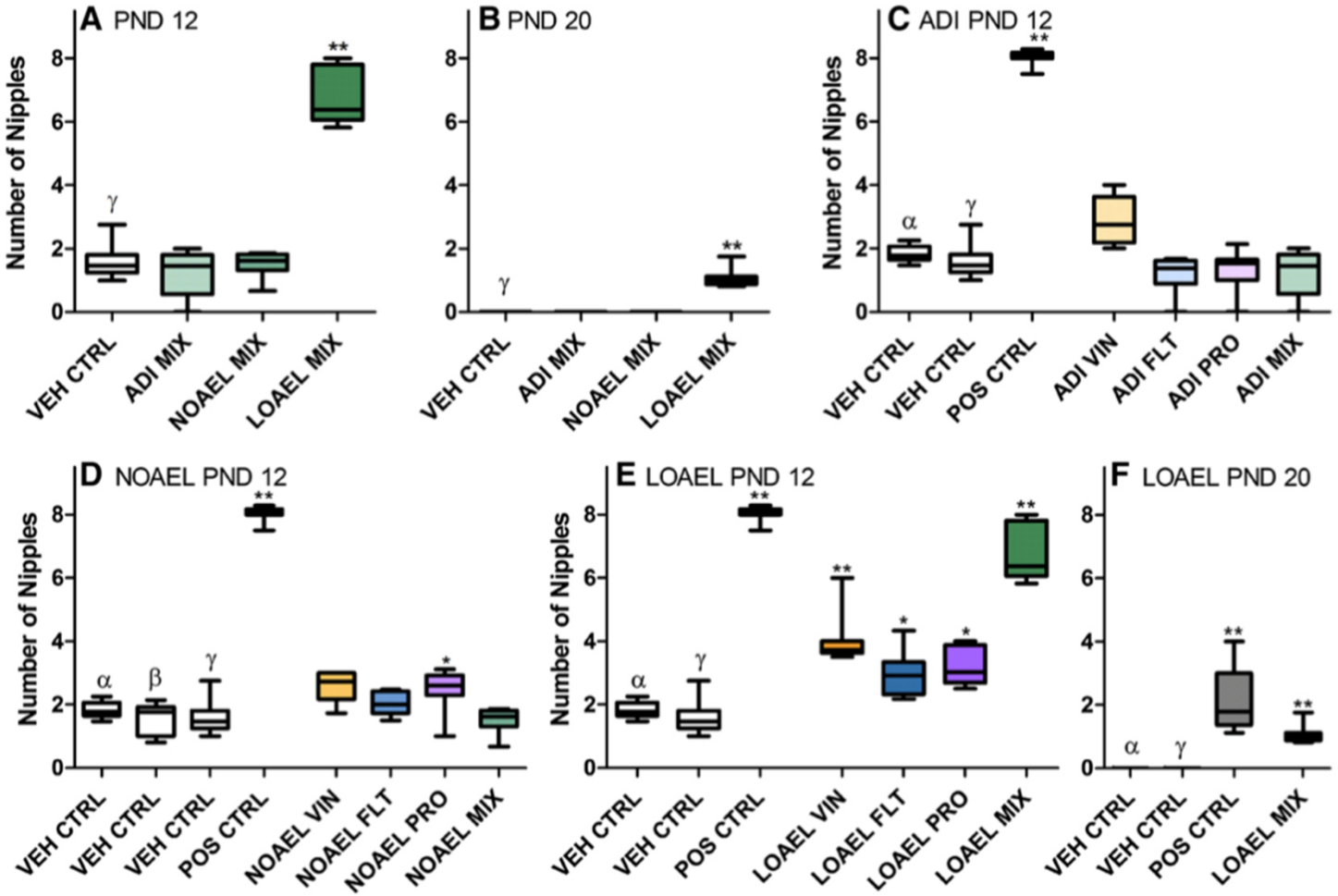
CASUS I

Combined exposure to low doses of three anti-androgens *in vivo*

Dose–response relationship of combined exposure to low doses of three anti-androgens



Effect of mixtures of vinclozolin, futamide, and prochloraz on the number of nipples/areolae on the male pups.



Arch Toxicol
DOI 10.1007/s00204-017-2053-3

REPRODUCTIVE TOXICOLOGY

Investigations on the dose–response relationship of combined exposure to low doses of three anti-androgens in Wistar rats

Steffen Schneider[1] · Karma C. Fussell[2] · Stephanie Melching-Kollmuss[3] · Roland Buesen[1] · Sibylle Gröters[1] · Volker Strauss[1] · Xiaoqi Jiang[1] · Bennard van Ravenzwaay[1]

Nipple/areola counts appeared to be a sensitive measure of effect, in addition to male sex organ weights at sexual maturation, and finally gross findings. **The results indicate the absence of evidence for effects at low or very low dose levels.** No (adverse) effects were seen at the NOAEL dose. A non-monotonic dose–response relationship was not evident.

