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## **TECHNICAL REPORT No. 74**

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**QSARs in the Assessment of the  
Environmental Fate and Effects of  
Chemicals**

June 1998

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# QSARs in the Assessment of the Environmental Fate and Effects of Chemicals

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## SUMMARY

Quantitative Structure Activity Relationships (QSARs) are based on a comparison of the structure or some physico-chemical property of a substance ("descriptor") with a measured endpoint which may be another physico-chemical property or a biological effect. The issues that arise in the development, selection and use of QSARs are discussed together with appropriate examples.

The report describes how all QSARs should be based on a defined and measurable endpoint. A consistent dataset for that end point covering a well defined scope of chemical structures ("domain") is required from which a "training set" to be used for the development of the mathematical correlation is separated. The remainder of the dataset is the "test set" which is used to verify the mathematical correlation. Finally, a clearly described statistical process must be employed in order to determine the variability.

The principal lessons learnt are that the variability inherent in the measured endpoints and descriptors determines the variability in the prediction. How this variability will need to be addressed, will depend on the use of the prediction.

QSARs that are developed with an understanding of the relationship between the endpoint and the descriptors, i.e. "mechanistic" QSARs, have a number of advantages over those based only on a statistical relationship between the descriptors and the endpoint. These advantages may include being able to better understand how to investigate outliers, whether new chemicals are part of the domain of the original QSAR and if not, being better able to assess the potential for extrapolation.

From the above points it is clear that QSARs should only be used by experts, who need to understand more than the endpoint being predicted. They should also understand the descriptors used and their relevance to the chemicals in the QSAR's domain, and the use to which the QSAR is to be put. When used properly, QSARs are capable of highlighting new understandings and if the variability is accounted for, may help in the development of probabilistic risk assessments.

However, when QSARs are applied to substances outside of the domain for which they were developed, the uncertainties involved will often lead to the incorporation of added conservatism and increased error propagation within risk assessment models.

From the reviews carried out in this report there are a number of QSARs that need to be developed.

These are:

- metabolism in fish;
- bioconcentration including metabolism in organisms other than fish;
- microbial breakdown of chemicals;
- soil/water partitioning including kinetics;
- aquatic photolysis;
- effects on terrestrial organisms, sediment dwelling organisms and marine organisms.

One major area within the present EU risk assessment process which is poorly served by QSARs is the understanding of how chemicals behave within the terrestrial and sedimentary compartments and to what extent they are capable of expressing their intrinsic properties. Not only is there insufficient information for assessing these compartments, but as a result, there are few QSARs to predict the influence of bioavailability on toxicity.

It is important that QSARs that are recommended for use in the Regulatory area should be subject to constant improvement and refinement as more data become available. This is the case within the US - PMN<sup>1</sup> process, although it is also recommended that the improvement should be as transparent as possible, given commercial confidentiality.

It is the recommendation of this report that QSARs could be used to check the validity of data or to fill data gaps, for priority setting, risk assessment and classification. However, in such circumstances, there need to be appropriate mechanisms to allow for the generation of measured data when requested. Valid measured data, when available, should always take precedence over QSAR predictions.

QSARs may be defined as models, involving multi-stages, within the endpoint. Thus a fish toxicity QSAR includes uptake, distribution, metabolism and excretion. As such it is important that they are developed, selected and used by experts with a clear understanding of the uncertainty involved in their prediction and a good understanding of how to take this uncertainty into account, whether for risk assessment or classification.

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<sup>1</sup> Pre-Manufacture Notice



## 1. INTRODUCTION

Structure activity relationships (SARs) are based on a comparison of the structure or some physico-chemical property with a measured parameter or endpoint of a chemical. The endpoint being estimated may be another physico-chemical property or it may be a biological effect. When the result is expressed quantitatively the relationship is a Quantitative Structure Activity Relationship (QSAR).

One of the first examples of the use of QSARs in a regulatory scheme was as part of the US Toxic Substances Control Act (TSCA, 1976) for new chemicals. Section 5 of TSCA requires that a manufacturer provides a Pre-Manufacture Notice, (PMN), on the basis of which the EPA try to assess whether the substance is likely to give rise to a reasonable or unreasonable risk. There is no requirement to provide ecotoxicity data under the PMN, and it has been estimated that less than 15% of PMNs comprise such data (Auer *et al*, 1994). To overcome this lack of information, the EPA developed a series of ecological and ecotoxicological QSARs for specific chemical classes. The regulatory use of QSARs was then further increased under Commission Regulation (EC) No. 1488/94 laying down the principles for the assessment of risks to man and the environment of existing substances (EEC, 1994).

Outside of regulatory schemes, QSARs have been developed and used within narrow ranges of chemical classes. Furthermore, the use of QSARs may be considered as a process in itself and, thus involves expert judgement. This includes an understanding of the assumptions and methods that were involved in the development of the QSARs and which therefore underlie their use. Frequently, when extending their use into regulatory schemes, these assumptions have not always been properly taken into account.

Arising from these considerations, ECETOC has re-formed a task force (ECETOC, 1986; Turner *et al*, 1987) on this subject with the following terms of reference:

- compare the predictions obtained with QSARs to measured data using ECETOC databases and other sources of data and comment on the validity and applicability of such QSARs;
- identify and review software packages which are available for accessing and using appropriate QSARs;
- identify those aspects of environmental distribution, fate and effect where the further development of QSARs is desirable and feasible;
- provide a scientific basis for ECETOC's contribution to the European Community and European Community-European Chemical Bureau's activities in this area.

## 2. BACKGROUND

Structure Activity Relationships, SARs, are often developed, particularly in their use in drug design, based on a hypothesis regarding molecular and/or physico-chemical properties and the expression of a particular endpoint. SARs are non-quantitative and indicate the membership of a chemical in a particular grouping of chemicals, e.g. active/non-active. When a prediction about the level of activity needs to be made a Quantitative Structure Activity Relationship, QSAR, is required.

SARs and QSARs have historically been used in research programmes to help direct designing and screening of chemicals or to better understand their mechanism of action. They have been used with success as a research and development tool in improving efficacy and product innovation and are especially helpful when developing a hypothesis or a novel structure for bioactive molecules.

More recently QSARs have been developed and used to help predict the fate and effect of chemicals in the environment. This application of QSARs allows the environmental fate and effects of a chemical to be assessed on the basis of structure and simple physico-chemical parameters. This assessment is very useful for chemicals in the early stages of development, even prior to synthesis, and may be a fast and cost effective method of screening potential new structures. QSARs are proving to be useful in the design of new chemicals or alteration of existing structures which are capable of expressing an endpoint within a required range. There is a further use of QSARs within the context of the risk assessment process for new and existing chemicals. In this instance QSARs may be used either to validate historical data, or to add data points, post-base set (EEC, 1993a; 1993b).

QSARs may be developed based on a hypothesis regarding molecular and/or physico-chemical properties and the expression of the endpoint being studied. QSARs based on such an understanding are mechanistic and can be amended or updated as the understanding of the underlying science changes. QSARs based on a mathematical relationship with no such understanding are statistical. One advantage of a mechanistic QSAR, is that it is easier to understand the limitations of the relationship and how the domain of chemicals covered by the QSAR is limited.

Normally a QSAR will contain three different elements; the measured parameter or endpoint, a physico-chemical property or a structurally derived parameter (or descriptor) and a process for linking the first two elements, e.g. linear regression.

The measured endpoint will be either a physico-chemical property (e.g. solubility or octanol-water partition coefficient  $K_{ow}$ ) or a biological effect (e.g. fish toxicity or biodegradability of the chemical).

The descriptors used in QSARs may be either theoretically or experimentally derived, based on structure or a physico-chemical property. These are discussed in more detail in the OECD report on the application of QSARs in the estimation of the environmental fate of chemicals (OECD 1993a).

Examples of QSARs include the estimation of a chemical's octanol-water partition coefficient ( $K_{ow}$ ) from its structure or the toxicity of a chemical from its  $K_{ow}$  value. The general perception of QSARs is that they are an equation relating the descriptor to an endpoint. However, the use of the QSAR is a process during which important decisions are made and it is in that context that QSARs will be discussed in this report.

A QSAR is developed by measuring the endpoint of interest for a range of chemicals and measuring or calculating a physico-chemical property (the descriptor). These are then examined using statistical tools and a model is obtained. However, it is important to note that the reliability and accuracy when using QSARs is difficult to assess. The exact domain for which the model is valid, and chemical inclusion or exclusion rules need to be stated if QSARs are to be used with confidence. Re-formulation of QSARs for a wider range of physico-chemical characteristics maybe needed when QSARs are known not to be valid for the chemical and endpoint under consideration. Exploration and use of other descriptors in combination with good statistical analysis is required to ensure further success. There are clearly a number of stages in this process and these issues will be discussed in Chapter 3.

Numerous software packages and databases have been developed, which are able to help in the development of QSARs. These software tools will be considered in more detail in Chapter 3.

To give a better understanding of the QSARs in general use, either for regulatory purposes or chemical development a number of the more common QSARs are reviewed in Chapter 4. Reference is made, where available, to comparisons between predicted and measured data.

Impending changes in EEC legislation have accelerated the need to define the principles and practical considerations of the use of QSARs in priority setting and risk assessment. It is important to understand the limitations of this approach and to review whether and how this information should be used in the risk assessment. Chapter 5 will discuss in more detail how QSARs could be used within such a regulatory framework. The main areas to be covered will be for data checking, priority setting, risk assessment and classification purposes.

### 3. DEVELOPMENT AND SELECTION OF QSARs

#### 3.1 INTRODUCTION

This chapter will deal with the development and application of Quantitative Structure Activity Relationships (QSARs). A large number of QSARs have been described in the open literature (Lyman *et al*, 1982; OECD, 1992a; 1993a). All of these relate the endpoint, a biological or physical property, with a known physical-chemical property or the molecular structure of the substance under consideration. Normally QSARs are empirically derived, based on previous measurements of several similar compounds (Lyman *et al*, 1982; Suter II, 1993a).

Structure Activity Relationships (SARs) provide information on the capacity of the substance under consideration with respect to a certain quality (e.g. potential to hydrolyse or biodegrade) or a biological mode of action (e.g. non-polar narcosis; polar narcosis; etc.). This information can be helpful for the selection of appropriate quantitative structure activity relationships (QSARs). Normally SARs are a set of rules that are applied by an expert to the chemical and therefore, depend heavily on the expertise and experience of the expert (Suter II, 1993b; EEC, 1996).

There are two approaches used for the development of QSARs. Which approach is used depends on the nature of the scientific information available on which the QSAR will be based. In many cases the available test results are correlated with known descriptor(s) by statistical evaluations. This technique is called the statistical approach. This approach is used when the understanding of why the descriptor(s) describe(s) the endpoint is either unknown or poorly understood.

When there is clear evidence for understanding the mechanism of a group of substances, descriptors may be chosen that directly impact on the endpoint, such QSARs are mechanistic QSARs. For example, if the thermodynamic principles underlying a physical-chemical property are understood, it may be possible to derive the required descriptors and hence develop the QSAR. This approach, the thermodynamic approach, is a sub-set of the mechanistic method and may also be applied to QSARs for describing fate parameters, for example phase distributions.

One advantage of the mechanistic approach is that it is easier to assess a new substance and identify whether it behaves in the same way as those chemicals in the original domain of the QSAR. Also with mechanistically based effect-QSARs, with common modes of action, and non-specific mechanisms, it may be possible to extrapolate the QSAR from one species to another (Roberts, 1991).

The development of a QSAR starts with a description of the domain. This comprises the identification of a group of substances that behave in the same way with respect to the endpoint to be estimated (e.g. a common mode of action for an effect QSAR). This group of substances is called the *training set*. The training set is described by identifying a common sub-structure, the class of chemicals to which the training set belongs, structural rules or mechanistic information (EEC, 1994).

The selection of a training-set is dependent on the skills of the QSAR expert and thus requires a full range of multi-disciplinary scientific knowledge. In this selection, the *domain* of the QSAR is defined. This domain limits the QSAR's use to the endpoint to be described and the group of substances for which it is valid. Furthermore, it limits the valid range of the descriptor within the boundaries of the lowest and highest values of those in the training-set. It is also essential that for all the substances selected, data on the descriptor (e.g.  $K_{ow}$ , solubility) are available.

When a QSAR is developed, it has to be tested or verified. For this, a further set of substances, with known measured endpoints, that fulfil the requirements of the QSAR domain should have been identified. This group of substances is called the *test-set*.

The development of QSARs is described in more detail in Section 3.2. In this section, the mathematical techniques available for deriving QSARs and the criteria for defining these will also be discussed.

When a QSAR is required for the estimation of an endpoint, the first step is to assess the structural features of the chemical or assess its potential to act via a particular mode of action. The selection of appropriate QSARs is the subject of Section 3.3. Section 3.4 then describes the problems caused when misusing QSARs. These frequently arise from the extrapolation of the QSAR.

Case studies on the development of QSARs for solubility, biodegradation, and the acute aquatic toxicity of a surfactant are described (Section 3.5) to highlight some of the earlier arguments and discussions in the chapter.

Finally, the last three sections of this chapter will describe databases (3.6), programs that can be used to develop QSARs (3.7) and programs that use QSARs (3.8). Although a number of databases and programs are included, it is important to realise that this list is neither exhaustive nor a recommendation for use of these databases or programs.

## 3.2 PRINCIPLES FOR THE DEVELOPMENT OF QSARs

### 3.2.1 Definition of Endpoint

A well defined endpoint is essential for the establishment of a QSAR. Examples of such endpoints are melting-point and the 48h-EC<sub>50</sub> to *Daphnia*. In some cases the endpoint is not easily defined or is variable, being dependent on the experimental procedure used. Two examples have been chosen to illustrate this point. Biodegradation, in which the definition of the endpoint is very dependent on the methodology and is also very variable even when the same method is used. The soil-water partition coefficient,  $K_{oc}$ , is a derived parameter, of which the derivation only accounts for one source of the behaviour of chemicals.

#### **Biodegradation**

Biodegradability can be defined as the molecular degradation of a substance, resulting from the complex action of micro-organisms. It is one of the most important processes determining the fate of organic chemicals in the environment. Hence, biodegradation rates play an important role in the estimation of the fate of organic chemicals in the environment. However, as will be discussed later one problem with many regulatory studies is that they measure extent of biodegradation not rates.

In general two types of biodegradation processes are distinguished. Primary biodegradability occurs when an initial small alteration is made to the molecule, changing its physico-chemical properties and integrity. It is quantified by measuring the disappearance of the parent compound with a specific analytical method or by the disappearance of a physico-chemical effect. Information on kinetics of primary degradation is warranted for chemicals whose toxic or inhibitory effects are lost as a result of the first enzymatic or abiotic reaction. Although there are few QSARs based on primary biodegradation, this is probably not unreasonable. Thus for example, in risk assessments, the uncertainties created by the need to assess unknown metabolites, would certainly limit the value of a primary biodegradation prediction.

Ultimate biodegradability occurs when a chemical substance is broken down and all the organic carbon is converted into carbon dioxide, methane and/or incorporated into biomass materials. This leads to a complete conversion of the organic carbon with extensive mineralisation and transient metabolites. Methods providing evidence of ultimate biodegradability use endpoints which are related directly or indirectly to the measurement of the oxidation of the organic carbon:

- standard methods for ultimate biodegradability with measurements of carbon dioxide and/or methane production, oxygen uptake or dissolved organic carbon (DOC) disappearance, which allow the progress of the oxidation of the organic carbon to be followed. These are the

basis of the regulatory requirements on the biodegradability of industrial chemicals (OECD, 1993b; EEC, 1984; 1987);

- more research oriented studies with  $^{14}\text{C}$  labelled substances to follow the mineralisation of organic molecules at low concentrations in realistic matrices (e.g. Larson and Wentler, 1982; Federle and Itrich, 1997).

There are a number of different tests that attempt to measure biodegradation, however, many of these are carried out for regulatory purposes and give rise to a variety of endpoints. For example, the ready biodegradability test yields a value, (frequently expressed as % biodegradability) and a term, (not-) readily biodegradable. The former, indicates the extent to which the substance degraded, while the latter is a legal or regulatory term that indicates whether a chemical passes or fails the OECD ready biodegradability test (OECD 301A-F). The test substance is the only source of carbon and conditions for biodegradation are stringent. The test can produce false negatives with respect to biodegradation potential in the environment. Biodegradation tests commonly used to assess ultimate biodegradability in a regulatory context, differ in measured endpoint, source and concentration of inoculum and test substance concentration used.

Many factors reflecting the extreme difference in biodegradation mechanisms to the very specific environmental conditions of each phenomenon, affect the biodegradability of a substance in the environment. Structural features such as molecular weight, types of bonds and substitutions affect biodegradation rate of organic compounds (Alexander, 1981; Kelcka, 1985). Environmental factors affecting biodegradability include microbial activity and growth as determined by temperature, pH, availability of nutrients, moisture level and residence time of the microbial population in the environmental compartment of interest. Processes such as microbial adaptation and co-metabolism add to the complexity of biodegradation.

A summary of strengths and limitations of available biodegradation data with respect to quantity and quality of data available for QSAR development and relevance to prediction of biodegradation potential in the environment is given in Table 1 (Cowan *et al*, 1996). Lack of uniform endpoints, substrate to biomass ratio, and time allowed for acclimation across the tests are responsible for the limited size of available training sets (compared to those used in toxicology) for Quantitative Structure-Biodegradability Relationships (QSBRs). Within a specific test, intra- and interlaboratory variability in endpoint measured add to the difficulties in selecting a training set. For a specific standard biodegradation test method carried out at different laboratories or within a single laboratory discrepancies and large variability can be observed in the results (King and Painter, 1983; Kitano and Takatsuki, 1988). Although biodegradation tests have been standardised by the OECD, a deviation of 20 percent is considered acceptable when a test procedure is carried out by the same laboratory (OECD 1993c). This limits the development of QSARs for biodegradation and biodegradation kinetics. Therefore, success of future developments in the QSAR area with respect to

biodegradability will be dependent on the availability of high quality experimental data (Peijnenburg and Karcher, 1995) and our ability to use these data and extrapolate them to the real environment.

There is also a need to develop methods for the prediction of inherent soil degradation. However, at this stage this may not be realistic.

**Table 1: Summary of Strengths and Limitations in Biodegradation Test Data for Use in QSAR development**

Source of Biodegradation Data	Strengths	Limitations
Screening tests for ready biodegradability	Very abundant datasets for wide ranges of chemicals	Test conditions are stringent and not environmentally relevant  Many false negative results, especially if not acclimated or test substance is toxic at test concentrations  Scaling factors required to extrapolate biodegradation rates to those in the environment
Inherent tests	Provides for acclimation of microbial populations  Abundant datasets for a wide range of chemicals	Test conditions do not represent environmental conditions or that of activated sludge treatment plant  Endpoint (i.e., DOC removal or O <sub>2</sub> uptake) are indirect measures of biodegradation potential  Can not extrapolate biodegradation rates in environment from this data
Simulation tests	High quality data representing removal in activated sludge tests  Relevant acclimation in terms of biomass turnover times	Endpoint (i.e. DOC removal) is indirect measure of biodegradation potential  Unclear how to extrapolate biodegradation rates in environment from this data  Database is limited
Realistic tests	Environmentally relevant data on biodegradation potential and biodegradation rate in environmental compartment	Tests require C <sup>14</sup> labelled substance or specific analytical methods  Database is limited
Field tests		Confounding factors effecting biodegradation rate can not be controlled and are often not measured  Removal or loss may not always be due to biodegradation



**Organic carbon/water partition coefficient,  $K_{oc}$** 

Unlike other physico-chemical properties of a chemical, the partitioning between soil (or aquatic sediments or other solid matrices) and water is an operational definition that ultimately depends on the solid phase used in the test. The parameter most often directly measured when assessing the potential for a chemical to partition between soil and water is the soil-water partition coefficient,  $K_D$ .

The soil/water partition coefficient may be defined as :

$$K_D = \frac{W_a}{W_d}$$

where  $W_a$  = weight of material adsorbed to soil and  $W_d$  = weight of material dissolved in water.

Several methods are available for the measurement of this parameter, e.g. OECD 106 (1981) which is currently being revised. The basic technique is to equilibrate a chemical between water and a characterised soil, measuring the initial concentration of the chemical in the water, the concentration in the water after equilibration and again after the soil is further equilibrated with clean water. The difference between the first two measurements represents adsorption and the third measurement is an estimate of the desorption of the chemical in the particular soil-water system being examined.

There are a number of reviews of the estimation of this parameter, the way in which the  $K_D$  varies with soil types, the soil-water ratio and various chemical features, to which the reader is referred for further information (Lyman *et al*, 1982; Samiullah, 1990).

There are two dominant interactions of a chemical with soil, or any other solid particle (e.g. aquatic sediments or sludges from waste water treatment plants). Firstly, there are electrostatic interactions between the chemical and the mineral surfaces of the solids. These have, traditionally been difficult to assess or measure and hence most of the predictational approaches, described later, tend to ignore these effects. The second interaction is between the chemical and organic matter associated with the solids and is often characterised by an organic carbon measurement. This has then been used to normalise data generated on one chemical with different soils and has been found to reduce the variability frequently observed with  $K_D$ . This new parameter, the soil (organic carbon)-water partition coefficient,  $K_{oc}$ , is the usual parameter used to describe a chemical's potential to partition between water and soils (or sediments and sludges):

$$K_{oc} = \frac{K_D \cdot 100}{OC}$$

where OC = % organic carbon.

As the electrostatic interactions are ignored, QSARs based on  $K_{oc}$  data will only be capable of being used for non-ionic chemicals and for substrates in which the organic carbon is the dominant factor.

### 3.2.2 Variability of Measured Endpoints

QSARs are based on data that is variable, but the product of the QSAR is a point estimate. Thus the uncertainty involved in the original measurement has been lost in applying the QSAR. The error associated with the estimate is often not reported or may be difficult to assess. This needs to be considered when applying the QSAR and becomes crucial when multi-QSAR models are developed, thus increasing the potential for error propagation.

Thus it is important, to consider the accuracy of a QSAR prediction in relation to the intended use of the QSAR. For example, QSARs used in generic risk assessment may not need to be highly accurate since the procedures usually involve several generic assumptions which by definition cannot always be very precise. In this case effect QSAR predictions may not need to be more accurate than that required to estimate the order of magnitude of toxicity.

Other uses of QSARs may have the aim of indicating smaller differences in endpoint, such as in the ranking of chemicals. This is certainly feasible for more specific uses where the QSAR has been developed on the basis of more precise data, for example, from data generated by one laboratory using one test system.

One important source of variability in the data upon which some QSARs are developed is the source and age of the data. Thus if the data is obtained from different laboratories over a long period of time, changes in the protocol will result in variable data. One example of this is the data used for development of algal QSARs. The test, being multi-generational, produces both an  $EC_{50}$  or a NOEC, based on either biomass or growth-rate over 72 or 96 hours. QSARs have been developed which use data derived from these different time periods and approaches to describing the  $EC_{50}$  (Van Leeuwen *et al*, 1992). A further example is a QSAR used for the prediction of the NOEC of non-polar chemicals to *Daphnia magna*. This study is now a 21 d study, however, the QSAR is based on data from studies of 16 d (Verhaar *et al*, 1995).

The other very important source of variability is derived from uncertainty in the definition of the endpoint. This topic was discussed in more detail above (see Section 3.2.1).

Another source of variability is that arising from the possible presence of impurities in the chemical tested. Ideally a chemical should be 100% pure and its atomic identification and connections fully known. In practice a lower purity or active content is acceptable if the impurities are judged not to alter

the activity of interest. This judgement could be based on specific knowledge regarding a substance or on the significance of the concentration of the impurity.

All measured endpoints are associated with a degree of variability inherent in the methodology used to obtain the measurement. The variability in a specific predicted endpoint may differ with the scope of the QSAR. For example, an endpoint such as the acute toxicity to *Daphnia magna* measured in a single laboratory is often significantly less variable than that associated with the same endpoint and substance measured in many different laboratories. However, it is difficult to make such generalisations since the accuracy and precision of the QSAR will depend on the quality of the data and the range of structures used to derive the QSAR.

### **Variability in Ecotoxicity Endpoints**

Examples of a range of aquatic toxicity endpoints and their respective variability are given in Table 2. It is noteworthy that the protocols in inter-laboratory ring tests are generally followed closely whereas in routine use there are more differences between laboratories and this would be expected to result in wider variability.

Table 2 indicates that for a wide variety of aquatic toxicity test endpoints, the coefficient of variation typically falls in the range 10-80%. This means that, depending on the endpoint, 95% of values are within a factor 1.2 to 9. Assuming one structural parameter such as log  $K_{ow}$  is primarily used as the basis for predicting all of these endpoints, the most variable endpoint in Table 2, (*Daphnia* reproduction), can only be confidently estimated to the nearest order of magnitude. However predictions made with a lower level of confidence may be acceptable - 50% of the results for the *Daphnia* 21 day NOEC were within a factor of 2 and 80% within a factor of 4.

The intra-laboratory repeatability of the acute daphnia toxicity test, one of the most widely used toxicity screening tests, was found to be slightly better than the typical inter-laboratory variability found for the various methods in Table 2. For example in a large number of repeated tests with a wide range of surfactants the lowest  $EC_{50}$  was on average 87% of the highest  $EC_{50}$  for the same surfactant (Unilever Research, unpublished data). The coefficient of variation for this test system was about 26%. A similar value, 23%, was found for 96 hour  $LC_{50}$  tests with rainbow trout in the same laboratory. Comparable tests with two algal growth test species, however had coefficients of variation of 59-82%.

Variation in endpoint may be greater when there is significant variation in the method used and species tested. For example Reiff *et al* (1979) ring tested different fish acute toxicity test methods, allowing testing laboratories to test any species using their own test method. A total of eleven surfactants of diverse structure were tested to four species for 6, 48 or 96 hours. The coefficient of variation for the

dataset which includes the surfactants for which there were at least four 96 hour tests (see Table 2) ranged from 20 to 78%, however, all LC<sub>50</sub>s were within a range of one order of magnitude.

**Table 2: Variability of Inter-laboratory Ring Tests of Biological Endpoints**

Endpoint	Substance	% coefficient of variation (n)		Reference
Fish growth rate, r (28 day NOEC)	dichloroaniline	57	(9)	Ashley and Mallett, 1990
	LAS	33	(4)	
Fish mortality (3 species) (96 hour LC <sub>50</sub> )	8 surfactants	20-78	(4-5)	Reiff <i>et al</i> , 1979
Accumulation in fish (BCF)	Lindane	60	(22)	Kristensen and Nyholm, 1987
	TCP	50	(7)	
Daphnia reproduction (21 day NOEC)	dichloroaniline	53	(52)	OECD, 1995
	CdCl <sub>2</sub>	80	(30)	
	phenol	76	(16)	
Rotifer (freshwater) mortality (24 hour LC <sub>50</sub> )	CuSO <sub>4</sub>	49	(102)	Persoone <i>et al</i> , 1990
Rotifer (marine) mortality (24 hour LC <sub>50</sub> )	CuSO <sub>4</sub>	49	(96)	Persoone <i>et al</i> , 1990
Artemia mortality (24 hour LC <sub>50</sub> )	CuSO <sub>4</sub>	47	(112)	Persoone <i>et al</i> , 1990
Algal growth (96 hour EC <sub>50</sub> )	CdCl <sub>2</sub>	24	(50)	Thellen <i>et al</i> , 1989
	phenol	35	(50)	
Algal growth <i>Skeletonema costatum</i> (72 hour EC <sub>50</sub> )	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	44	(9)	ISO/DIS, 1994
	3,5-dichlorophenol	18	(7)	
Algal growth <i>Paeodactylum</i> <i>tricornutum</i> (72 hour EC <sub>50</sub> )	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	26	(10)	ISO/DIS, 1994
	3,5-dichlorophenol	8.6	(10)	
Bacterial growth (3 assays)	Various incl. metals, organics, pesticides	5-32	(11)	Reteuna <i>et al</i> , 1989
Protozoan growth (24 hour EC <sub>50</sub> )	Various incl. metals, organics	13-35	(9-38)	Dive <i>et al</i> , 1989

An indication of the order of variability in the *Daphnia* 48 hour EC<sub>50</sub> test resulting from a change in culture and test conditions and a change in *Daphnia* strain was reported by Garforth (1983). For six of the seven organic substances tested, including insecticides and surfactants, the 48 h EC<sub>50</sub> for one strain was within a factor of 2.5 of the other EC<sub>50</sub>. Considering the potential factors that could have caused changes in EC<sub>50</sub>, these data suggest that for some types of test substance at least, variability in this toxicity test can be low.

### ***Variability in Abiotic Endpoints***

The accuracy of a range of methods used to determine physico-chemical endpoints is given in the OECD Test Guidelines (OECD, 1982). The accuracy of selected methods is given below.

It should be borne in mind that solid substances may occur in amorphous and/or one or several crystalline forms (polymorphism). This may influence endpoints such as melting point, vapour pressure and solubility.

#### **Melting point**

There are two basic methods for the determination of the melting or freezing point. These are visual and thermodynamic.

The visual approaches are divided in capillary and hot stage and freezing methods. The maximum accuracy expected from a capillary method ranges from  $\pm 0.1$  K (photocell detection) to  $\pm 0.5$  K (melting point devices with a metal block). The respective range of accuracy of hot stage and freezing methods is from  $\pm 0.2$  K (melt microscope) to  $\pm 1.0$  K (Kofler hot bar).

The thermodynamic measurement register the change of enthalpy as energy absorption or emission during the phase transition. The techniques to register this are Differential Thermal Analysis (DTA) and Differential Scanning Calorimetry (DSC). These methods are applicable in the temperature range of 173 to 1273 K. The accuracy for both methods is  $\pm 0.5$  K up to 600 K and  $\pm 2.0$  K up to 1273 K. DTA and DSC are more extensively described in ASTM E 472-86, ASTM E 473-85, ASTM E 537-76 and DIN 51005.

#### **Boiling Point**

There are five methods described by OECD with a range of maximum expected accuracy from  $\pm 0.3$  K using photocell detection to  $\pm 2.5$  K using Ebulliometer (for boiling points  $> 375$  K).

#### **Vapour Pressure**

There are also several methods available to determine vapour pressure curves. The variabilities of these methods are given in Table 3.

**Table 3: Variabilities of Various Vapour Pressure Methods**

Method	Recommended Range	Repeatability	Reproducibility
Dynamic method	$10^3$ to $2 \times 10^3$ Pa	Up to 25%	Up to 25%
	$2 \times 10^3$ to $10^5$ Pa	1-5%	1-5%
Static method	10 to $10^5$ Pa	5-10%	5-10%
Isotensioscope	$10^2$ to $10^5$ Pa	5-10%	5-10%
Vapour pressure balance	$10^{-3}$ to 1 Pa	5-20%	Up to 50%
Gas saturation method	$<10^{-3}$ to 1 Pa	10-30%	Up to 50%

### Water Solubility

There are two methods commonly used to determine water solubility depending on the properties of the substance including their expected water solubility. These are the shake flask method and the column elution method. In a comparison of solubility measurements of six diverse substances, a number of laboratories obtained values that varied from 2.2 to 19 times for the elution method and 1.2 to 2 times for the shake flask method.

### Partition Coefficient (n-octanol/water)

Connell (1994) gives a useful overview of the measurement of the octanol-water partition coefficient ( $K_{ow}$ ) and its use in ecotoxicology. Investigations into the accuracy of  $K_{ow}$  measurements suggest that compounds with a log  $K_{ow}$  of less than 6 can be accurately determined but the variability of measurements above this value increases rapidly with increasing  $K_{ow}$  (Chessels *et al*, 1991).

An indication of the range of variability in  $K_{ow}$  determinations is given by OECD (1982) who report the results of interlaboratory comparison testing for six substances ranging in  $K_{ow}$  from about  $6 \times 10^{-2}$  to  $1 \times 10^5$ . The highest  $K_{ow}$  values determined for each substance were from 6.1 to 16 times greater than the respective lowest  $K_{ow}$ s.

In the OECD method 107, the quoted repeatability of a test is  $\pm 0.3$  log units. This would indicate a coefficient of variation of 30% at log  $K_{ow} = 1$  reducing to 6% at log  $K_{ow} = 5$ . A recent survey of published log  $K_{ow}$  values, which carefully reviewed the reported values to ensure only measured databased on OECD 107 was used (Makovskaya, 1995), showed a similar measure of variability (see Table 4).

**Table 4: Statistically Evaluated log  $K_{ow}$  Values of Aromatic Compounds**

Chemical	Mean log $K_{ow}$	Coefficient of variation (%)	N
Benzene	2.18	11	13
Chlorobenzene	2.83	6	13
m-Dichlorobenzene	3.5	1.5	8
o-Dichlorobenzene	3.4	1.7	8
p-Dichlorobenzene	3.4	2.2	10
1,2,3-Trichlorobenzene	4.08	1.0	6
1,2,4-Trichlorobenzene	3.98	2.9	8
1,3,5-Trichlorobenzene	4.11	2.3	7
1,2,3,4-Tetrachlorobenzene	4.55	1.3	6
1,2,3,5-Tetrachlorobenzene	4.58	2.0	7
1,2,4,5-Tetrachlorobenzene	4.56	1.5	8
Pentachlorobenzene	5.03	2.0	9
Hexachlorobenzene	5.39	9.1	11
Bromobenzene	2.99	0.4	5
Toluene	2.56	8.4	10
Ethylbenzene	3.14	0.3	5
n-Propylbenzene	3.65	2.6	7
Acetophenone	1.64	3.8	9
Benzoic acid	1.84	5.3	5
Biphenyl	3.85	7.2	9
Phenol	1.47	20.3	18
Pentachlorophenol	4.99	4.9	6
p-Nitrophenol	1.66	30	6
o-Methylphenol	2.00	2.5	5
p-Methylphenol	1.97	3.1	7
Aniline	0.93	4.0	6
p-Methylaniline	1.44	5.0	5

## Hydrolysis as a Function of pH

The coefficient of variation for the half-life of two substances, aspirin and diazinon, determined in the OECD/EEC Intercomparison Testing Programme, Part II, ranged from 0.3 to 126% and 11 to 128% respectively.

Table 5 gives the quoted variability of various physico-chemical parameters required when initially notifying a new chemical in the EU.

**Table 5: Variability of Physico-chemical Endpoints (OECD, 1982)**

Endpoint	OECD Method	Variability (CV %)	Comments
Vapour pressure	104	1-25	Dependent on method and range
Aqueous solubility	105	<15	Flask method
		<30	Column elution method
Adsorption/desorption	106	Not known	In ring tests values differed by over 100% at $K_{oc}$ of less than 1000
			At $K_{oc}$ of over 1000 the differences were over 1 order of magnitude
Hydrolysis	111	<2	In the ring test the CVs reported were 0.3 - 126%
Dissociation constant	112	$\pm 0.1$ log unit	Equivalent to 10% at $pK = 1$

## Conclusions

The above discussions clearly demonstrate the variability of the measurements upon which QSARs are based. This leads to two conclusions :

- the techniques used when deriving the relationship that forms the QSAR, must be sensitive to and capable of coping with, the observed variability;
- the impact of the variability on the use of the QSAR must be understood to ensure the QSAR is correctly used to the maximum of its potential.

### 3.2.3 Descriptors

When selecting descriptors upon which to base a QSAR, the role that these descriptors play, either in the way the chemical behaves or the way the endpoint is expressed, should be known. This is



increasingly important now that complex descriptors, based on molecular, electronic or quantum mechanical properties of a molecule are becoming easily available.

### ***Measured properties***

Descriptors based on measured properties have historically been the most favoured approach when generating QSARs. A number of reviews are available which describe many of these approaches including Clements *et al* (1993) and Verhaar *et al* (1995).

The most frequently used parameter, especially in effect QSARs, is  $\log K_{ow}$ . This is probably because  $\log K_{ow}$  which is a measure of hydrophobicity of substances is considered to reflect the ability of organic substances to partition and accumulate in organisms. However, the use of  $\log K_{ow}$  assumes that the behaviour of chemicals under consideration is properly modelled by this parameter. Hence if they partition in some other manner rather than passive diffusion or there is significant metabolism or the chemical has a specific mode of action, then  $\log K_{ow}$  is not a reasonable descriptor.

### ***Presence of sub-structures***

Early attempts to assess the environmental impact of chemicals by SARs used a limited approach based on analogues and chemical class similarities. More recently, QSARs based on the presence of sub-structures which indicate the potential for biological activity or the expression of a physico-chemical property have been developed.

Parameters may be calculated for whole molecules or well defined sub-structures which may be either functional units, e.g. a hydroxyl group, or a clearly defined part of the molecular structure.

