rivm
research for
man and environment

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

RIVM report 601503 016

Estimating the PTB-profile

D. Sijm, E. Hulzebos, W. Peijnenburg

July 1999

This investigation has been performed by order and for the account of the Directorate General for Environmental Protection, Directorate for Chemicals, External Safety and Radiation Protection, within the framework of RIVM-project 601503, "General Assistance for the National Policy towards Substances".

RIVM, P.O. Box 1, 3720 BA Bilthoven, tel.: 31 - 30 - 274 91 11; FAX: 31 - 30 - 274 29 71

Abstract

The aim of this paper is to make an inventory of 'simple' methods that estimate missing data on Persistence (P), Toxicity (T) and Bioaccumulative potential (B), for the purpose of a simplified hazard assessment. Since it is earlier recognised that for many substances experimental PTB-data are not available, estimation models may be the most quickly and cheapest, and relatively more reliable than expert judgement or default values. Preference should always be given to reliable experimental data. However, such data are not always available, and to obtain them is a costly and time-consuming process.

Each of the PTB-criteria includes one or more *properties*. Each property can be either quantified as a numerical *value* or it can be qualitatively assigned. The value can finally be compared to a *cut-off value*, which will then result in categorising the substance into a *PTB-class*.

PTB-properties are in reality no intrinsic properties, but can operationally be treated as such. Environmental conditions, such as temperature, which highly affects persistence, should however be taken into account when standard conditions are set to determine the PTB-properties. The PTB-criteria may be used for simplified hazard identification for the environment and for human health. For each substance a PTB-profile thus can be derived, that includes the PTB-properties, and, when compared to cut-off values for PTB, may results in a PTB-class.

The advantage of a more simple approach, such as using PTB-criteria, is that only information is required on properties that are related to physical-chemical properties of the substance. In particular, information on production volumes and use patterns may vary with time and may be difficult to obtain or to estimate. The disadvantage of this approach is that only selected criteria are included and that this may be insufficient for specific policy actions and it is insufficient for risk assessment, since environmental release is not included.

The aim of choosing cut-off values and using the PTB-criteria for policy purposes is currently still under discussion and is outside the scope of the present report, and thus will not be further evaluated here. The PTB-criteria, however, may be used as one of the steps to identify potentially hazardous chemicals.

Prior to estimating the PTB-properties, a distribution profile, i.e. the distribution of the substance over the three major environmental compartments, air, soil and water, needs to be estimated, to identify the potential environmental distribution. For example, it is not useful to estimate atmospheric degradation of a fully ionised substance, which will not partition into air. Furthermore, identification of the chemical class, i.e. organic chemical, metal, etc., is needed to use the appropriate method for deriving the PTB-properties. For those substances for which the PTB-properties can be estimated, the required information varies from only a SMILES-notation to detailed quantum-mechanical information.

Estimates of the PTB-properties seem feasible for many organic compounds. However, for many 'other' compounds, estimation methods have not been developed yet, or the existing methods will provide unreliable estimates. These 'other' compounds include metals, organometals, polymers, mixtures, many ionizable compounds, complex structures, etc. For the latter chemicals it is advised to use either expert judgement or to use a default value to obtain the PTB-properties. Expert judgement may use structural analogues and/or chemical classes. It is suggested to choose the default values as worst-case values, but the various stakeholders within the context of hazard identification should further discuss the value.

Flow-charts are provided in the report to guide the required steps which need to be followed to determine the PTB-properties. In step 1 the required information of the substance and its physical-chemical properties is used to determine a simple distribution profile. In step 2, the PTB-properties will be determined. The PTB-profile can be further used to determine the PTB-class in step 3, which is outside the scope of the present report.

Preface

We would like to acknowledge the stimulating comments from our colleagues within RIVM and from the Directorate General for Environmental Protection, Directorate for Chemicals, External Safety and Radiation Protection (VROM/DGM/SVS). In particular we acknowledge Jack de Bruijn (RIVM), Jasper Groos (VROM/DGM/SVS), Dick Jung (VROM/DGM/SVS), Hélène Loonen (RIVM), Theo Vermeire (RIVM), Arnold van der Wielen (VROM/DGM/SVS), and Peter van der Zandt (RIVM). Furthermore we would like to acknowledge the stimulating comments from Han Blok (Haskoning), Peter Okkerman (BKH), and Jay Niemelä (Danish EPA).

Contents

Samenvatting

Het doel van dit rapport is een overzicht te geven van 'simpele' methoden die een schatting geven van Persistentie (P), Toxiciteit (T) en Bioaccumulerend vermogen (B). Dit ten behoeve van een simpele 'hazard assessment'. Uit eerdere studies (BKH/Haskoning, 1998a-c) is duidelijk geworden dat vele experimentele data voor PTB-eigenschappen ontbreken. Schattingsmethoden zijn in principe het snelst en goedkoopst en relatief betrouwbaar ten opzichte van expert judgement of default waarden. Als er betrouwbare experimentele data zijn, moet daar de voorkeur aan gegeven worden. Het is echter een kostbare en tijdrovende zaak om die experimentele data te verkrijgen.

Elk PTB-criterium bevat een of meer *eigenschappen*. Elke eigenschap kan worden gekwantificeerd met een numerieke *waarde* of het kan kwalitatief worden benoemd. De waarde kan tenslotte worden vergeleken met een zogenaamde *'cut-off' waarde*. Tenslotte leidt dit vergelijk tot een categorisering van een stof in een PTB-klasse.

Hoewel PTB-eigenschappen geen echte intrinsieke eigenschappen zijn, worden ze operationeel wel als zodanig behandeld. Er zou bij het vaststellen van standaardcondities voor het bepalen van de PTB-eigenschappen rekening moeten worden gehouden met milieuomstandigheden. Zo beïnvloedt de temperatuur bijvoorbeeld in sterke mate de persistentie van een stof. De PTB-criteria kunnen worden ingezet ten behoeve van een versimpelde gevaarsinschatting van stoffen voor het milieu of voor de mens. Voor iedere stof zou een PTB-profiel gemaakt dienen te worden. Dit profiel zou de PTB-eigenschappen moeten bevatten. Bij de toetsing van deze eigenschappen aan referentiewaarden, komt de stof vervolgens in een bepaalde PTB-klasse terecht.

Het voordeel van een simpele benadering, zoals het gebruik van PTB-criteria, voor 'hazard assessment' is dat slechts informatie nodig is, die is gerelateerd aan fysisch-chemische eigenschappen van een verbinding. Ander type informatie, zoals bijvoorbeeld over productie volume en gebruik variëren sterk in de tijd en is moeilijk te verkrijgen of te schatten. Het nadeel van zo'n simpele benadering is dat slechts een enkele criteria worden gebruikt, hetgeen onvoldoende kan zijn voor de risicobeoordeling van een stof of voor bepaalde beleidsdoeleinden, omdat emissies naar het milieu niet worden meegenomen.

Het vaststellen van de referentiewaarden en het gebruik van de PTB-criteria is een beleidsmatige keuze en wordt niet in dit rapport behandeld. De PTB-criteria kunnen wel worden gebruikt bij een beleidsmatige keuze voor het identificeren van potentieel gevaarlijke stoffen.

Vóórdat de PTB-eigenschappen worden geschat dient een zogenaamd distributieprofiel te worden bepaald. Dit omdat het bijvoorbeeld niet zinnig is de atmosferische afbraak van een stof te bepalen die volledig is geïoniseerd en die niet in de lucht voorkomt. Dit profiel geeft aan hoe een stof zich verdeelt over lucht, water en bodem, bij evenwicht. Daarnaast is de identificatie van de stof van nut voor de keuze

van de juiste schattingsmethode. De minimale informatie die nodig is om een schatting te maken varieert van de SMILES-notatie tot gedetailleerde kwantum-chemische informatie.

Het schatten van de PTB-eigenschappen lijkt mogelijk voor vele organische verbindingen. Voor vele 'andere' verbindingen, echter, zijn nog onvoldoende schattingsmethoden ontwikkeld of de huidige methoden geven té onbetrouwbare schattingen. Deze 'andere' verbindingen bevatten metalen, organometalen, polymeren, mengsels, vele geïoniseerde verbindingen, complexe structuren, etc. Voor deze 'andere' verbindingen wordt aangeraden schattingen te baseren op expert judgement of gebruik te maken van 'default' waarden. De laatste zouden als 'worst-case' ingezet dienen te worden, maar de beslissing daarover is een beleidsmatige.

In het rapport worden stroomschema's gebruikt om aan te geven welke stappen te nemen zijn om de PTB-eigenschappen te bepalen. In de eerste stap dienen een aantal fysisch-chemische eigenschappen bekend te zijn, om een distributieprofiel op te stellen. In de tweede stap worden de PTB-eigenschappen bepaald. Het PTB-profiel kan dan verder worden gebruikt om de stof te classificeren in een bepaalde PTB-klasse. Dat laatste is een beleidsmatige keuze en wordt niet in dit rapport behandeld.

Extended summary

The aim of this paper is to make an inventory of 'simple' methods that estimate missing data on Persistence (P), Toxicity (T) and Bioaccumulative potential (B), for the purpose of a simplified hazard assessment. Since it is earlier recognised that for many substances experimental PTB-data are not available, estimation models may be the most quickly and cheapest, and relatively more reliable than expert judgement or default values. Preference should always be given to reliable experimental data. However, such data are not always available, and to obtain them is a costly and time-consuming process.

PTB-properties are in reality no intrinsic properties, but can operationally be treated as such. The PTB-criteria may be used for simplified hazard identification for the environment and for human health. For each substance a PTB-profile thus can be derived, that includes the PTB-properties, and, when compared to cut-off values for PTB, may results in a PTB-class.

The aim of choosing cut-off values and using the PTB-criteria for policy purposes is currently still under discussion and is outside the scope of the present report, and thus will not be further evaluated here. The PTB-criteria, however, may be used as one of multiple steps to identify potentially hazardous chemicals.

Prior to estimating the PTB-properties, a distribution profile, i.e. the distribution of the substance over the three major environmental compartments, air, soil and water, may be estimated, to identify the potential environmental distribution. For example, it is not useful to estimate atmospheric degradation of a fully ionised substance, which will not partition into air. Furthermore, identification of the chemical class, i.e. organic chemical, metal, etc., is needed to use the appropriate method for deriving the PTB-properties. For those substances for which the PTB-properties can be estimated, the required information varies from only a SMILES-notation to detailed quantum-mechanical information.

The advantage of a more simple approach, such as using PTB-criteria, is that only information is required on properties that are related to physical-chemical properties of the substance. In particular, information on production volumes and use patterns may vary with time and may be difficult to obtain or to estimate. The disadvantage of this approach is that only selected criteria are included and that this may be insufficient for specific policy actions and it is insufficient for risk assessment, since environmental release is not included.

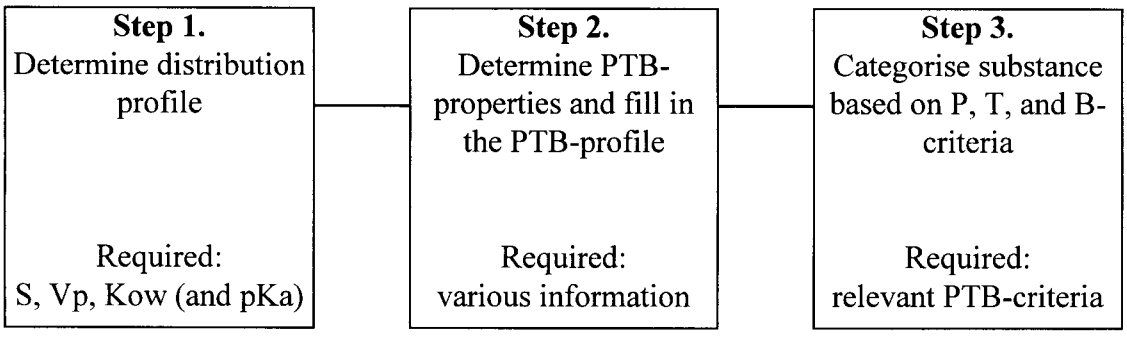
Estimates for the PTB-properties seem feasible for many organic compounds. However, for many 'other' compounds, estimation methods have not been developed yet, or the existing methods will provide unreliable estimates. These 'other' compounds include metals, organometals, polymers, mixtures, many ionizable compounds, complex structures, etc. For the latter chemicals it is advised to use either expert judgement or to use a default value to obtain the PTB-properties. Expert judgement may use structural analogues and/or chemical classes. It is suggested to

choose the default values as worst-case values, but the various stakeholders within the context of hazard identification should further discuss the value.

Flow-charts are provided in the report to guide the required steps which need to be followed to determine the PTB-properties (Scheme 1 and Table 1).

1. In step 1 the required information of the substance and its physical-chemical properties are used to determine a simple distribution profile.
2. In step 2, the PTB-properties will be determined.
3. The PTB-profile can be further used to determine the PTB-class in step 3. Further discussion needs to take place, which cut-off values to select for which relevant selection or prioritising actions in step 3. The latter is outside the scope of the present report.

Scheme 1. The step-wise approach to categorise substances following PTB-criteria.



For many organic substances, the selected 'simple' methods for persistence are (Table 2):

- Ready biodegradability, which can be estimated, using the ECB-model (Loonen et al., 1996) or the Syracuse estimation program, BIODEG. The latter may potentially provide too many false negatives, but is commercially available[1];
- Photodegradation in air, which can be estimated using the Syracuse estimation program, AOP;
- Hydrolysis in water, which can be estimated using the Syracuse estimation program, HYDRO.

For a worst-case approach, which will depend on the policy actions, the suggested cut-off values for persistency are whether a substance is not ready biodegradable (ECB-model), has an estimated time for ultimate biodegradation > months (BIODEG), has an atmospheric half-life time > 2 days, or has a half-life time for hydrolysis > 30 d.

For many organic chemicals, the selected 'simple' method for toxicity for the environment is (Table 2):

- Toxicity for the (aquatic) environment, which can be estimated from Kow, using QSARs, e.g. equations AQ-1 and AQ-2 (Chapter 3) for selected classes of organic chemicals, and, with a safety factor, for some other classes of organic chemicals.

[1] A recent study showed that the BIODEG program may improve when it includes the MITI-database and that it then no longer provides too many false negatives (Loonen et al., 1999).

A suggested cut-off value for a worst-case approach for toxicity, which will depend on the policy actions, is when a substance has an LC50 < 0.01 mg/L. The method will identify all organic substances with a log Kow > 7 as having an LC50 < 0.01 mg/L, which thus are very toxic for the aquatic environment, although it is recognised that substances with a log Kow > 7 will probably not show acute toxicity.

For mammalian toxicity, no 'simple' methods are available (Table 2):

- Mammalian toxicity, includes acute toxicity (oral, dermal, and inhalation), chronic toxicity, carcinogenicity, mutagenicity, and reproductive toxicity, and cannot be simply estimated since the currently available expert systems or (Q)SAR programs are not sufficiently validated and are not sufficiently reliable.

For organic chemicals, the selected 'simple' method for the bioaccumulative potential is (Table 2):

- Bioaccumulation in aquatic organisms, which can be estimated from Kow for organic chemicals, within a restricted log Kow range. The Syracuse estimation program, LOGKOW, can be used to estimate Kow. For ionised organic chemicals with a known pKa, estimates can be made for the unionised fraction. No estimates can be given for other classes of chemicals. The Syracuse estimation program BCFwin may also be used to estimate the BCF, but may underestimate secondary poisoning for substances with a very high Kow, i.e. log Kow > 7. Therefore, both BCF and Kow are relevant properties for estimating bioaccumulative potential.

A suggested cut-off value for a worst-case approach for bioaccumulative potential, which will depend on the policy actions, is when a substance has a BCF > 5,000 L/kg, or a log Kow > 5.

The commercially available Syracuse program, EPIWIN contains several of the models mentioned in Table 2, i.e. BIODEG, AOP, HYDRO, KOWWIN, and BCFwin. The estimations done by the model requires a SMILES-notation or a CAS-number as input, and very rapidly calculates the PTB-properties.

Table 1. The PTB-profile, which requires basic information and some physical-chemical properties of the substance as input to firstly determine the distribution profile. Secondly, the PTB-properties can be determined. When reliable experimental data are available, these should be selected. When cut-off values for the PTB-criteria are selected, the overall PTB-categorisation can take place.

Step 1. Determination of distribution profile		
Substance name:		
CAS no.:		
Chemical formula:		
Chemical structure:		
MW:		
Chemical class (according to Verhaar et al., 1992):		
Aqueous solubility:	mg/L	
Vapour pressure	Pa	
Log Kow		
PKa		
Distribution profile	Air: % Soil/sediment: % Water: %	
Step 2. Determination of PTB-properties		
PTB-property	*Value*	*Source*
P: biodegradation in water	Ready/not ready biodegradable $T_{1/2}$: hr/d/wk/months/yr.	Estimation / expert judgement / default / experimental
P: atmospheric photo-oxidation	$T_{1/2}$: hr/d/wk/months/yr.	Estimation / expert judgement / default / experimental
P: hydrolysis in water	$T_{1/2}$: hr/d/wk/months/yr.	Estimation / expert judgement / default / experimental
T: aquatic toxicity	LC50: mg/L EC50: mg/L	Estimation / expert judgement / default / experimental
T: acute mammalian toxicity	LD50 (oral): mg/kg LD50 (dermal): mg/kg LC50 (inhalation): mg/m^3	Estimation / expert judgement / default / experimental
T: chronic mammalian toxicity (prolonged exposure)	NOAEL (oral): mg/kg	Estimation / expert judgement / default / experimental
T: carcinogenicity / mutagenicity / reproduction toxicity		Estimation / expert judgement / default / experimental
B: bioaccumulation	BCF: L/kg Log Kow:	Estimation / expert judgement / default / experimental
Step 3. Determination of PTB-class		
PTB-profile:	$P_iT_iB_j$	
PTB-class:		

Table 2. Summary of estimation methods for PTB-properties, and for which class of chemicals they are feasible and reliable. If estimation models are not available, other approaches may be used to determine the PTB-properties, such as expert judgement or default values. If available, the recommended experimental methods are enclosed for comparison. 'Other' substances are defined in Figure 1.2 in Chapter 1.

PTB: property	**Recommended estimation model**	**Feasible for (reliability: + = good; +/- = fairly good)**	**Suggested other approaches**		**Experimental method**
			Expert judgement	**Default values**	
P: biodegradation in water	ECB-model or BIODEG	Organic substances (+)	'Other' substances	'Other' substances	OECD 301A-301F
P: atmospheric photo-oxidation	AOP	Organic substances (+/-)	'Other' substances	'Other' substances	not available
P: hydrolysis in water	HYDRO	Organic substances (+/-)	'Other' substances	'Other' substances	OECD 111
T: aquatic toxicity	KOWWIN & AQ1 or AQ2	Classes 1-4 organic substances (+)	'Other' substances	'Other' substances	OECD 201-203, 210-212
T: acute mammalian toxicity			Some specific 'other' substances	All substances	OECD 401-403
T: chronic mammalian toxicity			Some specific 'other' substances	All substances	OECD 407
T: carcinogenicity / mutagenicity / reproduction toxicity			Some specific 'other' substances	All substances	OECD 414, 451
B: bioaccumulation	KOWWIN and/or BCFwin	Organic substances (+)	'Other' substances	'Other' substances	OECD 305

1. INTRODUCTION

1.1 Summary

Because current risk assessment procedures are time consuming and may be complex processes, national and international actions currently take place to use PTB-criteria for more simplified hazard identification. Therefore, PTB-properties of substances are required, that in many cases are not available. The PTB-criteria are closely related to information that is needed for hazard identification, and thus is related to risk assessment procedures. However, it is a much simpler approach and cannot replace risk assessment. The aim of the present report is to make an inventory of 'simple' methods that may be used to estimate data on persistence, toxicity, and bioaccumulative potential of substances that lack experimental data on these properties.

1.2 National and international actions

General
Risk assessment includes effects and exposure assessment, which are combined in the risk characterisation to estimate the risk of a substance. Hazardous substances that are not emitted to the environment or to which man is not exposed will not pose a risk to man or environment. Substances that have a low toxicity and are accompanied with a high exposure to the environment thus may cause a risk to the environment. During the last two decades, legislation and several procedures in the EU has resulted in the ranking of substances and in decreasing the risk of substances for the environment and for workers, consumers, and man exposed via the environment.

For Classification and Labelling (CEC, 1993) of substances, experimental data on for example toxicity and persistency, are required.

For new chemicals, risk assessment will be carried out. With increasing tonnage, more information is required. For existing chemicals, first priority lists were established, which are based on production volume, and only a selected number of these were selected for which a risk assessment procedure is to be carried out.

Hazard identification of existing chemicals can be done when experimental test data are available. For most existing chemicals, these data are not available, and these chemicals are therefore not or insufficiently classified.

The Dutch government identified the problems associated with the insufficient number of experimental data on toxicity and persistence, which makes it difficult to establish a proper hazard identification of many substances. Earlier, the ministry of VROM proposed a system for priority setting of existing chemical substances (Van der Zandt and van Leeuwen, 1992), which was later adopted as EURAM, European Union Risk Ranking Method, by the European Chemicals Bureau, which includes

PTB aspects (Hansen et al., 1999). Recently, hazard identification solely based on persistent, toxic and bioaccumulative properties is investigated for the Dutch government.

International actions
Risk assessment (RA) guidance for new and existing substances is provided by the Technical Guidance Documents on Risk Assessment for new and existing substances (TGD, 1996). The European Union System for the Evaluation of Substances (EUSES) is often used for that purpose. The RA process for existing substances is a time consuming process and only has dealt with several tens of substances. Since there are many thousands of commercially available substances, other approaches are currently under discussion for 'simpler' hazard identification.

One of the possible approaches for very simple hazard identification is to identify substances that are Persistent, Toxic and Bioaccumulative (PTB-substances). Following PTB-criteria, substances may be selected from the chemical universe. The underlying result of taking solely P, T and B criteria was that an as simple as possible selection should be chosen, amongst others as a result of OSPAR agreements, i.e. the Esbjerg Declaration on the North Sea in 1995, and the OSPAR Convention in 1998. The outcome of these conventions is that the release of persistent, bioaccumulating and toxic substances to the North Sea should be eliminated within 25 years.

Persistent organic pollutants (POPs) are considered a subclass of PTB-substances that are prone to long-range atmospheric transport and deposition (Vallack et al., 1998). Under the auspices of the United Nations Economic Commission for Europe (UN-ECE) Convention on Long-Range Transboundary Air Pollution (CLRTAP), a protocol on POPs are defined as "a set of organic compounds that:
i) Possess toxic characteristics,
ii) Are persistent,
iii) Are liable to bioaccumulate,
iv) Are prone to long-range atmospheric transport and deposition, and
v) Can result in adverse environmental and mammalian toxicity effects at locations near and far from their sources" (UN-ECE, 1998).

The Netherlands
In the Dutch 3rd National Environmental Policy Plan (NMP, 1998) it is announced that new approaches towards regulating substances will be developed, in co-operation with European partners. One of these approaches deals with PTB-substances. For that purpose a project is started, which is called SOMS, which is a Dutch acronym for 'Strategy for dealing with substances'. The project is lead by the Ministry of Housing, Spatial Planning and the Environment (VROM). From available databases, VROM had earlier selected some substances that were shown to be persistent and toxic and bioaccumulative (BKH/HASKONING, 1998a-c). The latter reports showed that for many substances, values for the PTB-properties were missing. The present report is a follow-up for that action; i.e. to provide estimation models to estimate the various PTB-properties.

1.3 Simplified hazard identification

For hazard identification purposes one wants to identify whether or not a substance is hazardous to man (workers, consumers, via the environment (TGD, 1996)) or the environment. Below, the role of the PTB-properties in a simplified hazard assessment will be explained.

Toxicity is the 'easiest' property that will indicate the hazard of a substance, since the toxic concentration or dose indicates at which concentration or dose a substance exerts its toxic action. The lower the toxic concentration, the more toxic a substance is, i.e. even at low ambient or environmental concentration, the substance may pose a risk to man or the environment.

Persistence identifies whether or not a substance will be degraded in the environment, and thus indicates whether the substance will remain in the environment, i.e. it will not be degraded by biotic or abiotic transformation reactions. Persistence thus provides information on whether or not the ambient or environmental concentration will remain at the same level for prolonged time. If so, and if that concentration is close to the toxic concentration, the toxic effect will thus be exerted for a long time. It must be noted that even when a chemical is degraded fast, its metabolite(s) may pose a further risk to the environment. For example, the microbial conversion of chlorophenols into chloroanisoles result in substances, which are very persistent under aerobic conditions (Sijm et al., 1997).

Bioaccumulative potential indicates possible secondary poisoning, i.e. elevating the concentration in an organism above that in the ambient environment due to bioaccumulation processes. Thus, even if the environmental concentration is low, i.e. lower than a toxic level, organisms higher in the food chain may experience toxic effects by that substance, due to bioaccumulation and possibly biomagnification. Furthermore, bioaccumulative potential indicates whether chronic effects may occur. Chemicals with a strong bioaccumulative potential usually do not show acute effects, because their ambient concentration will be too low to cause these immediate effects. However, prolonged exposure to a low ambient concentration, may cause effects after long times. In the present study, bioaccumulative potential will be primarily related to the bioconcentration potential.

The PTB-properties can, to some extent, be related to risk assessment (RA). Within the European Union, RA makes use of the ratio Predicted Environmental Concentration (PEC) to Predicted No Effect Concentration (PNEC[2]). If the ratio PEC/PNEC (Equation 1.1) exceeds 1, there is reason for concern. If the ratio is smaller than 1, there is less reason for concern that the substance will cause harm to man or the environment.

[2] Within The Netherlands, the Maximum Permissible Concentration (MPC) is used analogous to the PNEC.

$$\mathbf{RA = \frac{PEC}{PNEC} = \frac{f(P, B, emission, etc)}{f(T, B, etc)}} \qquad \text{(Eq. 1.1)}$$

RA = risk assessment
PEC = predicted environmental concentration
f(...) = function of properties mentioned between brackets
P = persistence
T = toxicity
B = bioaccumulative potential

Toxicity (T), and to some extent bioaccumulative potential (B), would in this simplified comparison be related to the PNEC, while both Persistence (P) and Bioaccumulative potential (B) would be related to the PEC (Equation 1.1). However, one essential element is lacking for this simple hazard assessment, i.e. the environmental concentration. Persistence and Bioaccumulative potential indicate whether the concentration remains the same in time and the potential to result in elevated concentrations in organisms high in the food chain, respectively. They, however, do not indicate what the actual environmental concentration is, since that will depend on the amount of the substance that is released in the environment, and thus depends on production volume, use and emission patterns (Equation 1.1), i.e. extrinsic properties.

The advantage of a more simple approach, such as using PTB-criteria, is that only information is required on properties that are related to physical-chemical properties of the substance. In particular, information on production volumes and use patterns may vary with time and may be difficult to obtain or to estimate. The disadvantage of this approach is that only selected criteria are included and that this may be insufficient for specific policy actions, since no risk assessment is performed which includes environmental release estimation.

