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RIJKSINSTITUUT VOOR VOLKSGEZONDHEID EN MILIEU
NATIONAL INSTITUTE OF PUBLIC HEALTH AND THE ENVIRONMENT

RIVM report 601516.001

(Q)SARs for human toxicological endpoints: a literature search.

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February 1999

This investigation is performed by order and for the account of MAP Directie, within the framework of project 601516, Gaps in the risk assessment.

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Samenvatting

Het doel van dit rapport is het beschrijven van humaan toxicologische SARs (structuur-activiteitsrelaties) die beschikbaar zijn in de literatuur alsmede de SARs die gebruikt worden door de US EPA (Environmental Protection Agency). De implemtatie van het gebruik van SARs voor de effect assessment bij CSR is onderzocht. Structuur-activiteitsrelaties (SARs) correleren the moleculaire structuur met biologisch/chemische of fysisch-chemische activiteit. In kwantitatieve structuur-activiteitsrelaties (QSARs) zijn deze correlaties gekwantificeerd. De (Q)SARs worden grofweg verdeeld in "rule-based" SARs en statistische SARs. The "rule-based" SAR gebruikt vergelijkbare stoffen (verzameld in chemische klassen) die hetzelfde werkingsmechanisme hebben. Tevens worden beschrijvers (descriptoren) afgeleid voor dit werkingsmechanisme om het effect van andere vergelijkbare stoffen te voorspellen. De statistische SAR baseert zijn voorspelling op statistisch verkregen beschrijvers van meer heterogene groepen stoffen. De ontwikkeling van de SARs gedurende de jaren hebben beide typen SAR nader tot elkaar gebracht. De gevonden (Q)SARs hebben verder validatie nodig voordat ze gebruikt kunnen worden voor de effect assessment. De onderliggende QSAR methodologie kan geïmplemteerd worden. Het voorspellen van het effect van een stof vergelijkbaar met een al eerder geëvalueerde stof of een chemische klasse wordt gebruikt op een ad-hoc basis bij CSR. (Q)SAR methodologie, chemische klassen, SAR kenmerken zoals electronische effecten, zouden geïmplementeerd moeten op een meer systematische manier om de expertise over SARs en om de helderheid van de effect assessment te vergroten.

Summary

The goal of this report is to describe human toxicological SARs (structure-activity relationships) available in literature as well as the SARs used bij the US EPA (Environmental Protection Agency. The implementation of the use SARs for the effect assessment at CSR is investigated. Structure-activity relationships (SARs) correlate the molecular structure with biological/chemical or physico-chemical activity. In quantitative structure-activity relationships (QSARs) these correlations are quantified. The (Q)SARs described are roughly divided in rule-based SARs and statistical SARs. The rule-based SAR uses similar chemicals (gathered into chemical categories) having the same mechanism of action and descriptors for this mechanism to predict the effect of other similar chemicals. The statistical SAR bases its prediction on statistical derived descriptors of more heterogeneous groups of chemicals. The development of SAR during the years have brought both types of SARs together. The QSARs found need further validation before they can be used for the effect evaluation. QSAR methodology can be implemented. Predicting the effect of a chemical similar to an already assessed chemical or chemical category is used on an ad-hoc bases for the effect assessment at RIVM/CSR. (Q)SAR methodology, chemical categories, SAR features such as electronic effects should be implemented in a more systemic way to increase the expertise of using SARs and to increase the transparency of the effect assessment.

1. Introduction

1.1 Structure-activity relationships

Structure-activity relationships (SARs) have been developed and used for predicting physico-chemical, toxicological and ecotoxicological properties. SARs are used for prediction of properties or effects of chemicals for which no or limited data are available, for elucidating mechanisms of biological activity, for building models. SARs are a correlation between the molecular structure and biological/chemical or physico-chemical activity (van Leeuwen en Hermens, 1995). SAR development started from different point of views and working environments. The development of SARs found great support in the pharmaceutical and agricultural industries (Rekker, 1992, (http://www.ibmh.msk.su/separt/function/projects.htm). In these areas they are mainly used to find active chemicals which show little side effects and also to limit animal testing. Hansch and co-workers (1962, 1964, 1969) initiated the development and use of SARs for a large group of disciplines in science.

One of the promises of SARs is that they may be used to predict toxicological effects to limit the number of animal testing (Barratt, et al., 1995). The use of SARs may be helpful for estimating the effects of chemicals for which little, insufficient or conflicting data are available. In addition, with the SARs available from literature a part of the expert judgement may be captured and therefore the thinking and reasoning of the experts may be mimicked. The US EPA (Environmental Protection Agency) has developed and used SARs for the evaluation of the Pre Marketing Notifications (PMN), since for these notifications usually few or no data are available, except for the structural formula (Wagner et al., 1995).

Before giving the reader a too optimistic impression regarding the possibilities of using predictions instead of real experimental data, it must be emphasised that predicting models always have limitations. It is extremely important to know these limitations. Only when those limitations are acknowledged the use of predictions is legitimate. For estimates in any risk assessment scheme, it is essential to evaluate carefully the reliability of the predicted property (Nendza and Hermens, 1995).

Definition:
Chemical modelling is based on the premise that a chemical's structure, molecular constitution and charge distribution determines its properties. The rationale is the establishment of causal relationships between features of the chemical structure on the one hand and the observed effects on the other. The quantification of the activity measures on structural descriptors is termed quantitative structure activity relationship (QSAR).

1.2 Goal of this report

SARs are available for several human toxicological endpoints and were reviewed by Cronin and Dearden in 1995 (a-c). However, it is unclear if and how the available SARs can be used for the effect assessment of chemicals at RIVM.
The goal of this report is to describe available (Q)SARs and types of (Q)SARs for human toxicological endpoints and to identify possibilities on the use of (Q)SARs for the (regulatory) effect assessment of chemicals. The following questions are addressed in the discussion of this report (Chapter 7):

1) Should SARs be used for the effect assessment?
2) What type of SARs may be used for effect assessment of chemicals at CSR?)
3) Does the RIVM/CSR has the expertise to use SARs?

1.3 Method

A literature search was performed to screen the available (Q)SARs.
An on-line search in TOXLINE PLUS (1990-1998), Medline EXPRESS 1966-1998, SERLINE, 1998, CD-ROM "Silverplatter" and Internet was performed using a search profile with the following key words: structure-activity relations, quantitative structure-activity relations, SAR, QSAR.

A further selection was made in the literature found. All human toxicological data, especially those referring to effect assessment, were included. Pharmaceutical and pesticidal QSARs are often based on slight changes in a single molecular structure and were therefore regardes as not directly as relevant for predicting effect assessment of chemicals.

Additional information about structure-activity relations was submitted by RIVM and TNO experts. Information was also found during a working visit of the first author to the US EPA (12 and 13 May, 1998) and to the SETAC QSAR Workshop in Baltimore (16-20 May, 1998).

1.4 Structure of the report

In Chapter 2 the (Q)SARs are further defined. The main types in (Q)SAR methodology are presented. These main types are further discussed in Chapter 3, 4 and 5 considering the chemical categories, the computerised systems and the (Q)SARs for toxicological endpoints, respectively. In chapter 6, the results of the working visit to the US EPA will be described. The project in which the predictive method for the risk assessment of new chemicals of US EPA is compared to the EC risk assessment method based on a base set of toxicological data, is also described in Chapter 6 (US EPA/EC Joint Project, 1993, EPA, 1993).

2. The derivation of SARs

2.1 Mechanism of action of chemicals

Several stages in the biological activity of a chemical can be distinguished in the whole process of the chemical reaching the target organ. A structure-activity relationship (SAR) study seeks to identify the essential feature of chemical structure which determines biological activity. This essential feature of the chemical structure may be part of the molecular structure or one of its properties. This implicitly assumes a causal relationship between fundamental molecular properties and activity (Richard, 1995, Barratt et al., 1995).

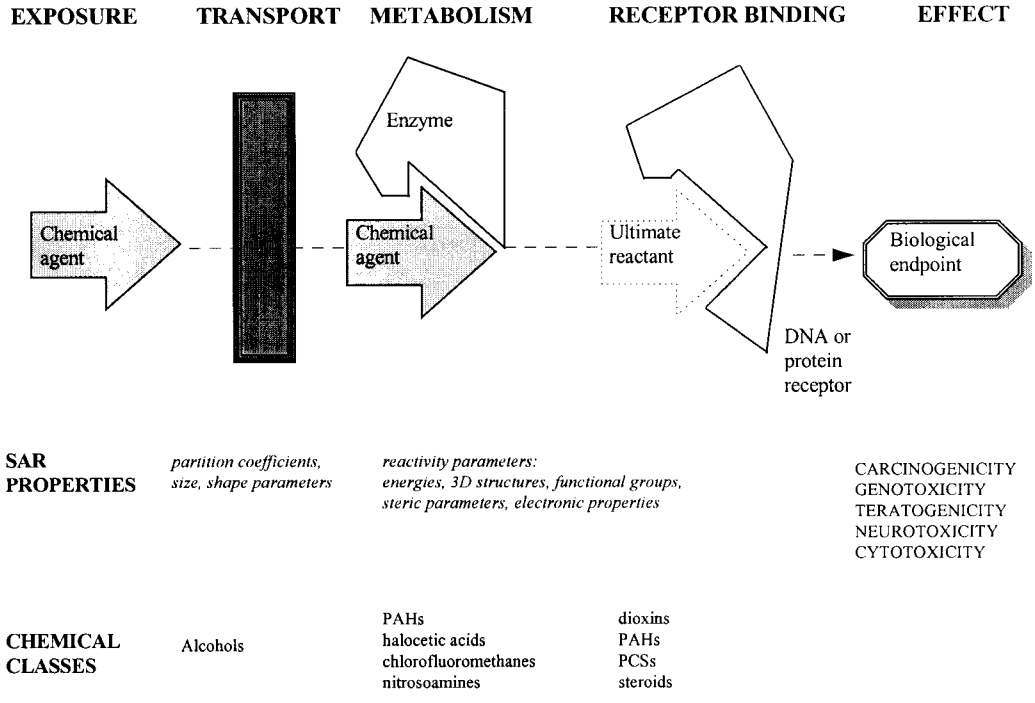

Fig. 1 Diagram representing various stages in a mechanism of action subject to SAR modelling and listing sample properties, chemical classes and biological endpoints. (Richard, 1995)

In Fig. 1 the whole process of biological action is shown schematically. It shows properties important for the various stages of biological action. It is often difficult (if not impossible) to determine the whole process of the biological action of a chemical. Therefore the determination of the essential feature of a chemical, which is responsible for the biological action, is as well difficult.
Structural activity relationships are often based on part of the process of biological action. The process of biological action can be divided in the uptake and distribution process (transport) and the reactivity process. These processes are called the toxico-kinetic and toxico-dynamic phase, respectively. Important properties of these phases used for structure-activity relations are described in 2.3. In 2.2 some properties and descriptors necessary for understanding SARs are elucidated.

2.2 Properties and descriptors for SARs

Fig. 1 shows (see also 2.3) that properties used for SAR can be divided in two types, those related to the toxico-kinetic and those to the toxico-dynamic phase. Properties used for SARs are estimated with different molecular structure properties. Fig. 2 shows two types of molecular properties: intrinsic, physico-chemical and biological activity. Molecular properties are often used as descriptors for SARs. Physico-chemical properties, for example partition coefficients are often used as descriptors for SAR properties. Connectivity indices are descriptors used for transport and steric properties.

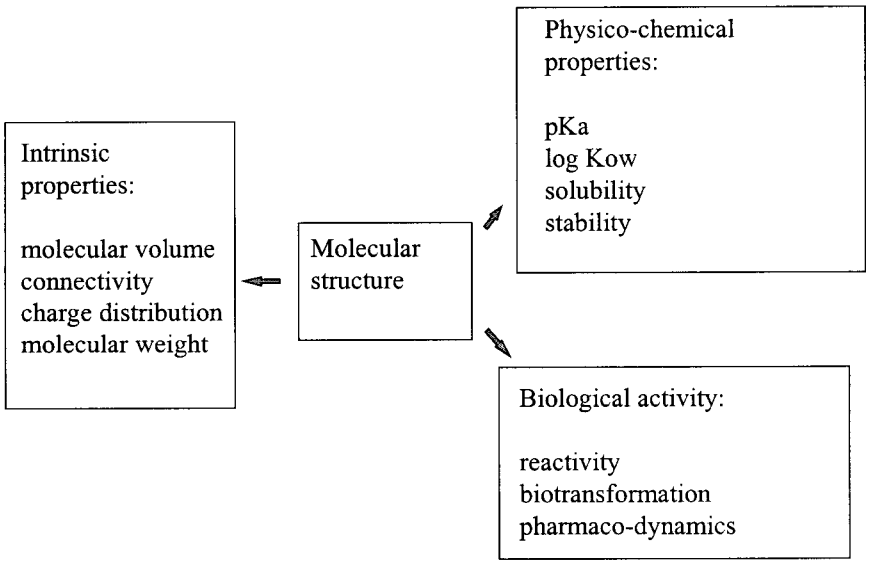

Fig. 2. Diagram of intrinsic, chemical and biological properties of molecules after Waterbeemd (1992).

