

# Computational Toxicity and QSAR

*Supa Hannongbua*

*Department of Chemistry, Faculty of Science*

*Kasetsart University, Bangkok 10900*

*fscisph@ku.ac.th*

**Background and Motivation**

**What is QSAR?**

**QSAR's Applications**

**Research Area in Thailand**

**Concept proposal**

**Computational Toxicity in Dyes and Cosmetics**



The European Chemicals Bureau provides technical and scientific support for implementation of certain EU legislation on dangerous chemicals and the preparation for REACH

**Action 1311**  
Assessment  
of Chemicals

**Action 1313**  
Support to REACH



EUROPEAN CHEMICALS BUREAU

<http://ecb.jrc.it>

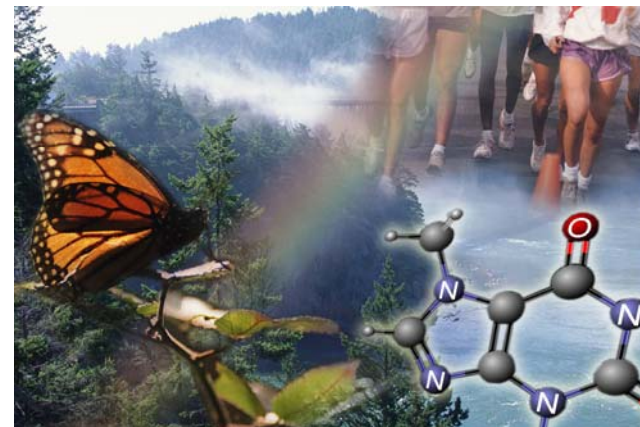
**Action 1314**  
REACH-IT  
&  
Informatics

**Action 1321**  
Computational  
Toxicology  
(QSARs)



## Actions in FP6 year 2006

- 1311 Assessment of Chemicals  
*New and Existing Chemicals\**  
*Biocidal Products*  
*Classification and Labeling\**  
*Testing Methods*  
*Export/Import*



**The Action mission** is to provide the scientific and technical support to the conception, development, implementation and monitoring of EU policies on dangerous chemicals.

# What is in silico / QSAR ?

- ❑ In silico and REACH
- ❑ What can be done in silico ?
- ❑ An example: in silico CYP inhibition
- ❑ What next ?

# Levels of testing

**in cerebro**



**in silico**



**in vitro**



**in vivo**

## (Q)SARs defined by ECB

Quantitative structure-activity relationships, collectively referred to as (Q)SARs, are theoretical models that can be used to predict the physicochemical and biological properties of molecules.

A **structure-activity relationship** (SAR) is a (qualitative) association between a chemical substructure and the potential of a chemical containing the substructure to exhibit a certain biological effect.

A **quantitative structure-activity relationship** (QSAR) is a mathematical model that relates a quantitative measure of chemical structure (e.g. a physicochemical property) to a physical property or to a biological effect (e.g. a toxicological endpoint).

## Possible applications for QSARs in the Regulatory Assessment of Chemicals

In principle, (Q)SARs could be used to supplement experimental data, or to replace testing:

### **Supplement to testing:**

- 1) To support priority setting of chemicals.
- 2) To guide experimental design (e.g. selection of tests /doses).
- 3) To provide mechanistic information.

### **Replacement of testing:**

- 4) To group chemicals into chemical categories.
- 5) To fill in data gaps for classification and labelling.
- 6) To fill in data gaps for risk assessment.

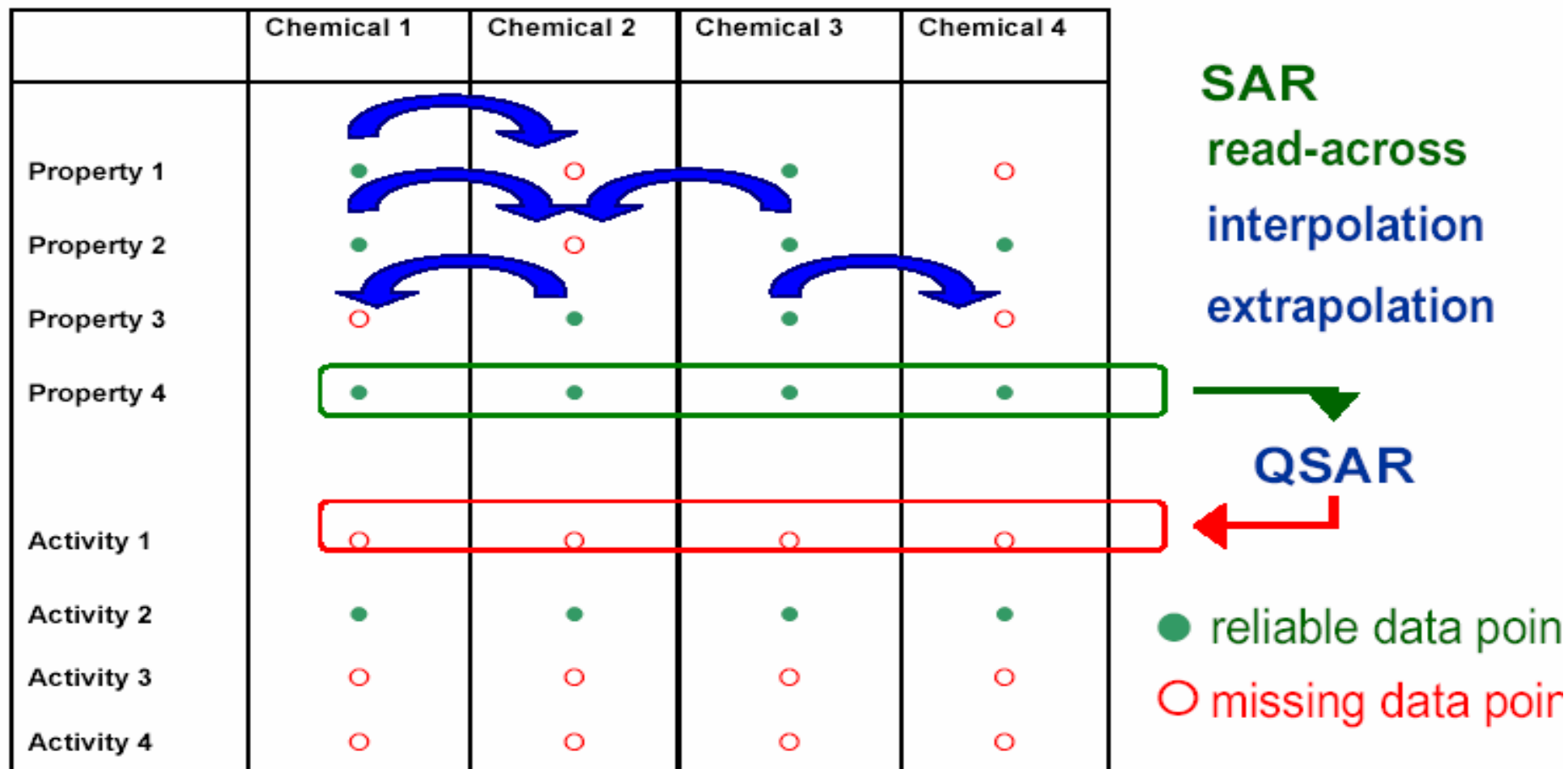
# Chemical categories and read-across

The underlying premise underpinning all structure-activity relationships (SARs) is the expectation that structurally similar chemicals are likely to have similar physicochemical attributes and biological effects. The approaches of read across and chemical categories are based on this similarity principle.

In the **read-across** or **analogue approach**, endpoint information for one chemical is used to make a prediction of the endpoint for another chemical, which is considered to be "similar" in some way. In principle, read-across can be used to assess physicochemical properties, toxicity, environmental fate and ecotoxicity, and it may be performed in a qualitative or quantitative manner.

ECB/JRC 2005

# The chemical category concept and supporting role of (Q)SARs



# Contents

- ❑ What is in silico / QSAR ?
- ❑ In silico and REACH
- ❑ What can be done in silico ?
- ❑ An example: in silico CYP inhibition
- ❑ What next ?

# The White Paper

## ❑ 3.2 Research and Validation

### ❑ Development of alternative methods

### ❑ Other research priorities

- improvement and simplification of risk-assessment procedures
- improvement and development of new toxicological and ecotoxicological methods
- particular research efforts need to be made for developing and validating in-vivo and in-vitro test methods as well as modelling (e.g. QSAR) and screening methods for assessing the potential of adverse effects of chemicals on endocrine systems of humans and animals.
- etc...