1.4 Earlier studies

Within national and international environmental policy plans, restrictive measures for PTB-substances are or will be taken. Criteria thus need to be developed with which it is possible to identify those PTB-substances. The Dutch Ministry of Housing, Spatial Planning and the Environment had asked BKH/HASKONING to give a survey of international actions dealing with PTB-substances, to select PTB-properties, to develop PTB-criteria, and further to select PTB-substances for the environment and for mammalian toxicity. This resulted in a series of BKH/HASKONING reports (BKH/HASKONING, 1995; BKH/HASKONING, 1998a-c).

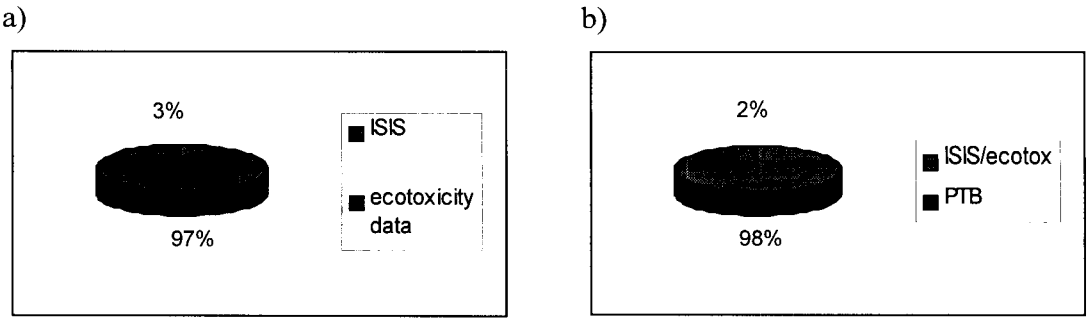


Figure 1.1. a) Percentage of substances with known ecotoxicity data; the substances are from the ISIS/Riskline database, that contains approximately 100,000 entries. b) Percentage of substances that could be identified as PTB substances by BKH/HASKONING (1998a-c), from substances with known ecotoxicity data.

The BKH/HASKONING reports and other studies (EPA, 1998) showed that many data are lacking for the more than 100,000 organic substances from the BKH/HASKONING database of existing chemicals, or for the High Production Volume Chemicals (HPVCs), respectively. For only ca. 3,600 substances, data on aquatic toxicity are available, and only 57 substances (Figure 1.1) were categorised as PTB-substances for the environment (BKH/HASKONING, 1998a). Based on mammalian toxicity, approximately 250 substances were categorised as PTB-substances (BKH/HASKONING, 1998c).

An evaluation of the limited number of intrinsic properties of a substance would potentially lead to a fast screening and selection process. Other (extrinsic) properties, such as production volume, use pattern, etc., are therefore not included in the BKH/HASKONING reports. Whether this is an effective approach needs further discussion. Also for which (policy) reason the selection process is to be used, requires further discussion.

The PTB-properties have been experimentally determined or estimated for organic substances (BKH/HASKONING, 1998a-c). However, not all substances are organic chemicals. Verhaar et al. (1992) recently showed that among 2000 High Production Volume Chemicals (HPVCs), approximately 45% could be classified as belonging to one of four organic chemical classes, but the remaining 55% could not be classified as such for several reasons (Figure 2.2). The aim of the study of Verhaar was to use QSARs (quantitative structure activity relationships) to estimate the aquatic toxicity of the HPVCs. Estimating aquatic toxicity was possible only when a chemical belongs to one of the four classes, and was not feasible when a chemical does not belong to those chemical classes. It would be interesting to know how many of the existing chemicals can be classified according to Verhaar et al. (1992), since for these chemicals the estimation of the toxicity and of other PTB-properties is most feasible. Those chemicals that could not be classified were metals, organometals, inorganic substances, polymers, petroleum products, mixtures, etc.

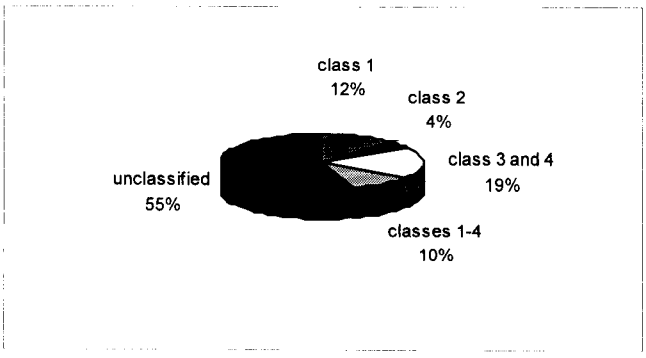


Figure 1.2. Percentage of High Production Volume Chemicals (total of 2000) in chemical classes 1, 2, 3 or 4 (all organic chemicals), and those that cannot be classified (metals, organometals, inorganic substances, polymers, petroleum products, mixtures, etc.) according to Verhaar et al. (1992). For classes 1 to 4, estimates of aquatic toxicity can be made, while for the unclassified substances no estimates of aquatic toxicity can be made.

Tyle and Niemelä (1998) recently suggested to solely use QSARs for selection of POPs, based on P and B properties. They thus selected 539 substances from a list of 166,075 substances. For persistence, the selected properties were biodegradation, photo-oxidation, and hydrolysis. In addition, they selected substances on their volatility. For bioaccumulation, the single property was an estimated BCF. Their motivation for not including toxicity was that toxicity data are scarce, and that substances with a high log Kow would be selected as toxic chemicals anyway.

There is thus a need to first determine or to estimate the individual PTB-properties, and in a later stage to use them for further selection procedures. There is thus also a need to further define how a PTB substance should be treated. The latter is beyond the scope of the present report and needs to be discussed elsewhere.

1.5 Definitions

The *PTB-criteria* relate to persistence, toxicity, and to bioaccumulation. *Persistence* will indicate whether the substance will remain in the environment, i.e. will not be degraded by biotic or abiotic transformation reactions. Persistence thus provides information whether or not the ambient or environmental concentration will remain at the same level for prolonged time. If so, and if that concentration is close to the toxic concentration, the toxic effect will thus be exerted for a long time.

Toxicity will indicate the hazard of a substance, since the toxic concentration or dose indicates at which concentration or dose a substance exerts its toxic action. The lower the toxic concentration, the more toxic a substance is, i.e. even at low ambient or environmental concentration, the substance may be hazardous to man or the environment. Toxicity includes toxicity for the environment and mammalian toxicity.

Bioaccumulative potential indicates possible secondary poisoning, i.e. elevating the concentration in an organism above that in the ambient environment due to bioaccumulation processes. Thus, even if the environmental concentration is low, i.e. lower than a toxic level, organisms higher in the food chain may experience toxic effects by that substance, due to bioaccumulation and possibly biomagnification. Furthermore, bioaccumulative potential indicates whether chronic effects may occur. Chemicals with a strong bioaccumulative potential usually do not show acute aquatic ecotoxicological effects, because their actual ambient concentration will be too low to cause these immediate effects. However, prolonged exposure to a low ambient concentration, may cause effects after long times. In the present study, bioaccumulative potential will be primarily related to the bioconcentration potential.

Each of the PTB-criteria includes one or more *properties*. Each property can be either quantified as a numerical *value* or it can be qualitatively assigned. The value can finally be compared to a *cut-off value*, which will then result in categorising the substance into a *PTB-class*. For example, for persistence, (at least) three properties can be distinguished: biodegradation in water, atmospheric photo-oxidation and hydrolysis in water. For biodegradation in water, a qualitative value may be 'ready biodegradable' or 'not ready biodegradable'. For atmospheric photo-oxidation a quantitative value for the half-life of a substance may be 15 days. When the cut-off value in the latter case is for example, 2 days, the substance will be considered persistent.

1.6 Scope of the report

To experimentally determine all the missing PTB-properties of the existing chemicals is a time-consuming and very costly exercise. Therefore, the aim of the present report is to evaluate the available methods that can be used to estimate the required PTB-properties as simple and reliable as possible. If no estimation method is available, then either expert judgement may take place or a default value may be chosen. Reliable experimental data will always prevail, but the starting point for the present report is that many experimental data will be missing. (Suitable) estimation methods are preferred over expert judgement and default values for reasons of reliability, costs, speed and validity (Table 1.1). How to further deal with the PTB-properties with regard to policy actions, is beyond the scope of the present report. This report, however, may serve as a discussion paper for these further actions.

Table 1.1 Relative reliability, costs, speed and validity of experimental data, estimation models, expert judgement, and default values. +++ = very good, + = good, - = bad.

	Experimental	Estimation model	Expert judgement	Default values
Reliability	+++	+	+	-
Costs	-	+++	+	+++
Speed	-	+++	++	+++
Validity	+++	+	+	-

Each of the following chapters will start with a short summary of its contents.

Chapter 2	starts with discussing the PTB-properties as intrinsic properties, and discusses the significance of other properties that are related to risk or hazard identification. Then, the PTB-criteria that are reported by BKH/HASKONING (1998a-c) are discussed. A final selection of a distribution profile and the most relevant PTB-properties are presented in the last section of the chapter.
Chapter 3	presents an inventory of 'simple' estimation methods for the PTB-properties. For persistence, aquatic toxicity, and bioaccumulative potential selected models are available and described. In addition to the estimation models, accepted experimental methods are provided for reasons of comparison. The chapter does not provide an exhaustive literature search and evaluation, but mainly relies on earlier review studies.
Chapter 4	summarises the physical-chemical properties or descriptors that are required either for determining the distribution profile (chapter 2), or as input for the estimation models (chapter 3). Also this chapter does not provide an exhaustive literature search and evaluation, but mainly relies on earlier review studies.
Chapter 5	provides a stepwise approach that needs to be followed to estimate or determine missing PTB-data. In step 1 the required information of the substance and its physical-chemical properties is used to determine a simple distribution profile. In step 2, the PTB-properties are determined. This PTB-profile can then be used to determine the PTB-class. A suggestion for the cut-off values for the PTB-criteria is enclosed. Further discussion needs to take place, which cut-off values to select for which relevant selection or prioritising processes in step 3. The latter is outside the scope of the present report.
Chapter 6	presents some final remarks and conclusions.
Appendix 2	presents a simple distribution model to estimate the distribution profile, and shows some examples for some selected chemical classes.
Appendix 3	presents the summary of (Q)SAR systems for mammalian related toxicological endpoints.
Appendix 4	presents a table from which to select the most relevant PTB-properties, based on the distribution profile, and shows the classification of chemicals.
Appendix 5	shows an example of how PTB substances may be categorised.
Appendix 6	presents a rough estimate of PTB-properties of substances for which no estimation is possible.

2. EVALUATION OF PTB-PROPERTIES

2.1 Summary

PTB-properties are in reality no intrinsic properties, because they depend on environmental conditions. However, they will be treated operationally as intrinsic properties. Other properties that are related to risk or hazard identification are briefly discussed in this chapter. Also PTB-criteria that are reported by BKH/HASKONING (1998a-c) are discussed. A final selection of a distribution profile and the most relevant PTB-properties are presented in the last section of the chapter.

2.2 General

PTB-properties are assumed to be intrinsic properties that may be used for hazard identification. First, some remarks will be made on the intrinsic properties, then on the simplified hazard identification, before other properties that are related to risk or hazard identification are briefly discussed.

2.2.1 Intrinsic properties

With regard to the intrinsic properties of substances, there are different views on how to define and use them. For clarity, it is useful to return to the strict definition of the intrinsic properties of a substance. Intrinsic properties would be those that would not be affected by the environment where the substance is in. For example, the molecular weight would not differ when the substance is in water, in air, or in an organism.

For persistence, both biodegradation in water and photo-oxidation in air are chosen as degradation processes. In general, degradation processes are second-order processes. With respect to biodegradability as an intrinsic property, it must be noted that biodegradation is not solely a property of the substance, since the environment will affect biodegradability. The biodegradation half-life depends on a biodegradation rate constant, which is dependent on temperature, the reactivity of the substance, and on whether or not the micro-organisms have the ability, i.e. contain or are able to induce the enzymes, to degrade the substance, and on the abundance of micro-organisms in water. For example, different species of micro-organisms may degrade a substance at various rates, from extremely slow to very fast. Furthermore, at low temperatures, biodegradation proceeds at a much lower rate than at higher temperature. Thus, biodegradation depends on extrinsic properties.

Also photo-oxidation is affected by its surrounding environment, since the half-life depends on a) the rate constant, which is dependent on temperature, on the reactivity of the substance, and on the latitude (solar flux), b) on the concentration of hydroxyl radicals in air. A recent example shows that the photo-oxidation half-life of a tetrachlorobiphenyl molecule may vary between 1 day and 6 years under tropical and polar conditions, respectively. The average ambient temperature and hydroxyl radical

concentrations in these tropical and polar environments are 30°C and $6 \bullet 10^6$ molecules/cm^3 and -20°C and $0.006 \bullet 10^6$ molecules/cm^3, respectively (Webster et al., 1998). Furthermore, photo-oxidation rate constants usually refer to gaseous substances. Many semi-volatile substances are associated to particles or aerosols that may affect the photo-oxidation half-life.

Both for biodegradation and photo-oxidation, the environmental conditions are significant and in most cases may be dominant for the actual degradation.

With respect to toxicity as an intrinsic property, it must be noted again, that it is not solely the properties of a substance that cause toxicity. For example, benzene is a carcinogen, because in higher organisms, an enzymatic conversion makes it carcinogenic. In lower organisms, which do not have the responsible enzyme, benzene is not a carcinogen. Thus, also toxicity depends on extrinsic properties.

PTB-properties may still be considered operationally as intrinsic properties, but these properties should be treated predominantly as relative and not as absolute values. In addition, some of the properties, such as the different toxicological endpoints, indeed require multiple endpoints.

2.2.2 Other properties related to risk or hazard identification

A few other properties are addressed that might be relevant in the selection of hazardous substances, in hazard identification or in risk assessment. These other properties are a simple distribution profile, long-range transport, production volume, and emission pattern.

Distribution profile
All substances will distribute over the different environmental compartments after being emitted into the environment. Some of them will mainly reside in water, while others mainly reside in air or soil. If a substance resides mainly in air, then the persistence criterion of biodegradation in water is not a very useful property. A substance that is ionised at pH 7, will probably mainly reside in the aqueous phase, and estimating atmospheric photo-oxidation will then not be very useful. Therefore, prior to estimating the PTB-properties, a distribution profile may be used to estimate the environmental distribution of a substance. A simple model, analogous to a Mackay level I multimedia model can be used, that only requires a few physical-chemical properties, a simple-defined air/water/soil multimedia model and some additional assumptions. The required physical-properties are the vapour pressure (Vp), the aqueous solubility (S), the octanol/water partition coefficient (Kow), and the pKa. The properties of the multimedia model can be chosen as being at a European scale or that for The Netherlands. The assumptions required are the following:
(i) there is an equilibrium
(ii) the soil/water partition coefficient can be estimated from the Kow, and
(iii) the air/water partition coefficient can be estimated from the ratio Vp/S.
A few examples of the simple model and the outcome of a series of chemical classes are provided in Appendix 2.

In addition to estimating the distribution profile from a steady-state situation, Wania and Mackay (1996) have recently proposed a simple scheme that predicts the global distribution of organic substances. The scheme is based on a few physical-chemical parameters: the subcooled liquid vapour pressure (P_L), the octanol-air partition coefficient (Koa), and a contaminant-specific condensation temperature (T_C), as shown in Table 2.1. Koa can be estimated from the ratio of the Kow and the Henry's Law Constant (He). He can be estimated from the ratio of the vapour pressure and the aqueous solubility.

The advantage of the steady-state distribution profile is that a simple calculation indicates which PTB-properties are relevant and which are not. However, the disadvantage of the distribution profile is that in reality also the route of entry into the environment will determine the overall environmental half-life or persistence (Webster et al., 1998). Thus, a soluble substance that will be degraded fast in water, but if emitted into the soil, may still have a long persistence in the environment, since it needs time to be released from the soil into the water before it is degraded.

Table 2.1. Global distribution of organic contaminants (adapted from Wania and Mackay, 1996).

	Low mobility	**Relatively low mobility**	**Relatively high mobility**	**High mobility**
Global transport behaviour	Rapid deposition and retention close to source	Preferential deposition and accumulation in mid-latitudes	Preferential deposition and accumulation in polar latitudes	World-wide atmospheric dispersion, no deposition
Log octanol-air partition coefficient (Koa)	<---------- 10 ---------- 8 ---------- 6 ---------->			
Log vapour pressure of subcooled liquid (P_L in Pa)	<---------- -4 ---------- -2 ---------- 0 ---------->			
Temperature of Condensation (T_C in °C)	<---------- +30°C ---------- -10°C---------- -50°C---------->			
Examples	PCBs: 8-9 Cl PCDDs: 4-8 Cl PAHs: > 4-rings Mirex	PCBs: 4-8 Cl PCDDs: 2-4 Cl PAHs: 4-rings DDTs, chlordanes	penta- and hexachloro-benzenes PCBs: 1-4 Cl PCDDs: 0-1 Cl PAHs: 3-rings HCHs, dieldrin	Mono- to tetra-chlorobenzenes PCBs: 0-1 Cl PAHs: 2-rings

Long-range transport
A distribution profile may estimate the distribution of a chemical at equilibrium, and does not take into account degradation processes in the environment. However, in the environment, degradation processes take place, and a global equilibrium will not likely be reached. It is thus very important to estimate whether or not a chemical can be transported globally. This transport will thus depend on a) the physical movement of the substance from the source where it is emitted to other parts of the earth, and b) its persistence in the environment (Table 2.1; van Pul et al., 1998).

In general, either air or water physically moves a substance from one place to another. In particularly, air transport is considered the most important route to distribute substances globally (Vallack et al., 1998; van Pul et al., 1998). A substance must have a certain volatility in order to be transported trough the air. However, if the substance is very volatile, then it will remain in the atmosphere and will, in general, not cause a risk for human beings and the environment. Ozone depletive substances or substances that affect global warming are notable exceptions. For the general approach, Vallack et al. (1998) have suggested to further assess substances for long-range transport that have a vapour pressure (Vp) < 1000 Pa. Since persistence in air is already included in the PTB-criteria, it will not be further discussed here.

The 1998 SETAC Pellston Workshop (SETAC, 1998) addressed the importance of long-range transport as well as the estimation of different degradation processes in the context of persistency. The results of the workshop are very useful in the context of PTBs, but are not available yet, and therefore cannot be incorporated in the present report.

Production volume and emission pattern
Production volume, usage and emission patterns determine where and how much of the substances are emitted into the environment. For example, if a substance is used in closed systems, it will not or only in small amounts be emitted to the environment, whereas a substance that has a widespread use in open systems will be emitted to the environment by many diffusive sources. As is shown earlier, the compartment in which the substance is emitted, significantly determines the environmental distribution and the environmental half-life for persistence. Then, a more complex, higher orders Mackay type of multimedia model is required to estimate environmental distribution and persistence (Webster et al., 1998).

A further discussion is required on how to use information on those extrinsic properties, since detailed information is probably difficult to obtain, while neglecting the information would be unrealistic. A further selection based on production volume may serve useful, preferably after determining the PTB-profile.

2.3 Evaluation of PTB-criteria as reported by BKH/HASKONING (1998a-c)

In general, BKH/HASKONING (1998a-c) has selected properties and cut-off values, which occur on international lists or within international actions, or have adjusted specific properties or cut-off values (see BKH/HASKONING, 1998a-c).

2.3.1 Persistence

In the BKH/HASKONING-study, persistence (P-property) for the environment is based on biodegradation in water and photo-oxidation in air. Only when a substance is found to be persistent for biodegradation, it was further evaluated to estimate its persistence in air. The cut-off values for the biodegradation in water is the probability for linear biodegradation, which is incorporated in the BIODEG model, is < 0.1, and the time required for ultimate biodegradation is months, or > months. The half-life for persistence in air, $t_{1/2}$ (photo-oxidation in air), is > 2 days.

It must be noted that:
- no other degradation processes in water are included, such as hydrolysis, photolysis, oxidation and reduction in water, anaerobic biodegradation, and biotransformation;
- no degradation processes in other environmental compartments are included, except for atmospheric oxidation for the environment;
- the rate of aerobic biodegradation in water as done by BKH/HASKONING is estimated using the BIODEG program;
- the cut-off value > months is usually not available in test results, and is only used in the BIODEG program. There are currently no standardised tests to measure the biodegradation half-life in water. It would be more pragmatic to stick to the cut-off value that is used in the OECD biodegradation tests, i.e. a substance is persistent when it is not ready biodegraded (60% of theoretical or biological oxygen demand, ThOD or BOD) within 28 days;
- the rate of photo-oxidation in air is assessed by BKH/HASKONING only when a chemical distributes for 10% or more in air, using a Mackay level I model, and then estimated using the AOP program;
- only the daytime oxidation rate constant is estimated, whereas the night-time NO_3 radical reactions (Sabljic and Güsten, 1990) are not taken into account.

The comments show that many properties have not been included. However, it can be expected that for the selected persistence properties, most data can be retrieved, whereas far less information will be available for other degradation processes. For example, far less data are available for the night-time NO_3 radical reactions, and for many substances the night-time NO_3 degradation is much less significant than OH degradation. Also, far less information will be available for other substances than for organic substances, e.g. that cannot be classified according to Verhaar et al. (1992). In some cases, one of the other degradation processes may dominate biodegradation or photo-oxidation. A case-by-case approach is required to show whether or not other

degradation processes are dominating, and what is the most relevant degradation process.

Biodegradation in water
In the BKH/HASKONING-study, the BIODEG program was used to estimate the half-life of a substance in water. BIODEG estimates the probability for the rapid aerobic biodegradation of an organic chemical in the presence of mixed populations of environmental micro-organisms. The BIODEG program is capable of estimating biodegradation in water for organic chemicals for which a SMILES notation is or can be made available. This limits the estimation of biodegradation for other classes of chemicals, such as polymers, organometals, etc. A more detailed description of BIODEG will be given in Chapter 4.

An attempt for validating BIODEG was provided by Rorije et al. (1997). They have studied the prediction of environmental degradation rate constants for High Production Volume Chemicals (HPVC) using QSARs. For more than 50% of the compounds no predictions could be made, since they were either ill defined or present as mixtures. For 930 of the 1073 HPVC compounds, biodegradation estimates could be produced, for which for 182 substances (20%) conflicting results from different models were obtained. External validation of the BIODEG estimates showed that of a set of 488 biodegradation test data from MITI, approximately 90% of the degradable substances were predicted as such by BIODEG. However, BIODEG misclassified 56% of the non-degradable substances. Therefore, a significant amount of potentially persistent chemicals are predicted by BIODEG to be degradable (EU, 1995a).

It must be noted that the MITI database contains conservative values, i.e. the test shows ready degradability results, which are difficult to compare to the BIODEG results. However, this external validation is one of the few available studies, and may indicate that BIODEG may overestimate biodegradability. This may imply that substances are predicted as being biodegradable, while they are persistent. Further validation studies are thus required to study the use of this and other estimation programs.

A recent study (Rorije et al., 1998) evaluated BIODEG, the OECD models on biodegradation (Degner et al., 1993), MULTICASE (Klopman et al., 1995) and a model of the European Chemicals Bureau (ECB-model) on biodegradation (Loonen et al., 1996). The study showed that the ECB model performed best in predicting overall degradable and non-degradable substances (Table 2.2). The most relevant in this is that ready biodegradability is predicted well. The model for a worst-case approach should predict the 'not ready biodegradable' substances as close to 100% as possible. As a worst-case approach, it is less a problem if a substance is actually 'ready biodegradable', but the model predicts it as being 'not ready biodegradable'. Thus, the most reliable model should predict 'ready biodegradable' in an accurate way, such as the ECB-model does (Table 2.2).

Table 2.2. Comparison of models in predicting biodegradability. Percentages of number of correct predictions are given. For BIODEG, 733 substances from the MITI database were taken, for OECD, ECB-model and MULTICASE, 894 substances were taken.

	BIODEG	BIODEG Updated	OECD (Degner et al., 1993)	ECB-model (Loonen et al, 1996)	MULTI-CASE (Klopman et al., 1995)
Ready biodegradable	68.1 %	88 %	81.9 %	88.2 %	84.8 %
Not ready biodegradable	76.0 %	88 %	88.2 %	80.0 %	72.0 %

'Ready biodegradable' thus seems to be best predicted by the ECB-model. When a substance is not ready biodegradable, however, it is not necessarily a persistent substance. Micro-organisms have a very strong ability to adapt to various substances, and to degrade them after prolonged times. Therefore, if a substance is predicted as not 'ready biodegradable', it will not necessarily be persistent in the long run. These microbial adaptation processes make it also difficult to estimate the biodegradation half-life, even qualitatively. Not only the total biomass is important in this case, also the number of micro-organisms that are able to degrade the substances is important, the so-called 'degraders'. If the number of 'degraders' in a micro-organism population is small, a substance will be persistent. The latest BIODEG-program has now included the MITI database and was further updated, which results in predicting 'ready biodegradable' and 'not ready biodegradable' at equal accuracy, i.e. > 88% (Loonen et al., 1999). Both the ECB-model and an upgraded version of BIODEG will thus be suitable to use for estimating aerobic biodegradation in water.

Photo-oxidation in air
In the BKH/HASKONING-study, the AOP (Atmospheric Oxidation Program) program is used to estimate atmospheric photodegradation. AOP estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. It also estimates the rate constant for the gas-phase reaction between ozone and olefinic/acetylinic compounds. The rate constants estimated by the program are then used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl reactions and ozone (Meylan and Howard, 1993).

Internal validation of AOP shows that for a list of 647 organic chemicals, over 90% of the estimated gas-phase hydroxyl radical rate constants are within a factor of two of the experimental values, while over 95% are within a factor of three of the experimental values. It must be noted that the 647 chemicals are all chemicals for which experimental values are available, which thus shows that AOP is fairly accurate. AOP seems to provide better results than the PCFAP program (Fate of Atmospheric Pollutants) of the US EPA GEMS (Graphic Exposure Modelling System) software, that estimates the same rate constants. For 617 of the 647

chemicals, PCFAP is within a factor of two for 49%, and within a factor of three for about 65%.

An attempt of external validation was provided by Rorije et al. (1997). For oxidation in the atmosphere, the Syracuse model AOP (hydroxyl radical and ozone reactions) and the MOOH-method (hydroxyl radical reaction) of Klamt (1993) were used. For the MOOH-method, semi-empirical calculation of molecular orbital energies are required using the AM1 (Dewar et al., 1985) parametrisation in the MOPAC program (Stewart and Coolidge, 1990; Stewart, 1990).

For 917 of the 1073 HPVC compounds, an estimation of the reaction rate constant for reaction with hydroxyl radical was possible using the AOP model, and for 864 compounds using the MOOH-method. The two models, however, give very different results (Rorije et al., 1997).

The AOP model seems to be the best recommended model, since it contains almost all the available experimental atmospheric photo-oxidation reactions, and predicts them very accurately. Still, the experimental database is small, and needs to be further extended.

Persistence; general
Whereas neither biodegradation in water nor atmospheric photo-oxidation will provide all information on the persistence of a substance in the environment, these properties are the most studied among the many possible degradation processes. Hydrolysis in water may be an additional process for which an estimate can be provided by the Syracuse software. Hydrolysis will result in degradation of the substance in water. It is assumed that the product will be less persistent, although exceptions may be possible. Whether or not the selected properties for persistence are relevant will amongst others depend on the environmental distribution of the substance.