Biological activity of molecules is caused by the interaction of the molecule with a biological target. If the biological activity is an unfavourable effect it is called toxicological action.

Another way of showing properties used for SARs is shown in Fig. 3. These properties were used for predicting carcinogenicity (Parry, 1994). From bottom to the top the properties of chemicals are derived from the whole of the molecular structure (structural analogues, chemical classes) or parts of the molecular structure to specific intrinsic properties derived from the molecular structure (K*e*).

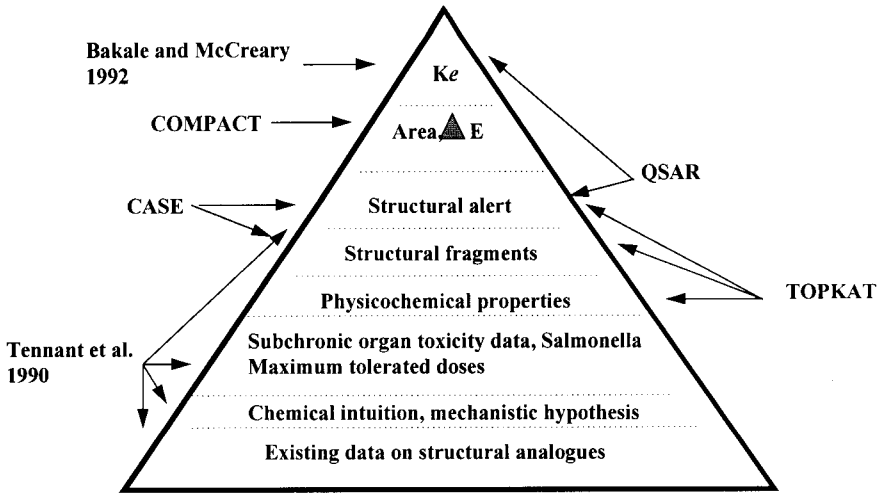

Fig. 3. Triangle of Richards after Parry (1994) showing structural activity features used by several expert groups predicting carcinogenicity.

For more information on SARs, the descriptors, their domains and their use, see Nendza (1998) who gives an overview of QSARs in ecotoxicology including a wide variety of QSAR descriptors.

2.3 SARs related to the mechanism of action

The process of biological action can be divided in the toxico-kinetic and toxico-dynamic phase (Fig. 1). Zomer (1989) described several theoretical chemical properties and descriptors often used in QSARs, which play a role in the biological action of a chemical. These will be summarised here.

Toxico-kinetic properties:
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. For exposure the stability and the volatility of the chemical are important. The stability of the molecule is important for the reactivity. If a chemical is very unstable in water and gives reactive molecules it will cause an immediate effect on the site of exposure . For example, NaOH is very reactive with water and will therefore cause a local effect. Stability is also an important property for estimating the binding of the molecule with receptors and other proteins. The volatility may give an estimation of the exposure target. Properties important for uptake are:

- molecular size (descriptors: molecular weight, molecular volume, refraction)
- lipophilicity (descriptors: log Kow, solubility in water)
- ionisation (descriptor: pKa/pKb)

Distribution is the process which determines the transport potential of the chemical to the receptor site. Similar chemicals with the same functional groups do not necessarily have the same transport mechanism or the same velocity transport. Important properties for distribution potential are:
- lipophilicity (descriptors: log Kow, solubility)
- protein binding
- kinetics (descriptor: uptake rate, enzyme activity, both are organism dependent)

Excretion is the process which determines the potential of the chemical leaving the target and the organism and will be determined by similar properties as mentioned at the distribution process.

Toxico-dynamic properties:
Biotransformation potential of the functional groups within a molecule is important as it gives information on the metabolites which may have other (new) functional groups. Biotransformation and the reaction of a chemical directly with its target depend on the reactivity of the molecule. The reactivity can be described with:
- Electronic properties effects, which cause reactivity.
- Steric properties, which are important for the reaction velocity.
- Kinetics which are important for the elimination rate of the chemical.

These electronic properties are divided in resonance and inductive properties and are difficult to separate. Structures related to resonance electronic properties are:
electron donating such as: O^-, S^-, NR_2, OR, OH, and Cl.
electronic withdrawing such as, NO_2, C=N, C-O-R, SO_2R, NO.

Inductive effects are a kind of electronic properties and are caused by polarisation of two atoms with different electron negativity binding:
Functional groups are:
electron donating such as: O-COO-, Cr^{3+}, CH_3, Deuterium.
electron withdrawing such as: NR^+, NO_2, SO_2, C=N, Cl and OR.

Steric properties are important properties for the reaction velocity. The reaction velocities were originally obtained from reaction-kinetics investigation. Taft (1952) quantified the theoretical energy relation between steric properties and reactivity for the first time but this is seldom used in QSAR equations.

Hansch et al. (1962, 1964 and 1969) concluded that uptake-distribution, electronic and steric properties are often sufficient to describe the toxicological effect.

2.4 Rule-based SARs

In the section above the use of SARs related to a general mechanism of action were described. SARs also often start from structural similarities between chemicals (Fig. 3). Similar chemicals, which are called structural analogues, used for SARs are presumed to have properties and reaction rates in common. Some structural analogues are gathered into chemical categories. In Fig. 4 examples of chemical categories are shown. It can be seen that

the categories can be either specific or wide. For example, the ethylene glycol ethers are a narrow group of chemicals but the cationic-dyes are wide.

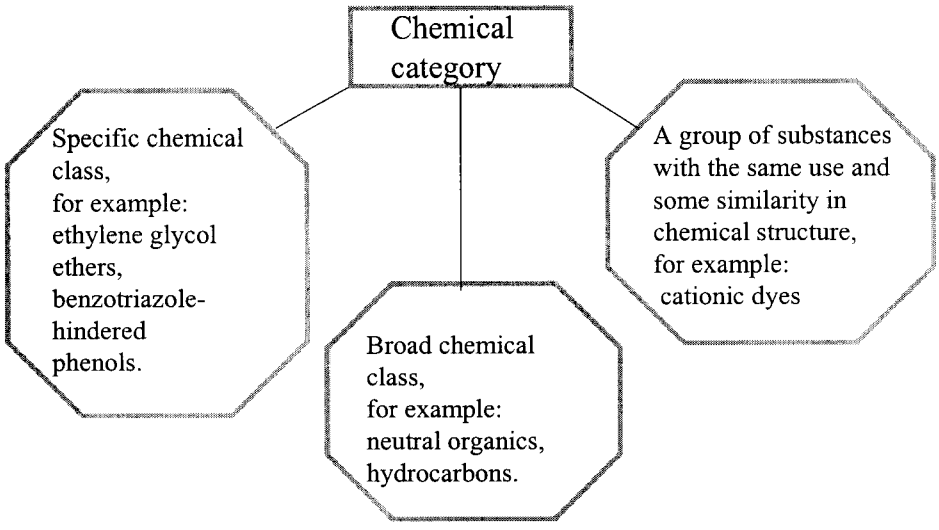

Fig. 4 Examples of chemical categories.

Another way of dividing chemicals into categories is described by Verhaar (1995). Chemicals are divided in types of ecotoxicological mechanistic action: narcosis, polar narcosis, reactive chemicals and specifically acting chemicals. Chemicals in these categories do not necessarily have structural features in common (Verhaar, 1995). The use of these categories for human toxicology as a framework should be considered as it shows the basic principles of QSAR methodology.

We focus in this report on the chemical categories with similar structural features and for which the same mechanism of action is assumed. Other methods not based on similar structural features are described in 4.3.2 and 5.1. In chapter 3 chemical categories, used for the prediction of human toxicological endpoints, are described. These categories are also the basis for rule-based SARs. Synonyms are mechanistic or empirically derived SARs. A rule-based SAR targets a single or a few stages in the overall mechanism where small changes in chemical structure elucidate the mechanism of action. This is why the restriction of a SAR investigation to a well-defined chemical class is central to the success of a rule-based SAR study, why SAR modelling in terms of chemical reactivity properties and insight into molecular mechanisms is important, and why development of a useful SAR is practicable and feasible (Richard, 1995).

2.5 The statistically based SAR

Two types of statistically based SARs will be introduced. The most used in ecotoxicology is the SAR based on "the linear free energy relationships" defined by Hansch and co-workers

(1962, 1964, 1968) and reviewed by Nendza and Hermens (1995, 1998). The other statistical SAR type is based on the occurrence of structural fragments in active and in-active chemicals.

1) The linear free energy approach
Biological action within a chemical category can also be determined in terms of a "continuous flow". Any alteration in the chemical structure should be reflected by a change in the degree of biological action. This function based on "linear free energy relationship" is generally described as:

T = f (S)

T being the measure of toxicity, for example, acute median lethal concentration (LC50). **S** is a set of numerical descriptors for one or more properties, and **f** a mathematical function. The equation may be quantified at any level of complexity ranging from a count of atoms or using log Kow as a single descriptor to sophisticated quantum mechanical indices. Depending on the variety within the chemical class more or less descriptors are necessary to calculate the toxicity.
A variety of statistical methods may be used, such as linear multiple regression analysis to neural networks to determine the explicit form of **f**. Statistical analysis is also often used to select the most important descriptor for a certain chemical category and/or toxicological endpoint.

The equation above is often transformed to a log equation, it then becomes:

Log **T** = a log (descriptors for distribution) + b log (descriptors for reactivity p) + c

A rationalisation of this equation can be given by considering that the activity of a biological active molecule depends on (Verhaar, 1995):
- the probability that a chemical reaches its (proposed) side of action
- the probability that the chemical will react with its (proposed) target at this site.

Especially in aquatic toxicology the effect concentration is often predicted with the above type of equation and using log Kow as a single descriptor. For example, the US EPA uses it for a wide variety of chemical classes in predicting the aquatic toxicity. They use the log Kow of these chemicals to predict the effect on aquatic organisms. This approach was studied for acute oral toxicity as well (Leegwater, 1989). For several other human toxicological endpoints this approach has been developed (see Chapter 5). Enslein and co-workers (Enslein et al, 1994) have used the approach in a computerised system TOPKAT for several endpoints, which will be further described in Chapter 4.

2) The presence of structural fragments.
Klopman and co-workers (1994) developed structure activity relations which are based on the presence of active and inactive substructures selected with statistical analysis. These substructures are selected without considering a mechanism of action. This model will be further described in Chapter 4.

3. Rule-based SARs

3.1 Chemical categories

Chemical categories for effect assessment can be wide or narrow (Fig. 4). They may have only a small molecular fragment in common, a so-called "structural alert" (see 3.2), or the similarities may be based on a large part of the molecule a "structural analogue" (see 2.4). Results of the literature search for chemical categories can be found in Appendix 1. Most information from this table is from the US EPA report (Moss, 1997) and is presented in alphabetic order. Appendix 1 is not intended to be a complete overview; it shows the possibility of listing chemical categories and their possible use for effect assessment.

Most of the presented chemicals have more than one relevant toxicological endpoint. For example, acrylamides have five reported endpoints, benzotriazole-hindered phenols have six reported endpoints, and ethylene glycol esters even have 12 reported endpoints. For a few chemical categories only one toxicological endpoint is considered e.g. ethers (narcotic potency), azo-dyes (carcinogenicity) and quinolones (antibacterial activity).
The boundaries of some molecular properties for toxicological activity are shown, for example:

1. Molecular weight (acrylamides, boron compound)
2. Details of structure formula (vinyl esters, quinolones)
3. Physical properties (log Kow: acetate esters, boiling point: ethers, halogenated hydrocarbons)

Sometimes the boundaries of more than one molecular property are given. At the other hand for some chemical categories the boundaries of molecular properties are not given at all (benzotriazole-hindered phenols, di-isocyanates). For chemicals with narcotic potential the boundaries are rather vague.

