Using (Q)SARs, TTC, molecular docking simulation and read-across as a first tier in mixture toxicity risk assessment

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Dutch Nat.Inst.Public Health & Environment (RIVM)

EUSAAT 2016, August 25

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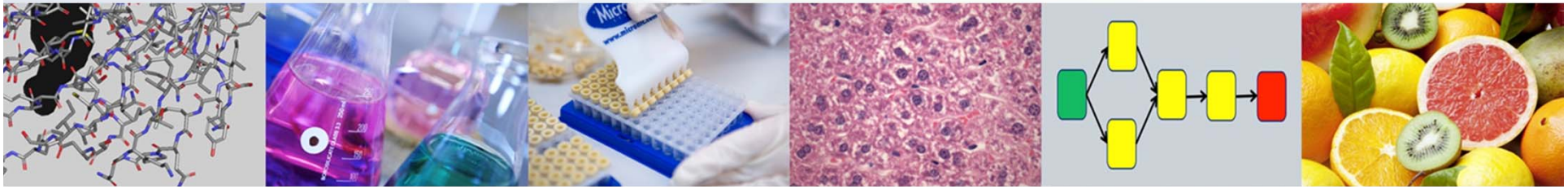
2. Tiered approach to Cumulative Risk Assessment
3. QSAR to determine which Common Assessment Group(s) apply
4. Assign a Relative Potency Factor (NOAEL / RA / TTC)
5. The MCRA tool (probabilistic cumulative risk assessment)
6. Refine the Cumulative Risk Assessment -> in vitro assays



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EuroMix

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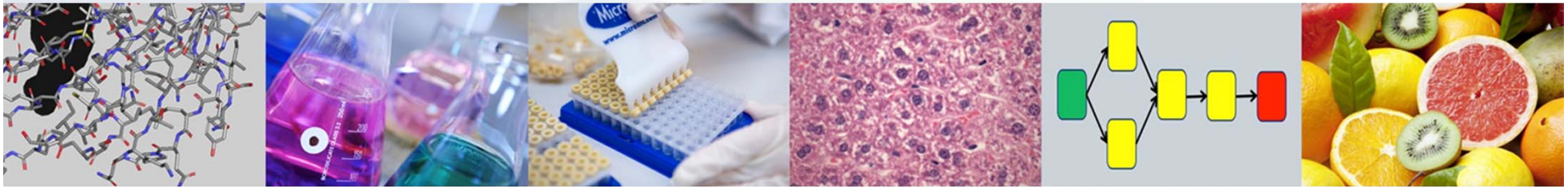
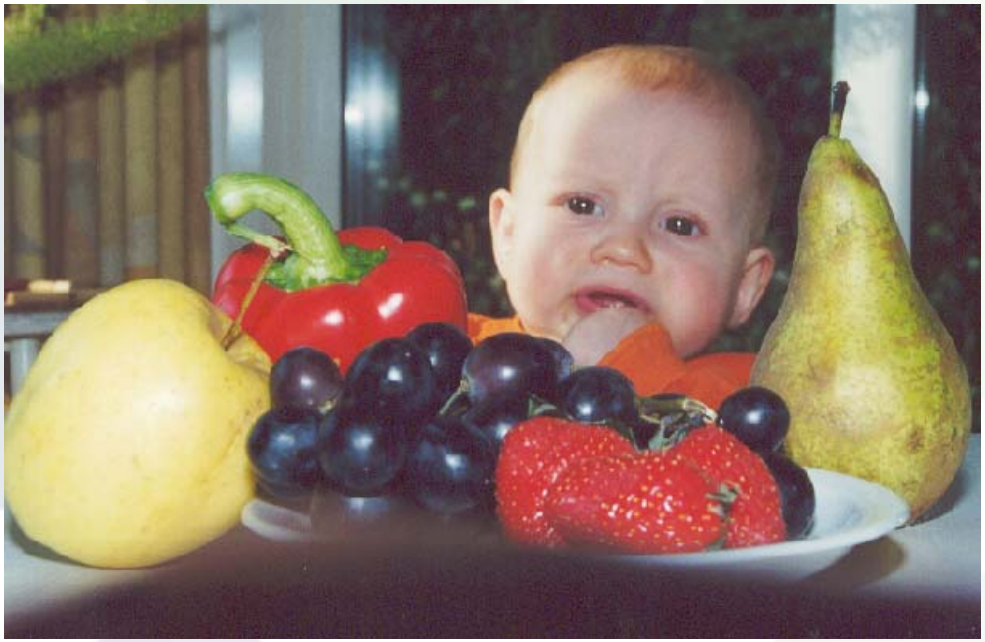


European Parliament asked for CRA



- Consumer groups
- European Parliament
- Regulation 396/2005, art 14
- EFSA colloquium and EFSA opinions
- EU funded project ACROPOLIS

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Directive EC 396/2005 and 1007/2009

Regulation EC 396/2005 on maximum residue levels (MRLs)

It is also important to carry out further work to develop a methodology to take into account cumulative and synergistic effects. In view of human exposure to combinations of active substances and their cumulative and possible aggregate and synergistic effects on human health, MRLs should be set after consultation of the European Food Safety Authority.

Regulation EC 1107/2009 on the placing of PPPs on the market

"…a PPP shall not have harmful effects on human health, including that of vulnerable groups, or animal health, taking into account known cumulative and synergistic effects where the scientific methods accepted by the Authority to assess such effects are available.."

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EFSA grouping chemicals into CAGs

Cumulative Assessment Groups (CAG)s

- Level 1: organ level
- Level 2: phenomenological endpoints
- Level 3: mode of action
- Level 4: mechanism of action

EFSA opinions to be expected in 2016/17

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LEVEL 1
EFSA grouping pesticides into CAG

16 organs identified

- Adrenal glad
- Bone marrow
- Bones/skeleton
- Cardiovascular system
- Eye
- Gallbladder
- Haematological system
- Kidney
- **Liver**
- Muscles
- Nervous system
- Parathyroid gland
- **Reproductive system**
- **Developmental toxicity**
- Spleen
- Thyroid
- Urinary bladder

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LEVEL 2
EFSA grouping into CAG

CAG 2A: hypertrophy

CAG 2B: fatty changes (steatosis)
90 pesticides identified which non-pesticide chemicals need to be included?