The study of Rorije et al. (1997) showed that other abiotic degradation processes, such as hydrolysis, oxidation and reduction in the aqueous phase, resulted in estimates for only 237 of 1074 HPVC compounds. These other reactions were facing missing data or time-consuming calculations were needed (Rorije et al., 1997). This shows that it seems not feasible to 'simply' estimate the persistence based on those other degradation processes.

The estimation of atmospheric oxidation using the Syracuse software programme, AOP, is probably the most state-of-the-art approach. The TGD (1996) does not include atmospheric degradation, and thus would adopt a worst-case situation that is different than using the PTB-criteria for hazard assessment. For biodegradation in water, the ECB-model (Loonen et al., 1996) is probably better than the BIODEG model. This ECB-model, however, is currently not public available yet. Therefore, until the ECB-model is available, BIODEG may be used, with knowledge of its limitations. It must be noted, however, that there are only few validation studies on the programmes, which makes it difficult to value the programmes. BIODEG may

provide false-negatives, i.e. identify poorly degradable substances as being fast degradable.

BIODEG and AOP only deal with organic chemicals, but neither can deal with non-organic substances, mixtures, metals, organometals or polymers.

Finally, further discussion is required on the applicability of the different properties for persistence. For example, substances that are very volatile need to be studied primarily for their persistence in the atmosphere, and less for biodegradability in water. Therefore, further information on the environmental compartment in which the substance will reside, must be taken into account when estimating its persistence.

2.3.2 Toxicity

In the BKH/HASKONING-study, toxicity (T-property) for the environment is based on acute aquatic toxicity, while that for mammalian toxicity is based on multiple endpoints (BKH/HASKONING, 1998a-c).

Ecotoxicity
Toxicity (T-property) for the environment is based on aquatic toxicity data for fish, invertebrates, algae and bacteria for three different cut-off values (BKH/HASKONING, 1998a-c):

LC50 < 1 mg/L	and/or	NOEC (L) < 0.1 mg/L
LC50 < 0.1 mg/L	and/or	NOEC(L) < 0.01 mg/L
LC50 < 0.01 mg/L	and/or	NOEC(L) < 0.001 mg/L

The BKH/HASKONING studies showed that almost no chronic toxicity data were found in the databases that they used, therefore, aquatic toxicity was almost entirely based on acute toxicity data.

The reason for choosing cut-off values that are different from e.g. classification and labelling is related the expected actual concentrations in the environment. Many of the substances that are or may be found in the environment, will have concentrations in water that are much below 100 mg/L. Therefore, cut-off values were chosen that could distinguish substances in more environmentally relevant concentrations.

It must be noted that:
- no other toxicity endpoints, such as embryotoxicity, carcinogenicity, endocrine disruption, etc. for those aquatic organisms are included;
- no toxicity endpoints for other aquatic organisms, such as bivalves, protozoa, etc. are included;
- no toxicity endpoints for other organisms, such as terrestrial, avian or mammals, are included;
- no other toxicity endpoints for those other organisms are included;
- only data from ISIS/Riskline have been used;
- only few data are available.

The comments show that many toxicity endpoints have not been included, but the importance of these comments is limited. It is to be expected that among the many environmentally related toxicity endpoints, the availability of data for aquatic toxicity will be highest. Much less information will be available for other endpoints and other organisms. However, in some cases, one of the other properties may be more relevant than the selected ones. It should be noted that also for deriving a Maximum Permissible Concentration (MPC) within the Dutch project "Setting Integrated Environmental Quality Standards", often only aquatic ecotoxicity data are used.

One further comment is on the choice of the cut-off values. For classification and labelling substances are called harmful, toxic and very toxic when the cut-off values are below 100 mg/L, 10 mg/L, and 1 mg/L, respectively (CEC, 1993). A more harmonised terminology and more harmonised cut-off values are preferred, even though the goals of the BKH/HASKONING-study and classification and labelling are different.

Mammalian toxicity
In the BKH/HASKONING-study, mammalian toxicity (T-property) is based on one or more of the following properties which are shown in table 2.3 (BKH/HASKONING, 1998c). A substance is selected as a T-substance, if the cut-off values of one or more of these aspects are met, which are in line with classification and labelling (CEC, 1993).

Table 2.3. Selected properties for mammalian toxicity (BKH/HASKONING, 1998c).

T-property	**T-cut-off value**		
ACUTE TOXICITY			
Exposure route	Sub-classification cut-off values		
Oral (LD50, rat, mg/kg) < 2000	Harmful (Xn) Toxic (T) Very toxic (T+)	200 < LD50 ≤ 2000 25 < LD50 ≤ 200 LD50 ≤ 25	R22 R25 R28
Inhalation (LC50, rat, mg/L, 4 h) < 20	Harmful (Xn) Toxic (T) Very toxic (T+)	2 < LC50 ≤ 20 0.5 < LD50 ≤ 2 LD50 ≤ 0.5	R20 R23 R26
Dermal (LD50, rat/rabbit, mg/kg) < 2000	Harmful (Xn) Toxic (T) Very toxic (T+)	400 < LD50 ≤ 2000 50 < LD50 ≤ 400 LD50 ≤ 50	R21 R24 R27
CARCINOGENICITY		R45 or R49 or IARC 1 or IARC 2a	
REPRODUCTION TOXICITY		R60 or R61 or R62 or R63	

It must be noted that:
- no other classifiable endpoints are included, such as irritation, sensitisation (R43), may cause serious damage to health after prolonged exposure (R48) and

mutagenicity (R40). In particular irritation and sensitisation are important for workers and consumers;
- no endpoint for systemic chronic toxicity is included, which will be more relevant than acute toxicity for environmental exposure;
- no other non classifiable toxicity endpoints are included, such as neurotoxicity, immunotoxicity, no observed adverse effect levels (NOAEL, that are partly included in R48), endocrine disruption, etc.
- only data from ISIS/Riskline have been used;
- only few data are available.

The comments show that for human toxicological endpoints, the database should have been screened for R48 and R40, since both classifications are important for long-term exposure. Irritation and sensitisation for workers and consumers are also important for short- and long-term exposure, but not for "man, exposed via the environment" (EUSES). For neurotoxicity and immunotoxicity, it is expected that much less information will be available. The latter two toxicological endpoints are considered important in the EU hazard assessment procedure (TGD, 1996), but since little information is available, and the information is difficult to retrieve from sub-acute studies, neurotoxicity and immunotoxicity are only taken into account on a case-by-case basis.

2.3.3 Bioaccumulative potential

The bioaccumulative potential (B-property) for the environment is based on either bioconcentration factor (BCF) in fish or a related value of Kow, while that for mammalian toxicity is solely based on Kow (BKH/HASKONING, 1998a-c). Three cut-off values are chosen:

log Kow ≥ 4	or	BCF ≥ 1,000
log Kow ≥ 4.5	or	BCF ≥ 3,000
log Kow ≥ 5	or	BCF ≥ 5,000

It must be noted that:
- the proposed cut-off values show no linear relationship between log Kow and log BCF. A linear relationship should have been appropriate in this log Kow range of 2 to 6, and therefore the rationale behind the log Kow and the corresponding BCF values is not clear;
- no BCFs of other aquatic organisms, such as mussels, are included;
- no bioaccumulation information for other organisms, such as mammals, terrestrial or benthic organisms, are included;
- the approach is suitable for organic substances, for which a SMILES notation is required, but is not suitable for other substances, such as metals, inorganic substances, polymers, organometals, etc.

The comments show that several properties have not been included, but the importance of these comments is limited. It is to be expected that in particular for organic chemicals a Kow can be retrieved, and subsequently, a BCF can be estimated.

One further comment is on the choice of the cut-off values. For classification and labelling a cut-off value for log Kow > 4 is used (OECD, 1998). An additional cut-off value would have resulted in a more harmonised approach.

The relationship between BCF and Kow is well studied for neutral organic chemicals, and the relationship is well established for those neutral, organic substances that have a log Kow in the interval 1 to 6. For substances with a log Kow > 6, another relationship may be used (see chapter 4). Organic chemicals that have a molecular weight of > 1000 g/mol, have a molecular diameter of >10 Å, or have a molecular length of > 5.6 Å, are considered to have a molecular dimension that is too big to let them passively diffuse over biological membranes (Opperhuizen, 1986). These limiting values may not be used to predict them as nonabsorbable for food-chain transfer, since in the gastro-intestinal tract other uptake mechanisms than passive uptake are possible, such as pinocytosis, etc. The Kow will generally overestimate the BCF of those chemicals that are biotransformed, but will provide no information whether the metabolite(s) that are formed show a lower bioaccumulation potential and are less toxic than the parent compound. In general, the metabolite(s) will be more hydrophilic and thus will have a lower bioaccumulative potential, but there are exceptions.

Since there is no relationship found and also not to be expected, based on mechanistic arguments, between e.g. the BCF of metals and Kow, in general Kow will not serve as an appropriate predictor of the BCF of metals and other substances, such as polymers, inorganic compounds, etc.

In many cases, the Kow will thus likely provide a worst-case estimate of the BCF for organic substances. For the other substances, either experimental BCF values or other estimation approaches are required.

2.3.4 General evaluation of the BKH/HASKONING-procedure

The BKH/HASKONING-procedure has resulted in two procedures, one for ecotoxicity and one for mammalian toxicity, to select substances that have met the cut-off values for the properties: Persistence and Toxicity and Bioaccumulative potential. The evaluation of the properties and cut-off values is shown in the previous sections. Below, the evaluation is shown on the entire procedure, the database that is used, and the substances that were used. The section ends with other properties that may be important within the procedure.

Procedure
The following questions will be addressed with regard to the procedure:
a) does the procedure result in a worst-case estimate of hazard assessment;
b) does the procedure need distinct approaches for mammalian toxicity and for the environment;

c) do substances need to meet all cut-off values for each of the PTB-criteria ?

Ad a:
In the BKH/HASKONING study, each of the PTB-criteria provides a different approach towards worst-case or best case with regard to hazard. For example, the Kow will, in general, result in a worst-case bioaccumulative potential for organic chemicals. Once a BCF is available the experimental value will prevail, and may result in a less stringent B-value. Analogously, the highest half-life of either biodegradation in water or photo-oxidation in air will be used for the P-property, which is a least stringent approach. However, for toxicity, if any one of the properties shows that a substance is toxic, it will be given the most stringent T-property. Whether this can be called a reasonable worst-case approach requires further discussion.

Ad b:
When a large number of substances need to be evaluated, one cannot always distinguish the most relevant protection level, either the environment or human health. Therefore, it may be more efficient to use one procedure. For each of the PTB-properties, the properties then need to be further defined. In particular for Toxicity, a substance may be called toxic if any of the underlying cut-off values (for the ecotoxicity and mammalian toxicity properties) is met. However, since PTB-properties may be used for different political or hazard assessment purposes, distinct procedures and PTB-properties may be used, which requires further discussion.

Ad c:
The BKH/HASKONING-procedure has resulted in a list of substances that were considered Persistent and Toxic and Bioaccumulative. The PTB-substances were primarily selected as potentially the most hazardous substances. If risk assessment was in place, the following two examples show that if any or not all of the PTB-criteria are met, the substance may still pose a risk.

The first example is PCBs, which will meet the Persistence and Bioaccumulative criteria, but will not meet the Toxicity criterion for ecotoxicity, since there are no experimental values showing that the LC50 of PCBs is less than 1 mg/L. However, the mammalian related toxicity criteria probably would have selected the PCBs as PTBs, in this case. Thus, whereas PCBs are PTB-substances and proved to be a risk for both the environment and human health, the BKH/HASKONING-study will not recognise PCBs as PTB-substances for the environment.

The second example is LAS, which fails to meet all the PTB-criteria, but still may pose an environmental risk. LAS is produced in very large amounts, is used widespread, and is emitted to the aqueous environment in large quantities. Only because wastewater treatment plants degrade so much of the LAS, the concentrations of LAS in receiving surface water do not pose a significant risk. However, in places where no wastewater treatment occurs, LAS may still pose a (local) risk.

There may be a need to identify very toxic substances, on the basis of toxicity alone, thus regardless of the P- and B-properties. There may also be a need to identify toxic

substances, in combination with P- and B-properties. In addition, there may be a need to identify persistent and/or bioaccumulative substances, regardless of the T-property. Tyle and Niemelä (1998) recently suggested using QSARs for selection of POPs and selected 539 substances from a list of 166,075 substances. The selection was entirely based on the P- and B-properties: biodegradation, photo-oxidation, bioaccumulation, volatility and hydrolysis. Their motivation for not including toxicity was that toxicity data are scarce, and that substances with a high log Kow would be selected as toxic chemicals anyway.

Thus, there is a need to first determine or to estimate the individual PTB-properties, and in a later stage to use them for further selection procedures. There is thus also a need to define where a substance needs to be placed in the hypothetical PTB-space, when not all criteria are met. For example, estimates or worst-case default values may be chosen for missing data. How to deal with the different PTB-properties will be further discussed.

Data: database, availability, and quality
The BKH/HASKONING-procedure has searched the ISIS/Riskline database for information on the PTB-criteria. The quality of the data in the database has, however, not been further evaluated. Whether or not this has led to false positives or to false negatives cannot be judged without a case-by-case evaluation of the data. BKH/HASKONING already pointed out that many data are missing, which requires further efforts to obtain estimates, experimental values, or defaults for the missing data.

Substances
The BKH/HASKONING-procedure has searched for data and followed the criteria for organic compounds, for which in many cases the CAS numbers were available, and for which a SMILES notation could be retrieved. However, there are many non-organic or other less defined substances, such as metals, inorganic compounds, organometals, mixtures, polymers, etc. In addition, for some of the organic compounds, no SMILES notation can be found when the (two- or three-dimensional) structure is too difficult to be translated into a linear code. As mentioned earlier, Verhaar et al. (1992) recently showed that among 2000 High Production Volume Chemicals, approximately 55% could not be classified as organic chemicals. It would be interesting to know how many of the existing chemicals can be classified according to Verhaar et al. (1992).

2.4 Final selection of PTB-properties

Based on the evaluation given in sections 2.1 and 2.2, the following is proposed (Table 2.4). It is suggested to first determine a distribution profile, and then to determine PTB-properties. This results in a PTB-profile of a substance. Persistent hydrophobic organic substances will reach relatively high concentrations in soil, sediment, and biota compared to those concentrations in air and water. However, the flux of the substances through and the transport by the latter two environmental

compartments requires (the estimation of) the PTB-properties in those compartments for further hazard or risk assessment.

Table 2.4. Selected properties, which are required to provide a PTB-profile.

Properties	Comments
Distribution profile	simple Mackay level I multimedia model, which helps to select most relevant PTB-properties
Persistence • Biodegradation in water • Photodegradation in air • Hydrolysis in water	one approach for human health and the environment
Toxicity for the environment • Acute aquatic toxicity Mammalian toxicity • Acute toxicity (oral, dermal, inhalation) • Chronic toxicity (prolonged exposure) • Carcinogenicity / mutagenicity / reproductive toxicity	two approaches, one for human health and one for the environment; further selection at later stage
Bioaccumulative potential • BCF • log Kow	one approach for human health and the environment

3. METHODS FOR DETERMINING PTB-PROPERTIES

3.1 Summary

Starting point of this chapter is to make an inventory of 'simple' estimation methods for the PTB-properties. Only selected methods are described that are taken from evaluation studies. Selected models are available for persistence, aquatic toxicity, and bioaccumulative potential. These models are described in the present chapter. In most cases these models are suitable for organic substances, and not for 'other' substances. For mammalian toxicity no estimation model has adequately been validated, and reliable methods seem not to be available, although much research is undertaken. In addition to the estimation models, accepted experimental methods are provided for reasons of comparison.
It is suggested to use:

- *The ECB-model or BIODEG for estimating biodegradation in water for organic substances.*
- *AOP for atmospheric photo-oxidation for organic substances.*
- *HYDRO for hydrolysis in water for organic substances.*
- *AQ-1 for aquatic toxicity for class 1 organic substances, AQ-2 for class 2 organic substances, and AQ-1 with an additional safety factor, for classes 3 and 4 organic substances. When it is assumed that an organic chemical has a molecular weight of approximately 250 g/mol, the corresponding log Kow values for class 1 organic substances are > 4.7, > 5.9, and > 7.1, respectively.*
- *No (Q)SAR system for mammalian toxicity.*
- *BCFwin for organic substances with a log Kow between 1 and 7, and KOWWIN for other organic substances.*

3.2 Introduction

In this chapter an overview will be given of methods that can be used to estimate or experimentally determine the selected PTB-properties. The required physico-chemical properties for the environmental distribution profile, i.e. aqueous solubility (S), vapour pressure (Vp), octanol/water partition coefficient (Kow) and the acid or base dissociation constant (pKa), are expected to be known, or to be relatively easily estimated or determined (see Chapter 5). First, a few related projects are mentioned, then the methods are summarised.

3.2.1 Related projects

In the past few years several attempts have been made to estimate missing data. Many individual papers and books showed the result of (Q)SARs, each having a specific applicability for estimating i) physico-chemical properties or ii) (eco)toxicological

endpoints. The following list shows the most relevant overviews of (Q)SAR or related studies and workshops, that are used in the present report.

- OECD (1993) - A workshop which was held in 1993 on the application of (Q)SARs to the estimation of properties important in exposure assessment. The relevance of the workshop to the present report is that it focuses on property estimation, such as physical-chemical data, degradation rate constants, sorption and accumulation. In addition, the workshop dealt with exposure modelling and computer programs that were used for property estimation.
- EU (1995a-b) - An EU-DG XII project was initiated to give an overview of structure-activity relationships for environmental endpoints. Relevant parts of that project for the present report are QSARs for ecotoxicity (280 models), for biodegradation (70 models), for chemical degradation in the gas phase (48 models), and for chemical degradation in the aqueous phase (65 models).
- Wilson et al. (1995) provide an overview of a workshop. The purpose of which was to review and assess the utility of physiologically based pharmacokinetic/pharmacodynamic (PBPK/D) modelling and structure-activity relationships (SAR) techniques as decision-support methodologies for making risk assessments and characterisations, following exposures to hazardous substances in the environment.
- The EU Technical Guidance Documents for the risk assessment of new and existing substances (TGD, 1996). The TGD describes evaluated QSARs that fit in the EU risk assessment framework, i.e. on exposure and effect assessment. The QSARs have also been incorporated in the European Union System for the Evaluation of Substances (EUSES).
- SETAC workshop (1998) on persistence and long-range transport.
- RIVM study: "A screening of (Q)SARs for human toxicological endpoints" by Hulzebos et al. (1999).

3.3 Persistence

For estimating the persistence of substances, many processes that are involved in biotic and abiotic transformation reactions in the different environmental compartments can be used. For the most simple approach, biodegradation in water, photo-oxidation in air, and hydrolysis in water are currently selected as properties for persistence.

With respect to biodegradation in water, two types of biodegradation can be distinguished: a) primary biodegradation, and b) ultimate biodegradation or mineralisation. Analogous to the EU QSAR study (EU, 1995a), a compound is considered to be readily biodegradable when the oxygen consumption in a ready biodegradability test is more than 60% of the theoretically possible oxygen consumption (ThOD), within a month (or 28 days). If this limit is not reached, the chemical is considered to be persistent. When biodegradability tests would have been extended to longer than 28 days, substances could become degraded, which makes them inherently biodegradable. However, for the simplest worst-case approach, the latter will not be included.

3.3.1 SAR and/or QSAR

Biodegradation in water
The following models will be briefly described: (i) the linear and non-linear model that are incorporated in the BIODEG program (Howard et al., 1992; Boethling et al., 1994; SRC, 1992), (ii) two of the models described in an OECD-report (Degner et al., 1993), and (iii) the ECB-model on biodegradation (Loonen et al., 1996). Those models are the best available according to an EU study (EU, 1995a; Rorije et al., 1998). Both BIODEG and the OECD models are reported in the TGD (1996).

Description: BIODEG (BIO-1)
Equation: Not given. The BIODEG program estimates the probability for the rapid aerobic biodegradation of an organic chemical in the presence of mixed populations of environmental micro-organisms. The outcome of the model can be translated to a half-life time in the range of days, weeks, months, etc.
Statistics/Validity: Internal validation of the model shows that for 186 chemicals, 97.3% was correctly predicted as "biodegrades fast". For 109 chemicals, 76.1% was correctly predicted as "does not biodegrade fast". For all the 295 chemicals, 89.5% was correctly predicted. External validation of BIODEG showed that of a set of 488 MITI (Japanese Chemicals Inspection and Testing Institute) biodegradation test data, approximately 90% of the degradable substances were predicted as such by BIODEG. However, BIODEG misclassified 56% of the non-degradable substances. Therefore, a significant amount of persistent chemicals are predicted by BIODEG to be degradable (EU, 1995a). It must be noted that the MITI database contains conservative values, i.e. the test shows ready degradability results, which are difficult to compare to the BIODEG results. However, this external validation is the only one available, and may indicate that BIODEG may overestimate biodegradability. This may imply that substances are predicted as being biodegradable, while they are persistent.
Descriptors: BIODEG estimates are based upon the molecular weight (MW) and 36 fragment constants that were developed using multiple and non-linear regression analyses. A discussion of the methodology is presented in Howard et al. (1992) and Boethling et al. (1994). Experimental biodegradation data were obtained from Syracuse Research Corporation's database of evaluated biodegradation data (Howard et al., 1987). The database currently contains a total of 295 chemicals, of which 186 were evaluated as "biodegrades fast" and 109 were evaluated as "does not biodegrade fast".
Domain: The relationship is restricted to a certain chemical domain: predictions will be provided for any compound that contains at least one of the 36 structural fragments. If no such structural element is present in the substance of concern, then BIODEG will not provide estimations. The program is capable of estimating biodegradation in water for organic chemicals for which a SMILES notation is or can be made available,

e.g. through a CAS library. This limits the estimation of biodegradation for other classes of chemicals, such as polymers, organometals, etc.
Experience: Requires some chemical background to identify organic substances and to convert chemical structure into SMILES
Costs: Approximately $ 1000 for software.

Description: The OECD models described by Degner et al. (1993) are restricted to specific chemical classes, i.e. for acyclic aliphatic (OECD model 75) and monocyclic aromatic (OECD model 78) compounds, respectively.
Equation: Not provided.
Statistics: Not provided.
Descriptors: The methods make use of structural fragments, but they are restricted to acyclic aliphatic and monocyclic aromatic compounds.
Domain: Acyclic aliphatic and monocyclic aromatic compounds.
Experience: Requires some chemical background to identify organic substances.
Costs: Not given.

Description: The ECB-model on biodegradation as described by Loonen et al. (1996) is not restricted to specific chemical classes, but can be used for organic substances. The outcome of the model is 'ready biodegradable' or 'not ready biodegradable'.
Equation: Not provided.
Statistics/Validity: A large number of descriptors is statistically adequately handled by using multivariate statistics for model fitting, in this case the Partial Least Squares (PLS) algorithm, instead of multilinear regression, as in the case of BIODEG. The model was created with 75% of the data, and then predicted the remaining 25%. This step was repeated three times. The predictive performance of the model was 83 to 87% correct of the predictions 'not-ready biodegradable' and 77 to 83% correct for predictions 'ready biodegradable'.
Descriptors: The ECB-model is a PLS model developed by Loonen et al. (1996). This model uses the largest set of MITI-I tested chemicals available (894 compounds) and a large set of predefined descriptor fragments (144 fragments).
Domain: Organic substances.
Experience: Requires some chemical background to identify organic substances.
Costs: Not given. (Estimates may become freely available from the ECB).

A decision tree for selecting the appropriate model is given in Rorije et al. (1997), where it is suggested to compare the outcome of two different models, i.e. BIODEG and one of the OECD models. In those cases that the two models can be applied, the outcome of the BIODEG model can be used as an addition to the appropriate OECD model. When the models disagree, the results are highly suspect.

Photodegradation in air
The recommended method (OECD, 1993) for estimating reaction rate constants for the reaction of organic compounds with hydroxyl radicals is given by Atkinson (1987, 1988). The computer program AOP is available for the calculation of hydroxyl radical

and ozone rate constants (SRC, 1990). The AOP model, which is originally developed by Atkinson (1987), is also reported in the TGD (1996).

Description: AOP (PHO-1)

Equation: Not given. The AOP (Atmospheric Oxidation Program) program estimates the rate constant for the atmospheric, gas-phase reaction between photochemical produced hydroxyl radical and organic chemicals. It also estimates the rate constant for the gas-phase reaction between ozone and olefinic/acetylinic compounds. The rate constants estimated by the program are then used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl reactions and ozone (Meylan and Howard, 1993).

Statistics/Validity: Internal validation of AOP shows that for a list of 647 organic chemicals, over 90% of the estimated gas-phase hydroxyl radical rate constants are within a factor of two of the experimental values, while over 95% are within a factor of three of the experimental values. The numbers of tested chemicals are all chemicals for which an experimental value is available, which thus shows that AOP performs very well. AOP shows better predictions than the PCFAP program (Fate of Atmospheric Pollutants) of the US EPA GEMS (Graphic Exposure Modelling System) software, that estimates the same rate constants. For 617 of the 647 chemicals, PCFAP is within a factor of two for 49%, and within a factor of three for about 65%. Larger deviations are apparent for several chemical classes: compounds with more than 3 halogen atoms on the same carbon atom, chemicals with NO_x groups, phosphates, small heterocyclic rings (epoxides and aziridines), nitroalkanes, and aromatics which are not benzene derivatives, and perhalogenated alkanes (EU, 1995b).
An attempt for external validation was provided by Rorije et al. (1997). They have studied the prediction of environmental degradation rates for 1073 High Production Volume Chemicals (HPVC) using QSARs. For more than 50% of the compounds no predictions could be made, since they were either ill defined or present as mixtures. For oxidation in the atmosphere, the Syracuse model AOP (hydroxyl radical and ozone reactions) and the MOOH-method (hydroxyl radical reaction) of Klamt (1993) were used. For the MOOH-method, semi-empirical calculation of molecular orbital energies are required using the AM1 (Dewar et al., 1985) parametrisation in the MOPAC program (Stewart and Coolidge, 1990; Stewart, 1990). For 917 of the 1073 HPVC compounds, an estimation of the reaction rate constant for reaction with hydroxyl radical was possible using the AOP model, and for 864 compounds using the MOOH-method. The two models, however, give very different results, which thus makes conclusions about external validation difficult.

Descriptors: The method is based on molecular fragment constants (13 parameters for reaction centers and 71 substituent constants).

Domain: The total hydroxyl reaction rate constant is constructed from rate constants of four important types of reactions: i) H-atom abstraction from C-H and O-H bonds, ii) addition of hydroxyl radicals to C-C double and triple bonds, iii) addition to aromatic rings, and iv) reactions with N, S, or P.
Experience: Requires high level of scientific expertise.
Costs: Approximately $ 1000 for software.

Hydrolysis in water
There is a recommended OECD method (OECD, 1993) for experimentally derive the hydrolysis rate constant. Furthermore, the Syracuse software model, HYDRO, estimates rate constants for selected chemicals.

