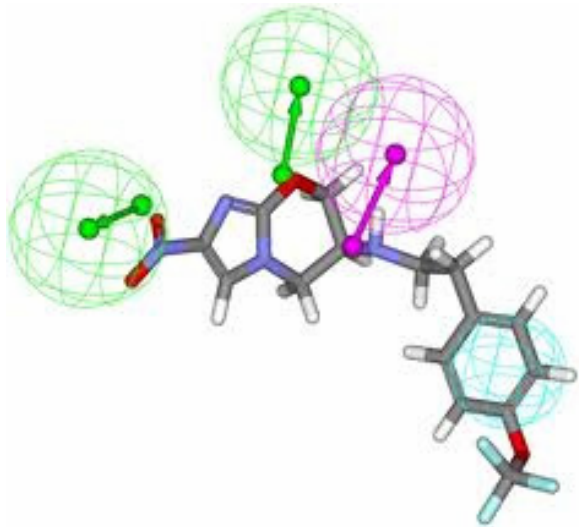


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# I metodi “read-across”: metodologie ed esempi applicativi



Giovanni Talamini  
IRCCS Fondazione Maugeri  
Università degli Studi di Pavia  
[giovanni.talamini@tossicologiapavia.it](mailto:giovanni.talamini@tossicologiapavia.it)

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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

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## 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).



# 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 1	Chemical 2
Structure	xxxxxxxxx	xxxxxxxxx
Property 1	●	○
Property 2	●	○
Activity 1	●	○
Activity 2	●	○

# 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.

	Chemical 1	Chemical 2	Chemical 3	Chemical 4
Structure	xxxxxxxxx	xxxxxxxxx	xxxxxxxxx	xxxxxxxxx
Property 1	● → ○	● → ○	● ? → ○	
Property 2	● → ○	● → ○	○ ← ? → ●	
Property 3	○ ← ? → ●	○ ← ? → ●	○ ← ? → ●	
Activity 1	● → ○	● → ○	● ? → ○	
Activity 2	● → ○	● → ○	○ ← ? → ●	
Activity 3	○ ← ? → ●	○ ← ? → ●	○ ← ? → ●	

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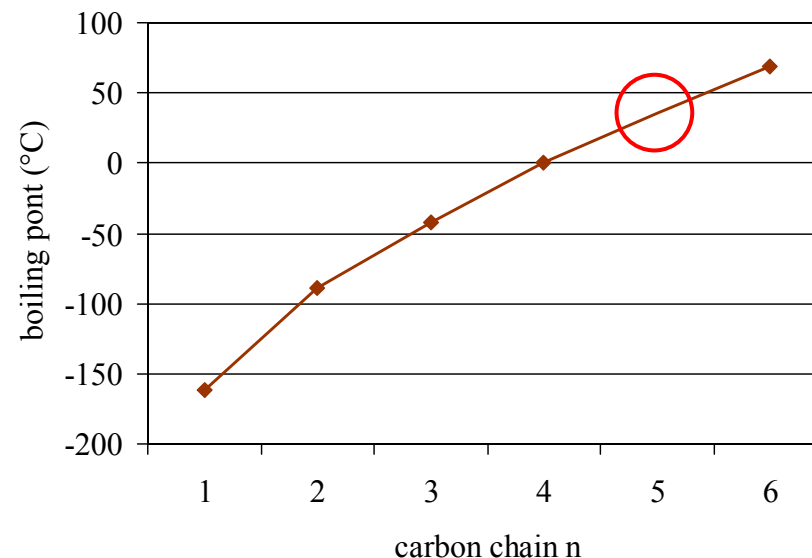
## Analogue vs category approach

- 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-chemical 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.