CAG 2C: cell degeneration/cell death

CAG 2D: inflammation

CAG 2E: foci of cellular alteration

CAG 2F: neoplasm

CAG 2G: lesion of biliary epithelium

CAG 2H: porphyria

CAG 2I: cholestasis

CAG 2J: karyocytomegaly

CAG 2K: inclusions

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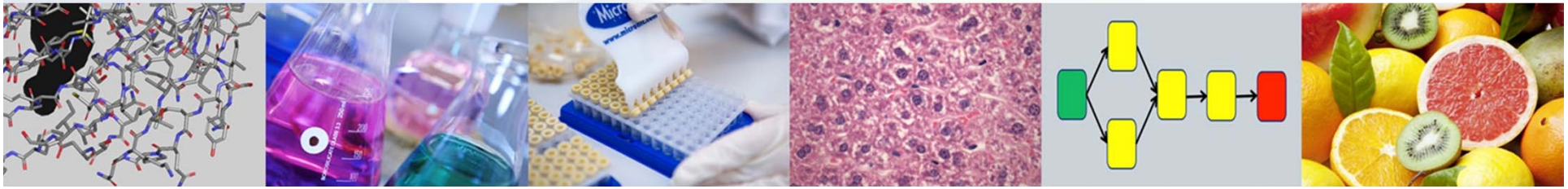
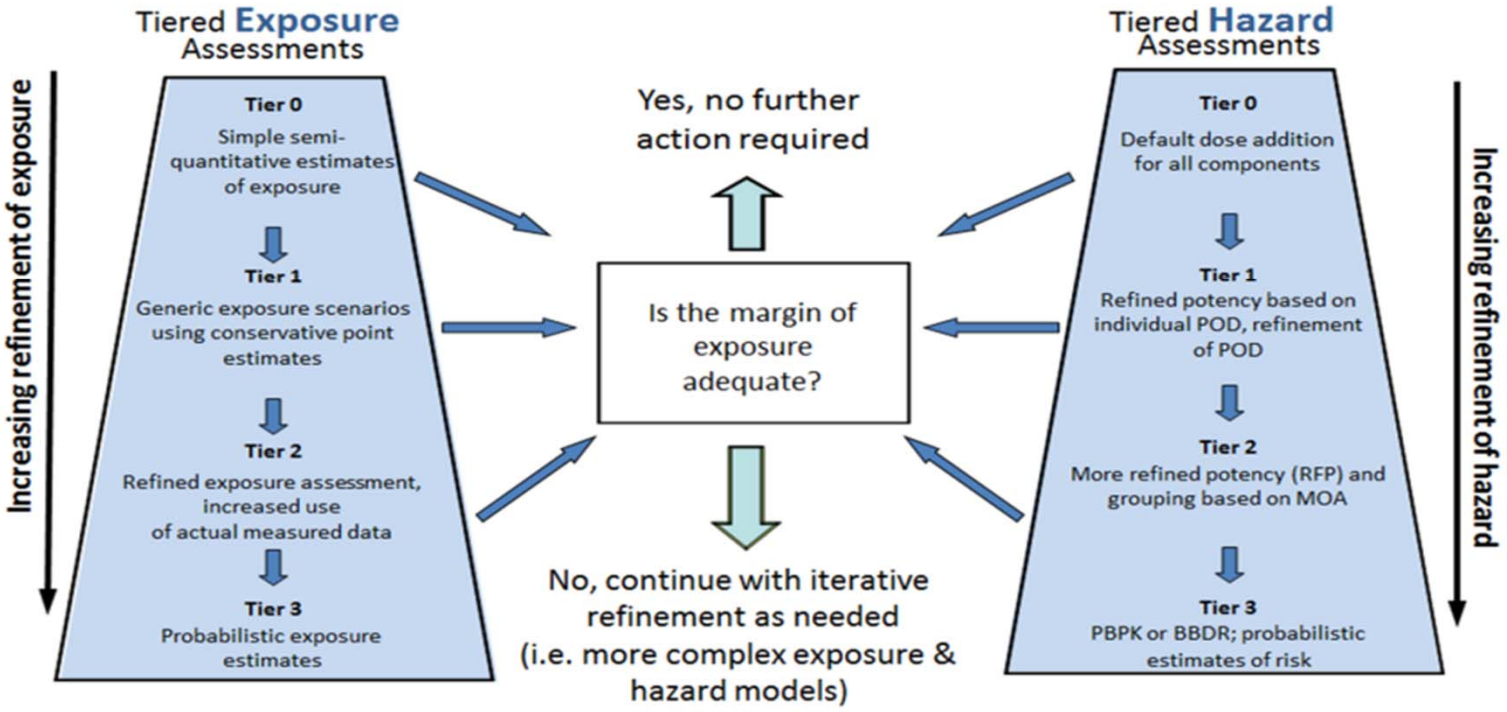


You can not test all the chemicals!

Framework for mixtures: Tiered approaches (WHO, Meek et al. 2011)



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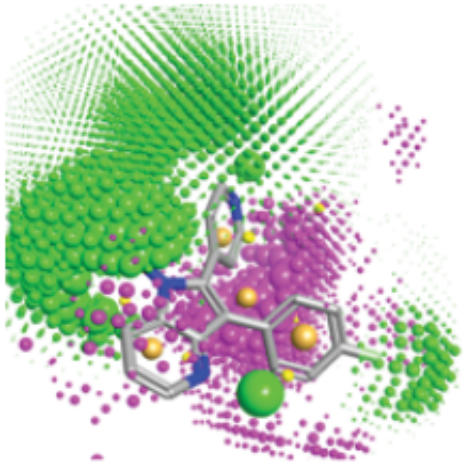
Concept testing strategy

