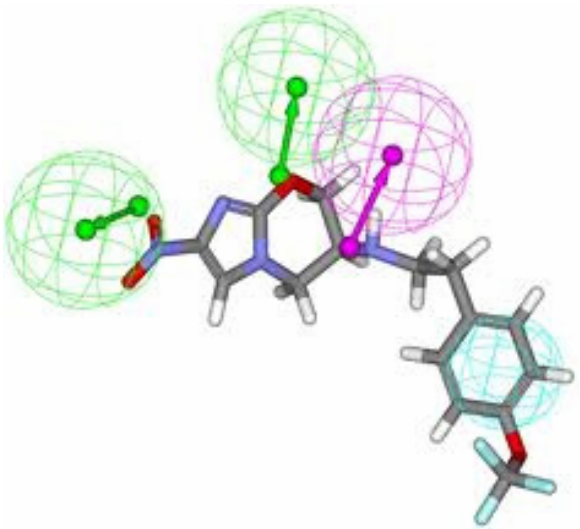
I metodi “read-across”: metodologie ed esempi applicativi



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Main approaches for generating non-testing data

- Grouping approaches
  - analogue approach
  - chemical category formation
- Structure-activity relationships
  - SAR
  - QSAR
- Expert systems

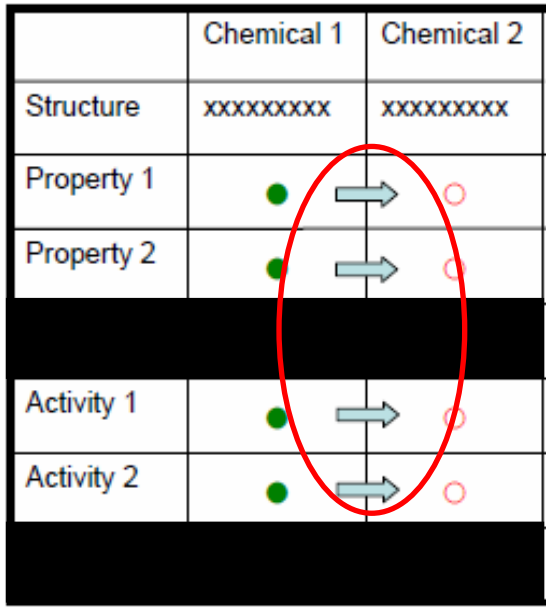
Read-across definition

- **Read-across**: technique used to predict endpoint information for one chemical by using data from the same endpoint from another chemical which is considered to be similar in some way (on the basis of structural similarity and similar properties and/or activities).

chemical source  chemical target

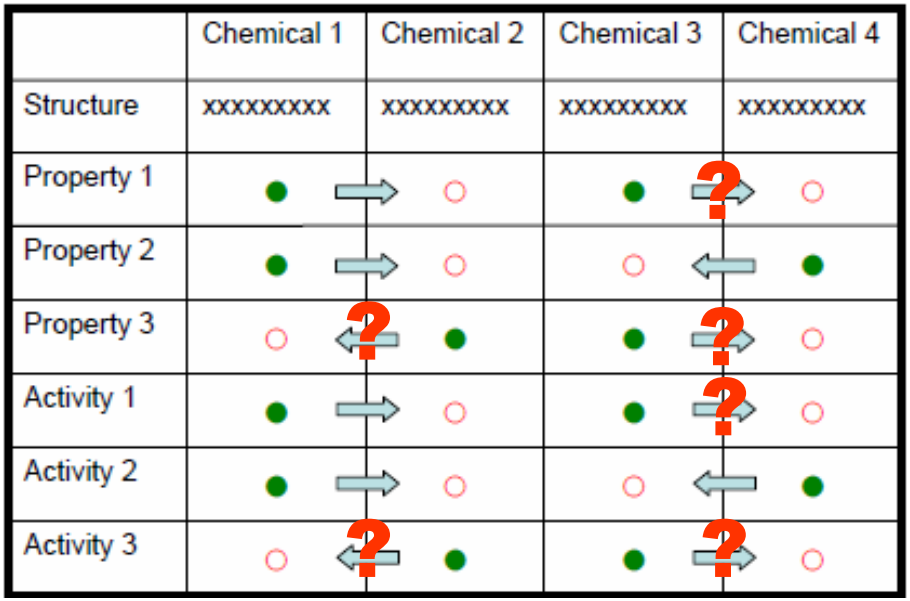
Analogue approach definition

- **Analogue approach**: In the analogue approach, endpoint information for one chemical is used to predict the same endpoint for another chemical, which is considered to be similar in some way (usually on the basis of structural similarity and similar properties and/or activities).