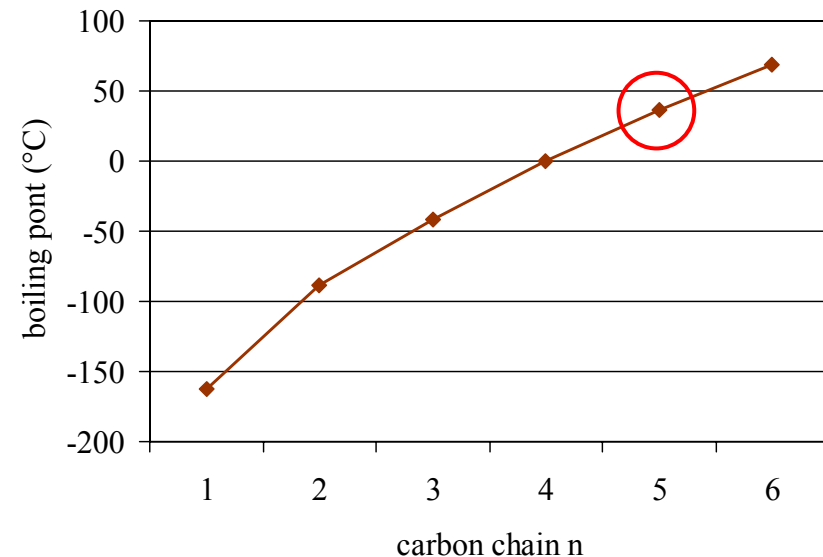
▪ methane	- 162 °C
▪ ethane	- 89 °C
▪ propane	- 42 °C
▪ butane	0 °C
▪ pentane	? °C
▪ hexane	69 °C



# Category approach for boiling point Trend analysis

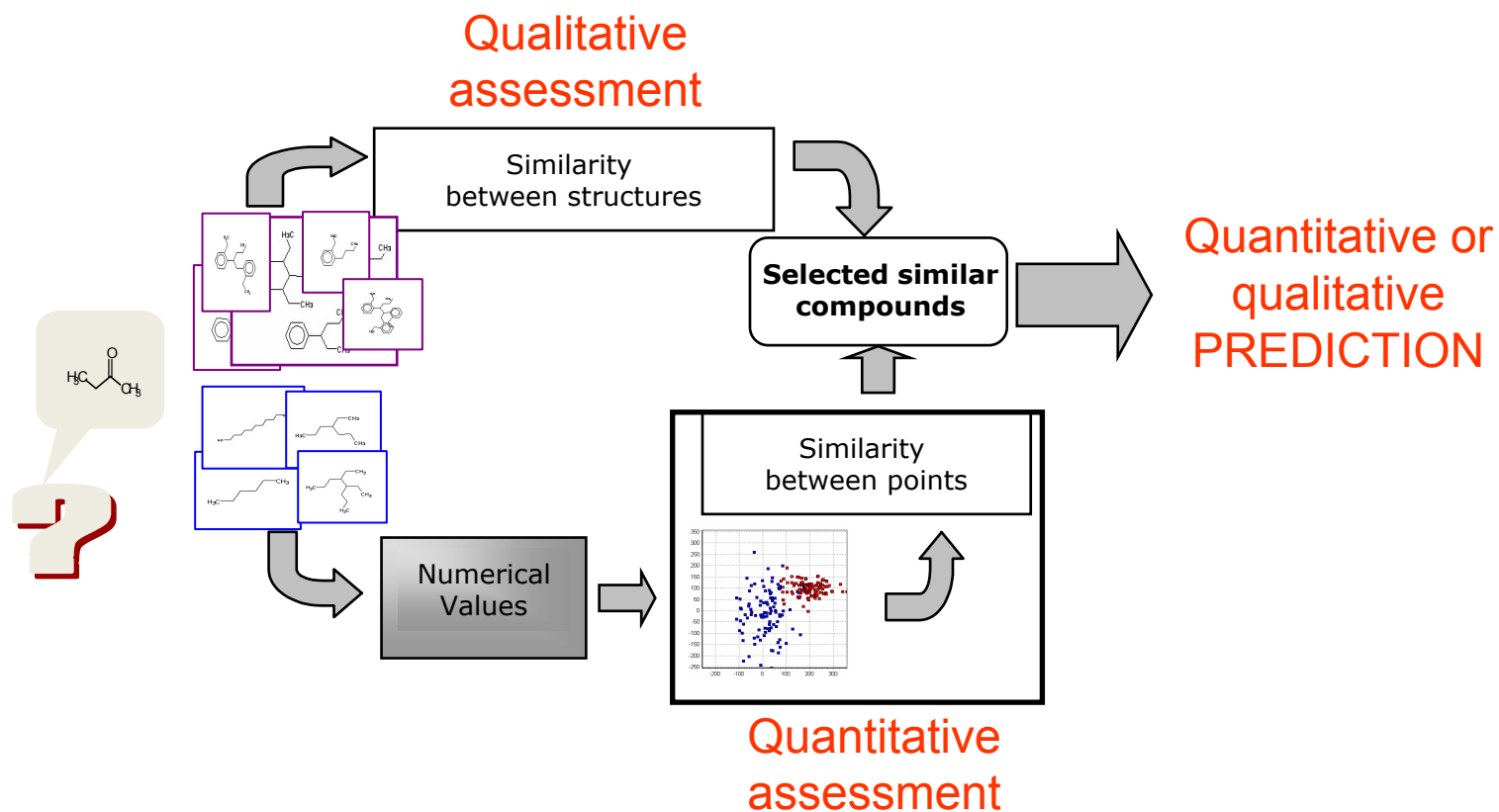
- When some chemicals in a category have **measured values** and a consistent trend is observed, missing values can be estimated by simple scaling from the measured values to fill in the data gaps.