This method has the distinct advantage that very large databases containing structures, are available allowing for the assessment of an extensive number of sub-structures and may also reduce the error of predictions based on one result per chemical. However, there are problems which the approach has difficulty in handling. For example, electronic interactions between sub-structures may vary and cannot always be anticipated. It follows from this that sub-structures which were not present in the original database will not be properly assessed. Therefore the approach is best applicable to chemicals containing sub-structures that have previously been evaluated.

An example of the use of sub-structures is that of Hansch and Leo (1979) when calculating the octanol-water partition coefficient,  $\log K_{ow}$ . This method, which became very widely used as the computer program, CLOGP, uses the presence and number of sub-structures to build up the  $\log K_{ow}$ . This approach is discussed further in Section 4.1.2.

Other examples include the development of a biodegradation QSAR (Howard *et al*, 1992), and the development of a fragmental approach using partial least squares, for use in classification of chemicals by Lindgren *et al* (1996).

### **Connectivity Indices**

The use of molecular connectivity indices (MCI) is extensively discussed (Kier and Hall, 1986) as these are the most successful of all such approaches based on topological information. They can be summarised as follows :

Path MCI - calculated from the non-hydrogen part of a molecule - which can be further divided into zero, first, second and higher order MCIs. The first group are assumed to relate to the bulk properties of a chemical, e.g. molecular volume and surface area. Thus Protic and Sabljic (1989) described a zero order valence MCI,  ${}^0\chi^v$ , which was used in the development of a QSAR for estimating the toxicity of some chemicals to fathead minnows and which they suggested was a good approximation for molecular volume. This is also supported by Govers *et al* (1984), who found excellent correlation with molecular weight for a series of PAHs. The higher order MCIs tend to become more related to local structural features and are then normally best used in combination with other parameters (Sabljic, 1991).

Cluster and path/cluster MCIs - These are strongly associated with branching in a molecule and may have some potential for QSARs requiring steric hindrance descriptors. This was noted by Kuenemann *et al* (1990) in the assessment of QSARs for biodegradation.

Chain MCIs - These are associated with rings and their substituents. However, although potentially useful for describing local properties and effects there have been few attempts to date, to use these in QSARs for ecotoxicity.

The principal advantages of MCIs are that they are relatively easy to obtain and can be calculated quickly, being based on structure. They are also very flexible, there being several MCIs available, capable of combining and thus incorporating local, as well as bulk properties of a chemical. However, it is this very flexibility that also tends to be used as a criticism of QSAR's based on MCIs. It is often difficult to know what a particular MCI actually corresponds to in a chemical. Hence it is difficult to propose a relationship based on possible behaviour and then relate that to a certain MCI or group of MCIs.

### ***Calculated Structural and Electronic Descriptors***

As the speed and availability of computers and software have increased, so has the use of calculated electronic descriptors. There are semi-empirical models now available that can calculate many electronic descriptors in minutes and even the more powerful and precise *ab initio* programs take only hours now instead of months for modest sized chemicals. These models calculate the electronic nature of chemicals and descriptors that can be measured and some that cannot be directly measured. The following is a partial list of descriptors that can be calculated: LUMO (lowest unoccupied molecular orbital) energy, HOMO (highest occupied molecular orbital) energy, dipole moment, molecular polarisability, solvent accessible surface area, atomic charge on an atom, nucleophilic and electrophilic superdelocalisabilities of bonds, atoms and molecules, heat of formation, and the change in free energy of reactions. Many of these descriptors are useful in predicting reactivity and since some chemicals are toxic because they react with cellular biochemicals to denature them, the descriptors can be used to predict toxicity (Purdy, 1991; Lewis, 1992; Verhaar *et al*, 1996). These descriptors have only started to be commonly used in the last five years and so there are not yet many QSARs using them, but the descriptors appear to provide tools to lump chemicals into larger classes than the traditional classes based on sub-structures. It is possible to use electronic descriptors to classify chemicals as to the mechanism by which they are toxic and in so doing allow the elimination of some testing. An advantage of this type of QSAR for chemicals with previously untested sub-structures is that the electronic or structural descriptors for those sub-structures can be obtained. However, when using a QSAR in this way it is important to remember that this is extrapolating the QSAR and may give rise to wrong values due to unexpected interactions.

As yet it does not appear that anyone has pushed this approach to the ideal of predicting chemicals with functional groups that have not yet been tested, but the approach should eventually be able to do this. The stage of the approach is probably too early yet to try this. The limiting factor is that not enough chemicals with a variety of functional groups and electronic natures have been tested.

#### **3.2.4 Criteria for Defining the Domain of QSARs**

The determination of a QSAR's domain has been mostly by expert judgement. The expert, after looking at the training set, can generally determine whether a new chemical will fit in the domain. However, the use of expert judgement does not always lead to unequivocal answers, so more uniform ways of determining the domain are needed. This is an emerging science. The need is recognised and researchers are now exploring methods.

### **Classification of Chemicals**

The most common classification of organic chemicals for QSAR training sets is according to functional group. Chemicals with the same functional group such as an alcohol, thiol, nitro, aldehyde, or other group are treated together. It is the most straightforward way for chemists to classify chemicals but it is the most difficult for the users. For instance the EPA system ECOSAR is a compilation of functional group QSARs, where the user has to choose which to use. If the chemical in question has more than one functional group, then the user has to run more than one QSAR, if available, and must then decide which is the most appropriate or accurate answer. This approach is flawed because the addition of new functional groups may lead to unexpected interactions between the functional groups. This can be expressed in an unusual or unexpected response of the test system to the chemical. For example programs that predict pKa based on one functional group give erroneous results when there are additional functionalities electronically coupled, such as two groups on an aromatic ring. Recently a pKa predicting program, SPARC (Hilal and Karickhoff, 1995), that considers interactions has become available and is much more accurate (see Section 4.1.1).

Another problem with classifying based on functional groups is that several chemicals for each of the functional groups have to be tested before QSARs for most organic chemicals can be developed. This is of course a time consuming and expensive exercise.

At least three sets of workers have tried to broaden the classification of chemicals beyond the presence of functional groups. The system ASTER developed at the US EPA Duluth laboratory contains rules for classifying chemicals into classes of toxic modes of action (Russom *et al*, 1991, Russom, 1994). The system SPARC does not classify but uses known electronic and other properties to compute physico-chemical properties. The third group of Verhaar *et al* (1992) propose classifying toxicants by mechanism of action. They have provided some extensive rules for what they refer to as Class 1 toxicants, but have only conceptualised the other classes which are quite similar to the classes in ASTER.

Verhaar *et al* (1992) (see also Clements *et al*, 1993; Veith and Mekenyan, 1993; Bradbury, 1994; Jäckel and Nendza, 1994) define class 1 chemicals as those that are "inert chemicals", and cause "narcotic-type" toxicity. These "inert chemicals" are not covalently reactive nor do they bind to active sites of enzymes or receptor molecules when considering overall acute effects. Narcosis is a non-specific mode of action and according to this approach a chemical will always be as toxic as is indicated by its hydrophobicity. Therefore the narcosis type of toxicity is called "baseline-toxicity" or minimum toxicity. Non-metabolised narcotic chemicals will always behave as such regardless of the duration of the test.

There will be some chemicals that based on short-term toxicity tests will be classified as narcotic, which either through their reactivity or metabolism will exhibit another mode of action in longer-term tests. Of

particular interest are those compounds that contain either soft or hard electrophilic sites, but act as narcotics in a 4-day bioassay, suggesting longer exposures at lower doses may initiate a different mode of action (Bradbury 1994).

Verhaar *et al* (1992) refer to class 2 as less inert chemicals. These are slightly more toxic than the baseline toxicity indicates. The type of toxicity may also be characterised as "polar-narcosis", and the characteristic property of these chemicals is their "hydrogen bond donor acidity". Examples are phenols and anilines.

The Class 3 group described by Verhaar, contained chemicals that exhibit enhanced toxicity, because they react with certain chemical structures found in biomolecules or they are metabolised to more toxic species (bioactivation). Such increases in toxicity generated by bioactivation have not been quantified for more than a few subclasses (Purdy, 1991; Mekenyan *et al*, 1993; Verhaar *et al*, 1996). Even simple acetylenic and allylic alcohols can be 10 to 5,000 times more toxic than narcotic alcohols (Veith *et al*, 1989; Mekenyan *et al*, 1993) due to metabolic activation to the corresponding  $\alpha,\beta$ -unsaturated aldehydes and ketones (Lipnick, 1985). Such metabolites are soft electrophiles (Pearson and Songstad, 1967; Klopman, 1974) and react rapidly with the soft nucleophiles such as glutathione (Friedman *et al*, 1965; Montellano and Mico, 1981; Deneer *et al*, 1988a; 1988b).

Class 4 of Verhaar *et al* (1992) are specifically-acting chemicals that exhibit severe toxicity due to specific interactions with certain receptor molecules, for example, organic phosphorus esters, which inhibit acetylcholinesterase.

### ***Explanation of "Outliers"***

Often when developing a QSAR there are chemicals that appear to be outliers. That is, a predictor can be found for the rest of the set, but not these outliers. These chemicals can be outliers for three reasons. First, their experimental data are inaccurate. Second, they actually belong to another class and were mistakenly included. Third, the best predictor(s) for the dataset was not found. This last reason is why one should be sceptical of QSARs that eliminate outliers.

There are two methods for rationalising the elimination of outliers, these are statistical and chemical.

*Statistical:* There are a variety of procedures (Section 3.2.6). They all require that some limit be set for identifying an outlier and its possible elimination from a QSAR training or test set. Judgement is used to set this limit. It may be set based on expert judgement and what is considered to be acceptable deviation from the model.

It is often assumed that an outlier does not belong with the rest of the chemicals because the data is faulty or developed in a different way. Ideally statistics alone should not be used to explain why a data point is an outlier. Reasons should be sought. A chemical should not be eliminated for statistical reasons alone, because it might be indicating that the chosen QSAR predictors are inadequate.

*Chemical:* Many times outliers belong to a different chemical class that is easily identifiable. Other times outliers indicate a yet unrecognised class that when identified can be used to set the chemical domain of the QSAR in question and serve as a starting point for a new QSAR on a previously unidentified class.

Outliers often indicate that a different mechanism of action is present or that another predictor(s) should be substituted for the original(s) or included. Outliers are warnings that there is more to understand about a problem.

### 3.2.5 Selection of the Training Set

Ideally QSARs are developed or created using a training set and then the applicability, precision and accuracy of the QSAR is evaluated with a test set of chemicals. It is best to start with a large dataset that is then divided into training and test sets. Rarely is a training set selected in an ideal manner. In some cases the QSAR training set comprises all the chemicals that have been tested because there are so few data.

A recent approach that may help in this field has been described by Erikson *et al* (1997). This involves the use of cluster analysis to assess how the chemicals in the full dataset are grouped. The training and test sets may then be based on representatives from these groups, or if no groups are identified selected from across the full dataset.

It is important in this process, that the selection should ensure all sub-structures of interest, or likely to have a significant impact on the results, are adequately represented in both sets. Ideally an algorithm, for establishing that representatives of structurally similar organic chemicals are in each set, should be used. Such algorithms are not yet fully developed, so an expert will normally do this, based on chemical knowledge. One recent approach that shows some promise, is that described by Lindgren *et al* (1996), in which the statistical technique of Partial Least Squares (PLS) is used to help characterise a group of chemicals. The approach depends on a matrix being developed, in which each line of the matrix comprises the presence or absence of a pre-defined fragment. The fragments used were obtained either from the CAS sub-structure dictionary (ACS, 1981), 157 ring fragments (Downs *et al*, 1989) or 149 sub-structures from CROSSBOW (Computerised Retrieval of Organic Structures Based on Wiswesser) (Eakin, 1974). The authors claim that the technique is simple and fast, and describe two applications of the approach.

As previously discussed, there are two approaches to developing QSARs for ecotoxicology and environmental fate:

1. develop QSARs for chemical classes (defined by functional group) based on a statistical relationship between a descriptor and the required endpoint (Clements *et al*, 1995);
2. develop QSARs based on mechanism of action (Bol *et al*, 1993).

The strategy for selecting ideal training sets for these approaches differ. For functional group classification, one attempts to select chemicals of a variety of sizes, octanol-water partition coefficients and the broadest range of responses (such as toxicity). In addition the largest variety of other functional groups or substructures that do not give rise to a response themselves should be included in some of the base structures. For example, if the functional group identifying the set is reactive, then examples of non-reactive functionalities should be part of some structures, but other reactive functionalities should not. Also functional groups that give rise to another mechanism of action should not be included. The ideal is to completely span the molecular space that it is possible for the class to occupy. For example, if the class is aromatic amines then primary, secondary and tertiary amines should be included, as well as various aromatic substructures and substituents such as halogens, alkyl groups, carboxy groups and others. In order to be broadly applicable, all possible sub-units and combinations need to be tested for the training set. For example, all three of the nitro-aniline isomers would need to be tested for many QSARs because there is a different electronic interaction for the ortho (the alpha-effect), meta and para substitutions.

For mechanism of action classes, much the same considerations apply, but in addition as many as possible functional groups that cause toxicity by the same mechanism should be included. This is done in order to find predictors that will apply to all of the functional groups in the training set, and also, one hopes to find predictors that will be good for untested functionalities that have the same mechanism of action. For example if the class is alkylators, then not only the commonly tested chemicals with leaving groups such as chlorine should be included, but also chemicals that contain other leaving groups. How many and what they should be are evolving questions which still require expert judgement. For example, Purdy (1996) showed that for alkylators the delocalisibility of the alkylating carbon and the pKa of the leaving group on the same carbon are good predictors. The pKa correlation with leaving group strength was first identified using the hydrolysis rate of chemicals. It was then used in the toxicity model and shown to be effective. Since it was based on almost a century of organic chemical reaction experience, the property did not have to be evaluated on all possible sub-units.

### 3.2.6 Quality of the Data and Statistical Models

In recent years many new statistical techniques have been developed and used to perform QSAR studies. In this and the following sections, an overview and discussion of the techniques available and their use for the evaluation of data for the establishment of QSARs is given.

To obtain successful results a multidisciplinary approach is required in which expertise in the field of the activity (e.g. ecotoxicology) and multivariate data analysis (MVA) is involved.

To achieve the full interpretative benefits, the choice, sources and size of errors in the input variables (also called descriptors) and activity measurements and the choice of the proper multivariate techniques are critical. This will be discussed in detail in the following sections.

#### *Properties of Data Used in QSAR Development*

The data used when developing QSARs in the environmental sciences frequently cause problems when being statistically assessed. These problems arise for the following reasons:

- Most biological endpoints are a complex expression of several mechanisms (sequential and/or parallel). This means:
  - the endpoint may be highly variable (see Section 3.2.2);
  - the activity may be non-linear with respect to the chemical's concentration, time or the descriptors;
  - there may be interactions involving the parameters or the mechanisms and the chemicals being assessed (Voyer and Heltshe, 1984).
- Most datasets are heterogeneous. This is in part caused by the problems above leading to a limited availability of good data. This results in:
  - the amalgamation of historical datasets to increase the number of data;
  - non-normally distributed datasets, for example across a chemical domain, as the substances have been selected from what was available, rather than a pre-defined training and test set (see Section 3.2.5).

Another problem is the increasing use of descriptors generated by molecular modelling and for these to be internally correlated.

Thus when examining data to be used for generating QSARs it is vital that statistical techniques are chosen that will highlight and/or cope with, the non-linearity or interactions and account for heterogeneity and non-normal distributions in the dataset.



The following sections will give some limited guidance of where and how, statistics may be used when generating QSARs and help address the issues raised above.

### ***Use of Statistics in QSAR Development and Evaluation***

A selection of the most common techniques currently applied when developing or assessing QSARs is given in Table 6. It is not the intention of this report to discuss these techniques in detail. There are a number of references given later which describe more fully the methods and how they are used. The discussions following will try to highlight the more important issues that relate particularly in the production of QSARs using these methods.

**Table 6: Available Statistical Techniques**

Linear	PCA-based	Non-linear	Clustering
Univariate Linear Regression (ULR)	Principal Component Analysis (PCA)	Artificial Neural Networks (ANN)	Principal Component Analysis (PCA)
Multiple Linear Regression (MLR)	Principal Component Regression (PCR)	Fuzzy regression	Fuzzy clustering
	Partial Least Squares (PLS)	Univariate Non-linear Regression (UNL)	Kohonen network (non) hierachical cluster analysis

Some of these methods, e.g. PCR, may be used as robust statistical techniques because they are insensitive to the presence of outliers or non-normally distributed data.

### ***Examination of Dataset***

This examination occurs both prior to describing a model and after the statistical development of that model. One purpose is to characterise the dataset and help in the initial choosing of the statistical model. A second purpose is to examine the dataset for confounding or interacting factors in the dataset that have arisen after the application of the statistical process. The characteristics of interest are:

- the identification of leverage points, i.e. those points which will have an unduly high weight on the statistical model;
- the number of data points versus the number of descriptors, as this will determine whether some statistical methods are inappropriate;
- the frequency distribution of the values of the descriptors and the endpoint which will help in the description of the frequency distribution, e.g. if normal, log-normal and the identification of outliers;

- when appropriate, assuming normal distribution and identifying outliers. It is important when outliers are identified that the reasons for this should be stated;
- the presence of correlated variables, as some techniques, for example multiple linear regression, do not cope well with such variables;
- the amount of variability in the data, either in the descriptors or endpoint. This is described later (see “Choice and application of statistical techniques”).

Classification and clustering are used to obtain an impression of outliers, grouping of the data and inter-dependencies of the descriptors.

### ***Cluster Analysis***

The purpose of classification and cluster analysis is to model in a broad sense, groupings (clusters) of the data. Cluster analysis depends upon the similarity that exists between objects and the similarity between the clusters formed by those objects. Thus when trying to obtain clusters the process is initiated by examining the similarity between the objects being examined. This similarity is frequently based on the distance between objects, measured either by Euclidean or Mahalanobis distance or correlation coefficient.

When analysing the clustering data there are two alternative approaches, hierarchical or non-hierarchical. Hierarchical methods rely on the sequential division of groups into smaller groups, until eventually the groups all contain one object. The results of such an approach are frequently displayed as a dendrogram. Non-hierarchical approaches are generally based on the plotting of two variables (or latent variables when using PCA) for each object. The groupings obtained may be assessed statistically, e.g. using confidence limits, or visually. In some cases the clusters may already be pre-defined and the purpose of clustering is then to see whether the data group in line with the pre-defined clustering.

Among the classification and clustering techniques currently used are Principal Component Analysis (PCA) and correlation studies. Other techniques which are being used and may provide more information are: K-nearest neighbour clustering (KNN), fuzzy clustering and Kohonen neural networks. A more detailed description, with relevant references, to these techniques is given below.

Further information may be obtained from Anderberg (1973) and Massart *et al* (1983).

### ***Choice and Application of Statistical Techniques***

Based on an examination of the dataset it has to be checked whether the appropriate statistical technique may be used. This requires expertise in statistics and multi-variable data analysis.

Table 7 gives some examples of links between properties of data used for QSAR development and the suggested methods.

**Table 7: Characteristic Properties of QSAR Data and Suggested Statistical Method**

Characteristic of dataset	Suggested method
Outliers Poor reproducibility - high variability Descriptors are derived from simulations	Robust regression (all techniques; see below for further explanation)
Non-linear relationships	Non-linear modelling (ANN, Fuzzy regression, UNL)
Interactions between descriptors	ANN, PLS
Limited data	Simple models, with few parameters (ULR, UNL, MLR)

The term “robust regression” indicates a class of regression techniques that can be applied to datasets which do not follow a normal error distribution. For each statistical technique, robust regression variants are available (Walczak and Massart, 1995)

Depending on the characteristics of the data, an appropriate technique must be chosen and in some cases adaptations need to be made. The selection process often is difficult and requires expertise. The difficulties involved in this process will be demonstrated with a few examples:

- some techniques have ‘hidden’ disadvantages with respect to properties of the data. PCA, PCR and PLS are regularly used but have a major and often unrecognised disadvantage. Principal components are linear combinations from the original variables  $q_i$  (in the form of  $b_1q_1 + b_2q_2 + \dots + b_nq_n$ ). This implies that PCA cannot deal with non-linearities and interactions between descriptors (for instance  $\dots q_1^{b_1} + q_2^{b_2} \dots$  or  $\dots b_1q_1q_2 \dots$ ). In some cases alternatives are available that are more suited to ecotoxicological data. The Kohonen network can be used for mapping, as an alternative for PCA and artificial neural networks can be used as alternatives for PCR and PLS;
- there is also sometimes a conflict between the characteristics of the data upon which a QSAR is to be based and the statistical method being used to develop the QSAR. The non-linearity and interactions of the data may require non-linear techniques, e.g. artificial neural networks. However, because some datasets are small, with respect to the actual number of endpoint data, simple models with less parameters are required, e.g. MLR, PCR, PLS. Alternatively, it might be useful to focus on special adaptations of the techniques (for instance reducing the number of parameters in ANN) to overcome these difficulties;
- one important assumption to be aware of when using these type of approaches for QSAR development in general, is the assumption that the experimental scatter arises entirely from

the endpoint being modelled. As previously described (Section 3.2.2), this is often not the case. The descriptors, especially those based on experimental parameters, will also contain uncertainty. To ensure that there are no serious limitations involved in this assumption it may be worth repeating the modelling process but with the two parameters (endpoint and descriptor) reversed. If the resulting line is similar then the model may be acceptable. However, if serious differences emerge the relationship should be more closely explored. Alternatively, general linear least squares methods may be used (e.g. Irvin and Quickenden, 1983). Recently, new developments have been made that allow for the determination of prediction confidence intervals in PCR and PLS, taking into account the errors in descriptor and endpoint data (Phatak *et al*, 1993). There have not been similar developments for ANN.

### ***Interpretation of the Model, Set-up of the Hypothesis and Validation of the Hypothesis***

At this stage after running the first statistical technique a number of parameters need to be assessed. These include:

- goodness of fit;
- ability of the model to predict the endpoint;
- the  $r^2$ ,  $q^2$  and J parameters;
- the presented outliers;
- the correlation of the descriptors.

Based on these the model may then be refined and the statistical process re-run.

This is again a point in the process when a multi-disciplinary approach needs to be applied. Thus the data and the statistical output need to be jointly assessed and the validity of the findings addressed using expert judgement.

### ***Statistical Techniques***

#### *Univariate regression (ULR, UNR)*

Univariate regression means one dependent response variable ( $y$ ) and one independent variable ( $x$ ). This is the simplest approach based on one variable or descriptor and an endpoint. The relationship obtained may be linear, curve-linear or non-linear.

*Linear univariate regression* assumes that the relationship being modelled is a straight line. It is the method most often applied to establish a QSAR.

The normal method of determining the line is to minimise the sum of the squares of the residuals between the measured points and the line. This is known as the method of least squares.

*Non-linear univariate regression models*, e.g. exponential functions, are less frequently used. This was partly because the computational methods were more difficult, requiring an iterative procedure. However, another reason is that the non-linear model is less constrained than the corresponding linear model. It is vital when assessing a non-linear model to examine the residuals across the full extent of the model to ensure that it has not been overfitted (Draper and Smith, 1981).

#### *Multiple Linear Regression (MLR)*

When the endpoint is to be modelled using more than one descriptor then multivariate techniques are applied. The technique of multiple linear regression is extensively covered elsewhere, e.g. Draper and Smith (1981) and Geladi and Kowalski (1986) and will not be further discussed. MLR suffers from the problems discussed above, including overfitting of the model and correlated variables.

Examples of relevant references are: de Bruijn and Hermens, 1991; Govers *et al*, 1991; Newsome *et al*, 1991; Vighi *et al*, 1991; Nevalainen and Kolehmainen, 1994.

#### *Principal Component Analysis and Principal Component Regression (PCA, PCR)*

PCA is a technique for dimension reduction and is based on linear combinations of the variables.

In Principal Component Regression (PCR) one obtains a reduced-order model by neglecting some components of the PCA modelling of the independent variables (X-matrix) and relating the maintained principal components to the dependent variables (Y-variables). The neglected components are usually dominated by non relevant information in the data. But it is possible that the neglected components contain some relevant information, this information is lost when the higher components are neglected. It is also possible that some noise is maintained in the model. In this situation a good model for the training set (dataset used to construct the model) is obtained but the model has a bad prediction ability for the test set. This effect is known as the overtraining effect.

When applying PCA it is difficult to identify outliers and hence the model will give undue weighting to these data points. Robust methods (Walczak and Massart, 1995) have been proposed in an attempt to overcome this problem (see also: Niemi, 1990; Kaiser and Esterby, 1991).

### *Partial Least Squares (PLS)*

Partial least squares (PLS) is a combination of MLR and PCR. It attempts to explain the variance in the independent variables and also tries to obtain a good correlation between the dependent and the independent variables. One major advantage of PLS is that it is very useful when co-linearity in the descriptors exists. To reduce the overtraining effect, a cross validation can be performed during the model constructing phase.

As for PCA, outliers are also a problem for PLS and again robust methods (e.g. Wakeling and Macfie, 1992; Griep *et al*, 1995) have been proposed. The final model is effected by the introduction of outliers. A better model will be obtained when the outliers are left out or become less important for the final model, i.e. when the model is made more robust. Some robust methods are proposed by different researchers, e.g. in the references mentioned below.

### *Artificial Neural Nets (ANN)*

Neural nets are used in many areas, such as pattern recognition, process analysis and non-linear modelling. In the following, the application in non-linear modelling will be described briefly.

An advantage of neural nets is that the neural net model is very flexible in contrast to the classical statistical models. The neural net 'learns' from examples by one of two different approaches, supervised or unsupervised learning. During supervised learning, the system is forced to assign each object in the training set to a specific class, while during unsupervised learning, the clusters are formed without any prior information. The first approach is commonly used in multi-layer feed-forward (MLF) networks. While the second approach is contained in Kohonen learning algorithms. The MLF neural nets are widely used for a variety of problems. An MLF neural net consists of three or more layers: one input layer, one output layer and one or more intermediate (hidden) layer. More detailed information is provided by Smits *et al* (1994), Xu *et al* (1994) and De Saint Laumer *et al* (1991). The Kohonen network is a self-organising feature map which is trained in an unsupervised manner. It maps vectors from a multi-dimensional space onto a low-dimensional space, thereby preserving the topology of the dataset as well as possible. More detailed information is provided by Kohonen (1982, 1984), Anderson and Rosenfeld (1988), Wasserman (1989), Beale and Jackson (1990), Hecht-Nielsen (1990); Freeman and Skapura (1991); Tetko *et al* (1995) and Zupan and Gasteiger (1993).

### *Fuzzy Clustering and Regression*

In contrast to traditional regression/classification techniques, fuzzy clustering or regression is capable of dealing with *probabilities* of finding objects belonging to certain classes, instead of classifying with

hard limitations (yes/no decisions) (Friederichs *et al*, 1996). The limiter functions (which have in most cases a sigmoidal shape), may hold all states or values between two extreme assertions.

#### *K-nearest Neighbour Clustering*

K-nearest neighbour clustering (KNN) determines the class of an object by assessing the class of a number of the closest neighbours to the object. The majority, sometimes weighted depending on distance, will determine the class of the object being assessed.

#### *Genetic Algorithms (GA)*

Genetic Algorithms (GA) are artificial intelligence techniques based on the theory of evolution that through the process of natural selection, formulae evolve to solve problems or develop control strategies. A useful, though somewhat oversimplified analogy is that a GA is an encapsulation of Darwinian evolution. GA can be used to search for a combination of features that perform optimally under some selection that is defined by the problem (which is analogous to the natural environment). GA have a number of unique features. First, a GA does not search for a single solution, but in fact maintains a set of perhaps thousands of solutions, referred to as a population. Second, the GA attempts to increase the "fitness" of this population at each generation. Each solution is evaluated as to its "fitness" based on some domain-specific function, then kept or discarded based on that evaluation. If discarded, that member of the population is replaced by a new solution which is created by a recombination of parts of existing good solutions.

This process is repeated thousands, perhaps millions of times, combining different aspects of good solutions, while searching for a combination of solution features that is optimal under the evaluation function imposed. The GA designer provides a function to evaluate the "fitness" of each individual solution; this fitness function is used to propagate "good" individuals into the next generation. A set of these fit individuals are chosen for a crossover operation, which recombines the strings of the parents into new children, trying to construct fitter solutions in the process. The mutation operator randomly alters some element of an individual (solution) in order to further enhance the population. Because genetic algorithms do not use statistical procedures, they are not limited by statistical assumptions. GA performance is, however, strongly influenced by design decisions made by the programmer. With a GA, the designer has more flexibility than is available with many other types of procedures. They can be modified to accommodate important characteristics of a system, explore alternate hypotheses, and elucidate underlying mechanisms simply by adding variables to the program or altering fitness criteria. GA readily lend themselves to the exploration of relationships between input and output data (i.e. QSAR development).

Forrest (1993) provides a brief but thorough introduction to genetic algorithms. Goldberg (1989) provides an easily understandable introductory text on genetic algorithm development, while Koza (1992) supplies a more advanced treatment of genetic algorithms and an excellent reference text.

#### *Black Box Warning*

For all statistical techniques the user must be aware of the fact that the models are mostly black box models. Only where a mechanism is known or assumed will the relationship within the QSAR be based on causality rather than correlation. In these latter cases any conclusions based on the model are related to and defined by the original dataset used to develop the model. This is especially true for small datasets where there are a limited number of and a large variability in the data.

### **3.2.7 Verification of QSARs**

Validation of environmental models is not possible. The term validation in the context of models usually refers to a process of showing how accurate and/or precise a model is, this should more correctly be referred to as *verification*.

An important aspect of this process is the use of a test set. This is a set of chemicals, ideally similar in all aspects to the training set, which have been similarly tested for the property of interest. This is done by division of the chemicals into the training and the test set (see Section 3.2.5).

One consideration that should be taken into account when assessing a QSAR is whether separate test and training sets were available. In many cases a QSAR is based instead on all the chemicals tested. In such circumstances, when the test and training sets are identical, there will be additional uncertainty involved in the estimate. This uncertainty should be accounted for when verifying the QSAR.

Other sources of uncertainty will arise from the variability of the endpoint, as previously described. There is also the process involved in generating the QSAR and the extent to which the variable or variables chosen for the QSAR are able to predict the desired property.

Normally when assessing the QSAR a correlation coefficient is established, together with the standard deviation from the mean and the F value. Also the number of chemicals used to establish and verify the QSAR needs considering.

It is also desirable in the report of a QSAR to include the standard error, the range of applicability and the chemical domain which the QSAR covers. An example of the type of data used for verification and its display may be seen in the report carried out as part of the EU DGXII research programme on QSARs (Verhaar *et al*, 1995).



Finally it is important to note that when assessing the accuracy of a QSAR, the use for which it was developed should be taken into account. A QSAR can not be better than the data from which it was developed.

### ***Cross-validation***

The usual unbiased estimate of residual variance,  $s^2$ , will tend to underestimate the true prediction error. The reason is that the same data is used to assess the model as that which was used to fit it, using parameters estimates that are fine-tuned to the particular dataset.

In order to get a more realistic estimate of prediction error, it is preferable to have a test sample that is separate from the training sample. Ideally, this would be in the form of some new data from the same population that produced our original sample. However, often additional data are not available. To overcome this, cross-validation uses part of the available data to fit the model and a different part to test it.

#### *'k-fold' cross-validation*

With smaller datasets, 'k-fold' cross-validation makes more efficient use of the available information.

The dataset is divided into k parts. Then the model is fitted k - 1 times thereby leaving out one dataset every time. Then the cross-validation estimate of prediction error (CV) is calculated. Often k=n is chosen, resulting in 'leave-one-out' cross-validation. For each observation i, the model is refitted by leaving the observation out of the data, and then computing the predicted value for the i-th observation. This is done for each observation and then the average cross-validation sum of squares CV is computed.

## **3.3 SELECTION OF QSARs**

One of the problems associated with selecting QSARs in environmental sciences is the vast number that are available. The issue to be discussed in this section is not whether there is a QSAR for an endpoint, but what should be considered when selecting an appropriate QSAR.

Most of the criteria have already been discussed in some detail and so will only be briefly referred to in this section.

- a) While there are many QSARs for particular endpoints, the first item to check is that the endpoint that is predicted by the QSAR is the endpoint required by the user.
- b) Is the chemical to be assessed within the domain of the QSAR?

- c) Associated with considerations of the endpoint are those of the methods used for generating the data. Are the data all from one method or several and if the later how comparable are they to each other?
- d) What is the reliability of the data used for generating the QSAR and what units are they expressed in?
- e) What are the descriptors that are used for the QSAR, are they easily available, are they appropriate for the chemical being assessed? It is particularly important in this assessment to understand whether the QSAR is based on an underlying mechanism which the descriptors support or whether it is strictly statistical. Thus if there is some supporting theory, it is possible to check that the descriptors and theory are equally applicable to the chemical under review. This then gives more confidence to the resulting endpoint.
- f) Other factors that should be considered include the statistical functions and whether outliers were assessed and disregarded during the development of the QSAR.

### 3.4 ABUSES OF QSARs

#### 3.4.1 Use of Inadequate Information

In the development and particularly in the application of QSARs it is essential to have identified the structural property or properties that are predictive of the endpoint of interest. If this relationship is not fully understood then it is possible that the domain of the QSAR will incorrectly exclude or include some chemicals. There are several possible causes of such errors:

*a) by missing a component in the generation or application of QSARs*

If a component of a chemical that causes the same effect as that predicted by the QSAR is not identified, then the QSAR prediction may be inaccurate when applied to chemicals not containing the component. This may be particularly relevant to chemicals that are not pure.

For example, the toxicity to fish of a set of predominantly single substances may be accurately predicted by a single QSAR. If, however, another substance in the same domain is synthesised by a modified process that results in a small impurity, structurally unrelated to the main component but being considerably more toxic to fish, then the QSAR may underpredict the toxicity.

This possibility is illustrated by the anionic surfactant alkyl sulphate. The acute toxicity values of alkyl sulphates to *Daphnia magna* are predicted by a simple QSAR using a single structural descriptor,  $K_{ow}$  (Roberts, 1991). However, commercial alkyl sulphates contain a low level of unsulphated alcohol which can also be toxic to *Daphnia*. It is essential to know how much alcohol is contained in the sample since

the alcohol, if soluble at toxic concentrations, is considerably more toxic than the alkyl sulphate and can account for the majority of the toxicity.

*b) by assigning faulty chemical structure in the generation or application of QSARs*

If a component is assigned a faulty structure, the QSAR is likely to predict an activity quantitatively different to that which is observed for a chemical with a correctly assigned structure.

A structure can be "faulty" despite correct allocation of chemical bonds. For example, if there is more than one component that could influence the magnitude of the observed endpoint and these components could interact with each other, then the endpoint predicted for substances containing single components may be different to those containing interacting components.

*c) by using inappropriate or misleading calculations*

It is generally agreed, although rarely specified, that in measuring the effect of a chemical it is at the molecular level that the chemical has an impact. It is important therefore that the calculations and relationships being developed should be based on effect concentrations expressed on a molar basis (McCarty and Mackay, 1993).

### **3.4.2 Interpolation/Extrapolation**

After a QSAR has been developed and verified, it can then be used to generate new data based on chemicals with an unknown effect. When assessing these new data, whether the data is interpolated, i.e. contained within the range of the original dataset, or extrapolated, i.e. outside the range of the original dataset will impact on the confidence placed on the prediction.

It is normally accepted that interpolated data will be safer, and less likely to be prone to unknown interactions or uncertainties than extrapolated data.

## **3.5 CASE STUDIES**

The following section attempts to highlight some of the previous discussion points by taking examples of QSARs. Each section will look at a specific QSAR and how it was developed.

### **3.5.1 Development of QSAR for Solubility**

Knowledge of a substance's aqueous solubility is essential when determining the potential environmental fate of a chemical. The measurement of aqueous solubility is part of the base-set information required for all new chemicals and those existing chemicals being assessed within the

Existing Substances Regulation. However, for a large number of chemicals it is not available or it may be necessary to evaluate several values obtained over a long period of time for the same substance and thus identify the "right" value. Thus a QSAR for predicting solubility is required.

When selecting such a QSAR the most immediate problem is deciding which one. There are a very large number of QSARs for aqueous solubility. For a comprehensive review the reader is referred to Lyman *et al* (1990). The methods described may be based on the prediction of solubility from another property, mostly log  $K_{ow}$ , or they may be based on various atomic or electronic properties. The example to be discussed in this section was reported by Nirmalakhandan and Speece (1988a and 1989).