Chemical category definition

- **Chemical category**: group of chemicals whose physico-chemical and human health and/or environmental toxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern as a result of structural similarity.



Analogue vs category approach

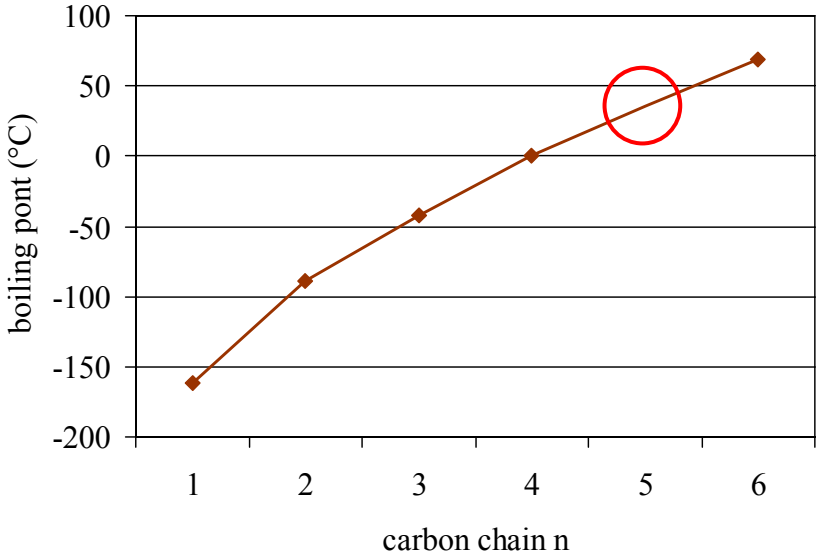
- It should be recognized that **the robustness of a category approach would be expected to be considerably greater than that of an analogue approach**, since the basis for evaluating any individual chemical in the category is greater, and there is usually more measured data available in such a wider approach.

- The category approach allows the interpolation/extrapolation of physico-chmical and (eco)toxicological properties by means of trend analysis.

Category approach for boiling point
Trend analysis

- When some chemicals in a category have **measured values** and a consistent trend is observed, missing values can be estimated by simple scaling from the measured values to fill in the data gaps.

▪ methane	- 162 °C
▪ ethane	- 89 °C
▪ propane	- 42 °C
▪ butane	0 °C
▪ pentane	? °C
▪ hexane	69 °C