▪ methane	- 162 °C
▪ ethane	- 89 °C
▪ propane	- 42 °C
▪ butane	0 °C
▪ pentane	36 °C
▪ hexane	69 °C



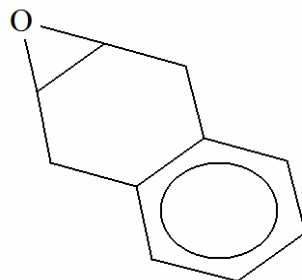


# SAR and QSAR models



# 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

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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

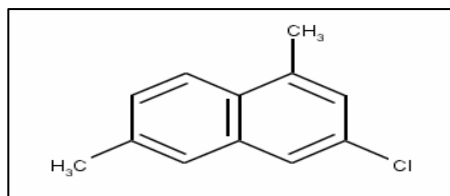
$$\text{Log 96-h LC50 (mmol/L)} = -0.5309 (\log Kow) + 0.0733$$

# QSAR definition

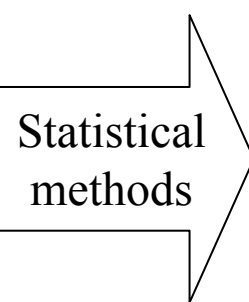
- **QSAR:** mathematical model (often a statistical correlation) relating one or more quantitative parameters derived from chemical structure to a qualitative/quantitative measure of a property or activity (e.g. a (eco)toxicological endpoint).



Motore	
	Manuale
Numero cilindri	6
Cilindrata	3.614 cm <sup>3</sup>
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)




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# 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

Structure	
— DNA binding by OASIS	Epoxides, Aziridines
— DNA binding by OECD	Arenes Epoxides MA: Direct Acting ... MA: P450 Mediate...
— Estrogen Receptor Binding	Mechanistic Domai...
— Protein binding by OASIS	Mechanistic Domai...
— Protein binding by OECD	Non binder, without...
	Protein alkylation b...
	Epoxides
	MA: Epoxides and ... Mechanistic Domai...

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

# Expert systems - rules system

DNA binding by OASIS (General Mechanistic) - Profiling Scheme Browser

Advanced

DNA binding by OASIS - Category definitions

- Acetoxy compounds
- Aldehydes
- alpha - Diketone derivatives
- Alpha, beta unsaturated aldehydes
- alpha,beta - unsaturated functional compounds
- Amines
- Azo compounds
- Beta lactones
- Butyrolactones
- Carbodiimides
- Diaminodiphenylmethane
- Dithiocarbamates
- Epoxides, Aziridines
- Halocarbons
- Haloisothiazolinone Derivatives
- Halotriazines and halopyrimidines
- Hydrazines
- Hydroxyl amines
- Isocyanates
- Isothiocyanates
- Monohaloarenes with Electron-Withdrawing Su...
- Nitro compounds
- Nitrogen Mustards
- Nitroso compounds
- N-Substituted p-phenylenediamines
- N-Trihalomethyl Diacetyl Amines
- o- and p-Aminophenols and p-Phenylenediamines
- Organic peroxides
- Organic Sulfonyl Halides
- Phosphates and Their Derivatives
- Polycyclic Aromatic Hydrocarbons (PAHs)
- Quinone methides
- Quinoneimine Derivatives
- Quinones
- Saturated sultones
- Some alpha, beta-Unsaturated Systems
- Sulfonates and sulfates
- Sulfonyl Azides
- Thiadiazole
- Thiols
- Ureides and Other Urea Derivatives