The method described is based on molecular connectivity indices,  $\chi$  (see 3.2.3) and a polarisability parameter,  $\Phi$ . These are both directly calculated from the molecular structure.

The model developed uses the parameters  $\chi^0$  and  $\chi^v$ , which are the zero-order simple and valence molecular connectivity indices respectively. They are calculated from a modified algorithm (Nirmalakhandan and Speece, 1988b) to that originally proposed by Kier and Hall (1986). The polarisability parameter  $\Phi$  was derived from that originally proposed by Ketelaar (Horvath, 1982).

The authors recognised the duplication of using connectivity indices which have been shown to encompass polarisability and a separate polarisability parameter. The polarisability parameter used was based on the following relationship :

$$\Phi = A(\text{no. of H}) + B(\text{no. of C}) + C(\text{no. of Cl}) + D(\text{no. of Br}) + E(\text{no. of double bonds})$$

in which A to E are estimated as 0.42, 0.93, 2.28, 3.34 and 0.58 respectively. In order to allow for the potential overlap between this parameter and the connectivity indices the coefficients, A-E, were left variable and statistically optimised during the study. This led to the development of a modified polarisability function.

The model was developed from a training set of 145 compounds and has been tested subsequently on an additional 325 compounds. The 470 compounds studied span more than 12 log units and cover liquid and solid alkanes, alkenes, alcohols, esters, ethers, cyclic organics, amines, aldehydes, ketones, nitro compounds, aliphatics and aromatics with halo substitutions, PNAs, PCBs, PCDDs *etc.* The unexplained variance in the model due either to faults with the model or the variability of the data available for solubility was only 2%. The QSAR equation and its associated statistics are as follows :

$$\log S = 1.543 + 1.638^0 \chi - 1.374^0 \chi^v + 1.003\Phi$$

with  $n = 470$ ;  $r = 0.990$ ;  $r^2 = 0.980$ ; SE (standard error) = 0.332.

The number of chemicals tested and the excellent correlation coefficients reported for this method make it widely applicable.

The authors described a number of approaches adopted in the two reported studies which demonstrate some of the methods used for developing a QSAR.

1. Starting with 16 descriptors, the authors tested for those that were highly correlated and removed them.
2. A step-wise multiple regression method was used to select the best correlation.
3. The number of variables used in each model tested was restricted such that the number of experimental points allowed a reasonable level of certainty of avoiding chance correlation. This was based on Topliss and Costello (1972).
4. A "jack-knife test" as described by Dietrich *et al* (1980) and Cornish-Bowden and Wong (1978), was used at various points in the studies to highlight outliers.
5. As a test for chance correlations, 20 regression runs were carried out on one sub-set of data, with the actual solubility data replaced by randomly generated values of a similar magnitude. The  $r$  of these runs never exceeded 0.585 and averaged 0.323. Similarly replacing the descriptors with random numbers yielded poor correlations.
6. By plotting the residuals between the measured and predicted data an outlier was confirmed. The residue of this outlier being four standard deviations from the mean of the residues.
7. A number of indicators of collinearity or multicollinearity are described.

### 3.5.2 Development of QSAR for Alcohol Ethoxylates

The work described here illustrates the application of an existing QSAR, rather than the development of a new QSAR, to the prediction of the acute toxicity of commercial alcohol ethoxylates (AEs) to *Daphnia magna*.

The aquatic toxicity of chemically unreactive non-electrolytes such as hydrocarbons, ethers, alcohols and ketones is well correlated with  $K_{ow}$  by the general narcosis equation (Könemann, 1981):

$$\log \frac{1}{EC_{50}} = 0.87 \log K_{ow} + 1.13$$

where  $EC_{50}$  is in moles/litre and the effect is lethality (7 or 14 day  $LC_{50}$  to guppies). This equation and similar ones have been found to apply for many compounds and for a variety of species (Sloof *et al*, 1983; Veith *et al*, 1983).

Consideration of the chemistry of AEs which are simple structures (alcohols ethoxylated with a number of ethylene oxide (EO) units, e.g.  $CH_3(CH_2)_nO(CH_2CH_2O)_mH$ ) suggested that they should act by a similar mode of action to other non-polar, unreactive organic chemicals and therefore that the QSAR developed by Könemann (1981) might be applicable i.e. that AEs would be included in the domain of the Könemann QSAR. Since the difference in sensitivity of a wide variety of aquatic organisms to AEs is relatively low, the fish  $LC_{50}$ s were expected to be similar to *Daphnia*  $EC_{50}$ s, i.e. both endpoints were expected to have a similar relationship with  $K_{ow}$  (similar slope but possibly different intercept).

In practice there were several difficulties in applying the QSAR to AEs: (i) the difficulty in measuring  $K_{ow}$  for surfactants, (ii) some key fragment values for calculating  $K_{ow}$  were considered unreliable, (iii) as was the modelling of isomerism in surfactant structures, (iv) some AEs have low solubility in water and (v) commercial surfactants are mixtures of homologues and isomers rather than pure compounds. These problems were overcome by proposing solutions and then comparing predictions of toxicity using the proposed solutions with measured toxicity obtained from 48 hour *Daphnia magna* toxicity tests.

#### ***Difficulty in Measuring $K_{ow}$***

$K_{ow}$  is difficult to determine experimentally for surfactants since a high proportion of the molecules become associated at the interface of the two liquids. Alternative methods to the conventional shake flask method have been investigated but are as yet not validated.

#### ***Calculation of log $K_{ow}$ for Alcohol Ethoxylates***

The Leo and Hansch fragment approach was used as a basis for calculating log  $K_{ow}$  for AEs. Although this approach has been shown to give accurate estimates of log  $K_{ow}$  for non-surfactant chemicals using generic fragment values, the difficulty of verifying values remains because of the lack of measured values for AEs.

Modified fragment values for EOs were derived from a series of measured log  $K_{ow}$  data for the compounds  $HO(EO)_nH$  ( $n=1-4$ ) quoted by Leo *et al* (1971). For this set of compounds the observed log  $K_{ow}$  increment is -0.1 for each EO unit.

The physical and toxicological properties of surfactants are influenced by the degree of branching in the alkyl chain whereas the fragment method for calculating  $K_{ow}$  is independent of such branching. Using aquatic toxicity data for a series of 20 pure isomers and homologues of linear alkyl benzene sulphonate,

Roberts (1989) derived a Position Dependent Branching Factor (PDBF) which has been found to apply to a wide range of surfactants including AEs (Roberts 1991).

### ***AEs with Low Solubility in Water***

During the application of the Könemann QSAR to AEs it was assumed that only the dissolved fraction of an AE test solution was available to cause a toxic effect. Since it is difficult to estimate this concentration and because it was considered likely that the low concentration of a poorly soluble AE would contribute little to the toxicity of a mixture containing soluble AEs, it was considered likely that AEs with predicted  $EC_{50}$ s above their solubility in water would not cause a toxic effect. Whilst it was recognised that this was not strictly correct, the resulting inaccuracies were considered to be within acceptable limits for the purpose of the QSAR. Subsequent work by Marshall and van Egmond (1995) suggests that undissolved chemicals are not toxic to fish in acute toxicity tests. This was further discussed by ECETOC (1996) who concluded that undissolved substances are generally not bioavailable.

### ***Calculation of Toxicities of Mixtures of AEs***

This calculation is based on the assumption that for mixtures of AEs toxicity is additive and that the toxicity of one structure is not modified by the presence of another. For example, a mixture of 10 AEs each at a concentration equal to 0.1 times its  $EC_{50}$  would be assumed to produce an effect equivalent to an  $EC_{50}$ .

### ***Verification***

Since the QSAR was applied to AEs and not derived specifically for this class of surfactants, a true training set was not involved. However, the applicability of the QSAR was established using a relatively small number of pure and commercial AEs. Subsequently the approach was applied successfully to many commercial AEs with predicted  $EC_{50}$ s typically within 50% of measured  $EC_{50}$ s. The relationship is described by the following :

$$\log EC_{50\text{observed}} = 0.91 \log EC_{50\text{predicted}} - 0.005$$

with  $r^2 = 0.953$  and  $n = 22$ .

### **3.5.3 Development of QSAR for Biodegradation**

In 1994, Boethling *et al* (1994) published four models to predict probability and rate of aerobic biodegradation. While no specific biodegradation endpoint is predicted, two of the models are

developed to predict the probability of biodegradation of chemicals and on this basis to classify them as "rapidly" or "not rapidly" biodegradable. The other two models provide a semi-quantitative prediction of primary and ultimate biodegradation rates. These models are present in BIODEG, a program developed by Syracuse (1994).

A linear and a logistic regression model calculate the probability of biodegradation based on presence of structural fragments and molecular weight and classify chemicals as "rapidly" or "not rapidly" biodegradable. The training set consists of 186 compounds that were classified as being "rapidly biodegradable" and 109 compounds classified as being "not-rapidly biodegradable". Classification was done after critical evaluation (Howard *et al*, 1987) of miscellaneous experimental data evaluated for consistency (Syracuse 1994). Chemicals in the "rapidly" and "not rapidly" biodegradation category are assigned an indicator variable with value of 1 and 0; respectively. This indicator variable serves as dependent variable in multiple linear and non linear regressions against 37 independent variables (36 fragments + molecular weight). Structural fragments (e.g. amide, ester, pyridine ring, ) were selected based on the general knowledge that they affect biodegradation. Regression coefficients in the linear model are estimated by the method of least squares. In the logistic model, regression coefficients are estimated using the maximum likelihood method. Fragment contributions are thought to be additive and fragment to fragment interactions are not considered. Because the approach had been verified before (Howard *et al*, 1992), verification with an independent set of data was judged not necessary by the authors.

The ultimate and primary biodegradation rate models present in BIODEG are based on a survey of 17 biodegradation experts conducted by the EPA in which experts were asked to evaluate 200 chemicals in terms of time required to achieve primary and ultimate biodegradability. The training set is given by Meylan and Howard (Syracuse, 1994). Primary and ultimate biodegradability were rated on qualitative scale using the terms 5-hours, 4-days, 3-weeks, 2-months, and 1-longer than months. Multiple linear regressions were performed using the mean scores for primary and ultimate biodegradation as dependent variables. The independent variables used are the same as for the linear and logistic model: counts of 36 structural fragments and molecular weight. Regression coefficients were estimated using the method of least squares. The primary or ultimate rating of a chemical is calculated by summing the values (fragment coefficients) of each fragment and then adding the summation to a constant coefficient value (3.8477 for primary and 3.1992 for ultimate biodegradability) that was determined for the entire dataset. Finally, the rating is converted back to the time required to achieve primary or ultimate degradation (i.e., predicted rating 3 = time required for biodegradation = weeks). Verification of the survey models occurred by comparison of survey scores to a limited set of measured biodegradation data. Direct comparison with estimates from the linear and logistic model are difficult as the training set and hence the domain, of both models partly differ.



The use of training sets with data from various biodegradation tests having different test conditions and endpoints and the use of an indicator value as biodegradation endpoint makes verification and appropriate use of above models difficult. While the domain appears to be defined by those compounds containing the structural fragments used in the training sets, it should be noted that for some descriptors the number of compounds in the training set is very low. Related to this is the fact that the database does not allow to check if values attributed by Boethling *et al* (1994) to selected fragments are statistically different from zero. A further disadvantage of the group contribution models used by Boethling *et al* (1994) is that interactions between fragments are not taken into account. As such, the chemical domain of the models is uncertain.

Using experimental data obtained in a biodegradation test designed by the Japanese Ministry of International Trade and Industry ("MITI-test"), the linear and logistic model incorporated in BIODEG were validated by Langenberg *et al* (1995). Their validation set consisted of 488 compounds of which 127 were readily degradable and 361 not-readily degradable. Overall, 56% of compounds were classified correctly as "rapidly (readily)" or "not-rapidly (not-readily)" biodegradable. This is below the OECD recommended critical 75% limit for QSAR performance for biodegradation. The performance was better for chemicals classified as not-readily than for those classified as readily biodegradable. Difference in predictability was shown to be highly dependent on the composition of the validation set which contained a much higher amount of non-biodegradables versus biodegradables as compared to the training set in BIODEG (Syracuse, 1994).

### 3.6 DATABASES

There is frequently the need to find data on the effect of, or the properties of, chemicals. Although this may be for product data sheet use or other chemical assessments, it is also a useful method to developing a QSAR. The need for a QSAR in such cases, is a wide range of well described data. It is preferable that the same methodology was used to generate the data, and the minimum of variation due to differing laboratories.

Table 8 describes sources of environmental data, with some indications of the type of data that is contained in the databases.

**Table 8: Databases Incorporating Environmental Information**

Database	Data	Comments
ENVIROLINE	Very wide range including air, water and land pollution and environmental impact of chemicals	
CA Search	Very wide database	Usually referred to as Chemical Abstracts
BIOSIS Previews	Life Sciences database	
EMBASE	Medical and Pharmaceutical database	
ECDIN	Data on 65,000 substances	
Environmental Fate Databases	Transport and fate of chemicals	Links 4 sub files : Datalog, Biolog, Chemfate and Biodeg
Hazardous Substances Database	Scientifically reviewed and edited data, includes biodegradation, ecotoxicity and log K <sub>ow</sub>	
LOG P Database	Log K <sub>ow</sub>	Contains over 30,000 data on 14,000 cpds.
AQUIRE	US EPA database for aquatic tox data, periodic updates	AQUatic Information REtrieval system - with simple quality rating system
ISHOW	US EPA database of phys chem data developed by Duluth Lab for QSAR studies	Information System for Hazardous Organics in Water - simple quality rating system
ENVIROFATE	EPA Office of Toxic Substances + SRC database of summary environmental fate and effects data	With simple quality rating system
BIODEG	EPA Office of Toxic Substances + SRC database of summary environmental fate and effects data	With simple quality rating system.
BIOLOG	Bibliographic database - containing references to papers covering biodegradation and bacterial inhibition studies.	No quality rating
DATALOG	Containing references to papers covering phys chem/occ health/ monitoring studies	Bibliographic database only - no quality rating
CHRIS	US Coastguard database containing full datasheets for materials transported by sea for emergency response	Chemical Hazard Response Information System - no quality rating
OHMTADS	US EPA database containing safety datasheets phys-chem/ecotox/environmental fate	Oil and Hazardous Materials/Technical Assistance Data System - no quality rating

**Table 8 (continued): Databases Incorporating Environmental Information**

Database	Data	Comments
TSCATS	Bibliographic references to data submitted to EPA under section 8d of TSCA	Toxic Substances Control Act Test Submissions database - Bibliographic - no quality rating.
RTECS	Data and bibliographic references to sources	Registry of Toxic Effects of Chemical Substances - National Institute for Occupational Safety and Health NIOSH
SIGEDB	Numerical database of phys chem/environmental fate data	In German
HEILBRON : Now Chapman & Hall Chemical Databases (CHCD)	Numerical/bibliographic data from C&H dictionaries	No quality ratings
Arizona DB	Aqueous solubility	10,000 data points - includes recommended values
CHEMINFO	Acute and chronic data	Limited to 600 chemicals
IUCLID	Any available data	On all HPVCs submitted by Industry
ECETOC - EAT	Aquatic acute toxicity	High quality but very limited

### 3.7 PROGRAMS FOR DEVELOPING QSARs

Increasingly the advent of the personal computer has led to the development of more programs offering a wider range of services, which may be used for the development of QSARs. These programs may be divided into three groups.

Firstly there are programs that estimate properties, to be used as descriptors, that may be used to generate the QSARs. These descriptors have been previously discussed, section 3.2.3. The comments made about the use and usefulness of the descriptors will apply, as will the comments that are made later (Section 4.2.1) about the estimations of those descriptors that are physico-chemical in nature, e.g.  $\log K_{ow}$ .

The next group of programs may be used to generate the QSARs using the descriptors and endpoint data. These are statistical programs that range from the simple linear regression available in, for example, Microsoft© Excel, to the complicated chemometric packages that are now widely available. Often these programs have been developed for other purposes, e.g. the exploration of multivariate analytical data, but the techniques lend themselves to the development of QSARs.

Finally there are programs whose primary use is to develop QSARs. The main features of these programs include the ability to input structures and endpoint data, generate the descriptors and then

statistically examine the relationships. In effect, they combine the two previous sets of programs into one program or series of linked modules.

Table 9 contains details of a limited series of such programs, giving the program name, input requirements and output available. The comments are based on those of the members of the Task Force, and were not the result of an extended or considered review of the programs. Where the table refers to a "chemometric package" this means that the program is essentially a chemometric program designed to run a wide range of the statistical procedures described in Section 3.2.6.

**Table 9: Programs for Development of QSARs**

Program	Manufacturer	Input	Output	Comments
MOPAC Pro	Adept Scientific plc	Structure	Semi-empirical quantum mechanical data	
GRAPH III	LPC	Structural data	Molecular connectivity indices	
SCAN FOR WINDOWS	Minitab Inc	Descriptors and endpoint data	Chemometric package	Runs under Windows, can cope with missing data, includes classification and regression analysis
UNSCRAMBLER	CAMO AS	Descriptors and endpoint data	Chemometric package	Runs under Windows, includes regression analysis, SIMCA and surface response mapping
Cerius <sup>2</sup> Quantum Mechanics Workbench and C <sup>2</sup> .QSAR+	Molecular Simulations Inc	Structures and end point data	Molecular descriptors and QSAR development	Many other modules also available to further extend the system
SciQSAR	SciVision	Structure and endpoint data	15 descriptors and regression analyses	Windows
HYPERCHEM	VCH	Structural data	Semi-empirical quantum mechanical data	Windows based, structure input rapidly changed to 3D several semi-empirical methods available, including Extended Huckel, AM1 and PM3
SIMCA	Umetri	Descriptors and endpoint data	Chemometric package	Windows includes numerous diagnostic and validation methods
SYBYL	Tripos	Structural data	Dependent on modules - very wide-ranging	Runs on UNIX systems, modular based. Has the potential to develop QSARs using wide range of generated or inputted data
CHEM-X	Chemical Design Ltd	Structure and endpoint information	Estimations of log K <sub>ow</sub> and statistical assessment versus endpoints	Variety of inter-linked modules, running on a wide range of computer platforms, develops QSARs
TOPKAT	Oxford Molecular	Structure and endpoint information	Predictions or QSAR development	Uses an internal database to check or develop QSARs, links with a wide range of other modules to generate descriptors and QSARs

### 3.8 PROGRAMS FOR USING QSARs

There are a number of programs that contain QSARs and whose prime purpose is to generate predictions. They are briefly described here and will be discussed in more detail in various sections in Chapter 4.

*CHEMEST* (Boethling *et al*, 1988) is a comprehensive software system for the estimation of chemical properties. It is commercially available and is largely based on the work of Lyman (1990a).

*EUSES* was developed based on the Technical Guidance Documents on risk assessment for new and existing substances (EEC, 1996). The system is intended as a regulatory tool to harmonise the hazard and risk assessment procedures. Different QSARs are used to fill in possible gaps in the data needed in the assessment process, are integrated parts of EUSES. The system is available to the public for a nominal payment.

The *SYRACUSE* Estimation Programs are a collection of stand-alone tools to estimate a variety of different physical-chemical and biodegradation endpoints (Meylan *et al*, 1992a; Meylan and Howard 1991a; 1993a; 1993b; 1994a; 1994b; 1994c; 1994d; 1995b; Boethling *et al* 1993). Additionally, there are two databases available which contain physical and environmental fate parameters for a large number of compounds. This commercial software system is widely used due to its availability on a number of common computer platforms.

*ASTER* (Russom *et al*, 1991) combines a comprehensive chemical database with a number of QSARs and was designed by the US EPA for risk assessment purposes. *ASTER* is available to international governmental agencies through different computer networks.

*ECOSAR* (Clements and Nabholz, 1994) was developed by the US EPA to estimate a number of ecotoxicity endpoints. It is a compilation of functional group QSARs on the basis of chemical classes. If the chemical in question has more than one functional group, the user has to choose which QSAR should be used.

### 3.9 SUMMARY

Within any process that needs information relating to the fate and effect of a chemical, including the regulatory needs, there is normally a use for QSARs. This often leads to two questions :

- is the data available as a measured endpoint ?
- is the measurement reliable ?

This is the point at which the QSAR process may be entered. This process should include the following steps:

### 3.9.1 Identification of QSARs

The first step is to identify whether there are any QSARs available for the required purpose. As part of this process the following actions are required :

- define the domain of chemicals of interest (see Section 3.2.4);
- assess available descriptors (see Section 3.2.3);
- define the endpoint (see Section 3.2.1).

If no appropriate QSARs are available, then in order to generate predictions one needs to be developed. This is more fully described in Section 3.2.

### 3.9.2 Description of Available QSARs

The next step is to assess those QSARs that are available. There are a number of parameters that need to be assessed only some of which will be mentioned here.

- accuracy: (is it sufficient for the purpose for which the QSAR is to be used ?);
- statistical parameters;
- domain: (is the QSAR extrapolating or interpolating ?);
- endpoint: (how close is it to that required, e.g. soil  $K_{oc}$  for sediment/water partition ?);
- nature of the QSAR: (is it mechanistic (preferred) or statistically based ?).

Again, if no appropriate QSARs are available one needs to be developed.

### 3.9.3 Use of QSARs

The issues involved in the use of QSARs are more fully discussed in Chapter 5. However, once the estimate has been generated the plausibility of that estimate needs to be assessed. If the value is not plausible then the process should be restarted as described in Section 3.9.1 and another QSAR investigated.

Finally, if a measurement is generated later, then it should be checked against the prediction made. If there are significant differences, both the measurement and QSAR need to be assessed, to identify reasons for the differences. Where appropriate the QSAR may need to be modified to reflect the incorporation of a new data point.

## 4. DESCRIPTION OF SELECTED QSARs

This chapter discusses various QSARs used to estimate the environmental fate and effects of a chemical. The endpoints considered, describe the physico-chemical behaviour of a chemical, its partitioning between different environmental media, its stability and effect on various organisms. In the discussion of the endpoints, a standard approach was adopted. First, an outline of the significance of the endpoint for environmental behaviour is given. Then, a selection of QSARs available for the estimation of the endpoint is described and their respective strengths and weaknesses are discussed. Many of the QSARs examined, especially those for physico-chemical properties are contained in one of five computer-based assessment systems described earlier (see Section 3.8).

### 4.1 ENVIRONMENTAL FATE PARAMETERS

#### 4.1.1 Physico-chemical Properties

##### *Melting Point*

The temperature at which, under constant pressure, the solid and the liquid phase of a substance are in equilibrium is referred to as the melting point. This parameter determines the physical state of a chemical and therefore its potential to disperse and to react in the environment as the substance. Although there is no basic difference in the specific reactivities of liquids and solids, they are only accessible to a reagent on their surface, therefore they show differences in the rates at which they react. Another implication of the melting point is that - as a general rule - the solubility of a substance increases as the melting point decreases. This feature is used by a number of QSARs for the estimation of solubility.

There are a number of QSARs for estimating melting point. The Lorenz/Herz approach (Gold and Ogle, 1969; Lyman, 1985) estimates the melting point from the boiling point of the compound of interest. Given that the boiling point is known, this provides a useful estimation tool. However, as pointed out by Boethling *et al* (1988), there is considerable uncertainty in the predictions. A comparison between estimated and measured data for 141 substances resulted in a mean error of 36° C.

In the second approach (Grain and Lyman 1983), the melting point is estimated from the molecular mass, the liquid density at the boiling point and a constant related to the chemical class. The applicability of this method is critically dependent on the availability of the above parameters. It is frequently noted that the class constant is a limiting factor of this method.



A recent development is an adaptation of the Joback method (Joback 1982; Reid *et al*, 1987). The original Joback approach is a group contribution method. It has been extended to include the same groups as used by Stein and Brown (1994) to estimate boiling points.

When tested on 388 compounds, the original Joback method had an absolute mean error of 23° C, a standard deviation of 25°C and a mean relative deviation of 11% (Joback 1982; Reid *et al*, 1987). In a comparison of 666 measured melting points to MPBPVP estimates (SYRACUSE, see later) an absolute mean error of 45° C and a standard deviation of 59° C are reported.

### *CHEMEST*

Two QSARs are available in CHEMEST, the Lorenz/Herz approach (Gold and Ogle, 1969) which estimates the melting point from the boiling point of the compound of interest, and the approach described by Grain and Lyman (1983). These have both been discussed above. It should be noted that these estimation methods are not widely accepted. Thus in its monograph on QSARs, the OECD (1993a) advised against the use of the CHEMEST procedures for melting point predictions.

### *EUSES*

No model is included in EUSES for estimating the melting point. Due to the importance of the melting point for environmental fate calculations, it is to be entered directly - no default values are provided by the system.

### *SYRACUSE*

The MPBPVP module of the SYRACUSE system estimates melting point by two different methods, the Gold and Ogle method and the adapted Joback method. The final estimate is computed as a weighted average of the output from each method with weights assigned according to the appropriateness of the methods to describe the chemical of interest. The ChemBase<sup>®</sup> Physical Properties DataBase<sup>®</sup> contains several hundred measured melting points.

### *ASTER*

No model is stored in ASTER for estimating the melting point. However, ASTER has a database which includes more than 6,000 measured melting points.

### ***Boiling Point***

The temperature at which a liquid's vapour pressure equals the atmospheric pressure is its boiling point. Much of what has been stated on the role of the melting point as a factor for environmental behaviour holds for the boiling point as well. The specific reactivities of liquids and gases are basically the same, but whereas liquids can only react at their interfaces to other phases, reactions with gases can occur throughout the volume they occupy. This means that potential reactants can access molecules of a given chemical much better in the gaseous phase than in the liquid phase. A further consequence of their physical differences is that the potential to disperse in the environment is fundamentally different for liquids and gases. Various QSARs use boiling point in the estimation of vapour pressure and water solubility.

There are a number of methods for estimating the boiling point. None of them require any input data beyond the structure of the compound of interest. Some of the main characteristics of the models are given in Table 10.

**Table 10: Characteristics of Several Models for Assessing Boiling Points**

No	Author	Applicability	Basis	Claimed Accuracy
1	Meissner (1949)	Organic compounds containing C, H, N, O, S and halides. Applicable to other compounds if their molar refraction is measured	Correlates boiling points with the parachor and the molar refraction. Adjustment is made for the chemical class	Mean error of ~2%, maximum error of ~8% in K
2	Forman and Thodos (1958, 1960)	Organic compounds containing C, H, N, O and halides	Estimates boiling points from the critical temperature and the boiling point/critical temperature ratio	Predictions not expected to be more accurate than 5 to 10 K
3	Miller (1980)	Most organic compounds	Estimates boiling points from the boiling point/critical temperature ratio, the critical pressure and the critical volume	Mean error of 23 K
4	Ogata and Tsuchida (1957)	Organic compounds containing C, H, N, O and halides of the form RX, where R is a limited number of hydrocarbon radicals with eight or less carbons	Estimates boiling points empirically	Errors <5 K for 98% of the chemicals
5	Somayajulu and Palit (1957)	Normal alkyl halides, acids, amines, ketones, aldehydes, benzenes, cyclohexanes and cyclohex-1-enes	Correlates boiling points with atomic number sum	Errors usually <5 K
6	Kinney (1938, 1940)	Alkanes, alkenes, alkynes, cycloalkanes and cycloalkenes	Correlates boiling points with 'boiling point number'	Errors usually <10 K
7	Stiel and Thodos (1962)	Alkanes only	Correlates boiling points with the number of carbons in saturated aliphatic hydrocarbons	Mean error ~0.5% in K

Sources: Rechsteiner (1990) and Boethling *et al* (1988)

It is obvious from the above table that only methods 1, 2 and 3 are applicable in a fairly general manner. The other methods are restricted to certain chemical classes. Meissner's method has been reviewed by Boethling *et al* (1988). He used Meissner's method for 179 chemicals and found a mean error of 23°C. The boiling points were underestimated for twice as many compounds as there were overestimations.

Another method which has been tested against a large number of measured boiling points is the method of Ogata and Tsuchida (1957). The authors found that the predictions were in excellent

agreement with the experimental data. For a group of 600 compounds, the deviations were  $<2^{\circ}\text{C}$  for 80% and  $<5^{\circ}\text{C}$  for 98% of the data, respectively.

Rechsteiner (1990) gives the following recommendations for selecting the appropriate estimation method:

- of the generally applicable methods (1, 2 and 3), 1 and 2 give more accurate estimates than 3. Of the former two, 1 is somewhat easier to use;
- method 3, due to its limited accuracy, should only be used for compounds which are not amenable to the other methods (e.g., nitro-containing compounds);
- methods 4, 5, 6 and 7, which are more specific with respect to chemical classes, yield better predictions than the general methods 1, 2 and 3. When a specific method is applicable to the compound of interest, it should be used in preference to other approaches.

The OECD (1993a) recommends method 1 for boiling point predictions. However, it is pointed out that this equation has been derived for monofunctional chemicals. When it is used for multifunctional compounds, prediction errors are expected to be much larger than indicated above.

The MPBPVP module of the SYRACUSE system uses an adaptation of the Stein and Brown (1994) method which is based on the Joback method (Joback, 1982; Reid *et al*, 1987) to estimate the boiling point. The Stein and Brown approach is a group contribution QSAR. The original method was extended in MPBPVP to include new functional groups and correction factors for specific chemical classes. The Stein and Brown method was derived from a training set of 4,426 organic compounds with an average absolute error of  $15.5^{\circ}\text{C}$ , a standard deviation of  $24.6^{\circ}\text{C}$  and an average error of 3.2% (Stein and Brown, 1994).

When the method was validated on a set of 6,584 compounds which were not part of the training set, the average absolute error amounted to  $20.4^{\circ}\text{C}$ , the standard deviation to  $38.1^{\circ}\text{C}$  and the average error to 4.3% (Stein and Brown, 1994).

#### *CHEMEST*

The system offers seven methods for estimating the boiling point (Table 10).

#### *EUSES*

None of the environmental fate modules included in EUSES relies on the boiling point, and thus the program does not use a QSAR.

## SYRACUSE

The MPBPVP module of the SYRACUSE system uses an adaptation of the Stein and Brown (1994) described above. There are several hundred measured data points stored in the ChemBase<sup>®</sup> Physical Properties DataBase<sup>®</sup>.

## ASTER

The model used for estimating boiling points is the Meissner method (Meissner, 1949). This method is also part of the methods available in CHEMEST and has been discussed in the corresponding section above. ASTER also has access to 3,000 measured boiling points in its database.

## **Vapour Pressure**

The pressure exerted by the vapour phase of a chemical which is in equilibrium with its condensed phase at a given temperature is called the equilibrium vapour pressure or vapour pressure for brevity. The vapour pressure determines the potential of a chemical to volatilise from its condensed or dissolved phases and to therefore exist as a gas. The boiling point can easily be derived from the vapour pressure: it is the temperature at which the vapour pressure equals atmospheric pressure. Therefore, the role of the vapour pressure as a factor for environmental behaviour is the same as that of the boiling point. An important partitioning parameter for the environmental behaviour of chemicals, Henry's law constant, is based on vapour pressure.

Two of the most common methods available rely on knowledge of the boiling point. Some of their characteristics are summarised in Table 11.

These methods require a minimum of experimental data and apply to almost any organic material, irrespective of its vapour pressure and physical state. Care must be taken when estimating the vapour pressure of a solid. Extrapolating a particular estimation method below the melting point yields the vapour pressure of the supercooled liquid rather than that of the 'crystalline' solid. Grain (1990) gives an equation to derive the vapour pressure of the solid from that of the supercooled liquid.

MPBPVP, the SYRACUSE program, estimates vapour pressure by three different methods: the Antoine method (Table 11), the modified Grain method (Lyman, 1985) and the Mackay method (Lyman, 1985).

All these estimation methods are based on the boiling point. The modified Grain method is a refinement of the modified Watson correlation (Table 11). The Mackay method applies to two chemical classes: hydrocarbons (aliphatic and aromatic) and halogenated compounds (aliphatic and aromatic).

The program calculates vapour pressures by all three methods. For liquids, it then suggests the modified Grain estimate, for liquids and gases the arithmetic average of the Antoine and the modified Grain estimates are given. The Mackay approach is not used in these suggestions due to its limited applicability.

**Table 11: Characteristics of Some Models for Assessing Vapour Pressure**

No.	Author	Applicability	Basis	Claimed Accuracy
1	Antoine (1888)	Liquids and gases, range of vapour pressures $1.33 \times 10^{-1}$ to $1.01 \times 10^5$ Pa	Estimates vapour pressure from the boiling point	Vapour pressure between $1.33 \times 10^{-1}$ and $1.33 \times 10^3$ Pa: mean error ~86%  Vapour pressure between $1.33 \times 10^3$ and $1.01 \times 10^5$ Pa: mean error ~3%
2	Watson (1943)	Liquids and solids, range of vapour pressures $1.33 \times 10^{-5}$ to $1.01 \times 10^5$ Pa	Estimates vapour pressure from the boiling point, allows for temperature dependence of the heat of vaporisation	Vapour pressure between $1.33 \times 10^{-5}$ and $1.33 \times 10^{-1}$ Pa: mean error ~47%  Vapour pressure between $1.33 \times 10^{-1}$ and $1.33 \times 10^3$ Pa: mean error ~39%  Vapour pressure between $1.33 \times 10^3$ and $1.01 \times 10^5$ Pa: mean error ~3%

Source: Grain (1990)

'Suggested' MPBPVP vapour pressures were compared to measured values for 805 compounds (all ranging between 20 and 30° C). All the boiling and melting points used for the vapour pressure estimates were estimated by MPBPVP (see sections above for a description of the methods used). Melting points are used by MPBPVP to convert a supercooled liquid vapour pressure to a solid-phase vapour pressure which is necessary for solids only. An  $r^2$  of 0.941 for the correlation between measured and estimated values was calculated. The standard deviation amounted to 0.717 and the absolute mean error of prediction to 0.476 mm Hg. All these measures are based on calculations on a logarithmic scale. The MPBPVP manual attributes a large fraction of the prediction error to poor boiling and melting point estimates and concludes that for maximum vapour pressure accuracy, experimental boiling and melting points should be used in the estimates.

#### CHEMEST

The two estimations used by CHEMEST are given in Table 11 above.

### *EUSES*

There is currently no method stored in EUSES for estimating vapour pressure. At present, the system suggests a default value of  $10^{-3}$  Pa when no measured vapour pressure is available.

### *SYRACUSE*

The three methods contained in MPBPVP are described above. The ChemBase<sup>®</sup> Physical Properties DataBase<sup>®</sup> contains several hundred measured vapour pressures.

### *ASTER*

The method provided for vapour pressure estimation is the modified Watson correlation (Table 11). ASTER also provides a total of 800 measured vapour pressures.

### ***Water Solubility***

The water solubility is a measure for the maximum amount of a chemical substance which can be dissolved in a given volume of water at a certain temperature. This is of major importance for the environmental fate of chemicals. The dissolved fraction of a chemical can disperse rapidly over large volumes of water. Furthermore, reactions in the dissolved phase can occur throughout the volumes over which the substances are distributed. Both factors are favourable to a high reactivity of dissolved substances. Water solubility is related to a number of important environmental partitioning parameters, e.g. soil or sediment adsorption coefficients, bioconcentration factors and Henry's Law Constant. Additionally, degradation processes such as hydrolysis or biodegradation mainly affect the dissolved fraction of a chemical, not its condensed or adsorbed fractions. However, depending on the substance, the water solubility will vary with changes to the local environment, e.g. pH or ionic strength. For this reason an extrapolation to environmental behaviour must be performed carefully.

Although the following sub-sections contain many of the regular QSARs used, it is important to note that they are mainly based on extrapolation from the octanol-water partition coefficient. Thus while they are probably adequate for most purposes, there will be chemicals for which these extrapolations are inadequate, when the octanol-water partition coefficient is unavailable, inadequately measured or inapplicable. In these circumstances, other QSARs should be considered e.g. Nirmalakhandan and Speece (1988a and 1989) described in Section 3.5.1.

*CHEMEST*

Two methods are provided. The first method is an assembly of 19 regression equations, 16 of which are solely based on a linear relationship between the logarithms of the solubility and the octanol-water partition coefficient. Two, use the melting point in addition and one equation does not rely on the octanol-water partitioning behaviour of the compound, but on its melting point and number of C atoms (Lyman, 1990b). The second method estimates the solubility from structural information alone (Irmann, 1965). Some main characteristics of both approaches are given in Table 12.

The methods cover a wide range of chemicals. However, as mentioned above, most of the equations in method 1 depend on the availability of octanol-water partition coefficients. In principle, this parameter can be estimated from structural information (see Section 4.1.2). Nevertheless, care must be taken when no measured values are available since the errors involved in both steps of such an estimation procedure may cause the final result to be substantially in error. Method 2 relies on structural information alone but is restricted to relatively narrow chemical classes.