## Use of QSARs under REACH

- ❑ Acceptance of QSAR results - BOTH positive and negative results will be accepted if
  - Models have been validated
  - Models are adequately documented and meet acceptance criteria for a given application
    - “fit for purpose” concept
  
- ❑ (Q)SARs may support grouping of chemicals Chemical categories and minimise testing
  
- ❑ Animal testing is conducted as a last resort

## Remarks

- Need to use (Q)SARs is explicit in legislative proposal for REACH
- In silico methods developing fast
- Predictive power already quite good in some (restricted) areas
- Future regulatory use of (Q)SARs will be controlled by various acceptance and validity criteria

# Alternative approaches can reduce the use of test animals

Examples of read-across under 793/93: Flame retardants

TCEP Tris(2-chloroethyl) phosphate 115-96-8

TCCP Tris(2-chloro-1-methylethyl)phosphate 13674-84-5

TDCP Tris[2-chloro-1-(chloromethyl)ethyl]phosphate 13674-87-8

V6 2,2-bis(chloromethyl) trimethylene bis[bis(2-chloroethyl) phosphate 38051-10-4

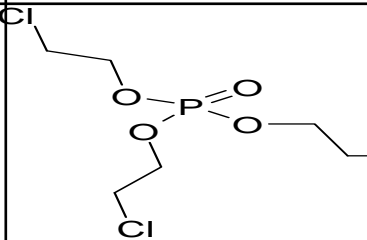
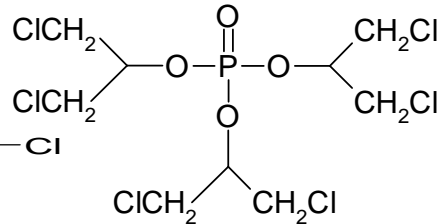
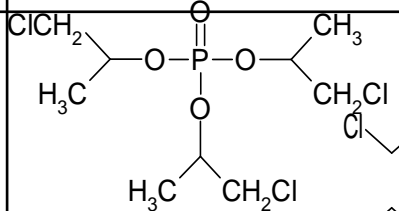
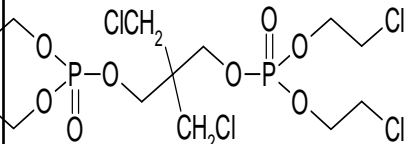
Rapporteur:  
Germany

ENV: UK  
HH: Ireland

## Basis for read across

- Structural analogy of chloroalkyl phosphate esters
- lack of data for some substances for some endpoints
- Decision needed for
  - Risk Characterisation and
  - Classification and Labelling

# Comparison phys-chem. Properties

Substance	TCEP	TCPP	TDCP	V6
Structural formula:				
Molecular formula:	C <sub>6</sub> H <sub>12</sub> Cl <sub>3</sub> O <sub>4</sub> P	C <sub>9</sub> H <sub>18</sub> Cl <sub>3</sub> O <sub>4</sub> P	C <sub>9</sub> H <sub>15</sub> Cl <sub>6</sub> O <sub>4</sub> P	C <sub>13</sub> H <sub>24</sub> Cl <sub>6</sub> O <sub>8</sub> P <sub>2</sub>
Molecular weight:	285.49	327.57	430.91	583
Melting point	<-70°C	<-20°C	<-20°C	FP < -50.5°C
Boiling point	320°C (decomp.)	Ca. 288°C (decomp.)	>200°C	252°C (decomp)
Vapor Pressure	1.14 x 10 <sup>-3</sup> Pa at 20°C	1.4 x 10 <sup>-3</sup> Pa	5.6 x 10 <sup>-6</sup> Pa at 25°C	2.75 x 10 <sup>-6</sup> Pa at 25°C
Water Solubility	7820 mg/l at 20°C	1080 mg/l	18.1 mg/l	232 mg/l
log Kow	1.78	2.68	3.69	2.83

# Read across for Sensitization

Substance	TCEP	TCPP	TDCP	V6
Study:	No study	Guinea pig, M&K	Guinea pig, M&K	Guinea pig, M&K
Results:		negative	negative	negative
Classification		No classification	No classification	No classification

## Conclusion:

TCEP is regarded as not being sensitizing on basis of read across to structural related substances (TCPP and TDCP) and no observation of sensitizing potential in workers exposed to TCEP.

# Read across for Carcinogenicity

Substance	TCEP	TCPP	TDCP	V6
Study:	2 y carc. Study in rat and mice	No study	rat, 2-y-carcinogenicity study	No study
Results:	Kidney tumours in two species and both sexes;		Renal cortical tumours, testicular interstitial cell tumours, hepatocellular adenomas and adrenal cortical adenomas	
Classification:	carc. Cat. 3 R40 agreed at TC C&L	Carc. Cat. 3 R40 proposed	Carc. Cat. 3 R40 agreed	

## Conclusion:

Read across to TCPP from TCEP and TDCP proposed. There are similarities in hydrolysis and one common metabolite. The NOAEL is taken from the study with TDCP. No read across for V6 as it is an alkyl bridged bis-phosphate ester which makes it probably a bulkier and less bioavailable molecule. (There were proposals from MS to consider V6 as two molecules of TCEP and to take over the same classification).

# (No) read across for Fertility

Substance	TCEP	TCPP	TDCP	V6
Study:	2 generation in mice	No study	12 weeks fertility study in rabbits, 2 y carcinogenicity study	No study
Results:	Impairment of fertility for both sexes: reduced sperm counts, reduced litter size	High dose 90 day study showed no significant effects	No effects in rabbits; significant effects on the male reproductive organs in the carcinogenicity study	
Classification:	repro. Cat. 2 R60 agreed at C&L		Repro Cat. 3 R62 agreed at TC C&L	

## Conclusion:

Read across was not regarded as appropriate on basis of the available data (V6 see also justification for carcinogenicity).

A new 2 generation study will be performed for TCPP and V6.

## (No) read across for Developmental Effects

Substance	TCEP	TCPP	TDCP	V6
Study:	developmental study in rats and mice	developmental study in rats	developmental study in rats	No study
Results:	No significant effects	No significant effects	No significant effects	
Classification:	No classification/no concern	No classification/no concern	No classification/no concern	

### Conclusion:

Read across was not regarded as appropriate for V6. Further testing will depend on the results of the 2 generation study.