Profile Description

### Aliphatic Epoxides, Aziridines and Epoxyethers

**Structural Alert group:**

(Epoxide)

(Aziridine)

(Epoxyether)

### A.1. Direct-Acting Mutagens: Direct Electrophilic Attack on DNA Bases via Strained Ring Opening Proposed

#### Case 1. Epoxides and Epoxyethers.

For these structural fragments *direct alkylation* mechanisms have been proposed. Epoxides such as ethylene oxide, propylene oxide and glycidol are known carcinogens that are widely used in industrial chemistry. Mutagenic and carcinogenic epoxides can also be formed metabolically from alkenes such as ethylene, butadiene, propylene and styrene and from vinyl monomers like acrylonitrile and acrylamide. All of the simple epoxides react with DNA at the most nucleophilic sites (ring nitrogens) to form 2-hydroxy-2-alkyl adducts. These are fairly unstable due to the presence of a charged quaternary nitrogen at the site of alkylation. They frequently undergo "depurination" to remove the charge. This leads to the formation of highly mutagenic sites. The final form of the adduct is uncharged and very stable. It is mutagenic and contributes to the hazards of exposure to simple epoxides [1]. Other sites and modes of interaction with DNA fragments are also given below [4]:

(DNA fragment)  
(dR - deoxyribose phosphate fragment)

The basic modes of interaction with DNA bases given below are principally the same:

09:51  
21/06/2012

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# 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**).

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# 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.



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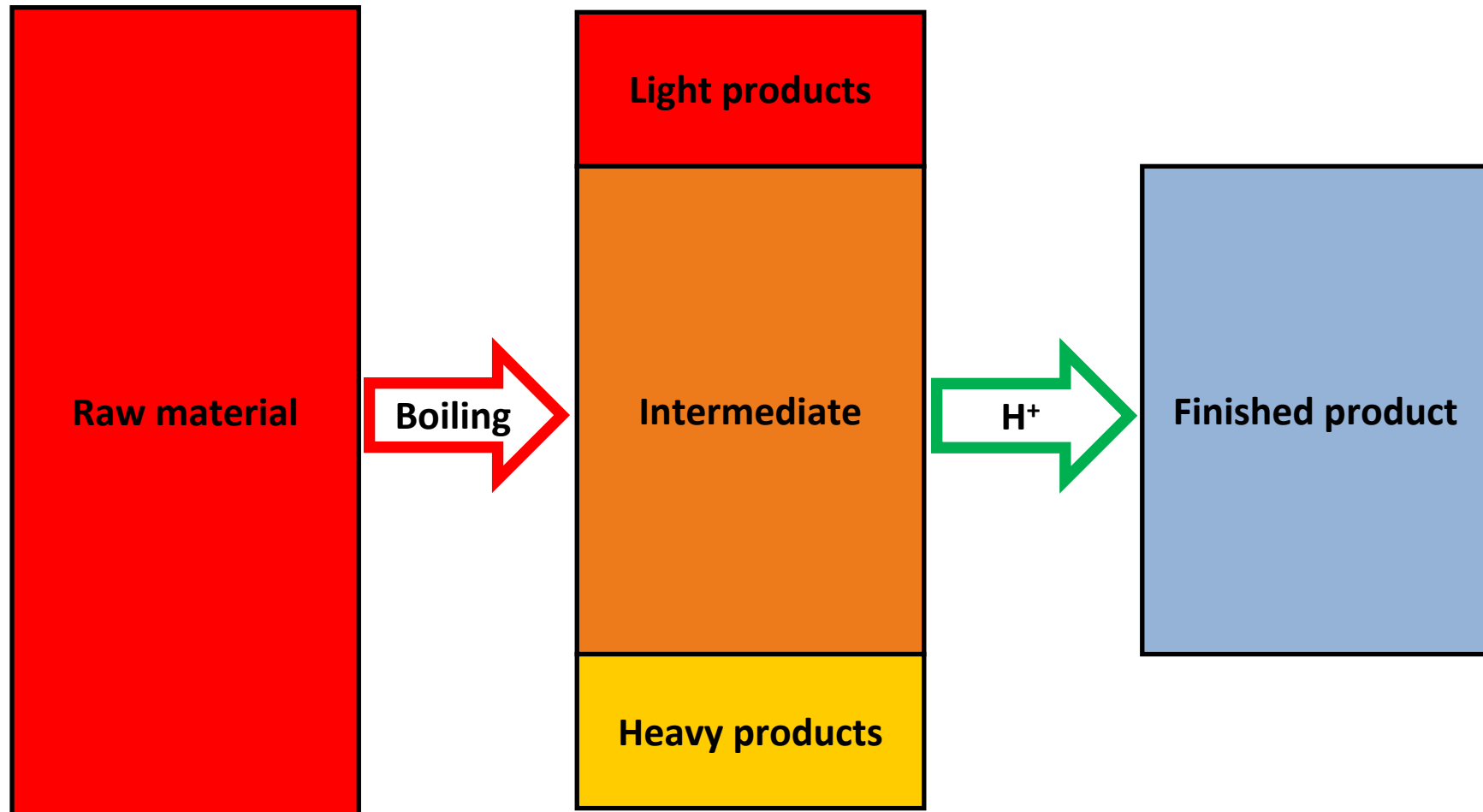
# Non-testing data and CLP Regulation