**Table 12: Characteristics of Several Models for Assessing Water Solubility**

No.	Author	Applicability	Basis	Claimed Accuracy
1	Various, assembled by Lyman (1990b)	A wide range of chemical classes	Estimates solubility from the octanol-water partition coefficient (and the melting point) or the melting point and the number of C atoms. Each equation refers to certain chemical classes	Predictions by most equations within a factor of ten of the measured values. Between 5 and 14% of the estimates in error by more than a factor of 100
2	Irmann (1965)	Chemicals with C, H, Cl, Br, I, and F only	Estimates solubility from atom types and structural elements present in the molecule. Adjustment is made for the chemical class	Estimates for 90% of the compounds within 15% of the measured values. Maximum deviation: factor 1.6

Source: Lyman (1990b)

Both methods provide estimates at  $-25^{\circ}$  C. Moreover, most equations were obtained from liquid chemicals. Use of these equations for solids will generally overestimate their solubility if the predicted solubility is not corrected. Various corrections have been suggested which depend on the melting point (e.g. Irmann, 1965).



### *EUSES*

Three estimation methods are suggested by EUSES. All of them are based on the octanol-water partition coefficient and formally are very similar to method 1 used in CHEMEST. For liquids or solids with a melting point, expressions by Isnard and Lambert (1989) are used. If the boiling point is unknown, an expression can be used which has been derived by Veith (RIVM *et al*, 1994).

According to the manual, different accuracies can be assumed for these estimation methods. Whereas for liquids, the error is expected to be similar to method 1 used in CHEMEST, larger uncertainties (up to a factor 5 of the measured values) are presumed for solids, especially if no melting point is available.

The OECD (1993a) recommends the use of the equations by Isnard and Lambert for solids and liquids as they are based on a large variety of substances with a wide range of octanol-water partition coefficients.

### *SYRACUSE*

The WSKOWWIN package by SYRACUSE provides two equations for aqueous solubility estimation. They are both based on the octanol-water partition coefficient. One equation is applicable to solids and requires the melting point of the compound to be known. Moreover, corrections are made for different chemical classes. The equation for solids was developed from a dataset containing 1,450 compounds. For these data, a log standard deviation of 0.41 and a log mean error of 0.31 was found (Meylan and Howard, 1994a). When the method was used to predict the solubility of 817 compounds which were not contained in this dataset, the log standard deviation of the predictions was 0.615, an  $r^2$  between the measured and the predicted values amounted to 0.902 (Meylan and Howard, 1994e). In addition to these estimation methods, the ChemBase<sup>®</sup> Physical Properties DataBase<sup>®</sup> contains several hundred measured solubilities.

### *ASTER*

ASTER uses one of the following algorithms developed by Veith.

If the program is not told whether the compound is a liquid or a solid the relationship is:

$$\log S = -1.23 \times \log K_{ow} + 0.79$$

where S is the molar water solubility.

If the chemical is known to be a solid then :

$$\log S = -1.16 \times \log K_{ow} + 0.705 - 0.009 \times (mp - 25)$$

where mp is the melting point in K.

If the chemical is known to be a liquid or a gas, then :

$$\log S = -1.16 \times \log K_{ow} + 0.705$$

### ***Acid dissociation constant (pKa)***

The acid dissociation constant is the pH at which a substance has undergone 50% dissociation. The dissociated chemical has different physico-chemical properties and reactivities compared to its associated counterpart. De-protonation of a chemical involves a change in the polarity of that chemical and, the aqueous solubility for the charged species can be several orders of magnitude higher than for the neutral molecule. As has been mentioned in the 'Water solubility' section, a number of partitioning and stability parameters are affected by such changes in solubility.

### ***CHEMEST***

The dissociation constant of an organic acid is commonly estimated by using linear free energy relationships, which as applied in this context, is basically a substituent-effect approach. Three methods are provided by CHEMEST, two of them based on Hammett and one on Taft correlations. Both approaches rely on substituent constants as well as specific reaction constants. Some main characteristics of the three methods and their ranges of applicability are given in Table 13 below.

**Table 13: Characteristics of Several Models for Assessing Acid Dissociation Constants**

No.	Applicability	Basis	Special features
1	Aromatic acids	Hammett correlation	
2	Aromatic acids	Hammett correlation	Accounts for 'through resonance' between reaction centre and electron-withdrawing substituents
3	Aliphatic acids	Taft correlation	Corrects for steric effects

Source: Harris and Hayes (1990)

Harris and Hayes (1990) give several sources of uncertainty in estimated values. These include:

- deviations from the basic linear free energy relationship assumptions of separability of substituent constants and reaction constants;
- difficulties in selecting appropriate parent compounds as well as extending the correlations beyond the range of substituents used in defining the reaction constant for the parent;
- that the available substituent and reaction constants have not been derived from one consistent set of measurements but from various sources.

Harris and Hayes compared some predicted and measured acid dissociation constants. They concluded that errors were smallest for aromatic species with a single acid functionality. Larger errors could be expected for aliphatic species and for compounds containing several acid functionalities. For 22 compounds belonging to various chemical classes, the errors in the estimated values ( $K_a$  values) ranged between 0.7 and 1600%, with only two errors >100%.

#### *EUSES*

No estimation methods for the acid dissociation constant are provided by the EUSES system.

#### *SYRACUSE*

No estimation methods are available. The ChemBase<sup>®</sup> Physical Properties DataBase<sup>®</sup> contains several hundred measured acid dissociation constants.

#### *ASTER*

Dissociation constants are estimated using an algorithm by Hunter (1988) which is based on Perrin *et al* (1981). The Perrin method calculates  $pK_a$  from the structure of the chemical, Hammett/Taft equations of analogous chemical classes and substituent constants. Approaches to attenuate effects of the reaction site and corrections for symmetrical reaction sites are used to refine the estimation method.

A number of measured  $pK_a$  were compared to predictions based on Hunter's algorithm. This resulted in a  $r^2$  of 0.65 and a standard deviation of 1.91  $pK_a$  units,  $n = 993$ . However, 85% of the estimates were within 1  $pK_a$  unit and 77% within 0.5  $pK_a$  units of their experimental values (Hunter, 1988).

More recently another program, SPARC, (SPARC Performs Automated Reasoning in Chemistry) that is used to estimate properties from structure has become available. The first version is available from the EPA and may be used to predict the  $pK_a$  of a chemical. The estimations have been found to be

excellent, thus 4,338 pKa values were used to test the predictability of the model. The resulting statistics were  $r^2$  of 0.99 with a standard deviation of 0.37 (Hilal and Karickhoff, 1995).

#### 4.1.2 Partitioning Parameters

##### ***Octanol-water Partition Coefficient ( $K_{ow}$ )***

A chemical's octanol-water partition coefficient,  $K_{ow}$ , describes its distribution between octanol and water after equilibration. The method (OECD 107) involves shaking a saturated solution of the substance in octanol or water with the other phase and analysing the concentration of the substance in the two phases after equilibration.

The experimental method has a number of problems which have been fully discussed (ECETOC, 1995) and the problems of the method will only be briefly discussed here. These are :

1. the method is unsuitable for surface-active molecules;
2. it is not applicable for ionic materials;
3. it has a limited range, up to  $\log K_{ow}$  of 6;
4. it is difficult to apply for very volatile substances.

As a result, a number of variants have been developed including the "slow-stir" method (Brooke *et al*, 1986) and estimation by HPLC (OECD 117). It is important when assessing data and QSARs describing  $\log K_{ow}$ , that the method used to obtain the values is ascertained.

There have been a number of different approaches to calculating  $\log K_{ow}$ , many of which have been reviewed and described in detail by Lyman (1990a).

The most frequently used method of calculating  $\log K_{ow}$  is by adding the hydrophobic fragments of molecules as developed by Hansch and Leo (1979). Lyman (1990a) found that this method gave  $\log K_{ow}$  values to  $\pm 0.12$  units for a variety of compounds. Meylan and Howard, (1995a), report that another fragment approach was able to predict  $K_{ow}$  within 0.8 log units for over 96% of an experimental dataset of over 8,000 chemicals and was considered to be statistically superior to other estimation methods.

Other methods of calculating  $\log K_{ow}$  such as correlation with parachor, molecular connectivity and water solubility have also been found to be accurate (McGowan, 1952; Murray *et al*, 1975; Chiou and Schmedding, 1982), however, the accuracy of calculated values declines as the  $\log K_{ow}$  exceeds 5.5 (Chessels *et al*, 1991).

One factor that must be highlighted when calculating  $\log K_{ow}$ , is that all the QSARs are based on chemicals which, in general, have measured  $\log K_{ow}$  values less than 6. Consequentially the prediction of  $\log K_{ow}$  values greater than 6 must be considered as an extrapolation and hence treated with caution.

#### *CHEMEST*

No estimation method available.

#### *EUSES*

There is no model in EUSES for estimating  $\log K_{ow}$  as it is part of the base-set for new substances and would also be required as a measured endpoint for any existing chemicals that were to be assessed.

#### *SYRACUSE*

The KOWWIN program is based on the group contribution method described by Meylan and Howard (1995b). The method uses fragments and correction values associated with each fragment. The method was based on the use of multiple regression of the original test set, which generated the fragments and correction factors. The fragments, of which there were initially 125, are used to re-construct a molecule. Depending on the fragments used and their interactions, a number of different factors may be applied. There were over 250 such correction factors. The original development was based on a training set of 2,351 compounds and yielded an  $r^2$  of 0.982, with a standard mean error of 0.16. A follow-up assessment on a test set of 6,567 chemicals gave an  $r^2$  of 0.944 and a mean error of 0.31.

#### *ASTER*

The estimation algorithm used in ASTER is CLOGP. This is a program described by Hansch and Leo (1979) and is one of the most widely used estimation programs. As with KOWWIN, the method is a group contribution method. However, the methods used for obtaining those contributions is very different. Hence the CLOGP approach utilises the changes that occur to the measured  $\log K_{ow}$  of a molecule when a group is added to a molecule.

In a validation check of the accuracy of the two methods, CLOGP and KOWWIN, Müller and Klein (1994) used 1,166 compounds. These were mainly simple organic molecules and although KOWWIN was better for chlorinated molecules and those containing phosphorous and sulphur, the two methods gave very similar results.

AUTOLOGP, a more recent calculational approach, has been reported by Devillers *et al* (1995a). This method has been developed using auto-correlation, Broto and Devillers (1990), and the fragments described by Rekker and Manhold (1992). The training set comprised of 800 heterogeneous substances. Although to date there has been little experience of the approach, it appears to be better with chemicals of high log  $K_{ow}$ , for example PCBs, PAHs, PCDDs and PCDFs.

### **Soil-Water Partition Coefficients**

The description of this parameter has been discussed previously (see Section 3.2.1). As already mentioned, the prediction of log  $K_{oc}$  can only be applied to non-ionic chemicals with matrices in which organic carbon is the dominant factor. This is discussed in more detail below.

Other than Lyman (1982), there have recently been a number of other reviews and some development of models for predicting  $K_{oc}$ . The most recent and relevant to this report are those by Güsten and Sabljic (1995a; 1995b) who reviewed 20 different relationships and suggested a strategy for their application and Watts *et al* (1995) as part of a review of QSARs for several physico-chemical properties. Within this work, predictions were generated using the Meylan and Howard method (see discussion below).

Some of the problems and concerns that need to be considered when developing or using QSARs for this endpoint were discussed by Karickhoff (1985). In this review it is noted that while the adsorption isotherm for non-ionics may be linear with low concentrations e.g. less than  $10^{-5}$  M  $l^{-1}$ , or below half the water saturation concentration, this is not necessarily the case with ionics.

Karickhoff also indicated three sets of conditions under which the role of non-hydrophobic interactions could be substantially increased, and hence QSARs based on log  $K_{ow}$ , more prone to inaccuracies. These are :

1. high sorbate polarity;
2. low organic carbon in the soil and/or high clay content;
3. low soil moisture.

The same review then compared several  $K_{ow}$  derived QSARs and showed that there were possible deviations caused by either non-hydrophobic interactions (leading to underestimation) at low  $K_{ow}$ s and steric or kinetic limitations (leading to overestimation) at high  $K_{ow}$ s.

More recently, experimental evidence has demonstrated the impact of organic matter and the inconsistent variation of adsorption of a chemical when the organic matter was removed (Fröbe *et al*,

1994). The conclusion was that the interactions of some chemicals with the mineral surface of the solid phase could be much stronger than the interaction with the organic phase.

Clearly there is a need for research to focus on these interactions and to begin the process of linking structure to ionic interactions in future QSARs for estimating soil sorption. One approach that may point towards one direction of research was described by Ames and Grulke (1995). This method assumed a structure for soil organic matter and calculated the solubility of non-ionic organic chemicals in this phase. The results were promising, although measured activity coefficients were needed to obtain reasonably accurate predictions. However, the use of a model structure might allow further modelling and refinements and thus extend the potential for a method of this nature.

The simplest and perhaps most widely used approach is that based on  $\log K_{ow}$  described by Karickhoff *et al* (1979):

$$\log K_{oc} = 1.00 \log K_{ow} - 0.21$$

Two other models of this nature that may be more widely applicable have also been reported. Means *et al* (1982) developed a relationship using substituted polyaromatic hydrocarbons and Schellenberg *et al* (1984) also developed a model, but for a series of chlorinated phenols. A more recent development to develop a general QSAR using  $K_{ow}$  was described by Bintein and Devillers (1994). Working with the original partition coefficient they were able to develop a QSAR using  $\log K_{ow}$  and  $pK_a$  for soils and sediments, with the introduction of a correction factor for acids or bases dependent on the difference between the  $pK_a$  and the soil pH.

As with the other endpoints, approaches based on structural sub-units or other molecular descriptors have also been developed. The use of topological indicators was described by Sabljic and Protic (1982; Sabljic, 1984; 1987; 1989) in a series of papers that utilise molecular connectivity indices.

Initially, Sabljic and Protic (1982) took Karickhoff's data of PAH  $K_{oc}$  values and established a relationship based on the second order valence molecular connectivity indices, (MCI). Later, Sabljic (1984) developed a first order MCI. The study was then extended to include chlorinated materials and then a wider set of chemicals, including alkanes, alkenes and chlorinated phenols (Sabljic, 1987). The relationship was found to be sufficiently robust that it was then further extended to a range of ionic materials. However all the  $K_{oc}$ s for these chemicals were over estimated. This led Sabljic to conclude, that (halogenated) hydrocarbons had optimal geometry and electron configurations for strong sorption to soil and the introduction of a polar sub-unit into a molecule led to a decrease in its  $K_{oc}$ . On the basis of this study, a set of semi-empirical coefficients were established to correctly predict the  $K_{oc}$  of these polar molecules, to account for the polar functional group.

The most recent method using group contributions was described by Meylan *et al* (1992b), which also incorporates molecular connectivity indices. Based on a dataset of 189 chemicals for the training set and 205 other chemicals for the test set, the initial approach divided the chemicals into two sets of non-polar and polar chemicals. A regression for the non-polar chemicals against the first order MCI was obtained and this was then corrected by the addition of group contributions obtained from the polar set of chemicals.

#### *CHEMEST*

No estimation method available.

#### *EUSES*

The model in EUSES for estimating  $\log K_{oc}$  is based on the Karickhof equation (Karickhof, 1981) as described above. However, there is also the opportunity to use either the estimated value obtained by HPLC or to use one of the equations described in Chapter 4 of the TGD.

#### *SYRACUSE*

The PCKOCWIN program is based on the group contribution method described by Meylan *et al* (1992a). The method has been described above.

#### *ASTER*

The estimation algorithm used in ASTER is based on that described by Lyman (1982). The method uses the  $\log K_{ow}$ , using the following relationship :

$$\log K_{oc} = 0.544 \times \log K_{ow} + 1.337$$

#### **Henry's Law Constant**

The Henry's Law Constant, H [Pa x m<sup>3</sup>/mole], is a property that represents the ability of a pure chemical to partition between air and water (Meylan and Howard, 1991a; 1991b). Therefore, it is relevant for environmental risk assessment.

Henry's Law correlates the partial vapour pressure of a solute x with its concentration in a solution (Atkins, 1982):



$$Vp_x = H_x \times [x]$$

The most reliable values for H are determined by direct measurement applying a proven experimental procedure (Mackay and Shiu, 1981).

As experimental data are relatively scarce, it is usually necessary to estimate H by one of several methods, such as the ratio of the vapour pressure and the solubility, these quantities being measured independently, and structure activity relationships (Lyman, 1985):

$$H = Vp / S$$

This equality is based on theory and assumes only that:

1. the solubility is <1 mole/l;
2. the total pressure is ca. 1 atmosphere, hence the vapour phase fugacity coefficient is 1.0;
3. no association of molecules in either solution or the vapour phase occurs.

The use of this equation varies, depending on the physical state of the chemical at environmental conditions. For gases, it is necessary to set Vp to 1 atmosphere. For liquids and solids, it is important to establish that the values of Vp and S are correct for the physical state at environmental conditions, ensure sometimes the Vp of solids is reported for a super cooled liquid.

Using the Vp/S ratio will always result in values for H that are lower than those obtained experimentally. It is roughly estimated that for S being 0.5, 1.0 and 2.0 mole/l, the calculated values for H will 10, 20 and 35% lower than measured values. Extrapolating these observations, the failure of the equation for infinitely miscible chemicals becomes obvious.

The above equation is the method of choice whenever reliable measured values of Vp and S are available and when  $S \leq 1$  mole/l. In other cases, the use of other estimation methods may provide a lower method error than the use of estimated values of Vp and S.

In 1975, Hine and Mookerjee described two methods for estimating H directly from the molecular structure. The first, uses group contribution for about 70 groups and the second, uses bond contributions for 34 different bond types. According to the authors, the group contribution method gives more reliable results than the bond contribution method.

The bond contribution method has been updated by Meylan and Howard (1991a) who also provide a computer program performing the respective calculations. On the basis of a training set of 345

chemicals they derived a QSAR with a correlation coefficient ( $r^2$ ) of 0.97, a standard deviation of 0.34 and a mean error of 0.21. These statistics apply to LWAPC values (log water to air partitioning coefficients), see also EUSES.

In the most recent version of the computer program HENRYWIN (Meylan and Howard, 1994c), the authors claim that the methodology has been upgraded by addition of new bond contributions and new correction values. However, no statistics on these improvements have been provided.

### *CHEMEST*

Two methods are provided. These are the Vp/S ratio and the Hine and Mookerjee Group contribution method.

### *EUSES*

The EUSES program, when no experimental H is entered, applies the Vp/S ratio. There are no limitations in the application of this method.

For the purpose of the exposure assessment within EUSES, the air-water partition coefficient ( $K_{aw}$ ) is used (RIVM *et al*, 1994). This coefficient, also known as the dimensionless Henry's Law Constant, is related to H according to:

$$K_{aw} = H / (R \times T)$$

where R is the gas constant (8.314 Pa x m<sup>3</sup>/mole x K) and T the absolute temperature in K.

### *SYRACUSE*

In the Syracuse property estimation package the HENRYWIN program based upon Meylan and Howard is provided.

### *ASTER*

The estimation of H in the ASTER package is based on the equation mentioned above, i.e. Vp/S ratio

### 4.1.3 Stability Parameters

#### *Aquatic photolysis*

In an aqueous system, a chemical may undergo a number of photolytic processes. Transformations which are caused by light absorption of a given substance are called direct photolysis. Indirect photolysis includes a number of different mechanisms, where it is not the substance of interest which absorbs light, but some other chemical moiety present in the system. These processes involve either energy transfer from an excited species to the substance of interest (sensitised photolysis) or chemical reactions of the substance of interest with highly reactive species (e.g. hydroxy-radicals, peroxy-radicals and singlet oxygen) that are formed in the presence of light. Photolytic reactions do not normally proceed to a complete mineralisation of the parent molecule. Nevertheless, its properties (toxicological and environmental fate parameters) can be drastically altered. Of interest for our discussion, are reactions that occur under natural sunlight conditions at the earth surface (i.e. at wavelengths between 290 and 600 nm). Though photolytic reactions may proceed solely or principally via indirect photolysis in natural water bodies, no efforts have been taken towards developing QSARs for indirect photolysis. Therefore the discussion here will be restricted to direct photolysis.

Generally, photolytic reactions involve large negative changes in standard free energy. Therefore, photolysis can be viewed as an irreversible process. Furthermore, most photolytic reactions can be assumed to be first-order or pseudo-first order.

None of the systems discussed here (CHEMEST, EUSES, SYRACUSE and ASTER) provide any QSARs for estimating rates of photolysis. This is because the methods developed up to the present are only applicable to a few chemical classes, whereas the software systems considered are generally applied to a wide range of chemicals.

The information needed to estimate rates of photolysis consists of the incident light intensity to which the compound of interest is exposed, its absorptivity as well as the quantum yield of the reaction. While the solar radiation characteristics on the earth surface as well as the UV-spectrum of the compound can be estimated or measured easily, the quantum yield is more difficult to measure.

Therefore, Peijnenburg *et al* (1992) have developed a QSAR which can be applied to mono- and disubstituted meta-halobenzenes and halophenols. The model uses as predictors, steric factors of the substituents, as well as the bond strength of the C-halogen bond, because its cleavage is the rate-limiting step of the reaction. The applicability of the approach was recently extended to multiple-substituted halobenzene derivatives (Stegeman *et al*, 1993). In addition to the above specified molecular descriptors, the authors used the inductive constants of the substituents to predict the quantum yield. The accuracy of both QSARs can be estimated from the data presented in the papers.

The mean errors amount to about 12 and 9% for Stegeman *et al* (1993) and Peijnenburg *et al* (1992), respectively. However, these data refer to the chemicals on which the models are based. Quantum yields predicted for chemicals which have not been involved in the development of the QSARs, would certainly deviate more from measured data. Given the limited applicability and validation of the method, there is a clear need for further work in this area.

### **Hydrolysis**

Reactions of a chemical with a water molecule, a hydroxide or a hydronium ion are commonly called hydrolysis. Due to its great abundance, water plays a pivotal role in the turnover of organic chemicals. In a hydrolysis reaction, the compounds are transformed into more polar products. These typically have different properties with respect to fate and effects than the parent compound. Though hydrolysis does not normally lead to complete mineralisation, its products are often of less toxicological concern compared to the starting compound. When considering environmental fate, the increased polarity of hydrolysis products will in general increase their aqueous solubility and therefore decrease the fractions residing in the adsorbed and gas phases.

Like photolytic reactions, most hydrolysis reactions involve large negative changes in standard free energy. In general, this causes hydrolytic processes to be irreversible. Furthermore, most hydrolysis reactions can be assumed to be first-order. However, the overall rate of hydrolysis includes contributions from acid- and base-catalysed, as well as neutral hydrolysis, depending on the prevailing pH conditions. Therefore, the task of predicting a hydrolysis rate, involves estimating the contributions of each of these mechanisms at the relevant pH.

### **CHEMEST**

The system provides seven methods for estimating hydrolysis rates which are all based on the application of linear free energy relationships. Harris (1990) gives an outline of the underlying theory. All methods are either based on Hammett or Taft correlations or on correlations with the pKa of the leaving group. Hammett correlations rely on Hammett's substituent constants as well as specific reaction constants, whereas Taft correlations use two parameters, which describe polar and steric effects of the substituents as well as two specific reaction constants. The range of application for each of the CHEMEST methods can be obtained from Table 14.

**Table 14: The Range of Application for each of the CHEMEST Methods**

No.	Applicability	Estimate	Basis
1	Ring-substituted benzamides, ethyl benzoates	Rate constant for acid-catalysed hydrolysis	Hammett correlation
2	Ortho-substituted benzamides	Rate constant for acid-catalysed hydrolysis	Taft correlation
3	Benzyl halides, dimethyl benzyl halides, benzyl tosylates	Rate constant for neutral hydrolysis	Hammett correlation
4	A number of benzene ring-substituted compounds	Rate constant for base-catalysed hydrolysis	Hammett correlation
5	Dialkyl phthalate esters	Rate constant for base-catalysed hydrolysis	Taft correlation
6	Aryl esters of methylphosphonic acid, a number of carbamates	Rate constant for base-catalysed hydrolysis	Correlation with pKa of leaving group

Source: Harris (1990)

All these correlations are based on rather limited datasets and cover only a small number of chemical classes. Though systematic comparisons between predictions based on the above methods and measured hydrolysis rates have not been done, Harris (1990) suggested that the estimated rates should be considered as order-of-magnitude estimates. If more precise statements are needed, measurements are inevitable.

### *EUSES*

The half-life for hydrolysis is one of the parameters used for calculating the overall degradation of a compound in surface water. However, no method is provided for predicting hydrolysis. If measured data are unavailable, no hydrolysis is assumed as a default.

### *SYRACUSE*

The HYDRO module of the SYRACUSE system estimates hydrolysis rates for selected chemical classes: esters, carbamates, epoxides, halomethanes and certain alkyl halides. The predictions rely on regression equations established for each chemical class and for acid- and base-catalysed hydrolysis (Meylan and Howard, 1993a). No methods are available for estimating neutral hydrolysis. The equations are formally similar or identical to the methods used by CHEMEST. They rely on Hammett and Taft constants, steric factors and, some equations, on further fragment specific parameters. An idea of the accuracy of HYDRO can be obtained from measured and predicted data for 70 compounds

which are given in the manual. The relative errors are below 50% in 25 cases and above 100% in 13 cases.

For situations where neutral hydrolysis is important, HYDRO will not give a sound indication of the overall hydrolysis rate.

The ChemBase Physical Properties DataBase contains several hundred measured hydrolysis rates.

### *ASTER*

The QSAR approaches described by Harris (1990) are used by ASTER for estimating hydrolysis rates. These methods are used by CHEMEST as well and have been discussed in the corresponding section above. One point to note, however, is that if the molecule contains unrecognisable fragments then a default of >1000 days is given.

### ***QSARs in Biodegradation***

Anaerobic biodegradability is frequently tested using the ECETOC test (ECETOC, 1988). Net biogas production in reactors containing the test substance is compared to biogas production in control reactors (not containing test substance). As with the ready biodegradability test (see Section 3.2.1 Endpoint Variability), this test is a screening test and uses an unrealistically high test substance to biomass ratio. Anaerobic testing with radiolabelled test compounds allows for realistic test substance and biomass concentrations to be used (Wagener and Schink, 1987; Nuck and Federle, 1996). However, currently few data have been produced using a consistent approach and this is reflected in the lack of QSARs for anaerobic biodegradation.

The rest of this section will therefore concentrate on aerobic biodegradation QSARs. The source of the problems to be expected with such QSARs have been discussed previously (see 3.2.1 Endpoint Variability).

Recent reviews on structure-activity relationships between biodegradation potential and/or rates of organic compounds and chemical structure parameters include those by Parsons and Govers (1990), Kuenemann *et al* (1990), Peijnenburg (1994), the OECD (1993c), and Peijnenburg and Karcher (1995). In general, two approaches may be distinguished (Peijnenburg, 1994):

1. A mechanistic approach (Parsons and Govers, 1990; Damborský, 1995) in which an attempt is made to identify the rate limiting step within the overall process.

2. A statistical approach in which large datasets containing results of various test procedures are used as a training set (Kuenemann *et al*, 1990; Howard *et al*, 1992; Boethling and Sabljic, 1989; Boethling *et al*, 1994).

Damborský (1995) reviewed key aspects of the mechanistic approach and provided a conceptual model on how to derive QSAR models for microbial degradation of organic compounds. The approach proposed to "compare various biological data measured with different species, under different conditions and at the different organisation levels in order to extract and quantify the mechanisms taking place during microbial biodegradation". This was recently applied to hydrolytic dehalogenation (Damborský *et al*, 1996) and provided insights into the rate limiting steps for this biodegradation process. Due to the complexity of most chemical degradation pathways and the fact that many non-specific enzyme systems are involved in biodegradation transformations, these type of models are specific to limited numbers of structurally related compounds.

A recent approach is to use knowledge of biodegradation pathways and patterns in physiology and enzymology to predict the biodegradability of chemicals in the environment (Klopman *et al*, 1995; Klopman 1996; Punch *et al*, 1996). With expert knowledge of enzyme substrate specificity and sequential steps in metabolism, chemical structures are modified to yield biodegradation intermediates/metabolites. This evaluation step can take into account the prevailing environmental conditions (Punch *et al*, 1996), for example, availability of various electron acceptors or molecular oxygen. Chemical structures are broken down into structural fragments and each target fragment is associated with a product fragment representing the most probable aerobic biodegradation metabolites. Currently, work is in process to 1) refine and extend the current biodegradation rules (Klopman, 1996; Punch *et al*, 1996), and 2) validate the biodegradability prediction systems (Punch *et al*, 1996).

In the models following the statistical approach, chemicals tested are typically subdivided into specific chemical classes and subsequently, structure activity models for estimating microbial degradability of homologous substances are developed. The outcome of these models is often a classification (yes/no biodegradable) or a ranking of biodegradation potential, resulting in a SAR. The "fragment" approach has been the most frequently adopted. It is based on the presence of certain substructures in a chemical which either decrease or increase biodegradation. Other approaches use physico-chemical properties, and/or geometric and topological descriptors in either regression or multivariate analysis, or neural network techniques including genetic algorithms. Basic principles underlying biodegradation, such as metabolic pathways, or theoretical models describing bacterial growth on substrates are generally ignored.

Published SAR models to date revealed limited suitability for predicting biodegradation. OECD (1993c) reviewed 78 different biodegradation SARs. Biodegradation parameters used as dependent

variables included biodegradation rate constants, percentage of biodegradation after a specified time, biodegradation half-life, and semi-quantitative estimates of biodegradation given by experts for molecules and molecular fragments. These have been correlated with physico-chemical (e.g. molecular weight,  $\log K_{ow}$ ), geometric (e.g. van der Waals radius), electronic (e.g. Hammett substituent constant), and topological (e.g. connectivity indices) descriptors (OECD, 1993c). Limited applicability of existing SAR models, could be traced back to deficiencies in databases used to develop them: lack of endpoint homogeneity, inconsistent test data, and use of restricted datasets.

In general, single descriptor models did not allow for an adequate relationship with biodegradation. Multiple descriptor models are often based on a limited number of chemicals in training set and a large number of descriptors leading to an overfitting of experimental data. Only a few models (models 74-78 in: OECD, 1993c) were found to provide an adequate level of agreement (> 75%) between estimated and experimental data. Their domain, remains restricted to aliphatic and monocyclic aromatic compounds and to qualitative predictions, discriminating between readily and not readily biodegradable (MITI I test) based on linear group or fragment contribution models.

Other published biodegradation models include those based on the application of neural networks (Cambon and Devillers, 1993; Schüürmann and Müller, 1994) or inductive machine learning (Gamberger *et al*, 1993). In the latter reference, existing biodegradation expert knowledge and evaluated biodegradation data are transformed from examples to a function (rule) of a logical if-then-else form. Prediction results were in good agreement with those obtained by a fragment contribution method (Boethling and Sabljic, 1989) and a neural network-based learning system (Cambon and Devillers, 1993) from the same set of biodegradation data.

Computer automated predictions of aerobic biodegradation of chemicals can be conducted by the EPA ASTER (Russom *et al*, 1991) and the BIODEG Probability (SYRACUSE, 1994) programs. The biodegradability QSAR used in ASTER was developed using multivariate statistics (PCA) based on biodegradation rate data for 287 chemicals (Niemi *et al*, 1987). The model estimates biodegradation by weighting various functional groups and substructures. Rate data are derived from the BOD<sub>5</sub> test (American Public Health Association, 1975) and an attempt was made to only include tests from acclimated activated sludge. The distinction between degradable and persistent was arbitrarily defined as a half-life of more or less than 15 days (16% Theoretical Oxygen Demand in a BOD<sub>5</sub> test). Chemicals were grouped using K-means clustering with eight principal components and physico-chemical parameters as clustering variables. The clustering variables were greatly influenced by outliers in the principal component space. Therefore, the database was divided in two groups and clustering conducted on the two groups. After the clusters were defined, discriminant function analysis, connectivity indices and  $K_{ow}$  were used to distinguish between degradable and persistent chemicals within a cluster. In a second step, results from the PCA analysis and literature on biodegradability were used to provide structural features associated with degradable and persistent



chemicals. Using this approach, 92% of the chemicals from the database were correctly identified as degradable or persistent. The model was subjected to a verification exercise (OECD, 1993c) using MITI data and provided 76% matching predictions. Of the compounds which were classified as readily degradable in the MITI test, 83% were correctly predicted and of those that were non-biodegradable, 70% were correctly predicted.

The BIODEG Probability Program developed by the US-EPA is available on-line or in PC-compatible format from Syracuse Research Corp. (1994). Its selection, development and use for quantitative structure biodegradation predictions was discussed in detail previously (see Section 3.4.2). Using a similar fragment approach, Loonen *et al* (1995) developed a model to predict whether a substance is ready or not ready biodegradable in the MITI I test. Fragments include simple substructures existing of one or two atoms as well as more complex substructures in order to take into account of fragment interactions and functional groups. Level of agreement between estimated and experimental data was 86 and 88% for ready and not ready biodegradable substances, respectively.

At present, (quantitative) structure biodegradability relationships predicting a test level, (non-) readily biodegradable in a specific laboratory test, are the best that may be achieved (Peijnenburg and Karcher, 1995). Predicting biodegradability and kinetics of biodegradation in the environment, solely on the basis of structure will remain difficult, as biodegradation is function of the chemical structure, its concentration, enzymes responsible, and a variety of environmental factors. Using structural features to predict the effects chemicals have on biodegradability, will need the quantitative measurement of the above variables - and their interactions - using adequate descriptors.

#### **4.1.4 Bioconcentration Factor (BCF)**

The following discussion will concentrate on the prediction of the bioconcentration of chemicals by fish. The main reason for this, is that there is very little work that has been carried out to develop QSARs for the bioconcentration of chemicals in other organisms, with perhaps, more recently, the exception of earthworms, for example Connell and Markwell (1990), sedimentary organisms (Markwell *et al*, 1989) and plants (Trapp and Matthies, 1995). It is probable, that as risk assessment procedures become more sophisticated and encompassing, that the uptake and potential for bioconcentration in other aquatic and sedimentary organisms will need to be assessed more realistically. This is clearly an area for further development.

There have also been a number of other reviews of the estimation of this parameter which should be consulted. These include Connell (1987), Bysshe (1990) and Kristensen and Tyle (1990).

The purpose of the estimation of bioconcentration is to assess whether there is any potential for the chemical to be accumulated in organisms and hence, for further transfer up a food chain.

The process recommended for assessing the potential impact of bioconcentration and bioaccumulation is a step wise approach and is fully described in ECETOC (1995).

It is important to note, that the predicted BCF will not take account of factors which will lead to reduced bioconcentration, e.g. metabolism, nor will they account for other mechanisms that may lead to increased bioconcentration, e.g. facilitated uptake.

A number of test guidelines are available for the direct measurement of bioconcentration, of which OECD 305E is the most widely applied. This guideline has recently been revised (OECD, 1996) and replaces the previous versions OECD 305A-E.

When assessing QSARs for bioconcentration it is important to critically evaluate the data being used for the generation of the QSAR. As well as assessing the quality of the data, see OECD 305, a parent compound bioconcentration factor (BCF) should be available.

The BCF is defined as the concentration in fish divided by the concentration in water at steady-state.

For the purposes of QSARs and in order to account for much of the variability noted in bioconcentration experiments, the BCF should be expressed in relation to the fish lipid content.

Although a wide range of descriptors have been suggested for predicting BCF, in a recent review (ECETOC, 1995), it was concluded that of those referenced in the TGD only those based on  $\log K_{ow}$  had been developed and validated. Although other properties may indicate a potential for bioaccumulation, there are to-date no QSARs for predicting BCF based on such properties with the exception of solubility. In general, QSARs based on solubility were no less accurate than those based on  $\log K_{ow}$ , when compared with the accuracy of the endpoint (Davies and Dobbs, 1984).

### ***Relationships between $K_{ow}$ and BCF***

There are at least three types of relationships between  $\log K_{ow}$  and BCF. These are linear, bi-linear and polynomial. The following discussions will examine each of these approaches.

#### *Linear Relationships*

A number of linear relationships have been developed, some of which are given in Table 15.

