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Establishing the level of safety concern for chemicals in food without the need for toxicity testing

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Food is chemicals

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proteinase inhibitor

methylbutanal

chaconine

oxalic acid

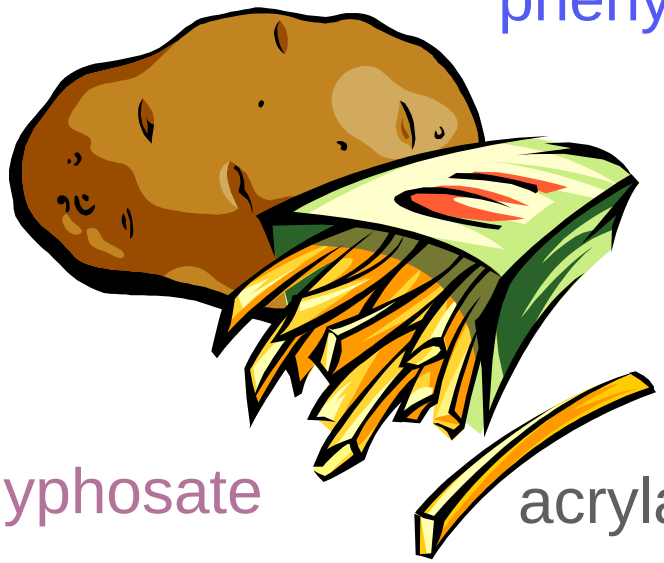
asparagine

iron

phenylalanine

DDT

deltamethrin



ascorbic acid

dimethylpyrazine

phytate

propham

lectin

styrene

potassium

glyphosate

globulin

acrylamide

furan

cadmium

folate

Isopropylthioxantone

lead

albumin

starch

anthocyanins

etc…

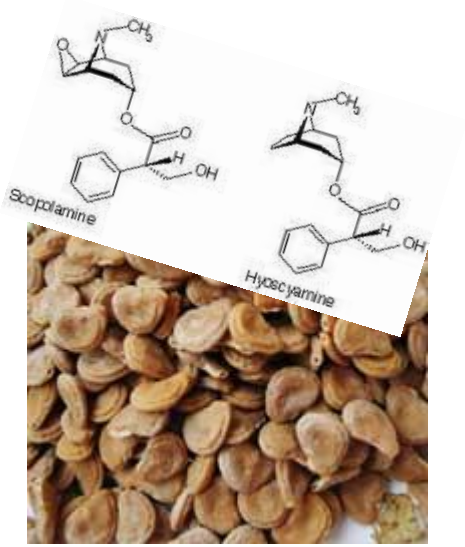
amylase

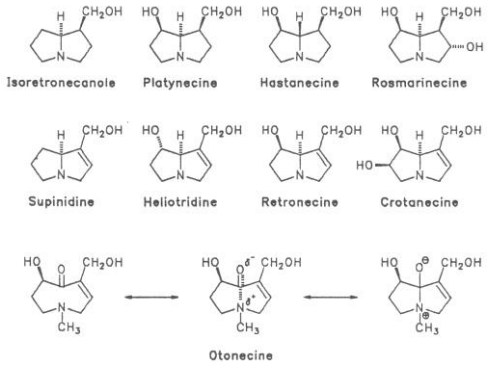
4-nonylphenol

hydoxymethylfurfural

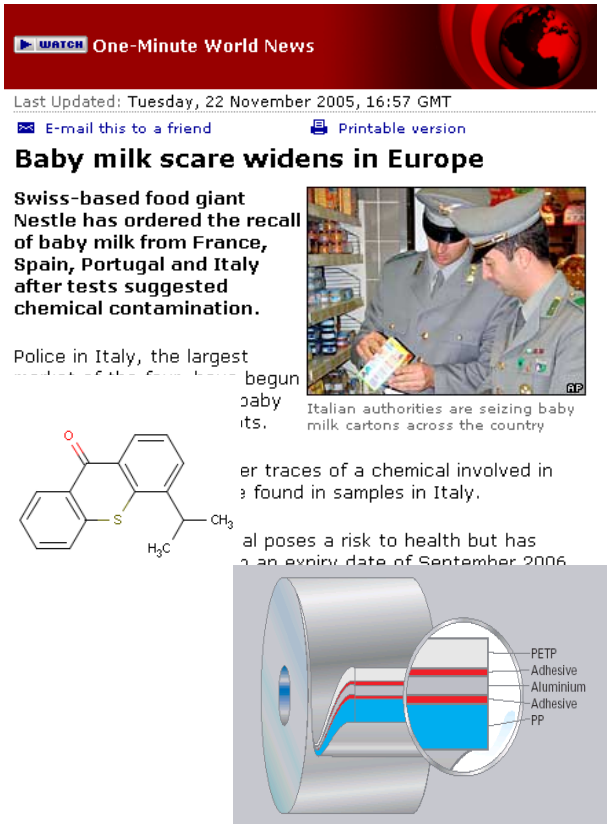
Other examples

200 tropane alkaloids

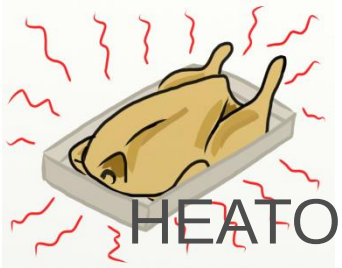




350 pyrrolizidine alkaloids



6000 substances potentially used in inks



HEATOX: 800 process contaminants

Current understanding

- 5 000 000 man made chemicals known
- 80 000 chemicals in commercial use today
- 100 000 naturally occurring substances