RIP 2

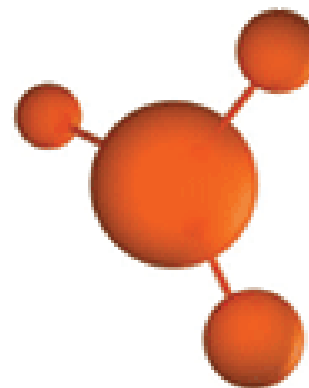


Actions in FP6 year 2006

**1314 - REACH-IT & Informatics (new in 2006)**

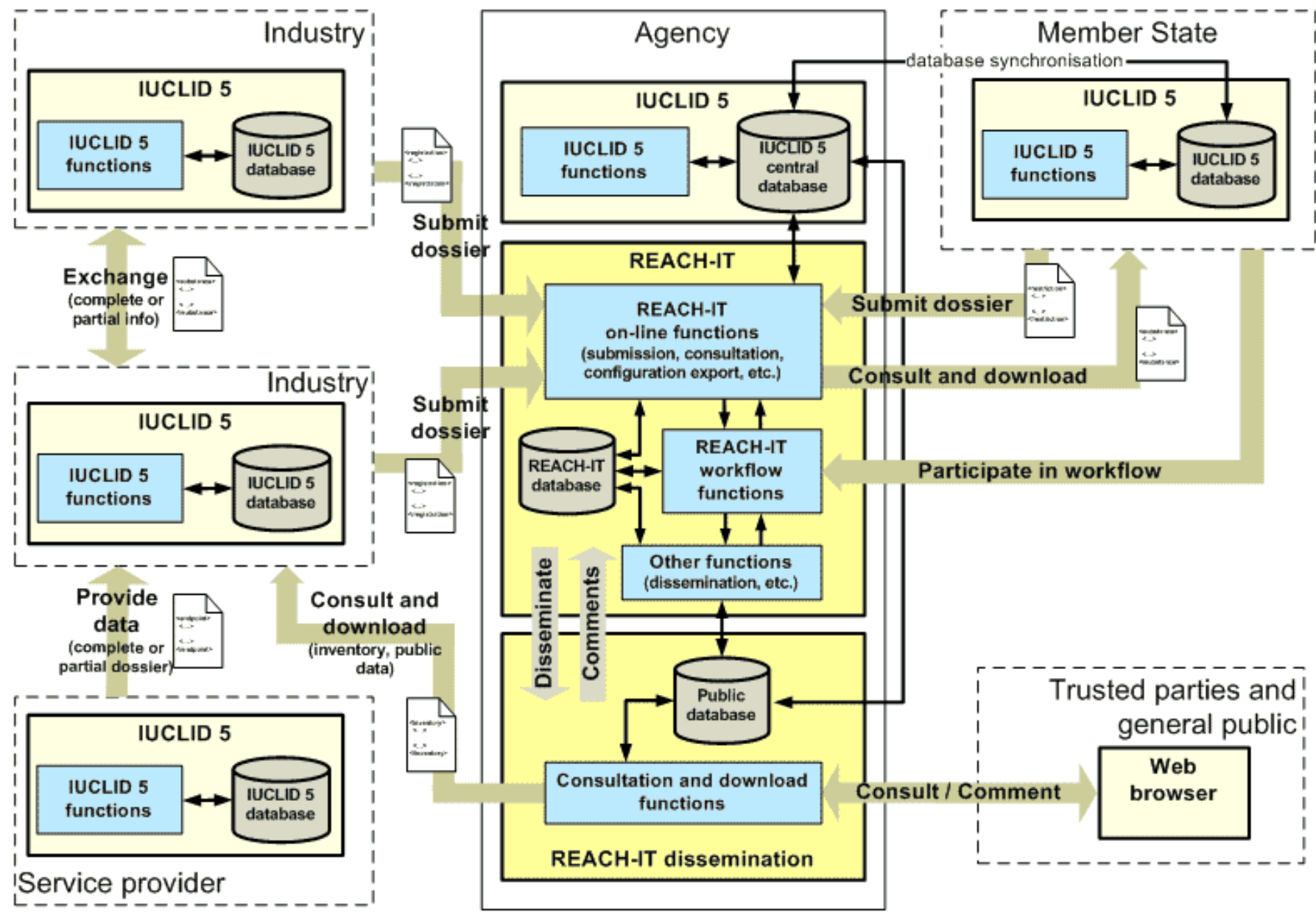


**Chemical databases,  
IT for registration,  
workflow for dossiers,  
global portal for sharing  
data**

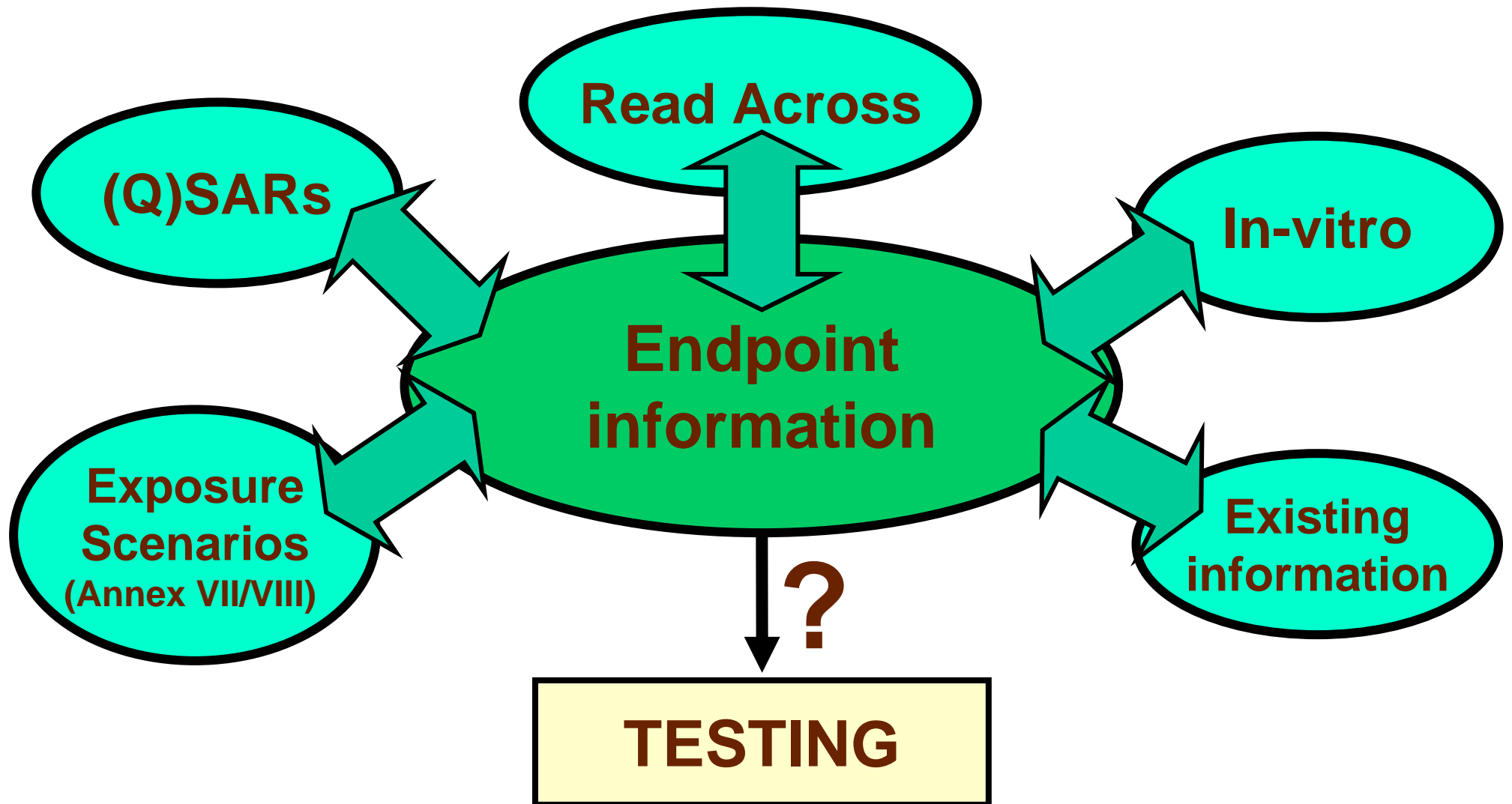


**IUCLID 5**  
INTERNATIONAL UNIFORM CHEMICAL INFORMATION DATABASE

# REACH-IT Architecture



# Intelligent Testing Strategies (ITS)





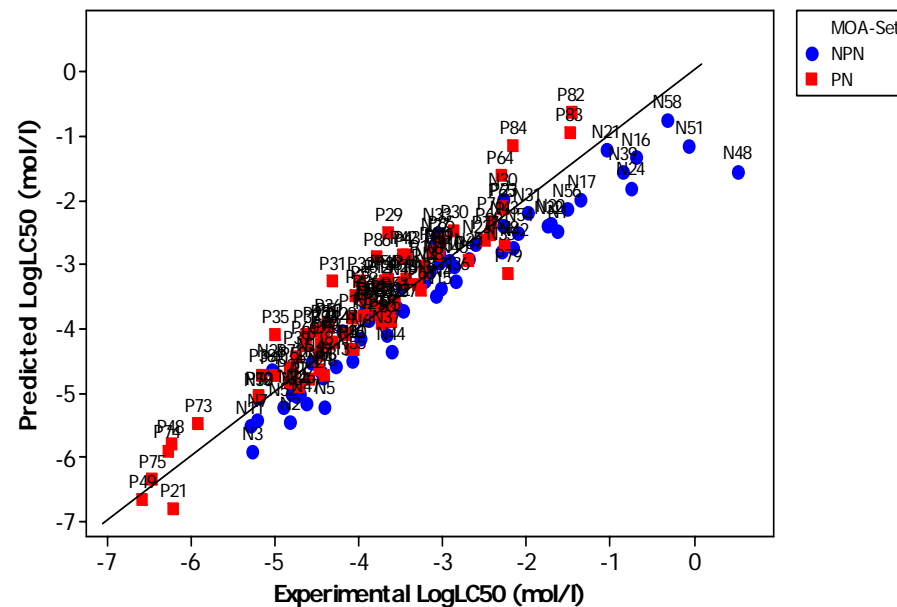
## Actions in FP6 year 2006

### Computational Toxicity (QSARs)

QSARs = Quantitative Structure Activity relationships



*Development, validation and implementation of (Q)SARs and other estimation methods for the assessment of chemicals*



# QSAR Action (Computational Toxicity)

**(Q)SARs:** theoretical models that can be used to predict the physicochemical and biological properties of molecules. They are sometimes called *in silico* models.

