

RIVM report 601516 008

**The application of structure-activity relationships
in human hazard assessment: a first approach**

EM Hulzebos, PAH Janssen, L Maślankiewicz
MCM Meijerink, JJA Muller, SMG Pelgrom, L
Verdam, TG Vermeire

March 2001

This investigation has been performed by order and for the account of Direction of RIVM, within the framework of project 601516, Risk assessment of Substances: Science and Market

Abstract

In this report an overview is given of structure-activity relationships (SARs) described in literature that can be helpful for the daily human hazard evaluation of chemicals. SARs describe the relation between molecular structure and biological- or physical-chemical activity of the chemical. Chemicals that share structural features are presented that can have an effect on the toxicological endpoints: irritation, sensitisation, neurotoxicity (acetylcholinesterase-inhibition), genotoxicity and reproductive and developmental. These SARs were discussed in a Workshop. The results of this Workshop, which was attended by RIVM and TNO risk assessors and experts, are presented also. One of the results is a list of structural features with possible effects on the above mentioned toxicological endpoints. It is proposed to test these SARs in the next two years on chemicals for which experimental data are available. This report may help to increase the quality of the hazard assessment.

Contents

Summary 7

Samenvatting 9

Introduction 11

Part 1 SARs for human hazard assessment 13

1 Skin irritation 13

1.1 Introduction 13

1.2 Mechanism of action 13

1.3 Structure-activity relationships 14

1.3.1 Software programmes 14

1.3.2 Classes of chemicals that share structural features 14

1.4 Evaluation and strategy 15

2 Sensitisation 17

2.1 Introduction 17

2.2 Mechanism of action 17

2.3 Structure activity relationships 18

2.3.1 Software programmes 18

2.3.2 Classes of chemicals that share structural features 19

2.4 Evaluation and strategy 19

3 Neurotoxicity: SARs for acetylcholinesterase-inhibition 21

3.1 Introduction 21

3.2 Mechanism of action 21

3.3 Structure-activity relationships 22

3.3.1 Software programmes 22

3.3.2 Classes of chemicals that share structural features 22

3.4 Evaluation and strategy 23

4 Genotoxicity and carcinogenicity 25

4.1 Introduction 25

4.2 Mechanism of action 25

4.3 Structure-activity relationships 25

4.3.1 Software programmes 25

4.3.2 Classes of chemicals that share structural features 26

4.4 Evaluation and strategy 27

5 Reproductive and developmental toxicity 29

5.1 Introduction 29

5.2 Mechanism of action 29

5.3 Structure-activity relationships 29

5.3.1 Software programmes 29

5.3.2 Classes of chemicals that share structural features 31

5.4	Evaluation and strategy	35
6	<i>Conclusions of the Workshop</i>	37
	<i>References</i>	39
	<i>Part 2 Classes of chemicals that share structural features of chemicals</i>	43
1.	Skin Irritation	45
2.	Skin sensitisation	49
3.	Neurotoxicity by acetylcholinesterase inhibition	51
4.	Genotoxicity and carcinogenicity	53
5.	Reproductive toxicity and developmental toxicity	55
	<i>Appendix I Participants on the Workshop: SARs in human toxicology</i>	67
	<i>Appendix II Mailing list</i>	69

Summary

The goal of this report is to present human toxicological structure-activity relationships (SARs) available in literature that are considered useful for the daily practice of hazard assessment of chemicals.

The present report consists of two parts. In the first part structure-activity relationships (SARs) for five human toxicological endpoints are described:

- *Irritation.* For this toxicological endpoint SAR-features that describe reactivity and skin penetration are the most important ones. Reactive chemicals, strong acids and cationic surfactants such as quaternary ammonium compounds (N^+) are corrosive or irritant by destroying cells immediately. Other (less) reactive chemicals such as organic acids, bases (amines), aldehydes, halogenated alkanes, alkenes and esters need to penetrate the skin first and can be cytotoxic for the cells below the upper layer of the skin.
- *Sensitisation.* Several classes of chemicals were derived from MultiCASE that share structural features and are suspected of sensitising potential. These classes include amines, aldehydes, halogenated alkanes and thioles. Software programmes such as MultiCASE and DEREK were reviewed and show a high percentage correctly predicted substances for positives and negatives (80-90% after internal validation by the authors). However, no external validation results are published.
- *Neurotoxicity.* Two classes of acetylcholinesterase-inhibitors, organophosphates and carbamates are described. Organophosphate esters with two small groups (methyl, ethyl or propyl) are expected to be acetylcholinesterase inhibitors. For the carbamates a CH_3 group connected to the N-atom of the carbamate-site (so not the ester site) is required to show this inhibition.
- *Genotoxicity/carcinogenicity.* Genotoxic structures are presented in the so-called 'supermutagen', which is an artificial chemical structure that includes all potentially genotoxic groups in the Ames Salmonella test and in chromosome aberration tests. Also toxicological information is included that is needed to predict carcinogenicity. Based on this information the percentage correct predictions were over 80% for both genotoxic and non-genotoxic carcinogens, tested in the NTP (National Toxicology Program of the US). This type of prediction was much better than from software programmes, which predicted carcinogenicity correctly for about 50%. Predictions by the US-EPA for genotoxicity, positives as well as negatives, based on the 'supermutagen', were over 80% correctly when they used test information from new chemicals in Europe.
- *Reproductive- and developmental toxicity.* Several groups of chemicals are presented that share structural features that may cause reproductive - and/or developmental toxicity. These groups are phthalates, ethylene glycolethers, carboxyl acids, sulphonamids, and others. Domains of some of these groups could be roughly established: for example for ethylene glycolethers, the methyl and ethyl derivatives are the most potent.

In a Workshop on these SARs, the main conclusion was that SARs, for the time being, should be used only for positive indication. This means that the absence of SAR indication does not exclude toxic properties. In addition, gaining experience with the use of SARs in hazard assessment of chemicals and continuation of the use of SARs is recommended.

In Part 2 a list of SARs is presented on the toxicological endpoints mentioned above. This list needs further investigation. It is proposed to test this list of structural features on chemicals for which test data are available.

Samenvatting

Het doel van dit rapport is om humaan-toxicologische structuur-activiteitsrelaties (SAR's) te presenteren die beschikbaar zijn in de literatuur en die gebruikt kunnen worden in de dagelijkse effect beoordeling van stoffen.

Het huidige rapport bestaat uit 2 delen. In het eerste deel worden de SAR's voor vijf humaan toxicologische eindpunten beschreven:

- *Irritatie*. Voor dit toxicologische eindpunt zijn SAR eigenschappen als reactiviteit en huid permeabiliteit de meest belangrijke. Reactieve stoffen zoals sterke zuren en cationische surfactanten zoals quaternair ammonium verbindingen (N^+) zijn corrosief of irritant door hun directe werking. Andere (minder) reactieve stoffen zoals organische zuren en basen (amines), aldehyden, gehalogeneerde alkanen, alkenen en esters moeten eerst door de huid heen dringen en kunnen dan cytotoxisch zijn voor de cellen onder de opperhuid.
- *Sensibilisatie*. Verschillende klassen van stoffen zijn afgeleid van MultiCASE en DEREK, die structurelementen gemeenschappelijk hebben en sensibiliserende eigenschappen hebben, zoals amines, aldehyden, gehalogeneerde alkanen en thiolen. Software programma's zoals MultiCASE en DEREK zijn geëvalueerd en voorspellen positieve zowel als negatieve sensibiliserende stoffen na interne validatie (80-90%). Externe validaties zijn nauwelijks gepubliceerd.
- *Neurotoxiciteit*. Twee klassen van acetylcholinesterase remmers, organofosfaten en carbamaten zijn beschreven. Organofosfaat-esters met twee kleine groepen (methyl, ethyl of propyl) zullen acetylcholinesterase remming vertonen. Voor de carbamaten een CH_3 -groep verbonden aan het N-atoom van de carbamaat-zijde (dus niet de ester zijde) is nodig om de remming te laten zien.
- *Genotoxiciteit/Carcinogeniteit*. Genotoxische structuren zijn gepresenteerd in de zogenoemde 'supermutagen', een kunstmatige structuur die alle potentiële genotoxische structuren bevat die positief scoren in de Ames Salmonella test en/of chromosoomaberratietest. Ook de toxicologische informatie is beschreven die nodig is om carcinogeniteit te voorspellen. Gebaseerd op genoemde informatie was het percentage correcte voorspelling meer dan 80% voor zowel genotoxische als niet genotoxische carcinogenen, die getest waren in het Nationale Toxicologische Programma van de VS. Dit type voorspelling was beter dan van de meeste software programma's, die zowel positieve als negatieve carcinogenen met circa 50% voorspelden. Voorspellingen door de US-EPA voor genotoxiciteit, met behulp van 'de supermutagen' waren meer dan 80% correct voorspeld gebruik makend van de testresultaten voor genotoxiciteit, aangeleverd in Europa voor Nieuwe Stoffen.
- *Reproductie- en ontwikkelings toxiciteit*. Verschillende groepen van stoffen met gemeenschappelijke structurelementen die mogelijk reproductie- en of ontwikkelings effecten hebben, zoals ftalaten, ethyleenglycolethers, carboxylzuren, sulfonamides en andere, zijn gepresenteerd. Van sommigen van deze groepen kon een indicatie aangegeven worden van de domeinen waarin deze stoffen werkzaam zijn: bijvoorbeeld van de ethyleenglycolethers zijn de ethyl en methylderivaten de meest potente.

In een Workshop over deze SAR's is geconcludeerd dat SAR's voorlopig alleen gebruikt kunnen worden voor positieve indicatie. Dit betekent dat de afwezigheid van SAR's toxische effecten niet uit sluit. Tevens is aanbevolen dat het gebruik van SAR's in effectbeoordeling van stoffen

In Deel 2 wordt een lijst van SAR's gepresenteerd gebaseerd op de toxicologische informatie hierboven genoemd. Voorgesteld wordt om deze lijst met structurelementen te testen met experimentele data.

Introduction

SARs are correlations between the molecular structure and biological or physico-chemical activity of chemicals. When these relations can be quantified they are called QSARs (quantitative structure-activity relationships). In ecotoxicology QSARs have been developed for a number of endpoints such as aquatic toxicity, biodegradation and bioconcentration (Nendza, 1998). However, in human toxicology the relations between physico-chemical properties, chemical structures and toxicological effects have been less developed and almost not quantified.

The use of SARs may be helpful for indicating possible health effects of chemicals. SARs may be valuable when little or insufficient data are available such as the non-assessed chemicals (Sijm et al. 1999) and new chemicals, but may also be relevant for other chemicals such as existing chemicals (TGD, 1996) and pesticides.

In 1997 a project was started in order to investigate the possible use of SARs in human hazard assessment and to gain insight in the potential use and limitations of (Q)SARs. The results were presented in Hulzebos et al. (1999). In the latter report an introduction to SARs in human toxicology is given and it was concluded that SARs found in literature are potentially useful.

The goal of this report is to present human toxicological structure-activity relationships (SARs) available in literature that are considered useful for the daily practice of hazard assessment of chemicals.

Risk assessors were invited to explore in detail SARs from scientific literature for a certain toxicological endpoint. Based on the report of Hulzebos et al. (1999) the endpoints explored were irritation, sensitisation, neurotoxicity, genotoxicity/carcinogenicity and reproductive – and developmental toxicity. Experts on these endpoints available at RIVM were invited to discuss the use of SARs found by the risk assessors at monthly meetings.

In Bilthoven at the RIVM on June 15, 2000, a Workshop entitled 'Structure-activity relations in Human Toxicology' was organised. These SARs were discussed with a broader group of toxicologists from RIVM and TNO.

The present report consists of two parts:

In Part 1, the SARs of five different toxicological endpoints are presented. In the final Chapter the conclusions of the Workshop are given.

In Part 2 a list of SARs is presented on the toxicological endpoints mentioned above. This list needs further investigation. It is proposed to test this list of structural features on chemicals for which test data are available.

Part 1 SARs for human hazard assessment

1 Skin irritation

1.1 Introduction

To evaluate the skin irritation potential standard tests according to OECD 404 (OECD, 1992) are performed. This Chapter will focus on skin irritation and corrosive properties of chemicals. The mechanism for irritation is explained in 1.2. Some physico-chemical properties and chemicals that share structural features are given to elucidate the use of SARs. The more specific SARs in literature for eye irritation, however, cannot be used directly for evaluation as for reactivity mainly dipole moments are used that are not available from the dossiers submitted. Respiratory irritation is not addressed nor is irritation in the intestinal tract. However it is expected that irritant chemicals will show effects when exposed via the inhalation or oral route, respectively. For corrosive chemicals concentrations via the inhalation and oral route need to be lowered to be able to distinguish between local and systemic effects.

Corrosive chemicals

According to OECD 404 (OECD, 1992), an irritation test need not be performed if the chemical is expected to be corrosive, that is when a chemical has a $\text{pH} < 2$ or $\text{pH} > 11.5$. Alternatively, an *in-vitro* test can be submitted if it is expected that the substance has corrosive properties. Based on a positive effect in an *in-vitro* test, the chemical can be classified as corrosive. In that case, neither a skin nor an eye irritation test is necessary. SARs can be used to predict the corrosive properties of chemicals and skin irritation tests can be restricted.

Doubtful test results

Sometimes test results are described with little detail or show inconclusive results. SARs can be useful to support the hazard assessment and classification and labelling, or used to support a decision for further testing.

1.2 Mechanism of action

The mechanism of irritation involves the exposure of the chemical to the skin and the cytotoxicity of the chemical for the cells at the exposure site. There are two possible ways (Barratt et al., 1995, 1998):

- 1) The chemical causes an immediate effect (e.g. strong acids or bases) and destroys the upper layer of the skin. For example, NaOH is very reactive with water and will therefore cause a local effect.
- 2) The first stage represents initial exposure, absorption of the chemical through lipid membranes and aqueous cellular compartments to the site of metabolism or receptor interaction and then shows reactivity. Properties important for skin permeability are:
 - molecular size (descriptors: molecular weight, molecular volume, refraction)
 - lipophilicity (descriptors: log Kow, solubility in water)
 - ionisation (descriptor: pKa/pKb)

However, it is not always possible to make a clear distinction between these two mechanisms as described below. Barratt (1995), Barratt et al. (1995, 1996, 1998) gave examples of the above two mechanisms. The SARs used by Barratt et al. (1995, 1996) for the analysis of skin corrosivity of organic acids, bases and phenols to rabbit skin involve knowledge about log Kow, molecular volume, melting points (descriptors for skin permeability) and pKa/pKb (descriptor for reactivity/electronic effects, for example strong H donors). The pKa/pKb descriptor has positive and negative effects on corrosivity. Ionisation lowers skin permeability and therefore dermal availability will be lower. However, strong H donors increase reactivity and will cause a high cytotoxic effect. Chemicals with lower log Kow values

and larger molecular volumes as well as chemicals with higher skin permeability and lower solubility are less likely to be found corrosive, unless they are particularly acidic or basic. Properties relating to skin permeability generally dominate over those, which determine reactivity. For example, short-chain aliphatic carboxylic acids (for example, hexanoic acid), which are regarded as relatively weak acids, are corrosive apparently by virtue of their relatively high skin permeabilities (Barratt et al, 1995).