Combined exposure at LOAEL level resulted in enhanced responses for anogenital index, number of areolas/nipples, delayed preputial separation and reduced ventral prostate weight in comparison to the individual compounds.

CASUS II

Acute oral toxicity of agrochemical formulations

GHS additivity formula: can it predict the acute systemic toxicity of agro-chemical formulations that contain acutely toxic ingredients?

Andrew Van Cott[a*], Charles E. Hastings[a], Robert Landsiedel[b], Susanne Kolle[b], Stefan Stinchcombe[b]

a. BASF Corporation, Research Triangle Park, NC. USA 27709
b. BASF SE Experimental Toxicology and Ecology, Ludwigshafen, Germany

Table 3: Predictivity of GHS Additivity formula for acute oral GHS Classification

$$\frac{100}{ATE_{mix}} = \sum_{n} \frac{C_i}{ATE_i}$$

ATE	= Acute Toxicity Estimate (e.g. LD_{50} /LC_{50})
C_i	= Concentration of ingredient i
i	= Individual Relevant ingredient from 1 to n
n	= Number of ingredients

Category (in vivo Data)	Classification (GHS Formula Prediction) 1	2	3	4	5	NC	Total
1	0	0	0	0	0	0	0
2	0	0	1	0	0	0	1
3	0	0	5	16	2	7	30
4	0	0	2	60	18	34	114
5	0	0	2	5	6	4	17
NC	0	0	0	11	8	29	48
							210

NC: Not Classified

100 additive
82 over-additive
28 under-additive

submitted manuscript

CASUS III

Local tolerance of agrochemical formulations *in vitro*

Regulatorily accepted but out of domain:

In vitro skin irritation tests for agrochemical formulations

Susanne N. Kolle*, Bennard van Ravenzwaay, Robert Landsiedel

BASF SE Experimental Toxicology and Ecology, Ludwigshafen, Germany

Table 3
Contingency table skin corrosion in vitro (OECD TG 431) vs. in vivo (OECD TG 404) classified according to UN GHS.

		In vivo (OECD TG 404) classified according to UN GHS		
		Not Corrosive	Cat 1	Sum
In vitro skin corrosion test (OECD TG 431)	NC	77 (70 + 7)	0 (0 + 0)	77 (70 + 7)
	Cat 1	4 (4 + 0)	0 (0 + 0)	4 (4 + 0)
	Sum	**81 (74 + 7)**	**0 (0 + 0)**	**81 (74 + 7)**

4 non-concordant
95% accuracy

Table 5A
Contingency table skin irritation in vitro (OECD TG 439) vs. in vivo (OECD TG 404) classified according to UN GHS.

		In vivo (OECD TG 404) classified according to UN GHS			
		Not Classified	Cat 3	Cat 2	Sum
In vitro skin irritation test (OECD TG 439)	NI	22 (19 + 3)	2 (1 + 1)	14 (14 + 0)	**38 (34 + 3)**
	Cat 2	10 (10 + 0)	6 (6 + 0)	11 (11 + 0)	**27 (27 + 0)**
	Sum	**32 (29 + 3)**	**8 (7 + 1)**	**25 (25 + 0)**	**65 (61 + 4)**

30 non-concordant
54%

Numbers provided in parenthesis are number of liquid plus solid formulations of the total tested.

http://dx.doi.org/10.1016/j.yrtph.2017.07.016

Conclusions from *Casus*

- from *Casus I*:
  There may be little toxicodynamic interaction of a.i. at NOAEL;
  Toxicodynamic interaction of a.i. at LOAEL may be small

- from *Causus II*:
  There is interaction of formulation‘s ingredients;
  most likely affecting toxicokinetics

- from *Casus III*:
  Current OECD *in vitro* methods may not be fit to capture these