Description: HYDRO (H-1)
Equation: Not given. The HYDRO program estimates aqueous hydrolysis rate constants at 25°C for selected chemicals classes, such as esters, carbamates, epoxides, halomethanes and selected alkylhalides.
Statistics/Validity: No information available.
Descriptors: HYDRO rate constant estimates are based solely upon the chemical structure of a compound and are calculated from regression equations derived from experimental hydrolysis data. (Mill et al., 1987).
Domain: The program is capable of estimating hydrolysis in water for organic chemicals for which a SMILES notation is or can be made available, e.g. through a CAS library. This limits the estimation of biodegradation for other classes of chemicals, such as polymers, organometals, etc.
Experience: Requires some chemical background to identify organic substances and to convert chemical structure into SMILES
Costs: Approximately $ 1000 for software.

3.3.2 Expert judgement

The QSAR models on biodegradation in water, photodegradation in air, and hydrolysis in water are in fact expert models. No further expert judgement systems will be described.

3.3.3 Experimental

Biodegradation in water

Description: Several OECD Guidelines (301 A-F) describe standardised tests for biodegradation, such as ready biodegradation test, using adapted sludge, and high biomass concentrations (OECD, 1992). The tests may take 28 days or longer.
Experience: Usually Good Laboratory Practice (GLP) is required.
Costs: $ 4,000 - $13,000 for each test.

Photo-oxidation in air

Description: Commercial laboratories provide tests to measure photodegradation in air. A standardised method is, however, not available.
Experience: Usually Good Laboratory Practice (GLP) is required.
Costs: $2,600 - $ 40,000

Hydrolysis in water

Description: The OECD Guidelines (111) describes standardised tests for hydrolysis in water.
Experience: Usually Good Laboratory Practice (GLP) is required.
Costs: $ 3,100 for each test.

3.3.4 Summary, persistence

Several methods are available to estimate or to measure biodegradation in water, atmospheric photo-oxidation and hydrolysis in water. The costs, applicability and reliability vary between the different methods. For neutral, organic substances costs are low, and applicability and reliability are high. For other substances, however, applicability and reliability of the (Q)SAR methods are low, while costs of the experimental methods are high.

It is suggested to use the ECB-model or BIODEG for estimating biodegradation in water for organic substances. For atmospheric photo-oxidation it is suggested to use AOP for organic substances, and for hydrolysis in water it is suggested to use HYDRO, for organic substances. For 'other' substances no estimation models are available.

3.4 Ecotoxicity

For estimating the ecotoxicity of substances, acute and chronic toxicity data for several aquatic and terrestrial species can be used. For the simplest approach, we suggest only to select acute toxicity data for fish as property for ecotoxicity. The available QSARs for fish, Daphnia and alga are highly correlated, therefore selecting QSARs for any of the three species, will not make much difference. When an experimental value is required, the simplest approach is again, to select one species, i.e. fish. With respects to time and costs, a Daphnia test may be preferred. That decision, i.e. on e.g. which experiment is to be preferred, is outside the scope of the present report. The following methods can be used to estimate aquatic toxicity, of which their merits are described.

3.4.1 SAR and/or QSAR

Prior to use of any QSAR, the chemical needs to be classified into class 1, 2, 3 or 4, or as not to be classified, according to Verhaar et al. (1992). The classification requires information on the molecular structure of the chemical. If a chemical cannot be classified according to Verhaar et al. (1992), a default value can be used, an

experimental value needs to be derived, or an expert judgement can be provided on a case-by-case basis. The TGD (1996) reports QSARs for non-polar (class 1) and polar (class 2) narcosis type of aquatic toxicity for fish, Daphnia, and algae.

Class 1 substances (Verhaar et al., 1992)

Description: From the EU-report (1995a) the following QSAR for 96-h LC50 (in mol/L) to *Pimephales* promelas is selected for class 1 chemicals
Equation: log (LC50) = -0.846•log (Kow) -1.39 (AQ-1)
Statistics: n=58, r^2 = 0.937, Q^2 = 0.932, s.e. = 0.36
Descriptors: The only required physico-chemical parameters are Kow and MW.
Domain: The applicability domain is: -1.24 < log(Kow) < 5.13. The chemical domain is for organic chemicals, classified as class 1 (Verhaar et al., 1992).
Experience: Requires some chemical background to classify substances as class 1.
Costs: Very low

For class 1 chemicals, all QSARs are based on Kow, therefore no distinction needs to be made between different aquatic species or between acute or chronic endpoints. Since the endpoint, LC50, is expressed in mol/L, the value needs to be recalculated using the molecular weight (MW) to relate it to a cut-off value of 1, 0.1 or 0.01 mg/L. When it is assumed that an organic chemical has a molecular weight of approximately 250 g/mol, the corresponding log Kow values are > 4.7, > 5.9, and > 7.1, respectively. It must be noted that it is recognised that substances with a log Kow > 7 will probably not show acute toxicity (Veith et al., 1983).

Class 2 (Verhaar et al., 1992)

Description: From the EU-report (1995a) the following QSAR for 96-h LC50 (in mol/L) to *Pimephales* promelas is selected for class 2 chemicals
Equation: log (LC50) = -0.725•log (Kow) -2.16 (AQ-2)
Statistics: n=86, r^2 = 0.902, Q^2 = 0.897, s.e. = 0.33
Descriptors: The only required physico-chemical parameters are the Kow and MW.
Domain: The applicability domain is: -1.31 < log (Kow) < 6.21. The chemical domain is for organic chemicals, classified as class 2 (Verhaar et al., 1992).
Experience: Requires some chemical background to classify substances as class 2.
Costs: Very low.

For class 2 chemicals, all QSARs are based on Kow, therefore no distinction needs to be made between different aquatic species or between acute or chronic endpoints. Since the endpoint, LC50, is expressed in mol/L, the value needs to be recalculated using the molecular weight (MW) to relate it to a cut-off value of 1, 0.1 or 0.01 mg/L.

Other classes (Verhaar et al., 1992)
No QSARs are available for other classes in general, although for some specific chemical classes, QSARs are available. For classes 3 and 4, it may be suggested to assume that they are class 1 chemicals, estimate the LC50 using equation AQ-1, and

use a safety factor of between 10 and 10,000 on top of that estimated LC50. This since Verhaar et al. (1992) concluded that toxicity of those classes is between 10 and 10,000 times higher than that of class 1. The decision on the use and the magnitude of the safety factor, are not scientific, but arbitrary decisions.

3.4.2 Expert judgement

For chemicals that cannot be classified into one of the 4 classes according to Verhaar et al. (1992), but have a molecular structure that is highly related to a chemical belonging to one of the 4 classes, i.e. structural analogues, expert judgement may indicate the aquatic toxicity.

ASTER (Assessment Tools for the Evaluation of Risks) is a QSAR based expert system that was developed by the US EPA at Duluth. This predictive tool benefits from integration with the AQUIRE (AQUatic toxicity Information Retrieval system) database of toxic effects. The QSAR program predicts various physico-chemical properties of a molecule and then uses this information in QSARs to provide predictions of various fathead minnow, sheepshead minnow and *Daphnia magna* toxicity endpoints, biodegradability, bioaccumulation and various fugacity calculations. There are different QSAR models for different types of compounds (such as non-polar narcotics, polar narcotics, anilines, etc.) so these predictions are likely to be more reliable than for instance those of TOPKAT are as they relate directly to mode of action. Also the program will identify structural alerts associated with mutagenicity and carcinogenicity in a similar manner to DEREK (Cronin and Dearden, 1995c).

The Fraunhofer-Institute for Environmental Chemistry and Ecotoxicology has developed an expert SAR program for the German Federal Environmental Agency. The SAR model comprises more than 90 estimation models for endpoints considered relevant in environmental assessment. It predicts many physicochemical properties such as log Kow, solubility, boiling point etc., utilising external programs such as MEDCHEM for log Kow calculations. Using these predicted constants it provides estimates of toxicities to various trophic levels in the environment (e.g. fish, *Daphnia*, *Tetrahymena*, bacteria) as well as biodegradation and accumulation data from QSAR models. Also included are models for mutagenicity and rodent acute toxicity. Undoubtedly there is considerable overlap between SAR and ASTER (Cronin and Dearden, 1995c).

3.4.3 Experimental

Description: A 96-h acute toxicity test to fish can be performed according to OECD Guideline 203 (OECD, 1992). The test takes 4 days, acclimation takes two weeks, and the number of fish to be used is at least 50.
Experience: Usually Good Laboratory Practice (GLP) is required.
Costs: $ 8,000

3.4.4 Summary, aquatic toxicity

The ecotoxicity of class 1 and class 2 chemicals are highly related to the single descriptor, Kow. For classes 3 and 4, it may be suggested to assume they are class 1 chemicals, estimate the LC50 using equation AQ-1, and use a safety factor of between 10 and 10,000 on top of that estimated LC50. This since Verhaar et al. (1992) concluded that toxicity of those classes is between 10 and 10,000 times higher than that of class 1. The decision on the use and the magnitude of the safety factor, are not scientific, but policy decisions. Organic chemicals with a log Kow > 7 always is selected as very toxic. The ecotoxicity of other classes of chemicals, such as the polymers, etc. cannot be estimated.

For other classes of substances, other descriptors may be used. The EU reports (EU, 1995a; EU, 1995b) provide more detailed information on both the various classes of chemicals and the descriptors for aquatic toxicity. For the use selecting the simplest model, those models are outside the scope of the present report.

It is suggested to use AQ-1 for class 1 organic substances, AQ-2 for class 2 organic substances, and AQ-1 with an additional safety factor, for classes 3 and 4 organic substances.

3.5 Mammalian toxicity

For estimating the mammalian toxicity of substances, toxicity data for several endpoints can be used, i.e. those that were suggested by BKH/HASKONING (1996): acute toxicity (oral; dermal; inhalation), carcinogenicity, and reproductive toxicity. According to the TGD (1996), (Q)SARs may be used as a contributing factor for the risk assessment for human health for certain purposes and for certain endpoints. Most (Q)SARs which are used for toxicity endpoints are of the expert judgement type. The TGD currently does not recommend any defined (Q)SARs for mammalian toxicity endpoints. The following methods are described of which their merits are provided.

3.5.1 SAR and/or QSAR

A few examples are given below of SARs for specific classes of chemicals. Then, a series of SARs are shown that has a more general applicability. A different approach than in sections 4.1 and 4.2 is taken to describe the individual SAR methods, since the present SARs have been much less thoroughly evaluated as those in the previous sections.

Specific classes
A simple SAR procedure is used by the Center for Food Safety and Applied Nutrition (FDA, USA) to search for certain functional groups in additives that are known to occur in toxic substances. Also to sort the additives in 3 structure categories, each which are then further refined into levels of concern based on anticipated exposure. For each level of concern different levels of testing are recommended (Scheuplein, 1995).

Richard (1995) lists a series of examples that relate carcinogenicity to computed properties:

- for PCB and dioxins, 3D distances, hydrophobicity, electron affinities, entropies, polarisabilities and molecular electrostatic potential (MEP);
- for PAHs, stability of carbo-cation of bay-region diolepoxide (ΔE); and
- for chloroethanes and alkylnitrosamines, ΔH_f radical intermediates.

However, the predictability needs major improvements and better databases.

General applicability

In addition to the two methods above that are designed for specific groups of chemicals, other systems were designed for broader applicability, i.e. for various substances and toxicological endpoints. These systems can be divided in rule-based systems and statistical programs.

Rule-based systems create rules derived from human knowledge, obtained through experts. Statistical procedures use methods like linear multiple regression to identify structural entities with a specific toxic effect, and a mathematical function describing the relationship between the effect and the structural moiety. Both, rule-based systems and statistical procedures have their advantages and disadvantages. It is difficult to translate the implicit toxicological knowledge of experts into explicit rules, and a rule-based system is fundamentally subjective. However, the process of analysis of a rule-based system is rather transparent. Statistical methods use relationships that may be statistically sound, but could be without scientific meaning. They need relatively extensive training sets of data to formulate the various QSARs and algorithms. They should not be used outside the strict boundaries that are produced by the training set and calculated confidence intervals. The process of generating databases and relationships, however, may reveal new insights in toxicological mechanisms.

A different way of how the programs can be distinguished is the molecular level on which their predictions are based. In Figure 3.1, Parry (1994) shows the evaluation of several computer programs and expert views (Tennant et al., 1990) that predict the carcinogenic potential for a series of chemicals, tested by the NTP program. From the bottom to the top the carcinogenic properties of the chemicals are derived from the entire molecular structure (structural analogues, chemical classes), from fragments to intrinsic molecular properties (area, etc.) which are derived from the molecular structure.

A series of programs are evaluated in Appendix 3, which shows for each program, its name and type, its toxicological endpoint(s), its level of expertise that is required, a short description, its performance, and its output. The programs can be further categorised based on their prediction of toxicological endpoints:

- RASH, DEREK, HAZARDEXPERT, COMPACT, Oncologic and the program developed by Purdy (1996) are the rule-based programs. RASH and Oncologic only predict carcinogenicity. Purdy (1996) predicts carcinogenicity and skin sensitisation. DEREK and HAZARDEXPERT predict a variety of toxicological endpoints.

- TOPKAT, MULTICASE (TOXAlert), Progol and FALS are statistical programs. Progol and FALS only predict carcinogenicity. TOPKAT and MULTICASE predict a variety of toxicological endpoints.

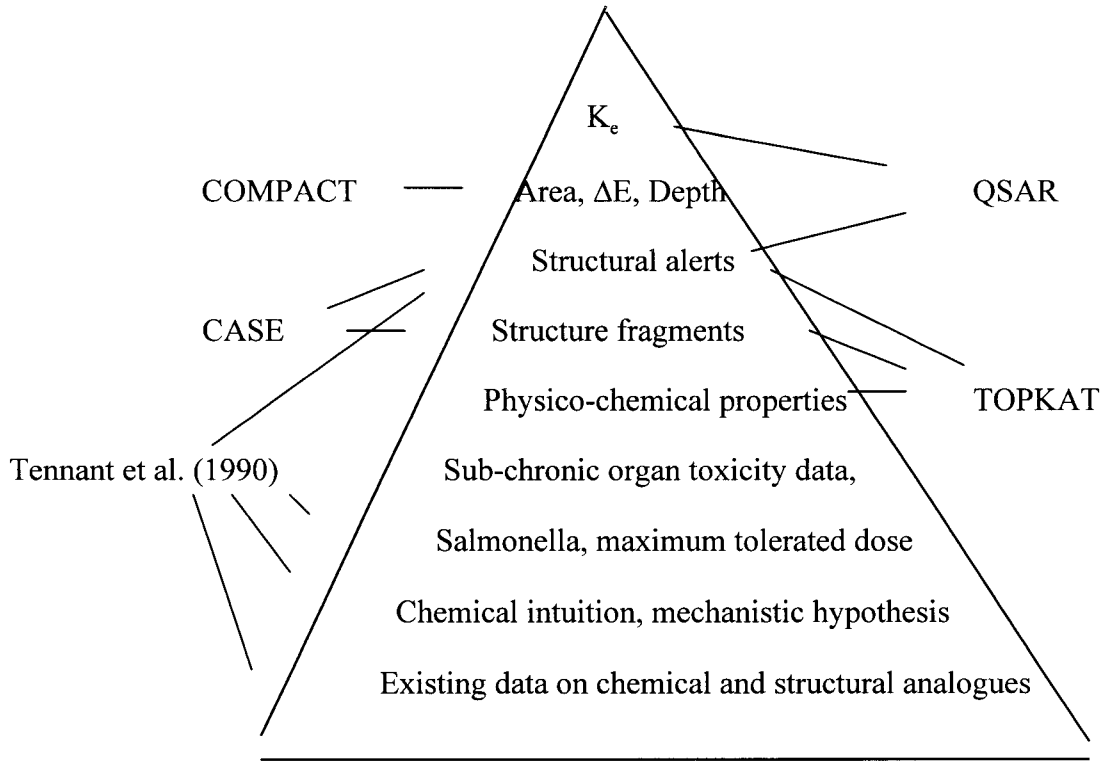


Figure 3.1. Prediction of chemical carcinogenicity according to Parry (1994).

The programs sometimes offer good performance statistics (Appendix 3). However, only few independent validation and performances studies have compared some of these programs. These comparisons showed that the programs generally present performances that are much worse, i.e. sometimes little better than random (Parry, 1994). Therefore an extensive validation is required before selecting or using any of those programs.

Only one blind trial was found in which the programs were to predict toxicological endpoints for chemicals for which the outcomes were unknown. Within the US National Toxicology Program (NTP) an extensive cancer bioassay program is set up, meant to test a few hundred substances. Tennant et al. (1990) started to predict the outcome of the first series of 44 chemicals to be tested. Other groups were asked to do the same. Some years later the predictive methods were evaluated when the outcomes of the NTP bioassays were available.

In 1994, Parry (1994) evaluated the programs, of which the results are shown in Table 3.1. It can be seen that the expert judgement of Tennant et al. (1990) shows the best predictions. According to Parry (1994) this is partly due to that they took into account short-term toxicity testing, since that seems to be the main difference between the prediction of the expert judgement and the programs (Parry, 1994).

Based on the carcinogenicity blind trial, it was stated that programs based on a single QSAR-rule would give less reliable predictions than programs that use more properties. In addition, rule-based QSARs programs performed better than statistical methods (Bristol et al., 1996). It should be noted, however, that statistical programs have learned from the rule-based programs and the other way around. The current differences will be less than described in Parry (1994) and Bristol (1996).

Table 3.1. Number of correct predictions of total of 44 substances that were tested in NTP cancer bioassay program (Parry, 1994). The different models are further described in Appendix 3, except Tennant et al. (1990), K_e and QSAR.

	Tennant et al. (1990)	K_e	DEREK	COMPACT	CASE	TOPKAT	RASH	QSAR
Carcinogenic in 2 species (n=5)	5	5	4	3	4	3	3	4
Probable genotoxic (n=9)	9	4	7	7	6	4	4	5
Species specific carcinogens (n=8)	6	3	4	1	3	2	4	1
Carcinogens in male rodents (n=3)	3	1	0	1	1	0	2	0
Carcinogenic in female rodents (n=2)	1	1	0	1	0	0	0	1
non-carcino-genic n=7)	6	4	5	2	4	0	6	1
Correct predictions (%)	88.2	52.9	58.8	44.1	52.9	26.5	55.9	35.3

In Appendix 3 a few selected programs are described in some more detail. No preference yet can be made for any of the programs, since further validation studies are required prior to choosing one.

In general, different systems have been developed for predicting a toxicological endpoint for mammalian toxicity. However, no system has been intensively studied for validation purposes, which makes it impossible to select one or more of the systems for predicting the various toxicological endpoints for substances. The following further explains this statement.

Hydrophobicity
Hansch et al. (1995) have evaluated the use of QSARs for the purpose of toxicology. They have a database of 6000 QSARs from which they can combine individual QSARs for different purposes, using descriptors, selected chemicals, selected (biological) endpoints, or any combination of those. In many cases hydrophobicity plays a role in enzyme-mediated reactions or even in mutagenicity. However, other parameters also play a significant role. For example, QSARs for mutagens fall into 3 clusters: dependent, non-dependent and partially dependent on hydrophobicity (Kow). It will be hard to anticipate all of the possible functional groups that can react with DNA and result in mutagenicity, and the role of hydrophobicity. One simple relationship between hydrophobicity and toxicity for one, let alone different classes of chemicals is therefore not possible.

LD50
The mammalian LD50 test is one of the most controversial toxicity tests, and many toxicologists question its validity and significance (Cronin and Dearden, 1995a). This since the LD50 is considered a whole body phenomenon and it is difficult to model and it may require parameters encoding information on metabolism, bioaccumulation, excretion, etc. However, the LD50 is still in many cases an obligate test. With this in mind much effort is being put into finding alternatives, especially *in vitro* alternatives. The problem of obtaining accurate and reliable biological data is especially pertinent to the QSAR analysis of mammalian acute toxicity data. Many QSAR studies have used data from commercial databases, of which the data are commonly not checked. This may be suitable for hazard assessment, but it is difficult to see how these data may be used satisfactorily in QSAR analysis.

The only analysis of a truly heterogeneous data set is that by Enslein et al. (1983b), who report a model based on 2066 chemicals and is based on whole molecule parameters and the presence of sub-structural features. Approximately 50% of the compounds were predicted within a factor of two. Their model is available in the TOPKAT (TOxicity Prediction by Komputer-Assisted Technology) software.

Developmental or teratogenic toxicity
Relatively little work has been performed in the QSAR analysis of developmental or teratogenic toxicity. This whole area is hindered by a lack of quality *in vivo* toxicity data to model, and by the mechanisms of action not being fully understood (Cronin and Dearden, 1995b).

Carcinogenicity
With respect to carcinogenicity, it has been appreciated that genotoxic and non-genotoxic mechanisms of carcinogenicity exist. The genotoxic carcinogen is defined as being one that "induces mutations of relevance to the aetiology of cancer by virtue of its ability to interact with DNA, its associated maintenance enzymes, or the metaphase spindle apparatus". A non-genotoxic carcinogen is defined as "being innocent in these respects, but is capable of causing changes in a tissue or the organism that result in the production of mutations relevant to carcinogenesis" (Cronin and Dearden, 1995b).

Non-genotoxic carcinogens
Little or nothing has been achieved in the area of QSARs for non-genotoxic carcinogens, a scientific area where thus much more effort needs to be applied. The areas of carcinogenicity and mutagenicity predictions by (Q)SAR methods are closely entwined. Predicting mutagenicity is based on a) qualitative description of molecular substructures that account for electrophilic reactivity, or b) quantitative description of the molecule in terms of its hydrophobicity or molecular orbital configuration or both (Cronin and Dearden, 1995b).

The expert system DEREK contains 'structural alerts' from 301 compounds, but it must be noted that it does not account for the molecular environment a substructure may be in, e.g. shielded by other un-reactive fragments, etc. (Cronin and Dearden, 1995b). Klopman et al. (1990) describe the use of the CASE methodology to analyse the Gene-Tox data base of *Salmonella typhimurium* mutagenicity results; 29 activating and 3 inactivating structural alerts were identified which correctly predicted the probability of carcinogenicity of 93,7% of the known mutagens and non-mutagens in the database. Later work using the MULTICASE (MULTIple CASE) algorithm showed further improvement in the prediction of mutagenicity (Klopman and Rosenkranz, 1992; Klopman and Rosenkranz, 1994a-c; Mersch-Sunderman et al., 1994). For some specific classes, E_{HOMO}, E_{LUMO}, Kow or a combination of the three is correlated with mutagenicity. However, the predictions are made for congener groups with a distinct mechanism of action, but for a general prediction, the equations are invalid.

Other non-lethal mammalian toxicological endpoints
For other non-lethal mammalian toxicological endpoints, such as irritation and respiratory allergy, the lack of quality *in vivo* toxicity data and the mechanisms of action not being fully understood hinders the development of QSARs. More work is required to make expert systems sufficiently accurate for reliable toxicity prediction (Cronin and Dearden, 1995c).

QSAR analysis in the area of (non-)lethal mammalian endpoints, such as skin sensitisation is bound to be dogged by the problem of lack of descriptors for reactivity. Thus the structural alert approach in combination with an expert system may be the best way of proceeding. This is also indicated by the success of the use of structural features in multivariate QSAR analysis (Cronin and Dearden, 1995c).

Expert systems
The most well known and wide-ranging QSAR based expert system is TOPKAT, developed and marketed by Health Designs Inc. The TOPKAT software contains QSARs that will provide predictions for mutagenesis, carcinogenesis, teratogenicity, rat oral LD50 and maximum tolerated dose, mouse inhalation, LD50, skin and eye irritation, *Daphnia magna* and fathead minnow toxicity, as well as a measure of biodegradability and interspecies extrapolations (Cronin and Dearden, 1995c).

The COMPACT (Computer-Optimised Molecular Parametric Analysis of Chemical Toxicity) procedure has been developed to discriminate between compounds likely to cause carcinogenesis on the basis of their putative ability to interact with a family of P450 cytochromes important in the toxicity process. This discrimination is based on the planarity of the molecule coupled with a low energy of activation, molecular features that are considered to be important in the binding of compounds with P450. This approach has shown considerable success in the modelling of over 100 carcinogens (Cronin and Dearden, 1995c).

QSAR predictions allow a level of confidence to be applied to the estimate. The database on which the model has been based can be searched so that the user can check whether the compound is adequately "covered" and so whether the estimate of toxicity is valid (Cronin and Dearden, 1995c).

3.5.2 Expert judgement

Many of the SAR and QSAR programs are in fact based on expert judgement (Appendix 3).

3.5.3 Experimental

Description: Guidelines for acute oral toxicity, acute dermal toxicity, and acute inhalation toxicity are described in the OECD Guidelines 401, 402, and 403, respectively (OECD, 1992).
Experience: Usually Good Laboratory Practice (GLP) is required.
Costs: acute oral: $4,500
acute dermal: $ 2,000 - $3,700
acute inhalation: $ 13,000 - $15,900

Description: Guidelines for chronic toxicity (prolonged exposure) are described in the OECD Guideline 407 (OECD, 1992).
Experience: Usually Good Laboratory Practice (GLP) is required.
Costs: Approximate costs of a 28-d repeated dose test in rat are $ 60,000.

Description: Guidelines for carcinogenicity are described in the OECD Guideline 451 (OECD, 1992).
Experience: Usually Good Laboratory Practice (GLP) is required.
Costs: Approximate costs of a 2-year test in rodents are $ 0.5 - 3 million

Description: Guidelines for reproductive toxicity are described in the OECD Guideline 414 (OECD, 1992).
Experience: Usually Good Laboratory Practice (GLP) is required.
Costs: $ 75,000

3.5.4 Summary, mammalian toxicity

Mammalian toxicity can be assessed for various endpoints. For many of them estimation programs are being developed, but they have been insufficiently evaluated for use. Further validation tests are required prior to choose one of the methods for estimation. Expert judgement and experiments will thus serve as the best methods to provide the toxicity properties.

Although much research is in progress, and several expert systems and computer programs have been developed, no single method has undergone a severe validation test. Therefore, the validity of the different methods cannot be provided. It must thus be recommended not to use any of the systems, until some further validation testing has been performed. Worst-case default values may be chosen for missing data.

3.6 Bioaccumulative potential

For estimating the bioaccumulative properties of substances, bioconcentration factors for different aquatic species can be used. For the simplest approach, we suggest to only select bioconcentration factors for fish as property for bioaccumulative properties. The following methods can be used of which their merits are described. The TGD (1996) suggest to use model B-1 for substances with a log Kow < 6, and to use model B-3 for substances with a log Kow > 6.

3.6.1 SAR and/or QSAR

In many cases BCF is related to Kow for non-ionic organic substances, for which the rationale is that the lipid tissue of the fish is the principal site for bioconcentration.