The form of the QSAR is:

$$\log \text{BCF} = a + b \log K_{ow}$$

**Table 15: Examples of Linear Relationship Between BCF and  $K_{ow}$** 

a	b	n	log $K_{ow}$ range	Domain	$r^2$	Reference
-0.7	0.85	55	1.0-7.0	Mainly non-polar and polar aromatics plus some surfactants		Veith <i>et al</i> , 1979
-1.32	1.00	44	1.35-6.0	Mainly non-polar and polar aromatics		Mackay, 1982
-0.63	1.02	11	3.4-5.5	Chlorobenzenes	0.98	Oliver and Niimi, 1983
-0.4	0.79	122	1.0-6.9	Not defined	0.86	Veith and Kosian, 1983
-1.30	0.98	20		Chlorinated organics	0.81	Davies and Dobbs, 1984
0.61	0.89	18	0.9-6.7	Chlorobenzenes	0.90	Chiou, 1985

It is important to note that linear relationships are only valid for  $\log K_{ow} < 6$ . Above this value, these predictions will become increasingly inaccurate. This is due to the limited availability of the chemical as well as the increasing difficulty in crossing membranes (Opperhuizen *et al*, 1985, Anliker *et al*, 1988).

The QSAR described by Veith *et al* (1979) has a number of issues which should be carefully assessed when considering its use. These include :

1. The  $\log K_{ow}$  values used include those based from estimations using HPLC. This may be acceptable for those chemicals that fitted the domain of the method used, however, in a number of cases the values were obtained by extrapolation. Thus the applicability of the method is unknown and the accuracy of the extrapolation uncertain.
2. A number of the bioconcentration values used were from alternative sources and probably should be examined independently of the experimental values used.
3. The data for BCF's in rainbow trout have been adjusted by the addition of 0.5 to allow for the difference between trout and bluegill. This was based on a bioconcentration experiment carried out on 1,2,4-trichlorobenzene and hexachlorobenzene to fathead minnow, green sunfish and rainbow trout. The trout bioconcentration values obtained were, for these two chemicals, approximately one third the value of those in the other two species.
4. Three chemicals were left out because they gave low BCFs. This is an example of exclusion of chemicals from a domain without sufficient reasoning. The assessment of unknown chemicals becomes increasingly uncertain when such exclusions are not described, as it is also uncertain whether or not the unknown chemical fits the domain of the QSAR.

Mackay (1982) critically evaluated the Veith data, either altering the log  $K_{ow}$  to more accurate values or removing unsuitable chemicals e.g. surfactants. This later exercise is clearly an example of changing or reducing the domain of the chemicals to which the QSAR applies. Further results were added, applying to chemicals that fitted the re-defined domain, mainly restricted to non-polar aromatics. Based on this work, the regression was slightly improved and the slope set to 1.

Oliver and Niimi (1983) measured the bioconcentration of 12 chlorobenzenes by rainbow trout (*Oncorhynchus mykiss*), although the bioconcentration value for hexachlorobenzene was omitted from the QSAR, as the fish may not have reached equilibrium with the aquatic concentrations. It is interesting to note that the measured BCFs obtained in this study were higher than those described by Veith *et al* (1979). The values obtained by Oliver and Niimi were very similar to those described by Veith *et al* (1979) for fathead minnows. This underlines the concerns mentioned above, about the correction of the rainbow trout data by Veith and his co-workers.

Veith and Kosian (1983) after carrying out a wide sweep of available data from the literature developed a very general QSAR. The domain was general and also covered a wide range of different fish species. There are a number of sub-sets described and the changes to the correlations are discussed in more detail in the paper.

Davies and Dobbs (1984) took a number of test data and checked them against certain criteria described in the paper. The initial QSAR was general, however, they then found a better relationship for the hydrocarbons and chlorinated hydrocarbons, which they suggested as being due to the stability of these compounds compared to the other test chemicals. The final relationship was very close to that of Mackay (1982), but as the same data are used (Veith *et al*, 1979) this is to be expected.

Chiou (1985) measured the partition coefficient of 38 organic substances in a water-triolein system. These were then compared with bioconcentration factors and a similar slope to that found by others using log  $K_{ow}$  obtained.

Although there are a wide range of linear relationships available, it is apparent that they mainly fall into the same general area. Using the Mackay model, for log  $K_{ow}$  of between 3 and 6 is probably the best compromise for all chemicals.

However, the prediction of a BCF for a chemical using a specific QSAR, based on the same class of chemicals should be used when available, e.g. for chlorobenzenes, using Oliver and Niimi (1983).

### *Bi-linear Relationships*

It is well established that a linear model of bioconcentration is inaccurate when predictions are based on chemicals with  $\log K_{ow}$ s greater than 6 (Bintein *et al*, 1993). One approach to improve the predictions was to adopt a bi-linear model which thus allowed for a reduced bioconcentration at high  $\log K_{ow}$ . This was done by Nendza and Russom (1991) who took the most conservative data available and derived a mathematical description of the worst case:

$$\log BCF = 0.99 \log K_{ow} - 1.47 \log (4.97 \times 10^{-8} K_{ow} + 1) + 0.0135$$

The principle difficulty with such a worst case model is that it is not a predictive tool for proper assessment of the endpoint and as such, for example, cannot be used for confirming experimental data. This model is very conservative and structured such that it is not possible to test its validity as a predictor of bioaccumulation potential.

An alternative bi-linear model was developed by Bintein *et al*, 1993. This was based on a training set of 154 chemicals and tested with a small set of 29 chemicals:

$$\log BCF = 0.91 \log K_{ow} - 1.975 \log (6.8 \times 10^{-7} K_{ow} + 1) - 0.786$$

This model does give more accurate assessments of the potential for molecules with high  $\log K_{ow}$  to bioconcentrate. In a recent study carried out by Devillers *et al* (1995b), this model gave the most realistic results for BCFs predicted for compounds with  $\log K_{ow}$  above 6 and an assessment of the mean square errors showed these to be lower than the other models assessed in that study (Veith *et al*, 1979; 1980; Mackay, 1982; Isnard and Lambert, 1988; Connell and Hawker, 1988).

### *Polynomial Relationships*

Alternative approaches to the bi-linear model to account for the lack of linearity above  $\log K_{ow}$  of 6 have resulted in polynomial relationships. These take the general form of:

$$\log BCF = a \log K_{ow} + b (\log K_{ow})^2 \dots$$

There needs to be more work to establish whether these relationships are suitable for use in a general context and the degree of accuracy of such models. The Connell and Hawker model (1988)

$$\log BCF = 6.9 \times 10^{-3} (\log K_{ow})^4 - 1.85 \times 10^{-1} (\log K_{ow})^3 + 1.55 (\log K_{ow})^2 - 4.18 \log K_{ow} + 4.79$$

tends to predict higher BCFs than the polynomial of Bintein *et al* (1993). The assessment of the Bintein bi-linear model gave a more accurate assessment of BCF (Devillers *et al*, 1995a) especially for chemicals with a log  $K_{ow}$  of >6, than the polynomial model of Connell and Hawker.

#### *Plateau Relationships*

A different approach to the bi-linear and parabolic models is the plateau relationship (Spacie and Hamelink, 1982). In summary, this approach predicts that bioconcentration will rise in a linear manner, with respect to increasing log  $K_{ow}$ , until a maximum value is reached, at which point the bioconcentration then remains the same regardless of log  $K_{ow}$ .

Although a complete assessment of this model has not been undertaken, it is clear that the accuracy of such a model will be considerably worse than that of the bi-linear or polynomial models and therefore it is not recommended.

The main problems when assessing QSARs for chemicals with a log  $K_{ow}$  of greater than 6 arise from two sources of uncertainty. Firstly, there are very few accurate measures of log  $K_{ow}$  for these chemicals, the reasons for this are briefly discussed in Section 4.1.2 and in ECETOC (1995). Secondly, accurate measures of the bioconcentration of chemicals with such high log  $K_{ow}$ s are very difficult to obtain.

Other than work on these two areas, there are two other issues that need to be further understood in order to properly evaluate highly lipophilic chemicals. The potential for a chemical to be bioconcentrated will depend not only on the uptake rate, but also depuration and metabolism. This later is still only poorly understood and there needs to be research into the metabolic pathways and the potential for prediction of whether chemicals will be metabolised. This issue is general to all chemicals regardless of their potential for bioconcentration, however, it will have a major impact on the actual bioconcentration for those chemicals that have a high predicted potential for bioconcentration. Finally the issues relating to uptake and availability and diet are of crucial importance for highly lipophilic chemicals. Until these issues have been more extensively researched it will always be difficult to develop mechanistic QSARs for the bioconcentration of such chemicals.

#### ***Other uses of BCF QSARs***

As the BCF QSARs are used to estimate the total concentration of a chemical in a fish, then it follows that it may be possible to use the BCF QSARs to estimate the total body burden of a fish. This may be particularly useful for chemicals with high log  $K_{ow}$ , i.e. greater than 6, when the predicted BCF may be used to extrapolate or check chronic toxicity using the chronic critical body burden concept, where

the experimental determination of these values may be complicated, (McCarty and Mackay, 1993). This use of BCF QSARs has only recently been proposed and there is still much work required before it can be shown to be generally applicable. However, a method has been suggested as to how critical body burdens might be used in the future for risk assessment (ECETOC, 1995). This will be further discussed below, Section 4.2.1.

#### *ASTER*

The estimation used by ASTER is based on that described by Veith and Kosian (1983).

#### *CHEMEST*

There is no estimation approach available.

#### *EUSES*

Chapter 4 of the recently amalgamated technical guidance document contains a section on the QSARs available for bioconcentration. The recommended model for  $\log K_{ow}$  up to 6 is Veith *et al* (1979), while for chemicals with  $\log K_{ow}$  greater than 6, a parabolic equation, re-calculated from that described by Connell and Hawker (1988), is recommended.

#### *SYRACUSE*

No estimation program available.

## **4.2 AQUATIC TOXICITY**

The following section will discuss the QSARs available for the prediction of ecotoxicity for freshwater organisms only. This is due to the limited database of test results on marine organisms. Hence there are very few marine effect QSARs currently available. In this context, however, a review of the sensitivity of marine and freshwater organisms based on ECETOC's aquatic toxicity database ECETOC (1993c) concluded that for all fish, 91% and 93% of all the substances  $EC_{50}$  and NOEC values were within a factor 10 (Hutchinson *et al*, 1998). For all invertebrates the corresponding values were 33% and 83% respectively. However, these were data from a limited database and the need for additional high quality data was highlighted.

### 4.2.1 Introduction

There are several good overviews of the major developments in the prediction of the biological effects of substances on aquatic organisms by the use of structural descriptors (Lipnick, 1988; Hermens, 1989; Blum and Speece, 1990; Van Leeuwen *et al*, 1990; Verhaar *et al*, 1992; Donkin, 1994). A wide range of biological effects and species have been investigated. This chapter is not intended to give a comprehensive review of these aquatic toxicity QSARs, but is intended to provide an overview of the types of QSAR used in the prediction of toxicity endpoints, most commonly used in environmental risk assessment schemes. These endpoints include the acute and chronic toxicity of substances to fish, *Daphnia* and algae.

The key parameter used in the vast majority of aquatic toxicity QSARs is the hydrophobicity parameter, n-octanol-water partition coefficient ( $K_{ow}$ ), which indicates the tendency of substances to escape from water and partition into a more lipophilic phase such as a biological membrane (Calamari and Vighi, 1984, Könemann, 1981). Donkin (1994) gives a good overview of the reasons for the dominance of  $K_{ow}$  in aquatic toxicity QSARs.

For highly lipophilic substances, long exposure is often required for the test organism to achieve a steady state body concentration. Therefore, chronic toxicity may be observed even if exposure to medium, saturated with the test substance causes no acute toxicity (McCarty, 1986). Generally, given the availability of an appropriate QSAR, when  $\log K_{ow}$  is 5 to 6 or less, valid predictions can be obtained for estimating acute toxicity.

QSARs can be used to predict the chronic toxicity of substances with  $\log K_{ow}$  up to 8, if the duration of exposure is at least as long as the time required for the substance to reach steady state concentrations in the organism. Generally, when  $\log K_{ow}$  is greater than 8, no adverse effects from aqueous exposure to neutral organics would be expected at saturation, even under long term aqueous exposures (Bradbury, 1994).

The value of QSARs for predicting the toxicity of narcotic substances is not just based on the relatively good correlations obtained with individual species endpoints. Since a wide range of aquatic species tend to have similar sensitivities to substances with this mode of action, some QSARs are predictive of similar endpoints in several species (Donkin, 1994). This is less applicable for substances with specific modes of action (Jäckel and Nendza, 1994). Kaiser and Esterby (1991) investigated the relationship between  $K_{ow}$  and the acute toxicities of 267 substances to six aquatic and one terrestrial species using correlation, principal component and cluster analysis techniques. Species sensitivity was highly correlated for fathead minnow, golden orfe and *Daphnia* acute toxicities and inhibition of luminescence in *Photobacterium*. However, a lower collinearity was found for algal toxicity data.



Despite the success of  $\log K_{ow}$  based QSARs in predicting the toxicity of substances with a narcotic mode of action, some workers tend to avoid its use because of the variability in measured/reported values (Sabljic, 1991) or because it is difficult to measure (for example for surface active substances, (OECD, 1984)). Sabljic has used a topological index, the molecular connectivity index (MCI) to develop QSARs, based only on information encoded in structural formulas (Sabljic and Piver, 1992). Banerjee and Williams (1993) reported that for some  $K_{ow}$  based QSARs for nitro compounds a significant improvement was obtained if an additional hydrophobicity term is included to account for the solubility of the substance in octanol.

QSARs based on  $K_{ow}$  are generally considered to predict the minimum or baseline aquatic toxicity of substances (Donkin, 1994). The toxicity of substances with a specific mode of toxic action is often under-predicted by such QSARs. For such substances, additional structural descriptors may be required. These can be used as the only structural descriptor in the QSAR or in conjunction with  $K_{ow}$  (Calamari and Vighi, 1984; Chester *et al*, 1992). For example, descriptors of electrophilicity for aromatic chemicals such as superdelocalisability descriptors and LUMO (Section 3.2.3) energy, have been used together with  $\log K_{ow}$  to explain the variation of acute toxicity of substituted benzenes, phenols and anilines to fish (Veith and Mekenyan, 1993). The use of only non-hydrophobic descriptors tends to narrow the domain of the QSARs and the range of species to which they apply.

Descriptors other than  $K_{ow}$  should be used with care, particularly if the mechanism of toxicity is unknown. Also the use of a QSAR as a "black box" to link the toxicity of a group of substances to such alternative descriptors, should also be carefully assessed, as the scope of the domain or the cause of the toxic effect may be unknown.

Donkin (1994) discusses the use of QSARs for several classes of substances that are more toxic than predicted by baseline narcosis. These are briefly reviewed below.

*Neurotoxins:* Substances in this class, which includes many pesticides, tend to interfere with the function of the nervous system. There are a number of modes of action involved and consequently the domain of many of the QSARs is restricted to a narrow range of chemical structures (Donkin, 1994).

*Esters with  $\log K_{ow} < 4$ :* The mode of action is unknown but may be related to the hydrolysis rate of the ester (Veith and Broderius, 1990).

*Respiratory uncouplers:* The mode of action blocks the electron transport and ATP synthesis in mitochondria. Substances with this mode of action include many substituted phenols and anilines. Since the substances must partition into the organism to inhibit the formation of adenosine triphosphate (ATP),  $K_{ow}$  is highly predictive of their toxicity. However, QSARs with domains that include a wide range of substituted phenols require additional electronic descriptors (Lipnick *et al*, 1986).

*Reactive compounds:* There are a wide range of electrophilic substances in this class. They have the potential to react with negatively charged groups (e.g. hydroxy, sulphhydryl) and are therefore likely to be best predicted by QSARs that include a descriptor for reactivity. The reaction rate constants with 4-nitrobenzyl pyridine have been used as one such descriptor (Hermens *et al*, 1985, Deneer *et al*, 1988a). However, not all reactive substances appear to require the inclusion of a descriptor for reactivity. Deneer found that for a series of aldehydes, a QSAR using  $K_{ow}$  as the only structural descriptor was not improved when a reactivity descriptor was added (Deneer *et al*, 1988b). This was considered to be due to the toxicity of these substances being limited by the rate of uptake into the fish, a process strongly correlated with  $K_{ow}$ .

Some unreactive substances can be transformed into reactive ones by metabolism. Several QSARs have been developed to predict their toxicity to fish (Deneer *et al*, 1987, Veith *et al*, 1989).

Verhaar *et al* (1995) proposed four main categories of toxic mode of action: non-polar narcotics, polar narcotics, reactive substances and specifically-acting substances. An external validation of the classification system using the high-quality aquatic toxicity data of the EAT database (ECETOC, 1993b) was made by Verhaar *et al* (in preparation) which is summarised in Appendix B. Although QSARs are available for the prediction of the first two classes, there are few available for reactive and specifically-acting substances. Verhaar *et al* (1996) suggest that quantum mechanical calculations on reaction transition states can be used to predict the reactivity of sets of substances and these could then be used to develop aquatic toxicity QSARs.

Arising from these considerations, there is a growing amount of information which suggests that substances with the same mode of toxic action tend to cause a given effect at similar internal body concentrations or critical body residues, CBRs. McCarty and Mackay (1993) proposed the following relationship between CBR and toxic endpoint:

$$CBR = BCF \times LC_{50}$$

where BCF (bioconcentration factor) =  $C_f/C_w$ ;

$C_f$  = concentration of the substance in the organism at steady-state;

$C_w$  = mean concentration of the substance during the exposure period in the water phase.

By considering the QSARs relating (i) BCF and  $K_{ow}$  and (ii)  $LC_{50}$  and  $K_{ow}$ , McCarty and co-workers predicted a lethal CBR of about 2 mmol/kg for substances with a non-polar narcotic mode of action (McCarty, 1986, McCarty and Mackay, 1993). For non-lethal endpoints lower CBRs are predicted.

For substances acting by a specific mode of action CBRs are expected to be lower than those acting by non-polar narcosis, i.e. less than 0.5 mmol/kg. It is possible that each specific mode of action may have

its own narrow range of CBRs. However, further research is required to assess if those differences are significant, and can be used to enhance the interpretation or prediction of the effects of specific substances.

The potential use of CBRs in environmental risk assessment is discussed by ECETOC (1995).

#### 4.2.2 EU/US-EPA Regulatory Aquatic Toxicity QSARs

The US EPA have developed the QSARs in ECOSAR on the basis of chemical classes with mode of toxic action playing a secondary role, although initial development was based solely on an understanding of mode of toxic action (Clements *et al*, 1995). The change in approach was made for pragmatic reasons - chemical classes are more easily determined than mode of toxic action. Whilst categorising substances on the basis of mode of toxic action is clearly the most appropriate scientific approach to selecting suitable toxicity QSARs, recognition of the actual mode of action of a substance requires considerable expertise. It is therefore open to contention. This is largely avoided by taking the chemical class approach. Hence, substances are categorised into one of 47 chemical classes and then the appropriate QSAR selected to predict the required toxicity endpoints (Clements *et al*, 1995).

In contrast to the US EPA approach, the EU Technical Guidance Document on the use of QSARs in risk assessment is based on a mode of toxic action approach (EEC, 1996). QSARs are recommended for predicting the toxicity of substances that act by non-polar narcosis (endpoints predicted are acute EC<sub>50</sub>s for fish and *Daphnia*, chronic NOECs for fish and *Daphnia* and algal EC<sub>50</sub>s) or polar narcosis (endpoints predicted are acute EC<sub>50</sub>s for fish and *Daphnia*). Although polar narcotic substances act by a relatively non-specific mode of toxic action, they are significantly more toxic than predicted by non-polar narcosis. Substances in this category tend to have a strong hydrogen-bond donating group. No QSARs are recommended for the prediction of the toxicity of substances that act by specific modes of action, due mainly to the limited database of available effect data.

All of the recommended QSARs have been obtained from linear regression analysis of data from OECD test methods or similar. Seven QSARs are used. In all cases, the only structural descriptor is K<sub>ow</sub> (Verhaar *et al*, 1995). The chemical domains for the polar and non-polar narcosis QSARs are specified, but both are only valid for substances with log K<sub>ow</sub> values in the range 1 to 6 (Verhaar *et al*, 1992, 1995). The predicted endpoints for 95% of the training set were within a factor of 3 of the measured value.

#### **Acute Fish LC<sub>50</sub> and Daphnia EC<sub>50</sub>**

The acute toxicity to fish is usually determined over a 96 hour exposure period under static, semi-static or flow through conditions. Several fish species are recommended in methods used in the notification of substances, the most commonly used include rainbow trout, fathead minnow and zebrafish. The LC<sub>50</sub>

is the concentration of the test substance lethal to 50% of an exposed group of fish. All the QSARs referred to are for freshwater fish.

The acute toxicity to *Daphnia* is usually determined over a 48 hour exposure period under static or semi-static conditions. The test is started with young Daphnids less than 24 hours old. The EC<sub>50</sub> is the concentration of the test substance that immobilises 50% of an exposed group of *Daphnia*.

Given the importance of these two endpoints in environmental risk assessment, it is interesting to compare the QSARs used by the US EPA and the EU (Clements and Nabholtz, 1994; EEC, 1996). This can be done by considering the QSARs that are applied to substances classed as having a narcotic mode of action by both regulatory agencies (Tables 16 and 17).

**Table 16: European QSARs Predicting 96 and 48 Hour L(E)C<sub>50</sub> of Narcotics to Fish and *Daphnia* (Verhaar *et al*, 1995) (concentrations in mole/l)**

Species	QSAR	Domain
Fish	$\log LC_{50} = -0.846 \log K_{ow} - 1.39$	Non-polar narcotics
Fish	$\log LC_{50} = -0.725 \log K_{ow} - 2.16$	Polar narcotics
<i>Daphnia magna</i>	$\log EC_{50} = -0.941 \log K_{ow} - 1.32$	Non-polar narcotics
<i>Daphnia magna</i>	$\log EC_{50} = -0.555 \log K_{ow} - 2.79$	Polar narcotics

The similarity in the QSARs used to predict the acute toxicity of non-polar narcotics is demonstrated by predicting the toxicity of a suitable substance using the appropriate QSAR. If this is done with n-octanol (log K<sub>ow</sub> 2.97, MW 130) then LC<sub>50</sub>s fall in a narrow range 9.98 to 16.3 mg/l. A comparison of the predicted acute toxicities of polar narcotics such as substituted phenols also suggests similar results. For example the predicted LC<sub>50</sub>s of 2,4,5-trichlorophenol (log K<sub>ow</sub> 3.72, MW 198) fall within a narrow range, 2.11 to 2.76 mg/l.

These simple comparisons suggest, that as long as a substance lies within the appropriate domain of these QSARs and the limitations are not exceeded, (in most cases log K<sub>ow</sub> must be <5 and the substance must be soluble at the predicted LC<sub>50</sub>), then both regulatory agencies would predict the substance to have a similar toxicity.

**Table 17: QSARs Predicting 96 and 48 Hour L(E)C<sub>50</sub> of Narcotics to Fish and *Daphnia* Used by the US EPA (ECOSAR) (concentrations in mmole/l)**

Species	QSAR	Domain	Reference
Fish	$\log LC_{50} = 0.399 - 0.616 \log K_{ow}$	Chlorinated phenols (polar narcotics)	Clements and Nabholtz, 1994
Fish	$\log LC_{50} = 0.72 - 0.64 \log K_{ow}$	Aliphatic amines (polar narcotics)	Nabholz and Platz, 1990
Fish	$\log LC_{50} = -0.94 \log K_{ow} + 1.75$	Neutral organics (non-polar narcotics)	Veith <i>et al</i> , 1983
Fish	$\log LC_{50} = 0.956 - 0.739 \log K_{ow}$	Anilines (non-polar narcotics)	Veith and Broderius 1987
Fish	$\log LC_{50} = -0.94 + 0.94 \log K_{ow} + 0.94 \log (0.000068 K_{ow} + 1) - 1.25$	Non-polar narcotics	
<i>D. magna</i>	$\log EC_{50} = 1.72 - 0.91 \log K_{ow}$	Neutral organics (non-polar narcotics)	Hermens <i>et al</i> , 1984
<i>D. magna</i>	$\log EC_{50} = -0.451 - 0.409 \log K_{ow}$	Phenols (polar narcotics)	
<i>D. magna</i>	$\log EC_{50} = -0.524 - 0.584 \log K_{ow}$	Aliphatic amines (polar narcotics)	Nabholtz and Platz, 1990
<i>D. magna</i>	$\log EC_{50} = -1.623 - 0.271 \log K_{ow}$	Anilines (polar narcotics)	Kühn <i>et al</i> , 1989; Sloof <i>et al</i> , 1983

Of course there are many types of substance that will not fall within the domains of the non-polar or polar narcosis QSARs compared above. In particular, many new substances are designed to have specific reactivities or functions and these may also have specific modes of toxic action to aquatic organisms. The development of additional QSARs may be required if the toxicity of such substances is to be predicted from structural descriptors. A brief description of some other QSARs is given below.

A well cited QSAR for a series of 50 industrial pollutants considered to act by non-polar narcosis was derived by Könemann (1981). This QSAR uses  $\log K_{ow}$  (up to  $\log K_{ow} < 6$ ) as the structural descriptor. This was found to give a better prediction of acute toxicity to guppies (*Poecilia reticulata*) than HPLC retention indices, solubility or molecular connectivity indices (Table 18).

Devillers *et al* (1987) presented an autocorrelation descriptor based on the Bondi volume, to construct a QSAR for fathead minnow (96h LC<sub>50</sub>) using a heterogenous set of 30 organic substances. They also developed a QSAR using 57 organic substances from many different chemical classes to predict toxicity to *Daphnia magna* (24h EC<sub>50</sub>). The latter included the Van der Waals volume with other autocorrelation vectors such as the connectivity and electronegativity. The QSARs had excellent predictive power for the substances tested. However, the QSARs were not predictive of all chemical classes; the fathead minnow QSAR did not predict toxicities of some small linear molecules such as

methanol and the *Daphnia magna* QSAR could not be applied to small branched or large linear molecules.

Hermens *et al* (1984) developed a QSAR to predict the acute toxicity of chloro and alkyl-anilines to guppies. They found that using a descriptor for electronic effects (Hammett constant), in addition to two descriptors of hydrophobicity, calculated  $K_{ow}$  and P constants, predicted toxicity was better correlated to measured values.

The acute toxicity to guppies of a series of 21 substituted phenols was well predicted by a QSAR based on  $K_{ow}$  (Saarikoski and Viluksela, 1982). However, since the toxicity of phenols is dependent on the degree of ionisation, the pH of the test medium can influence the relationship.

**Table 18: QSARs Predicting Acute Toxicity to Fish and *Daphnia***

Species	Endpoint	QSAR	Domain	Reference
<i>P. reticulata</i>	7-14d LC <sub>50</sub> (mmol/l)	$\log 1/LC_{50} = 0.871 \log K_{ow} - 4.87$	Non-polar narcotics	Könemann, 1981
<i>P. reticulata</i>	LC <sub>50</sub>	$-\log LC_{50} = (0.39 \pm 0.05) \log K_{ow} + (3 \pm 0.4) \log K_1 - 2.25$	Epoxy compds	Deneer <i>et al</i> , 1988a
<i>P. reticulata</i>	LC <sub>50</sub>	$\log 1/LC_{50} = 0.407 \log K_{ow} + 0.846 \Sigma \sigma - 3.17$	Chloro/alkyl- anilines	Hermens <i>et al</i> , 1984
<i>P. reticulata</i>	LC <sub>50</sub>	$-\log LC_{50} = (0.36 \pm 0.04) \log K_{ow} - 2.54$	Aldehydes	Deneer <i>et al</i> , 1988b
<i>Daphnia magna</i>	24 h EC <sub>50</sub> (mmol/l)	$\log 1/LC_{50} = 14.718V_o - 3.375V_3 + 0.349E_2 - 0.220E_4 - 3.902$	Organics	Devillers <i>et al</i> , 1987

The acute toxicity of commercial anionic and non-ionic surfactants have been predicted by adaptation of polar and non-polar narcotic QSARs developed for non-surfactants (Roberts, 1989; 1991). For both class of surfactant the structural descriptor was  $\log K_{ow}$  calculated from structural fragments. This suggests that these types of surfactants act by the same mode of action as the majority of other industrial substances, i.e. by non-specific narcosis mechanisms. The QSARs are given in Table 19.

**Table 19: QSARs Predicting Acute Toxicity of Surfactants**

Endpoint	QSAR	Domain	Reference
48h EC <sub>50</sub>	$\log 1/EC_{50} = 0.87 \log K_{ow} + 1.13$	non-ionic surfactants	Roberts 1991
48h EC <sub>50</sub>	$\log 1/EC_{50} = 0.63 \log K_{ow} + 2.52$	anionic surfactants	Roberts 1991

### **Algal Growth Inhibition**

Algal growth inhibition is determined using exponentially growing cultures of algae under static test conditions. The test duration is usually 72 or 96 hours. This allows growth over several generations and is therefore, truly a chronic test. Algal growth is assessed directly from cell counts or indirectly from spectrophotometric, colorimetric or fluorimetric measurements. However, as discussed previously (Section 3.2.1) this QSAR is based on varying time-scales and possibly different endpoints (EbC<sub>50</sub> and ErC<sub>50</sub>). This may be a problem for some substances.

**Table 20: QSARs Predicting Algal Growth**

Species	Endpoint	QSAR	Domain	Reference
<i>S. capricornutum</i>	72/96 h EC <sub>50</sub>	$\log EC_{50} = -1.0 \log K_{ow} - 1.23$	Non-polar narcotics	Van Leeuwen <i>et al</i> , 1992

### **Chronic Toxicity to Fish**

There are several methods for assessing the chronic toxicity of substances to fish. Of these, two are routinely used in regulatory assessment schemes:

- Inhibition of growth. Juvenile fish, ideally in exponential growth, are exposed to the chemical under semi-static or (usually) flow-through conditions. The weight and/or length of the fish is determined at the start of the test and typically again after 2 and 4 weeks. The key endpoint is the NOEC for growth inhibition although survival and behavioural effects may also be assessed.
- Effects on early life stages. Fertilised eggs are exposed to the test substance under semi-static or (more usually) flow through conditions. The duration of the test depends on the endpoints and species selected and a typical test can last from 6 to 55 days. These include survival and development of the eggs, hatching success, development, survival and growth of the fry. For each endpoint the NOEC is determined.

Van Leeuwen *et al* (1990) derived a Log K<sub>ow</sub> based QSAR for predicting the chronic toxicity of a series of chlorobenzene and aniline derivatives to the early life stages of two species of fish. The sensitivity of both zebra fish and fathead minnows to the chlorobenzenes were found to be similar and were predicted by the same QSAR. The chloroanilines were more toxic than predicted by baseline narcosis.

**Table 21: QSARs Predicting Chronic Toxicity to Fish**

Species	Endpoint	QSAR	Domain	Reference
<i>P. promelas</i> , <i>B. rerio</i>	28-32d NOEC	$\log\text{NOEC} = -0.9 \log K_{ow} - 2.3$	Non-polar narcotics	Verhaar <i>et al</i> , 1995
<i>P. promelas</i> , <i>B. rerio</i>	28d NOEC	$\log 1/\text{NOEC} = 1.06(\pm 0.09) \log K_{ow} - 4.57$	Chlorobenzenes	Van Leeuwen <i>et al</i> , 1990
<i>P. promelas</i> , <i>B. rerio</i>	28d NOEC	$\log 1/\text{NOEC} = 0.66(\pm 0.05) \log K_{ow} - 2.05$	Chloroanilines	Van Leeuwen <i>et al</i> , 1990

### **Daphnia Reproduction**

Effects on *Daphnia* reproduction are usually determined over 21 days exposure which is sufficient for <24 hour old Daphnids to mature and produce about 5 broods of offspring. Exposure is normally under static-renewal conditions although flow through tests are also feasible. Endpoints include survival and growth of the parental generation but the key endpoint is the NOEC for reproduction. As previously discussed (3.2.2) this QSAR is based on historical data and includes 16 and 21 day data.

**Table 22: QSARs Predicting Daphnia Reproduction**

Endpoint	QSAR	Domain	Reference
16d NOEC	$\log\text{EC}_{50} = -1.05 \log K_{ow} - 1.85$	Non-polar narcotics	Verhaar <i>et al</i> 1995

## **4.3 THE TERRESTRIAL ENVIRONMENT**

### **4.3.1 Bioaccumulation in Terrestrial Species**

The bioaccumulation in terrestrial species has been less intensively studied than for fish. In 1990, Connell and Markwell derived a QSAR for the bioconcentration (BCF) in earthworms.

$$\log(\text{BCF}) = 1.0 \times \log K_{ow} - 0.6$$

In their work they correlated the interstitial water concentration with the concentration in worms. The QSAR is based on literature data on 32 compounds that are known to be relatively persistent in soil. In total, 100 data points were used to generate the QSAR, and some duplication in the training set was observed. The QSAR has a reported *r* of 0.91. The domain of the QSAR is  $\log K_{ow}$  1 to 6.



The drawback of their method is that the BCF is in many cases generated by calculating the pore water concentration assuming an organic carbon fraction ( $f_{oc}$ ) of 4%. Hence the true BCFs for these data points that form the training set of the QSAR are not known accurately.

The above QSAR is also recommended in chapter 4 of the TGD (EEC, 1996). In that chapter, it is advocated that it should be used to derive a first estimation of the BCF. The reason for using this QSAR, although it is not adequately validated, is that it is in agreement with the equilibrium partitioning theory, developed by Di Toro *et al* (1991) for the assessment of sediments.

### 4.3.2 QSARs for Terrestrial Effects

There would appear to be no adequate QSARs available in the literature that correlate effects in terrestrial species with any physico-chemical parameter.

However, in their evaluation of toxicity and bioaccumulation of chlorophenols to earthworms, Van Gestel and Ma (1988) come to the conclusion that for earthworms, the toxicity and bioaccumulation can be predicted. Unfortunately, no equation was presented or derived.

## 4.4 THE ATMOSPHERE

The largest environmental compartment by volume to be considered, when assessing the impact of chemicals, is the atmosphere (Mackay, 1991) or more specifically its lower layer, the troposphere. For volatile substances, it is this compartment that serves as the major sink. These substances and others are introduced into the atmosphere during production, use and disposal directly (OECD, 1992b) and, due to their physico-chemical properties, by volatilisation from soil and aquatic systems (Müller and Klein, 1991).

This section will deal with the fate and the potential effects that can be addressed by means of existing SAR methodology. The assessment of partitioning and transport, as mentioned in the TGD (EEC, 1996), will not be discussed as these are beyond the scope of this document. These issues are also under consideration by the ECETOC-TF "Exposure Modelling".

### 4.4.1 Atmospheric Fate

Organic substances emitted into the troposphere are removed by direct photolysis and by chemical reactions with a number of reactive intermediate species, including OH, HO<sub>2</sub> and NO<sub>3</sub> radicals and ozone (Atkinson, 1988). Other removal, but not degradative, processes of environmental importance are wet and dry deposition and transport to higher atmospheric layers.

For the majority of the organic substances present in the troposphere, reaction with the OH radical during daylight hours is thought to be the dominant atmospheric removal process which leads to a variety of reaction products, frequently forming acids, aldehydes and ketones. Subsequent reactions lead ultimately to mineralisation forming oxidation products such as carbon dioxide, water, hydrogen chloride, etc. (ECETOC, 1983). In the case of hydrocarbons the first reactions in this degradation sequence are, for aliphatic substances, the abstraction of a hydrogen atom and, for aromatics and other unsaturated substances, the addition of the OH radical.

The rate at which organic compounds are transformed by indirect photodegradation depends on the rate constant of reaction of the compound with the sensitiser, e.g. the hydroxyl radical, and on the concentration of both reactants. Usually the hydroxyl radical concentration is assumed to have an approximately constant value, and the reaction is described by pseudo-first-order kinetics.

The concentration of hydroxyl radicals in the troposphere varies depending on factors such as the ozone concentration and available solar light. Crutzen (1982) reported the calculated, yearly averaged, global concentration of hydroxyl radicals in the troposphere to be  $0.5 \times 10^6$  molecules/cm<sup>3</sup>, with a range of 0.3 to  $1 \times 10^6$  molecules/cm<sup>3</sup>. A more recent evaluation, based on 7 year measurements, indicates this concentration to be  $0.77 \times 10^6$  molecules/cm<sup>3</sup> as a global annual average, with a range of 0.48 to  $1.04 \times 10^6$  molecules/cm<sup>3</sup> (Prinn *et al*, 1987). In 1995, Prinn *et al* published the extension of this project covering the period from 1978 to 1994. In this publication it was concluded that little change in the OH-radical concentrations had been observed (Prinn *et al*, 1995).