3.2 Structural alerts

A "structural alert" is a small fragment of a molecule (approximately 3-7 atoms) which is thought to be responsible for certain reactivity. The alert can be specific for endpoints such as mutagenicity or sensitisation (Karlberg et al., 1994) or may account for certain chemical activity, such as reactivity from strong H-donors.
For mutagenicity a theoretical structure was developed showing all possible mutagenic structural alerts, the so called "polycarcinogen" and is described below (Fig. 5, Miller and Miller, 1977).

The model "polycarcinogen" (also called "supermutagen") was based on the electrophilic theory of chemical carcinogenesis (F ig. 5). They identified the substructures in a parent molecule or its metabolite which were able to bind covalently with the nucleophiles

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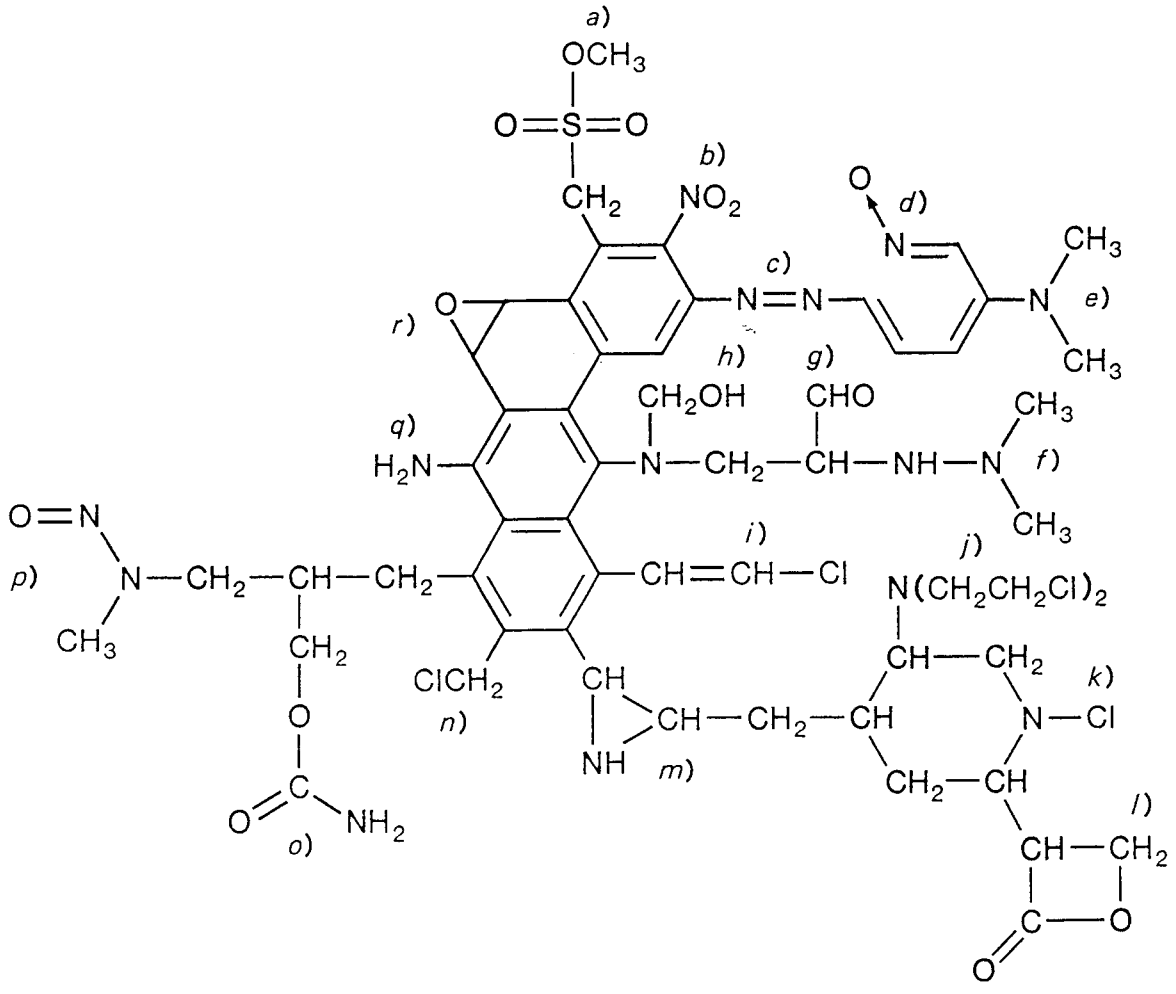

Fig. 1. Major structural units (letters) which led to a chemical being classed as structure–activity positive in Table 1. The substituents are as follows: (a) alkyl esters of either phosphonic or sulphonic acids; (b) aromatic nitro groups; (c) aromatic azo groups, not per se, but by virtue of their possible reduction to an aromatic amine; (d) aromatic ring *N*-oxides; (e) aromatic mono- and dialkylamino groups; (f) alkyl hydrazines; (g) alkyl aldehydes; (h) *N*-methylol derivatives; (i) monohaloalkenes; (j) a large family of *N* and *S* mustards (*β*-haloethyl); (k) *N*-chloramines (see below); (l) propiolactones and propiosultones; (m) aromatic and aliphatic aziridinyl derivatives; (n) both aromatic and aliphatic substituted primary alkyl halides; (o) derivatives of urethane (carbamates); (p) alkyl *N*-nitrosamines; (q) aromatic amines, their *N*-hydroxy derivatives and the derived esters; (r) aliphatic and aromatic epoxides. Qualifications or refinements of these units are discussed in the Methods section of this paper. The *N*-chloramine substructure (k) has not yet been associated with carcinogenicity, but potent genotoxic activity has been reported for it (discussed in Ashby et al., 1987). Michael reactive *α*,*β*-unsaturated esters, amides or nitriles form a relatively new class of genotoxin (e.g., acrylamide). However, the structural requirements for genotoxicity have yet to be established, and this unit is not shown in the figure.

Fig. 5. "Polycarcinogen" of Miller and Miller (1977) showing structural alerts, indicated by (letters), not red) responsible for the covalent binding with DNA.
u=aliphatic nitrogroup.

in DNA, RNA and proteins. These substructures were gathered in an theoretical molecule a so-called "polycarcinogen". This "polycarcinogen" was used to predict the outcome of the Salmonella tests of 222 chemicals tested for the (NCP/NTP) program (Ashby and Tennant, 1988). Circa 90% correlation was found between the prediction made by structural alert and the Salmonella assay.

Use of "polycarcinogen" in effect assessment
The model "polycarcinogen" for carcinogenic potency can lead to identification of potentially DNA-reactive chemicals. It may be used in a two-step decision tree:
Step one:

- Comparison of the (sub)structure of a new chemical substance with substructures of the "polycarcinogen". Basic chemical knowledge is required to compare the "new" chemical with the structural alerts.
- If this comparison gives a negative result, further analysis does not have to be performed. Otherwise go to step two.

Step two:
More precise comparison of the whole chemical structure of a new chemical compound (and not only substructures) with the data given in Ashby and Tennant (1988) might submit useful information:

- the "new" chemical is only similar in a small substructure.
- the "new" chemical is similar to one already found to be a proven carcinogen, according to the data of Ashby and Tennant (1988).
- if the "new chemical and the proven carcinogen are almost identical, data on relevant species (rat or mouse) and organs in which tumours occur, can be found in Ashby and Tennant (1988).

4. Computerised QSAR Programs

Several developed SARs have been computerised. In this chapter these programs will be described. In 4.1 the computerised programs are listed and categorised. Some examples of rule-based and statistical computerised programs will be described (4.2 and 4.3, respectively).

4.1 Types of SAR programs

In Appendix 2 the available software dealing with the prediction of toxicity (especially carcinogenicity) on the basis of the molecular structure of chemicals or other properties is summarised. This Appendix 2 shows the names of the programs, the Internet sites, the toxicological endpoints the programs predict, the level of expertise that is required for using the programs, the scope of the programs, a short description and the output file of the programs.

The programs can be categorised for their toxicological endpoints:
The rule-based programs which predict mainly carcinogenicity are RASH, COMPACT, the model of Purdy et al. (1996) and Oncologic. DEREK and HAZARDEXPERT predict for a variety of toxicological endpoints, see Appendix 2.
The statistical programs Progol and FALS predict carcinogenicity only. TOPKAT, MultiCASE (TOXAlert) predict for a variety of toxicological endpoints and for some ecotoxicological endpoints, see Appendix 2.

Rule-based vs. statistical programs
Both methods have their advantages and disadvantages. It is difficult to translate implicit knowledge of experts into explicit rules and therefore a rule-based program depends on the knowledge of the experts. At the other hand the process of analysis of a rule-based program is rather transparent. Statistical methods use relationships that may be statistically sound, but may lack scientific meaning. The statistical programs need relatively extensive training sets of data to formulate the various QSARs and algorithms. They should not be used outside the strict boundaries that are produced by the training set and calculated confidence intervals. The statistical programs may reveal new insights in toxicological mechanisms of action.

Accuracy, sensitivity, specificity
Accuracy can defined as the ratio of the number of correct predictions (active and non-active) and the number of chemicals analysed.
Sensitivity can be defined as the ratio of the number of correctly predicted active chemicals and the total number of active chemicals analysed.
Specificity can be defined as the ratio of the number of correctly predicted inactive chemicals and the total number of inactive chemicals analysed.

Validity
The authors of the programs sometimes present rather impressive performance statistics. However, the few independent validation and performances studies that analysed currently purchasable programs in a comparative fashion, generally present performances that are

generally lower (sometimes little better than random; Parry, 1994). Therefore extensive validation before purchase should be considered.

Only one real blind trial was found in which the programs were used to predict carcinogenicity for chemicals for which the outcomes were unknown. When the US National Toxicology Program (NTP) had developed an extensive cancer bioassay program Tennant et al. (1990) started to predict the outcome of the first 44 chemicals to be tested. Other groups were asked to do the same. Some years later the predictions were evaluated when the outcomes of the NTP bioassays were available. Parry (1994) evaluated the programs.

The program evaluation is shown in Table 1 (Parry (1994). It shows that the prediction of

Table III. Number of correct predictions

	Tennant *et al.*	K_c	DEREK	COMPACT	CASE	TOPKAT	RASH	QS.
1. Carcinogenic in both species, *five chemicals* (30, 38, 39, 43, 44)	5	5	4	3	4	3	3	4
2. Probable genotoxic carcinogens, *nine chemicals* (36, 37, 38, 39, 40, 41, 42, 43, 44)	9	4	7	7	6	4	4	5
3. Species specific carcinogens, *eight chemicals* (11, 16, 17, 20, 21, 26, 28, 32)	6	3	4	1	3	2	4	1
4. Carcinogen in male rodents, *three chemicals*, (11, 16, 32)	3	1	0	1	1	0	2	0
5. Carcinogenic in female rodent, *two chemicals*, (21, 26)	1	1	0	1	0	0	0	1
6. Non-Carcinogenic *seven chemicals*, (1, 4, 5, 8, 10, 19, 24)	6	4	5	2	4	0	6	1

Table IV. Predictions

	Tennant *et al.*	K_c	DEREK	COMPACT	CASE	TOPKAT	RASH	QS.
Number of correct predictions	30	18	20	15	18	9	19	12
%	88.2	52.9	58.8	44.1	52.9	26.5	55.9	35.

Table 1. Table showing the predictions of several SAR systems and of experts (Tennant et al., 1990), from Parry (1994),.

Tennant et al. (1990) gave very accurate predictions, for the carcinogens and probable genotoxic carcinogens. They also scored good for non-carcinogens. The computerised programs performed hardly better than random (Parry, 1994). According to Parry (1994) the accurate prediction of Tennant et al. (1990) was partly because they took into account short-term toxicity testing as this seemed to be the main difference between the prediction of the experts and that of the programs (Parry, 1994). Based on the carcinogenicity blind trial Bristol et al. (1996) concluded that programs based on a single QSAR-rule predicted less well than programs which used more properties. According to these authors, rule-based QSARs programs performed better than statistical methods (Bristol et al., 1996). It should be noted, however, that statistical programs have learned from the rule-based programs and the

other way around. Statistical programs start using expert rules and use chemical categories. Statistics becomes important in rule-based programs to select the best descriptor and to find the best relation between toxicity and descriptors. The outcome of the next chemicals of the NTP program will show if the predictability of the programs have increased.

Some additional remarks:
The correctness of the various algorithms and QSARs are a point of concern. Whether the output should be qualitative ("concern" vs. "no concern") or quantitative should also be determined. Some programs only determine parts of the molecular structure that represent a reason for concern, whereas other programs consider the molecule as a whole and some consider the parent chemical while others consider also the metabolites. Some programs demand powerful non-windows computers.

Below some programs are described in more detail. These examples are selected, because some more information was available.