1.3 Structure-activity relationships

1.3.1 Software programmes

No software programmes were tested.

1.3.2 Classes of chemicals that share structural features

Barratt et al. (1995, 1998) described several general chemicals that share structural features, which are illustrated below. Examples of these classes are given in Part 2 of this report.

For chemicals that share structural features described below the reactivity and skin permeability potential cannot always be distinguished.

- 1) Strong (in) organic acids, bases and oxidising agents are expected to show reaction 1. They have low skin permeabilities by virtue of their high polarities (electronic effects). They are possibly corrosive, because they are able to erode the stratum corneum and cause cytotoxicity to the cells under the stratum corneum. Often these type of chemicals will have either low pH (acids, < pH2) or high pH (bases, pH > 11.5) values in water.
- 2) Organic acids, bases, phenols, oxidising agents/electrophiles are expected to show reaction 2. The corrosivity of organics is postulated to result from the chemical first penetrating the stratum corneum and then killing the cells underneath. According to Barratt (1998) fatty acids with alkyl chains up to and including C8 for rat skin *in-vitro* and rabbit *in-vivo* are corrosive. For human skin this alkyl chains of C6 are corrosive. Fatty acids with longer alkyl chains will not be corrosive for rat, rabbit or human skin, but may be irritants.

Organic acids can be divided in:

- R-COOH
- Phenols including electron withdrawing groups such as halogen > nitro
- Acrylic acids, CH₂=CH-COOR (Greim et al., 1998)
R = H
R = C_nH_{2n+1} (after hydrolysis of the ester)
- Methacrylic acid, CH₂=C(CH₃)-COOR
R = H
R = C_nH_{2n+1} (after hydrolysis of the ester)

Methylacrylate and ethylacrylate probably also have irritating properties because of the activity of the double bond with SH groups of the mucous membranes.

No further criteria can yet be given on the size of the R groups but it is supposed they can be fairly large.

Organic bases are often amines. Greim et al. (1998) investigated the irritation potential of the aliphatic amines:

- Primary aliphatic amines, R-NH₂ (corrosive)
- Secondary aliphatic amines: R₁-NH-R₂ (corrosive)
- Tertiary aliphatic amines: R₁-NR₂-R₃ (when two alkyl chains are at least > butyl, no eye irritation is expected).

No further criteria can yet be given on the size of the R groups but it is supposed they can be fairly large.

Organic oxidising agents/electrophiles (Barratt et al, 1995, 1998):

Oxidising agents/electrophiles are those chemicals that take up electrons from their surroundings.

- Aromatic amines, the more electron withdrawing groups on the benzene-ring the more oxidising it is, such as halogen > nitro as electron withdrawing groups
 - Aromatic nitro compounds
 - Sulfonamides: R1-SO₂-NH-R2
 - Sulfones: R1-SO₂-R2
- No further criteria can yet be given on the size of the R groups but it is supposed they can be fairly large.
- Aldehydes: $\begin{array}{c} \text{H}-\text{C}=\text{O} \\ | \\ \text{R} \end{array}$
 - Halogenated alkanes,
Halogenated esters
 - Halogenated alkenes.

- 3) Anionic and cationic surfactants have low skin permeabilities due to charged head groups and long molecular volumes. As a category, anionic and non-ionic surfactants appear to be non corrosive. Cationic surfactants are stronger surfactants than anionics and therefore the cationic surfactants appear to be more cytotoxic. Corrosivity may result from solubilisation of the stratum corneum. Cationic surfactants are very often quaternary ammonium salts (Barratt, et al., 1995)
- N⁺- R_{1,2,3} (with at least one R-group being small, methyl or ethyl). Salts of quaternary ammonium compounds are likely to be less corrosive as the positive charge of the nitrogen-atom (causing the reactivity) is more neutralised.

4) Neutral non-electrophilic organics

Special groups of neutral organics that need special attention are the organic solvents. Organic solvents cause defatting of the human skin, This was discussed in a working group of the ECB (ECBI/22/96, the ad hoc Working group on defatting chemicals, meeting at the Chemicals Inspectorate, Solna, 15 May, 1997). In human skin they extract the intercellular lipids from the skin leading to loss of barrier properties and water retention of the skin, which is assumed to be the mechanism behind these adverse effects.

SARs for skin permeability

Chemicals showing reaction 2 will show cytotoxicity if the chemical can permeate the skin. The SARs from Barratt (1995) and Vermeire et al. (1993), indicate that permeability can be considered minor if log Kow < -1 and > 6 or if the molecular weight is > 700. Chemicals with negligible permeability will therefore not show irritant effects. For corrosive properties, DeVito (2000) restrict these boundaries even more to log Kow values between 1 and 2, whereas the molecular weight should be below 200.

1.4 Evaluation and strategyIrritant chemicals:

Irritant chemicals will have reactive properties and/or skin permeability will be substantial. Reactivity is expected if:

- local effects in the acute dermal test up to 2000 mg/kg bw are observed and
- physico-chemical properties indicating irritancy and
- structural features that indicate irritation, are available

Not every reactive chemical may be included in the list of chemicals that show structural features for irritancy. Defatting chemicals are not included, as they do not show the above described features.

Therefore, in addition to the reactivity properties, SARs on skin permeability can estimate absorption potential on the basis of with log Kow and molecular weight. Absorption can be considered considerable:

- if log Kow > -1 and < 6 or
- if molecular weight is < 700 (Vermeire et al., 1993, DeVito, 2000).

Based on reactivity and skin permeability, irritation effects can be expected.

Corrosive chemicals

Chemicals that show structural features for irritancy have a high reactivity, so that corrosivity or irritation can be expected as given in 1.3. In addition, the boundaries given by DeVito (2000) for high skin permeability (log Kow between 1-2) can be kept in mind but testing of this assumption will still be necessary.

Defatting chemicals

This group of chemicals will not have reactive structural features, but will be assessed in view of their skin permeability potential.

Doubtful tests skin irritation test: classification for irritation or not

The results of the skin irritation test may be poorly described and the irritation potential cannot be evaluated properly. Also the test animals may not be observed long enough for determining the reversibility of the effect when the observation period was < 14 days.

The arguments used for non-irritant chemicals can also be used to decide if a new test needs to be requested or that the substance need or need not be classified based on SARs.

2 Sensitisation

2.1 Introduction

The assessment of the sensitising potential of substances is based on studies with the substance or mixture of substances concerned. Sometimes these studies are lacking or the quality of the studies is not sufficient to make a decision about sensitisation. In some of these cases the assessment of the sensitising potential can be based on SARs. This relation is restricted to the delayed type hypersensitivity of the skin by organic substances because literature data are available for this type of hypersensitivity only. The use of structure activity relationships for the assessment of the skin sensitising potential of organic substances will be discussed and the strategy for the use will be established.

In addition to the SARs on sensitisation as described in Hulzebos et al. (1999), a literature research with Winspurs was performed to investigate whether there were more recent articles on this subject. The relevant articles are incorporated in this Chapter.

2.2 Mechanism of action

The mechanism of sensitisation of the skin exists of two parts, as depicted in figures 1a and 1b (Anonymous 1999).

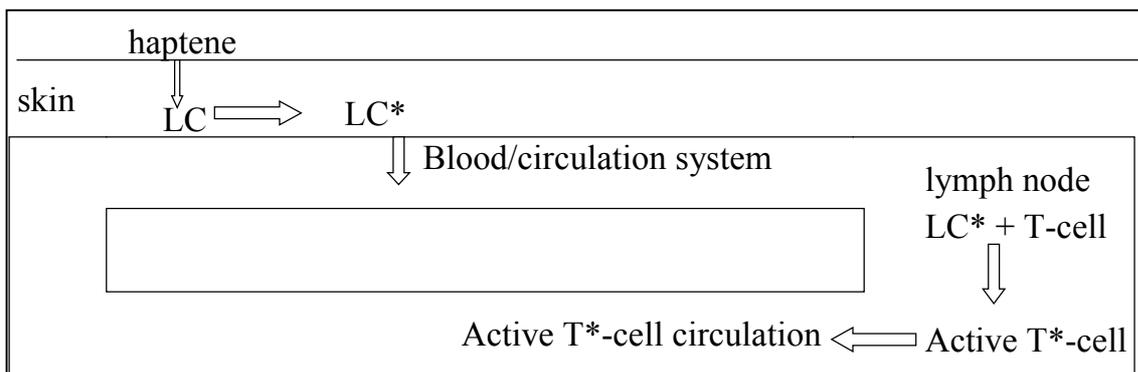


Figure 1a. Induction. LC = Langerhans cell, LC* = haptene bound to Langerhans cell (activation), T-cell = T-lymphocyte, * = activation

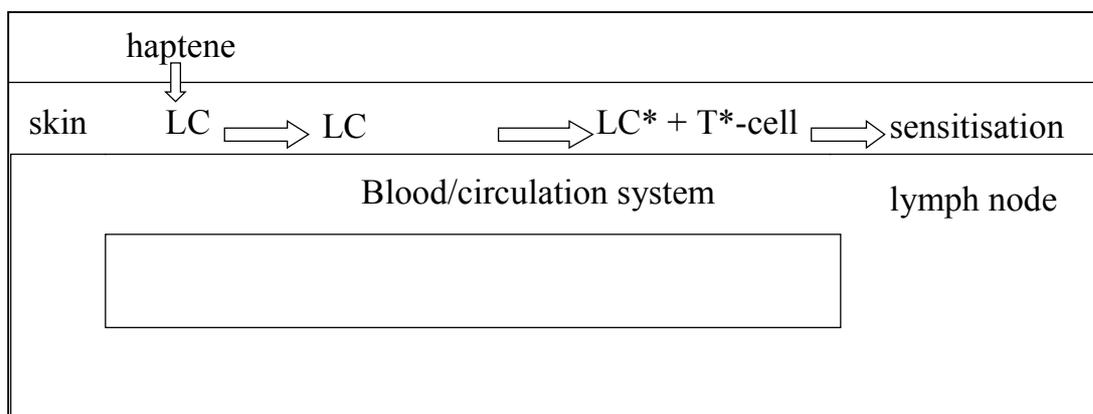


Figure 1b. Elicitation. LC = Langerhans cell, LC* = haptene bound to Langerhans cell, activated, T-cell = T-lymphocyte, * = activation

Induction

- The haptene (the substance) penetrates the skin and binds possibly after metabolic activation, to the Langerhans cells (LCs).

The LCs with the haptene (LCs*) leave the epidermis and migrate via the lymphatic system to the draining lymph node. During maturation, the LCs* become fully active antigen-presenting dendritic cells. The LCs* activate T-cells which proliferate and enter the blood circulation.

Elicitation

The haptene (the substance) penetrates the skin and binds to the LCs. Specific T-cells recognise the haptene bound to the protein on the LC and initiate a Th1-type cytokine cascade. After 24 to 72 hours this results in typical delayed type contact allergic reactions. For a more detailed description of this process reference is made to EHC 212 (Anonymous 1999).

2.3 Structure activity relationships

2.3.1 Software programmes

Structure activity relationships (SARs) for skin sensitisation have been described (Anonymous 1999, Graham 1996, Hostynek 1997, Magee 1994, Rosenkranz 1999). In these articles the relation between the sensitising potential of substances and a number of physical and chemical parameters were incorporated in a mathematical model. Examples of these models are models that take into account parameters for penetration (e.g. log P (partition octanol/water), molecular refraction) and reactivity with proteins (e.g. substructures, water solubility and molecular weight) (Hostynek 1997, Magee 1994). Other examples are (statistical) models (e.g. CASE/MultiCASE), in which the computer programme first splits-up the substance in substructures and then determines the correlation of a fragment with the sensitising properties of all substances in the database of the model. In addition to these structural fragments, modulators (e.g. molecular weight, log P, water solubility, HOMO and LUMO) are correlated with the sensitising properties of the substances in the database. Subsequently, the sensitising potential of a substance can be predicted stochastically (yes/no) or even (semi-) quantitative using the parameters of the substance. The authors of the articles on computer models have tested several substances and they have compared the predictions with the results of one or more tests for sensitisation. The results are shown in table 1.

Table 1. The comparison of some computer programmes that predict sensitisation, which was determined in sensitisation tests. ? = no data are available

Model (reference)	n in test/ N in data base	Not usable	Sensitivity (+/+)	Specificity (-/-)	Concordance
Discriminant (Hostynek 1997)	74/72	12	90%	100%	92%
Ranking (Hostynek 1997)	74/89		95%	60%	88%
MultiCASE (Gealy 1996)	30/1140		70%	85%	80%
MultiCASE (Gealy 1996)	30/1004		64%	93%	83%
MultiCASE (Graham, 1996)	103/931	11	78%	96%	90%
MultiCASE (Johnson 1997)	46/412		80%	91%	86%
DEREK(Barratt, 1994)	?/294	?	98%	82%	?
Classification model of ACD (Magee, 1994)	81/71		79%	88%	85%

Most published models have a concordance (% correctly predicted substances) of 80-90%. From the models it can be deduced that the presence or absence of certain substructures in the molecule concerned is the most determining parameter.

The use of models has the following disadvantages:

- the models have to be purchased and considerable costs are involved.
- the models are not yet externally validated.

In addition, it needs to be determined what kind of validation is necessary for evaluating chemicals in regulatory frameworks.

2.3.3 Classes of chemicals that share structural features

A second possibility of working with SARs is the use of “structural alerts” (substructures) or classes that share structural features, which are described in the models and that correlate with substances that are positive in sensitisation studies (see Part 2). This method has the following limitations:

- substructures that are not correlated with sensitisation are not mentioned in publications and therefore it is not possible to identify non-sensitising structural alerts
- it is most likely that not all structural alerts for sensitisation are known
- the sensitising potency is not only determined by the structural alerts but also by other chemical and physical characteristics (modulators) that are not incorporated in this approach
- not all structural alerts can be used without the programme e.g. distance between atoms is sometimes necessary and this information is not easily available)
- the models and therefore the structural alerts are not yet externally validated.