Propaedeutic

Substance interactions on a molecular biology level

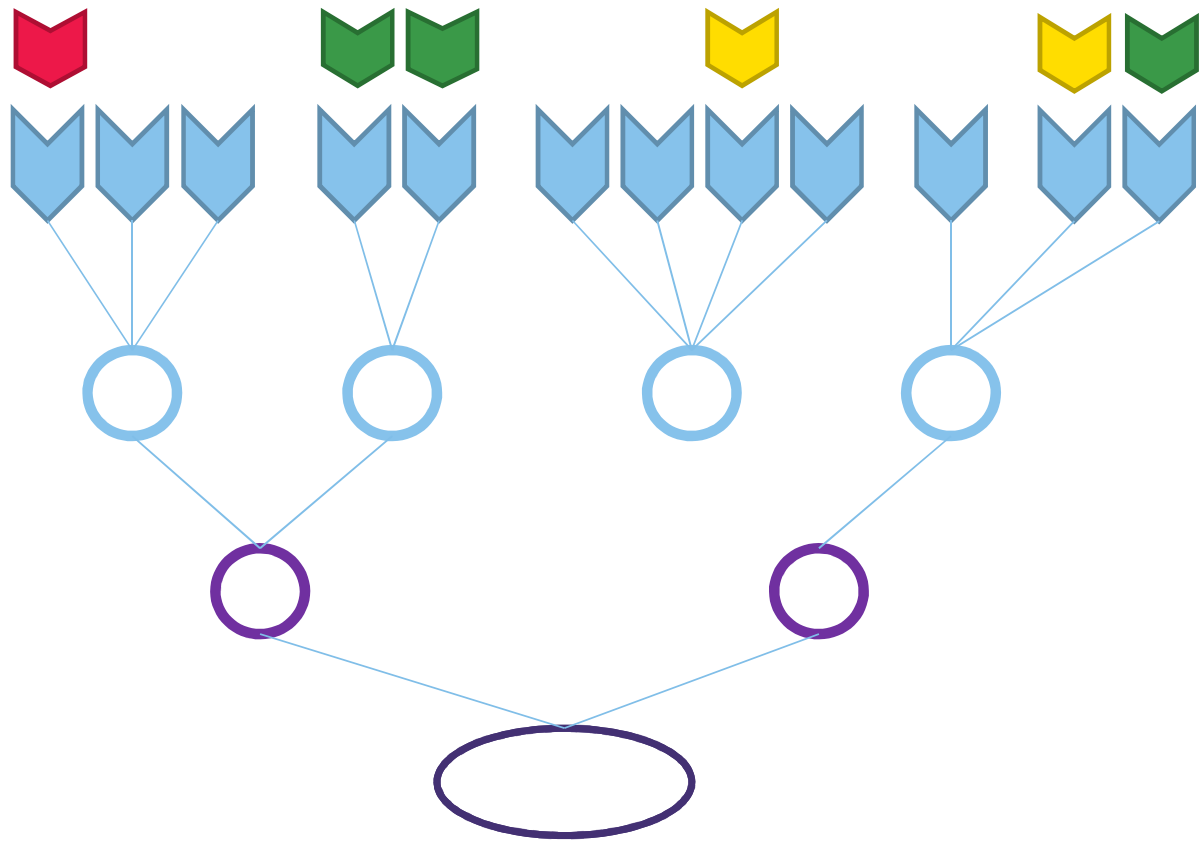
Multiple substances activate multiple pathways, which may interact