QSARs can be used, in combination with other types of information, to minimize testing (e.g. animal testing)

This does not imply that a single QSAR replaces a single test



## (Q)SARs and REACH: Use of Animals

### Additional animals



3.9 million  
2.6 million  
2.1 million

### Use of (Q)SARs, read-across



Minimal use  
Average use (likely scenario)  
Maximal use

Animal-saving potential: **1.3-1.9 million animals**

Van der Jagt *et al.* (2004).

Alternative approaches can reduce the use of test animals under REACH.

<http://ecb.jrc.it>

## (Q)SARs and REACH: Testing Costs

### Additional cost



2.3 billion Euro

1.5 billion Euro

1.1 billion Euro

### Use of (Q)SARs, read-across



Minimal use

Average use (likely scenario)

Maximal use

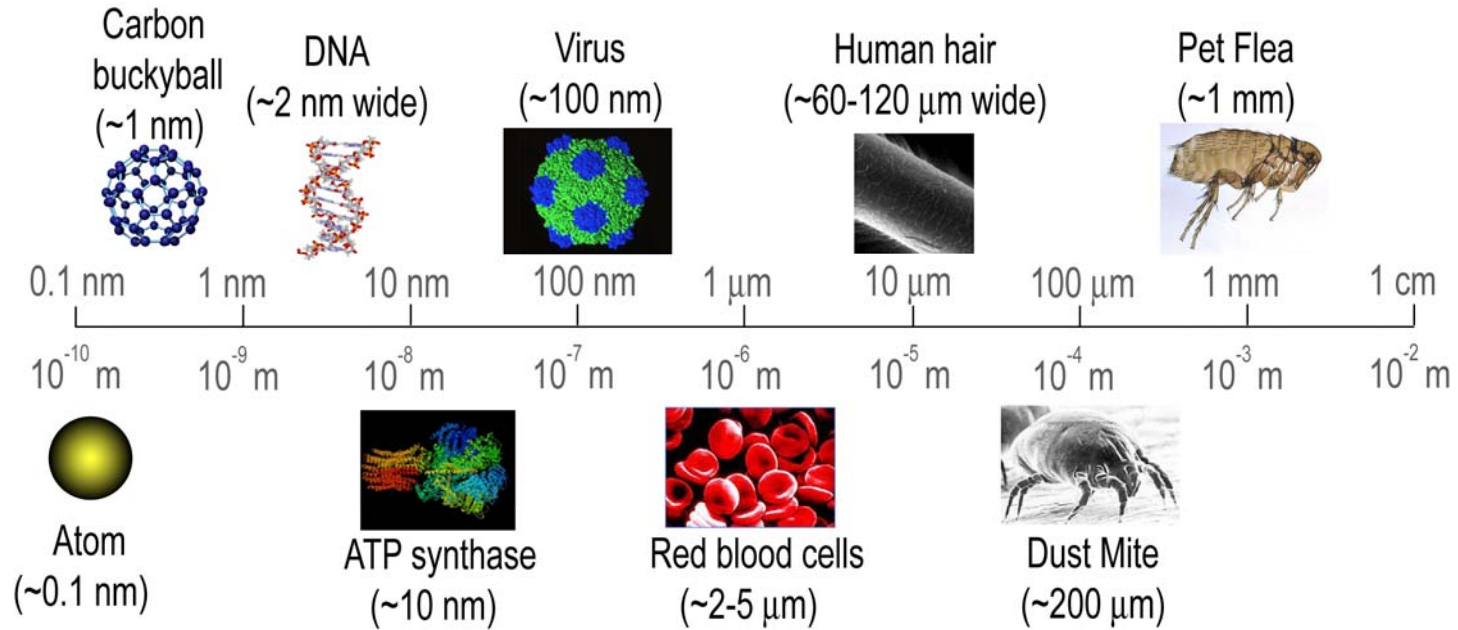
Cost-saving potential: **€ 800-1130 million**

Pedersen *et al.* (2003).

Assessment of additional testing needs under REACH.

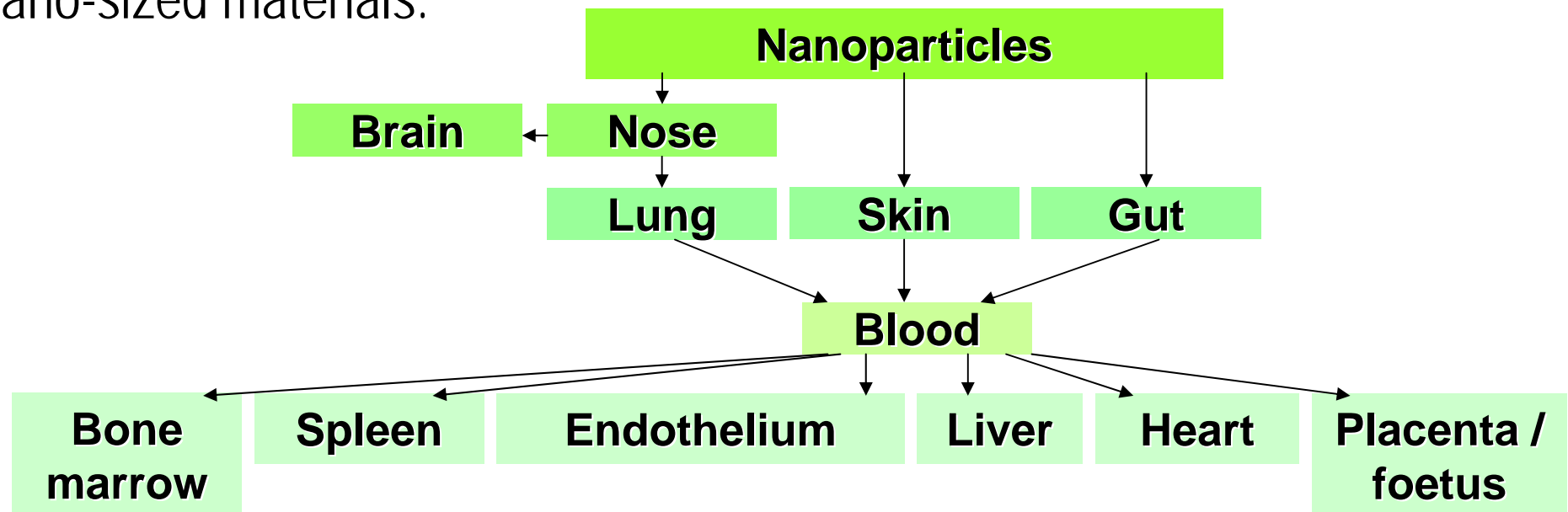
<http://ecb.jrc.it>

# Computational Nanotoxicology



## Nanotoxicology

- **Increasing importance of nanotechnology**
- **Unique risks:** adverse effects of nano-particles and materials cannot always be predicted from the known toxicity of the corresponding bulk material.
- **Limited understanding** of the potential toxicity of nano-sized materials.
- **Nanotoxicology:** addressing the special toxicity that may be associated with nano-sized materials.



**Background and Motivation**

**Why Computational Toxicity?**

**What is QSAR?**

**QSAR's Applications**

**Research Area in Thailand**

**Computational Toxicity in Dyes and Cosmetics**

**Concept proposal**

**3D-Molecular Database for substances in Dyes and Cosmetics**

# QSAR's Applications

**QSAR for Drug Design (Since 1960, C. Hansch) (Most)**

**QSPR for Materials (Inorganic, etc.)**

**QSAR for Agriculture and Toxicology (Pesticide, etc.)**

## BASIC CONCEPTS

Structure-activity relationships (SARs) and quantitative structure-activity relationships (QSARs), collectively referred to as (Q)SARs, are theoretical models that can be used to predict the physicochemical, biological and environmental fate properties of molecules.

A chemical category is a “family” of chemicals that have been grouped together because they share similar chemical structures or physicochemical properties, and are consequently considered to share similar environmental, ecotoxicological or toxicological properties.

In the read-across (analogue) approach, endpoint information for one chemical is used to make a prediction of the endpoint for another chemical, which is considered to be “similar” in some way.