Description: The most general relationship for the log Kow range of 1.0 - 6.9, is that of Veith and Kosian (1983)
Equation: $\log (BCF) = 0.79 \cdot \log (Kow) - 0.40$ (B-1)
Statistics: $r^2 = 0.86$, $n = 122$
Descriptors: Kow
Domain: log Kow range of 1.0 - 6.9
Experience: Requires some chemical background to identify substances as non-ionic organic substances
Costs: Very low

Description: Nendza (1991) has established a non-linear relationship between BCF and Kow, for broader ranges of log Kow of 1.0 - 11.2
Equation: $\log (BCF) = 0.99 \cdot \log (Kow) - 1.47 \cdot \log (4.97 \cdot 10^{-8} \cdot Kow + 1) + 0.0135$

(B-2)

Statistics: n = 132
Descriptors: Kow
Domain: log Kow range of 1.0 - 11.2
Experience: Requires some chemical background to identify substances as non-ionic organic substances
Costs: Very low

Description: A parabolic equation was recalculated from Connell and Hawker (1988) as described in the TGD (1996), between BCF and Kow for fish, for the log Kow range of > 6, but with an upper limit for the log Kow of 10
Equation: $\log (BCF) = -0.20 \cdot \log (Kow)^2 + 2.74 \cdot \log (Kow) - 4.72$ (B-3)
Statistics: n = 43, $r^2 = 0.78$
Descriptors: Kow
Domain: log Kow range of 6 - 10
Experience: Requires some chemical background to identify substances as non-ionic organic substances
Costs: Very low

Description: BCFwin (B-4)
Equation: Not given. The program estimates the BCF from the molecular structure of the compounds, which must be given as the SMILES notation. The model uses a fragment constant methodology, which is based on fragments and correction factors. BCFwin recognises substances that are likely to be biotransformed, and which results in decreased BCF values (Meylan et al., 1997). A more extensive description is given by Meylan et al., 1999).
Statistics: n = 694
Descriptors: Fragment constants and interaction terms.
Domain: log Kow range of 0 to 11.
Experience: Requires some chemical background to identify organic substances and to convert chemical structure into SMILES.
Costs: Approximately $1000 for software.

The only required physico-chemical property for models B-1 to B-3 is the Kow, while for B-4 the SMILES notation is required. The chemical domain is for non-ionic organic chemicals. It must be noted that the equations may hold for non-ionic organic chemicals, but not for those outside the log Kow domain, and also not for ionizable or charged substances, and for metals and metalloids. Furthermore, chemicals with a relatively large molecular size (diameter > 10 Å or length > 5.6 Å) or molecular weight (> 1000 g/mol), or chemicals that are likely to be biotransformed do not seem to accumulate in fish.

In addition to the QSARs, i.e. B-1 to B-3, the BCFwin program that is incorporated in the Syracuse software provides some additional features. The EPA already uses this program, that estimates BCF based on log Kow. Estimates will be given also outside the log Kow range of 1-11 and biotransformation is partially included, the latter

process that will decrease the BCF (reference not available, yet). The drawback of the model is that the estimated log BCF will in general result be low for very hydrophobic chemicals, i.e. with a log Kow > 8. This implies that for deriving PTB-properties, the potential to accumulate through the food chain is estimated as very low. Therefore, the log Kow should be provided in addition to the estimated BCF, when BCFwin is used.

Whereas it is suggested to correct the Kow of ionizable substances for pH, we suggest not doing so for BCF. The reason for that is that in addition to the non-dissociated fraction of ionizable substances, the dissociated fraction may also contribute to bioconcentration processes. Escher and Schwarzenbach (1996) for example showed that the octanol/water partitioning of a series of substituted phenols highly depended on pH, but the biomembrane/water partitioning much less depended on pH. Only taking into account the bioaccumulative potential of the non-dissociated fraction could highly underestimate actual bioaccumulation. Thus, we propose to estimate the BCF of the non-dissociated ionizable substance, irrespective of its pKa, or pKb, and irrespective of the actual pH as a first estimation.

3.6.2 Expert judgement

For chemicals for which no estimation can be given, but that have a molecular structure that is highly related to a chemical for which an estimate can be given, expert judgement may indicate the Kow or BCF. The BCFwin model may also be called expert judgement.

3.6.3 Experimental

Description: Semi-static or flow-through bioconcentration tests can be performed that may take days to weeks according to the OECD Guidelines 305A to 305E (OECD, 1992).
Experience: Usually Good Laboratory Practice (GLP) is required.
Costs: $47,000 - $ 60,000

3.6.4 Summary, bioaccumulative potential

For many neutral, organic substances, BCF and Kow can be estimated using QSARs. The Syracuse program, BCFwin, also recognises organic substances that may be biotransformed, and result in decreased BCF values. BCFwin may potentially underestimate bioaccumulation through the food chain for extremely hydrophobic substances, i.e. with a log Kow > 7. For other substances, no QSARs are available. However, for several metals, BCF data will be available. For polymers, it may be expected that their BCF will be extremely low, since they are high molecular weight compounds. For mixtures, the BCF of the most hydrophobic and the most hydrophilic compound in the mixture may be estimated, which will provide the range of BCFs that can be expected. For inorganic compounds and organometals, only few data will be available.

It is suggested to use KOWWIN for organic substances that will result in an estimated log Kow. For organic substances with a log Kow between 1 and 7, BCFwin is recommended.

4. Relevant descriptors for estimating PTB-properties

4.1 Summary

The present chapter summarises the physical-chemical properties or descriptors that are required either for determining the distribution profile, as described in chapter 3, or as input for the estimation models, as described in chapter 4. Molecular structure, molecular weight (MW), Koa and the SMILES-notation of many substances can probably be obtained easily. Only in a few cases, they cannot easily be determined, e.g. MW or SMILES for polymers or mixtures, or they are not relevant, e.g. log Kow for metals and organometals. MO energy calculations require experts to interpret the results. pKa values can most reliably be obtained from experiments, but there are some software programs available (PALLAS, ACD/pKa, and SPARC). It is suggested to use:

- *KOWWIN to estimate log Kow for organic substances.*
- *WS-KOW to estimate aqueous solubility (S) for organic substances.*
- *MPBPVP to estimate vapour pressure (Vp) for organic substances.*
- *HENRY to estimate the Henry's law constant (He) for organic substances.*

4.2 Introduction

For the PTB-criteria that have been mentioned in the previous chapters, some can be relatively easy estimated using QSARs, other require expert judgement, and for some, no estimation methods are available. The present chapter summarises the physical-chemical properties or descriptors that are required for determining the distribution profile (chapter 3), or as input for models (chapter 4).

4.3 Molecular structure

Molecular structure is the key information that is required to categorise a substance into a chemical class, and to further determine or estimate physical-chemical properties.

Description: From the molecular structure and formula information on molecular fragments, size and other, e.g. the SMILES-notation, can be derived. The molecular structure and formula can be derived from the manufacturer or when other relevant information, e.g. the CAS no., is available from e.g. the manufacturer or the Chemical Abstract Services (Internet site: http://www.cas.org). It must be noted that even if the molecular structure is known, not always the SMILES-notation can be provided.

Experience: Requires some chemical background

Costs: Low

4.4 Molecular weight (MW)

Molecular weight is used in some of the estimation models, such as BIODEG.

Description: When the molecular formula or molecular structure is available, the molecular weight can be simply calculated.
Experience: Requires limited chemical background.
Costs: Low.

4.5 SMILES

The Simplified Molecular Input Line Entry System (SMILES) can be used as input into models and describes the molecular structure of a substance. The programs are able to derive structural fragments from the SMILES-notation.

Description: There are databases that provide the SMILES-notation from the CAS number, and some software packages can derive the SMILES-notation from a given molecular structure.
Experience: Requires some chemical background.
Costs: Low.

4.6 Molecular orbital (MO) energy calculations

MO energy calculations are used to estimate e.g. atmospheric photo-oxidation or pKa by some estimation models. Furthermore, these calculations are used in some human and aquatic toxicity related expert systems.

Description: MO energy calculations are used to determine the energy of the Highest Occupied Molecular Orbital (E_{HOMO}), and the Lowest Unoccupied Molecular Orbital (E_{LUMO}). E_{HOMO} and E_{LUMO} can be used to estimate oxidation reaction rates in the atmosphere (persistence in the atmosphere). The semi-empirical calculation of molecular orbital energies can be done by the AM1 (Dewar et al., 1985) parametrisation in the MOPAC Version 6.00 program (Stewart and Coolidge, 1990; Stewart, 1990).
Experience: Highly trained experts should perform these calculations and their interpretation.
Costs: Approximately $ 2000 for software.

4.7 n-Octanol/water partition coefficient (Kow)

The n-octanol/water partition coefficient Kow is a key parameter in studies on environmental fate and toxicology for (non-ionic) organic substances. Log Kow is

related to ecotoxicity, aqueous solubility, soil/sediment sorption and bioconcentration, and together with the Henry's law constant it is further being used in the estimation of the extent of volatilisation from or sorption to soil and vegetation. The TGD (1996) discusses the use of three QSARs for estimating Kow, i.e. CLOGP, KOWWIN, and AUTOLOGP, but does not specifically conclude which model should be preferred.

4.7.1 SAR and/or QSAR

Various models are being used to predict the Kow for organic chemicals from fragment constants and interaction terms. Furthermore, books are available that describe methods to estimate aqueous solubility, such as Lyman et al. (1991) and Verschueren (1983). Examples for automatic calculation are MedChem (1989), AUTOLOGP™ (Devillers et al., 1997), and KOWWIN (SRC, 1997). Only KOWWIN will be described, since both internal (Meylan and Howard, 1995) and external (Schüürmann et al., 1995) validation showed that this is the most accurate estimation program currently available.

Description: KOWWIN (KOW-1)

Equation: Not given. The program estimates the logarithmic octanol/water partition coefficient of organic compounds. KOWWIN requires only a chemical structure to estimate a log Kow. The program uses a fragment constant methodology. Coefficients for 135 individual fragments and groups, and 255 correction factors were derived by multiple regression of more than 2400 reliably measured log Kow values and comparisons between predicted and experimental values (Meylan and Howard, 1995). Since the log Kow for ionizable compounds will depend on pH of the environment, and on the pKa of the substance, the User's Guide provides the following equation to correct for ionisability:
log Kow (corrected) = log Kow (at pH 7.4) + log $(1+10^{pKa-7.4})$.

Statistics/Validity: Internal validation of the 2413 compounds showed a correlation coefficient of 0.981, a standard deviation of 0.219 and the absolute mean error of 0.161.
External validation showed that the Syracuse model predictions were most accurate (Schüürmann et al., 1995) among different prediction models for Kow. Furthermore, Meylan and Howard (1995) showed for 8900 substances that for the relation between estimated and experimentally determined log(Kow) values the cross-validated regression coefficient $Q^2 = 0.954$, and the standard deviation is 0.42 log units (factor 3).

Descriptors: Fragment constants and interaction terms.

Domain: (Mainly non-ionic) organic substances, and log Kow values between -3 and 7

Experience: Requires some chemical background to identify organic substances and to convert chemical structure into SMILES.

Costs: Approximately $ 1000 for software.

Although some of the available models claim to accurately predict the Kow for ionizable compounds, it is advised to regard these predictions as highly suspect. Also

suspect are predictions that result in log Kow values higher than ca. 7 or smaller than ca. -3, for substances with MW higher than ca. 500, or for substances with structures that have many different substituents, such as organic dyes. Predictions for metals, metalloids or organometals are not possible, not relevant or very unreliable.

4.7.2 Expert judgement

The models that are described under the QSAR models can be regarded as expert systems.

4.7.3 Experimental

Description: The Kow can be experimentally measured by the shake-flask method, the "slow-stirring" method, the HPLC method, the generator column method or by the ratio of the solubilities in n-octanol and water. Relevant OECD Guidelines are 107 and 117 (OECD, 1992).
Experience: Usually Good Laboratory Practice (GLP) is required.
Costs: $3,750 - $ 8,000

4.8 Aqueous solubility (S)

The aqueous solubility (S) is a key parameter in studies on environmental fate and on toxicology for all substances. S has been related to ecotoxicity, Kow, soil/sediment sorption and bioconcentration.

4.8.1 SAR and/or QSAR

Various models are being used to predict the S for organic chemicals from fragment constants and interaction terms. Furthermore, books are available that describe methods to estimate aqueous solubility, such as Lyman et al. (1991) and Verschueren (1983). The Syracuse program WS-KOW provides one of the best estimates for S for organic chemicals.

Description: WS-KOW (S-1)
Equation: Not given. The program estimates the aqueous solubility of organic compounds using the compound's log Kow, molecular weight (and if available, melting point), and corrections, following state-of-the-art relationships (Meylan et al., 1996). WS-KOW requires only a chemical structure, i.e. a SMILES notation, to estimate S. Correction terms are available for 15 structure types. The equations were derived from a database that contained 1450 compounds with measured log Kow, aqueous solubility and melting point.
Statistics/Validity: Validation of 817 compounds showed a correlation coefficient of 0.902, a standard deviation of 0.615 log units and the absolute mean error of 0.480 log units.
Descriptors: Equations and correction terms.

Domain: (Mainly non-ionic) organic substances.
Experience: Requires some chemical background to identify organic substances and to convert chemical structure into SMILES.
Costs: Approximately $ 1000 for software.

Predictions for metals, metalloids or organometals are not possible, not relevant or very unreliable.

4.8.2 Expert judgement

The models that are described under the QSAR models can be regarded as expert systems.

4.8.3 Experimental

Description: S can be experimentally measured following the relevant OECD Guideline 105 (OECD, 1992).
Experience: Usually Good Laboratory Practice (GLP) is required.
Costs: $3,200

4.9 Vapour pressure (Vp)

The vapour pressure (Vp) is a key parameter in studies on environmental fate for all substances. Vp and the Henry's law constant are used in the estimation of the extent of volatilisation from or sorption to water, soil and vegetation.

4.9.1 SAR and/or QSAR

Various models are used to predict Vp for organic chemicals from fragment constants and interaction terms. Examples for automatic calculation are MPBPVP (Syracuse software).

Description: MPBPVP (Vp-1)
Equation: Not given. The program estimates Vp from the (estimated) boiling point of organic compounds by three separate methods. MPBPVP requires only a chemical structure to estimate Vp.
Statistics/Validity: Internal validation of 805 compounds with known Vp values showed a correlation coefficient of 0.941, a standard deviation of 0.717 and the absolute mean error of 0.476.
Descriptors: Fragment constants and interaction terms.
Domain: (Mainly non-ionic) organic substances.
Experience: Requires some chemical background to identify organic substances and to convert chemical structure into SMILES.
Costs: Approximately $ 1000 for software.

Predictions for metals, metalloids or organometals are not possible, not relevant or very unreliable.

4.9.2 Expert judgement

The models that are described under the QSAR models can be regarded as expert systems.

4.9.3 Experimental

Description: The Vp can be experimentally measured following the relevant OECD Guideline 102 (OECD, 1992).
Experience: Usually Good Laboratory Practice (GLP) is required.
Costs: $1,550

4.10 Henry's law constant (He)

The Henry's law constant (He) is an additional key parameter in studies on environmental fate for all substances. The Henry's law constant is being used in the estimation of the extent of volatilisation from or sorption to water, soil and vegetation.

4.10.1 SAR and/or QSAR

Various models are used to predict He for organic chemicals from fragment constants and interaction terms. Examples for automatic calculation are HENRY (Syracuse software). Furthermore, books are available that describe methods to estimate He, such as Lyman et al. (1991) and Verschueren (1983). The most often used approach for estimating He is to take the ratio of the vapour pressure (Vp) and the aqueous solubility (S): He = Vp/S. Since He is usually estimated from S and Vp, no experimental methods are described. The TGD (1996) describes HENRY as a suitable QSAR.

Description: HENRY (H-1)
Equation: Not given. The program estimates He of organic compounds. HENRY requires only a chemical structure to estimate He. The program uses a fragment constant methodology following a 'bond contribution method' and a 'group contribution method' (Meylan and Howard, 1991).
Statistics/Validity: Internal validation of 345 compounds showed a correlation coefficient of 0.97, a standard deviation of 0.34 and the absolute mean error of 0.21.
Descriptors: Fragment constants and interaction terms.
Domain: (Mainly non-ionic) organic substances, and log Kow values between -3 and 7
Experience: Requires some chemical background to identify organic substances and to convert chemical structure into SMILES.
Costs: Approximately $ 1000 for software.

Predictions for metals, metalloids or organometals are not possible, not relevant or very unreliable.

4.10.2 Expert judgement

The models that are described under the QSAR models can be regarded as expert systems.

4.11 Octanol/air partition coefficient (Koa)

The n-octanol/air partition coefficient (Koa) is a key parameter in studies on environmental fate (non-ionic) organic substances. Log Koa is used in the estimation of the extent of volatilisation from or sorption to soil and vegetation. Although there are some experimental methods available, usually Koa is estimated from the ratio of Kow and He: Koa = Kow/He.

4.12 pKa

The acid dissociation constant - but in this context, also the base dissociation constant pKb - can be most reliably determined experimentally. However, there are a few software programs that may be useful and that will be briefly described below. It must be noted that this overview is not an extensive one.

- PALLAS estimates the pKa of chemicals, based on the chemical structural formulae. The program uses Hammett and Taft equations to perform the calculations. A test on the accuracy of the program showed that the comparison of predicted and experimental pKa resulted in a r^2-value of 0.897 for 433 acidic and basic drugs. The program is available from CompuDrug Chemistry Ltd. A free demo version can be obtained from the internet site: http://www.compudrug.com/pkalc.html.
- ACD/pKa algorithm estimates pKa. It is based on an internal database of over 8,900 structures with over 23,000 experimental pKa values under different temperatures and ionic strengths. The Hammett-type of equations that are used cover over 1,500 combinations of over 650 of the most popular ionizable functional groups. When the Hammett equation parameters are not available, the modified Jaffe method is used. Estimations of 5- and 6-member (poly) heterocyclic systems are within ±0.2 or better. The program is available from Advanced Chemistry Development, Inc., Toronto (Canada), internet site: http://www.acdlabs.co.uk/contact.html.
- SPARC estimates pKa. It analyses the chemical structure relative to a specific reactivity, and builds on energy differences between the LUMO (Lowest Unoccupied Molecular Orbital) and the HOMO (Highest Unoccupied Molecular Orbital) state. It further takes into account differential resonance, electrostatic and solvation effects. The average deviation of the estimated pKa values from the experimental values was 0.33 for more than 3500 compounds. Information on the

program is available from the internet site:
http://www.als.com/nalp/appls/sparc/pka.html.

4.13 Summary, descriptors

Molecular structure, molecular weight (MW), Kow, S, Vp, He, Koa and the SMILES-notation of many substances can probably be obtained easily. Only in a few cases, they cannot easily be determined, e.g. MW or SMILES for polymers or mixtures, or they are not relevant, e.g. Kow for metals and organometals. MO energy calculations require experts to interpret the results. pKa values can most reliably be obtained from experiments, but there are some software programs available (PALLAS, ACD/pKA, and SPARC).

The commercially available Syracuse program, EPIWIN, contains several of the models mentioned in chapters 4 and 5, i.e. BIODEG, AOP, HYDRO, KOWWIN, WS-KOW, MPBPVP, HENRY, and BCFwin. The estimations done by the model requires a SMILES-notation or a CAS-number as input, and very rapidly calculates the PTB-properties. The costs for the model are approximately $5,000.

5. SCHEMES TO ESTIMATE THE PTB-PROFILE

5.1 Summary

This chapter provides a stepwise approach that needs to be followed to estimate or determine missing PTB-data. In step 1 the required information of the substance and its physical-chemical properties is used to determine a simple distribution profile. In step 2, the PTB-properties are determined. This PTB-profile can then be used to determine the PTB-class. A suggestion for the cut-off values for the PTB-criteria is enclosed. Further discussion needs to take place, which cut-off values to select for which relevant selection or prioritising processes in step 3. The latter is outside the scope of the present report.

5.2 Introduction

This chapter provides a stepwise approach (Figure 5.1) to estimate or determine missing PTB-data for simplified hazard identification purposes.

Flow-charts are provided to guide the required steps which need to be followed to determine the PTB-properties. In step 1 the required information of the substance and its physical-chemical properties is used to determine a simple distribution profile (section 5.3). In step 2, the PTB-properties will be determined (section 5.4). All the PTB-properties must be gathered in the PTB-profile, which can then be further used to determine the PTB-class. A suggestion for the cut-off values for the PTB-criteria is enclosed in section 5.5. Further discussion needs to take place, which cut-off values to select for which relevant selection or prioritising processes in step 3. The latter is outside the scope of the present report.

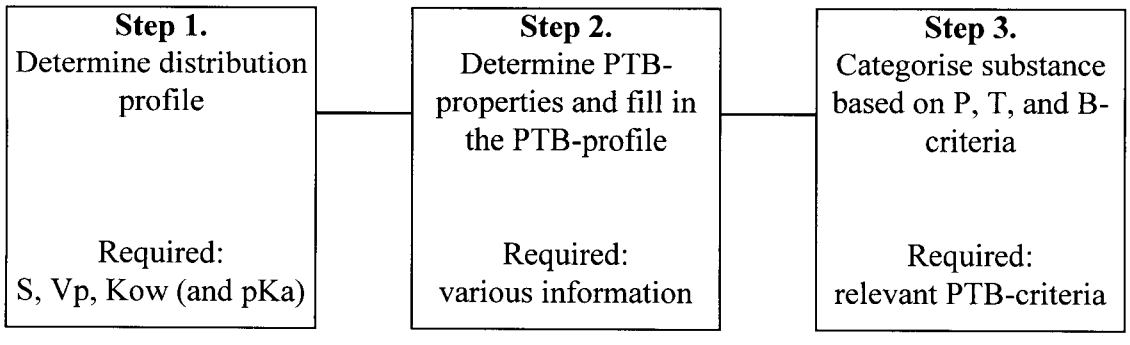


Figure 5.1. The step-wise approach to categorise substances following PTB-criteria.

5.3 Determination of distribution profile (step 1)

In step 1, the required information of the substance and its physical-chemical properties is used to determine a simple distribution profile (Table 5.1). When the distribution profile is determined (Appendix 2), the most relevant PTB-properties can be selected. Appendix 4 (Table A) provides the information to select the required information. Information of the chemical class (Appendix 4, Table B) will be used in step 2.

Table 5.1 Determination of distribution profile, which includes information on the substance and its physical-chemical properties.

Step 1. Determination of distribution profile	
Substance name:	
CAS no.:	
Chemical formula:	
Chemical structure:	
MW:	
Chemical class (according to Verhaar et al., 1992):	
Aqueous solubility:	mg/L
Vapour pressure	Pa
log Kow	
PKa	
Distribution profile	Air: % Soil/sediment: % Water: %

5.4 Determination of PTB-properties (step 2)

In step 2, the relevant PTB-properties are determined, for which Table 5.2 serves as guidance for which properties, values need to be filled in. The source of each value may be indicated, i.e. experimentally derived, estimated, expert judgement or default value. Flow-charts for the individual PTB-criteria are given in Figures 5.2 to 5.5 for persistence, aquatic toxicity, mammalian toxicity, and bioaccumulative potential, respectively. Table 5.3 summarises the required descriptors for estimating the distribution profile and the PTB-properties.

Table 5.2 Determination of the PTB-properties.

Step 2. Determination of PTB-properties		
PTB-property	*Value*	*Source*
P: biodegradation in water	Ready/not ready biodegradable $t_{1/2}$: hr/d/wk/months/yr.	Estimation / expert judgement / default / experimental
P: atmospheric photo-oxidation	$t_{1/2}$: hr/d/wk/months/yr.	Estimation / expert judgement / default / experimental
P: hydrolysis in water	$t_{1/2}$: hr/d/wk/months/yr.	Estimation / expert judgement / default / experimental
T: aquatic toxicity	LC50: mg/L EC50: mg/L	Estimation / expert judgement / default / experimental
T: acute mammalian toxicity	LD50 (oral): mg/kg LD50 (dermal): mg/kg LC50 (inhalation): mg/m^3	Estimation / expert judgement / default / experimental
T: chronic mammalian toxicity (prolonged exposure)	NOAEL (oral): mg/kg	Estimation / expert judgement / default / experimental
T: carcinogenicity / mutagenicity / reproduction toxicity		Estimation / expert judgement / default / experimental
B: bioaccumulation	BCF: L/kg Log Kow:	Estimation / expert judgement / default / experimental

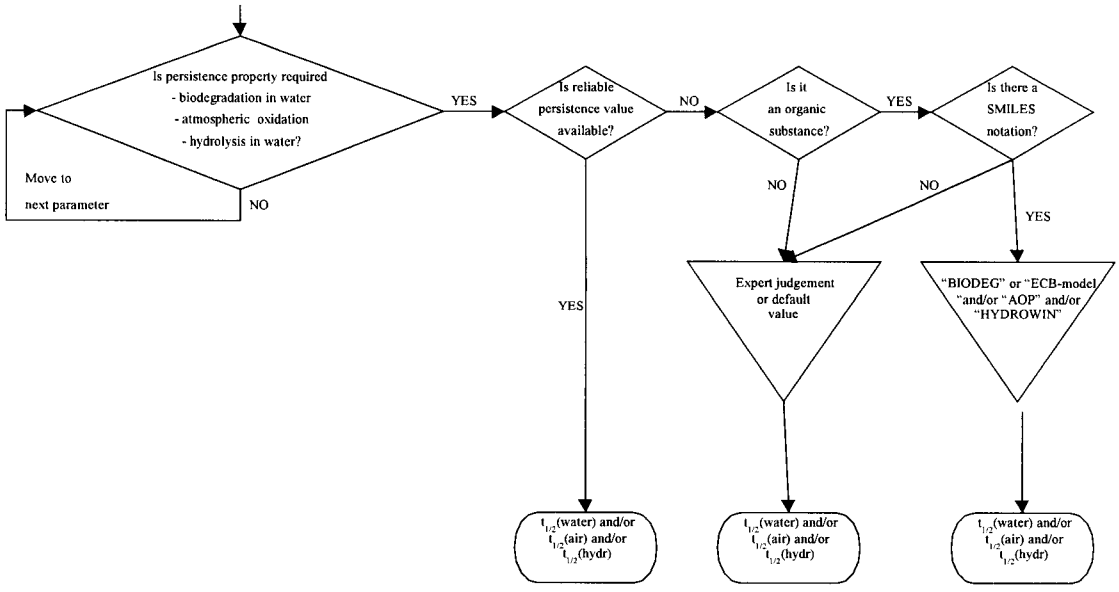


Figure 5.2. Scheme for determining persistence. A half-life is obtained for biodegradation in water, atmospheric photo-oxidation, hydrolysis in water, or all three properties. For each half-life the scheme should be followed. The half-life will be compared to the cut-off values in step 3. For the ECB-model the half-life should be read as 'ready biodegradable' or 'not ready biodegradable'. Experimental values will only be obtained from the literature, but will not be determined as a required part of the scheme.