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Trend analysis

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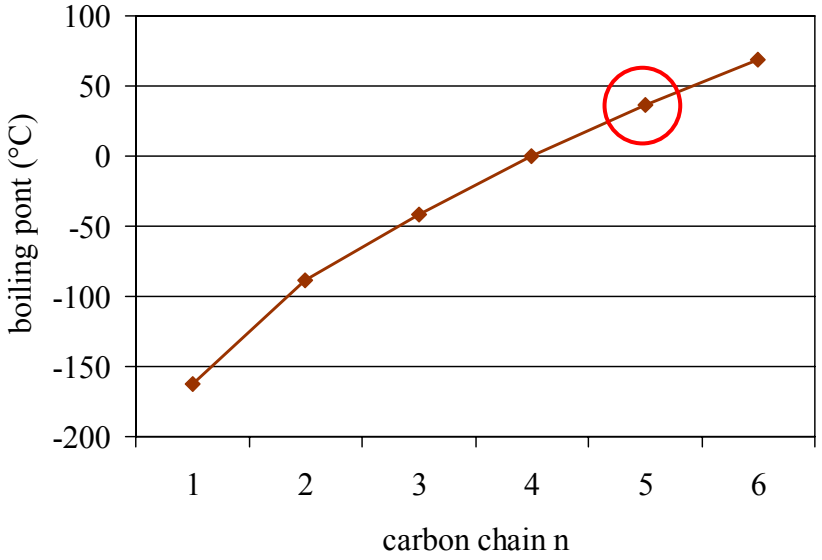
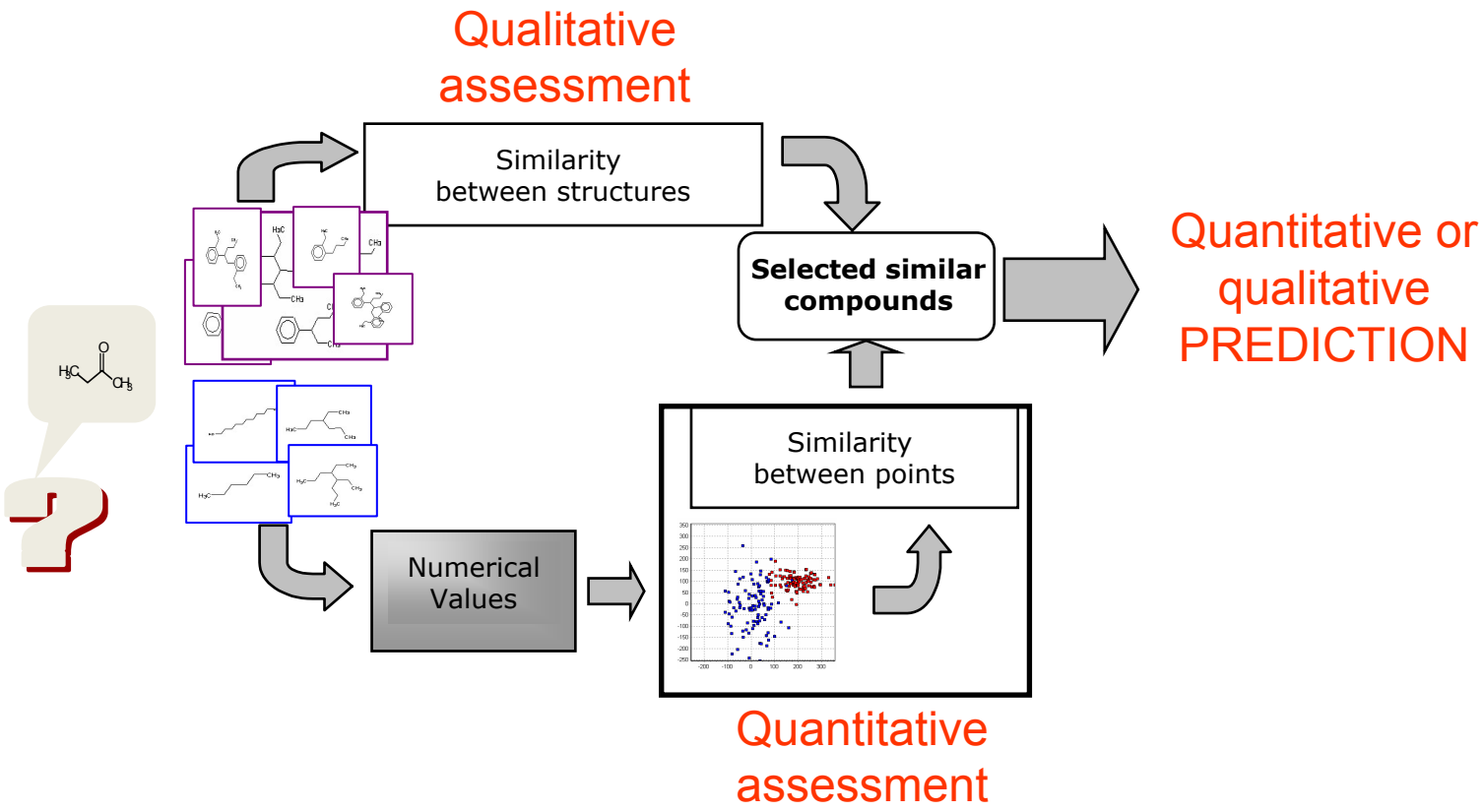
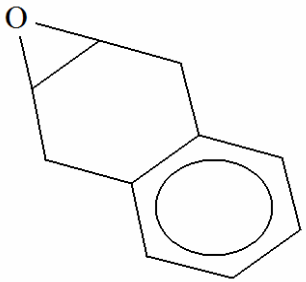


SAR and QSAR models



SAR definition

- **SAR**: qualitative relationships that relates a (sub)structure to the presence or absence of a property or activity of interest.