# QSAR's Applications

## INFORMATION ON CHEMICALS PROVIDED BY (Q)SARs

(Q)SARs can be used to provide the following types of information which may be useful for regulatory purposes:

- 1) physicochemical properties; (**~100 calculated properties**)
- 2) (eco)toxic potential and potency;
- 3) environmental distribution and fate; and
- 4) biokinetic processes.

In the hazard and risk assessment process, (Q)SARs are generally used in combination with other sources of information on chemicals, either to prioritise chemicals for further assessment, or to replace testing.

***15 published QSARs for drug design by Hannongbua, S. et al.***


**Example: QSAR of Polyaromatic Hydrocarbons (PAHs)**

## QSAR for Materials (Inorganic)

- Inorganic cations toxicity - application of QSAR analysis. *Ind. Environ. Xenobiotics, Proc. Int. Conf.* 1981, Meeting Date 1980, 83-6.
- Enache M.; Dearden J. C.; Walker J. D. QSAR analysis of metal ion toxicity data in sunflower callus cultures (*Helianthus annuus* Sunspot). *QSAR & Combinatorial Science*, 2003, 22:234-240.
- QSAR in toxicology. III. Its use in the determination of the toxicity of inorganic cations. *Ceskoslovenska farmacie*. 1981, 30:7-10.
- Use of structure-activity relationships to estimate toxicity of inorganic cations. *Experientia. Supplementum*. 1976, 23:83-4.

Ecotoxicology and Environmental Safety **49**, 293–301 (2001)

Environmental Research, Section B

doi:10.1006/eesa.2001.2074, available online at <http://www.idealibrary.com> on  IDEAL<sup>®</sup>

# Use of the Ciliated Protozoan *Tetrahymena pyriformis* for the Assessment of Toxicity and Quantitative Structure–Activity Relationships of Xenobiotics: Comparison with the Microtox Test

P. Bogaerts,\* J. Bohatier,\*<sup>†,1</sup> and F. Bonnemoy\*

\*Laboratoire de Biologie Comparée des Protistes, UPRESA 6023, Université Blaise Pascal, 63177 Aubière Cedex, France; and <sup>†</sup>Laboratoire de Biologie Cellulaire, Faculté de Pharmacie, Université d'Auvergne, 28 Place Henri Dunant, 63001 Clermont Ferrand Cedex, France

Received October 17, 2000

## The aim of the research

- To investigate the toxicological effects of 13 inorganic and 21 organic compounds are evaluated using the *pyriformis* FDA esterase test, the conventional *pyriformis* population growth impairment assay and the luminescent inhibition test (Microtox test).
- To Study the relationships between the toxic effects of the substances tested and the ion characteristics of the metal ions or the hydrophobicity (quantified by the 1-octanol/water partition coefficient,  $\log K_{ow}$ ) of the organic compounds.

# Methods

- **Calculations and Statistical Analyses**

The relative toxicity of the tested substances was quantified by the determination of the  $IC_{50}$

For each toxicant and each assay, the concentrations were transformed into logarithms, and the  $IC_{50}$  was determined by regression analysis.

The relationships between the *Tetrahymena* 1-h  $IC_{50}$ , 9-h  $IC_{50}$ , and the Microtox 30-min  $EC_{50}$  were determined by the Spearman rank correlation coefficient ( $P < 0.05$ ).

The relationship between toxicity results and ion characteristics or lipophilicity of organic substances was tested using mean square root linear regression analysis ( $P < 0.05$ ).

Statistical analyses were computed with Stat View SE 1.03 software.

# Results

**TABLE 1**  
**Comparative Toxicity Values Using the *T. pyriformis* Enzymatic Test, the *T. pyriformis* Proliferation Rate Test, and the Microtox Test**

Compound	Chemical	<i>T. pyriformis</i>				Microtox	
		1-h IC <sub>50</sub>		9-h IC <sub>50</sub>		30-min EC <sub>50</sub>	
		mg/L	μM	mg/L	μM	mg/L	μM
1	Hg <sup>2+</sup>	0.016 <sup>a</sup>	0.080	2.00	9.97	0.11 <sup>b</sup>	0.55
2	Cd <sup>2+</sup>	0.011 <sup>a</sup>	0.098	3.00	26.69	8.30 <sup>b</sup>	73.84
3	Cu <sup>2+</sup>	0.25 <sup>a</sup>	3.93	67.60	1,063	0.43 <sup>b</sup>	6.76
4	Zn <sup>2+</sup>	0.20 <sup>a</sup>	3.06	75.40	1,153	0.30 <sup>b</sup>	4.59
5	Cr <sup>6+</sup> (as CrO <sub>3</sub> )	18.25 <sup>a</sup>	350.96	12.00	230.77	5.72	110.00
6	Cr <sup>6+</sup> (as K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> )	32.60	626.92	5.60	107.69	9.10 <sup>b</sup>	175.00
7	Mn <sup>2+</sup>	26.95 <sup>a</sup>	490.53	210.00 <sup>a</sup>	3,822	15.00	273.02
8	Fe <sup>3+</sup> (FeCl <sub>3</sub> , 6H <sub>2</sub> O)	2.50 <sup>a</sup>	44.76	> 50		28.00	501.34
9	Fe <sup>3+</sup> (FeSO <sub>4</sub> , 7H <sub>2</sub> O)	> 100		52.20	934.64	81.00	1,450
10	Pb <sup>2+</sup>	0.10 <sup>a</sup>	0.48	> 100		0.09 <sup>b</sup>	0.43
11	Co <sup>2+</sup>	2.95	50.06	41.20	699.13	16.00	271.51
12	Ni <sup>2+</sup>	2.09 <sup>a</sup>	35.60	90.00 <sup>a</sup>	1,532	25.70	437.74
13	As <sup>5+</sup>	0.47	6.27	3.90	52.05	7.94	105.98
14	Carbaryl	6.58	32.70	94.00	467.19	0.49 <sup>b</sup>	2.43
15	Malathion	5.61	16.98	32.00	96.88	109.20	330.61
16	Parathion ethyl	3.06	10.50	4.35	14.93	5.99 <sup>b</sup>	20.56
17	Parathion methyl	4.34 <sup>a</sup>	16.49	5.90 <sup>a</sup>	22.41	0.43 <sup>b</sup>	1.63
18	Lindane	8.95 <sup>a</sup>	30.77	3.50 <sup>a</sup>	12.03	11.02 <sup>b</sup>	37.89

# Results

**TABLE 2**  
**Relationship between Bioassays**

	FDA-Pop	FDA-Microtox	Pop-Microtox
Whole substances	0.64*	0.60*	0.65*
Metal ions	0.43	0.74*	0.47
Organic substances	0.77*	0.45	0.57*

\* $P < 0.05$ .

# Results

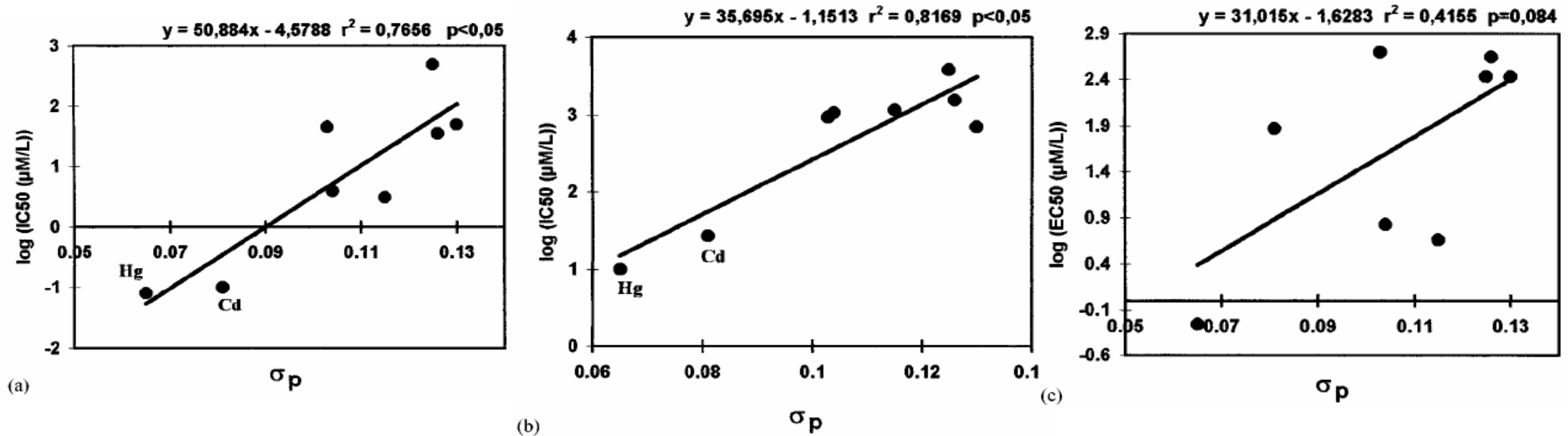


FIG. 2. Relationship between the 1-h  $\text{IC}_{50}$  *T. pyriformis* (a), 9-h  $\text{IC}_{50}$  *T. pyriformis* (b), 30-min  $\text{EC}_{50}$  Microtox values, and the softness index of tested metal ions.