From an extensive review of published observed values Hewitt and Harrison (1984) concluded that the 24-hours averaged hydroxyl radical concentration in the lower troposphere, i.e. in the mixing layer, generally ranges from 0.3 to  $3.0 \times 10^6$  molecules/cm<sup>3</sup> with a typical average concentration of  $1 \times 10^6$  molecules/cm<sup>3</sup> during the winter and of  $2 \times 10^6$  molecules/cm<sup>3</sup> during summer.

Atkinson (1985) published an extensive review of measured rate constants of reaction of organic compounds with hydroxyl radicals. On the basis of these data he developed an estimation method for hydroxyl radical rate constants that is considered to be accurate to within 50% (Atkinson 1985, OECD, 1986 and OECD, 1992b). Based on these data and assuming an average hydroxyl radical concentration in the mixing layer of  $1 \times 10^6$  molecules/cm<sup>3</sup>, the rate of reaction and the half-life of the compound in the troposphere at 10 to 30° C can be estimated from the structure.

The approach presented above is available in a computerised form, the "Atmospheric Oxidation Program" (Meylan and Howard, 1993b, 1994d). The claimed accuracy of this program, and thus the approach, presented in the user's guide, is that 91 % of the calculated hydroxyl radical rate constants for a test-set of 509 substance are within a factor of two of the experimentally determined values.

This was supported by an independent evaluation by Müller and Klein (1991) on an earlier version of the AOP-program using a test set of 369 substances which indicated a similar accuracy.

Taking into account the validations of the above SAR methodology it is seen as a very valuable tool for the initial evaluation of the abiotic degradation of organic atmospheric contaminants.

Similar approaches have been developed and reviewed, for the reactions with ozone (Atkinson and Carter, 1984; Meylan and Howard, 1993c) and nitrate radicals (Müller and Klein, 1991; Wayne *et al*, 1991, Atkinson, 1991). The methods are well described and referenced in these papers and therefore not discussed here in detail.

Compared with the absolute removal rates of organic substance by OH radicals, atmospheric ozonolysis and reactions with NO<sub>3</sub> are in most cases less important. However, for unsaturated compounds these should not be overlooked. Therefore, the calculation of the rate constants of ozonolysis of these substances is included in the aforementioned AOP-program.

A statistically significant correlation between measured nitrate radical reaction rates and measured Ionisation Energies ( $E_{i,v}$ ) has been published by Sabljic and Güsten (1990). Based on these findings, Müller and Klein (1991) developed a QSAR for which no measured data is necessary. They have correlated measured nitrate radical reaction rate with quantum chemically calculated HOMO and LUMO energies. The accuracy of this method is reasonable for an initial assessment on substances without any measured information.

Finally, substances may be subject to direct photolysis (De Leeuw, 1993). In comparison with the processes mentioned above, this removal process is regarded as less important for the majority of organic substances. For materials in the troposphere, the UV-B and UV-A regions, wavelengths of 290 – 450 nm, are important. At present, however, there are no SARs that estimate the removal rates by direct photolysis.

#### **4.4.2 Atmospheric Effects**

##### ***Biotic Effects***

There are no QSARs available for predicting the atmospheric effect of chemicals on organisms. As part of the EU risk assessment process, an effects assessment is made based on mammals (EEC, 1996). This should then be based on the results of the inhalation tests that, when applicable, are part of the acute mammalian base set. However, atmospheric concentrations will not normally be high enough to cause any short-term effects.

There have been few studies that have attempted to address the impact of organic chemicals on terrestrial invertebrates and plants, via atmospheric exposure. Thompson and Carmichael (1989), describe an experiment in which emergent seedlings of *Sorghum bicolor* and *Brassica napus* were exposed to 1,1,1-trichloroethane vapour in sealed vessels. One attempt to develop a QSAR is described by Bacci *et al* (1990) in which accumulation by azalea leaves was correlated to log  $K_{ow}$  of trifluralin, hexachlorobenzene, mirex, thionazin and sulfotep.

### **Abiotic Effects**

In the TGD on Risk Assessment of Existing Substance the following abiotic atmospheric effects are considered:

- global warming;
- ozone depletion in the stratosphere;
- ozone formation in the troposphere and
- acidification.

The assessments of these four issues are discussed below. The approach adopted for consideration of the four endpoints follows the same pattern. A qualitative assessment is first carried out, to investigate whether the issue is of real concern for the substance under consideration. This may then be followed by a more quantitative assessment. However, even these initial quantitative assessments are of a qualitative nature, as they only address the potential of a molecule to cause or contribute to the effect being considered. For the absolute quantification of each effect, additional evaluations are needed.

For each of the topics this can be done by several techniques. As the issues are global, it is strongly advocated that the method applied follows a global consensus model. When available, a quantitative model is given.

### **Global Warming**

The potential impact of a substance on global warming is based on its IR absorption characteristics and total atmospheric life time. This is as presented as the Global Warming Potential (GWP) (Houghton *et al*, 1990, 1992 and 1995). The GWP is an indication of the potential that a molecule has in contributing to the global warming relative to a reference substance.

The reference molecule selected by the Intergovernmental Panel on Climate Change (IPCC) is carbon dioxide. Hence, the GWP of CO<sub>2</sub> is set at one. The real contribution to any assumed climate change of a substance is a function of the GWP and the relative abundance in the atmosphere of the

substance under consideration compared to CO<sub>2</sub>. In view of this international consensus, other approaches should base any assessment models using GWP, on the CO<sub>2</sub> standard.

In general when a substance has an atmospheric life time less than two years, its global warming potential can be neglected (De Leeuw, 1993). The life time can be established as a function of deposition and the atmospheric degradation, the latter described in Section 4.4.1.

For substances with a longer life time a first indication of the GWP, relative to trichlorofluoromethane, can be obtained by:

$$GWP = \frac{\tau_{(x)}}{\tau_{(CFC11)}} \times \frac{M_{(CFC11)}}{M_{(x)}} \times \frac{S_{(x)}}{S_{(CFC11)}}$$

where  $\tau$  is the atmospheric life time,  $M$  is the molecular weight and  $S$  is the IR absorption strength of respectively substance  $x$  and CFC11, trichlorofluoromethane (De Leeuw, 1993). According to the author this equation gives GWP-values within a factor of two of values obtained by complex models for CFCs and chlorinated hydrocarbons. For other substances this SAR has not been verified and should be used with care.

In addition, to prevent confusion between a European and a global standard, adjustment of the GWP-values, obtained by the above SAR, to CO<sub>2</sub> is necessary.

### ***Ozone Depletion in the Stratosphere***

The capacity of a substance to scavenge stratospheric ozone is expressed as its Ozone Depletion Potential, ODP (Houghton *et al*, 1995). This is a relative measure of the ability of a bromine or chlorine containing molecule to destroy stratospheric ozone. The reference molecule for this effect is CFC11.

A substance may have a negative influence on the stratosphere if it contains chlorine or bromine and has a tropospheric life time sufficiently long to allow transport into the stratosphere. Thus a qualitative assessment that establishes if the substance contains chlorine or bromine should be the first step.

If so, the atmospheric life time has to be estimated using the SAR methodology of Section 4.4.1. According to De Leeuw (1993), an atmospheric life time of less than one year leads to an ODP of almost zero, and in such cases, no further assessment should be undertaken.

When a substance does not fulfil the above criteria, the ODP can be estimated by applying a SAR developed by De Leeuw (1993).

### ***Ozone Formation in Troposphere***

The formation of ozone at tropospheric level is regarded as an environmental problem. Above certain concentrations it can cause human health effects (Doull *et al*, 1980) and it can be harmful to crops and trees (Heck, *et al*, 1983). These higher concentrations may occur during episodes of certain atmospheric conditions (De Leeuw, 1993). Furthermore, ozone is a greenhouse gas and increases in its concentrations in the troposphere to date, have contributed to the greenhouse effect (UK-DoE, 1993).

With respect to episodic ozone, Derwent and Jenkins (1990) have developed the concept of the Photochemical Ozone Creation Potential (POCP) index. The POCP value for a given hydrocarbon assesses its ability to form ozone relative to ethene for an identical atmospheric emission. By definition the POCP value of ethene is 100. The POCP concept is widely accepted and advocated by the IPCC as the indicator for ozone formation hazards (Houghton *et al*, 1995).

Although the POCP concept gives insight in the potential impact of individual hydrocarbons in ozone formation and it may provide a guidance on regional and national emission control strategies for volatile organic compounds, the applicability in effects assessment of substances is limited (De Leeuw, 1993). This is due to the amount of information that is needed on the atmospheric fate of a substance and atmospheric conditions necessary for the elaborate computation of a POCP. This information will normally not be available from standard laboratory studies.

For the purpose of an initial effects assessment, the complexity of the POCP index can, according to De Leeuw (1993), be circumvented by using a reactivity scale based on the rate constant of the reaction with OH radicals.

However, an attempt to verify this by means of the data on 12 substances, as presented in his paper, by regression techniques, gave an  $r^2$  of only 0.379. Therefore, this method of approach needs extensive additional verification and potential adjustment, before using it in a formalised atmospheric risk assessment scheme.

### ***Acidification***

During the atmospheric oxidation processes, substances that contain Cl, F, N or S may form acidifying compounds, e.g. HCl, HF, NO<sub>2</sub> and SO<sub>2</sub>.

To incorporate this feature into an effect assessment, De Leeuw (1993) has proposed the concept of an Acidification Potential (AP). The AP is defined as the number of potential acid equivalents per mass unit compared to that of a reference compound, preferentially SO<sub>2</sub>. However, the approach has limited scientific rational and is unverified. Consequently it is of limited value and should not be used within any formalised risk assessment scheme.

## 5. REGULATORY USE OF QSARs

In the regulatory context, QSARs are used in the US as part of the US Toxic Substances Control Act (TSCA) for new chemicals in order to complement the information provided by a manufacturer in the frame of a Pre-manufacture Notice (PMN). In the EU the use of QSARs is encouraged as a supporting tool in the process of risk assessment of substances but is limited to (1) data evaluation; (2) decision support for further testing; (3) selection of input parameters/data which are needed in the risk assessment; and (4) identification of (other) potential concerns (EEC, 1995).

This chapter discusses the ways in which QSARs are used in support of these regulatory processes. However, the main emphasis will be on their use within the European legislative framework.

### 5.1 DATA GENERATION AND DATA CHECKING

When using QSARs, as previously mentioned (Section 3.9), there are two parts to be considered, the generation of the data and the data checking process. In this section recommendations on how QSARs could be used, with examples of QSARs being used for these purposes, will be described.

#### 5.1.1 Data generation

##### ***Butadiene***

When the risk assessment for butadiene was done within the context of the Existing Chemicals Regulation, it became obvious that there were no valid aquatic toxicity test results available. This lack of experimental data for 1,3-butadiene was not surprising given the physical nature of the substance, with its combination of very high vapour pressure and flammability.

In the risk assessment two approaches were considered. Firstly the toxicity was estimated using QSARs and secondly, data from structurally similar substances was used. This section will only consider the data generation.

In Chapter 4 of the Technical Guidance Documents (EEC, 1995) equations for estimating various toxicity endpoints are given (see Section 4.2.2). The toxicity data for 1,3-butadiene was derived using a log  $K_{ow}$  value of 1.99. The equations used were considered suitable for non-polar narcotic chemicals for which there is some evidence (Bol *et al*, 1993).



- 72 h EC<sub>50</sub> for algae = 32.6 mg/l
- 48 h EC<sub>50</sub> for *Daphnia* = 44.9 mg/l
- 96 h for freshwater fish (Fathead minnow) = 42.8 mg/l
- 21 day NOEC for *Daphnia* reproduction/growth = 9.2 mg/l
- 28 day NOEC for *B. rerio* and *P. promelas* = 4.5 mg/l

In the risk assessment these data were then compared with those for 1,3-pentadiene and 2-methyl butadiene which had been reviewed as part of the OECD HPV programme. The actual experimental results indicated that the QSAR results were conservative, i.e. indicating a higher toxicity for 1,3-butadiene, than would have been expected.

### 5.1.2 Data checking

When checking experimental data using QSARs, it is important to ensure that the chemical is within the domain of the QSAR and that the endpoint is comparable with that required. Furthermore, the accuracy of the QSAR needs to be established. It is also necessary to know either the variability within the endpoint being checked, or the general variability of the endpoint when measured by the method referenced (see Section 2.1). Finally, it is especially important to be aware of the required precision and accuracy relative to the use of the QSAR.

There are then a number of approaches that could be used, to check whether the data point being assessed is valid. These are discussed below and then case studies, showing the approaches are described.

Ideally, both experimental value and prediction should have confidence limits which, when they overlap, would confirm the reliability of both points. This approach depends upon the confidence interval of the estimates from a QSAR being defined or capable of being defined. If the experimental value is outside the confidence interval both the QSAR and the experimental value need to be carefully examined. This approach is essentially that described in Chapter 4 of the amalgamated Technical Guidance Document for Risk Assessment of New and Existing Substances (EEC, 1996). However, it is unclear in the TGD whether the confidence interval used, is from the QSAR predictions or the methodology of the required endpoint. One disadvantage of this approach is that, by definition, some data points will be outside the confidence limits, (in chapter 4 of the TGD, 5% of the data will be outside the confidence limits of the generated QSAR) and hence, of apparently doubtful value, even though they may have been used to generate the QSAR.

An alternative approach, would be to check if the experimental data point is an outlier from the test chemicals used to generate a QSAR. In this approach, the dataset that was used to generate a QSAR is taken and the experimental point being assessed is added. Then a test for outliers made.

This approach is very dependent on the expertise of the user and requires a good understanding of the statistics associated with acceptance or rejection of outliers.

The final approach, described here, is to obtain the degree of variability reported for the method used to generate the measured data point. Thus for log  $K_{ow}$  the OECD method refers to a variability of  $\pm 0.3$  (see Section 3.2.1). This can then be used to compare the measured and predicted values. If they are within this variability, the predicted and measured values may be accepted as essentially the same and hence, the measured value would be used. This is a very stringent approach but nevertheless leads to high level of confidence in the generated data.

### ***Vinyl acetate***

Another chemical which has been assessed within the EU risk assessment process is vinyl acetate. The issue with the data available for vinyl acetate related more to the validity of the available historical physico-chemical values. Table 23 compares the measured data with those points for which the data were estimated.

Many of the historical data would, on the basis of this comparison, appear to be acceptable. There is a difference for the solubility value, which would need to be more fully understood. However, the effect of the change in solubility at this level, on the environmental behaviour and the risk assessment of vinyl acetate is slight.

**Table 23: Experimental and Estimated Physical/Chemical Values for Vinyl Acetate**

Property	Experimental value	Reference	Estimated value	Reference
Melting point	-93° C	BUA, 1993	-83.5° C	Syracuse
Boiling point	72 - 73° C at 1013 hPa	BUA, 1993	76° C	Syracuse
Vapour Pressure	120 hPa at 20° C	BUA, 1993	103 hPa at 25° C	Syracuse
Log $K_{ow}$	0.73 (Shake-flask)	BUA, 1993	0.72 0.697	Syracuse ASTER
Water solubility	23 g/l at 20° C	BUA, 1993	30.3 g/l 67.8 g/l	Syracuse ASTER
Hydrolysis ( $t_{1/2}$ )	11 d at 20°C and pH 7	Mabey and Mill, 1978	141 d at pH 7 and 25°C	Syracuse
	7.3 d at 25°C and pH 7	Skrabal and Zahorka, 1927		
	9.3 - 17 d at pH 7	Grant and Pullinger, 1979	>1000 d at pH 7 and 25°C	ASTER
	8.3 d at pH 7	Lijinsky, 1988; Lijinsky and Reuber, 1983		

The hydrolysis is more difficult to understand and its impact on the environmental assessment may be relevant. As mentioned in the review section on hydrolysis, ASTER uses a default if the chemical contains sub-structures that are not recognised. Discussions with the EPA would suggest that this is the case for vinyl acetate.

### **Alkylbenzenes**

Solubility of linear alkyl benzene (LAB; MW=236 and average alkyl chain = 11.6) in de-ionised water is reported to be 41 µg/l at 27° C (Gledhill *et al*, 1991). It was determined as the sum of the C9 to C13 peak areas in a gas chromatographic analysis. No experimental data on single LAB homologues are available. With increased alkyl chain length the water solubility is expected to decrease.

Aqueous solubility of the C10 to C14 LAB homologues as calculated using the ASTER and SYRACUSE programs are listed in Table 24. It appears that the values are at variance and that both measured and predicted values would need to be further investigated, prior to their use in risk assessment.

**Table 24: Calculated water solubility of LAB homologues**

LAB homologue (CAS number)	Calculated water solubility (ASTER) in µg/l	Calculated water solubility (SYRACUSE) in µg/l
C10 (104-72-3)	1.92	18.5
C11 (6742-54-7)	4.98 x 10 <sup>-1</sup>	3.30
C12 (123-01-3)	1.29 x 10 <sup>-1</sup>	1.01
C13 (123-02-4)	3.31 x 10 <sup>-2</sup>	2.09 x 10 <sup>-1</sup>
C14 (1459-10-5)	8.48 x 10 <sup>-3</sup>	4.11 x 10 <sup>-1</sup>

### **Toluene**

One of the first substances, for which fate and effect data had to be provided in the frame of the European risk assessment process was toluene (CAS No. 108-88-3). This substance has been extensively studied. Therefore, it was not surprising that the amount of data found in the open literature and in industrial databases led to several thousands of entries. To reduce the number of entries to a manageable size only validated data were submitted to the HEDSET for toluene.

An example, to demonstrate how this was achieved, is found in the entries for the acute toxicity to fish. A literature search revealed 230 citations covering different studies for this endpoint. It became

evident that in several studies, no corrections for volatilisation had been made. This resulted in effect concentrations much higher when compared with studies with appropriate corrections.

To be able to select the most appropriate data points, it was decided that in addition to the normal quality criteria as GLP and analytical determination of the test concentrations in spent medium, a comparison would be made between the reported measured data and effect estimations derived with the ECOSAR-program (Clements and Nabholz, 1994) and the QSARs of Verhaar *et al* (1992). The results of these are shown in Tables 25-26.

On this basis, 14 relevant freshwater and 1 marine acute fish LC<sub>50</sub> values were selected, which were all in the same order of magnitude as the calculated ones. The lowest reported measured freshwater LC<sub>50</sub>, 96 h *Carassius auratus*, of 13 mg/l was measured in a flow-through system and is very close to the calculated values of 16.8 mg/l (ECOSAR) and 21 mg/l (EEC, 1996).

The marine value 96 h LC<sub>50</sub> *Oncorhynchus kisutch*, of 6.3 mg/l also is close to the estimated value of 5.3 mg/l (ECOSAR).

**Table 25: ECOSAR Evaluation of Toluene**

Organism	Duration	Endpoint	mg/l
Daphnid	48 h	LC <sub>50</sub>	18.8
Green Algae	96 h	EC <sub>50</sub>	12.2
Fish	14 d	LC <sub>50</sub>	33.7
Fish [SW]	96 h	LC <sub>50</sub>	5.3
Daphnid	16 d	EC <sub>50</sub>	1.3
Mysid	96 h	LC <sub>50</sub>	3.0
Earthworm	14 d	LC <sub>50</sub>	360
Fish [FW]	96 h	LC <sub>50</sub>	16.8
Green Algae	>96 h	ChV	1.8
Fish	>14 d	BCF	49.3
Fish	>14 d	ChV	2.4

**Table 26: Evaluation of Toluene According to the EU Technical Guidance Documents, Chapter 4**

Non-polar narcosis	mg/l
96 h LC <sub>50</sub> fish	21
28-32 d NOEC fish ELS	1.9
EC <sub>50</sub> <i>Daphnia magna</i>	13.4
16 d NOEC <i>Daphnia magna</i>	2.1
EC <sub>50</sub> Algae	12.2
Polar narcosis	mg/l
96 h LC <sub>50</sub> fish	7.4
48 h EC <sub>50</sub> <i>Daphnia magna</i>	4.9

## 5.2 PRIORITY SETTING

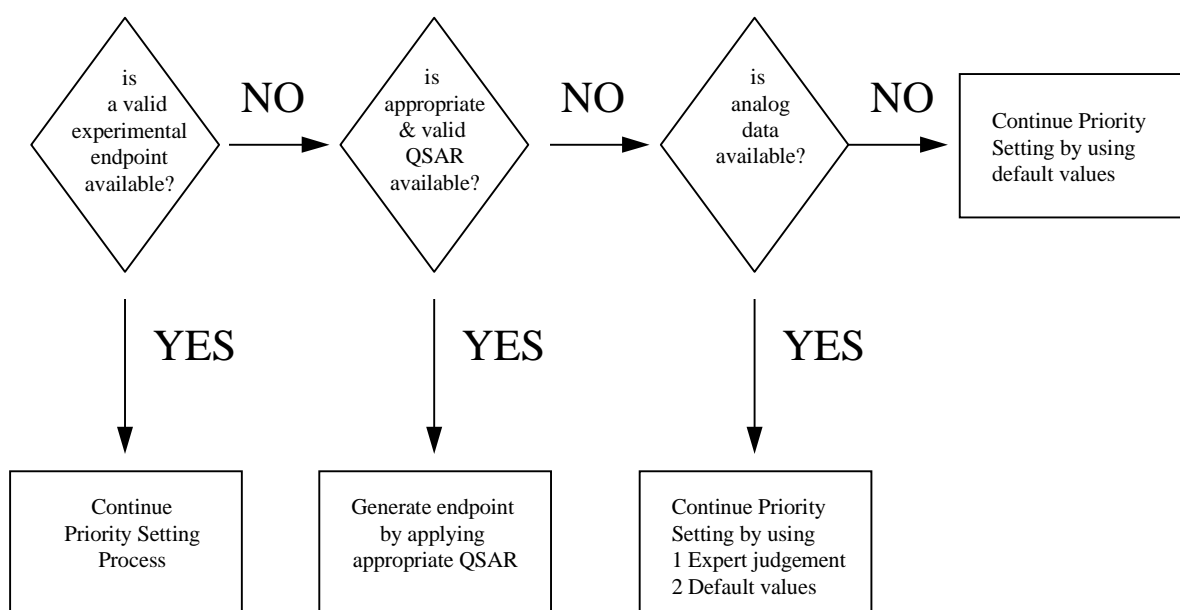
The EC Council Regulation EEC/793/93 on the Evaluation and Control of the Risks of Existing Substances requires that priority lists be established for risk assessment. Originally, it had been the intention of the Commission to derive such priority lists by using the submitted ecotoxicological, toxicological and physico-chemical substance data of high production volume chemicals to rank the substances according to their relative risk based on an automated informal priority setting system (IPS method).

One of the problems, which became apparent when IPS was run, however, was that the defaults were so stringent that substances with limited datasets were given the highest priority. While there may be some logic in this, the concern expressed by member states, was that there were other chemicals of a high perceived risk which would not be assessed in the immediate future as a result of the priority setting process.

The best way of avoiding this use of stringent defaults would be to employ appropriate QSARs to fill in the data gaps (see Figure 1). While there is bound to be some inaccuracies with an approach of this nature, as a screening tool the approach would be a reasonable use of QSARs.

The next development needed for this approach is to link the QSARs and the databases which contain the data of the substances electronically and to have a process that can group similar chemicals for assessment via the QSARs. This is now being addressed within the EU by the European Chemicals Bureau.

**Figure 1 : Proposed Use of QSARs in Priority Setting (Feijtel, 1995)**

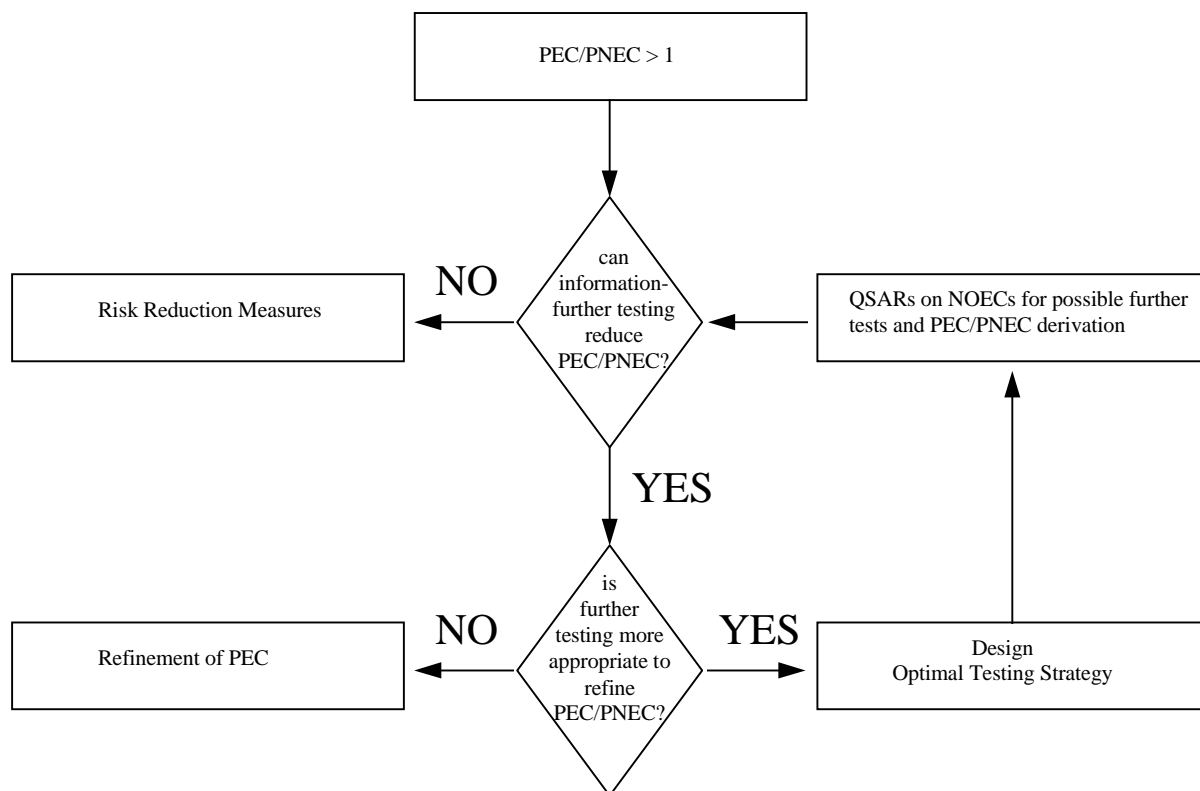


## 5.3 RISK ASSESSMENT

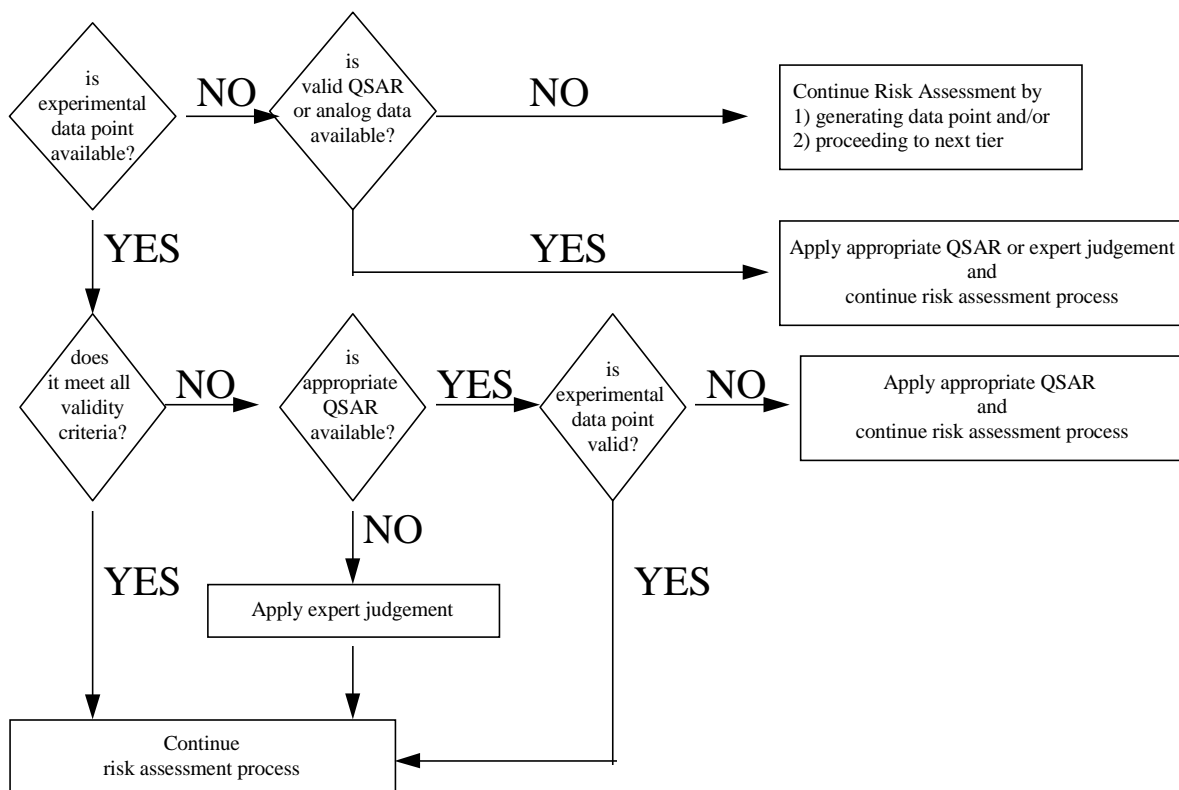
### 5.3.1 General Methods of Use of QSARs within EU Technical Guidance Documents

The explicit use of QSARs within the risk assessment TGD is currently restricted to that shown in Figure 2.

**Figure 2: Present Method of Use of QSARs in the EU Risk Assessment Process (Feijtel,1995)**



The use of QSARs should, however, be extended beyond that of data checking and this is shown in Figure 3, below. The principle expansion of the use of QSARs is for the prediction of endpoints beyond the initial screening risk assessment. This, with proper assessment of the uncertainties involved, would lead to a more rapid risk assessment and ensure that focus was maintained on those aspects of the risk assessment which were of real concern.

**Figure 3: Recommended Use of QSARs in Risk Assessment (based on Feijtel, 1995)**

### 5.3.2 Use of QSARs in Extrapolation of Laboratory Data to Field Fate and Effects

The assessment of whether a substance presents a risk to organisms in the environment, is based on a comparison of the predicted environmental concentration (PEC) with the predicted no effect concentration (PNEC) to organisms in ecosystems. This assessment can be performed for different compartments (e.g. air, water and soil) and on different spatial scales (local, regional) (ECETOC, 1993a; ECETOC, 1994). The assessment of exposure and effect concentrations is implicitly or explicitly based on use and/or integration of QSARs.

What is poorly understood, is the uncertainty of extrapolation to the “real” world of laboratory data. This is true of all extrapolations, however, many of the methods use QSARs and models in a non-transparent manner, thus making it difficult to assess the validity of the extrapolation process.

The following sections will briefly describe some of the underlying assumptions within these extrapolations. There will, where appropriate, be some discussion of the impact these assumptions have on the risk characterisation in the different environmental compartments.



### **Partitioning parameters**

#### *Octanol-water Partition Coefficient, $K_{ow}$*

The octanol-water partition coefficient ( $K_{ow}$ ) of a chemical is an important parameter used when estimating its potential for bioconcentration. This arises from the ability of octanol to act as a satisfactory surrogate for lipid in fish tissue. Similarly, within the risk assessment process the  $K_{ow}$  is used to describe partitioning onto sludge, soil, sediment and suspended matter, affecting the bioavailable concentration in water, pore water and/or soil solution. It is therefore important to recognise that this physico-chemical parameter plays a crucial role in both the exposure and effect assessment, and that for each additional calculation step, error and uncertainty is introduced.

The validity of  $\log K_{ow}$  as a key controlling parameter for fate, accumulation and effect models, is in principle restricted to a limited range of non-ionic lipophilic substances. Most QSAR relationships fail to include key biological processes (e.g. metabolism), and little or no insights are currently available in the limitations and uncertainties of applying these QSARs in exposure models assessing multiple transfers from primary to secondary and tertiary compartments.

#### *Organic Carbon - Water Partition Coefficient, $K_{oc}$*

The organic carbon-water partition coefficient, is used at several stages in the risk assessment to obtain the solids-water partition coefficient in the various compartments. The range of organic carbon covered by this assumed relationship, is 2% in soils to 10% in suspended matter. It is also known that the range of organic carbon varies from <1% in the environment to 50% in sewage works (EEC, 1996). Even for non-ionic substances, the ability of the  $K_{oc}$  parameter to normalise for the behaviour of substances over such a wide range of organic carbon must be doubtful. This extrapolation will not account for ionic interactions which occur between the substance and the matrix and the impact of the soil pH on those interactions.

It is also clear from the TGD that the extrapolation is of an equilibrium, which assumes that partitioning is a one off event and takes no account of desorption or the kinetics of the underlying processes. It is vital that the impact of these assumptions be more fully understood if a proper risk assessment is to be carried out.

#### *Henry's Law Constant, $H$*

There are a number of problems with the extrapolation of Henry's law constant within the environmental risk assessment. These were outlined by Suntio *et al* (1988).

H is very temperature sensitive, thus values change diurnally and seasonally. As a rule of thumb, H increases by a factor of 2 for an 8° C temperature rise. It is also important to realise that only the dissolved fraction of a substance exerts a partial pressure. Thus undissolved material or adsorbed material will not contribute to the distribution of a chemical between air and water.

Hence, the presence of dissolved electrolytes, organic matter, detergent and emulsified materials which will effect the 'solubility' of the chemical in water (or more strictly its activity coefficient ( $\gamma$ )) will lead to an altered H.

These factors will clearly have a major impact on the extrapolation to the real environment and it is important to understand the impact for each chemical.

#### *Degradative Processes*

The fate of chemicals in the environment may also be influenced by the presence of other chemicals. Such chemicals may influence toxicity or degradability. For example, some chemicals can become (better) degradable after having formed a complex with another chemical or ion. Fe-EDTA is an example of improved degradability by photolytic reactions or catalysed oxidation (Lockhart and Blakeley, 1975; Frank and Rau, 1990).

#### *Aquatic Risk Characterisation*

In order to display toxic effects to environmental organisms, substances must be bioavailable. Laboratory tests are usually designed in such a way that there is a maximum of bioavailability. In the "real" environment, however, the presence of other substances or materials may considerably influence bioavailability. Humic acid, for example, is a chemical that is widely present in natural surface waters as a result of the degradation of organic detritus. It is well known that humic acid can modify the toxicity of certain chemical structures by forming complexes. The US-EPA ecological effects test guidelines OPPTS 850.1085: Fish acute toxicity mitigated by humic acid, takes this into account and provides guidance to test chemicals in the presence and absence of humic acid.

Within the EU risk assessment this is more simplistically assessed by using the log  $K_{oc}$  to re-partition the chemical between sediments and water.

#### *Terrestrial Risk Characterisation*

The use of  $K_{ow}$  and  $K_{oc}$  for extrapolation into the terrestrial environment will initially lead to an underestimate of the extent to which organisms may interact with the chemical. Similarly there is likely to be an over-estimate of the long term availability of the chemical which will lead to a level of

residual chemical, but which may not be bioavailable. This is a research area that needs to be more fully investigated.

In the absence of available effects data for terrestrial species, the TGD suggests a screening approach. This method is called the equilibrium partitioning method and was developed for the assessment of the two phase sediment/water system (Di Toro *et al*, 1991). This method is used to calculate the  $PNEC_{soil}$  (mg/kg) of substances from the  $PNEC_{aq}$  for the aquatic environment.

In essence it means that the PNEC of the interstitial or pore water equals the concentration at which aquatic toxicity is observed. This assumption is experimentally proven for earthworms (Van Gestel and Ma, 1988). However, it is questionable if this assumption is also valid for soil dwelling species that are not exposed as extensively as earthworms to pore water. Therefore the advice is given in the TGD to test for soil toxicity when such a screening soil assessment gives PEC/PNEC ratio >1.

The TGD then suggests that for more lipophilic compounds the exposure through ingestion of soil will be larger than the exposure through pore water. To circumvent this, the calculated  $PEC_{soil}$  has to be multiplied by a factor of 10 for substances with a  $\log K_{ow} > 5$ .

However, this approach is contradicted by the results of Van Gestel and Ma (1988). In their work they demonstrated the linearity of toxic effects as a function of pore water concentrations. The influence of soil ingestion on both toxicity and bioconcentration of the amount absorbed to soil particle was negligible. Hence the uptake in their experiments is only a function of the bioavailable fraction in the pore water. Therefore, the enhancement with one order of magnitude of the  $PEC_{soil}$  is not supported by scientific research and should not be part of a science based risk assessment. This is clearly an area which needs investigation and a proper assessment through the correct use of new QSARs needs to be developed.