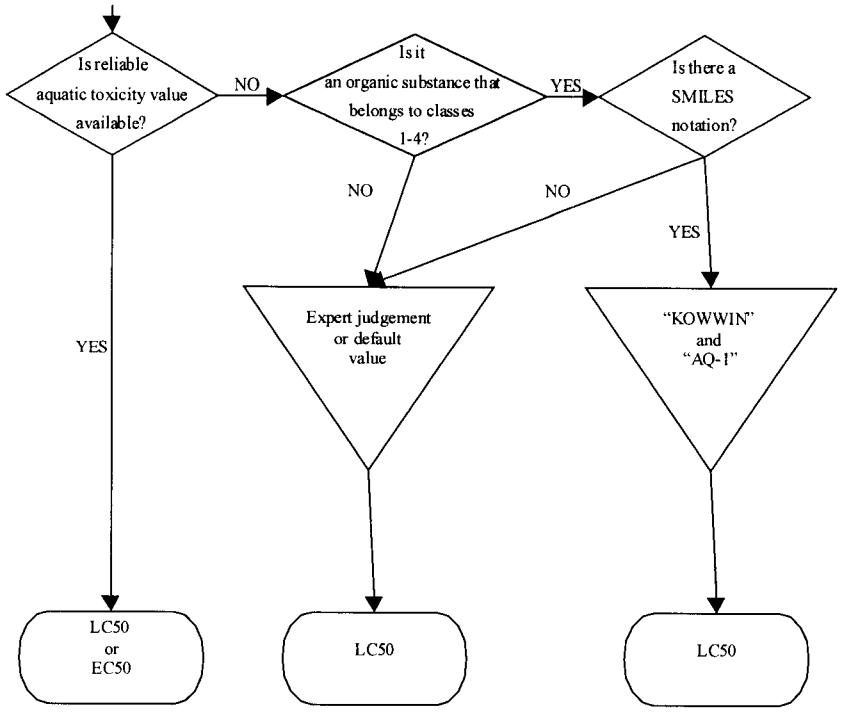


Figure 5.3. Scheme for determining aquatic toxicity. The outcome, LC50 or EC50 will be compared to the cut-off values in step 3. Experimental values will only be obtained from the literature, but will not be determined as a required part of the scheme.

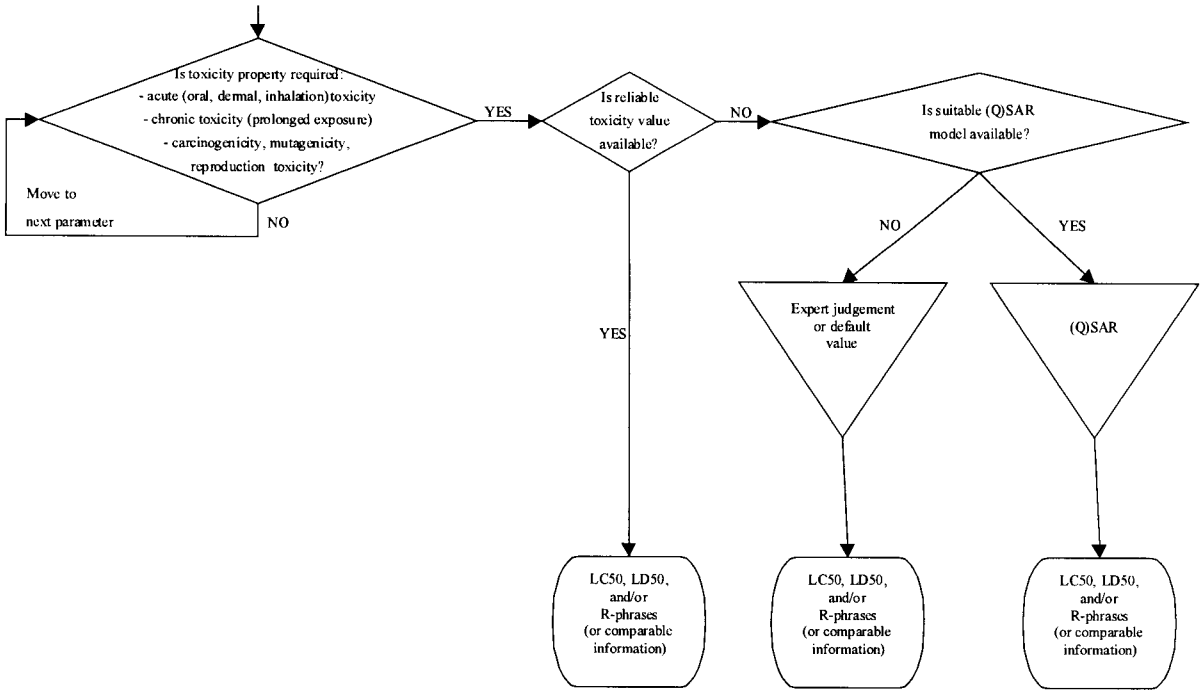


Figure 5.4. Scheme for determining mammalian toxicity. The outcome, LD50 or R-phrases will be compared to the cut-off values in step 3. Experimental values will only be obtained from the literature, but will not be determined as a required part of the scheme. A toxicity value is obtained from one or more of the following properties: acute toxicity (oral, dermal or inhalation), and carcinogenicity / mutagenicity / reproduction toxicity. For each property the scheme should be followed.

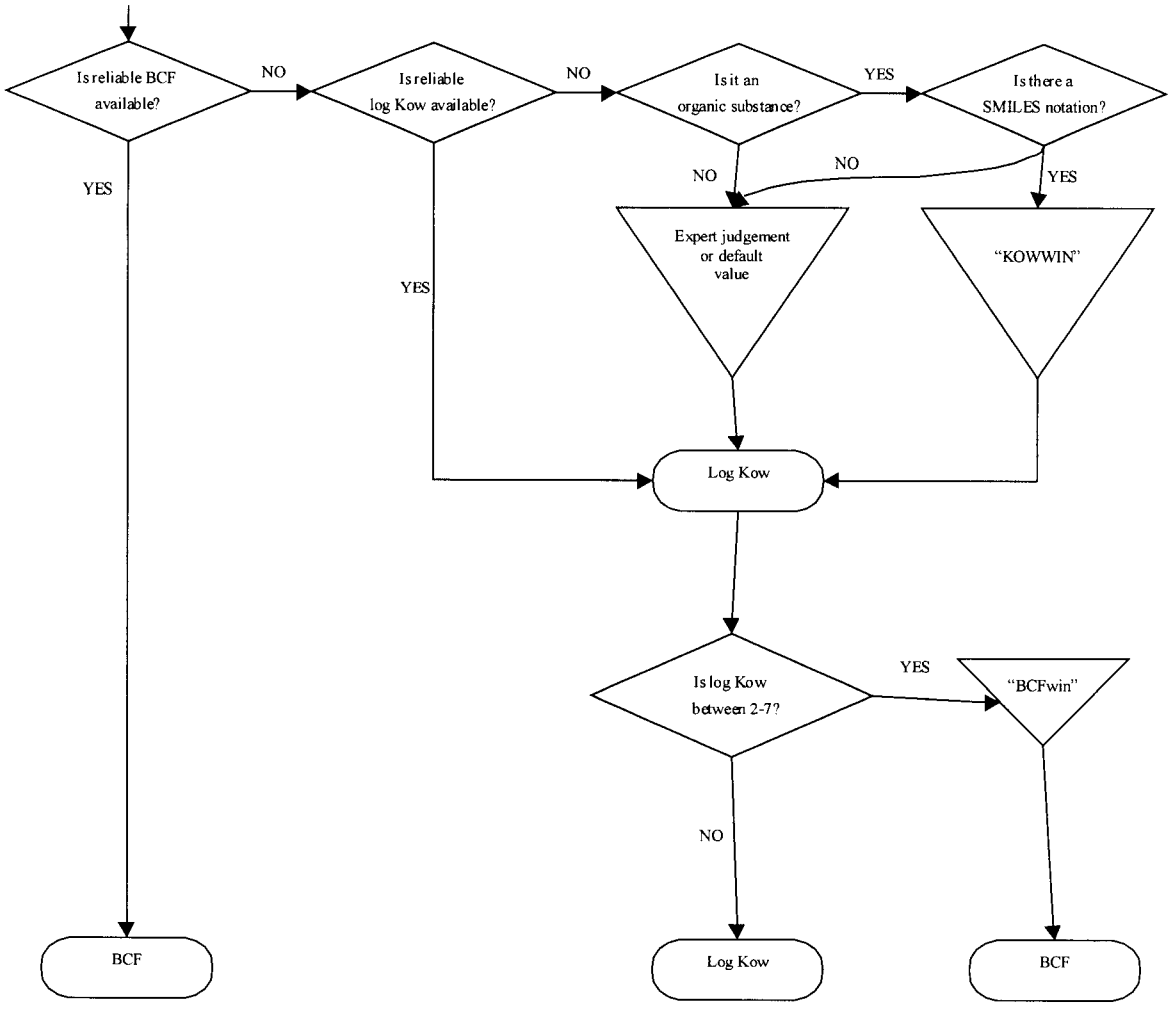


Figure 5.5. Scheme for determining bioaccumulative potential. The outcome, BCF or log Kow will be compared to the cut-off values in steps 3. Experimental values will only be obtained from the literature, but will not be determined as a required part of the scheme. The bioaccumulation potential is obtained from the bioconcentration factor (BCF) or from the octanol/water partition coefficient (Kow).

Table 5.3. Important descriptors for estimating the distribution profile and the PTB-properties.

Property	**Primary related descriptor**	**Secondary related descriptor**
Chemical class (Verhaar et al., 1992)	Chemical structure	
Distribution profile according to Mackay level I	S	Chemical structure SMILES CAS
	Vp	Chemical structure SMILES CAS
	Log Kow	Chemical structure SMILES CAS
	PKa	Experimental or chemical structure
Persistence	Biodegradation in water	Chemical structure SMILES CAS
	Atmospheric photo-oxidation	Chemical structure SMILES CAS
	Hydrolysis in water	Chemical structure SMILES CAS
Toxicity for the environment	Log Kow	Chemical structure SMILES CAS
Mammalian toxicity	Chemical structure	
Bioaccumulative potential	BCF	Chemical structure SMILES CAS
	Log Kow	Chemical structure SMILES CAS

5.5 Determination of PTB-class (step 3)

How a substance is categorised in a PTB-class depends on different factors, which should be based on decisions taken by the different stakeholders. The first step is to derive the PTB-properties, as is done in step 2 of Figure 5.1. The PTB-values then need to be compared to cut-off values, and further put into subclasses, for each property. Then, decisions need to be made how to compare the different properties for

a single criterion, i.e. P or T or B, which results in a $P_iT_iB_i$ class (i = 1 to n, n is the maximum number of subclasses). Finally, the $P_iT_iB_i$ class needs to be translated into a single PTB-class (Table 5.4).

Appendix 5 shows as an example that when the PTB-criteria are given equal weight, the classes may further depend on how each class is defined, e.g. as (P+T+B) or as (P x T x B). The number of substances within each class will depend on where the boundaries of each class are set, i.e. the actual cut-off values. A further discussion on these classes is required which should include the (policy) goal of the classes. If only a limited number of substances are to be selected in the highest class, that number will affect the boundary of the top class. The definition of each class, including its boundaries, thus highly depends on which choices are made. The latter is outside the scope of the present report, but should be discussed by the different stakeholders.

Table 5.4 Determination of the PTB-class, which requires information on the PTB-properties from step 2, and cut-off values.

Step 3. Determination of PTB-class	
PTB-profile:	$P_iT_iB_i$
PTB-class:	

A suggestion for the different cut-off values is given in the section below. The cut-off values are taken from different sources. No suggestion will be made on how to compare the individual cut-off values for properties for one of the PTB-criteria and for the combined PTB-criteria.

Factors that may be taken into account in selecting the cut-off values are current cut-off values from classification and labelling (CEC, 1993). In addition, environmental conditions may be taken into account, such as suggested by BKH/HASKONING for selecting cut-off values for ecotoxicity. Other examples are to take into account colder temperatures in Polar Regions, that may suggest taking stringent cut-off values for persistence, since at those colder temperatures biodegradation will proceed at a very low rate. Also as mentioned earlier, photo-oxidation reactions at Polar Regions and during night-time may occur at a very low rate. Furthermore, the biomass concentration in water, which will be much higher in coastal regions than in open oceans, may affect actual biodegradation rates in the environment. All these factors and arguments need to be included in further discussions on setting cut-off values.

Suggested classes for persistence:

- the ECB-model: 'ready biodegradable', and 'not ready biodegradable';
- the BIODEG model: '< days', 'days', 'months', and '>months';
- AOP: '<0.1 d', '0.1-1 d', '1-2 d', and '> 2 d';
- HYDRO: '< 1 d', '1-10 d', '10-30 d', and '> 30 d'.

Suggested classes for aquatic toxicity:

- EC50 or LC50 in mg/L: '< 0.01', '0.01-0.1', '0.1-1', and '> 1'.

Suggested classes for mammalian toxicity:

- acute toxicity
- oral: '< 25 mg/kg or R28', '25-200 mg/kg or R25', '200-2000 mg/kg or R22', and '> 2000 mg/kg';
- dermal: '< 50 mg/kg or R27', '50-400 mg/kg or R24', '400-2000 mg/kg or R21', and '> 2000 mg/kg';
- inhalation: '< 0.5 mg/L or R26', '0.5-2 mg/L or R23', '2-20 mg/L or R20', and '> 20 mg/L'.
- chronic mammalian toxicity: R48 (< 50 mg/kg body weight in 28-day study or < 150 mg/kg body weight in 90-day study)
- carcinogenicity / mutagenicity / reproduction toxicity:
- carcinogenicity / mutagenicity: 'yes or R45 or R49 or IARC 1 or IARC 2', and 'no';
- reproduction toxicity: 'yes or R60 or R61 or R62 or R63', and 'no'.

Suggested classes for bioaccumulative potential:

- BCF in L/kg: '< 1000', '1000-3000', '3000-5000', '> 5000';
- log Kow: '< 4.3', '4.3-4.7', '4.7-5', and '> 5'.

5.6 PTB-profile

Table 5.5 shows the summarising table of the PTB-profile and class.

Table 5.5. The PTB-profile, which requires basic information and some physical-chemical properties of the substance as input to firstly determine the distribution profile. Secondly, the PTB-properties can be determined. When reliable experimental data are available, these should be selected. When cut-off values for the PTB-criteria are selected, the overall PTB-categorisation can take place.

Step 1. Determination of distribution profile		
Substance name:		
CAS no.:		
Chemical formula:		
Chemical structure:		
MW:		
Chemical class (according to Verhaar et al., 1992):		
Aqueous solubility:	mg/L	
Vapour pressure	Pa	
Log Kow		
PKa		
Distribution profile	Air: % Soil/sediment: % Water: %	
Step 2. Determination of PTB-properties		
PTB-property	*Value*	*Source*
P: biodegradation in water	ready/not ready biodegradable $t_{1/2}$: hr/d/wk/months/yr.	estimation / expert judgement / default / experimental
P: atmospheric photo-oxidation	$t_{1/2}$: hr/d/wk/months/yr.	estimation / expert judgement / default / experimental
P: hydrolysis in water	$t_{1/2}$: hr/d/wk/months/yr.	estimation / expert judgement / default / experimental
T: aquatic toxicity	LC50: mg/L EC50: mg/L	estimation / expert judgement / default / experimental
T: acute mammalian toxicity	LD50 (oral): mg/kg LD50 (dermal): mg/kg LC50 (inhalation): mg/m^3	estimation / expert judgement / default / experimental
T: chronic mammalian toxicity (prolonged exposure)	NOAEL (oral): mg/kg	Estimation / expert judgement / default / experimental
T: carcinogenicity / mutagenicity / reproduction toxicity		estimation / expert judgement / default / experimental
B: bioaccumulation	BCF: L/kg log Kow:	estimation / expert judgement / default / experimental
Step 3. Determination of PTB-class		
PTB-profile:	$P_iT_iB_i$	
PTB-class:		

6. FINAL REMARKS AND CONCLUSIONS

6.1 Summary

The present report shows that for the PTB-criteria for the environment, a series of suitable estimation models are available. These models, however, are only to be used for selected organic substances. For 'other' substances, the models are not suitable. For the PTB-criteria for human health, the models for persistence and bioaccumulative potential should be the same as for those for the environment. However, for mammalian related toxicity endpoints, other models should be used. At present, no model seems to be suitable for reliably estimating the human toxicity endpoints.

6.2 Final remarks

6.2.1 PTB criteria

Persistence
For persistence, aerobic biodegradation in water, atmospheric photo-oxidation and / or hydrolysis in water are suggested as properties to identify persistence. As mentioned earlier, other processes may result in loss of a substance in the environment. However, only limited data will be available for those other processes. Therefore, the choice for the selected persistence properties will be the best feasible. It must be noted that precise estimation of persistence remains difficult, in particular for the marine environment. Other factors that make predictions difficult are the large differences in mean residence time of substances in various compartments, the different ambient temperatures at various geographic sites, the variety of biomass concentrations in different geographic sites, etc.

Toxicity
Although it can be discussed to separately estimate toxicity for the environment and mammalian toxicity, the estimates will provide different types of information. The toxicity properties for man and the environment may be used both or individually depending on the type step taken after determining the PTB-profile.

Ecotoxicity
Toxicity for the environment can be related to Kow for certain classes of organic chemicals. The toxicity estimate for neutral and polar organic chemicals will result in the so-called baseline toxicity. The toxicity property will then be compared to the toxicity cut-off value. Some substances will be more toxic than this baseline toxicity, and toxicity will then be underestimated. Following equation AQ-1, compounds with a log Kow > 7.1 will already show a baseline toxicity of < 0.01 mg/L, assuming that the molecular weight of the substance is approximately 250 g/mol. Thus, even when the actual toxicity is higher than the predicted toxicity, all substances with a log Kow > 7.1 will be selected as very toxic substances for the environment. It must be noted

that it is recognised that substances with a log Kow > 7 will probably not show acute toxicity (Veith et al., 1983).

Mammalian toxicity
In general, different systems have been developed for predicting a toxicological endpoint for human health. However, no system has been intensively studied for validation purposes, which makes it impossible to select one or more of the systems for predicting the various toxicological endpoints for substances. Although much research is in progress, and several expert systems and computer programs have been developed, no single method has undergone a severe validation test. Therefore, the validity of the different methods cannot be provided. It must thus be recommended not to use any of the systems, until some further validation testing has been performed.

Bioaccumulative potential
Since Kow is one of the key parameters for estimating bioaccumulation, the estimation of Kow is of relevance when data are missing. Rorije et al. (1997) have studied the prediction of Kow for 1074 High Production Volume Chemicals (HPVC) using QSARs. A prediction was possible for 996 out of these 1074 substances. Those compounds, for which no estimation could be made, were mainly inorganic substances or organometals. For 479 compounds a recommended literature value for Kow could be found, which showed a good comparison with the predicted Kow values ($r^2 = 0.969$). This shows that for organic substances the program that is used to estimate Kow is very suitable. Again, for other substances, such as metals, organometals, mixtures, polymers, etc., the program is not suited.

6.2.2 Miscellaneous

Industrial decision tools
The PTB-criteria were selected as the most important criteria that are related to hazard identification of substances. From the available knowledge and results from the literature, QSARs and other methods are identified which may help to estimate missing data. Additional information from within industry may be helpful in further identifying hazardous substances. Industry already makes use of expert knowledge and QSARs as decision tools to select substances prior to synthesis and production.

Validation of estimation methods
Whereas some individual estimation models have been evaluated and/or validated, the suite of models has not been validated. Even though if it will be very difficult to validate such complex models, the suite of models may be used to show if some well-known persistent organic pollutants (Vallack et al., 1998) will be identified as PTB-substances for the environment. Other models, in particularly those for mammalian toxicological endpoints, need to be further evaluated, and require further validation.

The commercially available Syracuse program, EPIWIN, contains several of the models mentioned in chapters 4 and 5. The estimations obtained from the model require a SMILES-notation or a CAS-number as input, and very rapidly calculates the PTB-properties. The costs for the model are approximately $5,000.

"Other" substances
Most of the QSARs and computer programs have difficulty with estimating properties for "other" substances, i.e. polymers, metals, organometals, inorganic substances, mixtures, petroleum products, etc. From expert judgement, rough estimates can be provided for some but not for all of the individual PTB-properties for those 'other substances (Appendix 6). This reflects the limitations of the models, and also the limitations of the current knowledge with respect to estimating properties. Whether models are required to obtain information on the PTB-properties may differ on a case-by-case basis. For example, for polymers, there is a guidance document that helps to decide which tests are relevant (EU, 1993).

It may be interesting to estimate the potential number of those "other" substances, compared to the number of organic substances, for which estimations can be made more easily, and further review the current knowledge on "other" substances.

Further steps
The PTB-criteria will serve as a useful step in hazard identification. As mentioned earlier, further steps are needed which will require possibly other criteria for distinct policy actions.

6.3 Conclusions

The present report shows that for the PTB-criteria for the environment, a series of suitable estimation models are available. These models, however, are only to be used for selected organic substances. For 'other' substances, the models are not suitable. For the PTB-criteria for mammalian toxicity, the models for persistence and bioaccumulative potential should be the same as for those for the environment. However, for mammalian related toxicity endpoints, other models should be used. At present, no model seems to be suitable for reliably estimating the toxicity endpoints.

References

1. Atkinson, R. (1987). Structure-activity relationship for the estimation of rate constants for the gas-phase reactions of hydroxyl radicals with organic compounds. Int. J. Chem. Kinet. 19, 799-828.
2. Atkinson, R. (1988). Estimation of gas-phase hydroxyl radical rate constants for organic chemicals. Environ. Toxicol. Chem. 7, 453-462.
3. Benfenati, E. and G. Gini (1997). Computational predictive programs (expert systems) in toxicology. Toxicol. Lett. 119, 213-225.
4. BKH/HASKONING (1995). Criteria voor zeer persistente toxische stoffen. R0216076/SRO.
5. BKH/HASKONING (1998a). Selection of PTBs phase 2. Selection of Toxic, Persistent and Bioaccumulative Substances, based on ecotoxicity data. M0216007/2471P.
6. BKH/HASKONING (1998b). Selection of PTBs phase 2. Selection of Toxic, Persistent and Bioaccumulative Substances, based on mammalian toxicity data. M0216007/2319P.
7. BKH/HASKONING (1998c). Selection of PTBs phase 3. Selection of Toxic, Persistent and Bioaccumulative Substances, based on mammalian toxicity data. First draft.
8. Boethling, R.S., P.H. Howard, W. Meylan, W. Stiteler, J. Beauman and N. Tirado (1994). Group contribution method for predicting probability and rate of aerobic biodegradation. Environ. Sci. Technol. 28, 459-465.
9. Bristol, D.W., J.W. Wachsman, and A. Greenwell (1996). The NIEHS predictive toxicology evaluation project. Environ. Health Persp. 104, 1001-1016.
10. CEC (1993). Commission of the European Communities. Commission Directive 93/21/EEC of 27 April 1993 adapting to technical progress for the eighteenth time Council Directive 67/548/EEC of the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Off. J. Eur. Communities, L110A.
11. Connell, D.W. and D.W. Hawker (1988). Use of polyniominal expressions to describe the bioconcentration of hydrophobic chemiclas by fish. Ecotoxicol. Environ. Saf. 16, 242-257.
12. Cronin, M.T.D., and J.C. Dearden (1995a). (Q)SAR in toxicology. 2. Prediction of acute mammalian toxicity and interspecies correlations. Quant. Struct.-Act. Relat. 14, 117-120.
13. Cronin, M.T.D., and J.C. Dearden (1995b). (Q)SAR in toxicology. 3. Prediction of chronic toxicities. Quant. Struct.-Act. Relat. 14, 329-334.
14. Cronin, M.T.D., and J.C. Dearden (1995c). (Q)SAR in toxicology. 4. Prediction of non-lethal mammalian toxicological endpoints, and expert systems for toxicity prediction. Quant. Struct.-Act. Relat. 14, 518-523.
15. Darvas F, and R. Eker (1996). Computer-assisted metabolic prediction: A help in development and design of pesticides. Pestic. Chem.: Adv. Int. Res., Dev., Legis., Proc. Int. Congr. Pestic. Chem., 7th. 287: Fed. Rep. Ger. Weinheim. ECBI/22/96-Add.10. 1996. Ad-hoc Working Group on Defatting Chemicals.
16. Degner, P., M. Müller, M. Nendza and W. Klein (1993). Structure-activity relationships for biodegradation. OECD Environment monographs No. 68, Paris, France.
17. Devillers, J., D. Domine, C. Guillon, S. Bintein and W. Karcher (1997). Prediction of partition coefficients (log P_{oct}) using autocorrelation descriptors. SAR QSAR Environ. Res. 7, 151-172.
18. Dewar, M.J.S., E.G. Zoebisch, E.F. Healy and J.J.P. Stewart (1985). AM1: A new general purpose quantum mechanical molecular model. J. Amer. Chem. Soc. 107, 3902-3909.
19. Enslein, K., T.R. Lander and J.R. Strange (1983a). Teratogenesis: a statistical structure-activity model. Teratog. Carcinog. Mutag. 3, 289-309.