# Results

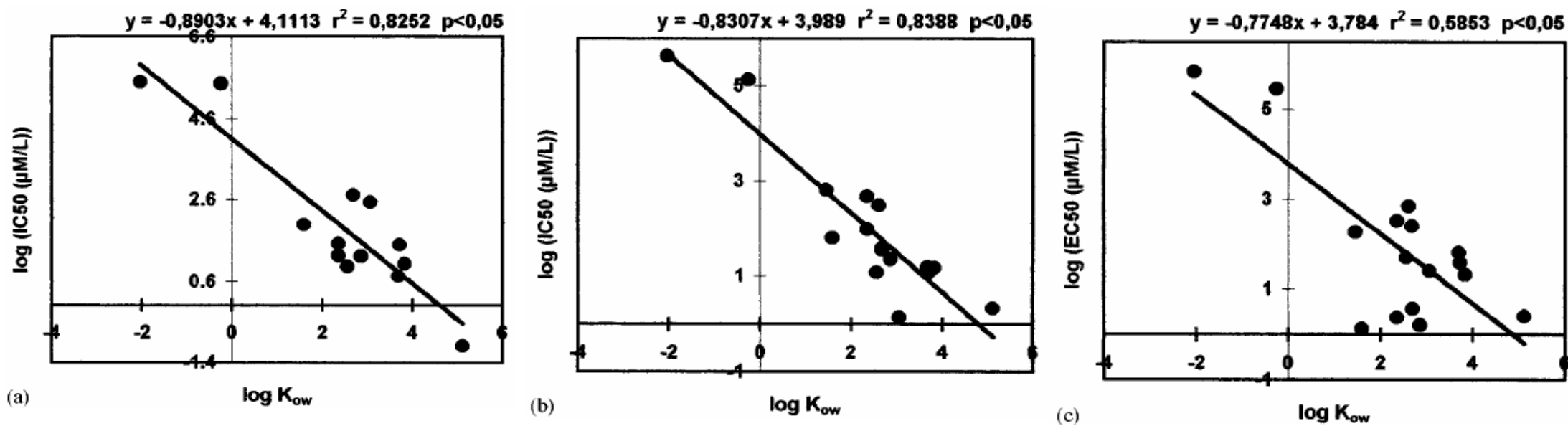


FIG. 4. Relationship between the 1-h  $IC_{50}$  *T. pyriformis* (a), 9-h  $IC_{50}$  *T. pyriformis* (b), 30-min  $EC_{50}$  Microtox values, and lipophilicity coefficient of tested organic substances.

# Conclusion

- Toxicology-based QSARs can be used for the prediction of the toxic potency of chemicals and the interpretation of mechanisms of action (Cronin and Dearden, 1995).
- The relative toxicity of metal ions and organic compounds determined with the two *Tetrahymena* biotests is predictable using two ion characteristics, the softness index  $\sigma_p$  and  $\chi_m^2 r$ , and the hydrophobicity coefficient  $\log K_{ow}$ , respectively.

Background and Motivation

Why Computational Toxicity?

What is QSAR?

QSAR's Applications

Research Area in Thailand

Compututational Toxicity in Dyes and Cosmetics

# **QSAR's application in Cosmetic**

# **Ranking of hair dye substances according to predicted sensitization potency: quantitative structure–activity relationships**

H. SØSTED<sup>1</sup>, D. A. BASKETTER<sup>2</sup>, E. ESTRADA<sup>3</sup>, J. D. JOHANSEN<sup>1</sup> AND G. Y. PATLEWICZ<sup>2</sup>

<sup>1</sup>The National Allergy Research Centre for Consumer Products, Department of Dermatology, University of Copenhagen, Gentofte Hospital, Denmark, <sup>2</sup>Safety and Environmental Assurance Centre, Unilever Colworth, Colworth House, Sharnbrook, Bedford, MK44 1LQ, UK, and <sup>3</sup>X-ray Unit (BioComputing) RIAIDT-Structural Studies Area, Edificio CACTUS, University of Santiago de Compostela, Santiago de Compostela, Spain

## The aim

Collect information on all hair dye substances used in permanent or temporary hair dyes in Europe and then to rank these substances according to their estimated potency, the perspective being to supplement the current diagnostic work-up for hair dye allergy with new potential allergens.

# Methods

- Prediction of sensitization potency

Structures identified were then imported into a molecular spreadsheet TSAR (Version 3.3, Accelrys Ltd., Cambridge, UK).

- Ranking the substances according to their sensitization potential

The TOPS-MODE QSAR model was used in order to estimate the likely sensitization potency

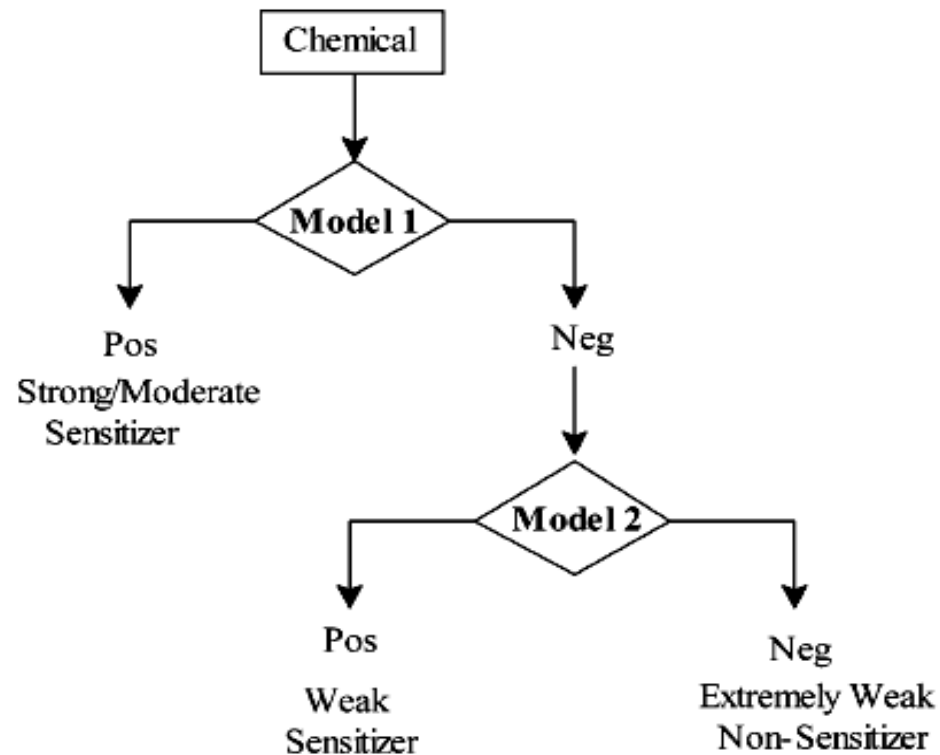
- Tonnage amount

The European Cosmetic Toiletry and Perfumery Association has generated tonnage data for use in the EU Commission for prioritizing hair dyes in risk assessment and risk management.

- Cluster analysis

The cluster analysis provided a means of grouping substances according to their chemical properties such that a representative diverse subset could be selected for further work.