In addition it needs to be determined what kind of validation is necessary for evaluation of chemicals in regulatory frameworks.

Since lists of substances with a clear sensitising potency were not found chemicals that share structural features were derived from the published structural fragments. Only structural fragments for which examples were provided in the literature are included as in the list in Part 2. Therefore, this list is only indicative.

2.4 Evaluation and strategy

To gain more insight in the structural features of sensitisation, for the time being, substances can be grouped in sensitising and non-sensitising chemicals and put in a working document. When sufficient substances are present, structural alerts as described in literature, can be tested for their sensitising potential using the substances in the working document. Risk assessors can make themselves familiar with working with these structural alerts. Additionally, models can be purchased. External validation can be performed using substances that are not already used in developing the model (but derived from other data available). After validation a list is present in which it is possible to work quickly and objectively with a reliability that is probably higher than in the approach of using only structural alerts of groups of substances.

It can be concluded that when dermal sensitisation studies are absent or equivocal, SARs, based on models or on chemicals, that share structural features, can be used but only to describe the substance as “suspected of sensitising potency”. As long as the SARs are not validated (and it needs to be determined what kind of validation is necessary for evaluation of chemicals in regulatory frameworks) this description can not be used in the hazard assessment.

When the SARs show a high sensitivity, that means determine positive sensitising chemicals, these SARs can be used in the hazard assessment procedure.

Default strategy: When assessing substances for which studies are given with equivocal results the positive results of the SARs will be used to describe the substances as sensitising.

3 Neurotoxicity: SARs for acetylcholinesterase-inhibition

3.1 Introduction

In the guidelines of OECD 407(1995) and EU for repeated-dose studies the determination of cholinesterase activity is not strictly recommended. In these guidelines determination of acetylcholinesterase (AChE) is considered necessary 'for substances in certain classes or on a case-by-case basis'. Such classes have not been specified in the guidelines. Insecticides with an organic-P-ester fragment or carbamate fragment in their chemical structure are generally known to be active as cholinesterase inhibitors. These two classes are further discussed here.

On-line searches in TOXLINE, from 1985 up to 2000 on, have been done. No appropriate data on structure-activity relationships for acetylcholinesterase have been found.

3.2 Mechanism of action

The mechanism of neurotoxicity for acetylcholinesterase-inhibition is more extensively described in Fukimo (1990) and CCRX (1984), and therefore only a short description is given here.

Acetylcholinesterase is mainly localised in the synapses of the somatic, autonomic and central nervous systems. This enzyme is involved in the decomposition of the neurotransmitter acetylcholine thereby limiting the degree and duration of postsynaptic activation. Inhibition of acetylcholinesterase results in overstimulation of cholinergic receptors by acetylcholine (cholinergic toxicity or cholinergic "crisis"). **Organic-P-esters** (OP-esters, organophosphates) as well as **carbamates** inhibit acetylcholinesterase (AChE) but with a slight difference in the mechanisms. For OP-esters the interactions are presented in figure 1. The compounds are reversibly bound to the cholinesterase (step 1). After elimination of the leaving group (step 2) a covalent bond between the OP-ester and the AChE is formed. The OP-AChE complex can be reactivated (step 3). The rate of this reactivation step can be low, which results in a long inhibition of the AChE enzyme. Inactivation occurs when another organic group leaves the OP-ester (step 4). This irreversible step is called "ageing". For some OP-esters the rate of the ageing step is much higher than the rate of the reactivation step so that the inhibition is irreversible.

The enzyme-carbamate bond will not age but will be broken (reactivation) under decomposition of the carbamate so that the AChE-inhibition by carbamates will be reversible.

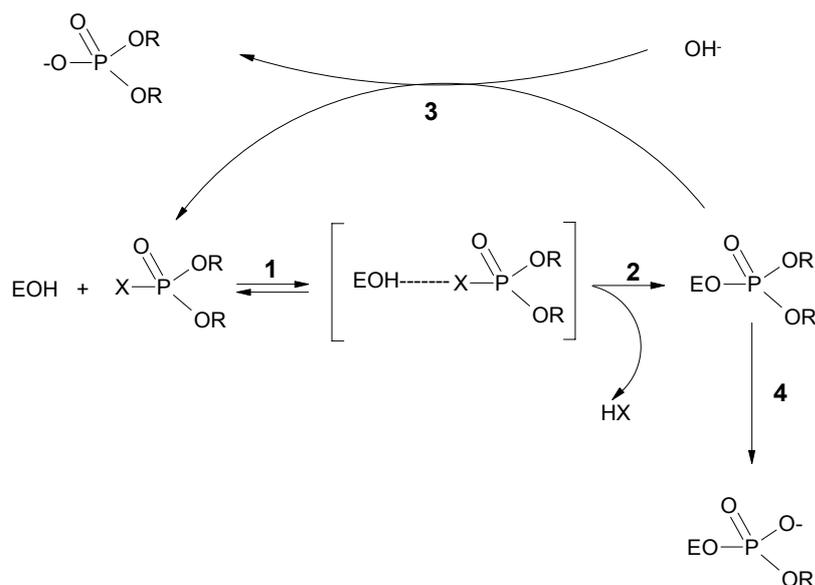


Figure 2. Schematic presentation of the interaction and enzymatic steps for an organophosphate-esters and acetylcholinesterase inhibition.

3.3 Structure-activity relationships

3.3.1 Software programmes

Software programmes were not tested. No specific literature data were available that described the prediction of acetylcholinesterase-inhibition in software programmes in TOXLINE.

3.3.2 Classes of chemicals that share structural features

3.3.2.1 Organic P-esters

The effect of organic P-esters was studied from the Pesticide Manual (Tomlin, 1997). Nearly all (approximately 70) organic P-esters, which have been reported in the Pesticide Manual (Tomlin, 1997), are insecticides and cause cholinesterase inhibition. All these P-esters possess the structural fragment:

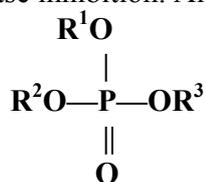


Figure 3. Organicphosphate-ester, where R^1 and $R^2 = \text{CH}_3-$, C_2H_5- and/or C_3H_7- and $R^3 =$ any organic group (See also Part 2). Replacement of one or more O-atom(s) with an S-atom occurs in many cases and/or with an N-atom in a few cases.

A few other organic P-compounds that cause cholinesterase inhibition are known as nerve gases (Clayton and Clayton, 1993 and Hawley, 1981) and have one OR-group replaced by an F-atom or CN-group. The substances from the Pesticide Manual were synthesised in order to cause cholinesterase inhibition. Little is known about the cholinesterase inhibiting potential of other organic P-compounds. NB. Some organic P-esters cause paralysis by primary axonal degeneration of peripheral nerves (“dying back”, delayed neuropathy). This effect, however, is not associated with cholinesterase inhibition. Therefore, absence of cholinesterase inhibition does not exclude peripheral nerve lesions.

When exposed to compounds with a similar structure additional effects can be expected, because they are working all as AChE-inhibitors.

3.3.2.2 Carbamates

In the Pesticide Manual (Tomlin, 1997) several carbamate compounds have been reported with different modes of action and application. Carbamates, that are insecticides, and reported as cholinesterase inhibitors and have the structural fragment described below. However, reviews on cholinesterase-inhibiting capacity of non-insecticidal carbamates are not found.

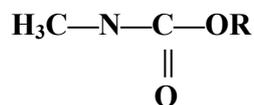


Figure 4. Carbamate, where R = any organic group (See also Part 2)

Possibility to reach the target organ

The target organs for cholinesterase inhibition are the central nervous system (CNS) and peripheral nervous system (PNS). The CNS is protected by the blood-brain-barrier, the PNS by a less effective perineurium. However, as many organic P-esters pass the blood-brain-barrier relatively well, the difference in exposure of CNS and PNS to a circulating P-ester will be small.

3.4 Evaluation and strategy

Organic P-esters

Organic P-compounds with the following structural fragment are suspected to give cholinesterase inhibition. Therefore, determination of the cholinesterase is needed for an adequate toxicological evaluation of these substances for a "weight of evidence approach"). The need for cholinesterase determination of other organic P-compounds should be judged on a case-by-case basis.

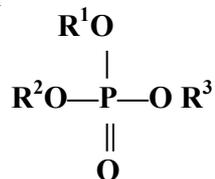


Figure 5. Organicphosphate-ester, where R^1 and $R^2 = \text{CH}_3-$, C_2H_5- and/or C_3H_7- and $X =$ any organic group. One or more O-atom(s) may be replaced with S and/or with N and the OX-group may be replaced with an F-atom or CN-group.

Carbamates

Carbamates with the following structural fragment or are suspected to give cholinesterase inhibition. Therefore, determination of the cholinesterase inhibition is needed for an adequate toxicological evaluation of these substances. The need for cholinesterase determination of other carbamates should be judged case-by-case.

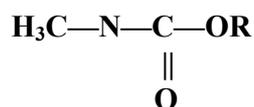


Figure 6. Carbamate, where R = any organic group.

Examples of these effects:

No investigations are known of non-pesticide (non-nerve gas) organic P-compounds and carbamates with respect to their cholinesterase inhibiting potential.

Published monographs available and New Chemicals database contain relevant toxicological information on few non-insecticide substances with the above structural fragments.

4. Genotoxicity and carcinogenicity

4.1 Introduction

With regard to carcinogenicity and genotoxicity of a substance the following toxicological information may be available:

- in-vitro* genotoxicity tests
- in-vivo* genotoxicity tests in experimental organisms
- DNA binding studies
- studies of genotoxicity effects in humans
- (toxicity)/carcinogenicity tests in experimental animals
- human epidemiological studies
- kinetic information
- information on mechanism of action

In many cases, however, the information available will be restricted to a few *in-vitro* genotoxicity tests, on the basis of which a first conclusion should be drawn on the genotoxic potential of a substance in humans. This information could also be used for predicting genotoxic carcinogenicity. (Q)SARs have been claimed to play a role in this assessment with regard to

1. the assessment of the data available;
2. the decision for further testing;
3. the identification of data gaps.

In this section the value of (Q)SARs for genotoxicity and carcinogenicity with regard to these aims is explored. Although various approaches have been explored further to the work by Hulzebos et al. (1999), this chapter concentrates on the most promising ones.

4.2 Mechanism of action

The mechanism of action of genotoxicity is determined mainly by

1. the electrophilicity of a substance which determines the potential of a substance to interact with the genetic material.
2. the potential of a substance to reach the genetic material in any target organ or tissue.

Genotoxicity can be one cause of carcinogenicity. Besides genotoxic carcinogens many carcinogens are known to act through non-genotoxic mechanisms of action, which are very diverse in nature. Both genotoxic and non-genotoxic mechanisms of action can be the subject of SAR-approaches and both will be considered here.

4.3 Structure-activity relationships

4.3.1 Software programmes

Several computerised (Q)SAR-programmes are available. These have been described in Hulzebos et al. (1999) and only a short summary will be provided here.

Rule based programmes are: RASH, COMPACT, the Purdy mode, OncoLogic, DEREK and HAZARDEXPERT. Statistical programmes are Progol, FALS, TOPKAT and MultiCASE. Only one real blind trial was performed in which the programmes were used to predict carcinogenicity for chemicals for which the outcome was unknown (Parry et al., 1994). The computerised programmes performed hardly better than random and therefore cannot be used, as yet, in day-to-day hazard assessment. OncoLogic, however, is a programme based on the knowledge of the US-EPA experts on carcinogenicity. It recognises that carcinogenicity is a multifactorial phenomenon. This computer uses the same functional

criteria as indicated by Tennant et al. (1990). The application of these criteria presently is most promising and will be described in section 5.3.2.

4.3.2 Classes of chemicals that share structural features

Genotoxicity

The available SARs concentrate on predictions on the basis of the electrophilic properties of the parent substance or metabolites thereof. Electrophilicity can be predicted from information on electronic properties (electron density, polarisability, electronegativity). This type of analysis has resulted in a theoretical structure showing all possible structural alerts for genotoxicity, the so-called supermutagen or polycarcinogen (Ashby et al., 1989), see Appendix III). This supermutagen can be used to identify potentially DNA-reactive, and therefore mutagenic, substances. Ashby and Tennant (1988) found 90% correlation between such a prediction of genotoxicity and the Salmonella assay.

Carcinogenicity

The available, most promising SARs recognise that carcinogenicity is a multifactorial phenomenon and that predictions must be based on a combination of structural, physico-chemical and biological descriptors. Three general categories are used to indicate either a predicted non-carcinogen, non-genotoxic carcinogen or genotoxic carcinogen. Three subcategories are used to reflect the level of uncertainty for substances suspected of having carcinogenic potential: unlikely, possible or probable carcinogen. According to Tennant et al. (1990) the prediction of carcinogenicity should be based on:

1. Assessment of chemical structure: identification of structural alerts on the basis of the supermutagen (Fig. 1) and estimation of the metabolism of the chemical.
2. Mutagenicity to Salmonella. Uncertainty is attached to using only the results of the Salmonella test as the sole source of genotoxicity information. It is desirable to have results of chromosome aberration tests.
3. Bioassay dose levels: the weight of evidence for carcinogenicity can be increased by an interpretation of the dose-levels used. It is assumed that low doses (< 20 mg/kg_{bw}/day oral equivalent) may reduce the potential for expression of detectable genotoxicity *in-vivo*. High doses (> 1000 mg/kg_{bw}/day oral equivalent) may increase the potential for chemically mediated disturbance in homeostasis with increased chance for non-genotoxic carcinogenesis. The large majority of genotoxic rodent carcinogens are active in the medium dose range (20 – 300 mg/kg_{bw}/day oral equivalent).
4. Sub-chronic toxicity data: subjective, selective use of toxicity data. Tennant et al. (1990) stated that no clear relationships have been established between sub-chronic toxicity and neoplasias arising from chronic exposure. The results can, however, be used selectively in conjunction with structural, mutagenic and dosage factors. For example: the presence of an acidic function within a molecule indicates a potential for the induction of rat peroxisome proliferation and, therefore, of a possible hepatocarcinogenic effect. This prediction would be strengthened by the observation of hepatocellular hypertrophy, which, in the absence of an acid function, would not in itself lead to a prediction of hepatocarcinogenesis.
5. Existing data on chemicals with a similar structure.
6. Properties of the test agent such as insolubility are sometimes used to modulate predictions, since they can provide insight in the bioavailability of the substance.

