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Estimation methods for bioaccumulation in risk assessment of organic chemicals

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PREFACE

We would like to thank Dick Sijm for his help with the fish model and comments on an early draft of this report. Furthermore, we would like to thank Theo Vermeire for his extensive commenting and Theo Traas for *ad hoc* advice on several occasions.

CONTENTS

ABSTRACT

In this study, the methodology for estimating bioaccumulation of organic chemicals is evaluated. This study is limited to three types of organisms: fish, earthworms and plants (leaf crops, root crops and grass). We propose a simple mechanistic model for estimating BCFs which performs well against measured data. To evaluate the dynamics of bioaccumulation, simple one-compartment models are selected and parameterised. For specific chemical properties, the concentration in the organism reacts slowly to changes in the environmental concentration. This does not generally affect the estimation of long-term average concentrations which are relevant for risk assessment.

SUMMARY

Chemicals may have negative effects on humans and other organisms through their diet. This phenomenon is known as "secondary poisoning" or "indirect exposure". To assess a chemical's potential to cause secondary effects, the EU Technical Guidance Documents (TGD) for new and existing substances include assessment procedures for several simple food chains. In this study, the methodology for estimating bioaccumulation of organic chemicals is evaluated. This study is limited to three types of organisms: fish, earthworms and plants (i.e. leaf crops, root crops and grass). Apart from evaluating the estimation of bioconcentration factors (BCFs) and bioaccumulation factors (BAFs), also the consequences of assuming steady state between organism and environment are evaluated.

A simple partition model is proposed for estimating BCFs or BAFs for all these organisms. This model is parameterised and compared to measured data. For fish, the performance of this approach is comparable to the QSAR regression advised by the TGD in the range log *Kow* 1-6. At log *Kow*>6, the TGD advises to use a polynomial equation for which the support is extremely limited and which may lead to serious underestimation of risk. Instead of using polynomial relationships, we advice to apply a constant BCF above log *Kow*=6. Since eel is much fatter than other species (about 8 times), a separate assessment for this species may be made. For earthworms, the partition model performs much better than the QSAR advised in the TGD. The QSAR was based on poorly evaluated data and overestimates BCF by a factor of 4. For plants, the theoretical model performs well against experimental data and is already advised by the TGD although slightly different parameter settings are appropriate.

To evaluate the dynamics of bioaccumulation, simple one-compartment models are used and parameterised. For fish, the kinetic parameters are estimated using an allometric model from the literature. For earthworms, an initial estimate is made on the basis of several experimental results and for plants, a mechanistic model from the literature is used. The concentration pattern in time in fish, earthworms and crops starts to deviate from the external concentration pattern when log *Kow*>4, log *Kow*>6 and log $K_{leaf\text{-}air}$>6 respectively. This does not significantly affect the long-term average concentrations (months to several years) in these organisms, but the different pattern can be important when more information on the toxicity mechanism of the chemical is known.

For crops, the scenario of using a 180-day average soil concentration is wrong because the concentration at the time of harvest is relevant. This scenario may be used when the following consequences are kept in mind. The TGD approach will overestimate concentrations in root crops and long-growing leaf crops (e.g. cabbage) when the chemical's half life in soil is short (a factor of 1.5-2 when the half life is 100 days, a factor of 20-100 when the half life is 20 days). At short half lifes in soil (20 days) and log $K_{leaf\text{-}air}$>6, the TGD approach will underestimate levels in short-growing crops (e.g. lettuce) by a factor of 2. When the half life in soil is larger than 100 days and log $K_{leaf\text{-}air}$>6, the assumption of continuous growth becomes crucial and it is better to use actual time-related growth information.

Finally, it should be noted that all BCF estimates are median case. We advice to include the uncertainty in the estimate in an uncertainty analysis. Furthermore, we recommend to investigate the possibilities of different exposure pathways in future research.

SAMENVATTING

Chemische stoffen kunnen negatieve effecten bij mensen en andere organismen veroorzaken via hun dieet. Dit fenomeen staat bekend als "secondary poisoning" of "indirecte blootstelling". Om een stof te beoordelen op potentiële secundaire effecten bevat de EU Technische Leidraad (TGD) voor nieuwe en bestaande stoffen methoden voor het doorrekenen van simpele voedselketens. In deze studie wordt de methodologie voor het schatten van bioaccumulatie van organische stoffen geëvalueerd. Deze studie beperkt zich tot drie organismen: vis, regenwormen en planten (verdeeld in bladgewassen, wortelgewassen en gras). Behalve het evalueren van de schattingsroutines voor bioconcentratiefactoren (BCF's) en bioaccumulatiefactoren (BAF's) worden consequenties van het aannemen van evenwicht tussen organisme en milieu geëvalueerd.

Een simpel partitiemodel wordt voorgesteld voor het inschatten van BCF's en BAF's voor al deze organismen. Dit model is geparameteriseerd en vergeleken met meetwaarden. Voor vissen presteert dit model vergelijkbaar met de QSAR-regressie zoals geadviseerd door het TGD in de range log *Kow* 1-6. Bij log *Kow*>6 adviseert het TGD een polynoom waarvoor de ondersteuning beperkt is en welke kan leiden tot serieuze onderschatting van het risico. In plaats van de polynoom adviseren wij een constante BCF toe te passen boven log *Kow*=6. Omdat paling veel vetter is dan andere soorten (ongeveer 8 keer) kan beter een aparte beoordeling voor deze soort gemaakt worden. Voor regenwormen is het partitiemodel veel beter dan de QSAR die door het TGD geadviseerd wordt. De QSAR was gebaseerd op slecht geëvalueerde data en overschat BCF met een factor 4. Voor planten is het theoretische model in overeenstemming met de gemeten waarden en is reeds geadviseerd door het TGD. Voor dit model zijn enigszins aangepaste parameterwaarden toepasselijk.

Simpele ééncompartimentsmodellen worden toegepast en geparameteriseerd om de dynamiek van bioaccumulatie te evalueren. Voor vissen is een allometrisch model uit de literatuur gebruikt om de kinetische parameters in te schatten. Voor regenwormen is een initiële schatting gemaakt op basis van een aantal experimentele resultaten en voor planten is een mechanistisch model uit de literatuur gebruikt. Het concentratieverloop in de tijd van vissen, wormen en planten begint af te wijken van het externe blootstellingspatroon als log *Kow*>4, log *Kow*>6 en log $K_{leaf\text{-}air}$>6 respectievelijk. Dit heeft weinig effect op de gemiddelde concentratie op lange termijn (maanden tot enkele jaren) in deze organismen, maar het veranderde patroon kan belangrijk zijn als informatie over het toxiciteitsmechanisme beschikbaar is.