# Results



**Figure 1.** Decision tree illustrating the classification scheme for models 1 and 2.

$$\begin{aligned}
 \text{class (1)} = & 1.331\mu_1^{\text{H}} - 0.00598\mu_4^{\text{H}} + 0.00781\mu_2^{\text{PS}} - \\
 & (2.1366 \times 10^{-4}\mu_3^{\text{PS}}) + 0.0755\mu_1^{\text{MR}} + 0.0319\mu_2^{\text{MR}} - \\
 & 1.1133\mu_5^{\text{Pol}} - 2.3797\mu_1^{\text{Ch}} + 0.1547\mu_3^{\text{Ch}} + 0.00425\mu_6^{\text{Ch}} + \\
 & 2.0932\mu_1^{\text{vdW}} - 0.8683\mu_2^{\text{vdW}} + 0.7954; \text{Wilks} - \lambda = \\
 & 0.61; F(12,63) = 3.39; D^2 = 2.52; p < 0.0007 \quad (6)
 \end{aligned}$$

$$\begin{aligned}
 \text{class (2)} = & 0.946\mu_1^{\text{H}} - 0.00468\mu_7^{\text{H}} - 0.894\mu_1^{\text{PS}} + \\
 & 0.1004\mu_2^{\text{PS}} - 0.0024\mu_3^{\text{PS}} + 0.0057\mu_3^{\text{Pol}} - 1.429\mu_1^{\text{Ch}} + \\
 & 0.0053\mu_8^{\text{Ch}} - 0.00111\mu_9^{\text{Ch}} - 5.309; \text{Wilks} - \lambda = \\
 & 0.38; F(9,26) = 4.63; D^2 = 8.76; p < 0.001 \quad (7)
 \end{aligned}$$

Table 2. Weak sensitizers

Number	INCI name	Case number	Predicted sensitization potency*	Tonnage amount /number used in oxidative hair-colouring products†	Tonnage amount /number used in direct hair-colouring products†	Cluster number‡
173	2,4-Diamino-5-methylphenetol HCl	113715-25-6	5.1			8
174	2-Amino-4-hydroxyethyl-aminoanisoie	83763-47-7	6.6	5		5
175	4,5-Diamino-1-methylpyrazole HCl		0.3			8
176	4-Methoxytoluene-2,5-diamine HCl		3.9			8
177	Acid Blue 1	129-17-9	36.4			1
178	Acid Blue 9	2650-18-2	61.7			9
179	Acid Green 25	4403-90-1	43.5			1
180	Acid Green 50	3087-16-9	41.3			1
181	Acid Red 51	16423-68-0	11.3			10
182	Acid Red 52	3520-42-1	32.8			1
183	Acid Violet 9	6252-76-2	37.5			1
184	Basic Blue 26 (CI 44045)	2580-56-5	18.4			7
185	Basic Blue 6	966-62-1	6.8			4
186	Basic Blue 7	2390-60-5	15.9	0.1	1	7
187	Basic Blue 9	61-73-4	6.9	0.1	0.1	4
188	Basic Brown 4	4482-25-1	19.7			8
189	Basic Green 1	633-03-4	15.1			4
190	Basic Red 2	477-73-6	19.1	1	0.1	4

*Table 4.* Literature overview of human evidence and local lymph node assay (LLNA) data on contact sensitization and hair dyes

Number	INCI name	LLNA	Human evidence based on patch-test data
<b>Strong or moderate sensitizers</b>			
16	2,4-Diaminophenol		1 case clinical relevant hair dye reaction (1).
25	2,7-Naphthalenediol		1 case clinical relevant hair dye reaction (64).
31	2-Aminomethyl- <i>p</i> -aminophenol HCL		1 case clinical relevant hair dye reaction (65).
34	2-Chloro- <i>p</i> -phenylenediamine		1 case clinical relevant eyelashes eyebrows dye reaction (35).
41	2-Nitro- <i>p</i> -phenylenediamine		1 case clinical relevant (66). 1 case clinical relevant eyelashes eyebrows dye reaction (35). A dentist has reactions on dyed hair from dental patients' hair (67). 2 hairdressers (68). 2 persons clinical relevant hair dye reactions (3). 4 hairdressers out of 103 tested (45). 5 cases clinical relevant hair dye reactions (1). 6 hairdressers out of 40 (9). 12 hairdressers' clients (5). 24 hairdressers (6). 93 patients out of 5202 (7).
55	4-Amino-3-nitrophenol		1 case clinical relevant hair dye reaction (10).
93	Basic Blue 99		1 case clinical relevant hair dye reaction (10,11).
109	Disperse Brown 1		8 patients in a trial (30).
110	Disperse Orange 3		1 case clinical relevant hair dye reaction (1,48,69). 3 persons clinical relevant hair dye reactions (3). 29 patients in a trial (30).
132	Hydroquinone	Positive (51–53,56,70,71)	1 case clinical relevant eyelashes eyebrows dye reaction (72).
139	<i>m</i> -Aminophenol	Positive (23,50-53,56)	2 cases of clinical relevant hair dye reactions at hair dressers' clients (34). 3 cases clinical relevant hair dye reactions (1).

## Conclusions

- The sensitization potential of each substance was then estimated by using a quantitative structure–activity relationship (QSAR) model and the substances were ranked according to their predicted potency.
- A cluster analysis by using TOPS-MODE descriptors as inputs helped us group the hair dye substances according to their chemical similarity. This would facilitate the selection of potential substances for clinical patch testing and would provide some clinical validation of the QSAR predictions.

# **QSAR's application in Dyes**



# Mutagenicity of aminoazobenzene dyes and related structures: a QSAR/QPAR investigation

Ashish Garg, Krishna L. Bhat, Charles W. Bock\*

*Department of Chemistry and Biochemistry, School of Science and Health and School of Textiles and Materials Technology, Philadelphia University, School House Lane and Henry Avenue, Philadelphia, PA 19144, USA*

Received 17 May 2002; received in revised form 10 June 2002; accepted 27 June 2002

---

## Abstract

Quantitative structure–activity/property–activity relationships (QSAR/QPARs) are developed that correlate the observed mutagenic activity of 43 aminoazobenzene derivatives with a variety of molecular descriptors calculated using quantum-chemical semiempirical methodology. Models based on multilinear regression techniques and artificial neural networks are presented that account for more than 80% of the variation in the reported relative mutagenicity of these compounds.

© 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** Aminoazobenzene dyes; Structure–activity relationships; Mutagenicity; Artificial neural networks; CODESSA

## The purpose of this paper

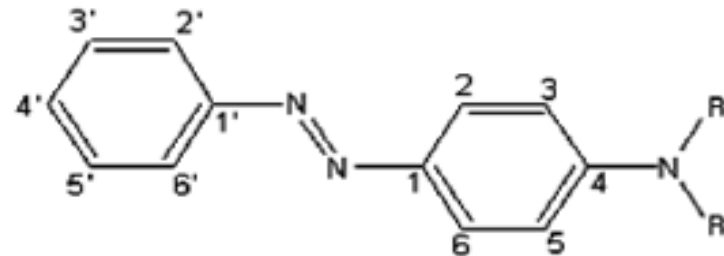
Derive quantitative structure-activity/property-activity relationships (QSAR/QPARs) for the mutagenicity (rev/nmol) of a variety of 4-aminoazobenzene (AAB), N-methyl-4-aminoazobenzene (MAB) and N,N-dimethyl-4-aminoazobenzene (DAB) derivatives in the *S. typhimurium* TA98 bacterial strain with S9 activation (TA98+S9); this particular bacterial strain is well known to detect frameshift mutagens.

# Methods

- The structures optimized at the semiempirical AM1 computational level as implemented in AMPAC 5.0.
- The CODESSA/AMPAC integrated software package was used to calculate hundreds (>300) of molecular descriptors.
- The entire collection of descriptors was then used in conjunction with the statistical facilities of CODESSA to develop multilinear regression models for the log of the measured mutagenicity (rev/nmol) in TA98+S9, logTA98.