4.2 Rule-based programs

4.2.1 Oncologic

The program was developed and based on the knowledge of the US EPA experts on carcinogenicity (Woo et al., 1997 and Appendix 2). Their program searches for structural moieties or fragments which may contribute to carcinogenic activity through a perceived postulated mechanism and evaluates the rest of the molecule to find whether the moiety or fragment contributing to carcinogenicity will be effective. Four functional criteria, are considered to further elucidate the carcinogenic potential:

1) data indicating effects on oncogenes and tumour suppressor genes
2) data indicating genotoxicity and/or ability of covalently binding to DNA
3) data indicating an epigenic mechanisms, including those which may cause endogenous or indirect genotoxicity
4) subchronic toxicity data/endpoints that may be indicative or suggestive of carcinogenic potential.

Based on the above considerations the carcinogenic potential is predicted. The program can handle a wide variety of chemicals including metals, fibres and polymers, but quite some expertise is needed to be able to use the program.

4.2.2 DEREK

DEREK (Deductive Estimation of Risk from Existing Knowledge) was developed by Sanderson and Earnshaw (1991) and updated by Ridings et al. (1996, see also Appendix 2). This program identifies toxicophores (segments of the molecule associated with a specific activity) and alerts the user to their presence, and gives references and examples. DEREK contains rules to identify toxicophores for adverse reproductive effects, carcinogenicity, irritancy, lachrymation, methaemoglobinaemia, mutagenicity, neurotoxicity, respiration and skin sensitisation. However, many of these areas are not comprehensively covered, notable exceptions being the rules for carcinogenicity and skin sensitisation (Cronin and Dearden, 1995c).

4.2.3 HAZARDEXPERT

HAZARDEXPERT developed by Smithing and Darvas (1992) shares many similarities with DEREK, in that it identifies toxic segments in a molecule and alerts the user. In addition, it provides species specific information across a range of trophic levels with different dosing regimes, whereas DEREK is designed to provide information on potential effect to humans. HAZARDEXPERT also calculates log Kow and pKa for each molecule and utilises this information to assess the relative bioavailability and bioaccumulation of a xenobiotic and how this will affect toxicity. It also contains a further expert system METABOLEXPERT that provides possible metabolites which can then be assessed in the HAZARDEXPERT system (Cronin and Dearden, 1995c).

4.3 Statistical programs

4.3.1 TOPKAT

TOPKAT is a computerised program that predicts several human toxicological endpoints such as irritation, sensitisation, LOAELs, reproductive and carcinogenicity toxicity. The program was developed by Enslein, Gombar and co-workers (1991, 1993, 1995 etc.). Their prediction methodology is based on the "linear free energy approach" which is a statistical method also often used in ecotoxicology (see 2.5).
TOPKAT QSARs are derived from databases with tested chemicals in which one or more toxicological endpoints are distinguished. The database is divided in a training set and a validation set. The training set consists of clear positive and clear negative and intermediate chemicals. The chemicals in the training set are scanned for reactivity properties, such as electron density charges, residual electronegativity and polarisability. For the uptake and distribution potential descriptors are used that quantify the shape of the molecule, some are atom specific and other correspond to the carbon skeleton of the molecule, such as connectivity descriptors. The latter are used to quantify topological features such as type and number of atoms and bonds and extent and position of branching and rings. Besides, the chemicals in the database from which the QSAR is derived, are used to serve as structural analogues to see whether there is a similarity between the chemical under investigation and the chemicals in the "library", the so-called Optimum Prediction Space (OPS). This use of the Optimum Prediction Space is well defined. If the chemical falls inside the probability of the outcome of the prediction is more realistic. From Internet a demo of TOPKAT is available which is summarised below.

A demo of TOPKAT can be downloaded from Internet. A "guided tour" is available for three example chemicals. For these examples predictions for three different toxicological endpoints can be calculated. For this program it is important to test a great number of chemicals to find the domains of this Optimum Prediction Space since the domains may contain a rather limited group of chemicals.
TOPKAT is not a very transparent system; you need to have a good knowledge of (Q)SAR theory and background is necessary to be able to understand the results. In addition, the program requires a SMILES notation input.

TOPKAT users
The TOPKAT program is used by the Canadian EPA for the effect assessment of chemicals. The Canadian EPA can only ask for testing (as the US EPA) if they consider a chemical "of

concern". They use TOPKAT to express their level "of concern" and were rather satisfied with the program. They admitted, however, that the assessment was not always transparent (personal communication with Mrs. Sitwell, Health Protection Branch, Ottawa, Canada). The Canadian EPA asks industry to submit tests based on the TOPKAT prediction.
The Danish EPA carries out a validity study on the mutagenicity prediction of TOPKAT and state a good prediction (personal communication, Dr. Jay Niemela, Danish EPA).

4.3.2 MultiCASE

This program originates from pharmaceutical industries for designing chemicals and was extended to human toxicological endpoints (Klopman et al., 1994a,b,c). They tried to uncover the relationship between chemical structure and biological activity without using mechanistic knowledge or known structures.

The first program they developed was called the CASE program in 1984 (Klopman, 1984). Klopman and co-workers filled the program with a training set when biological endpoints are set for the first time. This computer program analyses the biological activity of a given set of chemicals and identifies structural fragments of these chemicals believed to be responsible for the biological activity or for their in-activity. Biologically active fragments for a certain endpoint are called biophores, fragments for in-activity are called biophobes for a certain endpoint. A set of selected molecules showing a certain activity e.g. acids as well as non-acids. Both types of molecules are cut into fragments in a number of atoms. Once the training set is filled the program examines each of the fragments generated and evaluates its statistical distribution among the actives and non-actives in an attempt to identify those fragments that have the highest probability of being responsible for the observed activity.
The final outcome of such an analysis is an automatic selection, statistically sound, of the chemical structure most likely to produce activity. The CASE methodology has been extended during the years. The number of active and non-active fragments for certain biological endpoints has increased (Klopman et al., 1994a,b, 1995). In addition, for some endpoints, structural fragments determined by experts (biodegradation, Klopman et al., 1993) or rules for determining metabolisation abilities of chemicals have been included (Klopman et al., 1994).

The advantage of this program is that the selection of structural fragments of chemicals is solely based on probabilistic occurrence of active or inactive molecules. The selection of these fragments is not shadowed by expert knowledge which may overlook certain unknown fragments. Therefore this program may identify structural fragments, which were overlooked before by experts.
The disadvantage of this program is that structural fragments occurring in active molecules may not at all have a causal base. Molecules may have mutagenic properties and certain structural fragments as a combination, which may be a rather coincidental finding depending on the size and the heterogeneity of the training set. Therefore, the rules of logic should be watched. The fact that mutagenic chemicals have a certain structural feature in common does not imply that other chemicals with the same structural fragment are therefore mutagenic (most birds fly, but most flyers are not birds).

The information from Internet on TOXALERT is summarised below:
The TOXALERT program is based on the MultiCASE approach can be downloaded. For 15 chemicals toxicity can be predicted with existing and hypothetical structures. For predicting more chemicals the program can be restarted and another 15 chemicals can be tried. In the TOXALERT program structures are easily put in. The toxicity of the whole molecule is predicted and the biophores structures are made visible. The mechanism of action of these biophores may therefore be explained by toxicologists themselves. A marked option of this program is the use of exposure scenarios, doses and routes. It should be noted that the output is rather inaccurate (not the prediction) considering the number of spelling mistakes and the take over of the input structural formula to the output files. The transparency of the program is high.

5. Human toxicological endpoints

(Q)SARs for specific toxicological endpoints are described in this chapter. In developing (Q)SARs certain structural features and mechanisms of action of chemicals are elucidated for certain toxicological endpoints. This mechanism of action will be useful for the effect assessment and is therefore also included.

In three papers from Cronin and Dearden (1995a,b,c) an overview is given of (Q)SARs in human toxicology (and aquatic, not discussed here). This review from Cronin and Dearden (1995a,b,c) is based on articles about (Q)SARs until and including 1993. This review will not be extensively be summarised here. Other articles found but not cited in the overview of Cronin and Dearden (1995a,b,c) will be mentioned. Again it is pointed out that described literature is screened, only. However, it is thought that most relevant groups working in this area are mentioned.

5.1 Acute Mammalian toxicity

Hydrophobicity is commonly found to correlate well with acute aquatic ecotoxicity for chemicals showing non-polar narcosis. Several attempts have been made to use hydrophobicity as a the sole descriptor for acute mammalian toxicity. In the review of Cronin and Dearden (1995a) attempts were described to compare acute oral mammalian toxicity with acute aquatic toxicity. This lead to very dubious results. Some work has been done on the estimation of the oral toxicity for ketones for mice based on hydrophobicity (Cronin and Dearden 1995a). More reactive compounds cannot be modelled by hydrophobicity alone as is known in aquatic ecotoxicity as well (Verhaar, 1995). For a group of substituted anilines electric and steric properties were added to the equation to improve the QSAR (Cronin and Dearden 1995a).

Conclusion:
It seems to be quite difficult to predict a whole animal phenomenon such as is an LD50 value. For acute toxicity general QSARs do not seem to give useful predictions. QSARs for specific chemical classes may perform better. More validation, however, is necessary to establish the boundaries of the chemical classes.

5.2 Irritation

5.2.1 Skin irritation

The mechanism for skin irritation involves the exposure of the chemical to the skin. Thereafter two reactions are possible:

1) the reactive chemical causes an immediate effect (strong acids or bases) or
2) the chemical penetrates the skin first and causes an effect after passing the stratum corneum.

Barratt (1995, ECVAM workshop) did quite some work on skin corrosivity and gave some examples of the above mechanism. The SARs used by Barratt (1995, ECVAM, Barratt et al., 1996) for the analysis of skin corrosivity of organic acids, bases and phenols to rabbit skin

involved log Kow, molecular volume, melting points (descriptors for skin permeability) and pKa/pKb (descriptor for reactivity/electonic effects, for example strong H donors). The pKa/pKb desciptor have positive and negative effects on corrosivity. Ionisation lowers the skin permeability, but strong H donors increases the reactivity. The importance of these descriptors were detected with principal component analysis, a statistical method. Chemicals with lower log Kow values, larger molecular volumes were less likely to be found corrosive as well as chemicals with higher skin permeability and lower solubility, unless they were particularly acidic or basic. Properties relating to skin permeability generally dominated over those which determined cytotoxicity. For example, dinitrophenols were less corrosive than 2,4,6-trichlorophenol, because the ionised state of the dinitrophenols at physiological pH values lowered their effective partitioning into biological membranes. Barratt (1995) described several general chemical classes:

1) Inorganic acids, bases and oxidising agents were expected to have low skin permeabilities by virtue of their high polarities (electronic effects). They were possibly corrosive because they are able to erode the stratum corneum to get to the tissue beneath. For example, oxalic acid is very polar (little skin permeability) but very reactive (strong H donor, very low pKa).

2) Anionic and cationic surfactants have low skin permeability's due to charged head groups and long molecular volumes. As a category anionic surfactants do not appear to be 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.

3) Neutral organics: their skin permeability is generally greater than for inorganics or surfactants, because of their greater hydrophobicity. It is postulated that corrosivity for these chemicals result from the chemical first penetrating the skin; if it is sufficiently cytotoxic/reactive then the underlying cells are killed. For example, phenols show skin irritating properties, probably caused by their high skin permeability. Their cytotoxicity may be based on the uncoupling of the oxidative phosphorylation caused by the electron withdrawing effects.

Organic solvents cause defatting of the human skin which were 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). Organic solvent have special properties which solve fats. In human skin they extract the intercellular lipids from the skin and leading to loss of barrier properties and water retaining capacity of the skin, which is assumed to be the mechanism behind these adverse effects. This means that a delipidising effect occurs when the solvents can be highly dissolved in the skin lipids.

Conclusion: These mechanism of action define important properties for skin irritation.

5.2.2 Eye irritation

Cronin et al. (1994) used a number of chemicals for predicting the eye irritation potential. They could not find sufficient descriptors to describe the effect. They reasoned that the selected chemicals were reacting following different mechanisms of action and therefore were hard to quantify as a whole. The validity of the Draize eye irritation test was questioned. Therefore they found it also difficult to validate a QSAR with these data. Chamberlain and Barratt (1995) used the usual descriptors such as log Kow (passing a membrane) and dipole

moments (a measure for the charge distribution of the molecule and therefore a measur for reactivity) but also included an additional property for transport over the membrane. They calculated the cross-sectional area of the molecule. With the statistical use of principal component analysis and the use of a neural network they defined the importance of these descriptors. They found that chemicals with intermediate hydrophobicity, intermediate dipole moments and relative small cross-sectional width are eye irritants. Chemicals with zero or low dipole moments were not irritant to the eye (no reactivity, no irritancy). Posession of a large dipole moment, however will also tend to lower the polarity of a chemical (lower log Kow) and reduce its partition into membranes, and vice versa. The eye irritation potential of neutral organic chemicals was determined (in part) by the result of these two opposing features. (Chamberlain and Barratt, 1995, Barratt, 1997a and b).