#### *Atmospheric Risk Characterisation*

The above-ground effects assessments are of a qualitative nature as they are based on a relative scale correlated with a reference molecule. For the quantification of these, a number of additional elements would need to be established. Firstly, the amount of a substance actually present in the atmosphere would need to be known. Secondly, the contribution of that substance to the effect being assessed needs to be established. Thirdly, the size and the atmospheric conditions of the system that is to be assessed have to be defined.

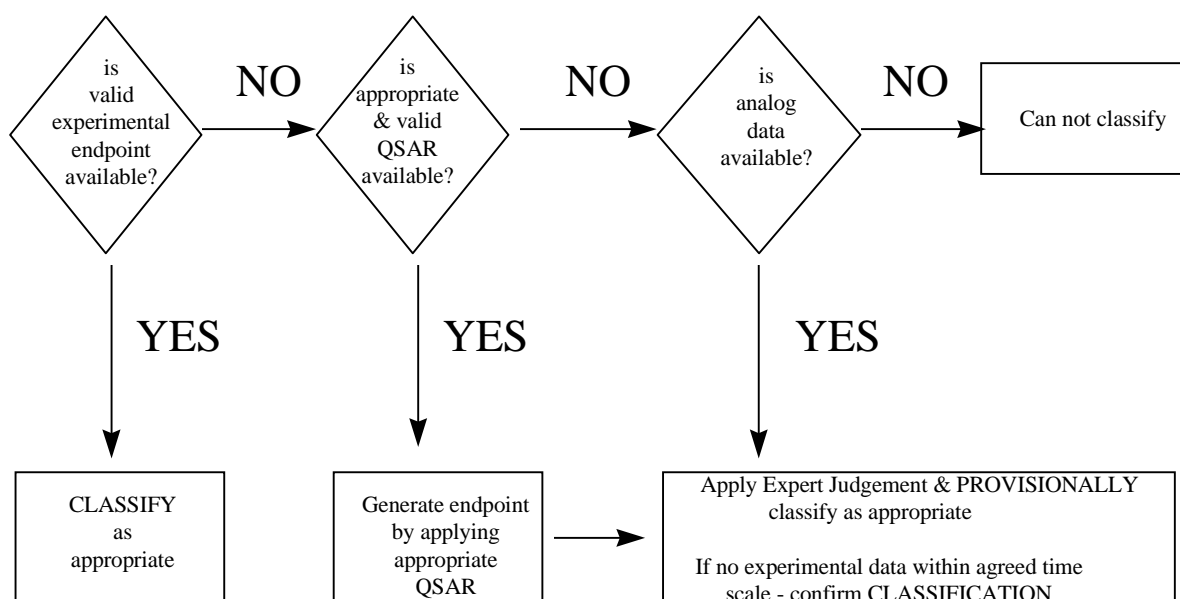
The risk characterisation scheme proposed by De Leeuw (1993) for new substances does not take these elements into account and is therefore of limited value.

## 5.4 CLASSIFICATION

The classification for substances and the corresponding Risk (R) and Safety (S) phrases has been published as part of the 12th Adaptation and in a revised form as part of the 18th Adaptation to Technical Progress of Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.

Although there is no reference to QSARs in any of these regulations, estimated values are being generated for existing chemicals in priority setting and risk assessment. Many of these chemicals will be on "Annex 1" already and are in the process of being evaluated as to whether they should be classified with respect to their environmental properties (EEC, 1992). However, many of the chemicals on "Annex 1" do not have relevant data, leading to the anomalous situation of similar chemicals being given different classifications, or more probably, some not being classified at all. The logical step would be to use estimated values to provide a provisional classification. Interested parties would be allowed to assess the QSAR data and the classification. If the data was acceptable then no further action would be necessary and, after a period of time the classification would stand. However, if there was some doubt over the data, new experimental data could be generated and a classification made on the basis of this data (Figure 4).

**Figure 4: Proposed Use of QSARs in Classification of Substances  
(based on Comber and Feijtel, 1998)**



## 6. CONCLUSIONS

When developing and using QSARs, there are a number of key points that should be addressed. The variability or uncertainty inherent in the original measurements upon which QSAR is based and which is therefore contained within the prediction, is frequently not known or available when predictions are made. When assessing chemicals and QSARs are used, either explicitly in generating a data point, or implicitly when describing their environmental behaviour, it is important that this uncertainty be properly assessed.

QSARs should generally not be used outside of their scope, but only for the purpose for which they were designed. This is particularly true for statistically derived QSARs, whereas QSARs based upon a mechanistic understanding may be used with more confidence when extrapolating beyond the original scope.

The sound principles and data upon which a mechanistic QSAR should be based are:

- well defined and measurable endpoint;
- a consistent dataset for that endpoint;
- a training set with a well defined chemical domain and relevant descriptors;
- a test set which is separate from the training set;
- the relevance of the descriptors should be appropriate for the scope of the QSAR;
- a clearly described statistical process.

From the above points it is clear that QSARs should only be used by experts, who need to understand more than the endpoint being predicted. They should also understand the descriptors used and their relevance to the chemicals in the QSARs domain, and the use to which the QSAR is to be put. Thus for example, the current methods for classifying chemicals on the basis of mode of action, which specify the QSAR to be used, require expert judgement.

Within any process, but especially that of risk assessment, it is, therefore, important, that QSARs should be used for which there is an understanding of how they were derived, how reliable they are and which were derived according to sound principles and data. In this way it becomes easier to begin the process of accounting for the uncertainties and moving towards a probabilistic risk assessment.

Problems with QSARs frequently arise when they are extended beyond their original purpose and unknown processes need to be taken into account. This explains the arbitrary use of a ten-fold factor

increasing the PEC above a trigger value of  $\log K_{ow}$  of 5 following the extension of equilibrium-partitioning theory into the terrestrial and sediment compartment risk assessments.

When using QSARs to predict an effect, it is important that other, related data, which would impact on the ability to express that property are taken into account. Thus, for example, when using a QSAR to predict the toxicity of a chemical, it is important to assess whether that toxicity would only occur above the water solubility and, thus, would not actually be expressed.

Given the current state of understanding of effect QSARs, they will be of limited applicability for predicting the effect of many of the new chemicals. One attempt to overcome this, is an improvement of the classification of chemicals, using their mode of action.

The current state of biodegradation (Q)SARs does not allow for their use within a regulatory framework. This is certainly the case for risk assessment, but is also probably true, for their use to support classification of chemicals. For example, within the EU system the classification R53, would not be safely applied using the current biodegradation QSARs.

The same arguments also apply to aquatic photolysis and  $K_{oc}$ , although in the case of the later property, there is a more telling need for a prediction which is capable of properly assessing a chemical's distribution between solid and water.

As there are currently no QSARs that adequately predict biotransformation or metabolism within organisms, QSARs for BCF may overestimate the actual bioconcentration of some chemicals.

One major area within the present EU risk assessment process which is poorly served by QSARs, is the understanding of how chemicals behave within the terrestrial and sedimentary compartments and to what extent they are capable of expressing their intrinsic properties. Not only is there insufficient information for assessing these compartments, but as a result, there are few QSARs to predict the influence of bioavailability on toxicity.

## 7. RECOMMENDATIONS

From the reviews carried out in this report there are a number of QSARs that need to be developed.

These are :

- metabolism in fish;
- bioconcentration including metabolism in organisms other than fish;
- microbial breakdown of chemicals;
- soil/water partitioning including kinetics;
- aquatic photolysis;
- effects on terrestrial organisms;
- effects on sediment dwelling organisms.

There is a need for continued work on the approaches to improve the predictive power of effect QSARs, by developing better classification schemes with regard to the mode of action of the substances.

There is also a clear need for QSARs for the effect for chemicals on marine organisms, and which account for the speciation of, for example, ionisable chemicals in marine waters.

Another need is for QSARs that will account for the impact of bioavailability of the effect on chemicals in the soil and sediment compartments.

It is important that QSARs that are recommended for use in the Regulatory area should be subject to constant improvement and refinement as more data become available. This is the case within the US - PMN process, although it is also recommended that the improvement should be as transparent as possible, given commercial confidentiality.

It is clear that uncertainty is involved in the measurement of properties and then in the development of QSARs. As such, processes for appropriately weighting, measuring and estimating the uncertainty in a QSAR prediction for any purpose, need to be developed.

The extrapolation from laboratory to the real world is difficult for any property, regardless of how the property was obtained. However, the implicit extrapolation or use of properties often involves QSARs. It is important then to realise that environmental conditions may deviate substantially from those included in the QSAR. Again an approach accounting for this needs to be developed.

It is the recommendation of this report that QSARs could be used to check the validity of data or to fill data gaps, for priority setting, risk assessment and classification. However, in such circumstances, there need to be appropriate mechanisms to allow for the generation of measured data when requested. Valid measured data, when available, should always take precedence over QSAR predictions.



## APPENDIX A: GLOSSARY OF TERMS

### ***Expert***

The term expert is used in many ways by different people. Within this report three terms in particular are used:

#### *Expert knowledge*

The special information and skills that a person working on QSARs has.

#### *Expert system*

A program of rules, summarising expert knowledge, which may be applied by someone without that expert knowledge.

#### *Expert judgement*

A process which is gone through that uses expert knowledge and possibly an expert system but extrapolates into an area of uncertainty. It is carried out by someone who has expert knowledge of the differing disciplines being used to arrive at the judgement.

### ***Structure Activity Relationship (SAR)***

A relationship between a structure and a biological or physico-chemical endpoint.

### ***Quantitative Structure Activity Relationship (QSAR)***

This is normally taken to mean a mathematical relationship between a descriptor and a biological or physico-chemical endpoint. However, within this report the whole process, including classification of the chemical under consideration, selection of the QSAR, as well as use of the QSAR is considered to be the QSAR.

### ***Model***

A series of algorithms, sometimes made of several sub-units called modules.

***Training/test set***

A set, or sub-set, of chemicals which are used either to develop a QSAR (training) or to check the validity of the QSAR (test).

***Domain***

A group of substances sharing certain structural and/or functional properties.

***Endpoint***

The property of a chemical being measured or predicted.

***Descriptor***

The property of a chemical used in a QSAR to predict an endpoint.

***Biodegradation***

Molecular degradation of a substance, resulting from the complex action of living organism.

## APPENDIX B: EXTERNAL VALIDATION OF AN AQUATIC TOXICITY CLASSIFICATION SYSTEM BY MEANS OF THE EAT DATABASE

The QSARs used within the EU Technical Guidance Documents (TGD) (see Section 4.2.2), and the approach used to define the domain of those QSARs, were based on the work of Verhaar *et al* (1992). This appendix provides a summary of a new article of Verhaar *et al* (in preparation) which uses the fish acute data in the ECETOC Aquatic Toxicity (EAT) database (ECETOC, 1993c) to validate that approach.

The authors first classified all of the substances in the EAT database according to the approach of Verhaar *et al* (1992), viz. non-polar narcosis (class 1), polar narcosis (class 2), reactive and specifically acting chemicals (classes 3 and 4, respectively). This yielded 39, 23 and 59 substances in classes 1, 2 and 3-4, with a total of 55 substances being non-classified. The non-classified chemicals were either inorganic salts or organo-metallics (31) or non-classifiable organics (24). Using the Verhaar QSARs, fish (*Pimephales promelas*) LC<sub>50</sub> values were then calculated.

The fish toxicity data (pertaining to 44 different fish species) for those classified substances were then retrieved from the EAT database. Finally, the two sets of data, measured (EAT) and calculated were compared. The results of this comparison were presented in graphical format.

The results showed that generally, the classification system provided adequate predictions of either the aquatic toxicity (class 1, "baseline toxicity") or the possible ranges of toxicity (class 2: five to ten times, classes 3 and 4: ten to ten thousand times more toxic than baseline toxicity) of organic substances.

The authors concluded that the previously published classification scheme for the assessment of fish toxicity generally performed as intended. Although the data of the EAT database were for a wide variety of different fish species, the agreement with the predicted *P. promelas* effect was good. The distribution of the data was noted by the authors to compare favourably to that of the original data upon which the QSAR was based.

Based on the analysis of a few outliers, the authors suggested that some of the compound groups that are currently predicted to belong to class 3, be reassigned to class 1, e.g. the aliphatic cyclic  $\alpha,\beta$ -unsaturated ketones. They also concluded that the classification approach, as originally described, would need to be altered to include organic esters and acids, these chemicals comprising the majority of the unclassified organic substances.

## BIBLIOGRAPHY

- Alexander M, 1981. Biodegradation of chemicals of environmental concern. *Sci.* 211, 132-138.
- ACS, 1981. CAS On-line screen dictionary for substructure search (2nd ed), Am. Chem. Soc., Ohio, USA.
- American Public Health Association, American Water Works Association and the Water Pollution Control Federation, 1975. Standard Methods for the Examination of Water and Waste Water, 14th ed. Am. Publ. Hlth Assoc., Washington, DC.
- Ames TT and Grulke EA, 1995. Group contribution method for predicting equilibria of nonionic organic compounds between soil organic matter and water. *Environ. Sci. Technol.* 29, 2273-2279.
- Anderberg MR, 1973. Cluster analysis for applications. Academic Press, New York, USA.
- Anderson JA and Rosenfeld E, 1988. Neurocomputing, Vol. I: Foundations of research. The MIT Press, Cambridge, Ma, USA.
- Anliker R, Moser P and Poppinger D, 1988. Bioaccumulation of dyestuffs and organic pigments in fish. Relationships to hydrophobicity and steric factors. *Chemosphere*, 17, 1631 - 1644.
- Antoine C, 1888. Tensions des vapeurs: Nouvelle relation entre les tensions et les températures. *Compt. Rend.* 107, 681-684.
- Ashley S and Mallett M, 1990. EEC Ring Test of a Method for Determining the Effects of Chemicals on the Growth Rate of Fish. Final Report to the EC. ECE ref. number B86614/88/37.
- Atkins PW, 1982. Physical Chemistry, second ed., Oxford University Press, Oxford, UK, 224.
- Atkinson R, 1985. Kinetics and mechanisms of gas-phase reactions with the hydroxyl radical with organic compounds under atmospheric condition, *Chem. Rev.* 85, 61-201.
- Atkinson R, 1988. Estimation of gas phase hydroxyl radical rate constants for organic chemicals. *Environ. Toxicol. Chem.* 7, 435-442.
- Atkinson R, 1991. Kinetics and mechanisms of the gas-phase reactions of the NO<sub>3</sub> radical with organic compounds under atmospheric conditions. *J. Phys. Chem. Ref. Data*, 20, 459-507.
- Atkinson R and Carter WPL, 1984. Kinetics and mechanisms of the gas-phase reactions of ozone with organic compounds under atmospheric conditions. *Chem. Rev.* 84, 437-470.
- Auer CM, Zeeman M, Nabholz JV, Clements RG, 1994. SAR - The US regulatory perspective. SAR and QSAR in *Environ. Res.* 2, 29-38.
- Bacci E, Calamari D, Gaggi C and Vighi M, 1990. Bioconcentration of organic chemical vapors in plant leaves: Experimental measurements and correlation. *Environ. Sci. Technol.* 24, 885-889.
- Banerjee S and Williams CL, 1993. Compatibility of functional groups in K<sub>ow</sub> based QSARs: Application to nitro compounds. *Environ. Toxicol. Chem.* 12, 1847-1849.
- Beale R and Jackson T, 1990. Neural computing: an introduction. Adam Hilger, Bristol PA, USA.
- Bintein S and Devillers J, 1994. QSAR for organic chemical sorption in soils and sediments. *Chemosphere* 28, 1171-1188.
- Bintein S, Devillers J and Karcher W, 1993. Nonlinear dependence of fish bioconcentration on *n*-octanol/water partition coefficient. SAR and QSAR in *Environ. Res.* 1, 29-39.
- Blum DJW and Speece RE, 1990. Determining the aquatic toxicity to aquatic species. The use of QSARs and surrogate organisms. *Environ. Sci Technol.* 24, 284-293.

- Boethling RS and Sabljic A, 1989. Screening-level model for aerobic biodegradability based on a survey of expert knowledge. *Environ. Sci. Technol.* 23, 672-679.
- Boethling RS, Campbell SE, Lynch DG and La Veck GD, 1988. Validation of CHEMEST, an on-line system for the estimation of chemical properties. *Ecotox. Environ. Safety* 15, 21-30.
- Boethling RS, Howard PH, Meylan WM, Stiteler W, Beauman J and Tirado N, 1993. User's guide for BIODEG. Syracuse Research Corporation; Environ. Sci. Center, Syracuse, NY, USA.
- Boethling RS, Howard PH, Meylan W, Stiteler W, Beauman J and Tirado N, 1994. Group contribution method for predicting probability and rate of aerobic biodegradation. *Environ. Sci. Technol.* 28, 459-465.
- Bol J, Verhaar HJM, van Leeuwen CJ and Hermens JLM, 1993. Predictions of the aquatic toxicity of high-production-volume-chemicals. Part A: Introduction and Methodology, VROM, Zoetermeer, NL.
- Bradbury SP, 1994. Predicting modes of toxic action from chemical structure: an overview. SAR and QSAR in *Environ. Res.* 2, 89-104.
- Brooke DN, Dobbs, AJ and Williams N, 1986. Octanol-water partition coefficient (P): Measurement, estimation and interpretation, particularly for chemicals with  $P > 10^5$ . *Ecotox. Environ. Safety* 11, 251-260.
- Broto P and Devillers J, 1990. Autocorrelation of properties distributed on molecular graphs. In: Karcher W and Devillers J (ed), *Practical applications of quantitative structure-activity relationships (QSAR) in environmental chemistry and toxicology*. ECSC, EEC, EAEC, Brussels and Luxembourg, 105-127.
- BUA, 1993. Beratergremium für umweltrelevante Altstoffe. Vinylacetat. BUA-Stoffbericht 116, S. Hirtzel Verlag, Stuttgart, D.
- Bysshe SE, 1990. Bioconcentration factor in aquatic organisms, In: Lyman WJ, Reehl WF and Rosenblatt DH (ed), *Handbook of chemical property estimation methods - Environmental behavior of organic compounds*, Chapter 5. Am. Chem. Soc. Washington DC.
- Calamari D and Vighi M, 1984. QSARS in Aquatic Toxicology: an experience. In: *Hommage Professeur René Truhaut, Monograph, Fac. Agrar. Univ. Milano, Italy*, 172-174.
- Cambon R and Devillers J, 1993. New trends in structure-biodegradability relationships. *Quant. Struct. Relat.* 12, 49-58.
- Chessels M, Hawker DW and Connell DW, 1991. Critical evaluation of the measurement of the 1-octanol/water partition coefficient of hydrophobic compounds. *Chemosphere* 22, 1175-1190.
- Chester NA, Haley MV and Landis WG, 1992. The aquatic toxicity of isopropylamine: Comparison of experimentally derived values with structure activity predictions. *Environ. Sci.* 1, 117-126.
- Chiou CT, 1985. Partition coefficients of organic compounds in lipid-water systems and correlations with fish bioconcentration factors. *Environ. Sci. Technol.* 19, 57-62.
- Chiou CT and Schmedding DW, 1982. Partitioning of organic compounds in octanol-water systems. *Environ. Sci. Technol.* 16, 4-10.
- Clements RG and Nabholz JV, 1994. ECOSAR: A computer program for estimating the ecotoxicity of industrial chemicals based on structure activity relationships. User's Guide. US EPA, Office of Pollution Prevention and Toxics (7403), Washington DC. EPA-748-R-93-002.
- Clements RG, Nabholz JV, Johnson DW and Zeeman M, 1993. The use and application of QSARs in the Office of Toxic Substances for Ecological Hazard Assessment of New Chemicals, ASTM STP 1179. Landis WG, Hughes JS and Lewis MA (ed), *Am. Soc. for Test. and Mat. Philadelphia*, 56-64.

- Clements RG, Nabholtz JV, Zeeman M and Auer CM, 1995. The application of structure activity relationships (SARs) in the aquatic toxicity evaluation of discrete organic chemicals. SAR and QSAR in Environ. Res. 3, 203-215.
- Comber MHI and Feijtel TCJ, 1998. Assessment and use of quantitative structure-activity relationships: A report from two ECETOC task forces. In: Quantitative Structure-Activity Relationships in Environmental Sciences - VII, Chapter 25, SETAC Press, 365-379.
- Connell DW, 1987. Bioaccumulation behaviour of persistent organic chemicals with aquatic organisms. Reviews of Environ. Contam. Toxicol. 101, 117-154.
- Connell DW, 1994. The octanol-water partition coefficient In: Calow P (ed), Handbook of Ecotoxicology, Vol. II, Blackwell Sci. Pubs., London, UK, 311-320.
- Connell DW and Hawker DW, 1988. Use of polynomial expressions to describe the bioconcentration of hydrophobic chemicals by fish. Ecotox. Environ. Safety 16, 242-257.
- Connell DW and Markwell RD, 1990. Bioaccumulation in the soil to earthworm system. Chemosphere 20, 91-100.
- Cornish-Bowden A and Wong JT-F, 1978. Evaluation of rate constants for enzyme-catalysed reactions by the jackknife technique. Biochem. J. 175, 969-976.
- Cowan CE, Federle TW, Larson RJ and Feijtel TCF, 1996. Impact of biodegradation test methods on the development and applicability of biodegradation QSARs. SAR and QSAR in Environ. Res. 5, 37-49.
- Crutzen PJ, 1982. The global distribution of hydroxyl. In: Goldberg ED (ed), Atmospheric Chemistry. Springer Verlag, Berlin, D.
- Damborský J, 1995. A mechanistic approach to deriving quantitative structure-activity relationship models for microbial degradation of organic compounds. In: Peijnenburg WJGM and Karcher W (ed), Proceedings of the workshop "Quantitative Structure Activity Relationships for Biodegradation", September 1994, Belgirate, Italy. Nat. Inst. of Publ. Hlth and Environ. Prot., Bilthoven, The Netherlands, Report no. 719101021.
- Damborský J, Manová K and Kutý MA, 1996. A mechanistic approach to deriving quantitative structure biodegradability relationships. In: Peijnenburg WJGM and Damborský J (ed), Biodegradability prediction. NATO ASI Series 2. Environment - Vol. 23, Kluwer, Dordrecht, NL, 75-92.
- Davies RP and Dobbs AJ, 1984. The prediction of bioconcentration in fish. Wat. Res. 18, 1253-1262.
- De Bruijn J and Hermens J, 1991. Qualitative and quantitative modelling to toxic effects of organophosphorous compounds to fish. In: Hermens JLM and Opperhuizen A (ed), QSAR in Environmental Toxicology-IV, Elsevier, Amsterdam, NL, 441-455.
- De Leeuw FAAM, 1993. Assessment of atmospheric hazards and risks of new chemicals: Procedures to estimate "Hazard Potentials". Chemosphere, 27, 1313-1328.
- De Saint Laumer, JY, Chastrette M and Devillers J, 1991. Multilayer neural networks applied to structure-activity relationships. In: Karcher W and Devillers J (ed), Applied multivariate analysis in SAR and environmental studies, Kluwer, Dordrecht, NL, 479-521.
- Deneer JW, Sinnige TL, Seinen W and Hermens JLM, 1987. Quantitative structure-activity relationships for the toxicity and bioconcentration factor of nitrobenzene derivatives towards the guppy (*Poecilia reticulata*). Aquat. Toxicol. 10, 115-129.
- Deneer JW, Sinnige TL, Seinen W and Hermens JLM, 1988a. A quantitative structure-activity relationship for the acute toxicity of some epoxy compounds to the guppy. Aquat. Toxicol. 13, 195-204.

- Deneer JW, Seinen W and Hermens JML, 1988b. The acute toxicity of aldehydes to the guppy. *Aquat. Toxicol.* 12, 185-192.
- Derwent RG and Jenkins ME, 1990. Hydrocarbon involvement in photochemical ozone formation in Europe, United Kingdom Atomic Energy Authority, Harwell, Oxfordshire, UK.
- Devillers J, Chambon P, Zakarya D, 1987. A predictive structure-toxicity model with *Daphnia magna*. *Chemosphere* 16, 1149-1163.
- Devillers J, Domine D, Karcher W, 1995a. Estimating n-octanol/water partition coefficients from autocorrelation method. *SAR and QSAR Environ. Res.* 3, 301-306.
- Devillers J, Bintein S and Domine D, 1995b. Comparison of BCF models based on log P. Conducted for: Ministère de L'Environnement, Bureau des Substances et Préparations Chimiques, CTIS, Report no. 95038, Lyon, F.
- Dietrich SW, Dreyer ND and Hansch C, 1980. Confidence interval estimators for parameters associated with quantitative structure-activity relationships. *J. Med. Chem.* 23, 1201-1205.
- Di Toro DM, Zarba CS, Hansen DJ, Berry WJ, Swartz RC, Cowan CE, Pavlou SP, Allen HE, Thomas NA and Paquin PR, 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ. Toxicol. Chem.* 10, 1541-1583.
- Dive D, Gabriel L, Hanssens O and Benga-Bengomme A, 1989. A bioassay using the measurement of growth inhibition of a ciliate protozoan: *Colpidium campylum* Stokes. *Hydrobiologica* 188/189, 181-188.
- Donkin P, 1994. Quantitative structure-activity relationships. In: Calow P (ed), *Handbook of ecotoxicology*, Vol. 2. Blackwell Scientific Publications.
- Doull J, Klaassen CD and Amdur MO, 1980. In: Casarett and Doull's *Toxicology; The basic science of poisons*, 2nd edition. Macmillan Publishing Co., NY, USA, 619-622.
- Downs GM, Gillet VJ, Holliday JD and Lynch MF, 1989. Computer storage and retrieval of generic chemical structures in patients. 10. Assignment of logical bubble-up of ring screens for structurally explicit generics. *J. Chem. Inf. Comput. Sci.* 29, 215-224.
- Draper NR and Smith H, 1991. *Applied Regression Analysis*. John Wiley and Sons, New York, USA.
- Eakin DR, 1974. The ICI Crossbow System. In: Ash JE and Hyde E (ed), *Chemical Information Systems*, Ellis Horwood Pub., Chichester, Chapter 14, 227-242.
- ECETOC, 1983. Experimental assessment of the photochemical phototransformation of chemicals in the atmosphere. Technical Report No. 7.
- ECETOC, 1986. Structure-activity relationships in toxicology and ecotoxicology: an assessment. Monograph No. 8.
- ECETOC, 1988. Evaluation of anaerobic biodegradation. Technical Report No. 28.
- ECETOC, 1993a. Environmental hazard assessment of substances. Technical Report No. 51.
- ECETOC, 1993b. Aquatic toxicity data evaluation. Technical Report No. 56.
- ECETOC, 1993c. Aquatic toxicity data evaluation. Appendix C: The Database. Technical Report No.56.
- ECETOC, 1994. Assessment of non-occupational exposure to chemicals. Technical Report No. 58.
- ECETOC, 1995. The role of bioaccumulation in environmental risk assessment: The aquatic environment and related food webs. Technical Report No.67.

ECETOC, 1996. Aquatic toxicity testing of sparingly soluble, volatile and unstable substances. Monograph No. 26.

EEC, 1984. Council Directive 84/449/EEC of 25 April 1984 adopting to technical progress for the 6th time Council directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Off. J. of the EC, L133/1.

EEC, 1987. Council directive 87/302/EEC of 18 November 1987 adopting to technical progress for the 9th time Council directive 67/548/EEC on the approximation of laws, regulations, and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Off. J. of the EC, L251/1.

EEC, 1992. Council Directive 92/32/EEC of 30 April 1992 amending for the 7th time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances ("7th Amendment"). Off. J. of the EC, L 154/1.

EEC, 1993a. Council Regulation (EC) 793/93 of 23 March 1993 on the evaluation and control of the risks of existing substances. Off. J. of the EC, L 84/1.

EEC, 1993b. Commission Directive 93/67/EEC of 20 July 1993 laying down the principles for assessment of risks to man and the environment of substances notified in accordance with Council Directive 67/458/EEC. Off. J. of the EC L 227/9.

EEC, 1994. Commission Regulation (EC) 1488/94 of 28 June 1994, laying down the principles for the assessment of risks to man and the environment of existing substances in accordance with Council Regulation (EEC) No. 793/93. Off. J. of the EC, L 161.

EEC, 1995. Technical Guidance Documents in support of the Commission Regulation of laying down the principles for the assessment of risks to

man and the environment of existing substances (EEC/1488/94).

EEC, 1996. Technical Guidance Documents in support of the Commission Directive 93/67/EEC on risk assessment for new notified substances and the Commission Regulation (EC) 1488/94 on risk assessment for existing substances. European Chemicals Bureau, Ispra.

Erikson L, Johansson E and Wold S, 1997. QSAR Model Validation. Proceedings of the 7th International Workshop on QSAR in Environmental Sciences, SETAC Press, Pensacola, FL, USA. Accepted for publication.

Federle TW and Itrich N, 1997. A comprehensive approach for assessing the kinetics and ultimate biodegradation of chemicals in activated sludge: Application to linear alkylbenzene sulfonate. Environ. Sci. Technol. 31, 1178-1184.

Feijtel TCJ, 1995. Evaluation of the use of QSARs for priority setting and risk assessment. SAR and QSAR in Environ. Res. 3, 237-245.

Forman JC and Thodos G, 1958. Critical temperatures and pressures of hydrocarbons. Am. Inst. of Chem. Eng. J. 4, 356-361.

Forman JC and Thodos G, 1960. Critical temperatures and pressures of organic compounds. Am. Inst. of Chem. Eng. J. 6, 206-209.

Forrest S, 1993. Genetic Algorithms: principles of natural selection applied to computation. Sci. 261, 872-885.

Frank R and Rau H, 1990. Photochemical transformation in aqueous solution and possible environmental fate of ethylenediamine tetraacetic acid (EDTA). Ecotox. Environ. Safety, 19, 55-63.

Freeman JA and Skapura DM, 1991. Neural networks: algorithms, applications, and programming techniques. Addison-Wesley, Reading MA, USA.



- Friederichs M, Fränzele O and Salski A, 1996. Fuzzy clustering of existing chemicals according to their ecotoxicological properties. *Ecological Modelling*, 85, 27-40.
- Friedman M, Cavins JF and Wall JS, 1965. Relative nucleophilic reactivities of amino groups and mercaptide ions in addition reactions with  $\alpha,\beta$ -unsaturated compounds. *J. Am. Chem. Soc.* 87, 3672-3682.
- Fröbe Z, Fingler S, Drevenkar V and Juracic M, 1994. Sorption behaviour of some chlorophenols in natural sorbents. 1. Validity of the partition model for sorption of phenolates. *Sci. Tot. Environ.* 155, 199-213.
- Gamberger D, Sekusak K and Sabljic A, 1993. Modelling biodegradation by an example-based learning system. *Informatica*, 17, 157-166.
- Garforth BM, 1983. Toxicity tests with *Daphnia magna*: acute toxicity of eight test materials to a newly-introduced strain of *Daphnia magna* in reconstituted fresh water. Shell Research Ltd. Report SBGR.83.100. Sittingbourne, UK.
- Geladi P and Kowalski BR, 1986. Partial least-squares regression: A tutorial. *Anal. Chim. Acta* 185, 1-17.
- Gledhill WE, Saeger VW, and Trehy ML, 1991. An aquatic environmental safety assessment of linear alkylbenzene. *Environ. Toxicol. Chem.* 10, 169-178.
- Gold P and Ogle G, 1969. Estimating thermophysical properties of liquids. Part 4 - Boiling, freezing and triple point temperatures. *Chem. Eng.* 13, 119 - 122.
- Goldberg DE. 1989. Genetic algorithms in search of optimization, and machine learning. Addison Wesley Longman Inc., Reading, Mass. 412p.
- Govers H, Ruepert C and Aiking H, 1984. Quantitative structure-activity relationships for polycyclic aromatic hydrocarbons: correlation between molecular connectivity, physico-chemical properties, bioconcentration and toxicity in *Daphnia pulex*. *Chemosphere* 13, 227-236.
- Govers HAJ, Luijk R and Evers EHG, 1991. Descriptors for isomer resolution of (bio-) distribution of chlorinated aromatic compounds. In: Hermens JLM and Opperhuizen A (ed), *QSAR in Environmental Toxicology - IV*. Elsevier, Amsterdam, NL, 105-119.
- Grain CF and Lyman WJ, 1983. Enhancement to CHEMEST program: estimation of melting points. EPA Contract 68-01-6271, Arthur D. Little Inc. Cambridge, Ma, USA.
- Grain CF, 1990. Vapor pressure. In: Lyman WJ, Reehl WF and Rosenblatt DH (ed), 1990. *Handbook of chemical property estimation methods. Environmental behavior of organic compounds*, Chapter 14. Am. Chem. Soc. Washington DC.
- Grant and Pullinger, 1979. Report No. 1932-51/1 for the Society of Plastics Industry, New York, Hazleton UK, Harrogate, 1-20.
- Griep MI, Wakeling IN, Vankeerberghen P and Massart DL, 1995. Comparison of semirobust and robust partial least squares procedures. *Chemometrics and Intelligent Laboratory Systems* 29, 37-50.
- Güsten H and Sabljic A, 1995a. QSARs for soil sorption. In: *Overview of structure-activity relationships for environmental endpoints*, Part 1: General outline and procedure. Report of the EU-DG-XII Project QSAR for predicting fate and effects of chemicals in the environment. Contract No. EV5V-CT92-0211.
- Güsten H and Sabljic A, 1995b. QSARs for soil sorption. In: *Overview of structure-activity relationships for environmental endpoints*, Part 2: Description of selected models. Report of the EU-DG-XII Project QSAR for predicting fate and effects of chemicals in the environment. Contract No. EV5V-CT92-0211.

- Hansch C and Leo A, 1979. Substituent constants for correlation analysis in chemistry and biology. John Wiley and Sons, New York, USA.
- Harris JC, 1990. Rate of hydrolysis. In: Lyman WJ, Reehl WF and Rosenblatt DH (ed), 1990. Handbook of chemical property estimation methods. Environmental behavior of organic compounds, Chapter 7. Am. Chem. Soc. Washington DC.
- Harris JC and Hayes MJ, 1990. Acid dissociation constant. In: Lyman WJ, Reehl WF and Rosenblatt DH (ed), Handbook of Chemical Property Estimation Methods, Environmental behavior of organic compounds, McGraw Hill Book Co., New York, USA.
- Hecht-Nielsen R, 1990. Neurocomputing. Addison-Wesley, Reading Ma, USA.
- Heck WW, Adams RM, Cure WW, Heagle AS, Heggstad HE, Kohut RJ, Kress KW, Rawlings JO and Taylor OC, 1983. A reassessment of crop loss from ozone, Environ. Sci. Technol. 17, 572A-581A.
- Hermens JLM, 1989. Quantitative structure-activity relationships of environmental pollutants. In: Hutzinger O (ed), Handbook of Environ. Chem., Vol 2E. Springer Verlag, Berlin, D, 111-162.
- Hermens JLM, Leeuwangh P and Musch A, 1984. Quantitative structure-activity relationships and mixture toxicity studies of chloro- and alkylanilines at an acute lethal toxicity level to the guppy (*Poecilia reticulata*). Ecotox. Environ. Safety 8, 388-394.
- Hermens JML, Busser F, Leeuwangh P and Musch A, 1985. Quantitative correlation studies between the acute lethal toxicity of 15 organic halides to the guppy (*Poecilia reticulata*) and chemical reactivity towards 4-nitrobenzylpyridine. Toxicol. Environ. Chem. 9, 219-236.
- Hewitt CN and Harrison RM, 1984. Tropospheric concentrations of the hydroxyl radical; A review. Atmos. Environ. 19, 545-554.
- Hilal SH and Karickhoff SW, 1995. A rigorous test for SPARC's chemical reactivity models: Estimation of more than 4300 ionization pK<sub>a</sub>s. Quant. Struct. Act. Relat. 14, 348-355.
- Hine J and Mookerjee PK, 1975. The intrinsic hydrophilic character of organic compounds. Correlations in terms of structural contributions. J. Org. Chem. 40, 292-298.
- Horvath AL, 1982. Halogenated hydrocarbons. Dekker, New York, USA, 260-262, 478-535.
- Houghton JT, Jenkins GJ and Ephraums JJ, 1990. Climate change; The IPCC Scientific Assessment. Cambridge University Press, Cambridge, UK.
- Houghton JT, Callander BA and Varney SK, 1992. Climate change 1992; The supplementary report to the IPCC Sci. Assess. Cambridge University Press, Cambridge, UK.
- Houghton JT, Meira Filho LC, Bruce J, Hoesung Lee, Callander BA, Haites E, Harris N and Maskell K, 1995. Climate change 1994; Radiative forcing of climate change and An evaluation of the IPCC IS92 emission scenarios. Cambridge University Press, Cambridge, UK.
- Howard PH, Hueber AE and Boethling RS, 1987. Biodegradation data evaluation for structure/biodegradability relations. Environ. Toxicol. Chem. 6, 1-10.
- Howard PH, Boethling RS, Stiteler WM, Meylan WM, Hueber AE, Beauman JA and Larosche ME, 1992. Predictive model for aerobic biodegradability developed from a file of evaluated biodegradation data. Environ. Tox. Chem. 11, 593-603.
- Hunter RS, 1988. Computer calculation of pK<sub>a</sub>. In: Turner JE, England MW, Schultz TW and Kwaak NJ (ed), Proceedings of the Third International Workshop on Quantitative Structure-Activity Relationships in Environmental Toxicology, May 22-26, 1988. Report No. CONF-880520-(DE8803180). National Technical Information Service, US Dept. of Commerce, Springfield, VA, USA, 22161, 33-42.