- Establish level of safety concern:
  - Incident/crisis management
  - Priority setting (for further testing or management)
  - Optimize the use of animals
  - Optimize R&D project management

Alternative solution:
In silico structure-activity relationship

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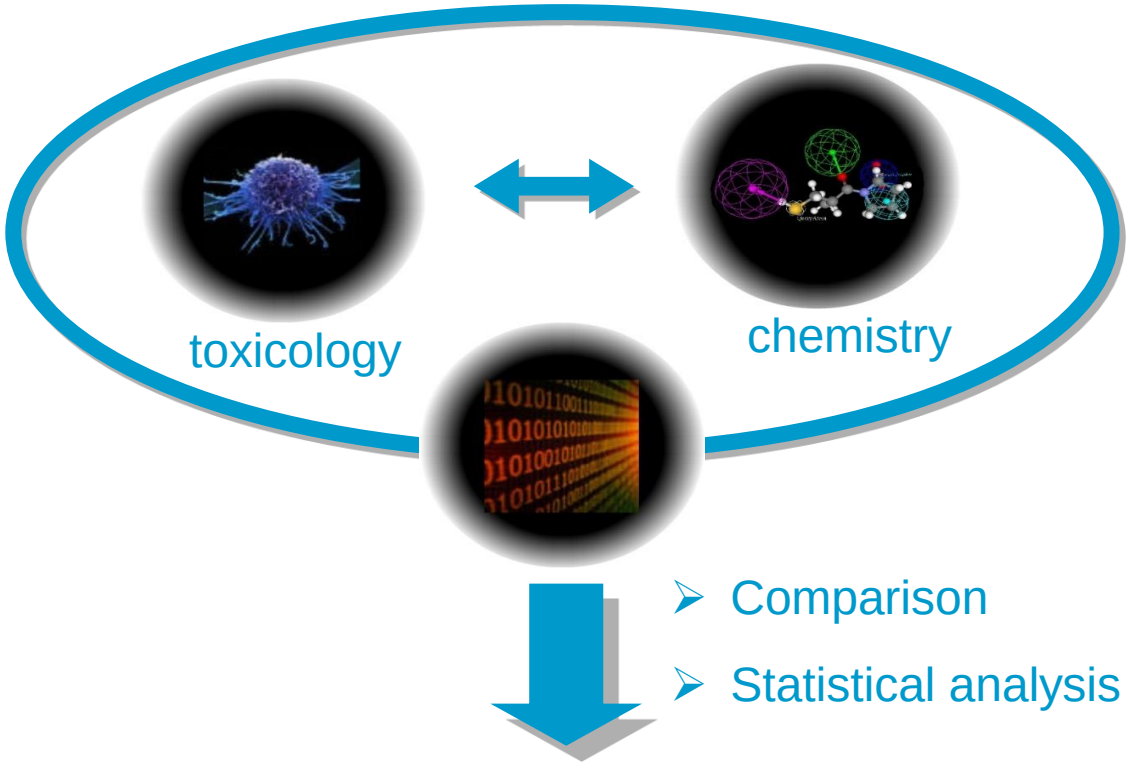
Structure determines toxicological properties:



- ➢ Comparison
- ➢ Statistical analysis

- ✓ Direct use of data from well characterized substances
- ✓ Predictive modelling

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The application of *in silico* methods are increasingly recognized

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OPTIONS TO FULFIL THE REACH INFORMATION REQUIREMENTS

Methods to avoid the use of animals

- Use of information on similar substances (Grouping and Read-across)
- Information combined together from various sources (Weight of evidence)
- Studies using cells, tissues or organs (*in vitro*)
- Computer modelling (QSAR)

Other justifications for omitting studies

- For example, low exposure considerations

Animal studies

- Results from existing studies
- Conduct new studies as a last resort to fill data gaps in the core data essential for registration
- Testing proposals for new studies of long-term hazards for example carcinogenicity or reproductive toxicity for substances at or above 100 tonnes *

ECHA

COSMOS

FDA

Use of computational tools in the field of food safety

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ABSTRACT

In this article we give an overview of how computational methods are currently used in the field of food safety by national regulatory bodies, international advisory organisations and the food industry. Our results show that currently the majority of stakeholders in the field of food safety do not apply computational methods on a routine basis, mainly because of a lack of in-house expertise. Some organisations, however, are very experienced in their use and have developed specialised in-house approaches. Despite this variable situation, computational tools are widely perceived to be a useful tool to support regulatory assessments and decision making in the field of food safety. Recognized, however, is a widespread need to develop guidance documents and software tools that will promote and harmonise the use of computational methods, together with appropriate training.