Voor gewassen is het middelen van de bodemconcentratie over 180 dagen verkeerd omdat de concentratie op het tijdstip van oogsten relevant is. Dit scenario kan wel gebruikt worden als de volgende consequenties beschouwd worden. De TGD-aanpak zal de concentraties in wortelgewassen en lang groeiende bladgewassen (bijv. kool) overschatten als de halfwaardetijd van de stof in de bodem kort is (een factor 1.5-2 bij een halfwaardetijd van 100 dagen, een factor 20-100 bij een halfwaardetijd van 20 dagen). Bij een korte halfwaardetijd in bodem (20 dagen) en een log $K_{leaf\text{-}air}$>6 zal de TGD-aanpak de niveaus in kortgroeiende gewassen (bijv. sla) onderschatten met een factor 2. Zodra de halfwaardetijd in bodem groter is dan 100 dagen en log $K_{leaf\text{-}air}$>6 wordt de aanname van continue exponentiële groei cruciaal en is het beter tijd-gerelateerde groei informatie te gebruiken.

Tenslotte moet worden opgemerkt dat alle BCF schattingen mediaan zijn. We adviseren om de onzekerheid in de schattingen mee te nemen in een onzekerheidsanalyse. Wij raden verder aan om de mogelijkheden van andere blootstellingsroutes in de toekomst te onderzoeken.

1. GENERAL INTRODUCTION

Chemical-risk assessment tries to protect humans and the environment from possible adverse effects caused by chemical substances. In general, environmental risk assessment is foremost concerned with direct exposure of the protection targets: aquatic organisms exposed to the dissolved water phase, terrestrial organisms exposed to porewater in soil, etc. Human exposure assessment is also primarily concerned with direct exposure: e.g. exposure of workers at a factory and through use of consumer products. Direct human exposure may also occur through the environment by ingestion of soil or water, uptake through the skin and inhalation of ambient air. Undoubtedly, direct exposure of organisms is an important pathway in risk assessment. However, for humans and other organisms this is not the only route of exposure as they may also be indirectly exposed via their diet. Indirect exposure involves more elaborate pathways as the chemical passes through the food chain. Organisms may be exposed to higher concentrations in their diet than occurring in the ambient environment. It is therefore conceivable that an assessment procedure aimed at protection of organisms through direct exposure may fail to protect organisms higher in the food chain.

Owing to considerable uncertainties in the assessment methodology, the results of an indirect-exposure assessment are generally viewed as "indicative". This is reflected in the EU Technical Guidance Document (TGD) supporting the risk assessment of new and existing substances (EC, 1996a). To assess a chemical's potential to poison organisms higher in the food chain, two example food chains are modelled: water → fish → predating bird or mammal, and soil → earthworm → predating bird or mammal. These calculations are only performed to give an *indication* that bioaccumulation may cause adverse effects.

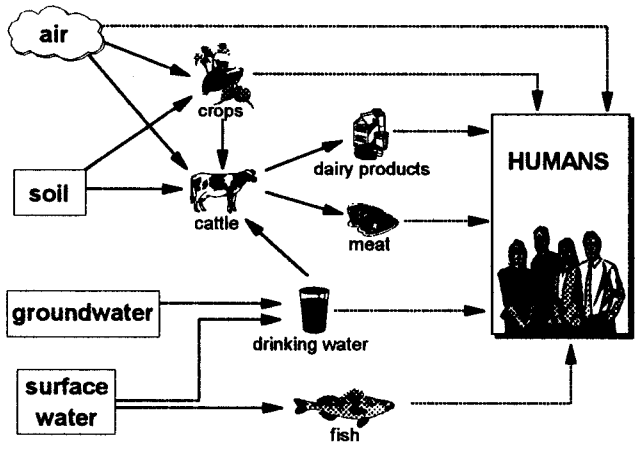

***Figure 1** Indirect exposure routes for humans as addressed in the TGD*

For human exposure through the environment a more detailed approach is followed where the modelled human individual is exposed via air, drinking water, fish, meat, milk and crops (Figure 1). As discussed in the TGD chapter on human-health risk assessment, there is no testing strategy triggered by the indirect exposure assessment. When the assessment indicates that there is "reason for concern" the assessment needs to be refined.

One of the most severe preconditions for risk assessment methodology in the framework of the TGD is the need for simple and generic methods. This is essential since the available data sets are small (e.g. the EC Base Set) and transparency in the assessment is required. However, risk assessment also requires efficient methodology. It is important to protect humans and organisms from possible effects caused by bioaccumulation but a worst case assessment may trigger too much false positives. The aim is therefore on a "reasonable worst case" assessment, indicating a protective approach, but not an unrealistic one. This need for simple methodology is also reflected in the forced time independence of the models described in the TGD. As environmental concentrations will usually vary in time, choices were made to arrive at fixed concentrations in the environment. As a result, indirect exposure of humans and predators is

assessed using averaged concentrations in the environmental compartments (annual average for surface water and air, 180-day average for soil) and assuming a steady state between the environment and the "prey organism"[1]. This approach is a pragmatic one, neglecting the kinetics of environmental fate processes and the kinetics of uptake and elimination from the prey organisms. For emissions which are continuous or diffuse, this assumption will generally be valid. For chemicals emitted as a result of batch processes, the validity of this steady-state assumption is questionable.

The purpose of this study is to evaluate the current methodology for bioaccumulation (model approach, parameter settings, exposure scenarios) and to propose alternative approaches if required and possible. A second purpose is to evaluate the consequences of assuming a steady state between the organism and its exposure media. The latter is done by comparing the steady-state approach to the results of (simple) dynamic modelling. This study departs from the methods and models described in the Technical Guidance Document (TGD) for new and existing substances (EC, 1996a) which are implemented in a computerised support system: the European Union System for the Evaluation of Substances (EUSES; EC, 1996b; Vermeire *et al.*, 1997). EUSES is an update of the USES system as already operational in the Netherlands (RIVM *et al.*, 1994; Vermeire *et al.*, 1994). The results of this study are however, not only applicable to this framework but are of more general interest in the practice of risk assessment and environmental quality. The evaluation is carried out in four steps:

1. Selecting simple steady-state and dynamic models for bioaccumulation in prey organisms. Chapter 2 gives a general introduction on bioaccumulation, Chapter 3 provides a general model for bioaccumulation which is specified and evaluated for the different prey organisms in the Chapters 4, 5 and 6.
2. Analysis of the dynamics in the local fate models (Chapter 7).
3. Comparing the results of dynamic simulation to the steady-state situation (Chapter 8).
4. Discussion and recommendations for updating or refining the methodology of the TGD (Chapter 9 and 10).