QSAR



priorities

TTC approach

Possibility of co-exposure

bioassay tool box

In vivo confirmation

Very large number of chemicals

Large number of chemicals and mixture experiment

Selected number of chemicals

High uncertainty

Refine and retain

Reduced uncertainty

Meeting

Date

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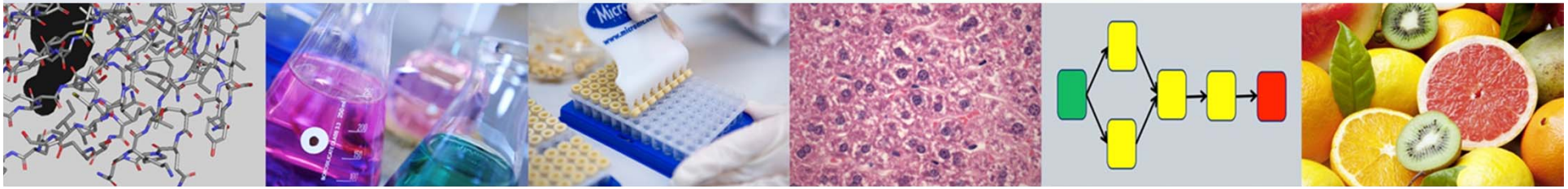

QSARs for CAG Liver Toxicity

Model	Sensitivity	Specificity	Accuracy
COSMOS LXR-binding QSAR model (PADEL descriptors)[a]	0.03	0.98	0.45
COSMOS LXR-binding QSAR model (RDKit descriptors)	0.69	0.48	0.60
COSMOS Nuclear Receptor model	0.31	0.80	0.53
DEREK Nexus (equivocal and above positive)	0.45	0.60	0.52
Fera QSAR model using CDK descriptors	0.85	0.24	0.58
MULTICASE Highest Consensus model (no call/out of domain as positive)	0.72	0.31	0.54
OCHEM AhR binding model	0.29	0.85	0.54
OCHEM PPARgamma model	0.30	0.86	0.55
OECD QSAR Toolbox HESS alerts	0.37	0.72	0.53
PaDEL-DD Predictor	0.78	0.16	0.50
Pizzo structural alert	0.46	0.90	0.66
Majority Consensus: Positive if 5 or more models positive	**0.63**	**0.64**	**0.64**

Bayesian statistics applied to QSAR battery

- Take into account strength (and weakness) of individual models
- Assessment of uncertainty in the CAG assignment

-> Substance A is part of CAG liver tox with XX% probability

- **RETAIN (and REFINE):**

  ALL substances are retained in the CAG, but with individual probability of contributing to the effect.

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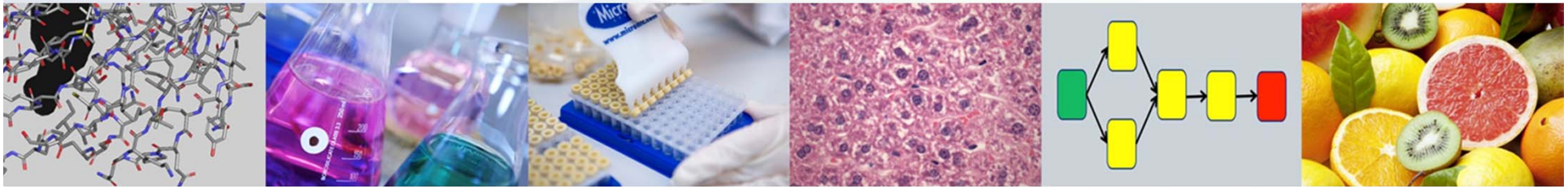
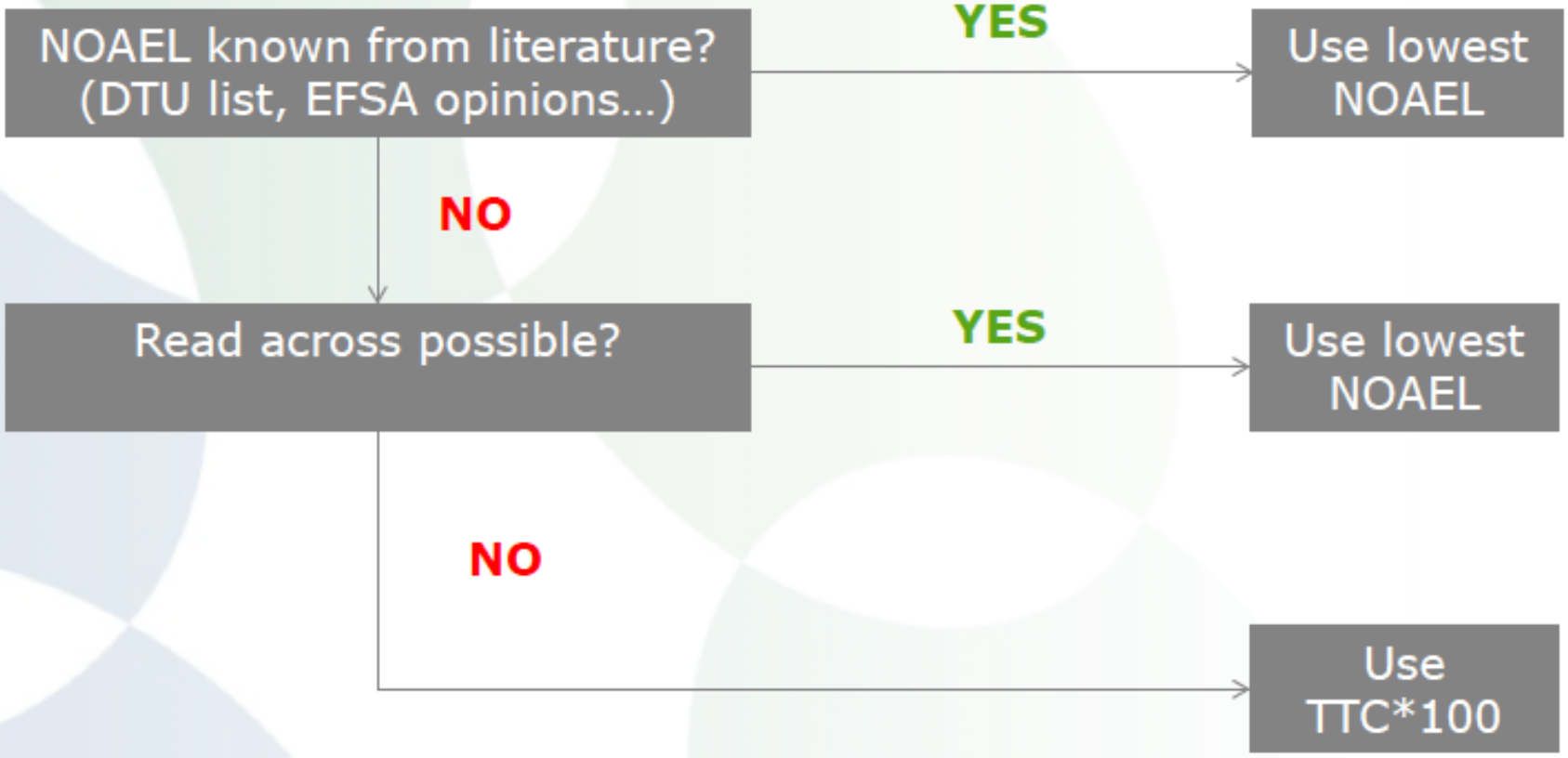