ECOSAR v1.00 Class(es) Found

Epoxides, mono

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Epoxides, mono	Fish	96-hr	LC50	8.751
Epoxides, mono	Fish	14-day	LC50	7.220
Epoxides, mono	Daphnid	48-hr	LC50	18.782
Epoxides, mono	Green Algae	96-hr	EC50	17.509

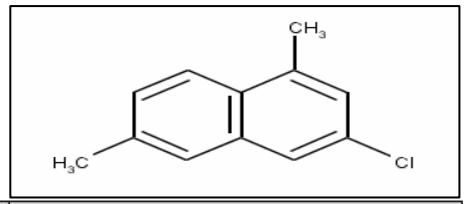
Epoxide, mono Equation

Log 96-h LC50 (mmol/L) = -0.5309 (log Kow) + 0.0733

QSAR definition

- **QSAR**: mathematical model (often a statistical correlation) relating one or more quantitative parameters derived from chemical structure to a qualitative/quantitative measure of a property or activity (e.g. a (eco)toxicological endpoint).

Motore	
	Manuale
Numero cilindri	6
Cilindrata	3.614 cm³
Motore/trazione	Posteriore a sbalzo
Potenza (DIN)	254 kW (345 CV)
Coppia massima	390 Nm
a giri/min	4.200-5.600 giri/min
Rapporto di compressione	12,5 : 1



Parameter	Value	Definition
MW	190,6800	Molecular weight
AMW	7,9450	Average molecular weight
Sv	16,2878	Sum of atomic van der Waals volumes (scaled on Carbon atom)
Se	23,6255	Sum of atomic Sanderson electronegativities (scaled on Carbon atom)
Sp	17,4261	Sum of atomic polarizabilities (scaled on Carbon atom)
Ss	26,4444	Sum of Kier-Hall electrotopological states
Mv	0,6787	Mean atomic van der Waals volume (scaled on Carbon atom)
Me	0,9844	Mean atomic Sanderson electronegativity (scaled on Carbon atom)
Mp	0,7261	Mean atomic polarizability (scaled on Carbon atom)
Ms	2,0342	Mean electrotopological state
nAT	24,0000	Number of atoms
nSK	13,0000	Number of non-H atoms
nBT	25,0000	Number of bonds
nBO	14,0000	Number of non-H bonds
nBM	11,0000	Number of multiple bonds
SCBO	19,5000	Sum of conventional bond orders (H-depleted)