Conclusion: These above described QSARs define important eye irritant properties

5.3 Sensitisation

5.3.1 Skin sensitisation

Generally the immunology and the mechanisms behind allergy are well understood. Karlberg et al. (1994) selected reactive centres (substructures) being responsible for sensitising activity. These reactive centres have been incorporated in the DEREK expert program (Chamberlain, 1997). Several QSARs were developed using statistics to discriminate between a variety of descriptors (based on a mechanism of action) which need to be calculated first, before using them for QSARs (Karlberg et al., 1994, Magee et al. 1994, Hostynek and Magee, 1997, Cronin (1996), Cronin and Dearden (1997). Graham. et al. (1996) selected substructures important for sensitisation based on MultiCASE approach (see 4.3.2).

Conclusion:
A list with reactive centres derived by Karlsberg (1994) or the DEREK expert program (see Appendix 2) is useful for the assessment of skin sensitisation. including multivariate QSAR analysis (Cronin and Dearden (1995c).

5.3.2 Respiratory Allergy

Respiratory allergy is a complex area of mammalian toxicology not only because of lack of consistent data, but also because of the lack of well defined and correctly identified respiratory allergens. However, Sarlo et al. and Gauggle et al. (as cited in Cronin and Dearden (1995c) described the use of structure-activity information, considering whether a chemical can modify carrier molecules and whether it belongs to a chemical category inducing hypersensitivity. This information was incorporated in a tiered approach to evaluate respiratory allergy. It should be noted that it is not yet clear why a chemical is a skin sensitiser and why a respiratory sensitiser.

5.4 Organ toxicity and determination of the NOAEL

QSAR models for predicting organ toxicity and the determination of NOAELS or LOAELS are very few, probably due to the complexity of the mechanism of action. The determination of NOAELS in 28-day and longer term studies is based on the toxicity on several organs. each of them having their own mechanism of action. In addition, the toxicity on an organ (e.g. liver, kidney) may be caused through several mechanisms of action. Developing general QSARs for all these different endpoints almost seems an impossible task. One of the problems is to select proper descriptors.

Mumtaz et al. (1995), related to the group of Enslein, Gombar and co-workers used an overall approach (see 4.3.1). They used databases of the US EPA and NCI/NTP (National Carcinogenic Institute/National Toxicology Program) of the US. They used topology-based methods as these were easier to calculate than molecular orbital methods and the outcomes of both methods were comparable. They found a 55% correct LOAEL prediction for the chemicals within a factor of two and a 93% correct LOAEL prediction for chemicals within a factor of five. It should clearly be stated here, as is also done by the authors, that data on the mechanism of action for the adverse effect is needed to determine causality.

Nephrotoxicity:
In a QSAR model the male rat nephrotoxicity: alpha-2μ globulin nephropathy, the mechanism of action of this specific nephrotoxicity was investigated Barratt (1994). A number of descriptors were selected which may be responsible for this specific effect. By principal component analysis the most important descriptors were selected. A wide range of aliphatic hydrocarbons could induce alpha-2μ globulin nephropathy. Hydrophobicity, metabolisation potential and binding of an electron negative atom were critical factors for binding.

Conclusion:
The only comprehensive QSAR program available for estimating organ toxicity is the TOPKAT model. The descriptors important for alpha-2μ globulin nephropathy may be used for the effect assessment.

5.5 Neurotoxicity

Cronin (1996) developed QSARs for the acute sub-lethal neurotoxicity of solvents. Earlier work on this subject indicated that hydrophobicity was one of the properties important for the effect. Also a number of other parameters was used: molecular volume, connectivity indices (for bulk and steric properties), melting point as a descriptor for aqueous solubility not interfering with log Kow, boiling point and others. Regression analysis and principal component analysis were used to find the most important descriptors. Log Kow and connectivity indices were important, so was melting point. A regression line with log Kow as the only descriptor was found and may be regarded as a kind of baseline toxicity. A comparison with the baseline of Könemann (1981) was also performed but was not satisfying. One of the problems is that not all the toxicity data on neurotoxicity are reliable and the mechanism is not fully elucidated and therefore establishing SARs is difficult.

Conclusion:
This model cannot be used at the moment since the mechanism of action is not sufficiently elucidated.

5.6 Reproduction and developmental toxicity

Effects of chemicals on the reproductive organs is of major interest at the moment, as part of the so-called endocrine disrupters issue. Though endocrine disrupters do not only involve reproduction toxicology, reproductive effects are dominating. The mechanism of action for reproductive toxicants is complex as several organs are involved, which are regulated by specific hormones. Slight changes in hormone levels may change the reproductive potential dramatically. Besides several mechanisms are involved inducing reproductive effects. Another problem of modelling developmental effects is the quality of the in-vivo data. There are a lot of studies available on developmental toxicity but not all were carried out properly or not all developmental endpoints were considered account.

QSARs were mainly developed for developmental toxicity and were reviewed by Cronin and Dearden, (1995b). Developmental toxicity is caused by several different mechanism of actions. Three categories of effects were identified:
1) nuclear (genotoxic) effects
2) cytoplasmatic (epigenic) events
3) whole embryo (organogenic) events.

QSAR modelling was done by Gombar et al. (1991) and was used for the TOPKAT program. They analysed a large heterogeneous database for their developmental effects. The chemicals were divided in main groups, heteroaromatics, carboaromatics, alicyclic compounds and acyclic compounds. The authors suggested that by splitting the data base in this manner common structural features may be related to specific modes of action and therefore the model predicted better. According to the authors the general specificity was over 90% and the sensitivity about 95%. In Cronin and Dearden (1995b) some other models were described for categories of chemicals: glycols, glycolethers, phenylhydantoins, phenols triazole alcohols and some short chain aliphatic acids.

Other, more qualitative information comes from experimental work in which congeneric series were studied. The retinoids and the glycol ethers are two group of chemicals on which quite some experimental work was carried out.

Retinoids belong to group of chemicals to which also vitamin A belongs. This group of chemicals is known for causing developmental effects. Studies examined the importance of the three main structural fragments of these retinoids (Saprano and Saprono, 1995). These three main fragments are:
1) the hydrophobic cyclohexenyl ring
2) the polar terminal group
3) the tetrahedral side chain as well as their specific-rigid three dimensional configuration.

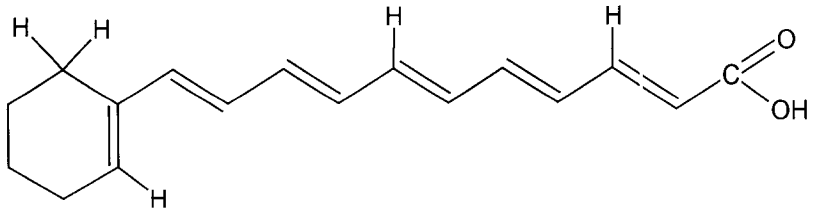

Fig. 6. Simplified molecular structure of a retinoid.

The terminal group being acidic or having the potential to metabolise to an acid is important for the developmental potential. The side chain is important, the length as well as the cis-trans configuration of the double bonds. Also the hydrophobic ring is required for the developmental potential: modification of the ring increases the developmental toxicity potential (Soprano and Soprano, 1995).

Glycol ethers are known for their developmental toxicity. Nagano (1983) studied some ethylene glycol alkyl ethers to determine the mechanism of action of this group of chemicals. The conclusion of the authors was that these chemicals probably cause their reproductive effects by their inhibitory effect on cell proliferation. However, the group is addressed being reprotoxic but not further boundaries are given.

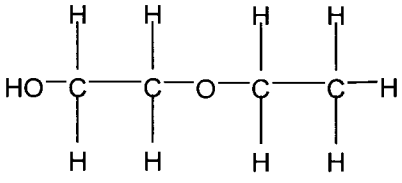

Fig. 7. Structure of ethylene glycol monoethyl ether

At the SETAC QSAR Workshop in Baltimore (16-20 May) several models were presented predicting reproductive effects. Most models presented were based on the same 40 chemicals known for their effects on the reproductive organs. Several 3D type of models were shown. Much attention was paid to the work of Mekenyan and co-worker who developed a technique to screen data sets of diverse structures for toxicological-active chemicals, with special reference to hormone receptor ligand binding affinity (Mekenyan et al., 1997). Further investigation is necessary to see if this is a useful approach.

Conclusion:
It is agreed with the conclusion of Cronin and Dearden (1995b) that most progress in reproduction and developmental toxicity is made by dividing chemicals into mechanistic categories. Thereafter reactivity and physico-chemical properties predict the developmental effect within one chemical category.

5.7 Mutagenicity and carcinogenicity

The carcinogenicity process can be subdivided in two mechanisms, the genotoxic and the non-genotoxic mechanism of action. Most carcinogenicity screening such as the mutagenicity tests and most modelling (except for the MultiCASE method), focus on a

genotoxic mechanism of action. Little work has been done on the prediction of the non-genotoxic effects of chemicals.

The predictions on the genotoxic mechanism of action of chemicals involve basically two properties of these chemicals:

1) Electrophilic properties causing electrophilic action of the chemical damaging the DNA. These electrophilic properties can be predicted qualitatively by the identification of substructures that account for electrophilic reactivity (see Fig 5). On the identification of substructures a lot of work has been done by Ashby and Tennant (1988) and Tennant et al. (1990).
   Electrophilicity of chemicals can also be measured by pulse conductivity (K_e) and may be calculated by several indicator variables as has been done by Benignini et al. (as cited by Cronin and Dearden, 1995b).
2) Distribution properties, which predict the potential of a chemical to reach the DNA.

The information derived for these endpoints were presented in section 3.2 on structural alerts and in Chapter 4 describing the computerised models.

Conclusion: Mechanistic features of chemicals about the uptake and distribution properties and reactivity combined with the results of available tests give the best predictive results but is not always available.

6. The US EPA Procedures

At the US EPA is the Environmental Protection Agency of the United States new and existing chemicals, pesticides and others are evaluated. In their evaluation procedure for new chemicals (Q)SARs are used for human and ecotoxicological endpoints for making a risk assessment. The general procedure of evaluating new chemicals is summarised in 6.1. In view of the experience with (Q)SARs special attention is paid to the use of (Q)SARs for human toxicological endpoints (6.2).
In 1992 the predictions of the US EPA scientists for several endpoints based solely on chemical structure were compared with the test results of the New Chemicals notified in the EC for which a base set test data were available (EPA, 1993). These results are described in 6.3.

6.1 US EPA procedure

The history of US EPA procedure

In the USA in 1976 the Toxic Substance Control Act ((TSCA) passed the Congress. The purpose of this act is "to protect human health and the environment by testing and necessary use restriction on certain chemical chemicals" (Di Carlo et al., 1985). Section 5 of this Act does not require any toxicity testing as a prerequisite for submission of a Premanufacturing/Premarketing Notice (PMN), but available data in possession and control of the submitter need to be presented in the time of the PMN submission. Some acute data and/or mutagenicity testing are available in 40% of the submissions. Few data are available on longer term or endpoint specific studies, including sub-chronic, developmental, reproductive studies. A chemical can only be considered of concern if the data provided are considered to be of concern, either because of the submitted and/or predicted intrinsic properties of the chemical or the estimated exposure in view of its use. (the chemical is innocent until it is proven guilty). These intrinsic properties consist of physico-chemical properties, acute and long-term aquatic toxicity and chronic, developmental and carcinogenic mammalian toxicity. Despite the few data submitted for a PMN the EPA wanted to perform a risk assessment for new chemicals. Therefore the Agency assembled a group of discipline experts, to systematically review all new chemical submissions, the so-called Structure Activity Team (SAT), who established a risk assessment based on the submitted data and predictions for physico-chemical, ecotoxicological and human toxicological endpoints (Wagner, 1995).

The history of the EC procedure

In the EC a new chemical needs to be notified since 1981. For new chemicals which are marketed and in the EC at > 1 ton a base set of data needs to be provided. These data include physico-chemical properties (e.g. melting and boiling point, water solubility, log Kow, vapour pressure), human toxicological data (acute oral and dermal toxicity, irritation studies, sensitisation, 28-day sub-acute test, two in-vitro mutagenicity tests) and ecotoxicological and environmental fate data (acute fish, Daphnia and algae test, biodegradability and a hydrolysis test). Based on these data the chemical can be classified and labelled according to the EC

Directive 92/32/EEC (1992). The EC notifications do have data on which the risk characterisation and risk assessment is based. Predictions are usually not necessary for the above described endpoints. If concern is raised based on these data further testing may be required.