Naturally, the kinetics of uptake and elimination in the "protection target" (humans or predators) can also play an important role in the final effect on the protection target (toxicokinetics). We, however, will focus on the dynamics of external exposure only. It should be noted that is not the primary purpose of this study to replace the current steady-state approach for bioaccumulation with a dynamic one. Dynamic models are, however, required to evaluate the impact of assuming an unrealistic steady state. On the basis of a comparison between steady state and dynamic models, it may be possible to advice simple "rules of thumb" to correct the steady-state models to yield more realistic results. Nevertheless, for more refined stages of risk assessment, dynamic models may prove useful.

Even though the exposure route for humans through consumption of meat and milk can be very important for hydrophobic compounds, there were insufficient data, models and time available to address cattle in this study (this route is subject of a more thorough investigation in 1997). Therefore, this study is restricted to bioaccumulation in fish, earthworms and plants.

[1] The term "prey organism" is used here to describe those organisms consumed by others (the "predators"). In the framework of the this study, we include in this term fish, earthworms, cattle and also crops.

2. INTRODUCTION ON BIOACCUMULATION

The chemical concentration in an organism depends on the concentration in its ambient environment, the concentration in its food[1], and the relative importance of all possible routes of uptake and elimination. The dominant processes affecting the concentration of the chemical in the organism are:

- uptake via diffusive mass transfer (e.g. through gills, skin or leaf surface);
- uptake via consumption of contaminated food;
- elimination via diffusive mass transfer (e.g. through gills, skin or leaf surface);
- elimination by biotransformation;
- dilution of the concentration by growth[2];
- transfer of the chemical to the organism's offspring.

2.1. Definitions

Three expressions are generally used to describe the net result of these routes of uptake and elimination:

1. Biomagnification is the increase of the concentration in an organism as a result of consumption of contaminated food. When steady state is reached between the concentration in the organism and in its food source (indicated by *Css*), the magnitude of biomagnification can be expressed by means of the biomagnification factor (BMF), which is defined as:

$$BMF = \frac{Css_{organism}\ (resulting\ from\ uptake\ via\ food)}{Css_{food\ source}}$$

2. Bioconcentration is the increase of the concentration in the organism as a result of diffusive mass transfer of a contaminant in the environment into an organism. When steady state is reached between the concentration in the organism and the environment, the magnitude of bioconcentration can be expressed by means of the bioconcentration factor (BCF), which is defined as:

$$BCF = \frac{Css_{organism}\ (resulting\ from\ diffusive\ uptake\ from\ the\ environment)}{Css_{environment}}$$

3. Bioaccumulation is the increase of the concentration in the organism as a result of both routes of uptake and all routes of elimination mentioned above. When steady state is reached, both between the concentration in the organism and the environment and between the concentration in the food and the environment, the magnitude of bioaccumulation can be expressed by means of the bioaccumulation factor (BAF), which is defined as:

$$BAF = \frac{Css_{organism}\ (resulting\ from\ all\ routes\ of\ uptake)}{Css_{environment}}$$

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[1] The term "food" is used here in the broadest sense as non-diffusive, one-way processes. Therefore, "food" may include soil solids for earthworms, algae for fish, but also porewater for the shoots of plants were translocation from root to shoot is mainly one-way.

[2] Dilution by growth is not an elimination process, but is comparable to the other processes in that it acts to diminish the concentration of the chemical within the organism. The *mass* of chemical in the organism is not affected by growth.

In risk assessment procedures, BMF, BCF and BAF are commonly used to estimate the concentrations in fish and earthworm species which are believed to be indicative for the food source of higher trophic levels in the aquatic and terrestrial food chain, respectively. Biomagnification and bioconcentration are usually studied in laboratory studies. To determine BMFs and BCFs in aquatic experiments, fish are either exposed to contaminated food (e.g. Sijm *et al.*, 1992) or to polluted water (e.g. Könemann & Van Leeuwen, 1980; Butte *et al.*, 1991; Zok *et al.*, 1991), respectively. In terrestrial experiments, BMFs can be determined by adding contaminated manure to a clean soil as a food source for earthworms (Belfroid *et al.*, 1994a). Separate BCFs can usually not be determined in laboratory experiments with earthworms, since these organisms will also ingest the contaminated soil[1]. Therefore, no distinction can be made between uptake from ingestion or from diffusive mass transfer when earthworms are exposed to contaminated soil, and it is only possible to determine BAFs in this kind of experiments. Estimates of earthworm BCFs in soil can be derived from bioconcentration experiments in water (Belfroid *et al.*, 1993; Lord *et al.*, 1980) by adopting the equilibrium-partitioning hypothesis that the uptake from passive absorption is mainly determined by the concentration in the interstitial water,.

Bioaccumulation is usually determined in (semi-)field experiments and monitoring programmes. In this kind of studies, it is possible to determine concentrations in the test species and in the ambient environment, whereas the relative contribution of the separate routes of exposure cannot usually be distinguished.

2.2. Estimation methods for fish

Traditionally, of all accumulation processes, bioconcentration in fish is studied best. In overview papers, many BCFs of organic compounds for several fish species have been collected (e.g. Neely *et al.*, 1974; Veith *et al.*, 1979; Kenaga & Goring, 1980; Mackay, 1982; Veith & Kosian, 1983; Oliver & Niimi, 1983; Chiou, 1985; Opperhuizen & Sijm, 1990). Usually, BCF is related to the octanol-water partition coefficient (*Kow*) but this is not the only possible descriptor (and is even irrelevant for chemicals like surfactants or metals). ECETOC (1995) gives a more detailed listing of alternative descriptors like molecular connectivity index or critical micelle concentration for surfactants. In most studies, linear relationships are derived for partitioning of a contaminant between water and fish by regression on the octanol-water partition coefficient (*Kow*), i.e. $\log(BCF) = a \cdot \log(Kow) + b$. This QSAR (quantitative structure-activity relationship) is based on the hypotheses that:

1. lipid tissue is the principal site for bioconcentration of organic pollutants, and;
2. fish lipid-water partitioning at steady state can be related to octanol-water partitioning.