Possibility to reach the target organ or tissue

The SAR predictions of Tennant et al. (1990) include an analysis of structural, physico-chemical and mechanistic data, which all together can inform on the distribution and metabolism of a substance. PBPK-modelling can add to the understanding of kinetic aspects.

Validation

Two validation exercises have been performed, one by Tennant et al. (1990, 1994) and one jointly by US-EPA and EC (USEPA/EC, 1993).

- 1) Tennant et al. (1990) predicted the outcome of carcinogenicity tests for 44 substances then in progress at the National Institute of Environmental Health Sciences in the US. These predictions have been compared to the final outcome of the bioassays for 40 substances (Ashby and Tennant, 1994). The percentage of correct predictions was 88.2% and the score was equally good for both genotoxic and non-genotoxic carcinogens and non-carcinogens.
- 2) US-EPA and EC compared the results of EC test data with the predictions made by the US-EPA on the basis of SARs. For genotoxicity 139 substances were found for comparison. There was disagreement in results for 20 of these substances: relative to the EC base set, 6 'false' negatives and 14 'false' positives were predicted. From the latter group, 12 EC-negatives were claimed to be due to insensitivity of the test methods used for specific classes of chemicals.

4.4 Evaluation and strategy

Genotoxicity and carcinogenicity can best be predicted using the method shown by Tennant et al. (1990). This approach could be applied in the hazard assessments done when evaluation chemicals in regulatory frameworks. The results show that such predictions are accurate enough to be of significant help towards our decisions with regard to the evaluation of the data, the testing strategy proposed and the identification of data gaps. However, the application of this method requires specific expertise with regard to the endpoints investigated.

As part of the evaluation, the supermutagen (Fig. 1) can be used to identify potentially mutagenic substances (Ashby and Tennant, 1989). Only limited chemical knowledge is required to be able to make such a prediction. The predictions can be used to support derogations for further testing or to focus the testing strategy. If the outcome of the prediction is not in agreement with test results, further evaluation is needed and may lead to additional testing requirements.

5 Reproductive and developmental toxicity

5.1 Introduction

The goal of this Chapter is to collect all available data on (Q)SARs for reproductive and developmental toxicity and to examine, if such an approach might be helpful/of use for the hazard assessment for chemicals. This Chapter presents data on classes of chemicals that share structural features, for which reproductive/developmental toxicity was proven, and also some information on statistical computer programmes built for developmental toxicity screening purposes.

Reproductive toxicity includes all effects from paternal or maternal exposure that deal with the conception, development, birth, and maturation of offspring to healthy adult life. Developmental toxicity covers any detrimental effect produced by exposures to developing organisms during embryonic stages of development (Niesink, 1999).

To evaluate the reproductive/developmental potential usually the standard studies according to OECD 414, OECD 415 and OECD 416 and OECD 422 (OECD, 1981, 1983, 1983, 1996) are carried out. Several classes of chemicals that share structural features, which can have potency for reproductive/developmental toxicity, are described.

In the following paragraphs, the mechanism, structure-activity relationships, examples of classes of chemicals that share structural features, conclusion and how to use data are described.

5.2 Mechanism of action

This paragraph deals with some general principles and underlying mechanisms of reproductive and developmental toxicity.

Reproduction toxicology examines all possible effects on the reproduction process, which means the effects on the reproduction organs and their hormonal working mechanisms, conception, implantation and embryo- and foetal development. Impairment of fertility represents disorders of male or female reproductive functions or capacity as well as postnatal effects.

Developmental toxicity is part of reproduction toxicology, which studies the harmful effects of chemicals when supplied during the most sensitive period of gestation. In general, developmental toxic compounds may induce four types of effects:

- death of pups,
- growth retardation,
- malformations, which can be structural (defect in normal appearance of the cell, tissues and organ systems), which cause functional defects in normal activity of cells, tissues and organ systems or functional disorders (e.g. metabolic, immunological) (Niesink, 1999, Thomson, et al., 1999).

The exact mode of action of reproductive or developmental toxic compounds is seldom known. These mechanisms of action are complex as several organs may be involved including their interaction. Specific growth factors and hormones may regulate this interaction.

5.3 Structure-activity relationships

5.3.1 Software programmes

5.3.1.1 CASE

CASE (Computer Automated Structure Evaluation selects its own descriptors from a learning set composed of active and inactive molecules. For references see Hulzebos et al. (1999). Descriptors can be divided into 1) biophores (activating fragment), 2) biophobes (deactivating fragment) and of 3) neutral

fragments. Once biophores and biophobes have been identified, unknown molecules can be analysed. The tested chemical is recognised as *active* when biophores are present and *inactive* either because of the presence of biophobes or because both biophores and biophobes are absent (Takihi et al, 1994).

The CASE programme selects a learning set from databases and groups chemicals in causing or not causing developmental toxicity. The programme identifies unique linear structural indicator variables (up to 6 atoms) through a recursive partitioning procedure that could or could not be associated with developmental toxicity. The biophores, biophobes and neutral fragments that give the highest two-tailed t-test with a 95% confidence interval (CI), are split into groups based on structure and developmental hazards. This partitioning procedure continues until no additional biophores, biophobes and neutral fragments are identified (Dawson, 1996).

With the use of CASE a database of chemical substances that show developmental toxicity in mouse, rat, rabbit and human was tested. The goal was to predict the potential for developmental toxicity of a chemical substance and to compare structure fragments that are associated with the different biological/toxicological endpoints. Developmental toxicity is classified as death of pups, retardation and structural and functional abnormalities. CASE identified 13 major structural fragments associated with developmental toxicity in mice, 15 in rats, 9 in rabbits and 7 in humans. These analyses indicate that there is indeed a structural basis for developmental toxicity, which may be used to predict the developmental hazard of untested chemicals (Linder et al, 1992).

In tables in Part 2 all structural fragments used as basis for the CASE programme are given. Using biophores and biophobes from CASE is complicated, because a two dimensional structure cannot easily be drawn from these fragments.

Conclusion: In case a substance is already suspected for developmental toxicity (for example from similarity with one of the chemicals that share structural features) biophores and biophobes can be used as an indication for choosing the test species for further testing.

5.3.1.2 TOPKAT

TOPKAT is a computational tool for toxicity assessment from a chemical structure. The programme estimates toxicological properties of a substance based on a training set (library with examples of clear positive, clear negative and intermediate compounds) and the structure descriptors, i.e. electronic attributes, transport attributes (log Kow) and molecular bulk attributes. Subsequently, the chemical under investigation is compared to the chemicals available in the database. If a similar chemical is available the prediction is considered to be more reliable and inside the so-called Optimum Prediction Space. A quantitative structure-toxicity relationship model is then performed (Gombar et al., 1995, Hardin, 1983). In TOPKAT it is unclear which properties are responsible for the predicted effects and is therefore not a very transparent system (Gezondheidsraad, 1985).

The use of the computer programmes "CASE" and "TOPKAT", could serve as an alerting function, for this specific toxicological endpoint. It should be noted that in the independent validation test for (carcinogenicity, the correct predictability of the computer models was around 40%. No external validation on the prediction of these programmes is found.

5.3.2 Classes of chemicals that share structural features

5.3.2.1 PHTHALATES

Phthalates, used as industrial plasticisers, have caused increasing concern due to estrogenic effects of some of these compounds, possibly affecting the human male reproductive health (sperm motility). In the toxicological studies with mice and rats effects on testis (reduced size and weight) and reduced number and weight of the pups were observed (Thomson et al., 1999).

A distinction should be made in clearly defined phthalates, e.g. DEHP, DBP and BBP and undefined (mixtures), e.g. DINP and DIDP. These abbreviations are described Part 2.

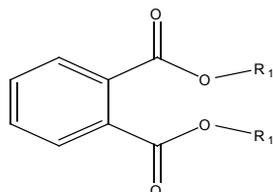


Figure 7. General structure of phthalates

The following conclusions can be drawn on the reproduction toxicity of phthalates:

- Ester moiety must be in the *ortho*-position (1,2) to be a testicular toxin
- Phthalate with both chain length of C₄-C₆ carbons may be a testicular toxin in the rat
- The more water-soluble phthalates tend to be the most active developmental toxicants (principally in mice and after high doses).

Chemicals showing the structures mentioned above are of concern and evaluation should focus on reproductive effects

5.3.2.2 GLYCOL ETHERS

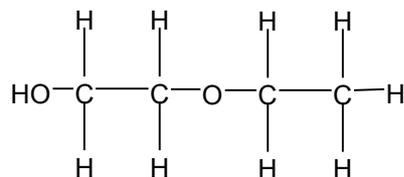


Figure 8. Structure of ethylene glycol monoethyl ether

The alkyl ether derivatives of ethylene, diethylene, and triethylene glycol are an important class of solvents with numerous industrial and consumer applications. These ethers and ethers that have acetates are used in surface coatings, cosmetics, liquid cleaning products, inks and dyes.

Four glycol ethers have been found to be developmentally toxic in experimental animals (see Part 2). Observations in various studies include testicular atrophy, degeneration of the germinal epithelium, infertility and abnormal sperm head morphology. Although not widely recognised, adverse effects on male reproductive system were first observed over 40 years ago. According to recent information, both sexes are potentially at risk, and exposure by oral inhalation and dermal route, may be hazardous (Hardin, 1983). Examples of glycol ethers showing this activity are given in Part 2.

The following conclusions can be drawn:

- 1) All ethylglycol ethers, especially methyl- and ethyl derivatives of ethyleneglycol, are developmentally toxic
- 2) Glycol ethers with a long C-chain inhibit this biological activity

5.3.2.3 CARBOXYLIC ACIDS

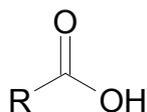


Figure 9. General structure of a carboxylic acid.
R can be an alkyl (C-C-C etc) chain with or without double bonds and with or without branching (side chains).

The developmental toxicity of carboxylic acids has received considerable attention since valproic acid (2-propylpentanoic acid) was implicated as a likely human developmental toxic. Carboxylic acids may pose a hazard to development upon accumulating in relatively early embryonic tissues.

Forty-five commercially available carboxylic acids were tested in the Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX) to evaluate the developmental toxicity of the acid to frog embryos. The compounds tested in this study were selected to relate measured developmental toxicity of the acids to frog embryos with molecular structure. Structure-developmental toxicity relationship for these carboxylic acids for *Xenopus* was performed based on the octanol/water partition coefficient (log Kow) and the ionisation constant (pKa) (Dawson, 1996).

An overview of examples is given in Part 2.

The developmental toxicity of the acids for *Xenopus* depends primarily on (Dawson, 1996):

- Carbon chain length, with acids containing five carbon atoms in the chain tending to be the most potent
- Unsaturation reduced the hazard in comparison with the corresponding unbranched saturated acids
- Developmental hazard was highest for 2-position branched compounds with 5- or 6-C chain, but was reduced for 2-position branched acids with 3- or 4-C chain
- Valproic acid showed twice higher developmental hazard than any other carboxylic acid tested.

Similar rules could be derived for mammalian developmental toxicity (Dawson, 1996).

Several carboxylic acids could not be used for testing in *Xenopus* due to not reaching equilibrium, to the high reactivity or more globular shape.

6.3.2.4 RETINOIDS

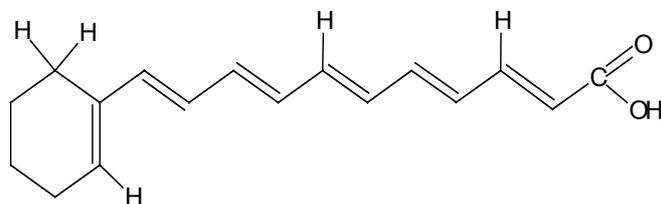


Figure 10. Simplified molecular structure of a retinoid.

Vitamin A is a lipid soluble compound available from naturally occurring dietary sources that consists of half retinol or retinol esters and half carotenoids. At physiological levels, retinoids are well known to stimulate growth of epithelial tissues and are essential to normal mammalian reproductive and embryonic development.

The extensive use of two synthetic retinoids (isoretinoin and etretinate) for treatment of dermatological disorders has reawakened the interest in the spectrum of developmental effects associated with structures that are similar to vitamin A. Isoretinoin (trade name Accutane) is the most problematic substance of this group, due to its therapeutic use at developmentally toxic levels for treatment of severe cystic acne.

Retinoids in humans may possibly cause the developing of the palate and limb with respect to their applicability to abnormalities of the central nervous system (CNS), both anatomic and functional. The characteristic pattern of malformations involves craniofacial, cardiac, thymic and CNS-structures (Lammer, 1985, Geelen, 1979).

The developmentally toxic effects of retinoids were also widely investigated in the experimental animals. The following effects were found:

- Exencephaly (severe malformation of the brain)
- Meningocele and meningoencephalocele (minor closure defects of the neural tube)
- Spina bifida
- Hydrocephallus
- Ocular malformations and malformations of the ear
- Craniofacial malformations
- Cleft palate
- Defects of the circulatory system and the respiratory system
- Urogenital defects
- Skeletal malformations.

At the higher dosages all of the retinoid compounds (this excludes beta-carotene) are developmentally toxic and this is currently believed to be due to their metabolism to all-trans retinoic acid and metabolites. Comparison study of the structure-activity relationship for the dose-response potency of retinol, all-trans retinoic acid and 13-cis retinoic acid showed, that retinol is approximately 1/4 as potent as all-trans retinoic acid and isotretinoin is approximately 1/8 as potent as all-trans retinoic acid (Adams, 1993). In Part 2 overview with chemical structures of retinoids and their developmental toxicity potency is given (Willhite, 1991).

5.3.2.5 SUBSTANCES DISTURBING HORMONAL BALANCE IN THE ORGANISM

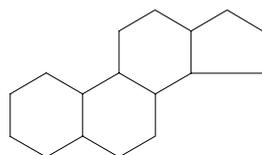
The so-called endocrine disrupters, that are a large, heterogeneous group of chemicals disturbing hormonal balance in the organisms, are presented in this paragraph.

Endocrine disrupters are chemicals (either natural or synthetic) which have the ability to alter the function of various processes under endocrine control. Another definition describes an endocrine disrupter as an exogenous agent that interferes with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and regulation of developmental processes.

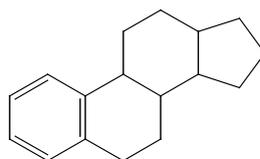
The effects caused by these chemicals include increase in the incidence of hormone-dependent cancers and abnormal reproductive system development (Hulzebos et al., 1999, and Zacharewski, 1998, Carney et al., 1997). Structural features for a generic SAR process to warn for potential endocrine modulating activity are given below.