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The food context

- ✓ "Global":
  - Broad chemical diversity
- ✓ Reliable/performant:
  - Protective,
  - Not overtly conservative
- ✓ Relevant:
  - Risk assessment
- ✓ Quantitative
  - NOAEL, LOAEL, TD50
  - Not only qualitative (yes-no answer)

Establish a margin of exposure (MoE)

Margin of exposure (MoE)

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Risk characterization: ***standard.***

$$ADI = \frac{NOAEL_{pivotal}}{UFs}$$

- Uncertainty factors (UFs):
  - Inter-species differences
  - Inter-individual differences
  - Limitations of the database

Margin of Safety (MoS) = ADI/Exposure

Risk characterization: ***alternative.***

$$MoE = \frac{\text{Tox.value}}{\text{Exposure}}$$

Predicted

- Uncertainties
  - Inter-species differences
  - Inter-individual differences
  - Extrapolation LOAEL/NOAEL
  - Exposure duration
  - ….

MoE = Margin of exposure

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QSAR
(quantitative structure-activity relationship)

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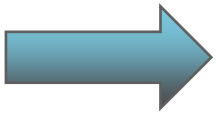
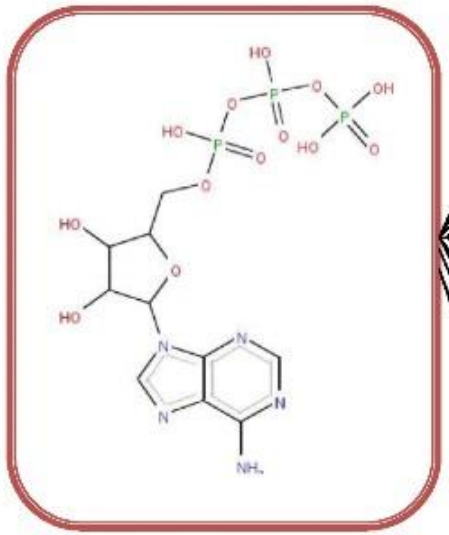
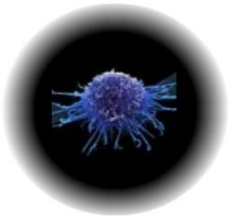
- Find a relationship (model) between the chemical structures of compounds and a given property

	LOAEL
comp 1	80
comp 2	20
comp 3	77
comp 4	30
comp 5	200
comp 6	5000
comp 7	3000
comp 8	20
comp 9	300
comp 10	4000
….	…..
comp n	30