Substances

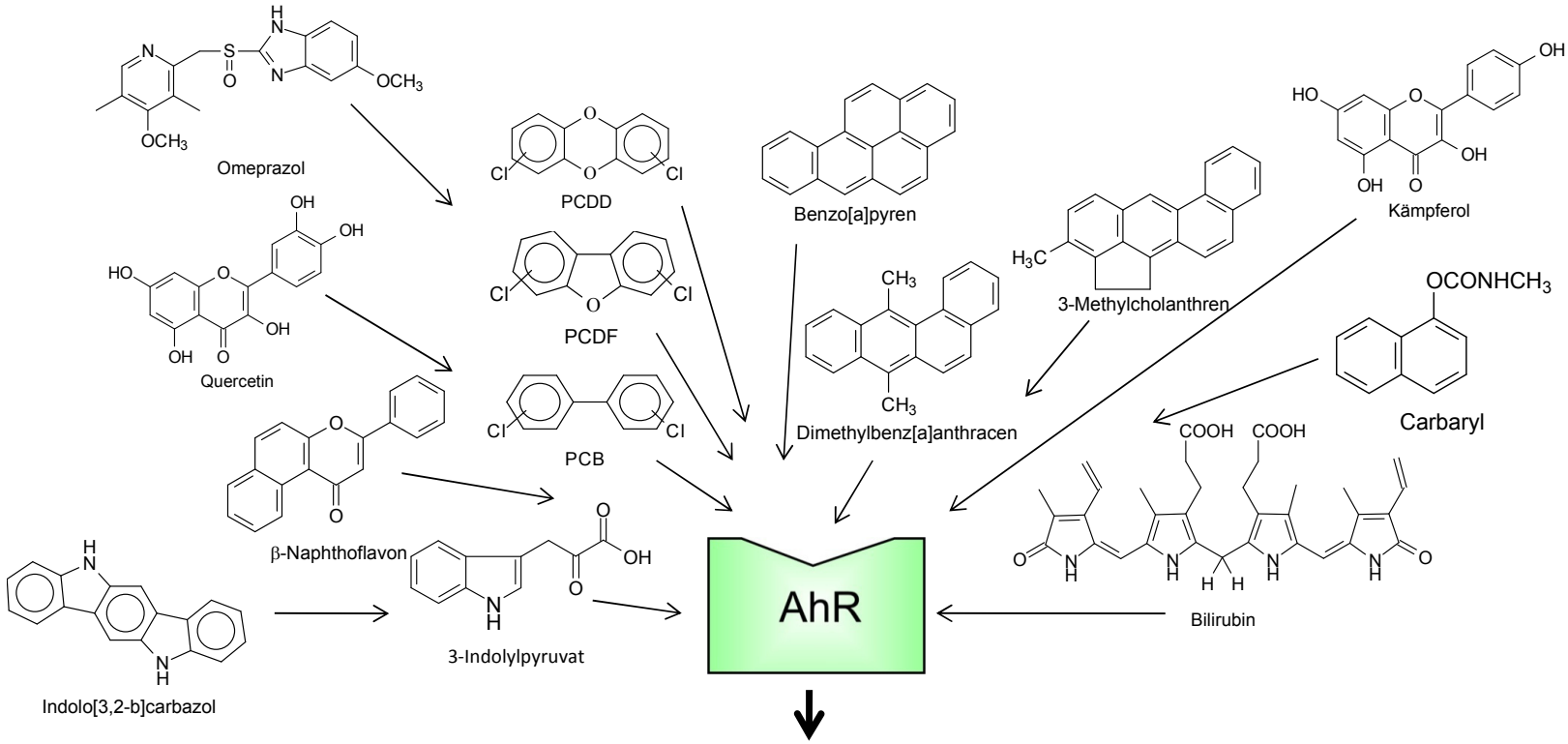
Receptors, Enzymes

Pathways

Effects

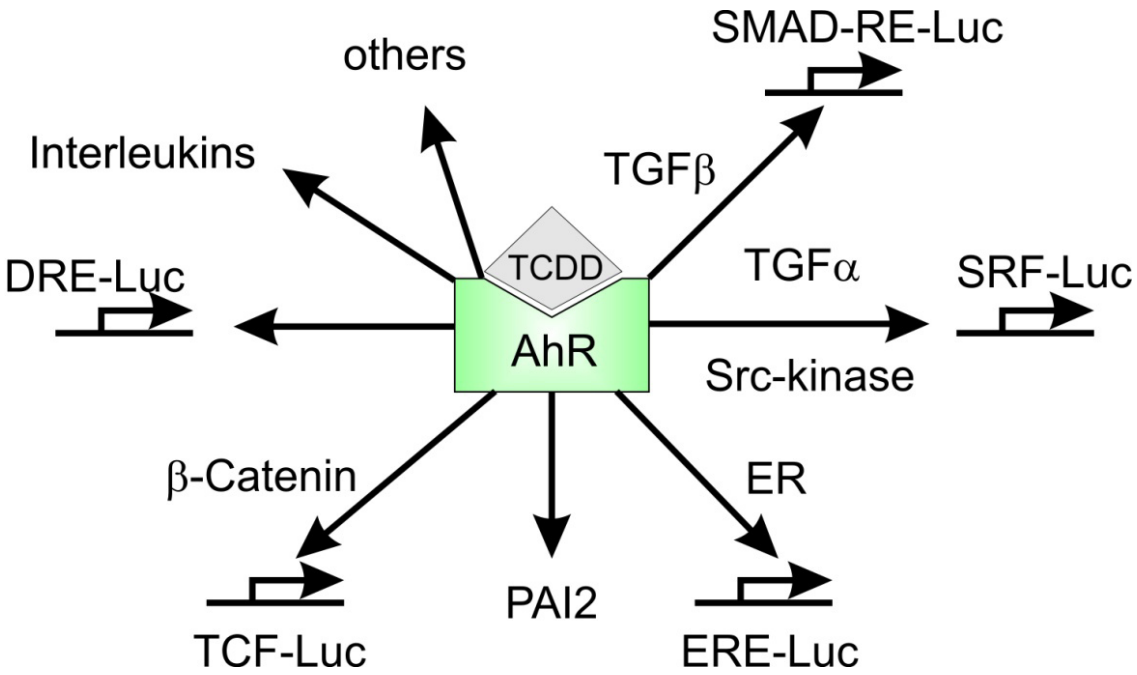


One receptor is affected by multiple substances



Adaptive and adverse responses

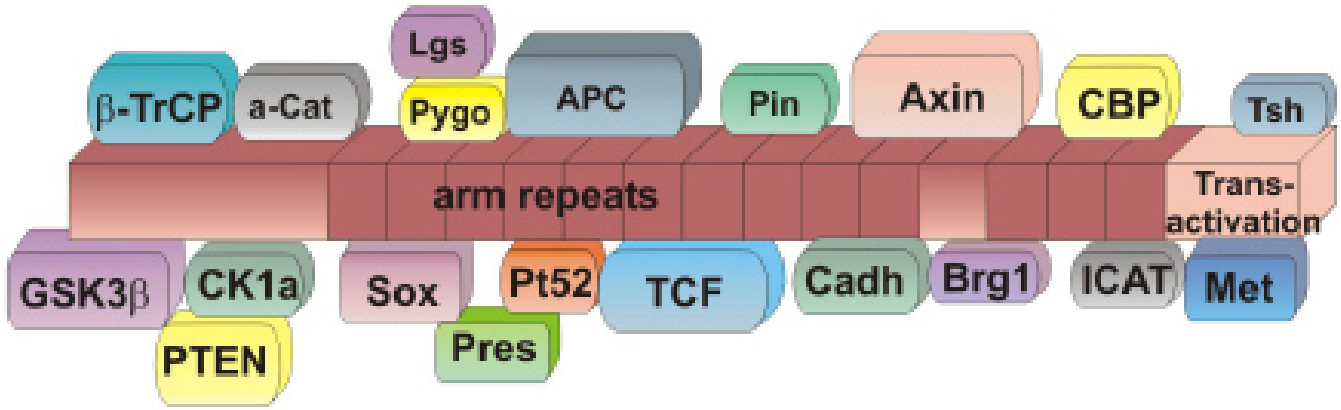
One receptor affects several signaling pathways



Which one is indicative of adversity?