# Results

**Table 1**  
Observed mutagenicity (rev/nmol) in the TA98 *Salmonella typhimurium* bacterial strain with S9 activation, calculated values [39] of logP and logS and melting points (°C), for derivatives of (A) AAB, (B) MAB, (C) DAB, and for (D) their metabolites



Compounds	Mutagenic activity in TA98 + S9 (rev/nmol) [Ref.]	LogP <sup>a</sup>	LogS <sup>b</sup>	Melting point (°C) [Ref.]
(A) AAB (R = R' = H)				
4'-NEt <sub>2</sub> -3-OMe-AAB	0.007 [42]	5.16	-3.41(-3.77)	147-149 [43]
2-OMe-AAB	0.010 [44]	3.87	-1.85(-2.43)	157-159 [3]
4'-OH-AAB	0.053 [45]	2.55	-0.66(-1.34)	180-181 [46]
3'-Me-4'-OH-AAB	0.059 [4]	3.01	-1.14	°
4'-OH-2',3-diMe-AAB (4'-OH-OAT)	0.112 [47]	3.47	-1.62	°
AAB	0.204 [42]	3.13	-1.05(-1.39)	124-125 [48]
3'-Me-AAB	0.240 [4]	3.59	-1.52(-1.57)	89-91 [46]

# Results

Table 2  
Quantitative structure–activity/structure–property relationships for the mutagenicity of the aminoazobenzene derivatives in Table 1<sup>a</sup>

QSAR/QPAR	<i>N</i>	<i>R</i> <sup>2</sup>	<i>s</i> <sup>2</sup>	<i>F</i>
<i>(A) BMLR equations</i>				
$\log \text{TA98} = 261.03(\pm 55.35) + 842.33(\pm 151.21)Q_1 - 1.06(\pm 0.21)Q_2 + 0.01(\pm 0.00)E_1$	3	0.66	0.28	25.22
$\log \text{TA98} = -2.03(\pm 1.41) - 4.23(\pm 0.60)E_2 + 22.54(\pm 3.05)Q_3 + 701.94(\pm 104.01)Q_1 - 0.40(\pm 0.07)H_1$	4	0.79	0.18	34.56
$\log \text{TA98} = -60.51(\pm 41.61) - 4.22(\pm 0.52)E_2 - 0.46(\pm 0.07)Q_4 + 573.29(\pm 95.55)Q_1 - 0.41(\pm 0.06)H_1 + 75.01(\pm 19.34)Q_5$	5	0.85	0.13	40.41
<i>(B) Heuristic equation</i>				
$\log \text{TA98} = 6.02(\pm 1.76) + 20.94(\pm 3.07)Q_3 - 7.55(\pm 1.52)G_1 - 0.29(\pm 0.09)O_1 - 3.81(\pm 0.66)E_2 + 436.31(\pm 90.05)Q_1$	5	0.80	0.18	28.96

<sup>a</sup> The molecular descriptors are identified in Table 3.

$G_1$  XY Shadow/XY Rectangle

$E_1$  WPSA-2 Surface weighted partial positive surface area

$E_2$  Polarity parameter/(distance)<sup>2</sup> = (Q<sub>max</sub>Q<sub>min</sub>)/(distance)<sup>2</sup>

$Q_1$  Average electrophilic reactivity index for a N atom

$Q_2$  Maximum electron-nuclear attraction for a C–C bond

$Q_3$  Minimum net atomic charge for a C atom

$Q_4$  Maximum electron-nuclear attraction for a C atom

$Q_5$  Maximum bond order of an N atom

$H_1$  Final heat of formation/number of atoms

$O_1$  LogP

# Results

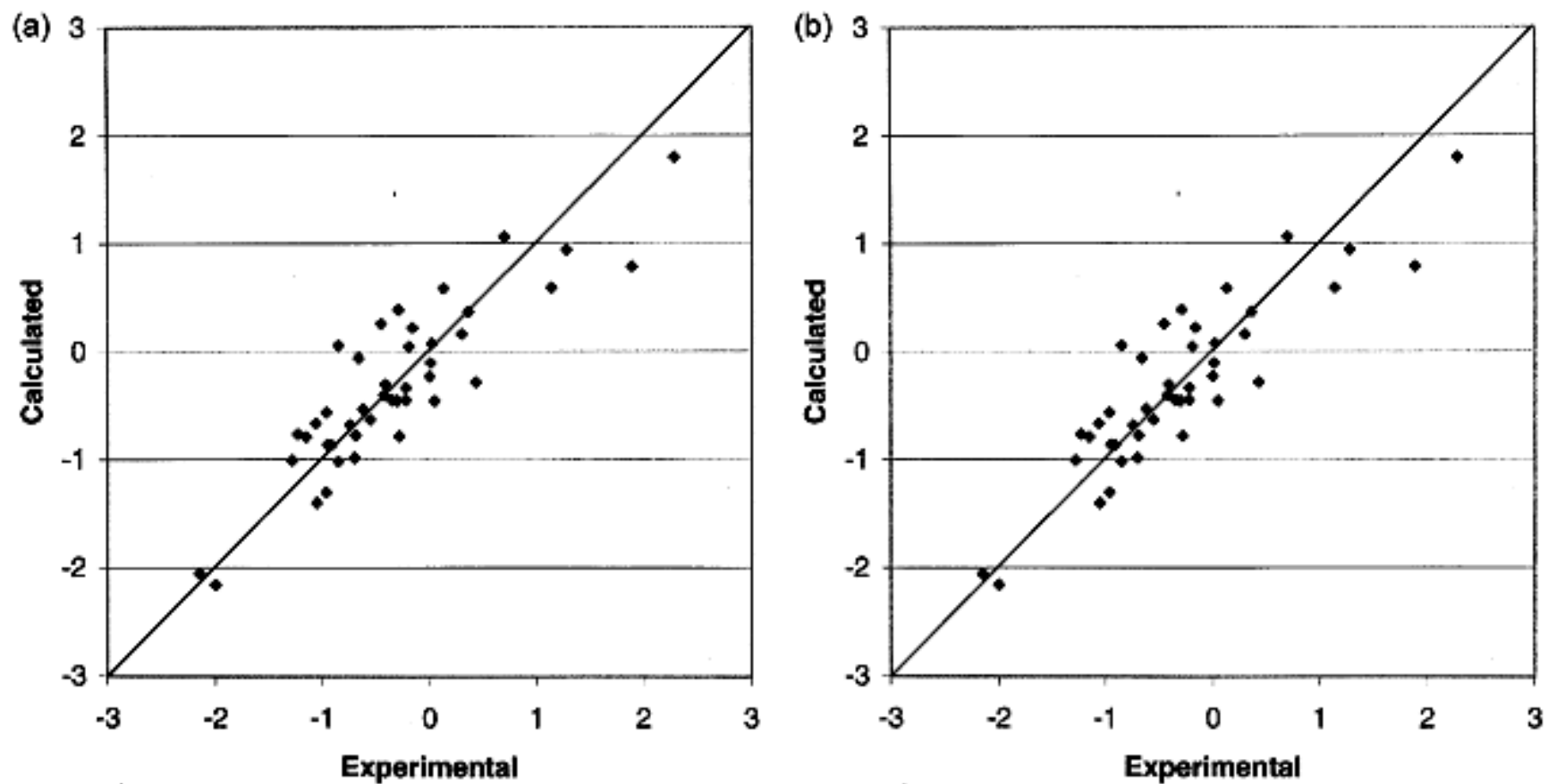


Fig. 2. Correlation Plots for the (A) 4-descriptor and (B) 5-descriptor BMLR equations in Table 2.

# Results

Table 4

Observed values of logTA98 and predicted values of logTA98 from the BMLR and heuristic QSAR/QPAR equations in Table 2 for the 43 compounds in Table 1

Compounds	Observed values of logTA98	Predicted values of logTA98			
		BMLR			Heuristic
		3-Descriptor	4-Descriptor	5-Descriptor	5-Descriptor
<i>(A) AAB</i>					
4'-NEt <sub>2</sub> -3-OMe-AAB	-2.15	-1.93	-2.06	-2.11	-2.32
2-OMe-AAB	-2.00	-1.41	-2.16	-2.17	-1.59
4'-OH-AAB	-1.28	-1.01	-1.00	-1.24	-1.03
3'-Me-4'-OH-AAB	-1.23	-0.93	-0.76	-0.96	-0.80
4'-OH-2',3-diMe-AAB (4'-OH-OAT)	-0.95	-0.26	-0.86	-0.87	-1.19
AAB	-0.69	-0.75	-0.78	-0.85	-0.41
3'-Me-AAB	-0.62	-0.61	-0.53	-0.55	-0.24
3-OMe-4'-N(CH <sub>2</sub> CH <sub>2</sub> OH) <sub>2</sub> -AAB	-0.41	-0.96	-0.31	-0.37	-0.36
3'-CH <sub>2</sub> OH-AAB	-0.22	-0.38	-0.34	-0.44	-0.08

# Conclusions

- Developing QSAR/QPARs that correlate the relative mutagenic activity of aminoazobenzene derivatives with various molecular descriptors can help identify factors that alter their relative mutagenicity.
- QSAR/QPAR studies can be useful in establishing biochemical mechanisms/interactions, and in developing combinatorial strategies for the synthesis of environmentally safe chemicals. Such studies involving aminoazobenzene derivatives are particularly important because of their widespread use in the textile industry.

Thank you for your attention