The US EPA procedure versus EC procedure

The risk assessment part of the PMN process at the US EPA is similar to the EC process for new chemical considering the risk assessment, which can be divided in three parts:
1) The effect assessment,
2) The exposure assessment and
3) Risk characterisation.

The main difference between the US EPA and the EC is that the US EPA needs to predict the possible effects whereas the EC has test data. This difference has caused a different US EPA and EC philosophy considering chemicals. In the EC the effect assessment is the first assessment and the philosophy exists that independent on the exposure potential of a chemical data on the intrinsic properties of the chemical should be available. These data are used for classification and labelling and risk characterisation. In the US process the exposure assessment is dealt with first. If the exposure is estimated to be low either because the tonnage level is low or because of the use pattern of the chemical (e.g. intermediate in closed systems), the chemical may be regarded of "no concern". Thereafter the bioavailability is regarded, for example, a chemical which cannot enter biological systems (very high molecular weight chemicals) are considered of "no concern" and further assessment is not performed.

The US PMN process

In the US process the Structure Activity Team (SAT) plays a crucial role in assessing the new chemicals. The PMN process can be divided into 3 parts: pre-SAT, SAT and post-SAT activities considering the effect assessment only. Experts peer review the basic information submitted in order to identify the structural formula and chemical name, the general chemistry, including process chemistry. During this peer review (so-called Chemical Review and Search Strategy, CRSS) already a first selection of chemicals is made for which no future evaluation is necessary (chemical of "no concern') or of chemicals for which there is high concern and regulatory action needs to be taken, immediately. For all other submitted chemicals a search strategy is executed by information specialists and the resulting data are included in the dossier that includes:
a) Chemical analysis
b) Identification of structural analogues
c) Literature search for toxicity data
d) Other environmental exposure estimations.

Database searches include internal US EPA confidential as well as publicly available databases. Data on structural analogues and the chemical itself, if any exist, are retrieved and added to the dossier. If a previous PMN chemical is identified as a suitable analogue, the record of the case is retrieved in order to review any data that may have been received initially in response to testing requirements.

After this first CRSS (Chemical Review and Search Strategy) meeting a SAT-meeting is planned 24 h later.

SAT-meeting.

In this SAT-meeting possible effects of the submitted chemicals are assessed. Expert scientist form a team, that does an initial review and evaluates the potential environmental fate, health and environmental effects of new chemicals. The scientific disciplines represented on this peer review are: chemistry, environmental fate, ecotoxicity, absorption/metabolism, mutagenicity, oncogenicity, developmental/reproductive toxicity, neurotoxicity, acute toxicity and subchronic/chronic toxicity. Every discipline presents the relevant parameters at the SAT meeting. In general the octanol/water partition coefficient, water solubility, the adsorption to sludge and sediment, percent removal from the STPs (Sewage Treatment Plants) (and ultimate biodegradation are always estimated and presented. The first two are used by the health and ecological assessors as factors for their estimation of the bioavailability. The other ones are used as indicators of potential environmental exposure. After the presentations of the different disciplines concerns and the level of concern are expressed. The concerns depends on the submitted toxicity data, strength of the analogues and their potency, known toxicity of certain chemical classes/moieties and most importantly, the knowledge and judgement of the discipline experts. When all the above information for each of the chemicals has been imparted to the chairperson, the meeting is completed. The chairperson is then responsible for the final phase, the post-SAT phase.

Post SAT activities

After the SAT-meeting the chairperson completes the effect assessment, which will be used by the exposure (consumer, engineers and environmental) assessors. Thereafter a risk characterisation is performed in the so-called FOCUS meeting.

The possible outcomes are:
1) drop the case from further review
2) hold it over for more investigation (standard review)
3) move directly toward a regulatory outcome for certain standard categories of chemicals.

If the risk assessment team decides that there is insufficient information , the chemical is placed into a more extensive review called a Standard Review. A standard review is indicated for circa 5% of the PMN chemicals.

6.2 Effect assessment of human toxicological endpoints

First the notified chemical is categorised in a chemical class and active and inactive structural fragments are also determined. Properties derived from the chemical structure include the potential of a chemical to hydrolyse and metabolise. The physico-chemical properties are predicted with several models that are included in the EPIWIN program (1997). Also the Clog P database is used. The outcomes of the programs should be in the domains of the models and confirmed by expert judgement. The prediction of the human toxicological endpoints starts with possible relevant exposure routes (oral, dermal and/or inhalator). The

experts predict the mechanism of action of the chemical and the likelihood of occurrence of this action based on the physico-chemical properties. Thereafter they search for structural analogues in confidential and non confidential databases for similar chemicals. When structural analogues are found, their structure, melting point, log Kow and molecular weight are used to compare the similarities of a new chemical with the analogue. Endpoints which are considered are:

1) chronic toxicity
2) reproductive and developmental toxicity and
3) carcinogenicity.

The assessment of the human toxicological endpoints is semi-quantitative only. After the assessment classes of concern (low, moderate or high concern)are provided. Based on these concern levels further testing is required. The US EPA experts developed a computerised carcinogenicity program called Oncologic (Woo et al. 1995 and 1997, see 4.3.1. The US EPA does not use the Oncologic model because all the rules used in this model are still available from an expert working at the Agency. For other endpoints human toxicological endpoints expert judgement is used.

Groups of structural analogues with known toxicological properties are combined to chemical categories. A set of 35 non-confidential chemical classes used for effect assessment are published (Moss, 1997, see Chapter 3).

6.3 US EPA/EC Joint Project.

In the US EPA/EC Joint Project (1993) a comparison was made between the US EPA predictive method and their SAR approach and the test data derived from the EC chemicals at base-set. In this US EPA/EC (1993) project report it is described for how many chemicals proper predictions for various toxicological endpoints have been made by the US EPA experts. For this comparison circa 300 chemicals were selected. The US EPA decided to leave those chemicals out for which they already had performed a risk assessment in the US. Therefore the outcome of the comparison would not be positively shadowed by chemicals assessed earlier at the US EPA. It should be noted that prediction of the acute oral toxicity, skin, eye irritation, sensitisation is not usually part of the routine evaluation of a new chemical in the US, but was considered in this US EPA/EC Joint project (1993).

Absorption:
The likely extent of absorption of a chemical via skin, lungs and gastro-intestinal tract is predicted by the US EPA experts as well as the exposure routes on the basis of the physico-chemical properties of the chemical, particularly the log Kow, which is usually a predicted value, and the physical form of the chemical. The absorption potential is qualified in terms of good, moderate, poor or no absorption. No cut-off values were mentioned in the report. However, if the EPA experts predict "no absorption", then the chemical is dropped from further review and needs not to be assessed further.

None of the chemicals classified in the EC for acute oral toxicity were predicted "no absorption" by the US EPA. Some of these chemicals were however predicted with "low concern" (12%) while in the EC they were classified with "harmful if swallowed".

The absorption rates of chemical estimates by the US EPA for the dermal and inhalation route could not be compared with the studies conducted in the EC as too few studies were available.

Structural analogues:
In the report the chemicals investigated were categorised in chemical classes. The predicted effects of these classes were based on confidential structural analogues and cannot be evaluated by others. Only in a single case the chemicals belonged to a category published by the US EPA (Moss, 1997). Therefore it was difficult to generalise the effect assessment of these derived classes.

In the US EPA/EC Joint project (1993) it was mainly discussed how well the predictions were. The first step in estimating effects is to predict the absorption potential of the chemcial. Absorption classes: good, moderate, low and no absorption were derived in the case of acute and systemic toxicity. However, the basis and domains of these classes were not given. Therefore the predictions of the US EPA are not tracable. The predictions of systemic, developmental toxicity, mutagenic and/or carcinogenic potential were based mostly on confidential structural analogues and are therefore not tracable. The results of human toxicological endpoints that can be detected with positive or negative are summarised below. During the US process the eye and skin irritation and sensitisation potential is not assessed. For acute effects the US predictions correspond to the EC results between 78-88% of the time. Eye irritation had the lowest correspondence between predicted and measure value and dermal irritancy the highest.

Skin irritation (n = 144):

	US SAR positive	US SAR negative
EC data positive	14	8
EC data negative	18	104

The US EPA showed a false negative prediction for 8/22 positive EC data and 18/122 false positives. For risk assessment purposes the false negatives cause more concern than the false positives. The false positives will be account restricting the exposure by means of classification in the EC and/or personal protection equipment.

Eye irritation (n = 144)

	US SAR positive	US SAR negative
EC data positive	26	13
EC data negative	18	87

The US EPA showed false negatives for 13/39 positive EC data and 18/115 false positives.

Skin sensitization (n = 144)

	US SAR positive	US SAR negative
EC data positive	9	19
EC data negative	4	108

The US EPA showed false negatives for 19/28 and false positives 4/112. For skin sensitization they underestimated the effect.

Mutagenicity (n = 139)

	US SAR positive	US SAR negative
EC data positive	12	6
EC data negative	14/2	107

For mutagenicity 139 chemicals were used for comparison. For the mutagenicity data set disagreement between the US EPA and EC (20 chemicals out of 139) could be attributed to the use of inappropriate analogues (3/21 chemicals), 2 out of 21 were due to the lack of positive analogue and weak or marginal positive response reported in the EC data and four were due absence of analogue mutagenicity data. The remaining 12 may be false negatives by the testing methods of the EC as the standard test procedures are known to be insensitive to specific classes of chemicals.

For systemic toxicity, exclusive of developmental and reproductive toxicity, neurotoxicity and carcinogenicity, the concern levels were scored as was the severity of the effect. The results of the comparison showed that for 57% for the 138 chemicals assessed the scores were identical and for 43% the scores disagreed. Further analysis revealed that the US tends to under-predict systemic toxicity, the effects as well as the severity observed in the 28-day sub-acute tests of the EC base set. The magnitude of the differences between the US predictions and EC data was rather small. The EC data suggest low-moderate concern while the US SARs predicted low concern. Toxicological endpoints not addressed in the base set (developmental and reproductive toxicity, neurotoxicity and carcinogenicity) but for which data were available were folded into the analysis. The US SARs predicted well in 78% of the cases. Those chemicals which had no test data for the mentioned endpoints were predicted by the US SARs. Of the 143 chemicals 66 had concerns: 32% developmental concerns, 9% had reproductive concerns, 23% had carcinogenicity concerns, 15% had neurotoxicity had concerns. The large number of chemicals that were predicted to have effects not addressed by the EC data suggests the chemicals notified in the EC need to be screened for these tests.

7. Discussion

The foregoing chapters will be discussed answering the following questions:
1) Should SARs be used for the effect assessment?
2) What type of SARs may be used for effect assessment of chemicals at CSR?
3) Does RIVM/CSR has the expertise to use SARs?

1) Should SARs be used in effect assessment?

Effect assessment of chemicals.
For the effect assessment of chemicals data are usually available in Europe. If there are more data necessary in view of a certain "concern" industry can be asked to submit these data. According to the Technical Guidance Document (TGD, 1996) further testing for chemicals may be initiated earlier if SARs are available, which show "concern" for a certain endpoint. (TGD: repeated dose studies, pg. 92, mutagenicity pg. 111, carcinogenicity, pg. 122, developmental toxicity, pg. 139). The TGD however does not give SARs for human toxicological endpoints (they do for some ecotoxicological endpoints and environmental fate). In the effect assessments of the EC for New Chemicals structural analogues are used to express "concern" for newly submitted chemicals, which are evaluated on a case by case base. For the regulatory assessment of chemicals the use of SARs are considered necessary to express levels of "concern" and therefore initiate further testing. It can also decrease the level of concern and limit animal testing. For other than regulatory submitted chemicals for which few or equivocal data are available SARs can be used. The TGD stimulates the use of SARs especially to express "concern" levels.
At CSR SARs are used on an ad-hoc basis. The TGD gives opportunities for the use of SARs more systematically. More expertise on the use of SARs is necessary. Using SARs will also lead to:
- A better understanding of structural features and mechanistic action of chemicals
- A more transparent assessment of chemicals.

Limitations and recommendations when using SARs
The use of SARs is only possible if expert judgement is included to see whether the used SAR is still applicable for a chemical under investigation. This expert judgement is necessary for all SARs. For chemical categories and/or structural analogues it should always be reasoned whether the chemical belongs to the category or not and whether the analogue is expected to have similar biologically and/or chemically properties. As the computerised programs are not validated the outcome of the predictions should be in agreement with the opinion of the experts.