Proteins as ‚nodes‘

β-catenin
(~1000 aa)



„sticky“ proteins will have multiple interaction partners
and their activity will be affected by many signaling pathways
(example β-catenin with its armadillo repeats)

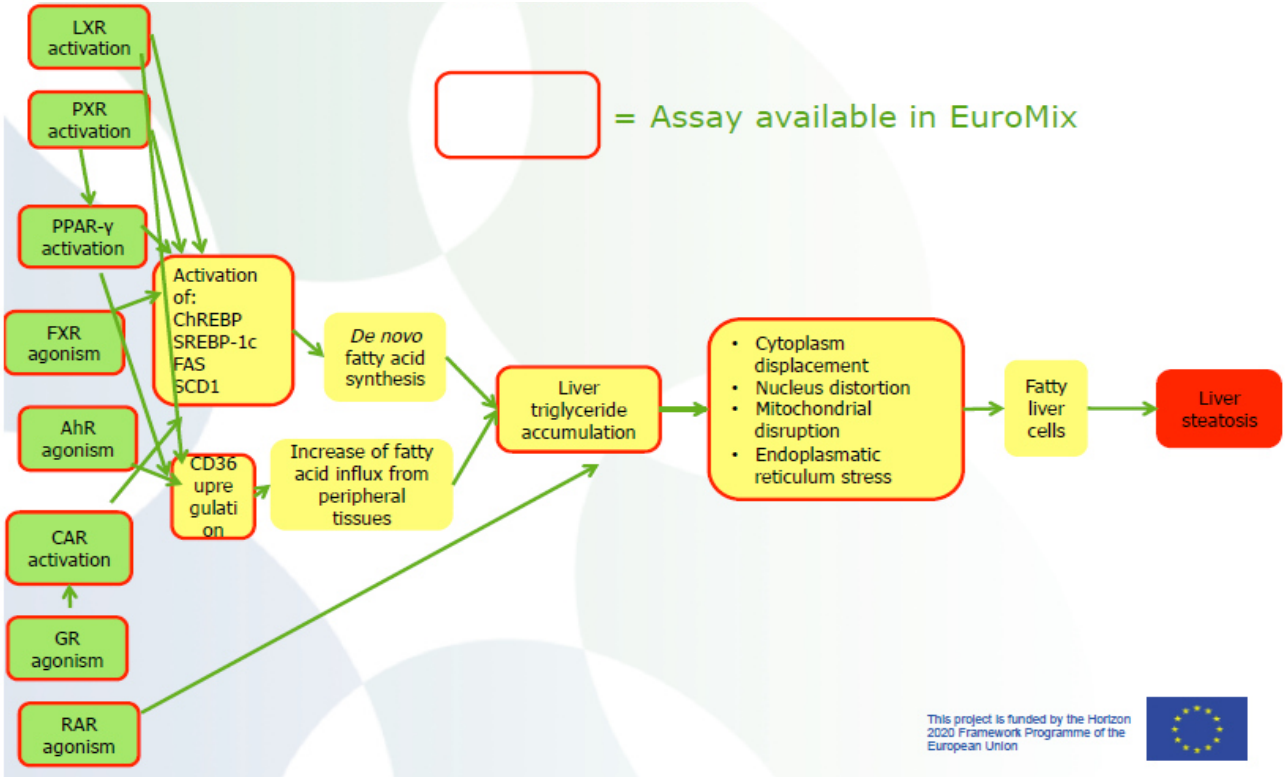
What to do
to address effects of mixtures *in vitro*

- Define ‘additivity’
- Define critical molecular or cellular targets
- Develop methods
- Verify

Status quo

Concepts and methods

EuroMix Project



- Adverse outcome: Liver steatosis = fatty changes
- Define AOPs
- Identify responses in key event assays and in MIEs
- Make an assumption on type of combination toxicity

Feasibility?