Statistical methods

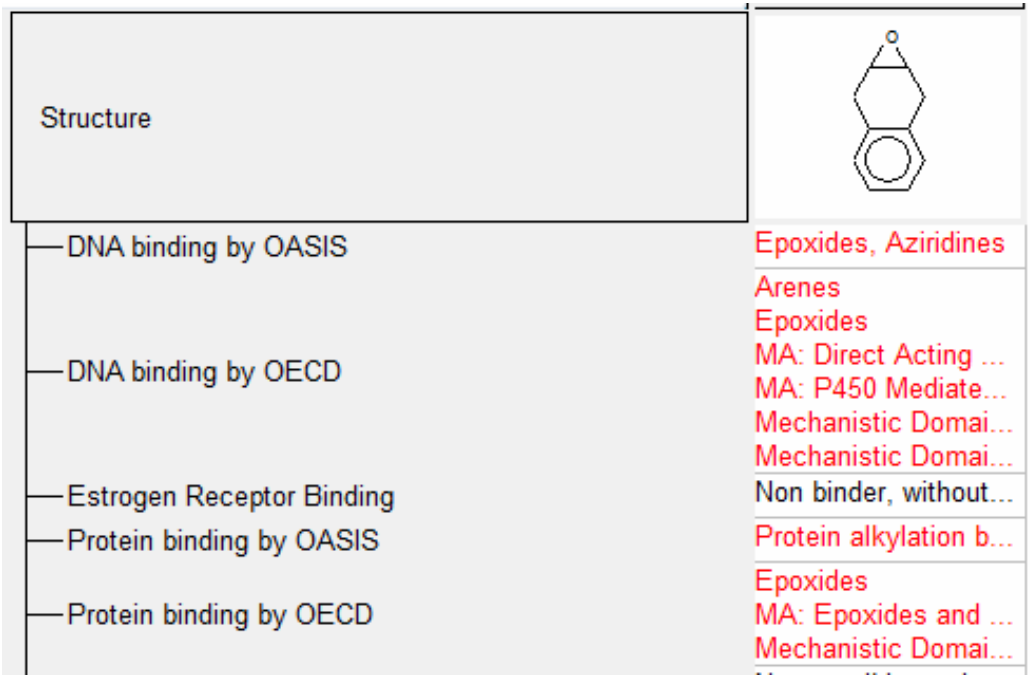
- Properties
- Toxicity
- Ecotoxicity

Expert systems

- **Expert Systems** attempt to formalize the knowledge of human experts, who assess the toxicity of a new compound, in computer program.