- 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

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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

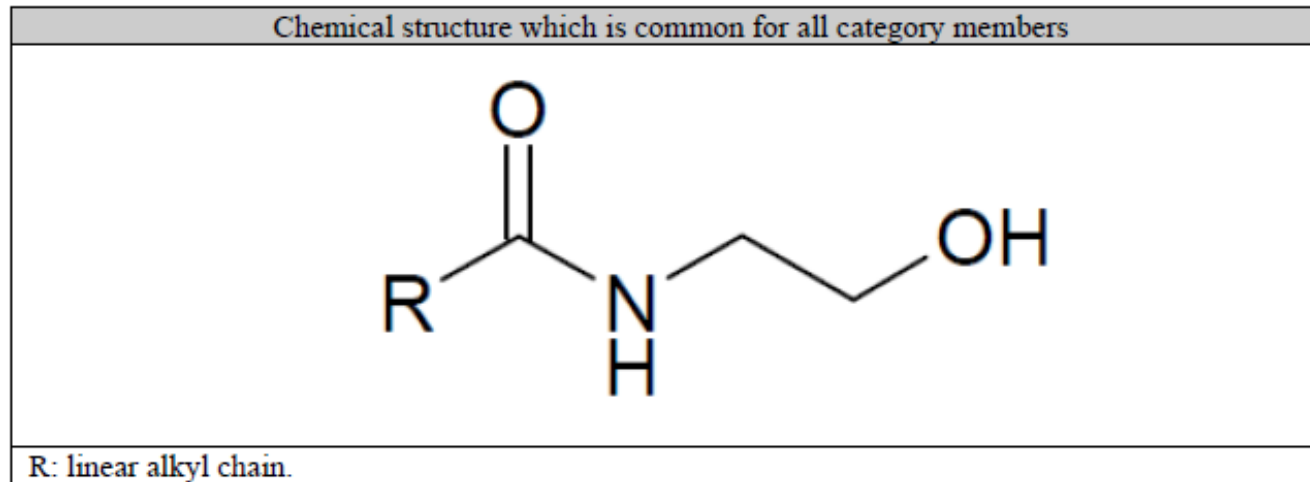
## Filling data gaps by experimental test data

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)	<b>Sens. (EXP)</b>	Sens. (EXP)
Repeated dose tox	Non toxic (EXP)	<b>inconclusive</b>	No data
Genotox.	Positive	<b>Positive (EXP)</b>	Negative

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Example applied to the hazard assessment of  
organic substance

# Category definition of fatty alkanolamides, monohydroxy-compounds



- Substance 1  $R = -(\text{CH}_2)_{10}-\text{CH}_3$
- Substance 2  $R = -(\text{CH}_2)_{12}-\text{CH}_3$
- Substance of interest  $R = -(\text{CH}_2)_{14}-\text{CH}_3$
- Substance 4  $R = -(\text{CH}_2)_{16}-\text{CH}_3$

**HAZARD?**

# 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	<b>read-across is not applicable</b>	
Skin sensitization		non-sensitizing in humans	non-sensitizing (read-across)	
Mutagenicity	non-mutagen in Ames test		non-mutagen (read-across)	

**Read-across** 

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# 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