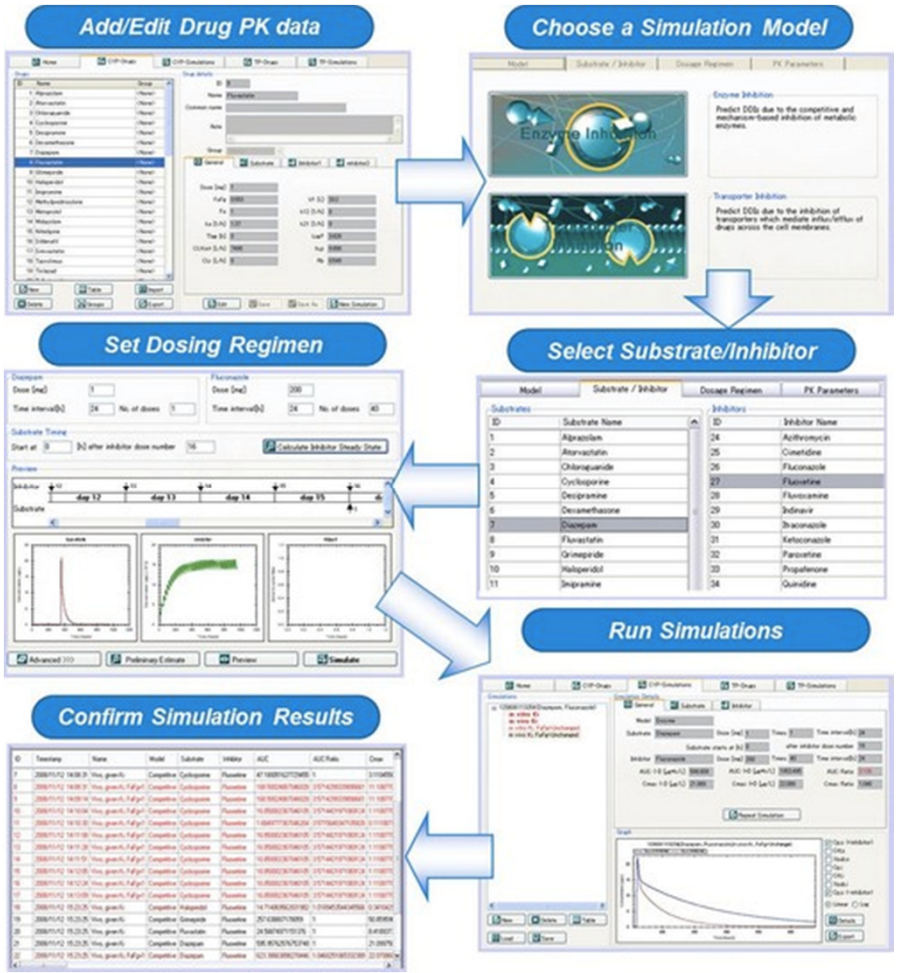
EuroMix

Toxicokinetic Interactions

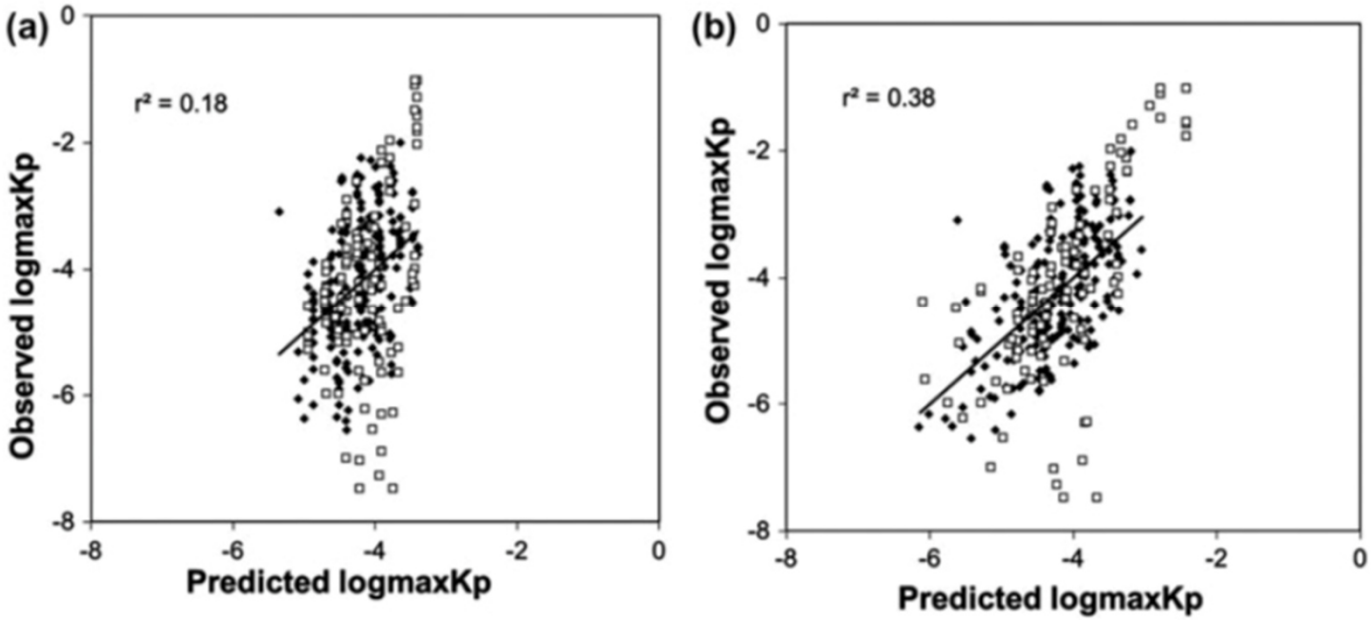
DDI Simulator quantitatively predicts the extent of drug-drug interactions arising from co-administration of drugs, an important study in drug development, through time course simulation of the concentrations of each drug in the body using physiologically-based pharmacokinetic (PBPK) mathematical models.