CAGS & RPFs (WP2): Assigning NOAELS for RPFs



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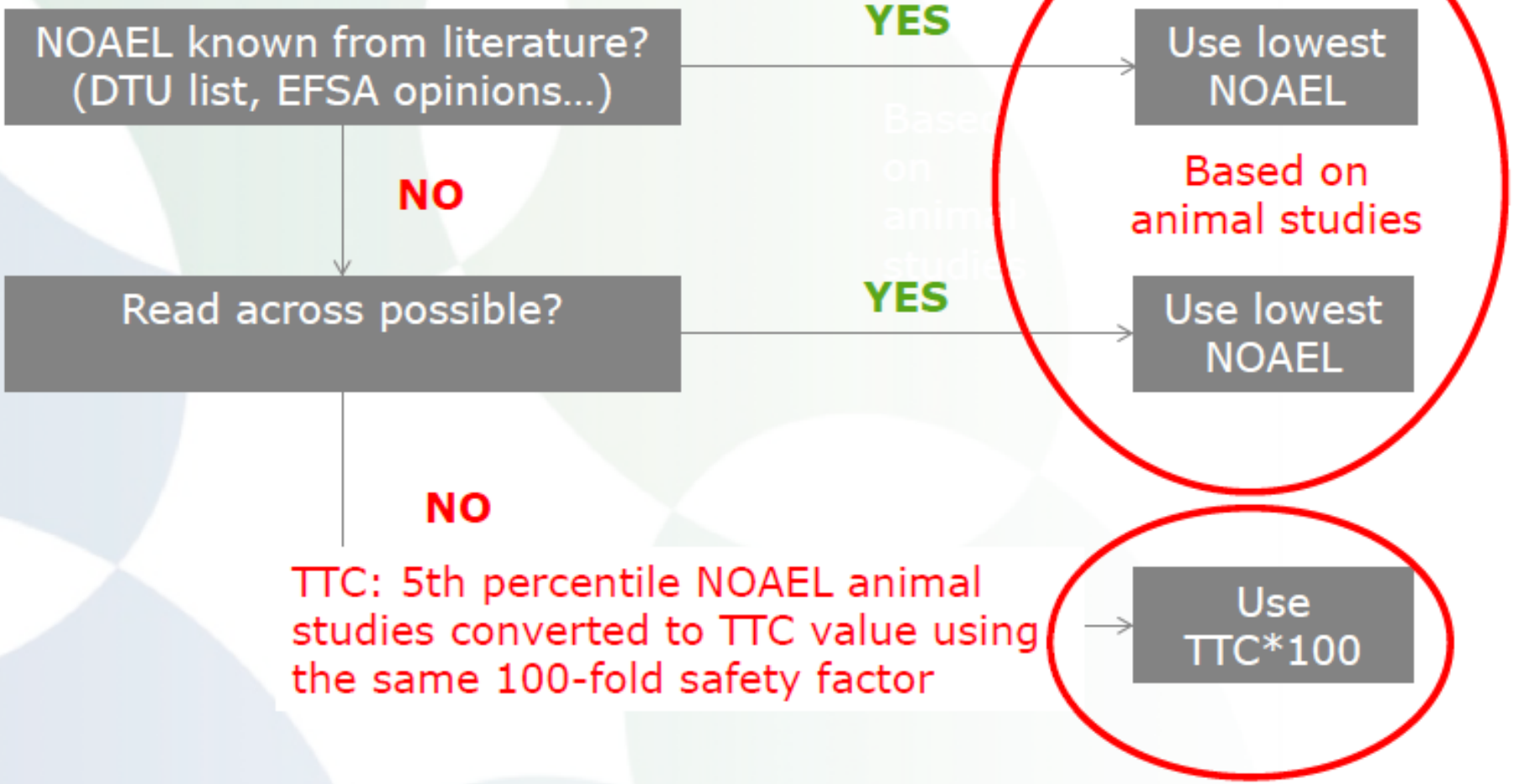
CAGS & RPFs (WP2): Assigning NOAELS for RPFs



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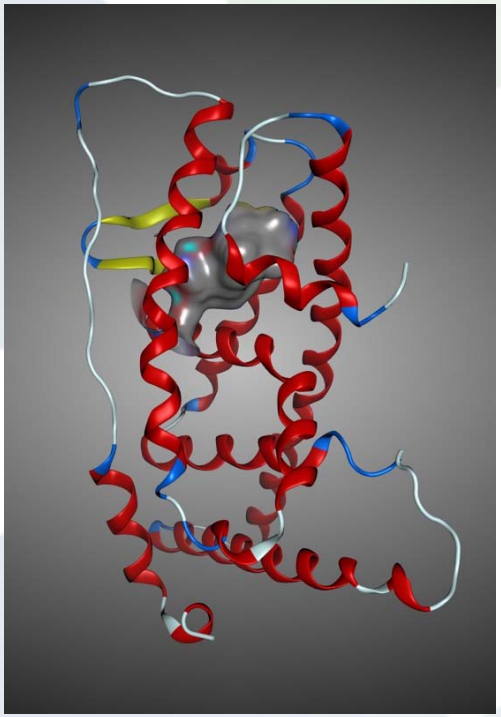
QSARs: Docking - Estrogenicity

3) Molecular docking of EUROMIX database on h-Estrogen Receptor alpha (h-ERa) LBD