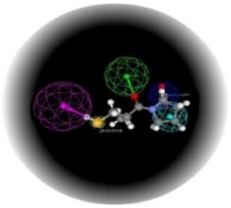




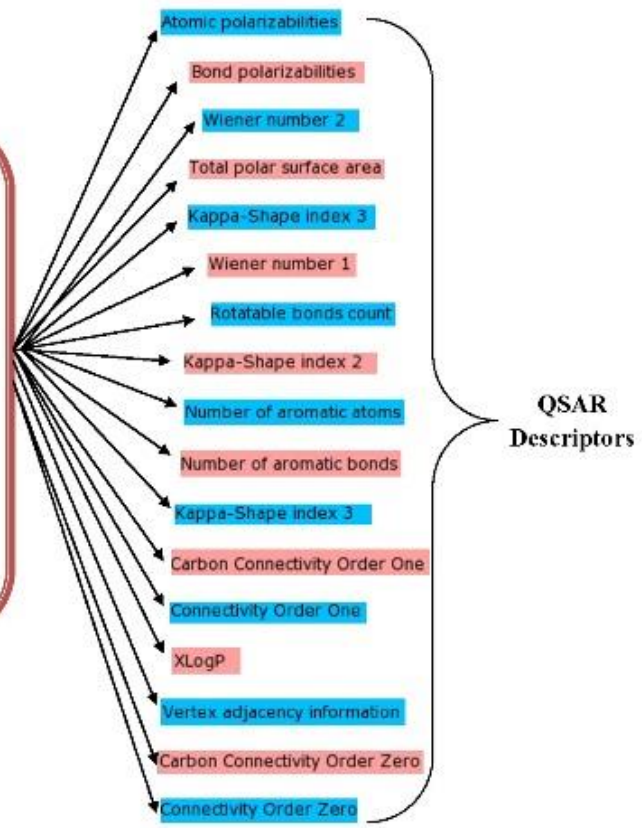
Chemical Compound



Biology/toxicology



chemistry



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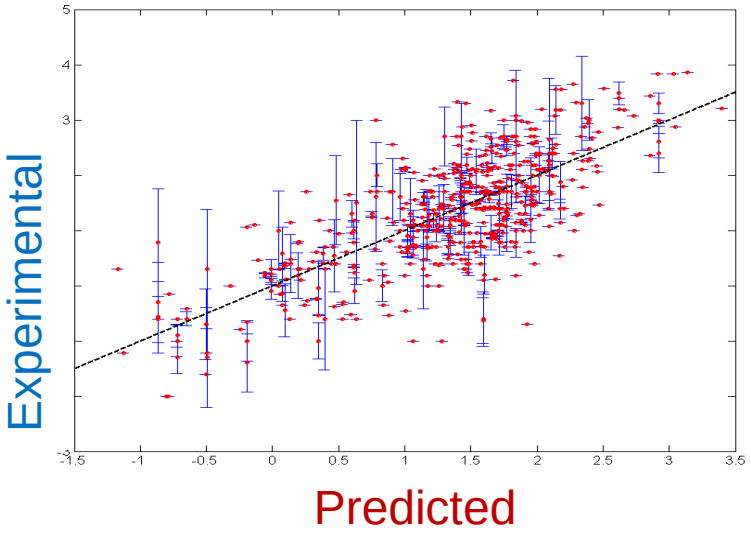
LOAEL-rat model

- 567 Lowest Observed Adverse Effect Levels, chronic, rat, 445 substances
- «Leave-one-out» cross-validation



average experimental variability	average model error
4.4	5.4

Prediction …		
Exp. error	10	100
64%	85%	99%

Mazzatorta, P.; et al., Modeling Oral Rat Chronic Toxicity. *J. Chem. Inf. Model.* 2008, 48, pp. 1949-1954.

Other chronic toxicity endpoints

- *MRTD (Human maximun recommended therapeutic dose):

	within applicability domain	all predictions
Mean error	0.47 (log)	0.59
Predictions within 1 log unit	89%	82%

Based 1300 drugs (clinical studies, US-FDA database). Chronic exposure. Surrogate of LOAEL in human. *(*Maunz, A.; Helma, C. Prediction of chemical toxicity with local support vector regression and activity-specific kernels. SAR and QSAR in Envir. Res. 2008, 1-38).*

- TD_{50} (carcinogenic potency)

Author (Method)		≤1-fold (%)	≤2-fold (%)	≤5-fold (%)	≤10-fold (%)
Bercu *et al. (VISDOM)*	**RAT**	**59**	**64**	**86**	**86**
	MOUSE	**66**	**81**	**88**	**97**
Contrera (SciQSAR)	**RAT**	**48**	**65**	**78**	**85**
	MOUSE	**56**	**75**	**78**	**97**
(Lazar)**	**RAT**	**43**	**57**	**71**	**76**
	MOUSE	**48**	**76**	**86**	**93**

*(**Lo Piparo, E.; Maunz, A.; Helma, C.; Vorgrimmler D.; Schilter, B. (2014). Automated and reproducible read-across like models for predicting carcinogenic potency. Reg. Pharm Toxicol., 70(1):370-378).*

Example:
Isopropylthioxanthone (ITX)

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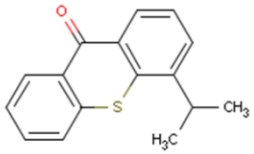
- Photoinitiator, UV-cured inks
- Detected in milk bricks: 70-600 ppb
- Calculated exposures: 3 et 50 μg/kg/d
- Non genotoxic



	TOPKAT	Rat LOAEL	Human LOAEL	*Experimental (LOAEL 28 d rat)
ITX (tox pred.)	**32 (mg/kg)**	**15 (mg/kg)**	**0.59 mg/kg**	**50 mg/kg**
MoE*	**700-10000**	**325-5000**	**12-200**	**1000-17000**

**MoE (margin of exposure) = ratio tox value/human exposure*

MoE interpretation:

- Rat LOAEL/TOPKAT :
  - Conversion LOAEL-NOAEL (3-10)
  - Interspecies differences (10)
  - Interindividual differences (10)

  } MoE>1000

- Human LOAEL:
  - Conversion LOAEL-NOAEL (10)
  - Interindividual differences (2-10)

  } MoE>100

MoE: >6000
(10x10x10x6)

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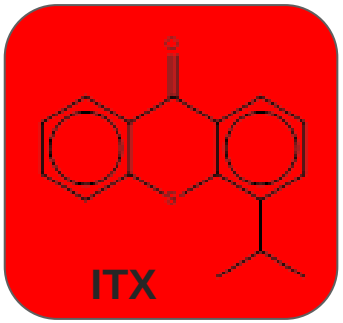
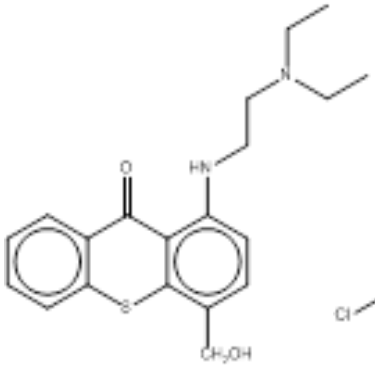
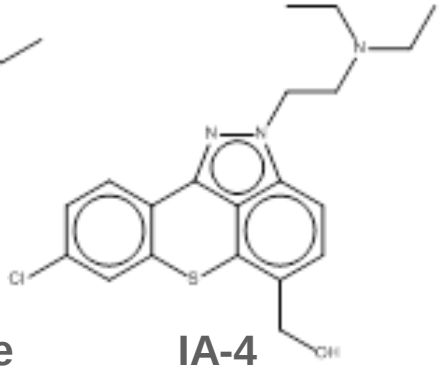
Schilter et al., Reg. Pharm. Toxicol. (2014).