薬物相互作用シミュレーションソフト

DDI Simulator ®



Prediction of barrier penetration of a.i. in complex mixtures



without ...

and with Mixture factor

SAR and QSAR in Environmental Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gsar20

In silico models to predict dermal absorption from complex agrochemical formulations

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What to do
to address effects of mixtures *in vitro*

- Define ‘additivity
- Define relevant concentration ranges
- Identify critical toxicokinetic interactions
- Define critical molecular or cellular targets
- Develop methods
- Verify!
  - Make it quantitative!

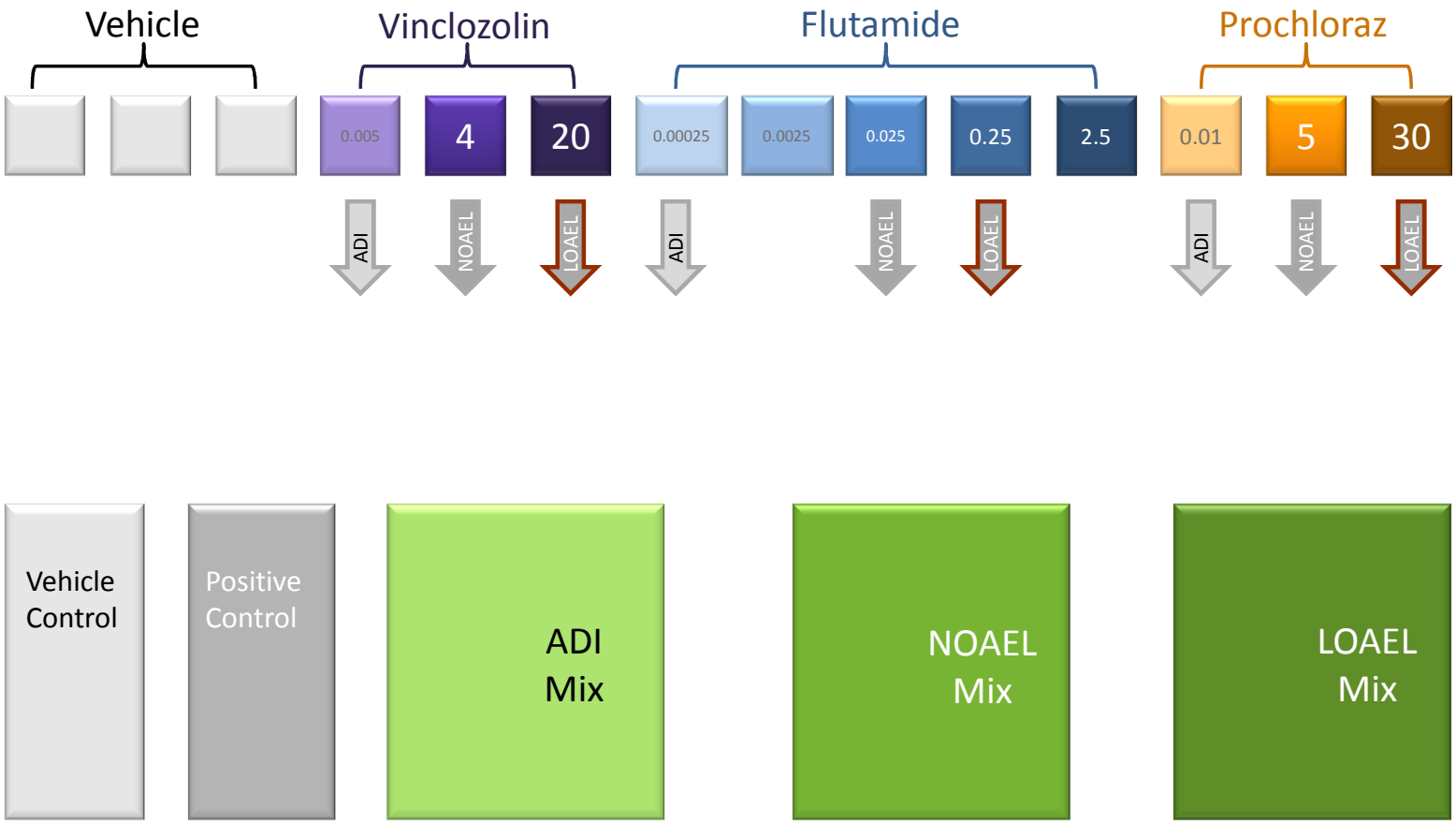
What to do to quantitatively address effects of mixtures *in vitro*