A unity slope (a=1) would reflect a strict dependence of BCF on partitioning solely, assuming that partitioning between octanol and water is completely comparable to partitioning between fish fat and water. For many data sets, however, significantly smaller slopes were found (i.e. $a \approx 0.6$). This finding seems to imply that BCFs increase less with increasing hydrophobicity of chemicals. For very hydrophobic chemicals (log *Kow*>6), a complete loss of linearity was found. Therefore, non-linear QSAR models were developed, such as polynomial and sigmoidal

[1] Exception is the work of Lord *et al.* (1980) where worms were exposed after "application of ligatures at both anterior and posterior ends of a worm to prevent uptake or excretion through the gut".

models (Könemann & Van Leeuwen, 1980; Connell & Hawker, 1988; Nendza, 1991). Although these models may describe the BCFs of specific, very hydrophobic, organic compounds more accurately, they are poorly supported by a mechanistic background. In fact there are, to our knowledge, no biological processes which act through a polynomial relationship. Furthermore, QSAR regressions in general have several drawbacks: different data sets will yield different relationships, extrapolation beyond the range of the training set[1] can lead to unacceptable results, and regressions provide little insight in the mechanism of uptake. The training set must be a representative sample from the type of chemicals for which the regression is intended (e.g. all organic chemicals) and should not be heavily based on a certain group of compounds. As an example, the decreasing BCF at high *Kow*s in the polynomial equation of Connell & Hawker (1988) is mainly based on BCF data for PCDDs and is therefore suspect (see Figure 10). In view of these limitations, a more mechanistical approach is preferred.

Various reasons were given by Nendza (1991) for the fact that BCFs no longer increase with increasing *Kow* for very hydrophobic compounds. Possibly:

1. Steady state was not reached during the experiment, resulting in an underestimation of the experimental BCF. The time required to reach steady state tends to increase with increasing hydrophobicity of the compound because the rate of passive excretion tends to decrease with increasing *Kow*.
2. Experimental difficulties. Hydrophobic compounds are difficult to test since they are less available for uptake, due to adsorption to suspended material or to the test container. Thus, in poorly conducted experiments, the concentration in the test water may be overestimated and the experimental BCF therefore underestimated.
3. The structural differences between fish lipids (which are mainly arranged in membranes) and octanol with little or no structure cause different solvent characteristics. Probably, solvent characteristics of octanol and fish lipids are comparable for small molecules but not for larger molecules. Permeability of membranes is hampered for chemicals with a cross section larger than 0.95 nm (Opperhuizen *et al.*, 1985), a length exceeding 5.3 nm (Opperhuizen *et al.*, 1987). Molecular weight is often used as descriptor for limited uptake e.g. >500 (Umweltbundesamt, 1990) or >700 g/mol (EC, 1996a). However, since oligomers with a molecular weight up to 1050 were found in fish tissue, it is unlikely that molecular weight is the only limiting factor (Opperhuizen *et al.*, 1987).
4. The rate of passive excretion tends to decrease with increasing *Kow*. Therefore, at high *Kow*, other processes like biotransformation and growth can have a significant effect on the elimination rate and thus on BCF even when their rate constants are very low. Biotransformation processes usually result in products which are more water soluble than the parent compound and hence have reduced BCF values.

And additionally:

5. Growth of the organism may have occurred in the experiments, diluting the concentration and resulting in a lower BCF (see for example Sijm *et al.*, 1992).

It should be realised that a QSAR is only applicable within the range of log *Kow* values which were originally used to derive the regression. As stated above, linear QSARs are only valid for (organic) chemicals with log *Kow*<6. As shown by Nendza (1991), the discrepancy between

[1] The term "training set" is used for the actual dataset which was used to derive the QSAR (usually by regression on a log-log scale).

actual BCFs and QSAR estimates is even more dramatic when polynomial models are extrapolated, beyond the range of the training set, to higher and lower *Kow* values. Despite the fact that these ratios only hold for a steady-state situation, BCFs (and BAFs) are commonly used for risk assessment purposes. The steady-state concentration in fish is calculated by multiplying the estimated or measured water concentration with the BCF.

2.3. Estimation methods for earthworms

As stated previously, bioconcentration for aquatic organisms (mainly fish and mollusc species) has been studied to a much greater extent than for other organisms. Earthworms play a central role in indirect exposure for the terrestrial ecosystem owing to their abundance and the fact that they comprise a large portion of the diet of many bird and mammalian species. Hence, soil→worm→predator food chains are incorporated in the derivation of Dutch soil-quality criteria (Romijn *et al.*, 1994) and EU risk-assessment guidances (EC, 1996a). In risk assessment practice, measured BCF data for earthworms are scarce and as a consequence, there is a clear need for descriptive estimation routines. QSARs for earthworms are given by Lord *et al.* (1980), Connell & Markwell (1990) and Belfroid *et al.* (1993). Connell & Markwell collected experimental BCF data for pesticides, whereas Belfroid and co-workers used BCFs from their own experiments for earthworms exposed to water only. Generally, uptake from the porewater phase of soil is regarded as the dominant exposure route for earthworms (Van Gestel & Ma, 1988; Belfroid, 1994)[1]. Therefore, the same type of log-linear QSAR is applied for BCF on porewater basis as a function of *Kow* as was done for fish. Little is known about bioaccumulation of extremely hydrophobic chemicals in earthworms but is seems likely that the observations of Section 2.2 also apply, at least to some extent, to earthworms.

2.4. Estimation methods for crops and grass

Plants or plant products, like vegetables, fruit and grains form the greater part of the food consumed by humans and cattle. When attempting to predict levels in plants tissues, several important conceptual problems need to be considered:

1. There are hundreds of different plant species forming the heterogeneous group of "food crops".
2. Different parts of the crop are consumed: roots, fruit, stems, leaves or even flowers.
3. Many crops are grown in controlled environments (greenhouses) or imported from abroad.
4. Plant exposure may take place through different routes: uptake and translocation from porewater, diffusive uptake from the gas phase of air, deposition onto the leaf surface, followed by absorption into the leaf.
5. Most crops are not eaten raw but are cooked, baked etc. This food processing may influence the chemical content.

[1] In her Ph.D. thesis, Belfroid (1994) discusses the validity of the equilibrium partitioning theory with respect to bioaccumulation and toxicity. In general, the assumption of porewater exposure was applicable to earthworms. Dietary exposure was important only for compounds with a very large *Kow* (generally log *Kow*>6) and in soils rich in organic matter.

6. Plants are have a high metabolic activity which cannot be predicted from simple physico-chemical properties. The rate of metabolism in plants is generally 25 times higher than biodegradation in soil (Severinsen & Jager, in prep.).

In view of these limitations, it is clear that a model can only provide a rough approximation of contaminant levels in crops. To address "point of concern" 2, the TGD makes a distinction between root and leaf crops. More elaborate modelling approaches also include stem and fruit compartments (e.g. the PlantX model as described by Trapp *et al.*, 1994). In general, the uptake of substances is viewed as a passive process, governed by physical sorption (for roots), the transpiration stream (for leaves), and passive diffusion (in case of uptake from air). Briggs *et al.* (1982) found that the BCF of roots increased with increasing hydrophobicity of the compound. For the efficiency of translocation from porewater to stems, an optimum *Kow* was found (Briggs *et al.*, 1982; Crowdy, 1973). It was suggested that at lower *Kow*, translocation is limited by the lipid membrane of the root whereas at higher *Kow* the rate of transport in the aqueous transpiration stream is limiting.