Read-across

- Unknown activity for a compound can be extrapolated from the activities of similar compounds

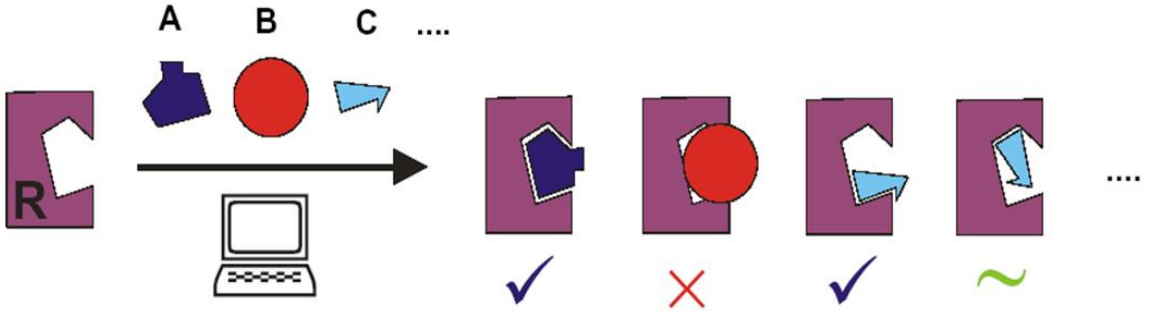


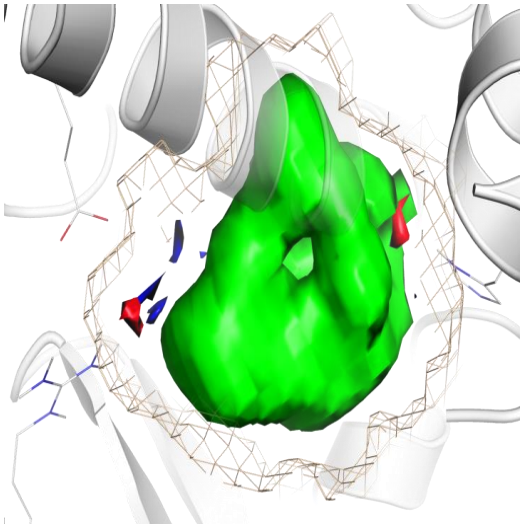




- **Establishing similarity is complex:**
  - Chemical structure
  - Physico chemical properties
  - Metabolism
  - Mechanism of action
  - Availibility of reliable data on analogues
  - ➢ *Based on expert judgments/choices*

Docking: predicting ligand-receptor interactions





Estrogen receptor α

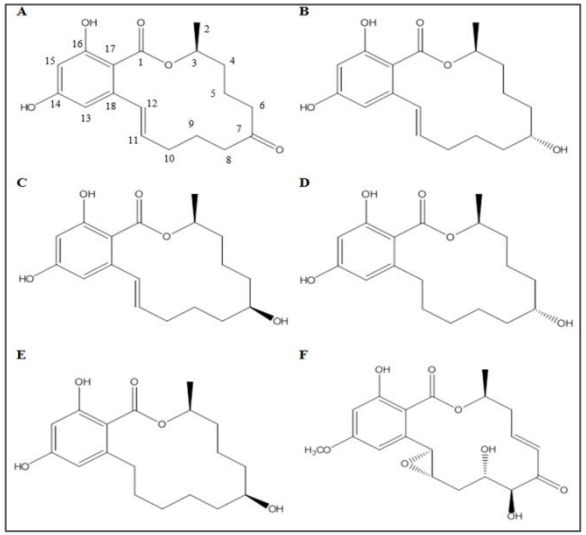


Figure 1. **A**: Zearalenone (**ZEN**). **B**: α-zearalenol (**αZOL**). **C**: β-zearalenol (**βZOL**). **D**: α-zearalanol (**αZAL**). **E**: β-zearalanol (**βZAL**). **F**: **Hypothemycin**.

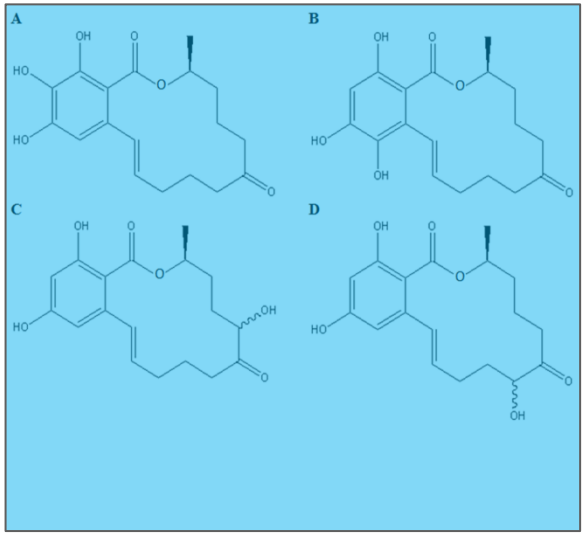


Figure 2. Oxidized metabolites of ZEN. **A**: 15-hydroxy-zearalenone (**15-OH-ZEN**). **B**: 13-hydroxy-zearalenone (**13-OH-ZEN**). **C**: 6-alpha/beta-hydroxy-zearalenone (**6α/β-OH-ZEN**). **D**: 8-alpha/beta-hydroxy-zearalenone (**8α/β-OH-ZEN**).