- **Define 'additivity'**
  define 'normal'
- **Define relevant concentration ranges**
  ADI, NOAEL, LOAEL? QIVIVE!
- **Identify critical toxicokinetic interactions**
  uptake and distribution (barrier) for formulations
- **Define critical molecular or cellular targets**
  identify critical 'nodes' since MIE may not be ideal
- **Develop methods**
  in vitro, *in silico*, alert list
  check for over-additivity at NOAEL only?
- **Verify!**
  In vitro = *In vivo* ? pertinent molecular/cellular changes


Thank You for listening
And
Keep loving Science!

BACKUP

Dose–response relationship of combined exposure to low doses of three anti-androgens



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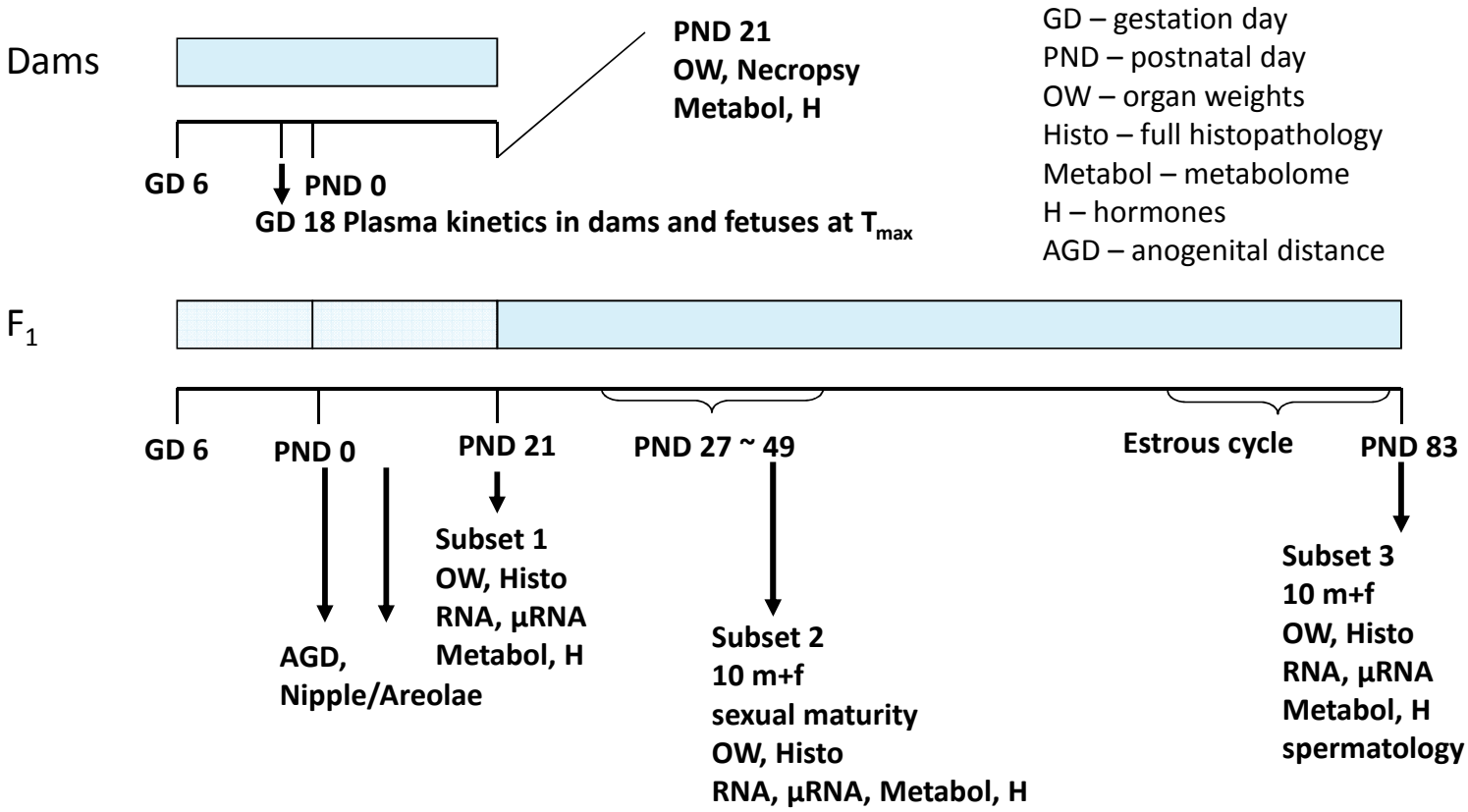

Differentiate

- **Differentiate**
  uptake and distribution vs. toxicodynamics (and metabolism?)
- **Differentiate effects:**
  local tolerance, acute systemic and repeated-dose effects
- **Differentiate mixtures:**
  multiple residues and formulations