1. Steroid nucleus

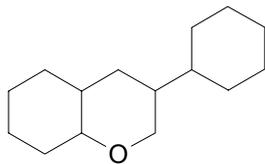
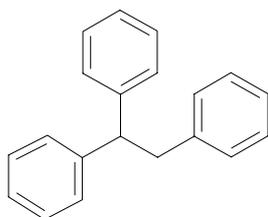
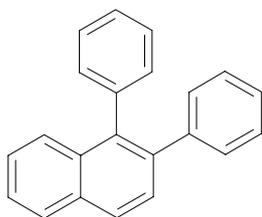
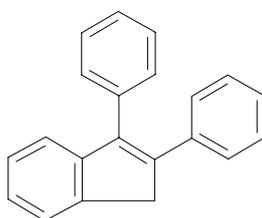
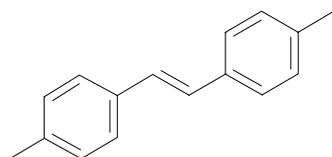
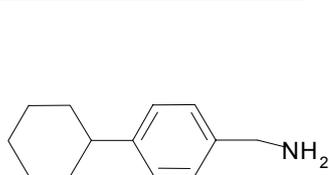
A. Cyclopentanophenanthrene - like derivatives



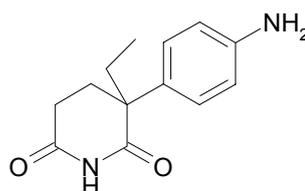
androgen



oestrogen

B. Flavone-like derivatives also called phytoestrogens**1) Non-steroid nucleus****A. Triphenylalkane, ene, anol derivatives****B. Diphenylnaphthalene derivatives****C. Diphenylindene derivatives****D. Biphenyl derivative variants (includes DES, DDT, bisphenol and PRDX)****E. Miscellaneous structures**

4-cyclohexylaniline



aminoglutethimide

Figure 11. Structural features that may disturb the hormonal balance of organisms

5.3.2.6 SULPHONAMIDES

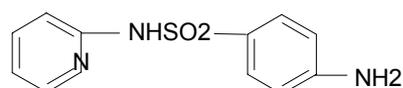


Figure 12. A structure of a sulphonamide: sulphapyridine

Sulphonamides are medicines used in treatment of rheumatoid arthritis, regional enteritis and other kinds of inflammations. Sulphasalazine (salicylazosulphapyridine) was recognised as a substance causing male infertility in humans already in 1979. This effect was completely reversible after drug withdrawal in the patient.

SARs given in this Chapter are based on only *one* study with rats. Fertility in this study was defined as a ratio of the number of foetuses and number of corpora lutea. Unfortunately, sulphasalazine (salicylazosulphapyridine) was not tested in this study.

Sulphonamides cause a decrease in the number of offspring. The action of sulphonamides is probably related to their ability to pass into the epididymal fluid. In rats sulphonamides do not alter testicular weight and histology, gonadotrophin and testosterone concentrations in the blood, or daily sperm production rate and sperm concentration in the epididymis. It might be concluded, that the site of action is post-testicular, possibly at the epididymis.

The reproductive toxicity (male infertility) of the sulphonamides primarily depends on:

- Presence of *para*-amino group in the benzene ring of benzene sulphonamides is required for maximum antifertility activity. Bromo- or nitro- analogues (at the *para*- but not at the *meta*- position of the benzene ring) were able to produce some antifertility, although to lesser degree than amino the group.
- Replacement of the pyridine ring by isoxazole or pyrimidine causes a complete loss of antifertility activity. Substitution of the pyridine ring by H- or simple alkyl group retained or enhanced antifertility activity.
- Substitution with a nitro-group in the *meta*- or the *ortho*-position in the benzene ring causes completely loss of in antifertility activity.
- Acetylation of amino group does not change the antifertility potency.

For an overview see Part 2. These are derived from Pholpramool, (1991).

5.4 Evaluation and strategy

For the hazard assessment of chemical substances a certain amount of data is available. From those data concern may be raised in relation to reproductive/developmental toxicity (e.g. indication from a 28-day toxicity study). SARs can be used to focus more on these effects.. In cases where there is a very reduced toxicity data set available or no indication from the available toxicity tests were found, an extra warning tool in the form of (Q)SAR might be useful to check for concern on reproductive- and developmental toxicity.

The difficulty with using (Q)SARs in relation to reproductive/developmental toxicity is that there are a variety of mechanisms underlying to these effects. This can not be related to one structural fragment. Presented classes of chemicals that share structural features may draw attention to the possible developmental toxicity concerns by the similarity of the investigated chemical with an example from one of the classes. Persons without any specialised training in SAR can use the described structures as early warning indicators.

Computer programmes (TOPKAT, CASE) are available, however as long as no external validation is available and it is yet unclear what kind of validation is necessary for chemicals evaluated in regulatory

frameworks, they should not be used for effect assessment. After external validation these computer programmes might be useful. The acquirement of such a programme has the advantage to build up a large databank with chemicals and/or structural fragments with known toxicological properties. Moreover, there are only a few general classes of chemicals that share structural features, which have been proven to be developmentally toxic. Several developmentally toxic compounds do not share the structural features presented here. CASE recognises in investigated chemicals small structure fragments, which might be responsible for reproductive toxicity.

Presented SARs may be applied to every day practice when evaluating chemicals in the following way:

1. A substance under investigation is compared with a class of chemicals that share structural features that are presented here.
2. If similarities are found it needs to be checked if the additional requirements for causing toxicity are also met.
3. If point 1 and 2 are applicable, further testing for developmental toxicity should be considered according to guideline requirements for specific frameworks and **taking into account extra indications on species specificity obtained from structure activity relationship.**
4. If the investigated chemical is of no concern, follow the **standard** test strategy for your framework.

In future one of the computer programmes might be used for the screening purposes (CASE, TOPKAT; see above for validation problems). The obtained results should be always critically evaluated by expert judgement.

6 Conclusions of the Workshop

In the Workshop on 'SARs in human toxicology', the SARs for different endpoints were presented as described in Chapter 2-6 and were discussed with risk assessors and experts from RIVM and TNO, at the RIVM on June 15, 2000. The conclusions on the separate toxicological endpoints were included in the Chapters describing these endpoints. General conclusions on working with SARs are presented here.

Conclusions

- 1) At the Workshop it was emphasised that SARs, being a relation between a structural feature and a toxicological effect, are already applied when evaluating chemicals on an ad-hoc basis. A more consistent use of SARs would fit in the normal procedure of evaluating chemicals.
- 2) SARs should be used for 'positive classification'. This means that if the chemical under investigation has no structural fragment related to the endpoint, this does not mean that the chemical is of 'no concern'. SARs need to be tested first on their potential to predict the effect or having no effect before they can be used for establishing 'no concern'.
- 3) SARs should be used in addition to the testing requirement of the framework in which the chemical is assessed. Test results usually overrule the SAR, because not the whole route between exposure and target organ can be fully covered by SARs based mainly on structural fragments. Therefore the normal procedure for testing chemicals should be carried out as it is outlined in the guidance documents for the different frameworks (e.g. TGD, 1996).
- 4) Additional criteria may need to be developed for the applicability of SARs of specific classes of chemicals that share structural features.
- 5) Gaining expertise on the use of SARs
 - a) In the Workshop it was emphasised that for a broader use of SARs more knowledge and expertise is necessary on chemical structures and their possible working mechanisms. First of all it is necessary to decide whether or not a chemical under investigation belongs to a specific structural class. This will often be a difficult task and may lead to arbitrary decisions. When chemists and toxicologists join together in these discussions the accuracy of these decisions would improve. This knowledge also becomes available for a larger group of risk assessors.
 - b) In the Workshop it was stressed that use of SAR software programmes may prevent re-inventing the wheel. Up to date toxicological and chemical knowledge is included and makes the evaluation less dependent on the expertise of individual risk assessors. Moreover, software programmes on SARs are more objective than the individual expertise. Another advantage may be that less knowledge on chemical structures is necessary for the application of SARs, as risk assessors need not determine the functionality of the structural fragment. It is essential to check whether or not the SAR can be used for the specific chemical and the specific endpoint.

Recommendations:

The participants at the Workshop proposed the following:

- 1) Incorporate the knowledge on classes of chemicals that share structural features and working mechanisms in the every day practice of evaluating chemicals. This can be done by comparing the predictions of the SARs with the reported test results for substances that are submitted to the 'toetsgroep (a group of experts in human toxicology, who peer review the advisory reports on human toxicology endpoints). The results can be discussed in this 'toetsgroep'.
- 2) Achieve more expertise on working with SAR software programmes.
- 3) Validate SAR software with experimental data.

References

- Adams J. Structure-Activity and Dose-Response relationship in the neural and behavioural teratogenesis of retinoids. *Neurotoxicology and Teratology* 1993;193 – 202.
- Anonymous EHC 1999; 212, IPCS.
- Ashby J, Tennant, RW, Zeiger E and Stasiewicz, S. Classification according to chemical structure, mutagenicity to Salmonella and level of carcinogenicity for a further 42 chemicals tested for carcinogenicity by the US National Toxicology Program. *Mutat. Res.* 1989;223: 73-103.
- Ashby J and Tennant RW. Chemical structure, Salmonella mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 222 chemicals tested in rodents by the US NCI/NTP. *Mutat. Res.* 1988;204: 17-115.
- Ashby J and Tennant RW. Prediction of rodent carcinogenicity for 44 chemicals: results. *Mutagenesis* 1994;9: 7-15
- Barratt MD. Quantitative structure-activity relationships for skin permeability. *Toxicology in Vitro* 1995; 9:27-37.
- Barratt MD, Castell JV, Chamberlain M, Combes RD, Dearden JC, Fentem JH, Gerner I, Giuliani A, Gray TJB, Livingstone DJ, Provan W, Rutten AAJJL, Verhaar HJM, Zbinden P. The integrated use of alternative approaches for predicting toxic effects. The report and recommendations of the ECVAM workshop 8. *ATLA* 1995;23:410-429.
- Barratt MD, Dixit MD and Jones PA. The Use of *In-vitro* Cytotoxicity Measurements in (Q)SAR Methods for the Prediction of Skin Corrosivity Potential of Acids. *Toxicology in Vitro* 10:283-290.
- Barratt MD. 1998. Integration of QSAR and *in-vitro* toxicology. *Environmental Health Perspective* 1996;106: 459-465.
- Barratt MD, Brantom G, Fentem JH, Gerner I, Walker AP, and Worth AP. The ECVAM International Validation study on *in-vitro* tests for skin corrosivity. 1. Selection and distribution of the test chemicals. *Toxicology in Vitro* 1998;2: 471-482.
- Barratt MD, Basketter DA, Chamberlain M, Admans GD and Langowski JJ. An expert system rule-base for identifying contact allergens. *Toxicology in vitro* 1994;8:1053-1060.
- Carney EW et al. Estrogen modulation: tiered testing for human hazard evaluation. *Reproductive toxicology* 1997; 11:879-892.
- CCRX. Evaluatierapport Cholinesteraseremmende Stoffen in Nederland. 1984 VROM, Den Haag.
- Chamberlain M. and Barratt MD. Practical Applications of (Q)SAR to In Vitro Toxicology Illustrated by Consideration of Eye irritation. *Toxic. in Vitro* 1995;9:541-547.
- Clayton GD and Clayton FE editors. *Patty's Industrial Hygiene and Toxicology*. Vol.II, Part A. 4th edition, New York: Johnson and Sons, 1993.
- Dawson AD. Developmental toxicity of carboxylic acids to *Xenopus* embryos: A Quantitative Structure-Activity Relationship and Computer Automated Structure Evaluation. *Teratogenesis, Carcinogenesis and Mutagenesis* 1996;16:109-124.
- DeVito S. Absorption through cellular membranes. In: *Handbook of Property Estimation Methods for Chemicals*. Environmental and Health Science, editors Boethling RS and Mackay D. London: Lewis Publishers, 2000
- ECETOC. Skin and respiratory sensitizers: Reference chemicals data bank. Technical Report No. 77. 1999
- Fukuto TR Mechanism of action of organophosphorus and carbamate insecticides. *Environmental Health Perspectives*;1990;87:245-254.
- Gealy R, Graham, Sussman NB, Macina OT, Rosenkranz HS and Karol MH. Evaluating clinical case report data for SAR modelling of allergic contact dermatitis. *Human and Experimental Toxicology* 1996;15: 489-493.
- Geelen JAG. Hypervitaminosis A induced teratogenesis. *CRC Critical Reviews in Toxicology*. 1979

- Gezondheidsraad advies inzake teratogeniteit van chemische stoffen. Uitgebracht door een commissie van de Gezondheidsraad. 11-3-1985.
- Gombar VJ et al. Assessment of developmental toxicity potential of chemicals by quantitative structure-toxicity relationship models. *Chemosphere* 1995;31:2499-2510, 1995.
- Graham C, Gealy R, Macine OT, Karol MH and Rosenkranz HS. QSAR for allergic contact dermatitis. *Quantitative Structure-Activity Relationships* 1996;15: 224-229.
- Greim HD, Bury HJ, Klimsch M, Oeben-Negele K, Ziegler-Skylakakis. Toxicity of aliphatic amines: structure activity relationship. *Chemosphere* 1998;271-95.
- Hardin BD. Reproductive toxicity of the glycol ethers. Review paper. *Toxicology* 1983;27:91-102.
- Hawley GG.. *Condensed Chemical Dictionary*, 10th edition. New York: Van Nostrand Reinhold Company, 1981.
- Hostynek JJ and Magee PS. Fragrance allergens: classification and ranking by QSAR. *Toxicology in vitro* 1997;11:377-384.
- Hulzebos EM, Schielen PCJI and Wijkhuizen-Maslankeiwicz L. (Q)SARs for human toxicological endpoints: a literature search. Bilthoven, RIVM, 1999 Report no. 601516.
- Johnson R, Macina OT, Graham C, Rosenkranz HS, Cass GR and Karol MH. Prioritising testing of organic compounds detected as gas phase air pollutants: structure-activity study for human contact allergens. *Environmental Health Perspectives* 1997;105(9):986-992.
- Lammer EJ.. Retinoic acid embryopathy. *The New England Journal of Medicine*. 1985;313(14):837 – 841.
- Linder RE et al. Endpoints of spermatotoxicity in the rat after short duration exposures to fourteen reproductive toxicants. *Reproductive Toxicology* 1992;6:491-505.
- Magee PS, Hostynek JJ and Maibach HI. A classification model for allergic contact dermatitis. *Quantitative Structure-Activity Relationships* 1994;13: 22-33.
- Nendza M. *Structure-Activity Relationships in Environmental Sciences*. Ecotoxicology Series No 6. London: Chapman and Hall, 1998.
- Niesink RJM et al. *Toxicology - Principles and Applications -*. 1999.
- OECD (Organization for Economic Cooperation and Development). *Guideline for Testing of Chemicals No 404, Skin irritation*, Paris, 1987.
- OECD (Organization for Economic Cooperation and Development). *Guideline for Testing of Chemicals No 407, repeated Dose 29-day Oral Toxicity Study in Rodents*, Paris, 1995.
- OECD (Organization for Economic Cooperation and Development). *Guideline for Testing of Chemicals No 414, Teratogenicity*, Paris, 1981.
- OECD (Organization for Economic Cooperation and Development). *Guideline for Testing of Chemicals No 415, One-Generation Reproduction Toxicity Study*, Paris, 1983.
- OECD (Organization for Economic Cooperation and Development). *Guideline for Testing of Chemicals No 416, Two-Generation Reproduction Toxicity Study*, Paris, 1983.
- OECD (Organization for Economic Cooperation and Development). *Guideline for Testing of Chemicals No 422, Combined repeated dose toxicity study with the reproduction/developmental toxicity screening*, Paris, 1986.
- Pholpramool C. Structural requirements of some sulphonamides that possess an antifertility activity in male rats. *Journal of Reproduction and fertility* 1991;92:169 – 178.
- Rosenkranz HS, Klopman G, Zhang YP, Graham C and Karol MH. Relationship between allergic contact dermatitis and electrophilicity. *Environmental Health Perspectives* 1999;107 (2):129-132.
- Sijm D, Hulzebos E, Peijnenburg W. Estimating the PTB-profile. Bilthoven: RIVM 1999 Report, 601503 016, 1999.
- Takihi N. Structural determinants of developmental toxicity. *Risk Analysis*; 1994;14:649 – 657.
- Tennant RW, Spalding J, Stasiewicz S and Ashby J. Prediction of the outcome of rodent bioassays currently being conducted on 44 chemicals by the National Toxicology Program. *Mutagenesis* 1990;5:3-14.