2) What type of SARs may be used in effect assessment?

The chemical categories of the rule-based SAR in Appendix 2 are fairly well defined and referenced and can be used for the effect assessment. The computerised programs all need validation. Among the statistical based computerised programs TOPKAT offers the most information. This program has the advantage that it uses mechanistic properties (active substructures and transport properties). It also compares input structures with database structures for finding structural analogues, which can be used as an internal validation.

However, it does not predict a causal relation (a mechanism of action) between structural features of the chemical under investigation and the predicted outcome.

Conclusions about the described SARs:

1) The (Q)SARs have not been externally validated. The only exception found is the prediction of the outcome of the carcinogenicity testing of the NTP (Parry, 1994). The uncertainty of the described SARs is high, they may predict false positives as well as false negatives. The development of SARs during the years have brought rule-based and statistical SARs together. Statistics become important for selecting descriptors for the rule-based SAR. Statistical SARs narrow the heterogeneous groups to relate more to the mechanism of action. Both are so improving their predictions.
2) (Q)SAR methodology, chemical categories and structural alerts for mutagenicity are necessary for understanding and predicting mechanism of actions of molecular structures. These can be implemented for effect assessment, but needs expertise.
3) The procedures and methods used by the US EPA for predicting human toxicological effect uses a lot of expert judgement and structural analogues of (non)-confidential databases for predicting effects but limits testing. The published chemical categories, the carcinogenicity model (and ecotoxicity models) are accessible for outsiders. The expert judgement on confidential structural analogues is not available.
4) The described SARs are developed for a select group of usually organic chemicals. These SARs can often not be used for other type of chemical such as metals, polymers or mixtures.

3) Does RIVM/CSR has the expertise for using SARs?

Before the described SARs can be used at CSR more knowledge and experience is required. Humane toxicologists and effect assessors at RIVM are hardly familiar with using SARs and therefore the outcome of SARs should be extensively explained why a certain effect is expected The mechanism of action should elucidated as much as possible: what kind of chemical is it, is absorption expected, does metabolisation occur, does the chemical has structures which indicate activity etc.

The use of models can only have good results if the user knows what to put in and can evaluate what comes out. Garbage in and garbage out accounts for the use of models in general and also for (Q)SAR models. Therefore only SARs should be used which can be understood and explained by toxicologists.

Recommendations

Despite the limitations of the available SARs, the use of SARs including expert judgement increases the quality and transparency of the results of the effect assessment. This is possible as SARs are already used on an ad-hoc bases. The expert judgement for SARs needs further development. As the use of chemical categories is close to the every day working practice of the effect assessor and experts, this way of working seems to be a good starting point. Besides, SAR methodology often start or end with chemical categories to increase the prediction of the SAR. At CSR a study program should be started to increase the knowledge on SARs. This program should start with general QSAR knowledge on the mechanism of action of chemicals and which structural features determine hydrophobicity, reactivity and steric effects and how these properties can be qualitatively or quantitatively be determined. Chemicals for which the effect assessment is performed can be used as examples as well as already chemical categories with a "known" mechanism of action. Also the QSAR programs described in literature give information on structural features and toxicological endpoints.

Some computerised programs can be validated with the extensive reliable data at CSR. The following programs are proposed for validation: TOPKAT, HAZARDEXPERT and DEREK. TOPKAT is proposed because it is based on a similar general methodology as used for most structure-activity relations in ecotoxicity. In addition, some validation is being carried out by the Danish governmental organisation involved in evaluation chemicals. HAZARDEXPERT and DEREK are proposed because these are rule-based programs, using chemical categories, having a more mechanistic approach, which may be more transparent.

If only one computerised program can be selected TOPKAT is proposed. It uses a similar methodology for human toxicology as for ecotoxicology and in ecotoxicology this methodology is widely used. In addition, TOPKAT can predict the whole range of human toxicological and ecotoxicological endpoints and therefore gives good opportunities for validations.

Acknowledgements

I would like to thank the people from the US EPA who made my visit possible: Dr. Oscar Hernandez who facilitated my visit. Dr. Tom McClintock who guided me through the human health section, the ecotoxicological section and introduced me to the experts working with PMN notifications. The discussions with you all introduced me to working with (Q)SARs. It helped me a great deal with insight in this field. Though not all the information is put into this report, the information is and will be used for the risk assessment for New Chemicals in the Netherlands and hopefully also for the risk assessment of other chemicals that need to be evaluated.

I want to thank Drs. Theo Vermeire for commenting on several drafts as well as Dr. Hans Könemann, Drs. Robert Luttik and Dr. Jack de Bruin for their comments. Especially I want to thank Dr. Dick Sijm for his positive and fruitful discussions and comments.

I want to thank Mw Ir. Annette Wilschut, Dr. Aldert Piersma, Drs. Arthur van Iersel, Dr. Jan van Benthem and Dr. Gerrit Zomer, Mw. Marian van Apeldoorn and Louwrens Verdam for submitting the literature and their comments on the subject.

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8. Appendix 1: Table with chemical categories

In this table chemical categories are listed, mainly derived from Moss (1997). See also chapter 3.

Chemical class	Biological endpoint	Properties required for toxicological action	Reference
Acetate esters	neurotoxicity, neurobehavioral potency	toxicity increases with increasing logP	Larsen P.B., Narcotic effects of organic solvent... Draft report of Institute of Toxicology Danish Veterinary and Food Administration, August 1997.
Acrylamides	carcinogen, heritable mutagens, reproductive tox., developmental toxi., neurotixines	only with M.W.< 5000; inhalation M.W.<1000; dermal M.W. <500	TSCA New Chemicals Program (NCP), US EPA, December 1997
Alcohols	narcotic potency	toxicity increases within homologous series up to 6 - 8 carbon atoms and then decreases for higher number of carbon atoms.	Larsen P.B., Narcotic effects of organic solvent... Draft report of Institute of Toxicology Danish Veterinary and Food Administration
Alkoxysilanes C-O-Si	lung toxicity from inhalation of vapors	Only with M.W < 5000, more than 25% M.W.< 1000 and/of more than 10% with M.W. <500 for methoxy and ethoxysilanes. For larger molecules M.W. <1000 is required to be toxic.	TSCA New Chemicals Program (NCP), US EPA, December 1997
Aliphatic acids	teratogenicity sedative/hypnotic activity, liver toxicity,	Teratogenicity: branched-chain pentanoates and hexanoates.	Di Carlo. F.J., Structure-Metabolism Relationship (SMR). Drug Metabolism Reviews, vol 17 , 1986.

Chemical class	Biological endpoint	Properties required for toxicological action	Reference
Aminobenzithiazole azo dyes	oncogenicity, mutagenicity	dimethyl aminostyryl benzothiazole, 4-ethyl-N,N-diethylaminoazobenzene	TSCA New Chemicals Program (NCP), US EPA, December 1997
	liver and thyroid	2-aminothiazole	
	neurotoxicity	chlorinated-2-aminobenzothiazole	
Anhydrides, Carboxylic acid	pulmonary sensitization	phthalic, trimellitic, isopropylidene bis(phthalic), sulfonyl bis(phthalic) anhydrides.	TSCA New Chemicals Program (NCP), US EPA, December 1997
	reproductive toxicity	maleic, succunuc and phthalic anhydrides M.W. < 500	
Azo dyes	carcinogenicity	Azo-dyes sulfonated on both sides of the azo linkage are NOT carcinogenic. Carcinogenic are compounds with a sulfonated half and an nonsulfonated half, compounds containing a free, alkylated or acetylated amine, compounds containing benzidine or 3,3'-disubstituted benzidine.	Brown M.A., Predicting of Azo Dye Toxiciry, Critical Reviews in Environmental Science and Technology, vol 23 (3), 249 - 324, 1993.

Chemical class	Biological endpoint	Properties required for toxicological action	Reference
Dianilines: - two phenyl rings with a binding C, O, N or S, - each terminal phenyl ring must have a primary amino group either meta- or para to the binding atom	carcinogens and mutagens	4,4'-methyldianiline, 4,4'-methylene-bis(o-toluidine), 4,4'-oxydianiline	TSCA New Chemicals Program (NCP), US EPA, December 1997
	retinotoxic agent	4,4'-methylenedianiline, 4,4;-oxydianiline	
	reproductive and systemic toxicant	4,4'-methylenedianiline	
Benzotriazole-hindered phenols	effects on liver, kidney, hematology, immune system. reproductive toxicity dermal sensitization		TSCA New Chemicals Program (NCP), US EPA, December 1997
Boron compound: borates, organoborates, borate esters, boron hydrides, boranes and boroxines.	reproductive toxicity, blood toxicity, neurotoxicity	M.W. < 1000	TSCA New Chemicals Program (NCP), US EPA, December 1997
Dichlorobenzidine based pigments	oncogenicity, mutagenicity		TSCA New Chemicals Program (NCP), US EPA, December 1997
Diisocyanates R-(N=C=O)>2	dermal and respiratory sensitization and pulmonar toxicity	M.W. <1000	TSCA New Chemicals Program (NCP), US EPA, December 1997
Epoxides /O\ -C---C---	cancer, reproductive effects	primary epoxides are the most toxic /O\ --C----C--- H H	TSCA New Chemicals Program (NCP), US EPA, December 1997

Chemical class	Biological endpoint	Properties required for toxicological action	Reference
Ethers	narcotic potency	Potency increases for increasing molecular size. For the substances with equal molecular weight Isomers having the longest straignt chain or the highest boiling point were the most potent.	Larsen P.B., Narcotic effects of organic solvent... Draft report of Institute of Toxicology Danish Veterinary and Food Administration
Ethylene glycol esters R-(OCH2CH2)n-OR	irritation of skin, eyes and mucosa, hemolysis, bone marrow damage, leukopenia of lymphocytes and granulocytes, kidney and liver damage, immunotoxicity, CSN depresion,		TSCA New Chemicals Program (NCP), US EPA, December 1997
	development and reproductive toxicants	short-chain only, shorter chain is more toxic, toxicity is reduced going from ethylene glycol to the triethylne glycol	
Halogenated hydrocarbons	narcotic potential	Potency increased with increasing boiling point. The introduction of a second halogen atom (Cl, Br, I) into a fluorocarbon increased the potency.	Larsen P.B., Narcotic effects of organic solvent... Draft report of Institute of Toxicology Danish Veterinary and Food Administration

Chemical class	Biological endpoint	Properties required for toxicological action	Reference
Hindered amines: amine with 2,2,6,6-tetramethyl-4-piperidinyl group, UV-stabilizer	immune system, liver, blood, male reproductive system, gastro-intestinal tract		TSCA New Chemicals Program (NCP), US EPA, December 1997
Hydrazines and related compounds	carcinogenicity, liver, kidney, blood, CSN depression, skin and eye irritation	there is a greater concern for chemicals with few substitutions on the functional group than for those with multiple substitutions.	TSCA New Chemicals Program (NCP), US EPA, December 1997
Hydrocarbons	narcotic potential (neurotoxic)	Potency increased with increasing molecular size. Alkylbenzenes>cycloalkanes>alkanes. The alkenes were for the lower members (<C7) more potent than the alkanes.	Larsen P.B., Narcotic effects of organic solvent... Draft report of Institute of Toxicology Danish Veterinary and Food Administration
Ketones	narcotic potential (neurotoxic)	Potency increases with increasing molecular size. Potency increases with logP.	Larsen P.B., Narcotic effects of organic solvent... Draft report of Institute of Toxicology Danish Veterinary and Food Administration
Naphthylamines, monosulfonated	corcinogenic	Concern is restricted to monosulfonated -naphthylamines where sulfonate or sulfatoethylsulfone group is on the ring distal to the -amino group.	TSCA New Chemicals Program (NCP), US EPA, December 1997

Chemical class	Biological endpoint	Properties required for toxicological action	Reference
Nickel Compounds Ni^{+2}	genotoxic, carcinogenic, neonatal mortality, fetotoxicity, dermatotoxicity	Any nickel compound that release Ni^{2+}	TSCA New Chemicals Program (NCP), US EPA, December 1997
Organotins: mon-, di-, tri-, and tetra-alkyl or phenyl oranotincompounds, including organotin esters/oxides.	irritating/corrosive to skin and eyes, neurotoxic, immunotoxic, effect on thymus, spleen, lymph nodes, bone marrow	Neurotoxicity: tri- and tetra substituted tins are more toxic than mono- and di- substituted compounds.	TSCA New Chemicals Program (NCP), US EPA, December 1997
Peroxides	carcinogenic		TSCA New Chemicals Program (NCP), US EPA, December 1997
Quinolones	antibacterial activity	Presence of fluorine at C-6 and cycloproplyl group linked at N-1 increase antibacterial activity	Bonelli D., The antibacterial activity of quinolones... Quant. Struct.-Relat. 10, 333-343 (1991)
Stilbene, derivatives of 4,4-bis(triazin-2-ylamino)	development/ reproductive toxicity concerns	Any water soluble (sulfonated) derivatives of 4,4-bis(triazin-2-ylamino)	TSCA New Chemicals Program (NCP), US EPA, December 1997
Triarylmethane Pigments/Dyes with Non-solubilizing groups	Oncogenicity, developmental and reproductive toxicity	di- and tri-amino substituted triphenylmethane and diphenylnaphthyl-methane	TSCA New Chemicals Program (NCP), US EPA, December 1997
Vinyl esters CH2=CH- and RCOO-	oncogenicity, neurotoxicity and reproductive toxicity	requirement for a vinyl group and an acid group	TSCA New Chemicals Program (NCP), US EPA, December 1997

Chemical class	Biological endpoint	Properties required for toxicological action	Reference
Vinyl sulfones and their precursors (sulfatoethyl-sulfonyl)	oncogenicity, mutagenicity		TSCA New Chemicals Program (NCP), US EPA, December 1997

9. Appendix 2: Table with Computerized QSARs

In this table computerized systems are listed. The numbered references mentioned can be found at the last page and are also included in the reference list. See also chapter 4.