20. Enslein, K., T.R. Lander, M.E. Tomb and P.N. Craig (1983b). A predictive model for estimating rat oral LD_{50} values. Princeton Scientific Publishers, Princeton, pp. 123.
21. Enslein, K. (1984). Estimation of toxicological endpoints by structure-activity relationships. Pharm. Rev. 36, 131s-135s.
22. Enslein K., V.K. Gombar, and B.W. Blake (1994). Use of SAR in computer-assisted prediction of carcinogenicity and mutagenicity of chemicals by the TOPKAT program. Mutat. Res. 305, 47-61.
23. EPA (1998). Chemical hazard data availability study. What do we really know about the safety of high production volume chemicals? EPA's 1998 baseline of hazard information that is readily available to the public. EPA's Office of Pollution Prevention and Toxics (April 1998).
24. EPIWIN 1997, Estimation program Interface for Microsoft Windows 3.1. Syracuse Research Corporation, North Syracuse, New Yersey.
25. Escher, B.I. and R.P. Schwarzenbach (1996). Partitioning of substituted phenols in liposome-water, biomembrane-water and octanol-water systems. Environ. Sci. Technol. 30, 260-270.
26. EU (1993). Guidance document for the implementation of Annex VII D of council Directive 67/548/EEC (Directive 93/105/EEC) and Requirements for spectral data.
27. EU (1995a). 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 # EV5V-CT92-0211.
28. EU (1995b). 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 # EV5V-CT92-0211.
29. Gombar, V.K., H.H. Borgstedt, K. Enslein, J.B. Hart and B.W. Blake (1991). A (Q)SAR model of teratogenesis. Quant. Struct.-Act. Relat. 10, 306-332.
30. Hansch, C., D. Hoekman, A. Leo, L. Zhang and P. Li (1995). The expanding role of quantitative structure-activity relationships (QSAR) in toxicology. Toxicol. Lett. 79, 45-53.
31. Hansen, B.G., A.G. van Haelst, K. van Leeuwen and P. van der Zandt (1999). Priority setting for existing chemicals: European Union risk ranking method. Environ. Toxicol. Chem. 18, 772-779.
32. Howard, P.H., A.E. Hueber and R.S. Boethling (1987). Biodegradation data evaluation for structure/biodegradability relations. Environ. Toxicol. Chem. 6, 1-10.
33. Howard, P.H., R.S. Boethling, W.M. Stiteler, W.M. Meylan, A.E. Hueber, J.A. Beauman and M.E. Larosche (1992). Predictive model for aerobic biodegradability developed from a file of evaluated biodegradation data. Environ. Toxicol. Chem. 11, 593-603.
34. Hulzebos, E.M., P.C.J.I. Schielen and L. Wijkhuizen-Masilankiewicz (1999). (Q)SARs for human toxicological endpoints: a literature search. RIVM report no. 601516 001, Bilthoven, The Netherlands.
35. Ioannides, C., D. Lewis and D. Parke (1994). The use of computers in the safety evaluation of drugs and other chemicals. Eur. J. Drug. Metab. Pharmacokinet. 19, 225-233.
36. Jones, T.D. and C.E. Easterly (1996). A RASH analysis of national toxicology program data: Predictions for 30 compounds to be tested in rodent carcinogenesis experiments. Environ. Health Persp. 104, 1017-1030.
37. Jones, T.D. and C.E. Easterly (1991). On the rodent bioassays currently being conducted on 44 chemicals: A RASH analysis to predict test results from the National Toxicology Program. Mutagenesis 6, 507-514.
38. King, R.D. and A. Srinivasan (1996). Prediction of rodent carcinogenicity bioassays

from molecular structures using inductive logic programming. Environ. Health Persp. 104 (Suppl. 5), 1031-1040.
39. Klamt, A. (1993). Estimation of gas-phase hydroxyl radical rate constants of oxygenated compounds based on molecular orbital calculations. Chemosphere 32, 717-726.
40. Klopman, G., M.R. Frierson and H.S. Rosenkranz (1990). The structural basis of the mutagenicity of chemicals in *Salmonella typhimurium*: the Gene-Tox data base. Mutation Res. 228, 1-50.
41. Klopman, G. and H.S. Rosenkranz (1992). Testing by artificial intelligence – computational alternatives to the determination of mutagenicity. Mutation Res. 272, 59-71.
42. Klopman, G., D.M. Balthasar and H.S. Rosenkranz (1993). Application of the computer-automated structure-biodegradation relationships for miscellaneous chemicals. Environ. Toxicol. Chem. 12, 231-240.
43. Klopman, G. (1994a). MultiCASE 1. A hierarchical computer automated structure evaluation program. Quant. Struct.-Act. Rel. 11, 176-184.
44. Klopman, G. and H.S. Rosenkranz (1994b). Approaches to SAR in carcinogenesis and mutagenesis. Prediction of carcinogenicity / mutagenicity using MULTI-CASE. Mutat. Res. 305, 33-46.
45. Klopman, G. and H. Rosenkranz (1994c). International commission for protection against environmental mutagens and carcinogens. Approaches to SAR in carcinogenesis and mutagenesis. Prediction of carcinogenicity / mutagenicity using MULTI-CASE. Mutat. Res. 305, 33-46.
46. Klopman, G. and H.S. Rosenkranz (1995). Toxicity estimation by chemical substructure analysis: the TOX II program. Toxicol. Lett. 79, 145-155.
47. Klopman, G., Z. Zhang, D.M. Balthasar and H.S. Rosenkranz (1995). Computer-automated predictions of aerobic biodegradation of chemicals. Environ. Toxicol. Chem. 14, 395-403.
48. Lewis D.F.V., C. Ioannides and D.V. Parke (1996). COMPACT and molecular structure in toxicity assessment: A prospective evaluation of 30 chemicals currently being tested for rodent carcinogenicity by the NCI/NTP. Environ. Health Persp. 104, 1011-1016.
49. Lewis, D.F.V. (1994). Comparison between rodent carcinogenicity test results of 44 chemicals and a number of predictive systems. Regul. Toxicol. Pharmacol. 215, 20:Pt. 1.
50. Lewis D.F.V. and G.R. Langley (1996). A validation study of the COMPACT and EffectExpert techniques with 40 chemicals. Mutat. Res. 369, 157-74.
51. Loonen, H., F. Lindgren, B. Hansen, W. Karcher, J. Niemelä, K. Hiromatsu, M. Takatsuki, W. Peijnenburg, E. Rorije and J. Struijs (1999). Prediction of biodegradability from chemical structure: modelling of MITI I data. *Submitted for publication.*
52. Loonen, H., F. Lindgren, B.G. Hansen and W. Karcher (1996). Prediction of biodegradation from chemical structure: Use of MITI data, structural fragments and multivariate analysis for the estimation of ready and not-ready biodegradability. In *Biodegradability Prediction* (eds. W.J.G.M. Peijnenburg and J. Damborsky) pp. 105-114, Kluwer Academic Publishers, Dordrecht, The Netherlands.
53. Lyman, W.J., W.F. Reehl and D.H. Rosenblatt (1991). Handbook of chemical property estimation methods. Environmental behavior of organic compounds. American Chemical Society, Washington, DC, USA.
54. Mackay, D., W.-Y. Shiu and K.-C. Ma (1992a). Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals. Volume I. Monoaromatic hydrocarbons, chlorobenzenes, and PCBs. Lewis Publishers, Boca Raton, USA.
55. Mackay, D., W.-Y. Shiu and K.-C. Ma (1992b). Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals. Volume II.

Polynuclear aromatic hydrocarbons, polychlorinated dioxins, dibenzofurans. Lewis Publishers, Boca Raton, USA.
56. Mackay, D., W.-Y. Shiu and K.-C. Ma (1993). Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals. Volume III. Volatile organic chemicals. Lewis Publishers, Boca Raton, USA.
57. Mackay, D., W.-Y. Shiu and K.-C. Ma (1995). Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals. Volume IV. Oxygen, nitrogen, and sulfur containing compounds. Lewis Publishers, Boca Raton, USA.
58. Mackay, D., W.-Y. Shiu and K.-C. Ma (1997). Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals. Volume V. Pesticide chemicals. Lewis Publishers, Boca Raton, USA.
59. Marchant, C.A. and R.D. Combes (1996). Artificial intelligence: the use of computer methods in the prediction of metabolism and toxicity. In *Bioact. Compd. Des.* (ed. M.G. Ford), pp. 153-162, Bios Scientific Publishers, Oxford, UK.
60. Marchant, C.A. and C.G. Derek (1996). Prediction of rodent carcinogenicity using the DEREK system for 30 chemicals currently being tested by the National Toxicology Program. Environ. Health Persp. 104 (Suppl. 5), 1065-1073.
61. MedChem (1989). MedChem Sofware Version 3.54. Daylight Chemical Information Systems Inc., Claremont, California, USA.
62. Mersch-Sunderman, V., H.S. Rosenkranz and G. Klopman (1994). The structural basis of the genotoxicity of nitroarenofurans and related compounds. Mutation Res. 304, 271-284.
63. Meylan, W.H. and P.H. Howard (1991). Bond contribution method for estimating henry's law constants. Environ. Toxicol. Chem. 10, 1283-1293.
64. Meylan, W.M. and P.H. Howard (1993). Computer estimation of the atmospheric gas-phase reaction rate of organic compounds with hydroxyl radicals and ozone. Chemosphere 26, 2293-2299.
65. Meylan, W.M. and P.H. Howard (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. J. Pharm. Sci. 84, 83-92.
66. Meylan, W.M., P.H. Howard and R.S. Boethling (1996). Improved method for estimating water solubility from octanol/water partition coefficient. Environ. Toxicol. Chem. 15, 100-106.
67. Meylan, W.M., P.H. Howard, D. Aronson, H. Printup and S. Gouchie (1997). Improved method for estimating bioconcentration factor (BCF) from octanol-water partition coefficient. Third update report - August 1997. SRC-TR-97-006 (EPA Contract No. 68-D5-0012).
68. Meylan, W.M., P.H. Howard, R.S. Boethling, D. Aronson, H. Printup and S. Gouchie. Improved method for estimating biooncentration/bioaccumulation factor from octanol/water partition coefficient. Environ. Toxicol. Chem. 18, 664-672.
69. Mill, T., W. Haag, P. Penwell, T. Pettit and H. Johnson (1987). Environmental fate and exposure studies development of a PC-SAR for hydrolysis: esters, alkyl halides and epoxides. EPA Contract No. 68-02-4254. Menlo Park, CA: SRI International.
70. MOLSTAC (1997). An extensive computerized catalog of substructure targets. Health Designs. Inc. Rochester;. NY 14604.USA Moss.
71. Moriguchi, I., H. Hirano and S. Hirono (1996). Prediction of the rodent carcinogenicity of organic compounds from their chemical structures using the FALS method. Environ. Health Persp. 104 (Suppl. 5), 1051-1058.
72. Nendza, M. (1991). QSARs of bioconcentration: validity assessment of log P_{OW}/log BCF correlations. In: Nagel, R., Loskill, R. (eds.), Bioaccumulation in aquatic systems, pp. 43-66. VCH Weinheim, Germany.
73. NMP (1998). Third National Environmental Policy Plan, Ministry of Housing, Spatial Planning and the Environment.

74. OECD (1992). OECD Guidelines for Testing of Chemicals. Paris, France.
75. OECD (1993). Application of structure-activity relationships to the estimation of properties important in exposure assessment. OECD Monographs No. 67, Paris, France.
76. OECD (1998). Harmonization of classification and labelling systems for chemicals. Approval of harmonized criteria for existing classification systems endpoints. 28th Joint Meeting, 4th-6th November 1998, Paris, France.
77. Opperhuizen, A. (1986). Bioconcentration of hydrophobic chemicals in fish. In T.M. Poston and R. Purdy (eds.), Aquatic toxicology and environmental fate, American Society for Testing and Materials, ASTM STP 921, Philadelphia, PA, USA, pp. 304-315.
78. Parry, J.M. (1994). Detecting and predicting the activity of rodent carcinogens. Mutagenesis 9, 3-5.
79. 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. Environ. Health Persp. 104 (Suppl. 5), 1085-1094.
80. Richard, A.M. (1995). Role of computational chemistry in support of hazard identification (ID): mechanism-based SARs. Toxicol. Lett. 79, 115-122.
81. Ridings J., M. Barratt, R. Cary, C. Earnshaw, C. Eggington and M. Ellis (1996). Computer prediction of possible toxic action from chemical structure: an update on the DEREK system. Toxicology, 106, 1-3.
82. Rorije, E., M. Müller and W.J.G.M. Peijnenburg (1997). Prediction of environmental degradation rates for High Production Volume Chemicals (HPVC) using Quantitative Structure-Activity Relationships. National Institute of Public Health and the Environment, Report No. 719101030.
83. Rorije, E., H. Loonen, M. Müller, G. Klopman and W.J.G.M. Peijnenburg (1998). Evaluation and application of models for the prediction of ready biodegradability in the MITI-test. Chemosphere (accepted for publication).
84. Sabljic, A. and H. Güsten (1990). Predicting the night-time NO_3 radical reactivity in the troposphere. Atmos. Environ. 24A, 73-78.
85. Sanderson, D. and C. Earnshaw (1991). Computer prediction of possible toxic action from chemical structure; the DEREK system. Hum. Exp. Toxicol. 10, 4.
86. Scheuplein, R. (1995). Information needed to support hazard identification and risk assessment of toxic substances. Toxicol. Lett. 79, 23-28.
87. Schüürmann G., R. Kühne, R. -U. Ebert and F. Kleint (1995). Multivariate error analysis of increment methods for calculating the octanol/water-partition coefficient.Fres. Environ. Bull. 4, 13-18.
88. SETAC (1998). Pellston Workshop "Criteria for persistence and long-range transort of chemicals in the environment".
89. Sijm, D.T.H.M., J. de Bruijn, P. de Voogt and W. de Wolf (1997). Biotransformation in environmental risk assessment. A SETAC-Europe Publication, SETAC-Europe, Brussels, Belgium.
90. Smithing, M.P. and F. Darvas F. (1992). HazardExpert. An expert system for predicting chemical toxicity. ACS Symp. Ser. 484 (Food Saf. Assess.), 191-200.
91. SRC (1990). Syracuse Research Corporation. Atmospheric Oxidation Program (AOP), Version 1.31. Syracuse, NY, USA.
92. SRC (1992). Syracuse Research Corporation. Biodegradation probability program (BIODEG), Version 3. Syracuse, NY, USA.
93. SRC (1997). Syracuse Research Corporation. LOGKOW for Microsoft Windows, Version 1.57. Syracuse, NY, USA.
94. Stewart, J.J.P. (1990). MOPAC: a semi-empirical molecular orbital program. J. Comput. Aid. Mol. Des. 4, 1-105.

95. Stewart, J.J.P. and M.B. Coolidge (1990). MOPAC 6.00 [A general molecular orbital package], Frank J. Seiler Research Laboratory, United States Air Force Academy, Co 80840: QCPE #455, USA.
96. Tennant, R.W., J.W. Spalding and S.J. Ashby (1990). Prediction of the outcome of rodent carcinogenicity bioassays currently being conducted on 44 chemicals by the US NTP. Mutagenesis 5, 3-14.
97. TGD (1996). Technical Guidance Document in support of the Commission Directive 93/67/EEC on risk assessment for new substances and Commission Regulation (EEC) No. 1488/94 on risk assessment for existing substances. ECB, Ispra, Italy.
98. TSCA New Chemical Program (1992). EPA, Washington DC, USA (http://www.epa.gov/opptintr/chemcat/chemcat.txt.)
99. Tyle, H. and J. Niemelä (1998). Use of QSARs for selection of POPs. Danish EPA, draft of November 1998.
100. UN-ECE (1998). Draft protocol to the Convention on Long-Range Transboundary Air Pollution on Persistent Organic Pollutants, (EB.AIR/1998/2), Convention on Long-Range Transboundary Air Pollution, United Nations Economic Commission for Europe.
101. Vallack, H.W., D.J. Bakker, I. Brandt, E. Broström-Lundén, A, Brouwer, K.R. Bull, C. Gough, R. Guardans, I. Holoubek, B. Jansson, R. Koch, J. Kuylenstierna, A. Lecloux, D. Mackay, P. McCutcheon, P. Mocarelli and R.D.F. Taalman (1998). Controlling persistent organic pollutants - what next? Environ. Toxicol. Pharmacol. 6, 143-175.
102. Van der Zandt, P.T.J. and C.J. van Leeuwen (1992). Proposal for priority setting of existing chemical substances. VROM 92408/b/9-92, 1502/033. Ministry of Housing, Physical Planning and the Environment, The Hague, The Netherlands.
103. Van Pul, W.A.J., F.A.A.M. de Leeuw, J.A. van Jaarsveld, M.A. van der Gaag and C.J. Sliggers (1998). The potential for long-range transboundary atmospheric transport. Chemosphere 37, 113-141.
104. Veith, G.D. and P. Kosian (1983). Estimating bioconcentration potential from octanol/water partition coefficients. In: Mackay, D. et al. (eds.), Physical behaviour of PCBs in the Great Lakes. Ann Arbor Science Publishers, Ann Arbor, MI, USA.
105. Veith, G.D., D.J. Call and L.T. Brooke (1983). Structure-toxicity relationships for the fathead minnow, *Pimephales promelas*: narcotic industrial chemicals. Can. J. Fish. Aquat. Sci. 40, 734-748.
106. Verhaar, H.J.M., C.J. van Leeuwen and J.L.M. Hermens (1992). Classifying environmental pollutants. 1: Structure-activity relationships for prediction of aquatic toxicity. Chemosphere 25, 471-491.
107. Verschueren, K. (1983). Handbook of environmental data on organic chemicals. Van Nostrand Reinhold, New York, USA.
108. Wania, F. and D. Mackay (1995). Tracking the distribution of persistent organic pollutants. Environ. Sci. Technol. 30, 390A-396A.
109. Webster, E., D. Mackay and F. Wania (1998). Evaluating environmental persistence. Environ. Toxicol. Chem. 17, 2148-2158.
110. Wilson, J.D., W. Cibulas, C.T. DeRosa, M.M. Mumtaz and E. Murray (1995). Decision support methodologies for human health risk assessment of toxic substances. Proceedings of the 1993 Decision support methodologies international workshop, Agency for Toxic Substances and Disease Registry, Atlanta, GA, USA, 18-20 October 1993. Toxicol. Lett. Special Issue, 79, Nos. 1-3.
111. Woo Y., D. LAI, J. Arcos, M. Argus, M. Cimino, S. DeVito and L. Keifer (1997). Mechanism-based structure-activity relationship (SAR). Analysis of carcinogenic potential of 30 NTP test chemicals. Environ. Carcinog. Ecotoxicol. Rev. 15, 139-160.

Appendix 1 Mailing list

1	Plv. DG Milieubeheer, dr.ir. B.C.J. Zoeteman
2	Directoraat Generaal Milieubeheer, Directie Stoffen, Veiligheid en Straling, hoofd afdeling stoffen, ir. J. van der Kolk
3-76	Dr. D.W.G. Jung (DGM-SVS)
77	Drs. A. van der Wielen (DGM-SVS)
78	Drs. K.A. Gijsbertsen (DGM-SVS)
79	Dhr. J. Groos (DGM-SVS)
80	Mevr. dr. M.E.J. van der Weiden (DGM-SVS)
81	Drs. D.A. Jonkers (DGM-DWL)
82	Mevr. D. Lerche (DGM-LE)
83	Dhr. H. Herremans (DGM-LE)
84-92	Dhr. P. van der Hoeven (RIZA, OVOC-secretariaat)
93	Dr. J.H.M. de Bruijn (RIVM-CSR)
94	Mw. P. Gingnagel (RIVM-CSR)
95	Mw. Ir. J.M.M. Herremans (RIVM-CSR)
96	Mw. Ir. M. Hof (RIVM-CSR)
97	Drs. J.A. Janus (RIVM-CSR)
98	Prof. Dr. C.J. van Leeuwen (RIVM-CSR)
99	Ir. J.B.H.J. Linders (RIVM-CSR)
100	Drs. T.G. Vermeire (RIVM-CSR)
101	Dr. W. Slooff (RIVM-CSR)
102	Mevr. Dr. A.P. van Wezel (RIVM-CSR)
103	Ir. P.T.J. van der Zandt (RIVM-CSR)
104	Dr.ir. D. van de Meent (RIVM-ECO)
105	Mevr. dr. H. Loonen (ECB, Italië)
106	Dr. B. Hansen (ECB, Italië)
107	Prof. Dr. D. Mackay (Trent University, Peterborough, Ontario, Canada)
108-110	Dr. R. Breton (Environment Canada, Hull, Quebec, Canada)
111	Dr. J. Buccini (Environment Canada, Hull, Quebec, Canada)
112	Dr. F. Wania (University of Toronto, Toronto, Canada)
113-115	Mrs. I. Grethe England (State Pollution Control Authority, Oslo, Norway)
116-118	Mr. Alf Lundgren (KEMI, Solna, Sweden)
119	Dhr. P. Benschop (RIZA)
120	Mr. H.S. Larsen (Danish EPA, Copenhagen, Denmark)
121	Mr. J.R. Niemelä (Danish EPA, Copenhagen, Denmark)
122	Mrs. E. Karhu (Finland)
123	Mr. B. Diderich (INERIS, Paris, France)
124-128	Mrs. Dr. C. Cowan (Proctor & Gamble Company, Cincinnati, Ohio, USA)
129	Mr. S.K. Sasnett (US EPA, Washington, DC, USA)
130	Dr. B. Boethling (US EPA, Washington, DC, USA)

131	Drs. H. Blok (Haskoning, Nijmegen)
132	Ir. P. Okkerman (BKH, Delft)
133	Dr. J.L.M. Hermens (RITOX, Utrecht)
134	Dr. J. Willis (UNEP)
135	Dr. C. Auer (US-EPA)
136	Dr. R. Visser (OECD)
137-156	Mr. S. Morishita (Secretay, OECD Task Force Environmental Exposure Assessment)
157	Depot Nederlandse Publikaties en Nederlandse Bibliografie
158	Directie RIVM
159	Hoofd Centrum voor Stoffen en Risicobeoordeling
160	Hoofd Laboratorium voor Ecotoxicologie
161	Hoofd Laboratorium voor Water en Drinkwateronderzoek
162	Hoofd Laboratorium voor Bodem en Grondwateronderzoek
163	Hoofd Laboratorium voor Afvalstoffen en Emissie
164	Hoofd Laboratorium voor Effectenonderzoek
165	Sectordirecteur Stoffen en Risico's
166	Sectordirecteur Milieuonderzoek
167-169	Auteurs
170-179	SBD/Voorlichting en Public Relations
180	Bureau Rapportenregistratie
181	Bibliotheek RIVM
182-196	Bureau Rapportenbeheer
197-225	Reserve exemplaren

Appendix 2 Simple distribution of chemicals over air, water and soil

Assumptions for the simple distribution of chemicals over air, water and soil:

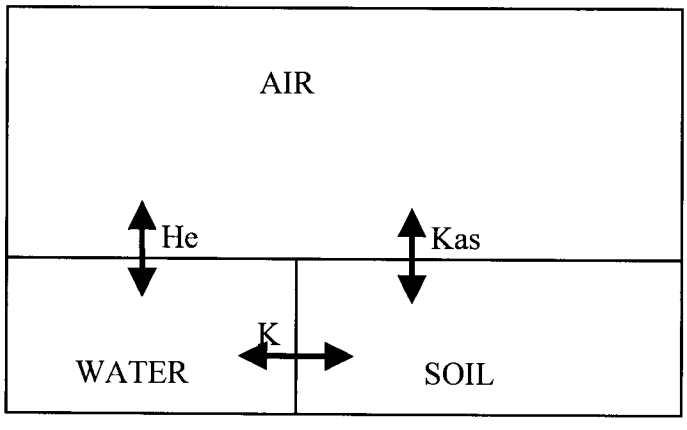
1) Only three environmental compartments: air, water and soil
2) Equilibrium exists between water, air and soil
3) Aqueous solubility (S, mol/m^3), vapour pressure (Vp, Pa) and octanol/water partition coefficient (Kow, no unit) are required parameters
4) Soil/water partition coefficient is derived from that from Kow and organic carbon content of soil (f_{oc} = 0.05)
5) Henry's law constants (He) is derived from ratio between Vp and S
6) Dimensions of environmental compartments derived from geographic area of 1.0 x 10^{11} m^2 (Europe):
   a) Air: height = 1000 m; volume = 1.0 x 10^{14} m^3
   b) Water: area = 1.0 x 10^{10} m^2; depth = 20 m; volume = 2.0 x 10^{11} m^3
   c) Soil: area = = 9.0 x 10^{10} m^2; depth = 0.1 m; volume = 9.0 x 10^9 m^3
7) For weak acids and bases, it is assumed that they are completely non-dissociated.

Equations

1) Kp = f_{oc} x 0.5 x Kow = 0.025 x Kow (L/kg)
2) He = Vp/S (Pa.m^3/mol)
3) Kas = He/Kp

NB when a different geographic area is taken, such as that of The Netherlands, no significant differences in the distribution profiles is found. In addition, when the volume of water increases or decreases by a factor of 10, the ranges of the distribution profiles will not change in the same order of magnitude. The main reason for this is the relatively large volume of air.
Dimension of environmental compartments derived from geographic area of 3.79 x 10^{10} m^2 (The Netherlands)

- Air: height = 1000 m; volume = 3.79 x 10^{13} m^3
- Water: area = 12.5% x area; depth = 3 m; volume = 1.42 x 10^{10} m^3
- Soil: area = 87.5% x area; depth = 0.2 m; volume = 6.64 x 10^9 m^3



Chemical class	S (mol/m^3)	Vp (Pa)	log Kow	Environmental distribution	
				Most volatile within range	Least volatile within range
Monoaromatics	$23\text{-}10^{-3}$	12700-0.2	2.1-4.6	Air: >99%	Air: >99%
Chlorobenzenes	$4\text{-}10^{-5}$	$1580\text{-}2\bullet10^{-3}$	2.8-5.5	Air: >99%	Air: >99%
PCBs	$10^{-2}\text{-}10^{-9}$	$1.3\text{-}5\bullet10^{-8}$	3.9-8.3	Air: >99%	Air: 6% Soil: 94%
PAHs	$1\text{-}10^{-7}$	$197\text{-}2\bullet10^{10}$	3.3-6.8	Air: >99%	Soil: >99%
Dioxins	$10^{-3}\text{-}10^{-10}$	$0.05\text{-}10^{-10}$	4.3-8.2	Air: >99%	Soil: >99%
Furans	$10^{-2}\text{-}10^{-9}$	$0.3\text{-}10^{-10}$	4.3-8.0	Air: >99%	Soil: >99%
Alcohols	misc. - 80	16000-10	-0.8-3.8	Air: >99%	Air: 89% Water:1% Soil: 10%
Aldehydes and ketones	misc. - 1	atm. -0.1	0.0-3.2	Air: >99%	Air: 91% Water: 3% Soil: 6%
Phenolic compounds	1000-0.04	130-0.001	1.0-5.8	Air: >98%	Air: 2% Soil: 98%
Carboxylic acids	misc. -0.4	$6000\text{-}10^{-4}$	-0.5-8.0	Air: >99%	Soil: >99%
Esters (including phthalic esters)	$4000\text{-}10^{-5}$	$80000\text{-}10^{-3}$	-0.3-9.8	Air: >99%	Soil: >99%
nitrogen and sulphur compounds	misc. $\text{-}10^{-3}$	atm. $\text{-}10^{-7}$	-2.1-5.8	Air: >99%	Soil: >99%
hydrocarbons (volatiles)	$60\text{-}10^{-3}$	atm. -0.05	2.0-6.2	Air: >99%	Air: 93% Soil: 7%
halogenated hydrocarbons (volatiles)	$160\text{-}10^{-3}$	atm. –11	0.9-5.0	Air: >99%	Air: >99%
Ethers	7600-0.1	71000-0.2	-0.3-4.3	Air: >99%	Air: 98% Soil: 2%
Herbicides	$5400\text{-}10^{-4}$	$1\text{-}10^{-7}$	-3-6.3	Air: 8% Water: 92%	Soil: >99%
Insecticides	$4500\text{-}10^{-7}$	$50\text{-}10^{-7}$	-1.0-6.9	Air: 85% Water: 15%	Air: 5% Soil: 95%
Fungicides	$36\text{-}10^{-3}$	atm. $\text{-}10^{-9}$	0.3-4.6	Air: >99%	Water: 2% Soil: 98%
Metals	$1000\text{-}10^{-10}$	$10^{-3}\text{-}10^{-20}$	0	Water: 99%	Air: >99%

Information on S, Vp and log Kow is obtained from Mackay et al. (1992a; 1992b; 1993; 1995; 1997), except for the metals.

Appendix 3 Summary of QSAR, SAR and expert systems for mammalian related toxicological endpoints

RASH

Rule: toxicity induced compensatory cell proliferation can serve as a upper-limit index of carcinogenicity promotion. Based on effects in short-term studies RASH predicts carcinogenic concern, taking into consideration the fact that high dosing normally gives false-positive results in carcinogenicity testing. Soon available. Internet site: http://www.esd.ornl.gov/iab/iab6-15.htm

DEREK

DEREK (Deductive Estimation of Risk from Existing Knowledge) identifies toxicophores (segments of the molecule associated with a specific activity) and alerts the user to the presence of the toxicophores, and further, gives references and examples. DEREK contains rules to identify toxicophores for adverse reproductive effects, carcinogenicity, irritancy, lachrymation, methaemoglobinaemie, mutagenicity, neurotoxicity, respiration and skin sensitisation. However, many of these areas are not comprehensively covered, notable exceptions being the rules for carcinogenicity and skin sensitisation (Cronin and Dearden, 1995c).

DEREK makes its toxicological predictions by comparing submitted structures with rules contained in the DEREK rule base. Internet site: http://129.11.12/LUK/derek.html

TOPKAT

TOPKAT (TOxicity Prediction by Komputer-Assisted Technology) includes different models that predict various toxicological endpoints. Enslein et al. (1983a-b), Enslein (1984), Gombar et al. (1991) started developing SARs based on databases in which one or more toxicological endpoints are distinguished. The database with tested chemicals is divided in a training set and a validation set. The training set consists of clear positive, clear negative and intermediate chemicals with regard to one or more toxicological endpoints.