- Technical Guidance Document in support of the Commission Directive 93/67/EEC on risk assessment for new substances and Commission Regulation (EEC) No. 1488/94 on risk assessment for existing substances, part 3. ECB, Ispra, Italy, 1996.
- Thomsen M et al. SAR/QSAR approaches to solubility, partitioning and sorption of phthalates. *Chemosphere* 1999;38(11):2613-2624.
- USEPA/EC. Joint project on the evaluation of (quantitative) structure activity relationships. Washington, USA, EPA, 1993.
- Van Och FMM, Slob W, de Jong, WH, Vandebriel, RJ and Van Loveren H. A quantitative method for assessing the sensitising potency of low molecular weight chemicals using a local lymph node assay: employment of a regression method that includes determination of the uncertainty margins. *Toxicology* 1999;146: 49-59.
- Vermeire T, Van de Poel P, Van de Laar RTH and Roelfezema H. Estimation of consumer exposure to chemical: application of simple models. *The Science of the Total Environment* 1992;135:155-176.
- Willhite CC. Structure-affinity relationship of retinoids with embryonic cellular retinoic acid-binding protein. *Toxicology and applied pharmacology* 1991;112:144 – 153.
- Zacharewski T. Identification and assessment of endocrine disrupters: limitations of *in-vivo* and *in-vitro* assays. *Environmental Health Perspectives* 1998;106 (2):577-82.

Part 2 **Classes of chemicals that share structural features of chemicals**

In this Part a list of classes of chemicals that share structural features having effects on irritation, sensitisation, neurotoxicity, genotoxicity and reproductive – and developmental toxicity, is presented. This list is distilled from literature data described in Part 1. Examples of chemicals are given that show effects on the endpoints mentioned. For example, primary amines are potentially irritating/corrosive, methylamine is given as an example of a corrosive chemical.

However, it is not yet clear when chemicals having these structural features do or do not show these effects. Therefore chemicals having these structural features need to be compared with test results to identify the potential use of this list for predicting effects.

The goal of this list is to present these structural features as a first step in testing structure-activity relationships (SARs) for human toxicological endpoints for risk assessors at CSR.

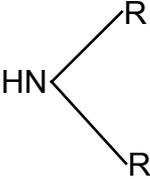
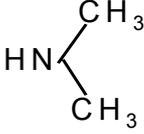
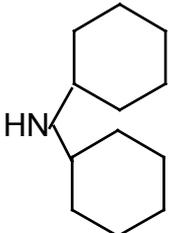
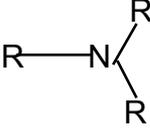
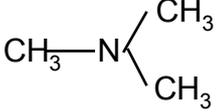
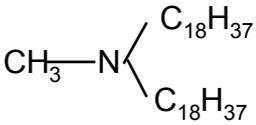
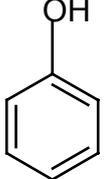
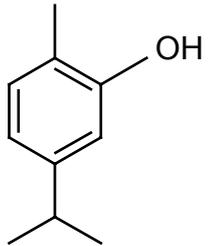
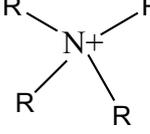
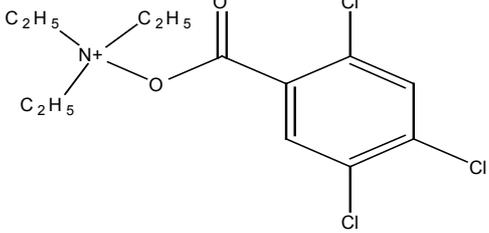
At this testing, in theory, the following 5 possibilities can be found:

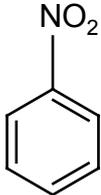
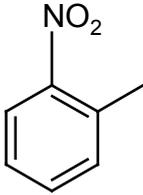
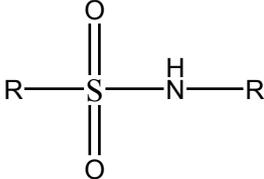
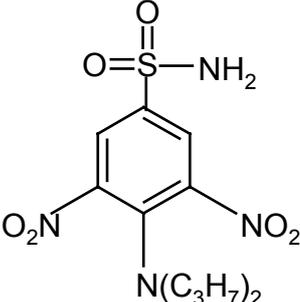
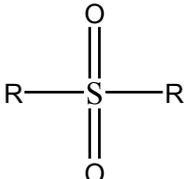
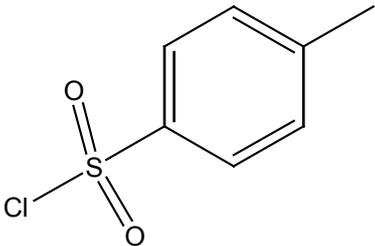
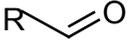
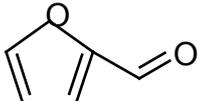
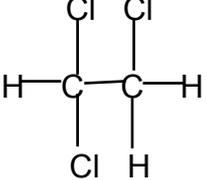
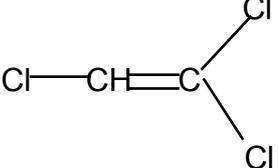
- 1) The chemical under investigation has a structural feature and shows the effect, this means a positive structural feature that is also shown in the test result (+ SAR/ + test result).
- 2) The chemical under investigation has a structural feature, but the surrounding of the structural feature may hinder the outcome of the SAR (steric hindrance). Also kinetic information based on physical-chemical properties may indicate that the structural feature cannot cause the effect on the target site. In these cases the outcome of the SAR is equivocal (+/-). The outcome of the test result may then give some further explanation about the most important feature for showing or not showing the effect: +/- SAR and +/- test.result.
- 3) The chemical under investigation has the structural feature but does not show the effect, this means, + SAR/ - test result.
- 4) The chemical under investigation has no structural feature but shows the effect, this means, - SAR/+test result.
- 5) The chemical under investigation has no structural feature and does not show effect, this means - SAR/- test result.

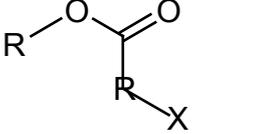
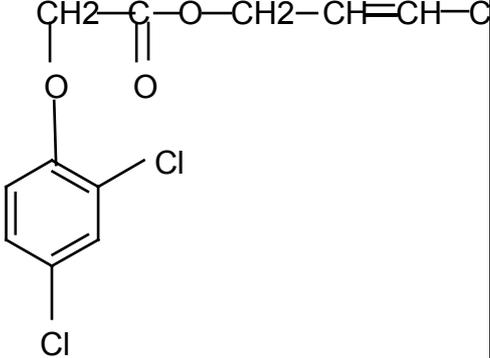
The outcome of this testing will revise this list and further identification of the potential of this list for the use of hazard assessment can be further explored.

1. Skin Irritation

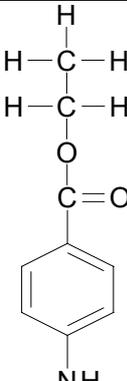
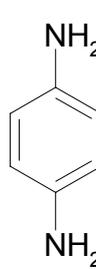
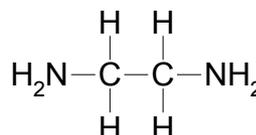
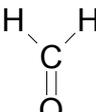
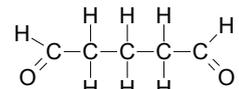
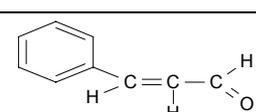
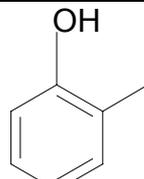
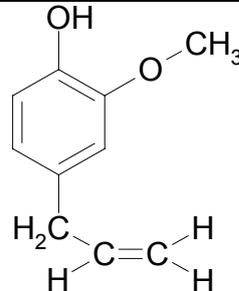
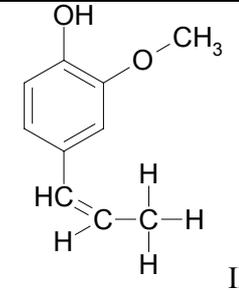
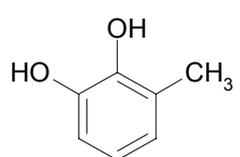
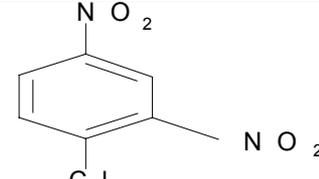
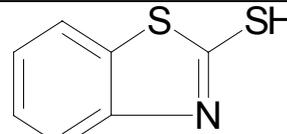
GENERAL STRUCTURAL FORMULA		EXAMPLES OF IRRITATING (R38) OR CORROSIVE COMPOUNDS (R34/R35)
ORGANIC ACIDS		
Saturated acids	$\text{R}-\text{C}(=\text{O})\text{OH}$	$\text{C}_5\text{H}_{11}-\text{C}(=\text{O})\text{OH}$ <p>Hexanoic acid Corrosive!</p>
Acrylic acids (unsaturated acids)	$\text{R}=\text{C}-\text{C}(=\text{O})\text{OH}$	$\text{CH}_2=\text{CH}-\text{C}(=\text{O})\text{OH}$ <p>Acrylic acid Corrosive!</p>
Methacrylic acids (unsaturated acids)	$\text{R}=\text{C}(\text{CH}_3)-\text{C}(=\text{O})\text{OH}$	$\text{CH}_2=\text{C}(\text{CH}_3)-\text{C}(=\text{O})\text{OH}$ <p>Methacrylic acid Corrosive!</p>
ORGANIC BASES, ALIPHATIC AMINES		
Primary aliphatic amines	$\text{R}-\text{NH}_2$	$\text{H}_3\text{C}-\text{NH}_2$ <p>Methylamine Corrosive!</p>
		$\text{CH}_3-\text{CH}(\text{CH}_3)-\text{NH}_2$ <p>Isopropylamine Corrosive!</p>
		$\text{CH}_3-(\text{CH}_2)_{13}-\text{NH}_2$ <p>Tetradecylamine Corrosive!</p>

GENERAL STRUCTURAL FORMULA		EXAMPLES OF IRRITATING (R38) OR CORROSIVE COMPOUNDS (R34/R35)
secondary aliphatic amines		 <p>Dimethylamine Corrosive!</p>
		 <p>Dicyclohexylamine, Irritant</p>
Tertiary aliphatic amines		 <p>Trimethylamine Corrosive!</p>
		 <p>1-Octadecanamine, N-methyl-N-octadecyl, Irritant</p>
Phenols		 <p>Carvacol Corrosive!</p>
CATIONIC SURFACTANTS		
Quarternairy ammonium salts		 <p>Triethylammonium (2,4,5-trichlorophenoxy)acetate, Irritant</p>

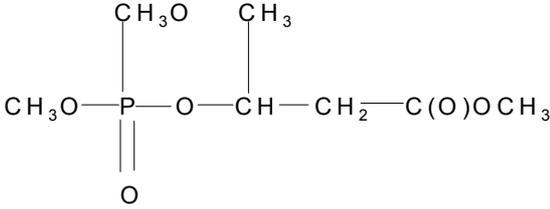
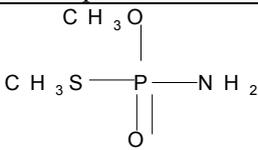
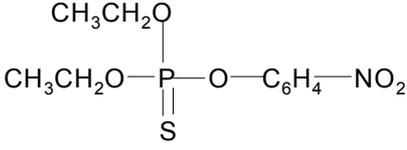
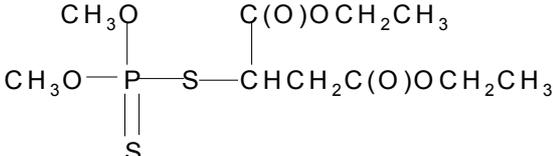
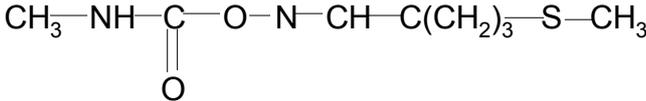
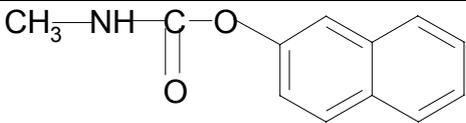
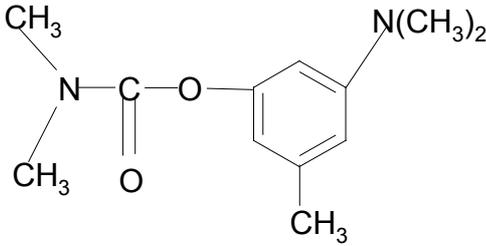
GENERAL STRUCTURAL FORMULA		EXAMPLES OF IRRITATING (R38) OR CORROSIVE COMPOUNDS (R34/R35)
OXIDIZING AGENTS		
Aromatic nitro compounds		 o-nitrotoluene
Sulfonamides		 4-(dipropylamino)-3,5-dinitrobenzene sulfonamide Irritant
Sulfones		 p-toluenesulfonylchloride, Irritant
Aldehydes		 2-furalaldehyde (Furfural), Irritant
Halogenated alkanes	$R-X$ Where X = Cl, Br or I	 1,1,2-trichloroethane, Irritant
Halogenated alkenes	$R=C(R)-X$ Where X = Cl, Br or I	 Trichloroethylene, Irritant