In the decision-support system USES (RIVM *et al.*, 1994), the relationships of Briggs *et al.* (1982, 1983) are used to describe uptake from soil into roots and translocation to shoots. A leaf-air partition coefficient (Riederer, 1990) and an aerosol-plant partition coefficient (McKone & Ryan, 1989) were used to describe uptake from air. These factors are comparable to the use of BCFs and BAFs for fish and earthworms. However, the problem with this type of approach for plants was two-fold:

1. A steady-state concentration ratio between leaves and porewater was defined. This is questionable since chemical transport is usually one way: the chemical in the transpiration stream will end up in the shoot where the water is transpired and the chemical remains. Most non-dissociating chemicals will not be transported with the phloem to the roots in significant amounts (Bromilow & Chamberlain, 1995).
2. Uptake from soil and air were calculated separately. Therefore, the effect of the route soil→plant→air could not be calculated.

The recently proposed model of Trapp & Matthies (1995, 1996) combines uptake from porewater with diffusive exchange with air into a simple one-compartment model. This model is also advised in the TGD (EC, 1996a) and is discussed in more detail in Chapter 6. Several limitations of this model were put forward by Trapp & Matthies (1995):

1. The model is limited to non-dissociating chemicals.
2. Concentrations are relevant for foliage. Levels in fruits may be largely deviating.
3. Exponential growth is assumed. This is only representative for crops harvested before maturation: green fodder, green vegetables, lettuce.
4. Transport of chemical with the phloem is neglected. For chemicals with certain properties (log *Kow* around -0.5, weak acids) this transport can become very important (Bromilow & Chamberlain, 1995).
5. Deposition by aerosols onto leaves is not included. According to the authors, it is unclear whether this process actually contributes to accumulation.
6. The model is parameterised for a "typical, generic plant" which is not a specific species but has some kind of average values.

3. A GENERAL APPROACH FOR BIOACCUMULATION

In this chapter, a general model is presented to describe bioaccumulation in a more mechanistic manner. QSAR approaches as discussed in the previous chapter are valuable in risk assessment but are only descriptive, not explanatory. It should be noted that the presented approach is not a new one; the same approach is used by several authors to describe the bioaccumulation process (e.g. Trapp & Matthies, 1995; Sijm & Van der Linde, 1995). Furthermore, the type of steady-state partitioning as described in Section 3.1 is also applied to describe chemical sorption in soil and sediments in the field of environmental chemistry. The dynamic description in Section 3.2 is the even more widespread linear one-compartment model. The approach is only written down in a more generic way to increase transparency and include all organisms (fish, worms, plant roots and leaves).

3.1. The steady-state situation

A general steady-state approach for neutral organic compounds is presented below. On a volume basis, the partitioning between organism and water can be described as a thermodynamical partitioning between the different compartments[1]:

$$K_{organism-water} = F_{water} + F_{fat} \cdot a \cdot Kow^{b}$$

With:

$K_{organism-water}$	organism-water partition coefficient	$[m_{water}^{3}.m_{organism}^{-3}]$
Kow	octanol-water partition coefficient	$[m_{water}^{3}.m_{octanol}^{-3}]$
F_{water}	volume fraction water in organism	$[m_{water}^{3}.m_{organism}^{-3}]$
F_{fat}	volume fraction fat in organism	$[m_{fat}^{3}.m_{organism}^{-3}]$
a	correction for sorption capacities fat and octanol	$[m_{octanol}^{3}.m_{fat}^{-3}]$
b	correction exponent for sorption capacities fat and octanol	[-]

Usually, the correction parameters *a* and *b* are set to 1 due to lack of knowledge of the actual processes. With this simplification, organism lipids and octanol are assumed to behave similarly in absorbing chemicals. The steady-state BCF (on a volume water per wet weight of organism) is then given by:

$$BCF = \frac{K_{organism-water}}{\rho_{organism}} = \frac{F_{water} + F_{fat} \cdot Kow}{\rho_{organism}}$$

With:

$\rho_{organism}$	bulk density of the organism	$[kg_{organism}.m_{organism}^{-3}]$
BCF	bioconcentration factor for the organism	$[m_{water}^{3}.kg_{organism}^{-1}]$

[1] It is important to note that this equation yields a concentration ratio on a *volume* basis (this parameter gets the symbol *K*). This is the case because *Kow has* got a unit: $(mol.m_{octanol}^{-3})/(mol.m_{water}^{-3})$ i.e. a ratio on volume basis. As a consequence, the fractions water and lipid in the organism need to be expressed on a volume basis also. This point was ignored by Sijm & Van der Linde (1995) and Trapp and Matthies (1995). After discussion with Stefan Trapp however, this author published a rectification in Environ. Sci. Technol. 30 (Trapp & Matthies, 1996). The point is mainly for consistency as the quantitative consequences are small. The way to avoid this problem is to specify the units (e.g. $[Kow] = m_{water}^{3}.m_{octanol}^{-3}$) and making the units consistent in the equation.

Fat fractions are usually expressed on a weight basis. These values cannot be used directly in the BCF equation presented above as *Kow* is expressed on a volume basis. Weight fractions can be translated to volume fractions by the following equation:

$$F_{fat} = WF_{fat} \cdot \frac{\rho_{organism}}{\rho_{fat}}$$

With:

F_{fat}	volume fraction fat in organism	$[m_{fat}^3.m_{organism}^{-3}]$
WF_{fat}	weight fraction fat in organism	$[kg_{fat}.kg_{organism}^{-1}]$
$\rho_{organism}$	bulk density of the organism	$[kg_{organism}.m_{organism}^{-3}]$
ρ_{fat}	bulk density of fat	$[kg_{fat}.m_{fat}^{-3}]$

as organism fat is assumed to act as octanol, the density of octanol may be assumed ($\rho_{octanol}$ = 827 kg/m^3). The fraction water of the organism may be assumed to partition 1:1 with the external water. This fraction will usually play a minor role in the bioaccumulation process but this term is nevertheless added for the sake of consistency. The water fraction is only important when log *Kow* is small, which also results in low BCFs. Therefore this term is of little interest from the point of view of a risk assessor[1].

Intermezzo: relation between the theoretical model and log-linear regressions.