- Hutchinson TH, Solbé J and Kloepper-Sams PJ, 1998. Analysis of the ECETOC Aquatic Toxicity (EAT) database. III. Comparative toxicity of chemical substances to different life stages of aquatic organisms. *Chemosphere* 36, 129-142.
- Irmann F, 1965. Eine einfache Korrelation zwischen Wasserlöslichkeit und Struktur von Kohlenwasserstoffen und Halogenkohlenwasserstoffen. *Chemie-Ing.-Techn.* 37, 789-798.
- Irvin JA and Quickenden TI, 1983. Linear least squares treatment when there are errors in both X and Y. *J. Chem. Educ.* 60, 711-712.
- Isnard P and Lambert S, 1988. Estimating bioconcentration factors from octanol-water partition coefficient and aqueous solubility. *Chemosphere* 17, 21-34.
- Isnard P and Lambert S, 1989. Aqueous solubility and n-octanol/water partition coefficient correlations. *Chemosphere* 18, 1837-1853.
- ISO/DIS, 1994. No. 10253.2.
- Jäckel H and Nendza M, 1994. Relative substructures in the prediction of aquatic toxicity data. *Aquat. Toxicol.* 29, 305-314.
- Joback KG, 1982. A unified approach to physical property estimation using multivariate statistical techniques. Steven Institute of Technology, submitted to the Department of Chemical Engineering for M.S. Degree at the Massachusetts Inst. of Technol. in June 1984.
- Kaiser KLE and Esterby SR, 1991. Regression and cluster analysis of the acute toxicity of 267 chemicals to six species of biota and the octanol/water partition coefficient. *Sci. Tot. Environ.* 109/110, 499-514.
- Karickhoff SW, 1981. Semi-empirical estimation of sorption of hydrophobic pollutants on natural sediments and soils. *Chemosphere* 10, 833-846.
- Karickhoff SW, 1985. Pollutant sorption in environmental systems. In: Neely WB and Blau GE (ed), *Environmental exposure from chemicals - Volume 1*. CRC Press Inc., Boca Raton, FL, USA.
- Karickhoff SW, Brown DS and Scott TA, 1979. Sorption of hydrophobic pollutants on natural sediments. *Wat. Res.* 13, 241-248.
- Kelcka GM, 1985. Biodegradation. In: *Environmental exposure from chemicals*. Ed. Neely WB and Blau GE. CRC, Boca Raton, FL, USA, 109-155.
- Kier LB and Hall LH, 1986. Molecular connectivity in structure-activity analysis. Res. Studio Press Ltd. Letchworth, UK.
- King EF and Painter HA, 1983. Ring-test programme 1981-82. Assessment of biodegradability of chemicals in water by manometric respirometry. Commission of the EC, DG XI, Report No. EUR 8631 EN.
- Kinney CR, 1938. A system correlating molecular structure of organic compounds with their boiling points. I. Aliphatic boiling point numbers. *J. Am. Chem. Soc.* 60, 3032-3039.
- Kinney CR, 1940. Calculation of boiling points of aliphatic hydrocarbons. *Ind. Eng. Chem.* 32, 559-562.
- Kitano M and Takatsuki M, 1988. Evaluation of the 1988 OECD-Ring test of ready degradability, 12th Sept. 1988, Chem. Inspect. and Test. Inst., Japan.
- Klopman G, 1974. *Chemical Reactivity and Reaction Paths*, John Wiley and Sons, NY, USA, 55-165.
- Klopman G, 1996. The Meta-Casetox system. In: *Biodegradability prediction*. In: Peijnenburg WJGM and Damborský J (ed), NATO ASI Series 2, Environment, Vol. 23, Kluwer, Dordrecht, NL, 27-40.
- Klopman G, Zhang Z, Balthasar DM and Rosenkranz HS, 1995. Computer-automated predictions of aerobic biodegradation of chemicals. *Environ. Toxicol. Chem.* 14, 395-403.
- Kohonen T, 1982. Self-organized formation of topologically correct feature maps. *Biol. Cybern.* 43, 59-69.

- Kohonen T, 1984. Self-organization and associative memory. Springer, New York-Berlin-Heidelberg.
- Könemann H, 1981. Quantitative structure-activity relationships in fish toxicity studies. Part 1: Relationship for 50 industrial chemicals. *Toxicol.* 19, 209-221.
- Koza JR, 1992. Genetic programming: on the programming of computers by means of natural selection. The MIT Press, Cambridge, Mass., 819p.
- Kristensen P and Nyholm N, 1987. Bioaccumulation of chemical substances in fish flow-through method. Final Report. EC Contract No. 84-B-6601-11-002-11-N.
- Kristensen P and Tyle H, 1990. The assessment of bioaccumulation. In: Nagel R and Loskill R (ed), Bioaccumulation in the aquatic systems: Contributions to the assessment; International workshop, Berlin, Germany. VCH, Berlin, 189-222.
- Kuenemann P, Vasseur P and Devillers J, 1990. Structure-biodegradability relationships. In: Karcher W and Devillers (ed), Practical applications of quantitative structure-activity relationships (QSAR) in environmental chemistry and toxicology. ECSC, EEC, EAEC, Brussels and Luxembourg, 343-370.
- Kühn R, Pattard M, Pernak K-D and Winter A, 1989. Results of the harmful effects of selected water pollutants to *Daphnia magna*. *Wat. Res.* 23, 495-499.
- Langenberg JH, Peijnenburg WJGM and Rorije E, 1995. On the usefulness and reliability of existing QSBRs for risk assessment and priority setting. In: Peijnenburg WJGM and Karcher W (ed). Proceedings of the workshop "Quantitative Structure Activity Relationships for Biodegradation", September 1994, Belgirate, Italy. Nat. Inst. of Publ. Hlth and Environ. Prot., Bilthoven, The Netherlands, Report no. 719101021.
- Larson RJ and Wentler GE, 1982. Biodegradation of detergent materials in natural systems at realistic concentrations. *Proceedings, Soap Cosmet. Chem. Spec.* 58, 33-42.
- Leo AJ, Hansch C and Elkins D, 1971. Partition coefficients and their uses. *Chem. Rev.* 71, 525-616.
- Lewis DFV, 1992. Computer-assisted methods in the evaluation of chemical toxicity. In: Lipkowitz KB and Boyd DB (ed), *Reviews in Computational Chemistry*, Vol. III, VCH Publishers, New York, 173-222.
- Lijinsky W, 1988. Chronic studies in rodents of vinyl acetate and compounds related to acrolein. *Ann. New York Acad. Sci.* 534, 246-254.
- Lijinsky W and Reuber MD, 1983. Chronic toxicity studies of vinyl acetate in Fischer rats. *Toxicol. Appl. Pharmacol.* 68, 43-53.
- Lindgren F, Nouwen J, Loonen H, Worth A, Hansen B, Karcher W, 1996. Environmental modelling based on a structural fragments approach. *Indoor Built Environ.* 5, 334-340.
- Lipnick RL, 1985. Validation and extension of fish toxicity QSARs and interspecies comparisons for certain classes of organic chemicals. In: Tichy M (ed), *QSAR in Toxicology and Xenobiochemistry*, Pharmacochimistry Library Vol. 8: Elsevier, Amsterdam, NL, 39-52.
- Lipnick RL, 1988. Quantitative structure-activity relationships and toxicity assessment in the aquatic environment. In: Richardson ML (ed), *Risk Assessment of Chemicals in the Environment*. Royal Soc. of Chem., 379-397.
- Lipnick RL, Bickings CK, Johnson DE and Eastmond DA, 1986. Comparison of QSAR predictions with fish toxicity screening data for 110 phenols. In: Bahner RC and Hansen DJ (ed), *Aquatic Toxicology and Hazard Assessment: Eighth Symposium*, ASTM, Philadelphia, USA, 153-176.
- Lockhart HB Jr and Blakeley R, 1975. Aerobic photodegradation of Fe(III)-(ethylenedinitrilo)

tetraacetate (ferric EDTA). Implications for natural waters. *Environ. Sci. Technol.* 9, 1035-1038.

Loonen H, Lindgren F, Hansen B and Karcher W, 1995. Prediction of biodegradation from structural fragments. Presented at the "US EPA-Japan-EU Trilateral QSAR meeting on fate and effects predictions for High Production Volume Chemicals", October 25-26 1995, Belgrate, Italy.

Lyman WJ, 1982. Adsorption coefficient for soils and sediments. In: Lyman WJ, Reehl WF and Rosenblatt DH (ed), *Handbook of Chemical Property Estimation Methods*, Chapter 4, McGraw Hill Book Co., New York, USA.

Lyman WJ, 1985. Estimation of physical properties. In: Neely WB and Blau GE (ed), *Environmental Exposure from Chemicals*, Vol. I, CRC Press, Boca Raton, FL, USA, 13-47.

Lyman WJ, 1990a. Octanol/water partition coefficient. In: Lyman WJ, Reehl WF and Rosenblatt DH (ed), 1990. *Handbook of chemical property estimation methods. Environmental behavior of organic compounds*. Am. Chem. Soc. Washington DC.

Lyman WJ, 1990b. Solubility in water. In: Lyman WJ, Reehl WF and Rosenblatt DH (ed), 1990. *Handbook of chemical property estimation methods. Environmental behavior of organic compounds*. Am. Chem. Soc. Washington DC.

Lyman WJ, Reehl WF and Rosenblatt DH, 1982. *Handbook of Chemical Estimation Methods; Environmental behaviour of organic compounds*. McGraw-Hill Book Company, New York, NY, USA

Lyman WJ, Reehl WF and Rosenblatt DH, 1990. *Handbook of chemical property estimation methods. Environmental behavior of organic compounds*. Am. Chem. Soc. Washington DC, USA.

Mabey W and Mill T, 1978. Critical review of hydrolysis of organic compounds in water under environmental conditions. *J. Phys. Chem. Ref. Data* 7, 383-415.

Mackay D, 1982. Correlation of bioconcentration factors. *Environ. Sci. Technol.*, 16, 274-278.

Mackay D, 1991. *Multimedia Environmental Models; The fugacity approach*. Lewis Publishers, Inc., Chelsea, Ma, USA.

Mackay D and Shiu WJ, 1981. A critical review of Henry's law constants for chemicals of environmental interest. *J. Phys. Chem. Ref. Data* 10, 1175 - 1199.

Makovskaya VV, 1995. Determination and estimation of octanol-water partition coefficients. Thesis, University of Northumbria, Newcastle, UK, in collaboration with Brixham Environmental Laboratory, Zeneca Ltd., Brixham, UK.

Markwell RD, Connell DW and Gabric AJ, 1989. Bioaccumulation of lipophilic compounds from sediments by oligochaetes. *Wat. Res.* 11, 1443 - 1450.

Marshall S and van Egmond RA, 1995. Bioavailability. UK Department of the Environment, Research Contract, Final Report.

Massart DL, Vandeginste BGM, Deming SN, Michotte Y and Kaufman L, 1983. *Chemometrics: a textbook*. In: Vandeginste BGM and Kaufman L (ed), *Data Handling in Sci. and Technol.*, Vol. 2. Elsevier, NL.

McCarty LS, 1986. The relationship between aquatic toxicity QSARs and bioconcentration for some organic chemicals. *Environ. Toxicol. Chem.* 5, 1071-1080.

McCarty LS and Mackay D, 1993. Enhancing ecotoxicological modeling and assessment. *Environ. Sci. Technol.* 27, 1719-1728.

McGowan JC, 1952. The physical toxicity of chemicals. II Factors affecting physical toxicity in aqueous solutions. *J. App. Chem.* 2, 323-329.

Means JC, Wood SG, Hassett JJ and Banwart WL, 1982. Sorption of amino- and carboxy-substituted polynuclear aromatic hydrocarbons by sediments and soils. *Environ. Sci. Technol.* 16, 93-98.

- Meissner HP, 1949. Critical constants from parachor and molar refraction. *Chem. Eng. Prog.* 45, 149-153.
- Mekenyan OG, Veith GD, Bradbury SP and Russom CL, 1993. Structure-toxicity relationships for  $\alpha,\beta$ -unsaturated alcohols in fish. *Quant. Struct.-Act. Rel.* 12, 132-136.
- Meylan WM and Howard PH, 1991a. User's guide for HENRY. Syracuse Research Corporation. Environ. Sci. Center, Syracuse, NY, USA.
- Meylan WM and Howard PH, 1991b. Bond contribution method for estimating Henry's law constant, *Environ. Toxicol Chem.*, 10, 1283 - 1293.
- Meylan WM and Howard PH, 1993a. User's guide for HYDRO. Syracuse Research Corporation. Environ. Sci. Center, Syracuse, NY, USA.
- Meylan WM and Howard PH, 1993b. User's guide for AOP. Syracuse Research Corporation. Environ. Sci. Center, Syracuse, NY, USA.
- Meylan WM and Howard PH, 1993c. Computer estimations of the atmospheric gas-phase reaction rate of organic compounds with hydroxyl radicals and ozone, *Chemosphere*, 26(12), 2293 - 2299.
- Meylan WM and Howard PH, 1994a. User's guide for  $K_{ow}$ . Syracuse Research Corporation. Environ. Sci. Center, Syracuse, NY, USA.
- Meylan WM and Howard PH, 1994b. User's Guide for MPBPVP for MS Windows 3.1, Syracuse Research Corporation. Environ. Sci. Center, Syracuse, NY, USA.
- Meylan WM and Howard PH, 1994c. User's Guide for HENRYWIN. Henry's Law Constant Program for Microsoft Windows 3.1<sup>®</sup> Syracuse Research Corporation. Environ. Sci. Center, Syracuse, NY, USA.
- Meylan WM and Howard PH, 1994d. User's Guide for AOPWIN. Atmospheric Oxidation Program for Microsoft Windows 3.1<sup>®</sup> Syracuse Research Corporation. Environ. Sci. Center, Syracuse, NY, USA.
- Meylan WM and Howard PH, 1994e. Validation of water solubility estimation methods using log  $K_{ow}$  for application of PCGEMS and EPI. Syracuse report prepared for RS Boethling (US-EPA). Syracuse Research Corporation. Environ. Sci. Center, Syracuse, NY, USA.
- Meylan WM and Howard PH, 1995a. Atom/fragment contribution method for estimating octanol-water partition coefficients, *J. Pharm. Sci.*, 84, 83-92.
- Meylan WM and Howard PH, 1995b. User's Guide for KOWWIN. Program for Microsoft Windows 3.1<sup>®</sup>. Syracuse Res. Corpor. Environ. Sci. Center, Syracuse, NY, USA.
- Meylan WM, Howard PH and Boethling RS, 1992a. User's guide for PCKOC. Syracuse Research Corporation. Environ. Sci. Center, Syracuse, NY, USA.
- Meylan WM, Howard PH and Boethling RS, 1992b. Molecular topology/fragment contribution method for predicting soil sorption coefficients. *Env. Sci. Technol.* 26, 1560-1567.
- Miller COM, 1980. Private communication, cited in Rechsteiner, 1990.
- Montellano Ortiz de PR and Mico BA. 1981. Destruction of cytochrome P-450 by allylisopropylacetamide is a suicidal process. *Arch. Biochem. Biophys.* 206, 43-50.
- Müller M and Klein W, 1991. Estimating atmospheric degradation process by SAR. In: Hermes JLM and Opperhuizen A, (ed), *QSAR in Environment Toxicology-IV*. Elsevier, Amsterdam, NL, 261-273.
- Müller M and Klein W, 1994. Anwendung von Struktur-Aktivitäts-Relationen für die Altstoffbewertung, UBA Report, 97316/34, Umweltbundesamt, Berlin, D.
- Murray WJ, Hall LH and Kier LB, 1975. Molecular connectivity III: Relationship to partition coefficients. *J. Pharm. Sci.* 64, 1978-1981.

- Nabholz JV and Platz RD, 1990. Generic environmental hazard assessment- Aliphatic amines. Environmental Effects Branch, Health and Environmental Review Division (TS-796), Office of Toxic Substances, US EPA, Washington, DC 20460, USA.
- Nendza M and Russom CL, 1991. QSAR modelling of the ERL-D fathead minnow acute toxicity database, *Xenobiotica* 21, 147-170.
- Nevalainen T and Kolehmainen E, 1994. New QSAR models for polyhalogenated aromatics. *Environ. Toxicol. Chem.* 13, 1699-1706.
- Newsome LD, Johnson DE, Lipnick RL, Broderius SJ and Russom CL, 1991. A QSAR study of the toxicity of amines to the fathead minnow. In: Hermens J and Opperhuizen A (ed), *QSAR in Environmental Toxicology - IV*. Elsevier, Amsterdam, NL, 537-551.
- Niemi GJ, 1990. Multivariate analysis and QSAR: Application of principal components analysis. In: Karcher W and Devillers J (ed), *Practical Applications of Quantitative Structure-Activity Relationships (QSAR) in Environmental Chemistry and Toxicology*, Kluwer, Dordrecht, NL, 153-169.
- Niemi GJ, Veith GD, Regal RD, and Vaishnav DD, 1987. Structural features associated with degradable and persistent chemicals. *Environ. Toxicol. Chem.* 6, 515-527.
- Nirmalakhandan NN and Speece RE, 1988a. Prediction of aqueous solubility of organic chemicals based on molecular structure. *Environ. Sci. Technol.* 22, 328-338.
- Nirmalakhandan NN and Speece RE, 1988b. QSAR model for predicting Henry's constant. *Environ. Sci. Technol.* 22, 1349-1357.
- Nirmalakhandan NN and Speece RE, 1989. Prediction of aqueous solubility of organic chemicals based on molecular structure. 2. Application to PNAs, PCBs, PCDDs etc. *Environ. Sci. Technol.* 23, 708-713.
- Nuck B and Federle TW, 1996. Batch test for assessing the mineralisation of <sup>14</sup>C-radiolabeled compounds under realistic anaerobic conditions. *Environ. Sci. Technol.* 30, 3597-3603.
- OECD, 1981. Guidelines for the testing of chemicals (including 1984 and 1987 updates), Paris.
- OECD, 1982. Guideline for the testing of chemicals, Paris.
- OECD, 1984. Guidelines for Testing of Chemicals, 107 "Partition Coefficient (n-octanol/water)", Paris.
- OECD, 1986. Draft OECD test guideline on photochemical oxidative degradation in the atmosphere. Revised by the Ad-Hoc Expert Meeting at Reichstag, Berlin, 6-8 October 1986.
- OECD, 1992a. Report of the OECD Workshop on Quantitative Structure Activity Relationships (QSARs) in aquatic effects assessment. OECD Environment Monograph No. 58, Paris.
- OECD, 1992b. The rate of photochemical transformation of gaseous organic compound in air under tropospheric conditions. OECD Environment Monographs No. 61, Paris.
- OECD, 1993a. Application of structure-activity relationships to the estimation of properties important in exposure assessment. OECD Environment Monograph No. 67, Paris.
- OECD, 1993b. OECD guidelines for the testing of chemicals. Paris.
- OECD, 1993c. Structure-activity relationships for biodegradation. OECD Environment Monograph No. 68, Paris.
- OECD, 1995. Draft Report of the final ring test of the *Daphnia magna* reproduction study, March 1995, Paris.
- OECD, 1996. Bioconcentration : Flow-through fish test. OECD Guidelines for Testing of Chemicals. Proposal for updating Guideline 305, Paris.

- Ogata Y and Tsuchida M, 1957. Linear boiling point relationships. *Ind. Eng. Chem.* 49, 415-417.
- Oliver BG and Niimi AJ, 1983. Bioconcentration of chlorobenzenes from water by rainbow trout: correlation with partition coefficients and environmental residues. *Environ. Sci. Technol.* 17, 287-291.
- Opperhuizen A, vd Velde EW, Gobas FAPC, Liem DAK and v d Steen JMD, 1985. Relationship between bioconcentration in fish and steric factors of hydrophobic chemicals. *Chemosphere*, 14, 1871 - 1896.
- Parsons JR and Govers HAJ, 1990. Quantitative structure-activity relationships for biodegradation. *Ecotoxicol. Environ. Safety.* 19, 212-227.
- Pearson RG and Songstad J, 1967. Application of the principle of hard and soft acids and bases to organic chemistry. *J. Am. Chem. Soc.* 89, 1827-1836.
- Peijnenburg WJGM, 1994. Structure-activity relationships for biodegradation: a critical review. *Pure and Appl. Chem.* 66, 1931-1941.
- Peijnenburg WJGM, de Beer KGM, de Haan MWA, den Hollander HA, Stegeman MHL and Verboom H, 1992. Development of a structure-reactivity relationship for the photohydrolysis of substituted aromatic halides. *Environ. Sci. Technol.* 11, 2116-2121.
- Peijnenburg WJGM and Karcher W, 1995. Proceedings of the workshop "Quantitative Structure Activity Relationships for Biodegradation", September 1994, Belgirate, Italy. *Nat. Inst. of Publ. Hlth and Environ. Protection, Bilthoven, The Netherlands, Report No. 719101021.*
- Perrin DD, Dempsey B and Sergeant EP, 1981. pKa prediction for organic acids and bases. Chapman and Hill Publ. Co., New York, NY, USA.
- Persoons G, Snell T, Blaise C, Janssen C and Van Steertegem M, 1990. Toxkits intercalibration exercise in Europe, the USA and Canada. Final report.
- Phatak A, Reilly PM and Penlidis A, 1993. An approach to interval estimation in partial least squares regression. *Anal. Chim. Acta* 277, 495-501.
- Prinn R, Cunnold D, Rasmussen R, Simmonds P, Alyea F, Crawford A, Fraser P and Rosen R, 1987. Atmospheric trends in methylchloroform and the global average for the hydroxyl radical. *Sci.* 238, 945-950.
- Prinn RG, Weiss RF, Miller BR, Huang J, Alyea FN, Cunnold DM, Fraser PJ, Hartley DE and Simmonds PG, 1995. Atmospheric trends and lifetime of CH<sub>3</sub>CCl<sub>3</sub> and global OH concentrations. *Sci.* 269, 187-192.
- Protic M and Sabljic A, 1989. Quantitative structure-activity relationships of acute toxicity of commercial chemicals on fathead minnows: Effect of molecular size. *Aquat. Toxicol.* 14, 47-64.
- Punch B, Patton A, Wight K, Larson RJ, Masscheleyn PH and Forney L, 1996. A biodegradability evaluation and simulation system (BESS) based on knowledge of biodegradation pathways. In: Peijnenburg WJGM and Damborský (ed), *Biodegradability prediction. J. NATO ASI Series 2, Environ., Vol. 23.* Kluwer, Dordrecht, NL, 65-73.
- Purdy R, 1991. The utility of computed superdelocalizability for predicting the LC<sub>50</sub> values of epoxides to guppies. *Sci. Tot. Environ.* 109/110, 553-556.
- Purdy R, 1996. A mechanism-mediated model for carcinogenicity: Model content and prediction of the outcome of rodent carcinogenicity bioassays currently being conducted on 25 organic chemicals. *Envir. Health Perspect.* 104, Suppl. 5, 1085-1094.
- Reid RC, Prausnitz JM and Poling BE, 1987. The properties of gases and liquids. 4th ed, Chapter 2. McGraw-Hill Inc., NY.
- Reichsteiner CE, 1990. Boiling point. In: Lyman WJ, Reehl WF and Rosenblatt DH, 1990. *Handbook of chemical property estimation*



methods. Environmental behavior of organic compounds. Chapter 12. Am. Chem. Soc., Washington DC, USA.

Reiff B, Lloyd R, How M, Brown D and Alabaster JS, 1979. The acute toxicity of eleven detergents to fish: results of an interlaboratory exercise. *Wat. Res.* 13, 207-210.

Rekker RF and Mannhold R, 1992. Calculation of drug lipophilicity. The hydrophobic fragmental constant approach. VCH, Weinheim, D.

Reteuna C, Vasseur P and Cabridenc R, 1989. Performances of three bacterial assays in toxicity assessment. *Hydrobiologica* 188/189, 149-154.

RIVM, VROM and WVC 1994. Uniform system for the evaluation of substances (USES), Version 1. Manual, Ministry of Housing, Spatial Planning and the Environment, The Hague Distribution-No. 11144/150.

Roberts DW, 1989. Aquatic toxicity of linear benzene sulfonates (LAS) - A QSAR analysis, *Comun. J. Com. Esp. Deterg.* 20, 35-43.

Roberts DW, 1991. QSAR issues in aquatic toxicity of surfactants. *Sci. Tot. Environ.* 109/110, 557-568.

Russom CL, 1994. ASTER, Version 1.0, A user's guide. US-EPA, Duluth, Minnesota, USA.

Russom CL, Anderson EB, Greenwood BE and Pilli A, 1991. ASTER: an integration of the AQUIRE database and the QSAR system for use in ecological risk assessments. *Sci. Tot. Environ.* 109/110, 667-670.

Saarikoski J and Viluksela M, 1982. Relationship between physico-chemical properties of phenols and their toxicity and accumulation in fish. *Ecotox. Environ. Safety* 6, 501-512.

Sabljić A, 1984. Predictions of the nature and strength of soil sorption of organic pollutants by molecular topology. *J. Agric. Food Chem.* 32, 243-246.

Sabljić A, 1987. On the prediction of soil sorption coefficients of organic pollutants from molecular structure: Application of molecular topology model. *Environ. Sci. Technol.* 21, 358 - 366.

Sabljić A, 1989. Quantitative modeling of soil sorption for xenobiotic chemicals. *Environ. Health Perspect.* 83, 179-190.a.

Sabljić A, 1991. Chemical topology and ecotoxicology. *Sci. Tot. Environ.* 109/110, 197-220.

Sabljić A and Protic M, 1982. Relationship between molecular connectivity indices and soil sorption coefficients of polycyclic aromatic hydrocarbons. *Bull. Environ. Contam. Toxicol.* 28, 162-165.

Sabljić A and Güsten H, 1990. Predicting the night-time NO<sub>3</sub> radical reactivity in the troposphere. *Atmos. Environ.* 24A, 73-78.

Sabljić A and Piver WT. 1992. Quantitative modeling of environmental fate and impact of commercial chemicals. *Environ. Toxicol. Chem.* 11, 961-972.

Samiullah Y, 1990. Prediction of the environmental fate of chemicals. Elsevier Appl. Sci., London, UK.

Schellenberg K, Leuenberger C and Schwarzenbach RP, 1984. Sorption of chlorinated phenols by natural sediments and aquifer materials. *Environ. Sci. Technol.* 18, 652-657.

Schüürmann G and Müller E, 1994. Back-propagation neural networks - recognition vs. prediction capability. *Environ. Toxicol. Chem.* 13, 743-747.

Skrabal A and Zahorka A, 1927. Die Kinetik der Verseifung von Vinylacetat. *Monatssch. Chem.* 48, 459-473.

Sloof W, Canton JH and Hermens J, 1983. Comparison of the susceptibility of 22 freshwater species to 15 chemical compounds: I. (Sub)acute toxicity tests. *Aquat. Toxicol.* 4, 112-128.

- Smits JRM, Melssen WJ, Buydens LMC and Kateman G, 1994. Using artificial neural networks for solving chemical problems. Part I. Multi-layer feed-forward networks. *Chemometrics and Intelligent Laboratory Systems* 22, 165-189.
- Somayajulu GR and Palit SR, 1957. Boiling points of homologous liquids. *J. Chem. Soc.* 1957, 2540-2544.
- Spacie A and Hamelink JL, 1982. Alternative models for describing the bioconcentration of organics in fish. *Environ. Toxicol. Chem* 1, 309-320.
- Stegeman MHL, Peijnenburg WJGM and Verboom H, 1993. A quantitative structure-activity relationship for the direct photohydrolysis of meta-substituted halobenzene derivatives in water. *Chemosphere* 5, 837-849.
- Stein SE and Brown RL, 1994. Estimation of normal boiling points from group contributions. *J. Chem. Inf. Comput. Sci.* 34, 581-587.
- Stiel LI and Thodos G, 1962. The normal boiling points and critical constants of saturated aliphatic hydrocarbons. *Am. Inst. of Chem. Eng. J.* 8, 527-529.
- Suntio LR, Shiu WY, Mackay D, Seiber JN and Glotfelty D, 1988. Critical review of Henry's law constant for pesticides. *Rev. Environ. Contam. Toxicol.*, 103, 1 - 59.
- Suter GW II, 1993a. *Ecological Risk Assessment*, Lewis Publishers, Chelsea, Michigan, USA, 94.
- Suter GW II, 1993b. *Ecological Risk Assessment*, Lewis Publishers, Chelsea, Michigan, USA, 185.
- Syracuse Research Corporation, 1994. BIOWIN, Biodegradation Probability Program for Microsoft windows 3.1. User's Guide, Syracuse, NY, 30p.
- Tetko IV, Livingstone DJ and Luik AI, 1995. Neural network studies. 1. Comparison of overfitting and overtraining. *J. Chem. Inf. Comput. Sci.* 35, 826-833.
- Thellen C, Blaise C, Roy Y and Hickey C, 1989. Round robin testing with *Selenastrum capricornutum* microplate toxicity assay. *Hydrobiologica* 188/189, 259-268.
- Thompson RS and Carmichael NG, 1989. 1,1,1-Trichloroethane: Medium-term toxicity to carp, daphnids, and higher plants. *Ecotox. Environ. Safety* 17, 172-182.
- Topliss JG and Costello RJ, 1972. Chance correlations in structure-activity studies using multiple regression analysis. *J. Med. Chem.* 15, 1066 - 1069.
- Trapp S and Matthies M, 1995. Generic one-compartment model for uptake of organic chemicals by foliar vegetation. *Environ. Sci. Technol.* 29, 2333-2338.
- TSCA, 1976. Toxic Substances Control Act. United States Publ. Law, 94-469,90 Stat 2003, October 11, 1976.
- Turner L, Choplin F, Dugard P, Hermens J, Jaechk R, Marsmann M and Roberts D, 1987. Structure-activity relationships in toxicology and ecotoxicology: an assessment. *Toxic. in Vitro* 1, 143-171.
- UK-DoE, 1993. Ozone in the United Kingdom 1993; Third report of the UK Photochemical Oxidants Review Group. Depart. of the Environ. London UK.
- Van Gestel CAM and Ma W-C, 1988. Toxicity and bioaccumulation of chlorophenols in earthworm in relation to bioavailability in soil. *Ecotox. Environ. Safety* 15, 289-297.
- Van Leeuwen CJ, Adema DMM and Hermens JLM, 1990. Quantitative structure-activity relationship for fish early life stage toxicity. *Aquat. Toxicol.* 16, 321-334.
- Van Leeuwen CJ, Van Der Zandt, Aldenberg T, Verhaar HJM and Hermens JLM, 1992. Application of QSARs, extrapolation and equilibrium partitioning in aquatic effects assessment: I.

- Narcotic industrial pollutants. *Environ. Toxicol. Chem.* 11, 267-282.
- Veith GD and Kosian P, 1983. Estimating bioconcentration potential from octanol/water partition coefficients. In: Mackay D, Peterson S, Eisenreich SJ, Simons MS (ed), *Physical behaviour of PCBs in the Great Lakes*. Ann Arbor Science Publishers, Ann Arbor, 269-282.
- Veith GD and Broderius SJ, 1987. Structure-toxicity relationships for industrial chemicals causing type (II) narcosis syndrome. In: Kaiser KLE (ed), *QSAR in Environmental Toxicology-II*, Elsevier, Amsterdam, NL, 385-391.
- Veith GD and Broderius SJ, 1990. Rules for distinguishing toxicants that cause Type I and Type II narcosis syndromes. *Environ. Health Persp.* 87, 207-211.
- Veith GD and Mekenyan OG, 1993. A QSAR approach for estimating the aquatic toxicity of soft electrophiles. *Quant. Struct. Act. Relat.* 12, 249-356.
- Veith GD, DeFoe DL and Bergstedt BV, 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. *J. Fish Res. Board Can.* 36, 1040-1048.
- Veith GD, Macek KJ, Petrocelli R and Carrol J, 1980. An evaluation of using partition coefficients and water solubility to estimate bioconcentration factors for organic chemicals in fish. In: Eaton JG, Parrish PR and Hendricks AC (ed), *Aquatic Toxicology*, ASTM STP 707, Philadelphia, USA, 116-129.
- Veith GD, Call DJ and Brooke LT, 1983. Structure-toxicity relationships for the fathead minnow, *Pimephales promelas*: Narcotic industrial chemicals. *Can. J. Fish. Aquat. Sci.* 40, 743-748.
- Veith GD, Lipnick RL and Russom CL, 1989. The toxicity of acetylenic alcohols to the fathead minnow, *Pimephales promelas*: Narcosis and proelectrophile activation. *Xenobiotica* 19, 555-565.
- Verhaar HJM, van Leeuwen CJ, Hermens JLM, 1992. Classifying environmental pollutants. 1: Structure-activity relationships for prediction of aquat. tox. *Chemosphere* 25, 471-491.
- Verhaar HJM, Mulder W and Hermens JLM, 1995. QSAR for ecotoxicology. In: *Overview of Structure-Activity Relationships for environmental endpoints, Part 1 : General outline and procedure*, Report of the EU-DG-XII Project QSAR for predicting fate and effects of chemicals in the environment, Contract No. EV5V-CT92-0211.
- Verhaar HJM, Rorije E, Borkent H, Seinen W and Hermens JLM, 1996. Modelling the nucleophilic reactivity of small organochlorine electrophiles: A mechanistically-based quantitative structure-activity relationship. *Environ. Toxicol. Chem.* 1011-1018.
- Verhaar HJM, Solbé J, Speksnijder J, van Leeuwen CJ and Hermens JLM, in preparation. Classifying environmental pollutants. 3. External validation of the classification system.
- Vighi M, Garlanda MM and Calamari D, 1991. QSARs for toxicity of organophosphorous pesticides to *Daphnia* and honeybees. In: Hermens J and Opperhuizen A (ed), *QSAR in Environmental Toxicology - IV*. Elsevier, Amsterdam, NL, 605-622.
- Voyer RA and Heltshe JF, 1984. Factor interactions and aquatic toxicity testing. *Wat. Res.* 18, 441-448.
- Wagener S and Schink B, 1987. Anaerobic degradation of nonionic and anionic surfactants in enrichment cultures and fixed bed reactors. *Wat. Res.* 21, 615-622.
- Wakeling IN and Macfie HJH, 1992. A robust PLS procedure. *J. Chemometrics* 6, 189-198.
- Walczak B and Massart DL, 1995. Robust principal components regression as a detection tool for outliers. *Chemometrics and Intelligent Laboratory Systems* 27, 41-54.
- Wasserman PD, 1989. *Neural computing: theory and practice*. Van Nostrand Reinhold, New York, USA.

Watson KM, 1943. Thermodynamics of the liquid state. Generalized prediction of properties. *Ind. Eng. Chem.* 35, 398-406.

Watts CD, Boxall ABA, Rogers H, Cousins IT and Dearden JC, 1995. Consultancy on the development of QSARs-Final Report to the Department of the Environment, DoE 4042, WRc plc, Medmenham, Marlow, UK.

Wayne RP, Barnes I, Biggs P, Burrows JP, Canosa-Mas CE, Hjorth J, Le Bras G, Moortgat GK, Perner D, Poulet G, Restelli G and Sidebottom H, 1991. The nitrate radical: Physics,

chem. and the atmos. *Atmos. Environ.* 25A(1), 1-206.

Xu L, Ball JW, Dixon SL and Jurs PC, 1994. Quantitative structure-activity relationships for toxicity of phenols using regression analysis and computational neural networks. *Environ. Toxicol. Chem.* 13, 841-851.

Zupan J and Gasteiger J, 1993. Neural networks for chemists: an introduction. VCH, Weinheim, D.

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