GENERAL STRUCTURAL FORMULA		EXAMPLES OF IRRITATING (R38) OR CORROSIVE COMPOUNDS (R34/R35)
Halogenated esters	 <p>Where X = Cl, Br or I</p>	 <p>Acetic acid (2,4-dichlorophenoxy), 4-chloro-2-butenlester, Irritant</p>

2. Skin sensitisation

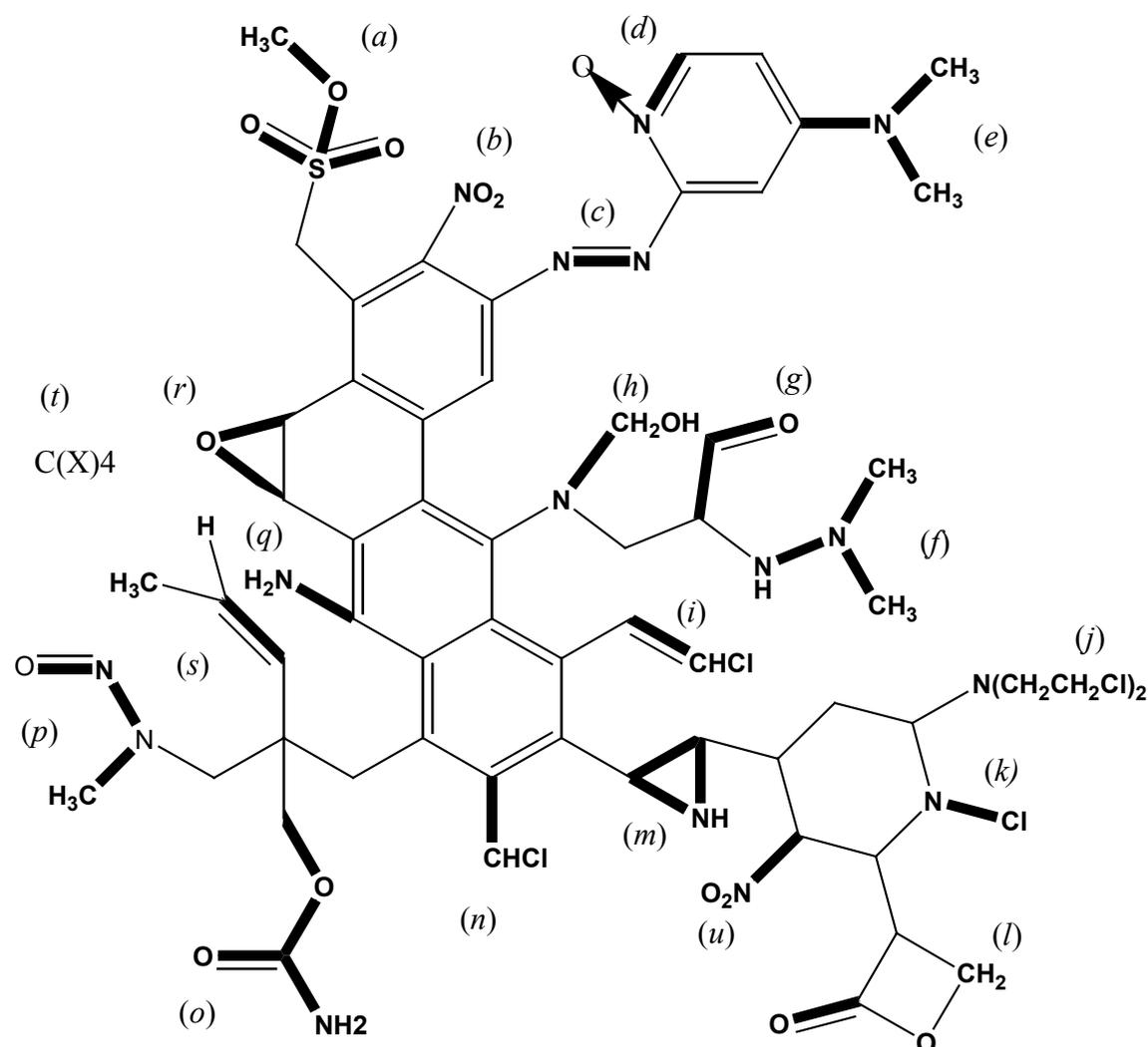
GENERAL STRUCTURAL FORMULA		EXAMPLES		
Amines	$R-NH_2$ Where R is aliphatic chain or (aromatic) ring	 Benzocaine (ethyl 1-aminobenzoate)	 p-Phenylenediamine	 Ethylenediamine
Aldehydes	$R-CHO$ Where R is aliphatic chain or (aromatic) ring	 Formaldehyde	 Glutaraldehyde	 Cinnamaldehyde
Substituted aromatic alcohols (phenols)		 Eugenol	 Isoeugenol II	 3-methyl catechol
Chlorides	$R-Cl$ Where R is aliphatic chain or (aromatic) ring	 DNCB (1-chloro-2,4-dinitrobenzene)		
Thioles	$R-SH$ Where R is aliphatic chain or (aromatic) ring	 MBT (2-Mercaptobenzothiazole)		

3. Neurotoxicity by acetylcholinesterase inhibition

GENERAL STRUCTURAL FORMULA	Examples
<p>Organic P-esters</p> $\begin{array}{c} \text{O} \\ \parallel \\ \text{R}_1\text{O}-\text{P}-\text{OR}_3 \\ \\ \text{OR}_2 \end{array}$ <p>where R₁ and R₂ = CH₃-, C₂H₅- and/or C₃H₇- and R₃= an organic group. One or more O-atom(s) may be replaced with S and/or with N and the OR₃-group may be replaced with an F-atom or CN-group</p>	<p></p> <p>Mevinphos</p> <p></p> <p>Methamidofos</p> <p></p> <p>Parathion</p> <p></p> <p>Malathion</p>
<p>Carbamates</p> $\begin{array}{c} \text{H}_3\text{C}-\text{N} \\ \diagdown \quad \diagup \\ \quad \quad \text{C}=\text{O} \\ \quad \quad \\ \quad \quad \text{OR} \end{array}$ <p>with R = organic group.</p>	<p></p> <p>Aldicarb</p> <p></p> <p>Carbaryl</p> <p></p> <p>Primicarb</p>

4. Genotoxicity and carcinogenicity

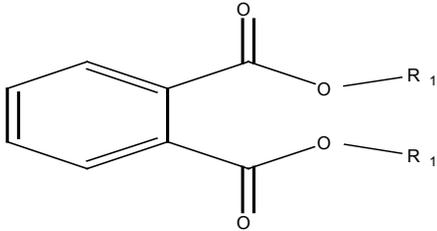
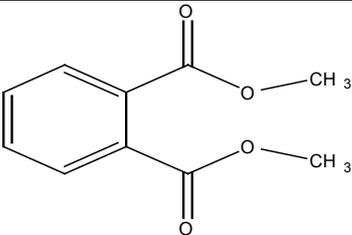
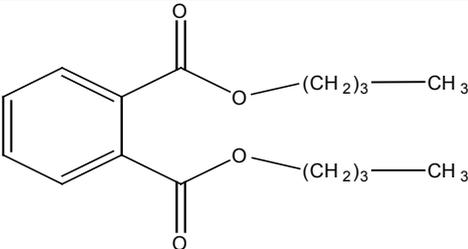
Structural alerts for genotoxicity, from Ashby and Tennant, 1989: The 'supermutagen', see Chapter 4.



(a)	Alkyl esters of either phosphonic or sulphonic acid	(n)	Both aromatic and aliphatic substituted primary alkyl halides
(b)	Aromatic nitro groups	(o)	Derivatives of urethane (carbamates)
(c)	Aromatic azo groups, not per se, but virtue of either possible reduction to an aromatic amine	(p)	Alkyl N-nitrosoamines
(d)	Aromatic ring N-oxides	(q)	Aromatic amines, their N-hydroxy derivatives and derived esters
(e)	Aromatic mono- and di-alkylamino groups	(r)	Aliphatic and aromatic epoxides
(f)	Alkyl hydrazines	(s)	?
(g)	Alkyl aldehydes	(t)	Halogenated methanes C(X)₄ X = H, F, Cl, Br, I (In any combination.)
(h)	N-methylol derivatives	(u)	Cyclic or alkyl nitrogroups
(i)	Monohaloalkenes		
(j)	A large family of N and S mustards (β -haloethyl)		
(k)	N-chloroamines		
(l)	Propiolactones and propiosultones		
(m)	Aromatic and aliphatic aziridinyl derivatives		

5. Reproductive toxicity and developmental toxicity

Phthalaten

GENERAL STRUCTURE	EXAMPLES
Phthalaten 	 Dimethylphthalaat (DMP)
	 Dibutylphthalaat (DBP)

Structural characteristics for selected phthalates

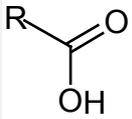
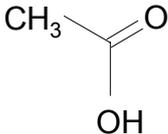
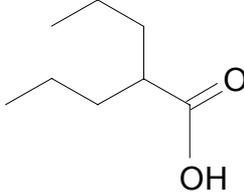
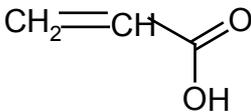
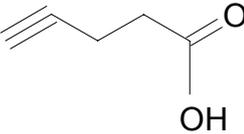
-PHTHALATE	ABBREV.	-R1	-R2	REPROTOX ¹⁾	DEVELOPMENTAL TOXI ²⁾
Dimethyl	DMP	-CH ₃	-CH ₃	-	+
Diethyl	DEP	-CH ₂ CH ₃	-CH ₂ CH ₃	+	+
Diallyl	DAP	-CH ₂ CH=CH ₂	-CH ₂ CH=CH ₂	No data	No data
Di-n-butyl	DnBP	-CH ₂ (CH ₂) ₂ CH ₃	-CH ₂ (CH ₂) ₂ CH ₃	+	+
Di-(2-ethyl-hexyl)	DEHP			+	+
Di-n-octyl	DnOP	-CH ₂ (CH ₂) ₆ CH ₃	-CH ₂ (CH ₂) ₆ CH ₃	+/- (testicular effects in 90-day study, not in testicular study)	No data
Di-n-decyl	DnDP	-CH ₂ (CH ₂) ₈ CH ₃	-CH ₂ (CH ₂) ₈ CH ₃	No data	No data
Butyl benzyl	BBP	-CH ₂ (CH ₂) ₂ CH ₃	-CH ₂ -phenyl	+	No data
Dimethoxyethyl	DMEP	-CH ₂ CH ₂ OCH ₃	-CH ₂ CH ₂ OCH ₃	-	+

- 1) Reproductive toxicity is characterised by, a.o. testicular atrophy in test species (sertoli cells function <); this effect is often accompanied by decreases in testicular zinc levels and increases in urinary zinc excretion. There are significant species differences between rat, mouse, hamster and guinea pig, however no conclusion can be drawn which species in general less sensitive. Fertility (number of litters, live pups/litter, pupweight) is an insensitive endpoint of reproductive toxicity, because of the variations in control (historical) values.
- 2) Increased incidences of malformations; in general the doses required are high and often maternally toxic as well. The most common gross abnormalities found in phthalate-treated animals had not tail, twisted hind legs and hematomas. The most common skeletal abnormalities are fused ribs, absence of tailbones, incomplete/missing leg bones.

Ethyleenglycolethers

GENERAL STRUCTURE	EXAMPLE OF ACTIVE SUBSTANCE [TESTICULAR TOXICANT AND/OR DEVELOPMENTAL TOXICANT]
$\text{R}-\text{O}-\text{R}-\text{OH}$ Where R = aliphatic chain	$\text{CH}_3-\text{O}-\text{C}_2\text{H}_4-\text{OH}$ Ethyleneglycolmonomethyl ether (EGME)
$\text{R}-\text{O}-\text{R}-\text{O}-\text{R}$ Where R = aliphatic chain	$\text{CH}_3-\text{O}-\text{C}_2\text{H}_4-\text{O}-\text{CH}_3$ Ethyleneglycoldimethyl ether (EGdiME)
$\text{R}-\text{O}-\text{R}-\text{O}-\text{R}-\text{OH}$ Where R = aliphatic chain	$\text{C}_2\text{H}_5-\text{O}-\text{C}_2\text{H}_4-\text{O}-\text{C}_2\text{H}_4-\text{OH}$ Diethyleneglycolmonoethyl ether (diEGEE)
$\text{R}-\text{O}-\text{R}-\text{O}-\text{R}-\text{O}-\text{R}$ Where R = aliphatic chain	$\text{CH}_3-\text{O}-\text{C}_2\text{H}_4-\text{O}-\text{C}_2\text{H}_4-\text{O}-\text{CH}_3$ Diethyleenglycoldimethyl ether (diEGdiME)

Carboxylic acids

GENERAL STRUCTURE	EXAMPLES
 <p>Saturated, unbranched carboxylic acids</p>	 <p>Acetic acid</p>
<p>Saturated, branched carboxylic acids</p>	 <p>2-Propylpentanoic acid (Valproic acid) POSSIBLY HUMAN TERATOGEN!</p>
<p>Unsaturated carboxylic acids</p>	 <p>Acrylic acid</p> <hr/>  <p>4-Pentynoic acid</p>