Collaboration with University of Parma (publication in preparation)

Docking to serve read-across

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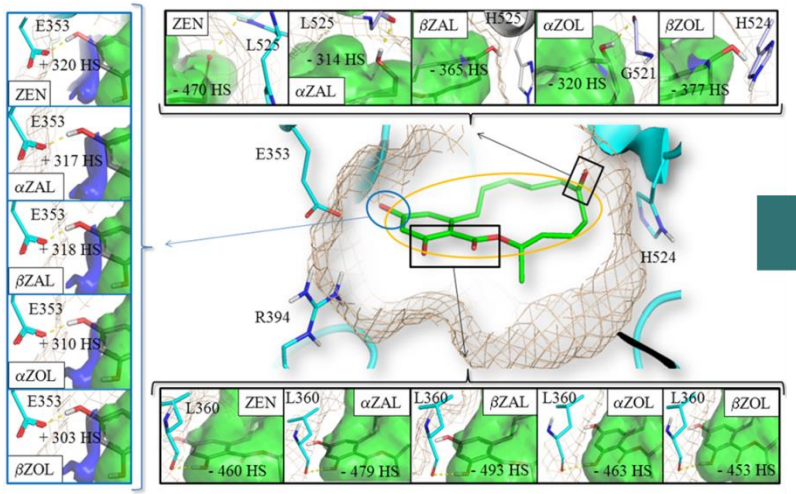
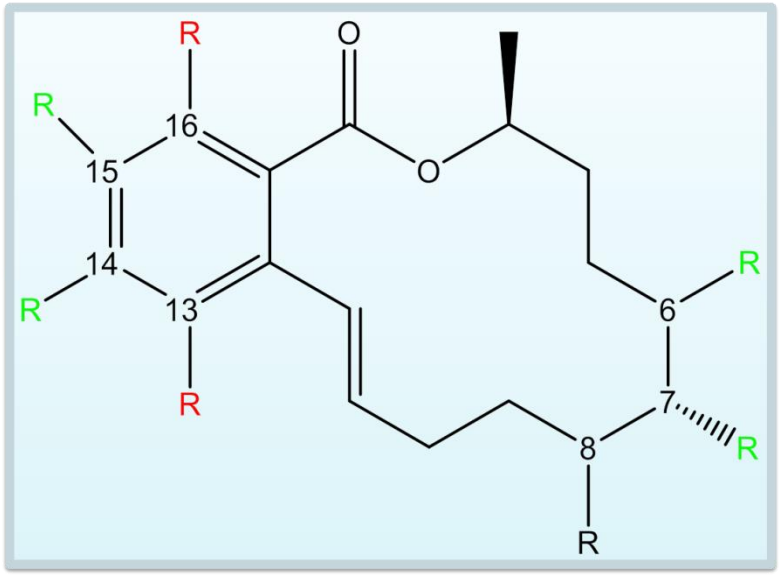
Table 1. In vitro results		
Compound	EC_{50} Redistribution Assay	EC_{50} CALUX Assay
Estradiol	0.14 nM	4.6 pM
hypothemycin	n. b.	n. b.
α ZOL	0.22 nM	9.6 pM
α ZAL	0.20 nM	19 pM
β ZAL	0.79 nM	280 pM
ZEN	4.27 nM	490 pM
β ZOL	48.69 nM	2500 pM

Table 2. *In silico* results of ZEN, reduced metabolites and positive (estradiol) and negative (hypothemycin) control		
Compound	Experimental rank	HINT score
Estradiol	1	1380.22
Hypothemycin	/	-1314.35
α ZOL	2	648.39
α ZAL	3	632.53
β ZAL	4	505.31
ZEN	5	499.77
β ZOL	6	469.55

Table 3. *In silico* results of oxidized metabolites		
Compound	HINT score	Predicted activity
13-OH-ZEN	-47.27	Negative
15-OH-ZEN	545.20	Positive
8aOH-ZEN	-37.74	Negative
6aOH-ZEN	197.87	Positive
8bOH-ZEN	212.76	Positive
6bOH -ZEN	280.99	Positive