The general expression for BCF is as follows (ignoring differences between octanol and lipids):

$$BCF = \frac{F_{water} + F_{fat} \cdot Kow}{\rho_{organism}}$$

At *Kow*>1000, the term F_{water} can be completely neglected compared to the product of F_{fat} and *Kow*, and BCF can be rewritten as:

$$BCF = \frac{F_{fat} \cdot K_{ow}}{\rho_{organism}}.$$

Log transformation results in:

$$\log(BCF) = \log(Kow) + \log\left(\frac{F_{fat}}{\rho_{organism}}\right)$$

i.e. a linear relationship, which is in agreement with the general form of the QSARs described in the previous chapter. This relationship shows a unity slope (assuming that partitioning between octanol and water is completely comparable to partitioning between fish fat and water). As discussed, this relationship holds only in case:

1. elimination by biotransformation is negligible;
2. elimination by growth dilution is negligible;
3. uptake of the chemical from food is negligible.

[1] This is acceptable for fish and earthworms. For plant leaves however, the water compartment may play an important role in the diffusive transfer with air. Especially for substances with a low Henry's law constant which prefer the water compartment to the ambient air and are rapidly taken up in the water phase of the leaf.

3.2. The dynamic approach

In this section, a generic one-compartment model for bioaccumulation in organisms is described. Chemical fluxes are governed by the concentration in the donating compartment (donor control). The general structure of the model is shown in Figure 2 ("environment concentration" may include possible food sources).

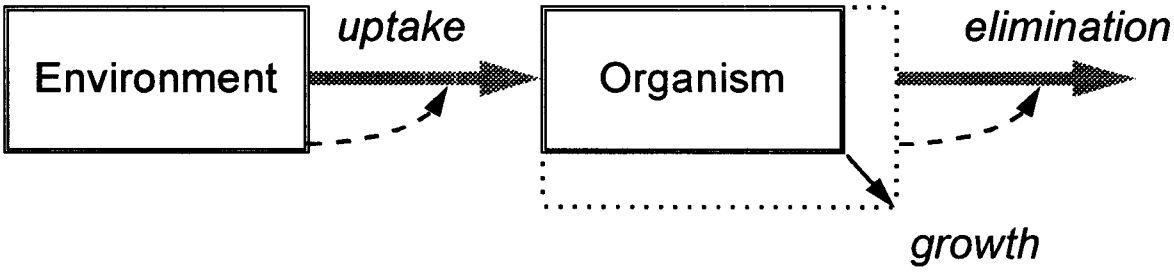

***Figure 2** Basic representation of a one-compartment uptake and elimination model. Thick lines represent a change in concentration, dashed lines represent linear dependence.*

A general one-compartment model has been widely accepted to describe the kinetics of bioaccumulation (e.g. Thomann, 1989; Ram & Gillett, 1993; Belfroid *et al.*, 1995a; Sijm *et al.*, 1992). This model can be described by the following linear differential equation:

$$\frac{dC_{organism}}{dt} = k_1 \cdot C_{environment} + k_f \cdot C_{food} - k_e \cdot C_{organism}$$

Where $C_{organism}$, $C_{environment}$ and C_{food} are the concentration in the organism, its environment and its food, respectively, k_1 is the uptake rate for diffusive mass transport, k_f is the uptake rate from food and k_e is the overall elimination rate constant. This elimination rate constant can be defined further as $k_e = k_2 + k_g + k_m$, with k_2, k_g and k_m being the rate constants for diffusive elimination, growth and metabolism, respectively. The dynamics of the uptake and elimination are assumed first order, and as a result, the rate constants are assumed constant in time and with concentration.

When steady state is reached, the quotient $dC_{organism}/dt = 0$. When uptake from food is relatively small compared to diffusive uptake from the environment, the term $k_f \cdot C_{food}$ can be ignored. Thus, $k_1 \cdot C_{environment} = k_e \cdot C_{organism}$ and BCF at steady state (*ss*) can be rewritten as:

$$BCF = \frac{Css_{organism}}{Css_{environment}} = \frac{k_1}{k_e} = \frac{k_1}{k_2 + k_g + k_m}.$$

This BCF formulation accounts for growth and metabolism. The elimination rate from the organism (k_e) may comprise elimination by diffusion, growth dilution, biotransformation. Until now, k_m has been neglected and set to zero. However, for some chlorinated organic pollutants, such as dibenzo-*p*-dioxins, dibenzofurans and specific PCB-congeners, higher elimination rates in fish were found than expected on the basis of (estimated) values of k_2 and k_g (Opperhuizen & Sijm, 1990; Sijm *et al.*, 1992). Due to this higher value of k_e, BCFs were accordingly lower than expected. This finding was attributed to biotransformation (i.e. k_m>0), which was demonstrated for these specific compounds in fish (e.g. Sijm & Opperhuizen, 1988; Boon *et*

al., 1989). A more in-depth discussion on metabolism is given in ECETOC (1995), including metabolic pathways, responsible enzymes, and the effects on bioaccumulation for different groups of compounds

The total elimination rate k_e also comprises dilution by growth. With increasing log *Kow* (log *Kow*>3), the rate of diffusive mass transfer (k_2) is expected to show an almost linear decrease (e.g. Sijm & Van der Linde, 1995). The rate of growth dilution is generally considered constant[1] and relatively low, therefore contributing little to the total elimination for most of the log *Kow* range. At high values of log *Kow* (e.g. for fish larger than 5), however, it is no longer negligible compared to the low values for k_2, whereas at log *Kow*>6 it can become more important than diffusive excretion (Sijm *et al.*, 1992). Assuming that the rate of biotransformation is also largely independent of *Kow*, this means that k_e will reach a constant minimum level of $k_e = k_g\ (+k_m)$ at log *Kow*>6. As a result, the BCF (defined as k_1/k_e) will also become constant for log *Kow*>6, since k_1 and k_e are both assumed constant within this *Kow*-range. This implies that, on theoretical grounds, no further increase in BCF is expected above log *Kow* of 6 (this is further worked out in Chapter 4).