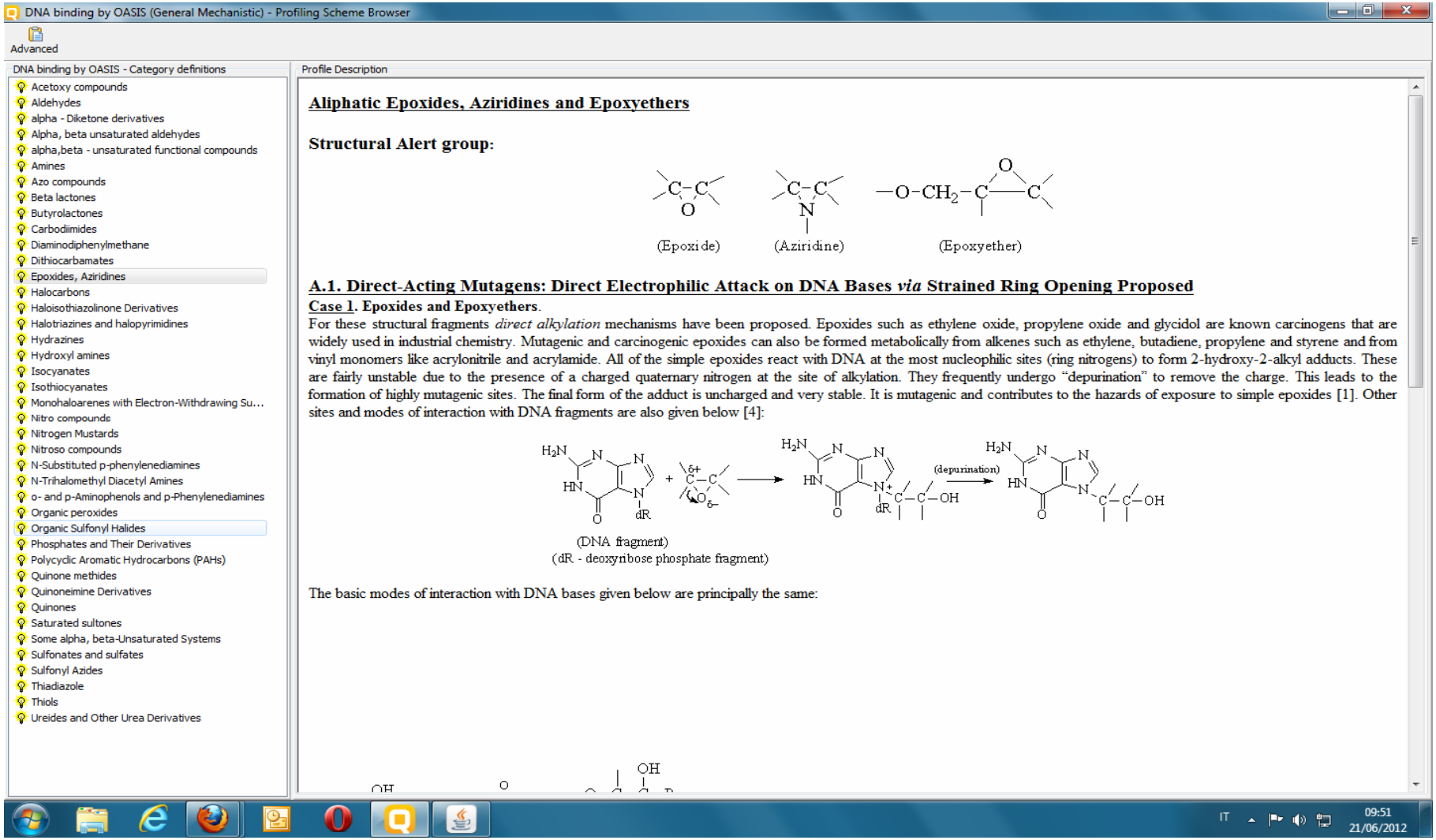
- These systems **screen the chemical molecules finding (sub)structures** which are recognized to be relevant for a specific endpoint on account of mechanistic information.