Collaboration with University of Parma *(publication in preparation)*

Summary

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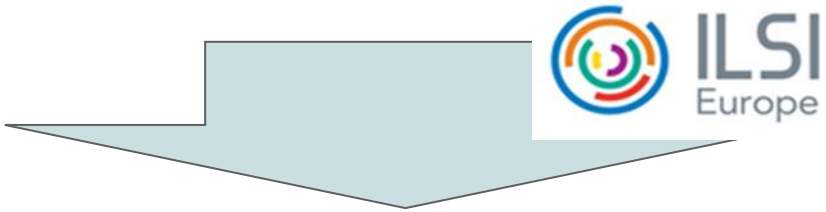
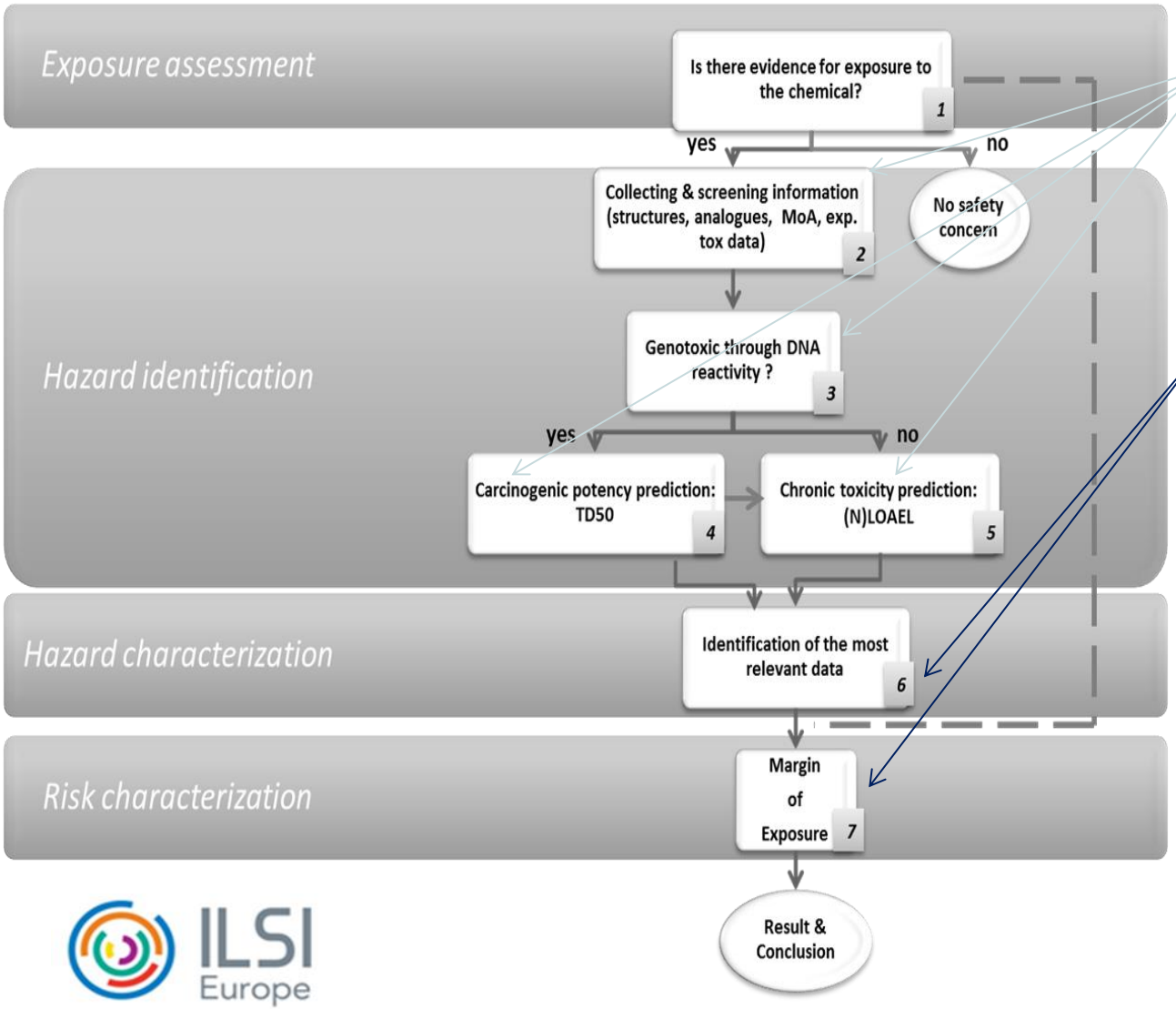
In silico tools are valuable to establish level of concern

- ✓ Require adequate information on structure.
- ✓ Many different tools available:
  - *Cover various modes/mechanisms of action and effects:*
    - *Yes/No*
    - *Ranking/potency*
  - *Provide quantitative information (for MoE)*
- ✓ Use models validated according to international standards



➢ **Integration of different tools**

A decision tree was developed to aid integrating exposure and predicted toxicological values(ILSI-Europe).



Predictions:

- *Read across*
- *QSAR*
- *Docking*

Weight of Evidence:

- *Quality*
- *Relevance*
- *Reliability*
- *Consistency*
- *….*

Level of confidence

Schilter B, Benigni R, Boobis A, Chiodini A, Cockburn A, Cronin M, Lo Piparo E, Modi S, Thiel A, Worth A "Establishing the level of safety concern for chemicals in food without the need for toxicity testing", Reg. Tox. Pharm., 2014, 68:275-298.