Compound	Binding free energy (kcal/mol)	dataset
Ethanol, 2-[2-(4-nonylphenoxy)ethoxy]-	-9.2	59_Easis.sdf
131860338 - Azoxystrobin	-8.4	271_PPP.sdf
17924924 - Zearalenone	-8.1	20_Mycotoxin.sdf
17 beta-estradiol	-8.1	Natural ligands

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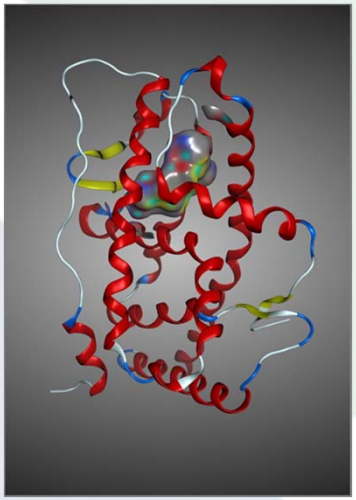
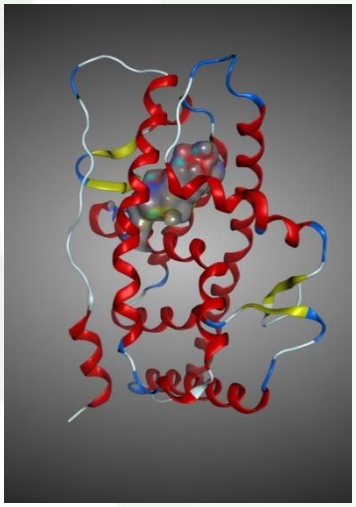
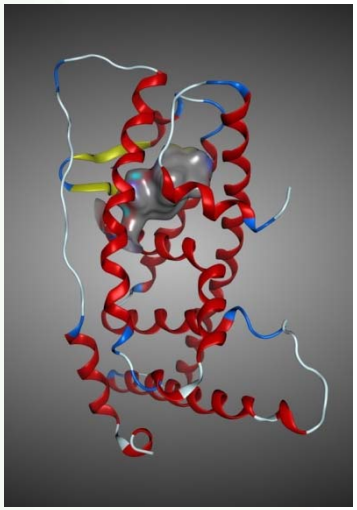
QSARs: Endocrine Docking models



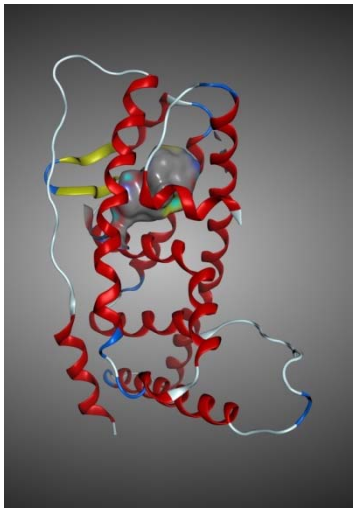
hPR



hAR



hER-a



hER-b

The following crystallographic structures of sex hormone receptor ligand binding domains were selected:

Receptor	PDB entry	Co-crystallized ligand	Natural hormone used as positive control
hPR	1ZUC	tanaproget	progesterone
hAR	2AM9	testosterone	dihydrotestosterone
hER-alpha	3UUD	estradiol	estradiol
hER-beta	3OLS	estradiol	estradiol

Meeting Date

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CAGs & RPFs

Overview of data used in calculations

A	B	C	D	E
idCompound	Name	idEffect	LimitDose	ModelCode
RF-00000451-ORG	Di-n-octyl phthalate	Liver2b	250	RA
RF-00000177-TOX	Fumonisin B1	Liver2b	37.2	RA
RF-00000210-TOX	Phomopsins	Liver2b	0.12	TTCx100
RF-00000078-ORG	HBCD alpha isomer	Liver2b	2.4	TTCx100
RF-00000079-ORG	HBCD beta isomer	Liver2b	2.4	TTCx100
RF-00000080-ORG	HBCD gamma isomer	Liver2b	2.4	TTCx100
RF-0135-001-PPP	Diflufenican	Liver2b	0.12	TTCx100
RF-0279-001-PPP	Mesotrione (RD)	Liver2b	0.12	TTCx100
RF-0343-001-PPP	Picloram	Liver2b	0.12	TTCx100
RF-0384-003-PPP	Quizalofop (including Quizalfop-P)	Liver2b	0.12	TTCx100
RF-0451-001-PPP	Ziram	Liver2b	0.024	TTCx100
RF-0011-001-PPP	Abamectin (RD)	Liver2b	0.25	NOAEL
RF-0014-001-PPP	Acetamiprid	Liver2b	7	NOAEL
RF-0112-001-PPP	Cypermethrin (RD)	Liver2b	1.9	NOAEL

Read across

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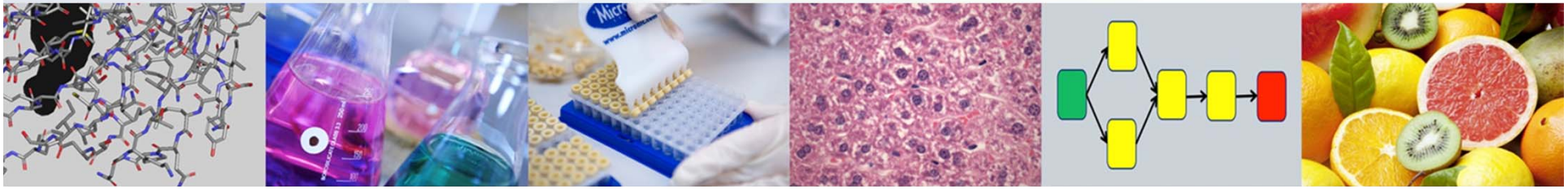

Hazard Quotient concept and mixtures (an example)