Name	type	Available	Internet	Toxicity-subdiscipline	Value/handling	Description	Perfor-mance[1]	Output	Lit.ref.
RASH (rapid screening of hazard)	Rule-based/statistical	Within soon	http://www.esd.ornl.gov/iab/iab6-15.htm	carcinogenicity	'to be used by the expert or non-expert' No data on computer requirements	Rule: toxicity induced compensatory cell proliferation can serve as a upper-limit index of carcinogenicity promotion. Based on effects in short-term studies RASH predicts carcinogenic concern, taking into consideration the fact that high dosing normally gives false-positive results in carcinogenicity testing.	Accuracy: 74% false pos.: 3/31 false neg.: 5/31	Produces limit values above which monitoring should commence	1, 2
DEREK (Deductive estimation of risk from existing knowledge)	Rule-based	Yes.	http://129.11.12/LUK/derek.html	mutagenicity, carcinogenicity, skin sensitisation, reproductive toxicity, irritancy, neurotoxicity	Readily, easy-to-use. Windows-based	DEREK makes its toxicological predictions by comparing submitted structures with rules contained in the DEREK rule base. Graphical interface	Accuracy: 59% false pos.: 4/37 false neg.: 11/37	Toxicological report	3-5
TOPKAT (Toxicity Prediction by Komputer-assisted Technology	QSAR/ proprietary algorithms /Optimum prediction space/statistical methods	Yes	http://www.oxmol.com/prods/topkat	carcinogenicity , ames mutation, oral LD_{50}, rat chronic LOAEL, skin sensitisation, developmental toxicity, irritancy, mouse inhalation, rat maximum tolerated dose and additionally some ecotoxicological tests	Users: US FDA, US EPA, industries, universities, test-labs (see ..../topkat/work for the complete list). At least 486, desirably pentium, Windows 3.1 en 95. Demo available	Models are generated using QSAR-techniques and propriety algorithms. TOPKAT determines an optimum prediction space, within which predictions are both robust and accurate. TOPKAT produces an identification of possible sites of toxicity. TOPKAT provides a user-friendly interface to guide users through a computer-based assessment of toxicity for a query structure, and validated pre-constructed QSAR models for rodent carcinogenicity etc. Input: SMILES notation of chemical.	Accuracy: 58% false pos.: 3/24 false neg.: 7/24	Toxicological report	6
COMPACT (Computer Optimized Molecular Parametric Analysis of Chemical Toxicity)	Modelling of planarity and electronic structure to predict interaction with P450 as a predictor of carcinogenicity	Not commercially (See [7])	-	Indirect carcinogenicity	Program not commercially available	Based on the presumption that to become reactive, electrophilic carcinogenic intermediates, compounds must be metabolised through oxygenation by P450 enzymes. Via a calculation of the molecular and electronic structure of the chemical (planarity, electronic structure; LUMO/HUMO, collision diameter), COMPACT determines whether the chemical will interact with cytochrome P450 subfamilies and hence be metabolised to form reactive intermediates that manifest toxicity. The one-rule-base (P450 metabolism) is a disadvantage. Program will not analyse non-organic compounds (e.g. fibers, metals) or direct-acting mutagens	Accuracy: 54% false pos.: 10/35 false neg.: 6/35 Accuracy: 70% false pos.: 3/31 false neg.: 9/31	unknown	8-10
(MULTI)CASE (computer-automated, structure evaluation)	Statistical (generates QSARs) biophore and modulator selection, computer-automated, structure evaluation, QSAR correlation	Via Multicase Inc.	http://cwgk4.chem.cwru.edu:443/biosoft.htm	depending on data-set (carcinogenicity mutagenicity, teratogenicity, biodegradation)	VAX/VMS workstation (MULTICASE is needed when using private databases in ToxAlert and CASETOX)	MULTICASE accepts series of compounds and quantitative or qualitative activity in tests performed under a common protocol. The program will evaluate the data set, identify biophoresand modulators and generate a QSAR correlation. Once this 'ad hoc dictionary is established, it may be used to analyse new molecules.	Accuracy: 49% false pos.: 9/35 false neg.: 9/35		11,12 (see Note)
CASETOX (MULTICASE derivative)	Statistical	Via Multicase Inc.	http://cwgk4.chem.cwru.edu:443/biosoft.htm	carcinogenicity, mutagenicity, irritation, teratgenicity, miscellaneous	VAX/VMS workstation	Uses prediction modules of MULTICASE. Most useful when investigating endpoints for which extensive, authorative MULTICASE databases are already available.	Not investigated	Toxicological report	

Name	type	Available	Internet	Toxicity-subdiscipline	Value/handling	Description	Perfor-mance[1]	Output	Lit.ref.
				toxicity, short term assays, biodegradation					
ToxAlert (MULTICASE derivative)	Statistical (based on the algorithms of the artificial intelligence program (MULTI(CASE)	Via Multicase Inc.	http://cwgk4.chem.cwru.edu:443/biosoft.htm	teratogenicity, carcinogenicity, biodegradation, chromosome aberrations, micronuclei, Salmonella mutation,	Windows-based. Input: structural formula. Windows 3.1, '95, NT	Based on the structural formula, partition coefficient, molecular weight, water solubility are calculated and used to predict toxicity. Prediction based on modules (training sets) for various kinds of toxicity ('the best databases for each generated toxicological endpoint as generated by MULTICASE'). Program will only analyse organics.	Not investigated	Toxicological report	
META (MULTICASE interfacable)	knowledge-based	Via Multicase Inc.	http://cwgk4.chem.cwru.edu:443/biosoft.htm	biodegradation	VAX/VMS workstation	META is a knowledge-based expert system, capable of predicting the sites of potential enzymatic attack and the nature of the chemicals. formed by such metabolic transformations. It operates from dictionaries of transformation operators, created by experts to represent known metabolic paths.	Not investigated	Unknown	
Purdy et al.	Hierarchical QSAR model (hybrid?)	Possibly	http://www.oxml.com (For QSARs only)	carcinogenicity, skin sensitisation	-	Uses a number of QSARS based around specific chemical reactivity mechanisms, thought to represent the mechanisms leading to carcinogenicity (SPARC, SRC, ProjectLeader/CAChe-Oxford Molecular Group) are some of the programs used to generate the analysis). A hierarchical model consisting of QSARs based mainly on chemical reactivity was developed to predict the carcinogenicity of organic chemicals to rodents. QSARs based on hypothesized mechanisms of action, metabolism, and partitioning. Predictors included octanol/water partition coefficient, molecular size, atomic partial charge, bond angle strain, atomic acceptor delocalizibility, atomic radical superdelocalizibility, the lowest unoccupied molecular orbital (LUMO) energy of hypothesized intermediate nitrenium ion of primary aromatic amines, difference in charge of ionized and unionized carbon-chlorine bonds, substituent size and pattern on polynuclear aromatic hydrocarbons, the distance between lone electron pairs over a rigid structure, and the presence of functionalities such as nitroso and hydrazine. Bias to the importance of e.g. epoxides as compared to SN2-reacting chemicals	Not investigated	Unknown	, p16, 13
OncoLogic	Rules of SAR-analysis/knowledge of mechanisms of action/human epidemiological studies	Yes	http://logichem.com	carcinogenicity	knowledge-based software developed in cooperation with US-EPA. Windows (?!). Demo program available	Classes of compounds analyzed: fibers, polymers, metals organic chemicals. Requires a considerable data input (e.g. physicochemical data, location of reactive centres).	Not investigated	Toxicological report, including data report and toxicological report. heuristically sound	No refs
HazardExpert	Rule-based learning system	Yes	http://.compudrug.hu/hazardtext.html	carcinogenicity, mutagenicity, teratogenicity, membrane irritation, neurotoxicity	MS Windows and UNIX. Window-based programs work in a PALLAS frame. 486 Windows 3.1 or Windows 95, 8 MB RAM	Knowledge base was developed based on a list of fragments reported by more than 20 lead experts. In combination with MetabolExpert, it predicts toxicity of both the parent compounds and metabolites. The expandable knowledge base is open to new information specific to each user.	Accuracy: 55% false pos.: 1/9 false neg.: 17/31	Toxicological report, easily interpretable results, including molecular weights, pKa and log P values	10,14,15

Name	type	Available	Internet	Toxicity-subdiscipline	Value/handling	Description	Perfor-mance[1]	Output	Lit.ref.
MetabolExpert (interfacable with HazardExpert)		Yes	http://.compudrug.hu/hazardtext.htlm	metabolism	MS Windows and UNIX. Window-based programs work in a PALLAS frame. 486 Windows 3.1 or Windows 95, 8 MB RAM	Knowledge-based prediction of metabolic trees and pathways of organic chemicals in mammals and plants. In combination with HazardExpert, toxicological potential of metabolites may be investigated.	Not relevant	Unknown	16,17
StAR	Rule-based	Not yet	http://129.11.12.1/iLUK/start21.html	carcinogenicity (and additional subdisciplines)	Data not available	The StAR project is developing novel risk assessment techniques that draw upon recent developments in mathematics and logic, and improved methods for communicating risks which incorporate results from psychological research into human risk perception and decision making under uncertainty. Based on the similarities in the syntax of the input rules, it is suspected that the method of King and Srinivasan is the basis of StAR. The program is heuristically sound	Data not available		No refs
Progol	Combined statistical and inductive logic programming methods	Yes	http://www.comlab.ox.ac.uk/oucl/groups, and Ashwin.srinavasan@comlab.oxford.ac.uk	carcinogenicity	DOS/Windows	King and Srinavasan embarked on the development of a method based on Progol, a machine learning program. Progol is the first inductive logic programming algorithm to use a fully relational method for describing chemical structure in SARs, based on using atoms and their bond connectives. Progol is well suited to forming SARs for carcinogenicity and is designed to produce easily understandable rules (structural alerts) for sets of non-generic compounds. Its process of data-set analysis is reminiscent of that of CASE/MULTICASE. Its accuracy was reported to be 63% (in this particular analysis the accuracy was comparable to that of programs like DEREK, CASE, COMPACT and TOPKAT.	Data not available. (authors own statement; 63%).	Decision carcinogen/non-carcinogen and an explanatory set of rules (which is not easily to interpret).	19
FALS (Fuzzy adaptive least squares)	Statistical pattern recognition method to generate QSARs	No	-	carcinogenicity	Not applicable	FALS is a pattern recognition method for correlating structure with activity rating. It was used to generate QSARs on the carcinogenicity of several chemical classes.	Not available	Not available	20

[1] As cited in 18 and 10

Note: Additional information is found in Zhang, Y.P. et al. (Env. Health Perspectives 104, Suppl. 5, 1045-105).

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10. Appendix 3: Abbreviations

CSR:	Center for Substances and Risk Assessment
EPA:	Environmental Protection Agency
NIEHS:	National Institute of Environmental Health Sciences
NCP:	TSCA New Chemical Program
NTP:	National Toxicology Program
PMN:	Premanufacturing/Premarketing Notice
QSAR:	Quantitative Structure Activity Relationships
SAR:	Structure Activity Relationships
SAT:	Structure Activity Team
TSCA:	Toxic Substances Control Act

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