Uncertainties

- Application of DT brings uncertainties similar to those associated with classical RA:
  - Exposure assessment
  - Hazard assessment (relevance for human, MOA)
- Relying on predicted tox values introduce additional uncertainties:
  - QSAR (performance of the models, domain of applicability)
  - Read-across (suitability of analogues, quality of tox data, extrapolation from analogues)
- Unlikely to significantly impact the "degree of conservatism" of the assessment:
  - Errors of the models not systematic (under- or over- estimate tox)
  - Different models based on independent training datasets
  - Different approaches involved (read-across, QSAR)
  - Introduction of mechanistic considerations

Safety assessment of packaging:
A role for *in silico* toxicology?

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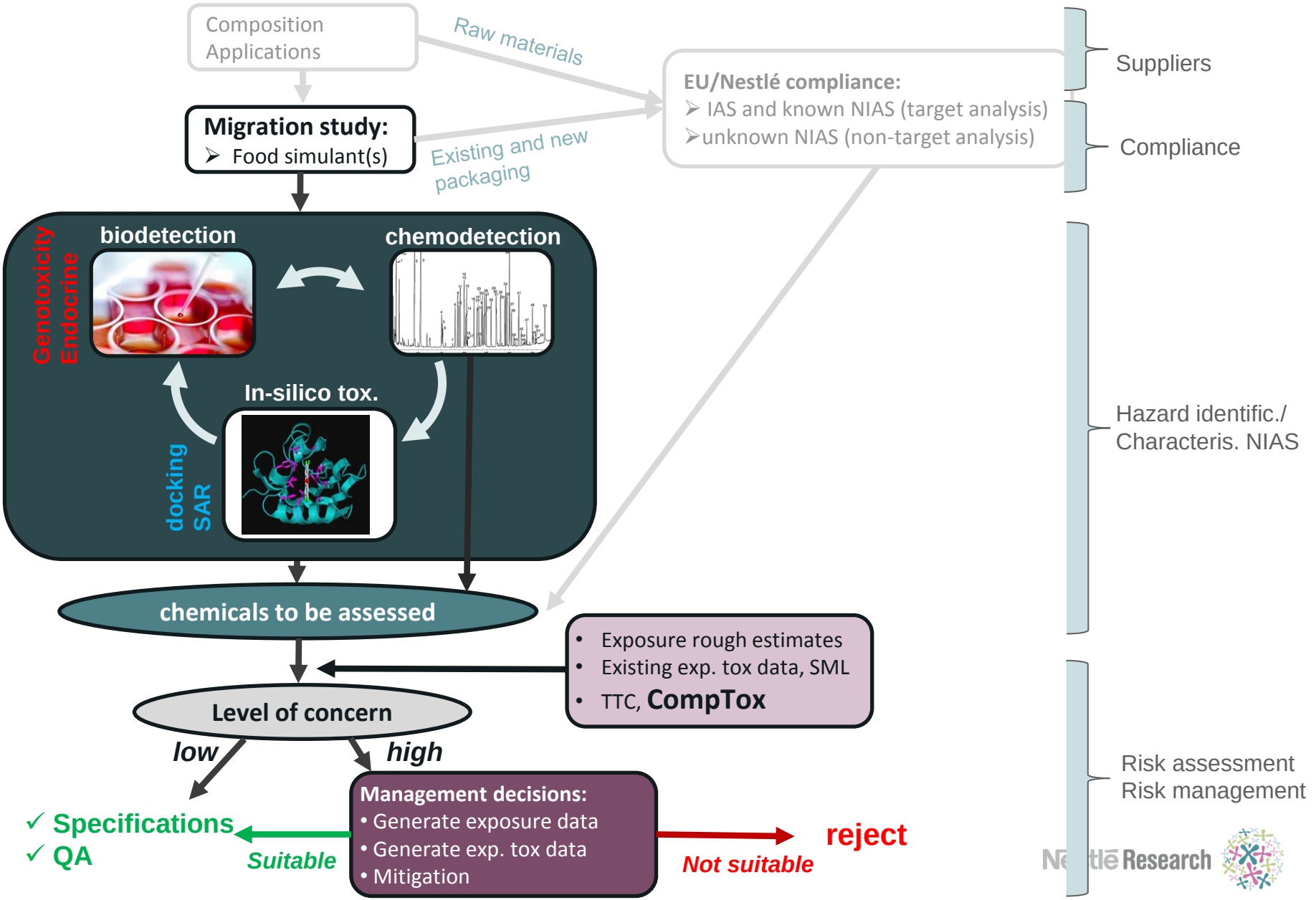

Conclusion/perspectives

- Hazard characterization can be conducted based on predictive models/approaches:
  - Several independent models should be applied in an integrated way
  - Models should be properly validated
- If properly applied, the ILSI-DT is likely to bring a reasonable degree of conservatism.
- The application of the ILSI-DT is likely to be a valuable tool allowing decisions while ensuring health protection.
- It can play a significant role in the safety assessment of packaging materials.
- Should be used to address the concern of large number of non-tested substances.

THANKS:

ILSI-Europe Expert group:

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