Expert systems - output



- **the expert based systems** are used to add mechanistic information

Expert systems - rules system



Non-testing data and REACH Regulation

- Whereas 1, 47
  - REACH should **promote** the development of alternative methods and it should **be implemented** through these methods.

- Article 13
  - **Information** on intrinsic properties of substances may be generated by means of alternative methods as **qualitative or quantitative structure-activity** relationship models or from information from structurally related substances (**grouping or read-across**).

Grouping and REACH Regulation

- Annex XI
- Substances whose are likely to be similar may be considered as a category.
  - The similarities may be based on:

    (1) a **common functional group**;

    (2) the **common precursors** and/or **common breakdown products;**

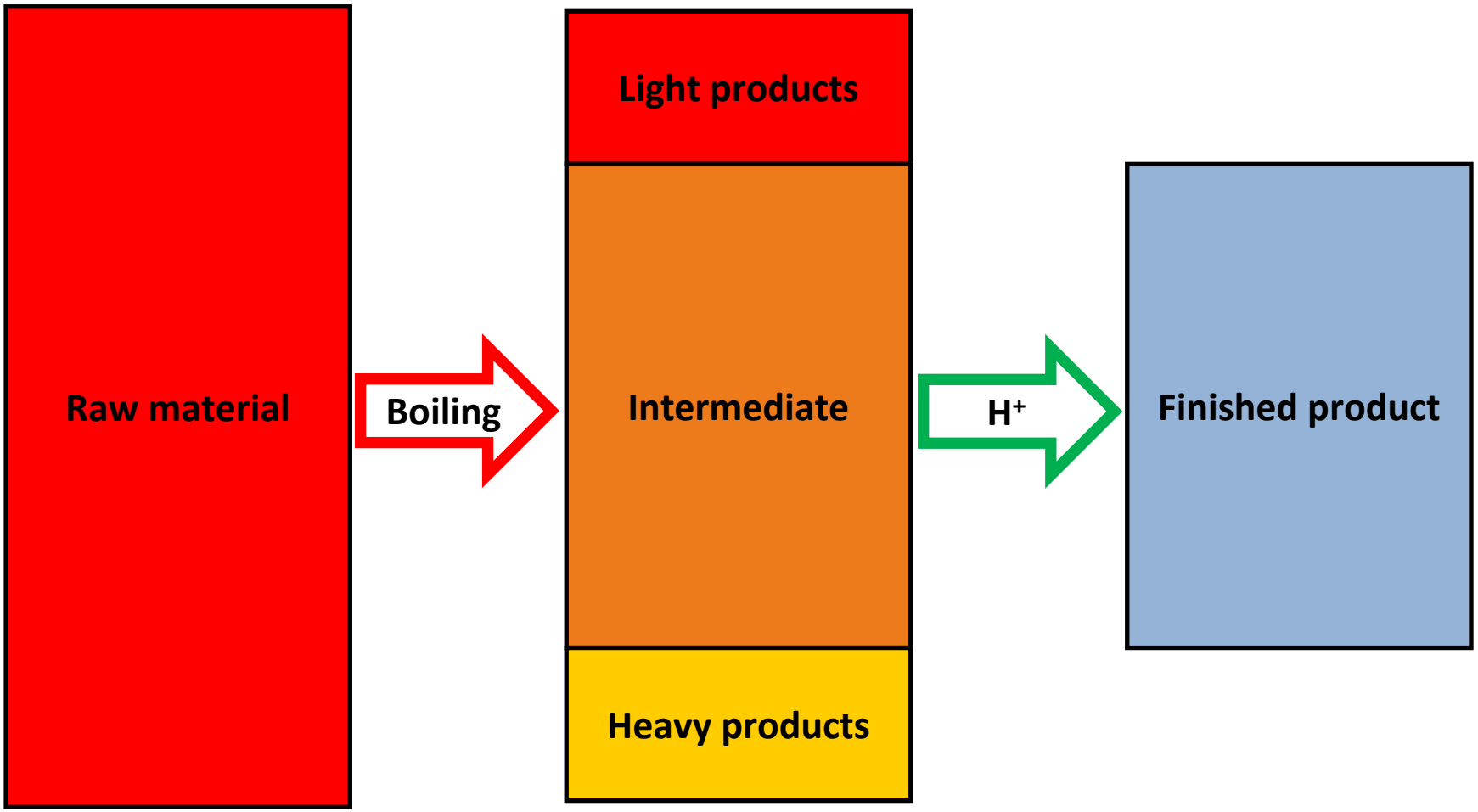
    (3) a **constant pattern** in the changing of the potency of the properties across the category.
- Results should be adequate:
  - for C&L and risk assessment;
  - for replacing the specific *in vivo* or *in vitro* data.