ACIDS	STRUCTURAL FORMULA
2-Methylpropionic	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_2\text{-CH-COOH} \end{array}$
2,2-Dimethylpropionic	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_2\text{-CH-COOH} \\ \\ \text{CH}_3 \end{array}$
2-Methylbutyric	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3\text{-CH}_2\text{-CH-COOH} \end{array}$
2-Ethylbutyric	$\begin{array}{c} \text{CH}_2\text{-CH}_3 \\ \\ \text{CH}_3\text{-CH}_2\text{-CH-COOH} \end{array}$
2,2-Dimethylbutyric	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3\text{-CH}_2\text{-C-COOH} \\ \\ \text{CH}_3 \end{array}$
3,3-Dimethylbutyric	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3\text{-CH-CH}_2\text{-COOH} \\ \\ \text{CH}_3 \end{array}$
2-Methylpentanoic	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3\text{-CH}_2\text{-CH}_2\text{-CH-COOH} \end{array}$
3-Methylpentanoic	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3\text{-CH}_2\text{-CH-CH}_2\text{-COOH} \end{array}$
4-Methylpentanoic	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3\text{-CH-CH}_2\text{-CH}_2\text{-COOH} \end{array}$
2-Propylpentanoic	$\begin{array}{c} \text{CH}_2\text{-CH}_2\text{-CH}_3 \\ \\ \text{CH}_3\text{-CH}_2\text{-CH}_2\text{-CH-COOH} \end{array}$

RETINOIDS

ED₅₀ (umol/kg) median effective single oral maternal dose administered for induction of teratogene

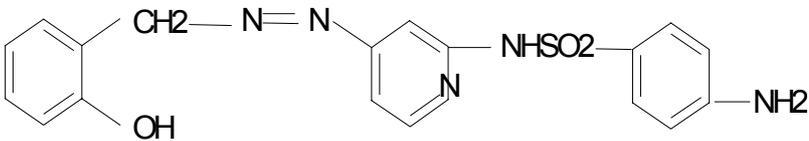
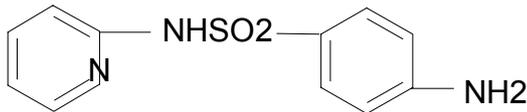
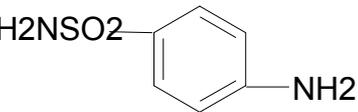
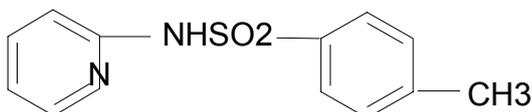
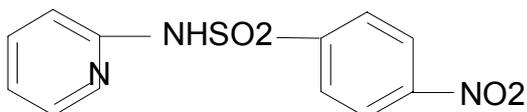
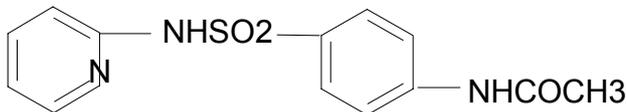
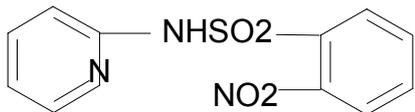
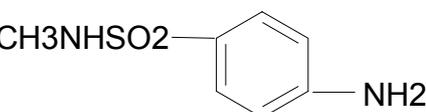
Common name	Examples	Structure	ED ₅₀ (umol/kg)
General structure			
All- <i>trans</i> -retinoic acid	(E)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid		35
SRI 2712-24	(E)-9-(2-ethyl-6,6-dimethyl-1-cyclohexen-1-yl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid		20
All- <i>trans</i> -retinal	(E)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenal		ND
All- <i>trans</i> -retinol	(E)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenol		76
13- <i>cis</i> -4-oxo-retinoic acid/Ro 22-6595	(2Z,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-3-oxo-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid		35
N-(2-hydroxyethyl) 13- <i>cis</i> -retinamide	N-1-(2-hydroxyethyl)(2Z,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenamide		Inactive
All- <i>trans</i> -retinylidene methyl nitron	(Z)-α-[(E)-2,6-dimethyl-8-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3,5,7-octatetraenyl]-N-methylnitron		130

Common name	Examples	Structure	ED ₅₀ (umol/kg)
hydroxenin	(Z)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)nona-2,4,6-triene-1,6-diol		>510
Ro 12-0995	Ethyl (E)-9-(2-chloro-3,6-dimethyl-4-methoxyphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoate		6
Ro 12-7554	Ethyl (E)-9-(2,6-dichloro-4-methoxy-3-methyl-phenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoate		ND
Ro 10-1770	(E)-3,7-dimethyl-9-[5,5-dimethyl-2-(1-methoxyethyl)-1-cyclopenten-1-yl]-2,4,6,8-nonatetraenoic acid		0.03
All-trans-5,6-epoxy-2-retinylidene - cyclohexane dione	2-[(E)-3,7-dimethyl-9-(2,2,6-trimethyl-5,6-oxacyclohex-1-yl)-2,4,6,8-nonatetraenylidene]-1,3-cyclohexanedione		ND
Ro 21-6667	(E)-8-(4-methoxy-2,3,6-trimethylphenylthio)-3,7-dimethyl-2,4,6-octatrienic acid		Inactive
SRI 5898-21	(E)-6-[2-(2,6,6-trimethyl-1-cyclohexen-1-yl)ethen-1-yl]-2-naphthalene-carboxylic acid		38
Ro 13-6307	(E)-7-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-3-methyl-2,4,6-octatrienic acid		1.95
Ro 13-2389	Ethyl(E)-7-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-3-methyl-2,4,6-octa-trienoate		2.3

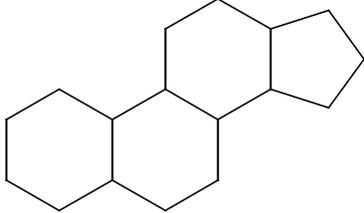
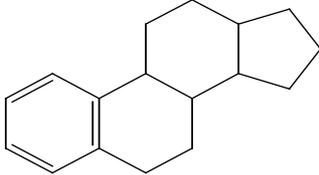
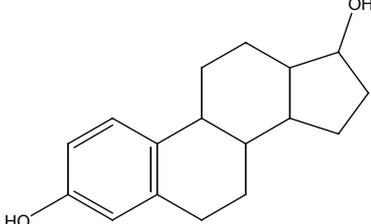
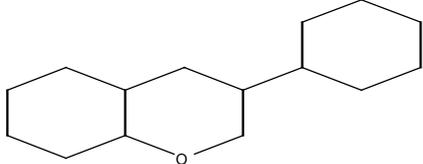
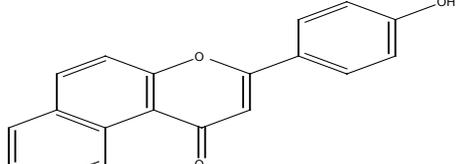
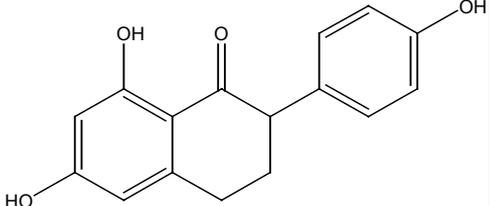
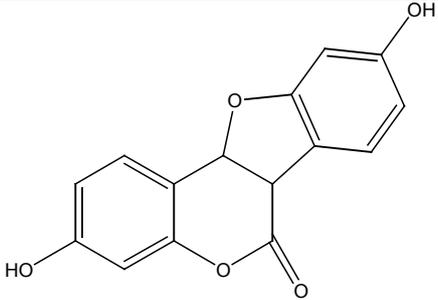
Common name	Examples	Structure	ED ₅₀ (umol/kg)
Ro 13-4306	(E)-3-methyl-7-(1,1,3,3-tetramethyl-5-indanyl)-2,4,6-octatrienoic acid		14.5
Ro 13-9272	(E)-5,6,7,8-tetrahydro-2-[1-(4-methylphenyl)propen-2-yl]-5,5,8,8-tetramethylnaphthalene		<0.11
Ro 13-8320	(E)-4-[2[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propen-1-yl]phenyl]methanol		0.085
Ro 15-1570	Ethyl(E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propen-1-yl]phenyl sulfone		82
Ro 13-6298	Ethyl (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propen-1-yl]benzoate		<0.053
Ro 15-1550	(E)-4-[2-(1,1,3,3-tetramethylindan-5-yl)-1-propen-1-yl]benzoic acid		ND
SRI 6409-94	N-(4-carbethoxyphenyl)-5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenecarboxamide		0.25
SRI 7323-78	(E)-6-[2-(4-carboxyphenyl)-1-propen-1-yl]-3,4-dihydro-4,4-dimethyl-2H--1-benzothiopyran		5
SRI 6153-40	6-(5,6,7,8-tetrahydro-8,8-dimethyl-2-naphthalenyl)-2-naphthalenecarboxylic acid		59

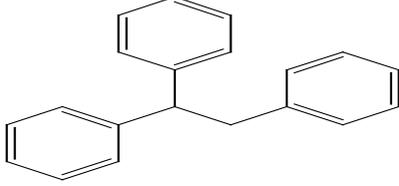
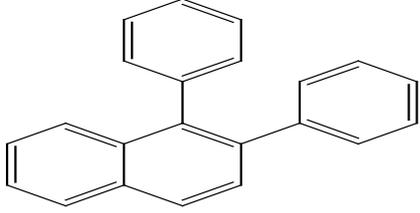
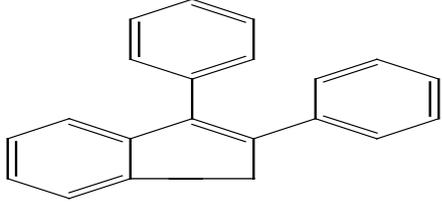
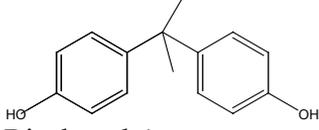
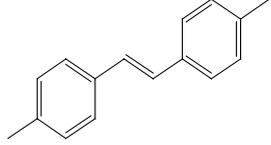
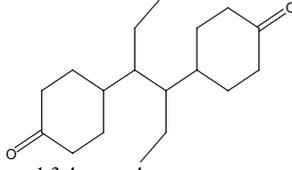
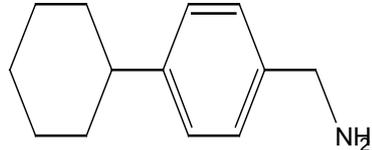
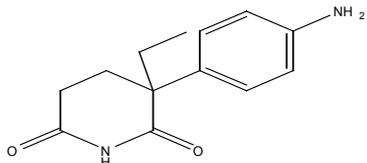
SULPHONAMIDES

Last column: Percentage inhibition fertility in study with rat at dose of 450 mg/kg/day after 5 weeks of treatment compared to the control.

Chemical name	Examples	% Inhibition of fertility
General structure	$R_1\text{-NHSO}_2\text{-R}_2$	
Salicylazosulphapyridine (Sulphasalazine)		100%
Sulphapyridine		25.9%
Sulphanilamide		18.0%
4-Methyl-N-2-pyridinylbenzenesulphonamide		89.4%
4-Nitro-N-2-pyridinylbenzenesulphonamide		56.2%
2-(N ⁴ -Acethylsulphanilamido-pyridine)		31.1%
2-Nitro-N-2-pyridinylbenzenesulphonamide		82.4%
N ¹ -Methylsulphanilamide		6.8%

GENERALISED STRUCTURES FOR SUBSTANCES DISTURBING HORMONAL BALANS IN THE ORGANISM

	General Structure	Examples
Steroid nucleus 1) Androgen	 <p>Cyclopentanephenanthrene</p>	
2) Estrogen		 <p>17-beta estradiol</p>
3) Flavone – like derivatives = phytoestrogenen		 <p>7,8-benzoflavone</p>
		 <p>coumestrol</p>
		 <p>genistein</p>

<p>Non-steroid nucleus: 1) Triphenylalkane, ene, anol derivatives</p>		
<p>2) Diphenylnaphthalene derivatives</p>		
<p>3) Diphenylindene derivatives</p>		
<p>4) Biphenyl derivative variants (including DES, DDT, Bisphenol and PRDX</p>		 <p>Bisphenol A</p>
		 <p>Diethylstilbenebistrol (DES)</p>
		 <p>d, 1,3,4-bis(4-oxocyclohexyl)hexane (PRDX)</p>
<p>5) Miscellaneous</p>		 <p>4-cyclohexylaniline</p>
		 <p>aminoglutethimide</p>

Appendix I Participants on the Workshop: SARs in human toxicology

Dick Sijm	RIVM/CSR
Etje Hulzebos	RIVM/CSR
Lidka Maslankiewicz	RIVM/CSR
Monique Meijerink	RIVM/CSR
Andre Muller	RIVM/CSR
Peter Janssen	RIVM/CSR
Theo Vermeire	RIVM/CSR
Louwrens Verdam	RIVM/CSR
Geert Janssen	RIVM/CSR
Marjan Draaijer	RIVM/CSR
Heleen van Groeningen	RIVM/CSR
Gerrit Wolterink	RIVM/CSR
Sjef Vos	RIVM/LPI
Hantze Stevenson	RIVM/LGM
Ferid Hajdarevic	RIVM/LGM
Cees de Heer	TNO/voeding
Annette Wilschut	TNO/voeding
Winfried Leeman	TNO/voeding

Appendix II Mailing list

- 1 RIVM: Directeur Generaal
- 2 Dr. D.W.G. Jung, DGM/SAS
- 3 Dr. H. Roelfzema, VWS/GZB,
- 4 Dr. C. De Heer, TNO/Voeding
- 5 W. Leeman, TNO/Voeding
- 6 Mw. Ir. A. Wilschut, SZW/Arbeidsinspectie, Roermond.
- 7 Sector Directeur Stoffen en Risico's
- 8 Hoofd Laboratorium Centrum voor Stoffen en Risicoanalyse
- 9 Hoofd Laboratorium Effectenonderzoek
- 10 Hoofd Laboratorium Pathologie en Immunotoxicologie
- 11 Hoofd Laboratorium voor Geneesmiddelenonderzoek
- 12 Hoofd Afdeling Voorlichting en Publicatie
- 13-22 Beoordelingsgroepen d.t.v. Mw. Drs. A.G.A.C. Knaap, Dr. F.X.R. van Leeuwen,
Drs. T.G. Vermeire, Mw. Dr. Ir. M.N Pieters
- 23- 30 Auteurs
- 31 Dr. B. Blaauboer
- 32 Dr. F. Hajdarevic
- 33 Mw. Ir. J.M.M. Herremans
- 34 Dr. A.A.J. van Iersel
- 35 Dr. C.J. van Leeuwen.
- 36 Dr. H. van Loveren
- 37 Drs. R. Luttik
- 38 Dr. A.H. Piersma
- 39 Dr. D.T.H.M. Sijm
- 40 Dr. W. Slooff,
- 41 Mw. Ir. H. Stevenson
- 42 Ir. P.T.J. Van der Zandt
- 43 Bureau Rapportenregistratie
- 44 Bibliotheek RIVM
- 45-70 Reserve exemplaren
- 71-90 Bureau Rapportenbeheer
- 91 Depot Nederlandse Publicatie en Nederlandse bibliografie