For fish and earthworms, the relative contribution of uptake by food to the process of bioaccumulation has, among others, been investigated by Thomann (1989) and Belfroid *et al.* (1994a, 1995a), respectively. The food uptake rate constant can be defined further as:

$$k_f = E \cdot f$$

with

k_f	uptake rate through food (fish, worms)	$[(mg.kg_{org}^{-1})/(mg.kg_{food}^{-1}).d^{-1}]$
E	uptake efficiency	[-]
f	feeding rate	$[kg_{food}.kg_{organism}^{-1}.d^{-1}]$

For fish, Thomann (1989) suggests that the value of E is a function of log *Kow*. According to the author, "there appears to be a general increase (...) up to log *Kow* $\approx$ 5.5 and then a decrease at higher log *Kow* values". Actually, this relationship seems less distinct than the author suggests. However, Thomann found that a BAF model including the assimilation efficiency as a function of *Kow* fitted field BAF data better than a model assuming E is constant over the *Kow*-range of the chemicals tested. Belfroid *et al.* (1995a) stress that for a single chemical E and f are not completely independent parameters. The uptake efficiency will mainly depend on the digestibility of the food. When the food is digested easily, E is high and an organism will eat less to be satisfied (f is small). When E is small, an organism needs more food to become satisfied (f is high). It is not clear, yet, whether both parameters are completely inversely related. Therefore, it is suggested that for k_f estimation, both parameters should be obtained from the same experiment.

Both Thomann (1989) and Belfroid *et al.* (1995a) find that uptake from food will only contribute to the total bioaccumulation for (very) hydrophobic compounds (log *Kow*>5). Uptake from food can only be included in bioaccumulation modelling when the concentration in food is known. For primary consumers (and primary producers) it is relatively easy to obtain

[1] This is in fact done to simplify modelling but is in general valid for younger organisms. For crops this assumption must be evaluated with more care as volumetric growth is minimised, or even stopped, during the flowering and seed filling stages. For grass which is grazed by cattle, the assumption of constant growth is more acceptable.

exposure estimates since these organisms usually have only a few food sources and their concentrations can relatively easily be estimated or measured. For instance, earthworms digest organic matter from soil after soil consumption and estimates of concentrations in soil (or litter) can be made. On the other hand, for a "generic fish" the main food source and it's concentration is not known. Thomann (1989), for example, describes a model for a four level aquatic food chain, including fish as third and fourth level organisms. To use these kind of models for risk assessment, specific information is needed about the level of the "generic" fish in the food chain and the bioaccumulation properties of its feed. This information is not available for screening-level risk assessments and cannot always be properly estimated.

The same approach to bioaccumulation as described earlier can be used to describe uptake by plants. Plants, however, do not generally consume "food" in the strictest sense. Translocation of the chemical to the shoot is not a diffusive process but is caused by an active transport mechanisms, the transpiration stream, moving one-way. The effect of translocation is therefore comparable to food uptake which is also non-diffusive and one-way. For plants, the factor $k_f \cdot C_{food}$ can therefore be replaced by $k_p \cdot C_{porewater}$ where k_p is the rate constant governing uptake and translocation from porewater to leaves.

Summarising, the following rate constants can be used to describe the process of bioaccumulation:

uptake		
k_1	diffusive uptake	$[(mg.kg_{org}^{-1})/(mg.m_{environ}^{-3}).d^{-1}]$
k_f	uptake through food (fish, worms)	$[(mg.kg_{org}^{-1})/(mg.kg_{food}^{-1}).d^{-1}]$
k_p	uptake from translocation of porewater (plants)	$[(mg.kg_{org}^{-1})/(mg.m_{porew}^{-3}).d^{-1}]$
elimination		
k_e	total elimination rate constant	$[d^{-1}]$
k_2	rate constant for diffusive excretion	$[d^{-1}]$
k_g	rate constant for dilution by growth	$[d^{-1}]$
k_m	rate constant for removal due to metabolism	$[d^{-1}]$

3.3. Multiple compartment models

In some cases, the one-compartment uptake and elimination model cannot sufficiently describe bioconcentration. This will usually be concluded when there are two or more stages in which the elimination rates differ. The most commonly applied multiple compartment model is the two-compartment model shown in Figure 3 with a "fast" initial compartment and a "slow" secondary compartment. Examples of the use of two-compartment models can be found in Könemann & Van Leeuwen (1980), De Wolf et al. (1994), Belfroid *et al.*, (1994b). Linear one-compartment models are, however, most abundant and seem to perform adequately for most compounds and species.

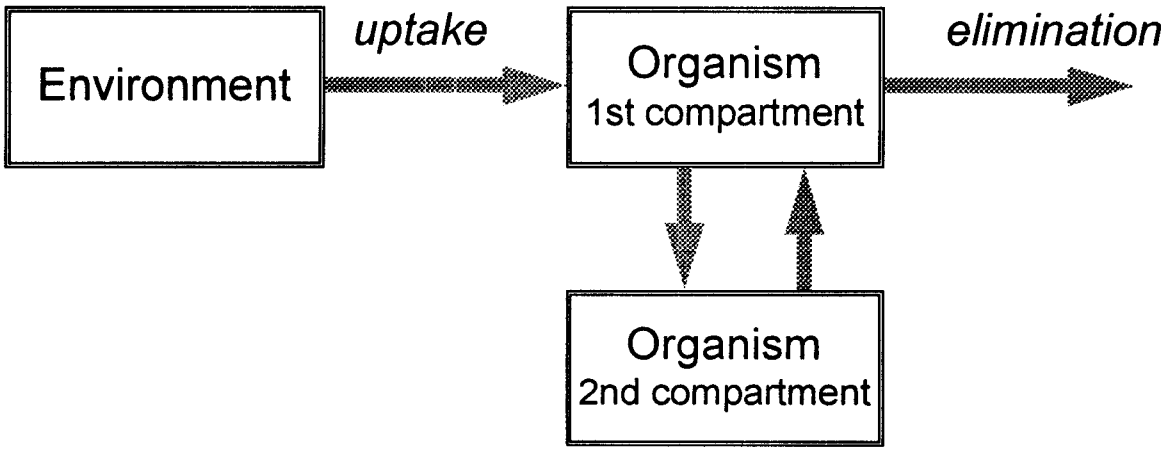

Figure 3 *Basic representation of a two-compartment uptake and elimination model. Thick lines represent changes in concentration.*

Even more complicated are physiologically-based models where the compartments represent actual tissues of the organisms (e.g. muscle, liver, fat, gills), and the blood flow between them. This type of model is generally beyond the scope of risk assessment and requires a large amount of experimental data. Examples can be found in Law *et al.* (1991) and Nichols *et al.* (1991). For plants, a multiple compartment model is described by Trapp *et al.* (1994) distinguishing roots, stems, leaves and fruit. This model can also be seen as a physiologically-based model.

3.4. Behaviour of one- and two-compartment models

In Figure 4, the general behaviour of a linear one-compartment model is plotted. Care must be taken when interpreting data on a logarithmic scale as this representation tends to clarify first-order elimination (straight line), but obscures first order uptake. It is therefore plausible that steady-state will be assumed erroneously (in this figure, 95% of steady state is achieved after 100 days but from the log scale it could be concluded that steady state was achieved within 25 days). This may for instance have been the case for the data of Belfroid *et al.* (1995b) where the authors report rapid steady state within 10 days whereas the elimination rates would suggest a period of 70 days to reach 95% of steady state.