Non-testing data and CLP Regulation

- Hazard classes for which non-testing data can be used under CLP Regulation:
  - Acute toxicity
  - Skin and eye corrosion/irritation
  - Skin and airways sensitization
  - Mutagenesis
  - Carcinogenesis
  - Reproductive toxicity
  - STOT SE (only for category 1 and 2)
  - STOT RE

Example applied to the hazard assessment of an UVCB substance

Intermediate in finished substance production



Data matrix
Filling data gaps by read-across

Hazard class	Raw material	Intermediate	Finished product
Acute oral tox.	>2000 mg/kg (EXP)	>2000 mg/kg (R-A)	>2000 mg/kg (EXP)
Acute dermal tox.	>2000 mg/kg (EXP)	>2000 mg/kg (R-A)	>2000 mg/kg (EXP)
Skin irrit.	Not irrit (EXP)	Not irrit (R-A)	Not irrit (EXP)
Eye irrit.	Not irrit (EXP)	Not irrit (R-A)	Not irrit (EXP)
Skin sensitiz.	Not sens. (EXP)	?????	Sens. (EXP)
Repeated dose tox	Non toxic (EXP)	?????	No data
Genotox.	Positive	?????	Negative

Reliable read-across in accordance with category trend

Extrapolation is not deemed as reliable

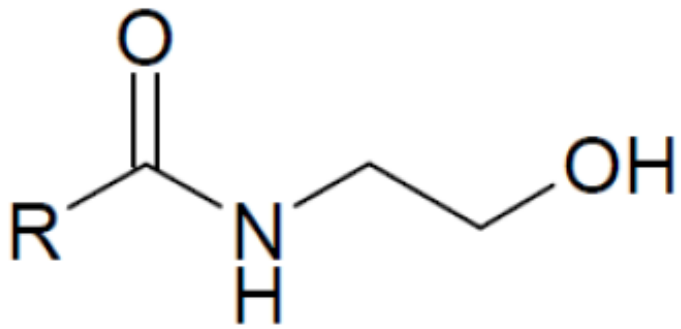
Trend analysis is not verified

Data matrix
Filling data gaps by experimental test data

Hazard class	Raw material	Intermediate	Finished product
Acute oral tox.	>2000 mg/kg (EXP)	>2000 mg/kg (R-A)	>2000 mg/kg (EXP)
Acute dermal tox.	>2000 mg/kg (EXP)	>2000 mg/kg (R-A)	>2000 mg/kg (EXP)
Skin irrit.	Not irrit (EXP)	Not irrit (R-A)	Not irrit (EXP)
Eye irrit.	Not irrit (EXP)	Not irrit (R-A)	Not irrit (EXP)
Skin sensitiz.	Not sens. (EXP)	**Sens. (EXP)**	Sens. (EXP)
Repeated dose tox	Non toxic (EXP)	**inconclusive**	No data
Genotox.	Positive	**Positive (EXP)**	Negative

Example applied to the hazard assessment of organic substance

Category definition of fatty alkanolamides, monohydroxy-compounds

Chemical structure which is common for all category members

R: linear alkyl chain.

- Substance 1 $R = -(CH_2)_{10}-CH_3$
- Substance 2 $R = -(CH_2)_{12}-CH_3$
- Substance of interest $R = -(CH_2)_{14}-CH_3$ **HAZARD?**
- Substance 4 $R = -(CH_2)_{16}-CH_3$

Data Matrix

Endpoint	Substance 1	Substance 2	Substance 3	Substance 3
MW	243	271	299	327
Boiling point (°C)	643	682	702	724
LogKow	3,24	4,22	5,21	6,19
Skin irritation	non-irritant in humans and mice	non-irritant in humans and mice	?	non-irritant in humans and mice
Eye irritation	data disregarded (not assignable reliability)	conflicting data	?	
Skin sensitization		non-sensitizing in humans	?	
Mutagenicity	non-mutagen in Ames test		?	

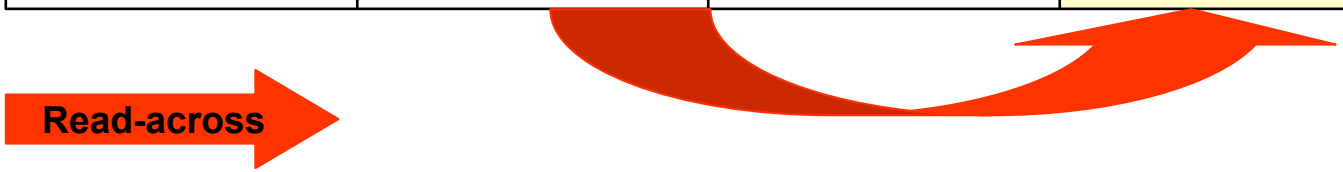
TREND →

Expert system assessment

Endpoint	Substance 1	Substance 2	Substance 3	Substance 3
DNA binding by OASIS	No binding	No binding	No binding	No binding
DNA binding by OECD	No binding	No binding	No binding	No binding
Protein binding by OASIS	No binding	No binding	No binding	No binding
Protein binding by OECD	No binding	No binding	No binding	No binding
Skin irritation/corrosion Inclusion rules by BfR	Inclusion rules not met	Inclusion rules not met	Inclusion rules not met	Inclusion rules not met
Eye irritation/corrosion Inclusion rules by BfR	Inclusion rules not met	Inclusion rules not met	Inclusion rules not met	Inclusion rules not met

Data Matrix

Endpoint	Substance 1	Substance 2	Substance 3	Substance 3
MW	243	271	299	327
Boiling point (°C)	643	682	702	724
LogKow	3,24	4,22	5,21	6,19
Skin irritation	non-irritant in humans and mice	non-irritant in humans and mice	non-irritant (read-across)	non-irritant in humans and mice
Eye irritation	data disregarded (not assignable reliability)	conflicting data	**read-across is not applicable**	
Skin sensitization		non-sensitizing in humans	non-sensitizing (read-across)	
Mutagenicity	non-mutagen in Ames test		non-mutagen (read-across)	



Consensus approach

- Each predictive toxicology technique has its own distinct advantages and weaknesses, an obvious approach is to **combine predictions from different methods**.

Grouping	→	definition of category
	→	identification of trends in the category
QSAR	→	statistical analysis
	→	information on reliability of the predictions
Expert system	→	MOA information