	General threshold (mg/kg bw/day)	Type of general threshold (ch	water conc (ug/l)	daily exposure (mg/kg bw)	HQ	
metal 1	0.00040		21	0.0006	1.5	
metal 2	0.02000		7	0.0002	0.01	
metal 3	1.00000		42	0.0012	0.0012	
drug 1	0.01667	Min Ther Dose/ UF	9	0.000257143	0.015428571	
drug 2	0.33333	Min Ther Dose/ UF	0.35	0.00001	0.00003	
drug 3	0.08333	Min Ther Dose/ UF	0.08	2.28571E-06	2.74286E-05	
drug 4	0.02000	Min Ther Dose/ UF	0.12	3.42857E-06	0.000171429	
pesticide 1	0.00020	TDI	8	0.000228571	1.142857143	
pesticide 2	0.05000	TDI	10	0.000285714	0.005714286	
pesticide 3	0.00007	TDI	0.01	2.85714E-07	0.004081633	
industrial chemical	0.07000	TDI	4	0.000114286	0.001632653	
analyte	0.00130	TTC	0.17	4.85714E-06	0.003736264	
analyte	0.00130	TTC	0.07	0.000002	0.001538462	
analyte	0.00780	TTC	0.01	2.85714E-07	3.663E-05	
analyte	0.00780	TTC	0.35	0.00001	0.001282051	
					HI	2.68774
					HQ max	1.5
					MCR	1.79182

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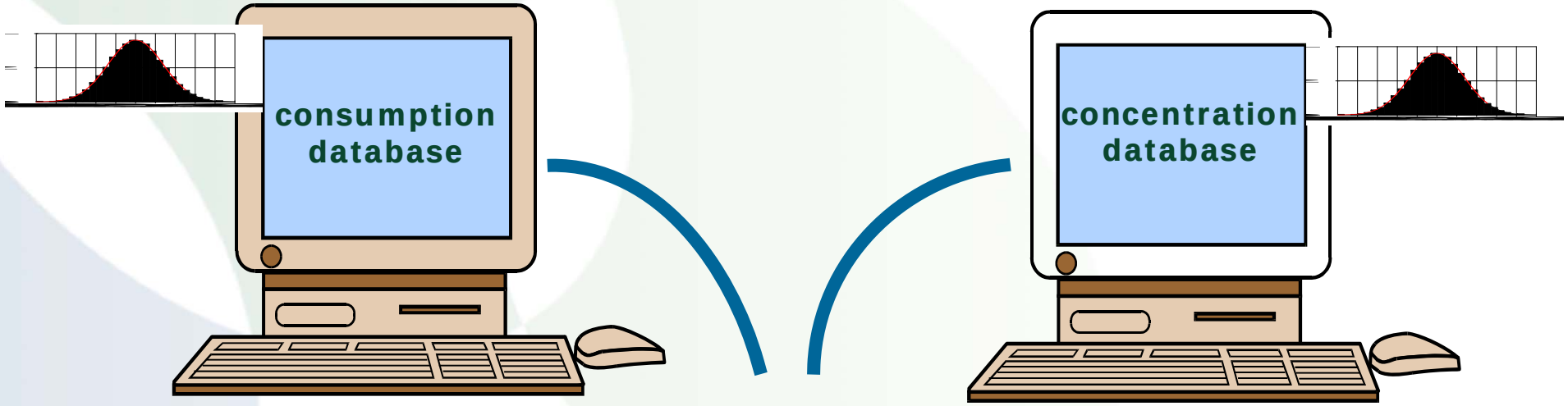
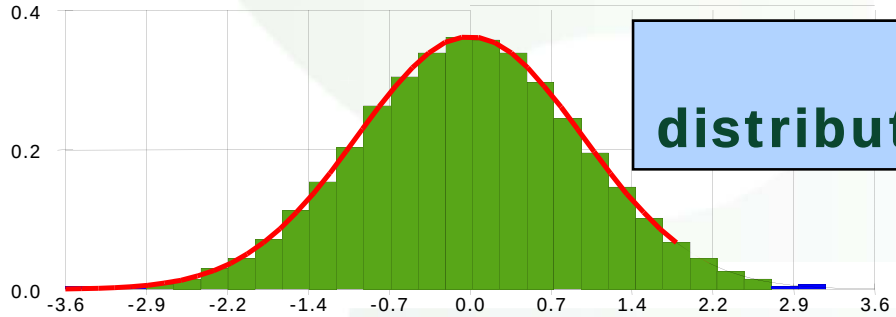
Exposure modelling: MCRA tool





Result: distribution of exposure

Random sampling from a concentration and a consumption database

van der Voet H, et al. (2015). The MCRA model for probabilistic single-compound and cumulative risk assessment of pesticides. Food and Chemical Toxicology, 79: 5-12.
MCRA tool online: https://mcra.rivm.nl/