The training set is first screened for toxicodynamic (reactivity) properties. From this training set, an analysis is made to identify which substructures in the MOLSTAC (1997) database also appear in the training set. The MOLSTAC system defines over 3000 molecular fragments representing chemically and biologically important functional groups, heterocyclic, aliphatic, and aromatic fused and unfused ring systems, electron-donating and -withdrawing groups and their molecular environments etc. The chemicals in the training set are also scanned for electronic descriptors, such as electron density charges, residual electronegativity and polarisability (intrinsic molecular properties). These descriptors are quantified. Both types of descriptors (fragments and electronic) will determine the reactivity of the chemical. It should be noted that in the latest TOPKAT model the MOLSTAC database is no longer used. Instead, the chemicals in the database from which the QSARs is derived are used to serve as structural analogues to predict the possibility that the tested chemical will have the similar toxicological activity.

For predicting the uptake by and distribution in the organism other descriptors are used. The molecular shape is important for membrane passage and for distribution. Several descriptors are used to quantify the shape of the molecule, some are atom specific and other correspond

to the carbon skeleton of the molecule, i.e. the molecular connectivity indices. Molecular connectivity descriptors are used to quantify topological features such as type and number of atoms and bonds and number and position of branching and rings. In this model the log Kow may be calculated from the descriptors that are described.

After testing the different descriptors for intercorrelations, they were selected by stepwise regression in order to select the best subset of descriptors of the training set for a specific toxicological endpoint. In addition, some other statistical analyses were carried out to reveal accurate separation of active and non-active chemicals.

The Danish EPA currently carries out a validity study using TOPKAT for the prediction of mutagenicity and state 79% good prediction (personal communication, Dr. Jay Niemela, Danish EPA). They compare the structures of chemicals with chemicals in the library of the TOPKAT program on which the QSAR is based for the specific endpoint. In this way the program gives a reliability indication of the prediction.

Models are generated using QSAR-techniques and propriety algorithms. TOPKAT determines an optimum prediction space, within which predictions are both robust and accurate. TOPKAT produces an identification of possible sites of toxicity. TOPKAT provides a user-friendly interface to guide users through a computer-based assessment of toxicity for a query structure, and validated pre-constructed QSAR models for rodent carcinogenicity etc.
Input: SMILES notation of chemical.
Internet site: http://www.oxmol.com/prods/topkat. Price: $ 80,000 (for all modules)

COMPACT

Based on the presumption that to become reactive, electrophilic carcinogenic intermediates, compounds must be metabolised through oxygenation by P450 enzymes. Via a calculation of the molecular and electronic structure of the chemical (planarity, electronic structure; LUMO/HUMO, collision diameter), COMPACT determines whether the chemical will interact with cytochrome P450 subfamilies and hence be metabolised to form reactive intermediates that manifest toxicity. The one-rule-base (P450 metabolism) is a disadvantage. Program will not analyse non-organic compounds (e.g. fibers, metals) or direct-acting mutagens. Not commercially available.

MULTICASE

Klopman and co-workers have developed a program of structural active and inactive fragments, not to model the mechanism of action of a substance, but simply to reveal the relationship between the chemical structure and the biological activity. They and their model are thus not hindered by mechanistical knowledge or known structures.

The first program they developed in 1984 was called the CASE program. CASE stands for Computer Automated Structure Evaluation. The CASE methodology has been expanded during the years as the number of known active and inactive fragments for the specific biological endpoints has increased (Klopman et al., 1994a; Klopman and Rosenkranz, 1994b; Klopman and Rosenkranz, 1995). In addition, for some endpoints, structural fragments determined by experts have been included (e.g. for biodegradation (Klopman et al., 1993)), or rules for determining the biotransformation potential of chemicals (Klopman and Rosenkranz, 1992).

The MULTICASE program selects its own descriptors automatically by fragmenting each molecular structure in logical subsets of a learning set composed of active and inactive molecules. The biological active fragments for a certain endpoint are called biophores, and

the fragments for inactivity for a certain endpoint are called biophobes. More than 70 toxicological endpoints can be evaluated, among them which are carcinogenicity and reproductive toxicity. The descriptors are normally linearly connected strings of atoms including, if necessary, a side chain and can be any length from two heavy atoms upward. Statistical analysis determines which fragments are associated with activity (biophores) or lack of activity (biophobes). Quantitative estimation of potency can be performed using multiple linear regression analysis including, if required, physicochemical parameters, such as log Kow. Once the system is trained, test compounds not included in the original CASE analysis can be submitted for qualitative as well as quantitative predictions. A comparative study showed that for the prediction of mutagenicity the best predictions were obtained with the MULTICASE software followed by CASE and CASE/GI (Graph Indices), respectively. MULTICASE is criticised in that there is no evaluation of the knowledge base, and so no mechanistic interpretation of toxicity is made (Cronin and Dearden, 1995c).

The advantage of this program is that the selection of the structural fragments of the chemicals is solely based on the probabilistic occurrence in active or inactive molecules. The selection of these fragments is not shadowed by expert knowledge that may overlook certain unknown (active or inactive) fragments.

The disadvantage of the program is that structural fragments occurring in active molecules may not at all have a causal relationship with the specific endpoint. Molecules may have mutagenic properties and some structural fragments, that may be coincidental, and may depend on the size and the heterogeneity of the training set. In addition, the rules of logic should be watched. Molecules having a specific (mutagenic) activity and having certain structural fragments is not per se the other way around (most birds fly, but most flyers are not birds).

The authors themselves conclude that predictions should be taken as a guide to rank and prioritise chemicals for evaluation rather than as a crystal ball to predict toxicity. Also, that interspecies differences translate into prediction uncertainties, that variable exposure regimens, coupled with the diversity of responses contributes to fuzziness of experimental input into the programs, and that the predictions are function of the nature, origin, diversity and size of the database.

MULTICASE accepts series of compounds and quantitative or qualitative activity in tests performed under a common protocol. The program will evaluate the data set, identify biophores and modulators and generate a QSAR correlation. Once this 'ad hoc' dictionary is established, it may be used to analyse new molecules. Program available via Multicase Inc. Internet site: http://cwgk4.chem.cwru.edu:443/biosoft.htm.

CASETOX

Uses prediction modules of MULTICASE. Most useful when investigating endpoints for which extensive, authoritative MULTICASE databases are already available. Available via Multicase Inc. Internet site: http://cwgk4.chem.cwru.edu:443/biosoft.htm.

ToxAlert

Based on the structural formula, partition coefficient, molecular weight and water solubility are calculated which are used to predict toxicity. Prediction based on modules (training sets) for various kinds of toxicity ('the best databases for each generated toxicological endpoint as generated by MULTICASE'). Program will only analyse organics. Available via Multicase Inc. Internet site: http://cwgk4.chem.cwru.edu:443/biosoft.htm.

META

META is a knowledge-based expert system, capable of predicting the sites of potential enzymatic attack and the nature of the chemicals. Formed by such metabolic transformations. It operates from dictionaries of transformation operators, created by experts to represent known metabolic paths. Available via Multicase Inc. Internet site: http://cwgk4.chem.cwru.edu:443/biosoft.htm

Purdy (1996)

Uses a number of QSARs based on specific chemical reactivity mechanisms, thought to represent the mechanisms leading to carcinogenicity (SPARC, SRC, ProjectLeader/CAChe-Oxford Molecular Group) are some of the programs used to generate the analysis). A hierarchical model consisting of QSARs based mainly on chemical reactivity was developed to predict the carcinogenicity of organic chemicals to rodents. QSARs based on hypothesised mechanisms of action, metabolism, and partitioning.

Predictors included:

- Kow
- molecular size
- atomic partial charge
- bond angle strain
- atomic acceptor delocalisibility
- atomic radical super-delocalisibility
- the lowest unoccupied molecular orbital (LUMO) energy of hypothesised intermediate nitrenium ion of primary aromatic amines
- difference in charge of ionised and unionised carbon-chlorine bonds
- substituent size and pattern on polynuclear aromatic hydrocarbons
- the distance between lone electron pairs over a rigid structure, and
- the presence of functionalities such as nitroso and hydrazine.

Bias to the importance of e.g. epoxides as compared to SN_2-reacting chemicals. Internet site: http://www.oxml.com (For QSARs only).

Oncologic

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

a) data indicating effects on oncogenes and tumour suppresser genes
b) data indicating genotoxicity and/or ability of covalently binding to DNA
c) data indicating an epigenetic mechanisms, including those which may cause endogenous or indirect genotoxicity
d) sub-chronic toxicity data/endpoints that may be indicative of or suggest carcinogenic potential.

Based on the above considerations a prediction is made. The program can handle a wide variety of chemicals including metals, but may need quite some expertise to be able to use the program.

Classes of compounds analysed: fibers, polymers, metals, and organic chemicals. Requires considerable data input (e.g. physicochemical data, location of reactive centres). Internet site: http://logichem.com

HAZARDEXPERT

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

Knowledge base was developed based on a list of fragments reported by more than 20 lead experts. In combination with MetabolExpert, it predicts toxicity of both the parent compounds and metabolites. The expandable knowledge base is open to new information specific to each user. Price: $ 4,250.
Internet site: http://.compudrug.hu/hazardtext.htlm

MetabolExpert

MetabolExpert provides a knowledge-based prediction of metabolic trees and pathways of organic chemicals in mammals and plants. In combination with HazardExpert, toxicological potential of metabolites may be investigated. Internet site:
http://.compudrug.hu/hazardtext.htlm. Price: $ 6,650

StAR

The StAR project is developing novel risk assessment techniques that build upon recent developments in mathematics and logic, and improved methods for communicating risks which incorporate results from psychological research into human risk perception and decision making under uncertainty. Based on the similarities in the syntax of the input rules, it is suspected that the method of King and Srinivasan (1996) is the basis of StAR. The program is heuristically sound. Internet site: http://129.11.12.1/|LUK/start21.html

Progol

King and Srinavasan (1996) embarked on the development of a method based on Progol, a machine learning program. Progol is the first inductive logic-programming algorithm to use a fully relational method for describing chemical structure in SARs, based on using atoms and their bond connectives. Progol is well suited to forming SARs for carcinogenicity and is designed to produce easily understandable rules (structural alerts) for sets of non-generic compounds. Its process of dataset analysis is reminiscent of that of CASE/MULTICASE. Its accuracy was reported to be 63% (in this particular analysis the accuracy was comparable to that of programs like DEREK, CASE, COMPACT and TOPKAT. Internet site:
http://www.comlab.ox.ac.uk/oucl/groups, and Ashwin.srinavasan@comlab.oxford.ac.uk

FALS

FALS is a pattern recognition method for correlating structure with activity rating. It was used to generate QSARs on the carcinogenicity of several chemical classes. Not available yet.

Remarks related to Appendix 3.

The table summarises the available software dealing with the prediction of toxicity (especially carcinogenicity) based on the molecular structure (and sometimes physico-chemical properties) of compounds. Some considerations, however, should be made.

6.3.1.1.1.1 Rule-based vs. statistical databases

Available programs may be divided in rule-based and 'statistical' programs. Rule-based systems create rules derived from human knowledge, obtained through experts. Statistical procedures use methods like linear multiple regression and two-group linear discriminant functions to identify structural entities with a specific toxic effect, and a mathematical function describing the relationship between the effect and the structural moiety. Both methods have their advantages and disadvantages. It is difficult to translate the implicit toxicological knowledge of experts into explicit rules, and a rule-based system is fundamentally subjective. However, the process of analysis of a rule-based system is rather transparent. Statistical methods use relationships that may be statistically sound, but could be without scientific meaning. They need relatively extensive training sets of data to formulate the various QSARs and algorithms. They should not be used outside the strict boundaries that are produced by the training set and calculated confidence intervals. The process of generating databases and relationships however may reveal new insight in toxicological mechanisms.

6.3.1.1.1.2 Fixed databases vs. learning databases

Some databases are fixed, i.e. they use a specific set of rules in which the user cannot introduce changes. Other databases either learn because they allow the user to introduce new rules, or produce their own rules when analysing sets of data.

6.3.1.1.1.3 Accuracy, sensitivity, specificity, validity

Accuracy is defined as the ratio between the sum of correct assignments (active and non-active) and the total number of checked compounds. Sensitivity is the ratio considering only active compounds, correctly assigned, and specificity is the ratio of inactive compounds, correctly assigned. Considerations of false positive and false negative predictions should be judged in relation to the intended use of the program. For example, if the program should rigidly identify potentially carcinogenic entities, a program producing a high percentage of false negative results is less suitable.

The authors of various programs sometimes present rather impressive performance statistics. However, the few independent validation and performance studies that analysed the currently purchasable programs in a comparative fashion, generally present performances that are lower (sometimes little better than random). Therefore, extensive validation before purchase should be considered.

6.3.1.1.1.4 Miscellaneous

Whether a program analyses the parent compound and/or its metabolites should be taken into account when choosing a specific program. The correctness of the various algorithms and QSARs and their interrelationships are a further point of concern. Whether the output of the programs should be qualitative (concern or no concern) or quantitative (up to the point where boundaries of uncertainty are given) should be determined as well. Some programs only identify moieties that represent a reason for concern, whereas other programs consider the entire molecule, introducing a more holistic approach. Some programs demand powerful non-windows computers.

Basically, an evaluation program should be
1. Heuristic, i.e. it should be able to deal both with theories and expert knowledge
2. Transparent, i.e. it should explain the rationale of the output
3. Flexible, i.e. it should be able to introduce new knowledge into its existing database.

Table A3.1. Summary of QSAR, SAR and expert systems for mammalian related toxicological endpoints.

Name	Type	Toxicity endpoint(s)	Level of expertise	Performance	Output	Ref.
RASH (rapid screening of hazard)	Rule-based/statistical	Carcinogenicity	'to be used by the expert or non-expert', no information on computer requirements	Accuracy: 74% false pos.: 3/31 false neg.: 5/31	Produces limit values above which monitoring should commence	1-2
DEREK (Deductive estimation of risk from existing knowledge)	Rule-based	Mutagenicity, carcinogenicity, skin sensitisation, reproductive toxicity, irritancy, neurotoxicity	readily, easy-to-use. Windows-based	Accuracy: 59% false pos.: 4/37 false neg.: 11/37	Toxicological report	3-5
TOPKAT (Toxicity Prediction by Komputer-assisted Technology	QSAR / proprietary algorithms / Optimum prediction space / statistical methods	Carcinogenicity, Ames mutation, oral LD50, rat chronic LOAEL, skin sensitisation, developmental toxicity, irritancy, mouse inhalation, rat maximum tolerated dose and some ecotoxicological tests	Users: US FDA, US EPA, industries, universities, test-labs (see ..../topkat/work for the complete list). At least 486, desirably pentium, Windows 3.1 en 95. Demo available	Accuracy: 58% false pos.: 3/24 false neg.: 7/24	Toxicological report	6
COMPACT (Computer Optimized Molecular Parametric Analysis of Chemical Toxicity)	Modelling of planarity and electronic structure to predict interaction with P450 as a predictor of carcinogenicity	Indirect carcinogenicity	program not commercially available	Accuracy: 54% false pos.: 10/35 false neg.: 6/35 Accuracy: 70% false pos.: 3/31 false neg.: 9/31	unknown	8-10
(MULTI)CASE (computer-automated, structure evaluation)	Statistical (generates QSARs) Biophore and modulator selection, computer-automated, structure evaluation, QSAR correlation	Depending on dataset (carcinogenicity, mutagenicity, teratogenicity, biodegradation)	VAX/VMS workstation (MULTICASE is needed when using private databases in ToxAlert and CASETOX)	Accuracy: 49% false pos.: 9/35 false neg.: 9/35		11
CASETOX (MULTICASE derivative)	Statistical	Carcinogenicity, mutagenicity, irritation, teratogenicity, miscellaneous toxicity, short	VAX/VMS workstation	Not investigated	Toxicological report	

Name	Type	Toxicity endpoint(s)	Level of expertise	Performance	Output	Ref.
		term assays, biodegradation				
ToxAlert (MULTICASE derivative)	Statistical (based on the algorithms of the program MULTICASE)	Teratogenicity, carcinogenicity, biodegradation, chromosome aberrations, micronuclei, Salmonella mutation,	Input: structural formula. Windows 3.1, Windows 95, Windows NT	Not investigated	Toxicological report	
META (MULTICASE interfaceable)	Knowledge-based	Biodegradation	VAX/VMS workstation	Not investigated	Unknown	
Purdy (1996)	Hierarchical QSAR model (hybrid?)	Carcinogenicity, skin sensitisation	-	Not investigated	Unknown	12
OncoLogic	Rules of SAR-analysis / knowledge of mechanisms of action / human epidemiological studies	Carcinogenicity	knowledge-based software developed in co-operation with US-EPA. Windows (?!). Demo program available	Not investigated	Toxicological report, including data report and toxicological report. heuristically sound	-
HazardExpert	Rule-based learning system	Carcinogenicity, mutagenicity, teratogenicity, membrane irritation, neurotoxicity	MS Windows and UNIX. Window-based programs work in a PALLAS frame. 486 Windows 3.1 or Windows 95, 8 MB RAM	Accuracy: 55% false pos.: 1/9 false neg.: 17/31	Toxicological report, easily interpretable results, including molecular weights, pKa and log P values	10,13,14
MetabolExpert (interfaceable with HazardExpert)		Metabolism	MS Windows and UNIX. Window-based programs work in a PALLAS frame. 486 Windows 3.1 or Windows 95, 8 MB RAM	Not relevant	Unknown	15,16
StAR	Rule-based	Carcinogenicity (and additional sub-disciplines)	data not available	Data not available		-
Progol	Combined statistical and inductive logic programming methods	Carcinogenicity	DOS/Windows	Data not available. (authors own statement; 63%).	Decision carcinogen / non-carcinogen and an explanatory set of rules (which is not easily to interpret).	17
FALS (Fuzzy	Statistical pattern	Carcinogenicity	not applicable	Not available	Not available	18

Name	Type	Toxicity endpoint(s)	Level of expertise	Performance	Output	Ref.
adaptive least squares)	recognition method to generate QSARs					

References for Appendix 3.

1. Jones, T.D. and C.E. Easterly (1991). On the rodent bioassays currently being conducted on 44 chemicals: A RASH analysis to predict test results from the National Toxicology Program. Mutagenesis 6, 507-514.
2. Jones, T.D. and C.E. Easterly (1996). A RASH analysis of national toxicology program data: Predictions for 30 compounds to be tested in rodent carcinogenesis experiments. Environ. Health Persp. 104, 1017-1030.
3. Marchant, C.A. and C.G. Derek (1996). Prediction of rodent carcinogenicity using the DEREK system for 30 chemicals currently being tested by the National Toxicology Program. Environ. Health Persp. 104 (Suppl. 5), 1065-1073.
4. Ridings, J., M. Barratt, R. Cary, C. Earnshaw, C. Eggington and M. Ellis (1996). Computer prediction of possible toxic action from chemical structure: an update on the DEREK system. Toxicol. 106, 1-3.
5. Sanderson, D. and C. Earnshaw (1991). Computer prediction of possible toxic action from chemical structure; the DEREK system. Hum. Exp. Toxicol. 10, 4.
6. Enslein K., V.K. Gombar, and B.W. Blake (1994). Use of SAR in computer-assisted prediction of carcinogenicity and mutagenicity of chemicals by the TOPKAT program. Mutat. Res. 305, 47-61.
7. Benfenati, E. and G. Gini (1997). Computational predictive programs (expert systems) in toxicology. Toxicol. Lett. 119, 213-225.
8. Lewis D.F.V., C. Ioannides and D.V. Parke (1996). COMPACT and molecular structure in toxicity assessment: A prospective evaluation of 30 chemicals currently being tested for rodent carcinogenicity by the NCI/NTP. Environ. Health Persp. 104, 1011-1016.
9. Ioannides, C., D. Lewis and D. Parke (1994). The use of computers in the safety evaluation of drugs and other chemicals. Eur. J. Drug. Metab. Pharmacokinet. 19, 225-233.
10. Lewis D.F.V. and G.R. Langley (1996). A validation study of the COMPACT and EffectExpert techniques with 40 chemicals. Mutat. Res. 369, 157-74.
11. Klopman, G. and H.S. Rosenkranz (1994b). Approaches to SAR in carcinogenesis and mutagenesis. Prediction of carcinogenicity / mutagenicity using MULTI-CASE. Mutat. Res. 305, 33-46.
12. 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. Environ. Health Persp. 104 (Suppl. 5), 1085-1094.
13. Smithing, M.P. and F. Darvas F. (1992). HazardExpert. An expert system for predicting chemical toxicity. ACS Symp. Ser. 484 (Food Saf. Assess.), 191-200.
14. Lewis, D.F.V. (1994). Comparison between rodent carcinogenicity test results of 44 chemicals and a number of predictive systems. Regul. Toxicol. Pharmacol. 215, 20:Pt. 1.
15. Darvas F, and R. Eker (1996). Computer-assisted metabolic prediction: A help in development and design of pesticides. Pestic. Chem.: Adv. Int. Res., Dev., Legis., Proc. Int. Congr. Pestic. Chem., 7th. 287: Fed. Rep. Ger. Weinheim. ECBI/22/96-Add.10. 1996. Ad-hoc Working Group on Defatting Chemicals.
16. Marchant, C.A. and R.D. Combes (1996). Artificial intelligence: the use of computer methods in the prediction of metabolism and toxicity. In *Bioact. Compd. Des.* (ed. M.G. Ford), pp. 153-162, Bios Scientific Publishers, Oxford, UK.
17. King, R.D. and A. Srinivasan (1996). Prediction of rodent carcinogenicity bioassays from molecular structure using inductive logic programming. Environ. Health Persp. 104 (Suppl. 5), 1031-1040.
18. Moriguchi, I., H. Hirano and S. Hirono (1996). Prediction of the rodent carcinogenicity of organic compounds from their chemical structures using the FALS method. Environ. Health Persp. 104 (Suppl. 5), 1051-1058.

Appendix 4 Relevant PTB-properties and classification of chemicals

Table A. Relevant PTB-properties who are required when substance is distributed over one or more environmental compartments.

	Property	AIR	WATER	SOIL	ALL
P-property	Biodegradation in water	-	+	+	+
	photo-oxidation in air	+	-	-	+
T-property	aquatic ecotoxicity [a]	+	+	+	+
	LD50, oral	+	+	+	+
	LD50, dermal	+	+	+	+
	LC50, inhalation	+	-	-	+
	carcinogenicity (or mutagenicity)	+	+	+	+
	reproduction toxicity	+	+	+	+
B-property	Kow/BCF [a]	+	+	+	+

[a] Although a substance that is primarily resided in another environmental compartment than water, information on aquatic toxicity and on bioaccumulative potential are still required to indicate the toxicity to the environment, and the potential for food-chain bioaccumulation, respectively.

Table B. Classification of substances according to Verhaar et al. (1992).

	Description	Important molecular structural features	Examples
Class 1	inert chemicals	no specific substituents	Polychlorinated benzenes
Class 2	less inert chemicals	hydrogen bond donor acidity	Phenols, anilines
Class 3	Reactive chemicals	reaction with biomolecules	Epoxides
Class 4	Specifically acting chemicals	specific knowledge on mechanism of action is required	OP-esters (acetylcholine esterase inhibitor), DDT (interaction with sodium channel regulating receptor)
Unclassified	all other		Metals, organometals, inorganic substances, polymers, petroleum products, mixtures

Appendix 5 Final categorisation of PTB-substances

P-criterion	T-criterion	B-criterion	PTB-category (3 classes, P x T x B)	PTB-category (3 classes, P+T+B)	PTB-category (5 classes, P+T+B)
1	1	1	A	A	I
1	1	2	A	A	I
1	1	3	A	A	II
1	1	4	A	B	II
1	2	1	A	A	I
1	2	2	A	A	II
1	2	3	B	B	II
1	2	4	B	B	III
1	3	1	A	A	II
1	3	2	B	B	II
1	3	3	B	B	III
1	3	4	B	B	III
1	4	1	A	B	II
1	4	2	B	B	III
1	4	3	B	B	III
1	4	4	B	C	IV
2	1	1	A	A	I
2	1	2	A	A	II
2	1	3	B	B	II
2	1	4	B	B	III
2	2	1	A	A	II
2	2	2	B	B	II
2	2	3	B	B	III
2	2	4	B	B	III
2	3	1	B	B	II
2	3	2	B	B	III
2	3	3	C	B	III
2	3	4	C	C	IV
2	4	1	B	B	III
2	4	2	B	B	III
2	4	3	C	C	IV
2	4	4	C	C	IV
3	1	1	A	A	II
3	1	2	B	B	II
3	1	3	B	B	III
3	1	4	B	B	III
3	2	1	B	B	II
3	2	2	B	B	III
3	2	3	C	B	III
3	2	4	C	C	IV
3	3	1	B	B	III
3	3	2	C	B	III
3	3	3	C	C	IV
3	3	4	C	C	IV
3	4	1	B	B	III
3	4	2	C	C	IV
3	4	3	C	C	IV
3	4	4	C	C	V
4	1	1	A	B	II
4	1	2	B	B	III
4	1	3	B	B	III
4	1	4	B	C	IV
4	2	1	B	B	III
4	2	2	B	B	III
4	2	3	C	C	IV
4	2	4	C	C	IV
4	3	1	B	B	III
4	3	2	C	C	IV
4	3	3	C	C	IV
4	3	4	C	C	V
4	4	1	B	C	IV
4	4	2	C	C	IV
4	4	3	C	C	V
4	4	4	C	C	V

Either the product (P x T x B) or the sum (P+T+B) of the PTB-criteria in the table were used to distinguish the different PTB-classes. Both methods assume that each criterion has equal weight!a: ≤ 4, b: 4-16; c: >16; A: ≤ 5; B: 6-8; C: > 8; I: ≤ 4; II: 5-6; III: 7-8; IV: 9-10; V: > 10

Appendix 6 Rough estimate of PTB-properties of 'other' substances

	Metals	**Polymers**	**Organometals**	**Homologue mixtures***	**Heterogenic mixtures****	**Inorganic compounds**
Molecular weight	Various	High	Various	Various	Various	Various
Kow	Low	Various	Various	Various	Various	Various
S	Various	Low	Various	Various	Various	Various
Vp	Low	Low	Low	Various	Various	Various
Distribution profile	Water and soil/sediment	Soil/sediment	Water and soil/sediment	All compartments	All compartments	Water and soil/sediment
Biodegradation	Low	Various	Various	Various	Various	Low
Photo-oxidation	Low	Various	Various	Various	Various	Low
Hydrolysis	Low	Various	Various	Various	Various	Low
Aquatic toxicology	Various	Low ?	Various	Various	Various	Various
Human toxicology	Various	Low ?	Various	Various	Various	Various
Bioaccumulation	Various	Low ?	Various	Various	Various	Various

* When 'boundaries' (smallest and biggest molecules from mixture, etc.) are known, an estimate of the extremes may give an indication of the different PTB-properties of the entire homologue mixture

** When the different sub-classes of the mixture can be identified, an estimate of the extremes may give an indication of the different PTB-properties of the entire mixture