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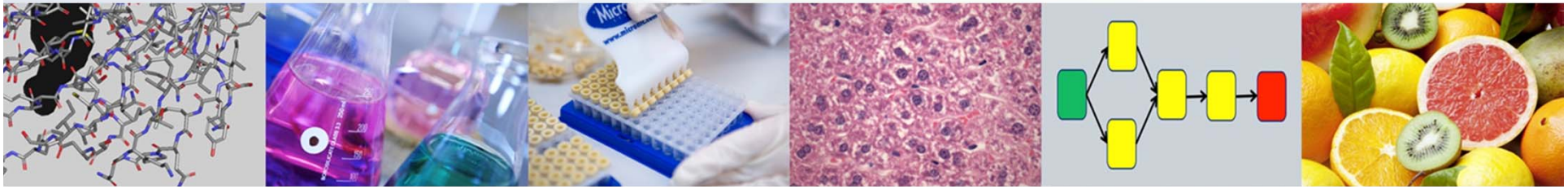
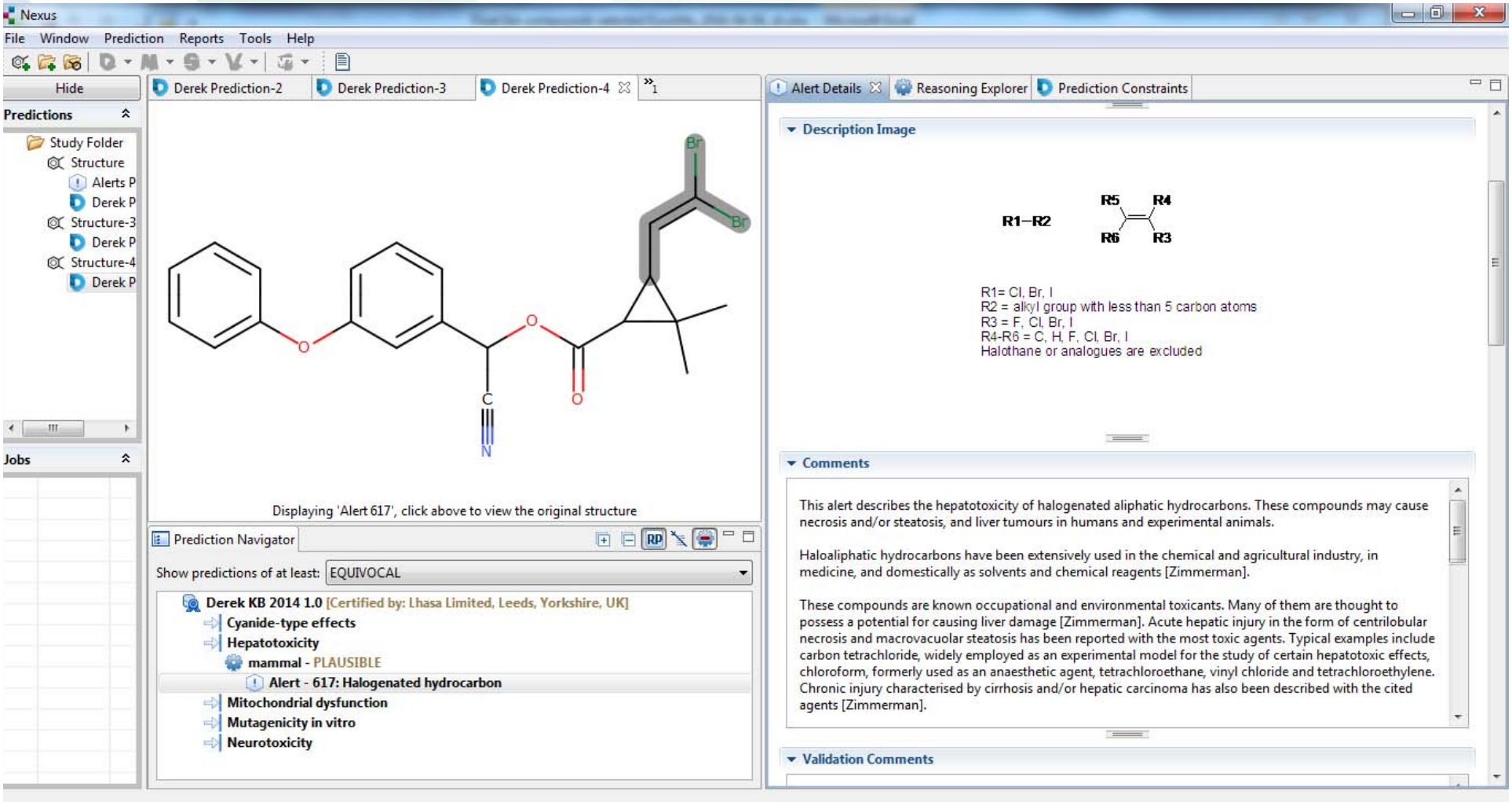


CAG Liver Toxicity - DEREK

Deltamethrin – CAG liver toxicity

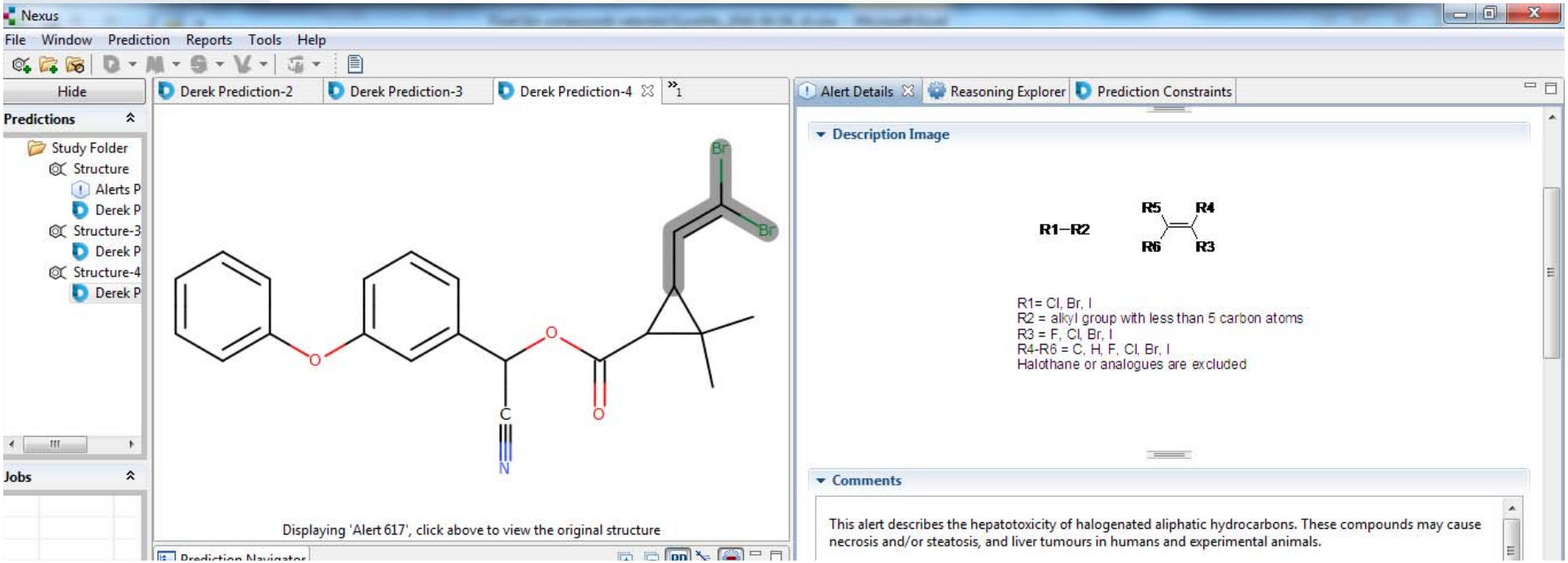
- Alert-617: Halogenated Hydrocarbon
- These compounds may cause steatosis and/or necrosis

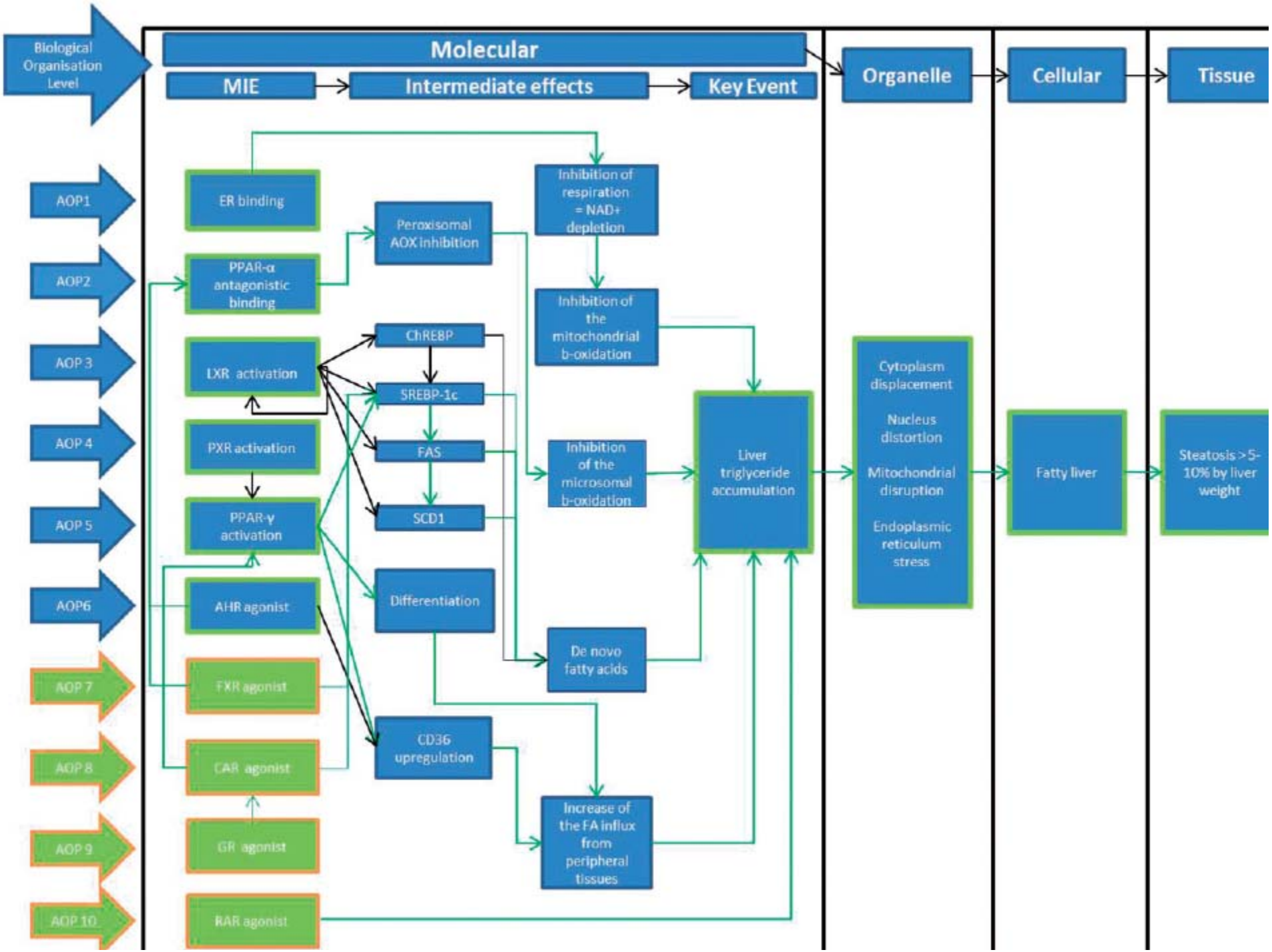


CAG Liver Toxicity - DEREK

Alert-617: Halogenated Hydrocarbon

- The hepatotoxicity is thought to require metabolic activation of the parent compound primarily mediated by CYP2E1. Formation of intermediates occurs via dehalogenation, reduction, or reductive oxygenation. These radical can covalently bind to cellular molecules, impairing crucial cellular processes such as lipid metabolism, form adducts with DNA or generate oxidative stress.




Biological Organisation Level
Molecular
MIE
Intermediate effects
Key Event
Organelle
Cellular
Tissue
AOP1
AOP2
AOP 3
AOP 4
AOP 5
AOP6
AOP 7
AOP 8
AOP 9
AOP 10
ER binding
PPAR-α antagonistic binding
LXR activation
PXR activation
PPAR-γ activation
AHR agonist
FXR agonist
CAR agonist
GR agonist
RAR agonist
Peroxisomal AOX inhibition
ChREBP
SREBP-1c
FAS
SCD1
Differentiation
CD36 upregulation
Inhibition of respiration = NAD+ depletion
Inhibition of the mitochondrial b-oxidation
Inhibition of the microsomal b-oxidation
De novo fatty acids
Increase of the FA influx from peripheral tissues
Liver triglyceride accumulation
Cytoplasm displacement
Nucleus distortion
Mitochondrial disruption
Endoplasmic reticulum stress
Fatty liver
Steatosis >5-10% by liver weight

Nuclear receptors associated with Hepatic Steatosis

Table 2. Summary of the effects on the liver following activation of nuclear receptors.

Nuclear receptor	Agonist effect on liver	Antagonist effect on liver
AHR	Induces hepatic steatosis	–
CAR	Induces hepatic steatosis	–
ER	Induces hepatic steatosis	Induces hepatotoxicity
FXR	Induces hepatic steatosis	–
GR	Induces hepatic steatosis	–
LXR	Induces hepatic steatosis	–
PPAR	PPAR γ induces hepatic steatosis	PPARα induces hepatic steatosis
PXR	Induces hepatic steatosis	–
RAR	Induces hepatic steatosis	–
RXR	Induces hepatic steatosis	–

COSMOS Nuclear receptor alert models:

Mellor et al. 2016, Chem.Res.Tox 29:203

Mellor et al. 2016, Crit.Rev.Tox. 46:2

Meeting Date

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Thanks to the EuroMix WP2 (in silico) team

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Ivano Eberini	Università degli Studi di Milano, Milano (IT)

and co-workers

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EuroMix participants

22 beneficiaries from 16 countries linked to international organisations including WHO, FAO and EFSA. EuroMix is coordinated by RIVM.