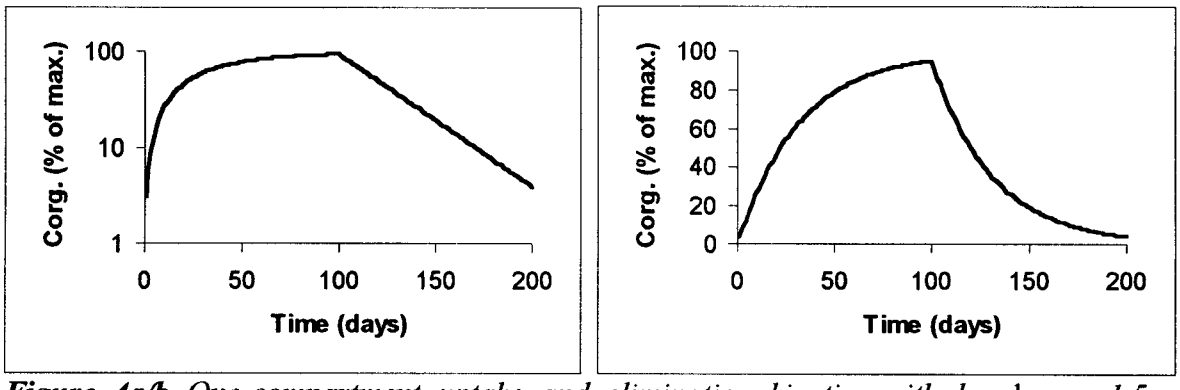

Figure 4a/b *One-compartment uptake and elimination kinetics with log* k_e *= -1.5 and exposure during 100 days. The left figure is plotted on a log scale, the figure right shows the same data on the original scale. After 100 days, 95% of steady-state is achieved.*

Figure 5 shows the behaviour of a linear two-compartment model. Figure a on the log scale reveals a two-phase elimination (two straight lines) which is not very clear from the original scale. Again, the log scale obscures the uptake phase. It is therefore difficult to distinguish between one-compartment and two-compartment uptake. Furthermore, from Figure 5b it can be concluded that although two-phase behaviour is eminent from Figure a, the first and rapid phase is in this case responsible for the main part of the body burden.

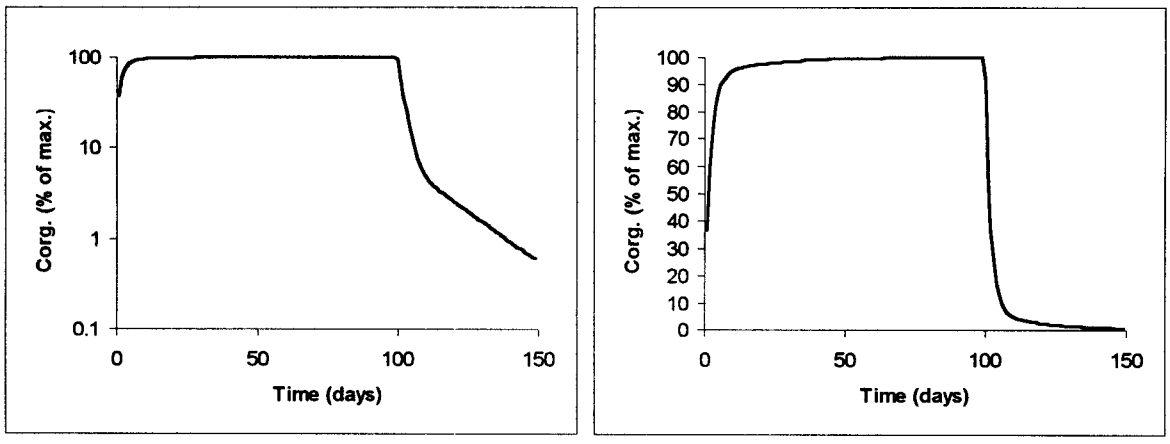

Figure 5a/b *Test runs with two-compartment behaviour. Left figure is plotted on a log scale, the figure on the right shows the same data on the original scale.*

In the following chapters, the general model of the previous chapter is specified and evaluated for the various prey organisms regarded: fish, worms, plant roots, and plant leaves. The theoretical steady-state approach is compared to measured data and the rate constants are specified. Furthermore, a limited sensitivity analysis is performed to indicate the relative importance of the parameters.

4. THE FISH MODEL

4.1. Dynamic model

Specification of the general model for fish gives the following differential equation:

$$\frac{dC_{fish}}{dt} = k_1 \cdot C_{water} + k_f \cdot C_{food} - (k_2 + k_g + k_m) \cdot C_{fish}$$

With:

C_{fish}	concentration in fish	$[mg.kg_{wwt}^{-1}]$
C_{water}	concentration in water (dissolved)	$[mg.m^{-3}]$
C_{food}	concentration in food	$[mg.kg_{food}^{-1}]$
k_1	rate constant for diffusive uptake	$[(mg.kg_{wwt}^{-1})/(mg.m_{water}^{-3}).d^{-1}]$
k_2	rate constant for diffusive losses	$[d^{-1}]$
k_f	rate constant for uptake through food	$[(mg.kg_{wwt}^{-1})/(mg.kg_{food}^{-1}).d^{-1}]$
k_g	rate constant for dilution by growth	$[d^{-1}]$
k_m	rate constant for losses due to metabolism	$[d^{-1}]$

The rate constant for food uptake can be further specified as:

$$k_f = E \cdot f$$

With:

E	uptake efficiency	[-]
f	feeding rate	$[kg_{food}.kg_{wwt}^{1}.d^{-1}]$

Uptake from food by fish is not regarded for the purpose of this study as it is difficult to take this into account in a generic model[1]. Nevertheless, food chain accumulation within the aquatic ecosystem remains a relevant point of concern, especially for very hydrophobic compounds. Model calculations of Thomann (1989) indicate that in the range log *Kow* 5-7, food chain accumulation can lead to elevated BAFs for top predators (up to two orders of magnitude higher than the BAF for an organism in the first trophic level).

4.2. Steady state

The partitioning between fish and water is described as a thermodynamical partitioning of the chemical between water and the constituents of the fish. The partition coefficient on a volume

[1] Since fish generally consume algae, crustaceans, insects or other fish, taking uptake via food into account requires more intricate food web modelling which is outside the scope of risk assessment in the initial or intermediate stage.

basis is based on the volume fractions of water and lipids in the fish[1]. The steady-state BCF in m^3/kg can then be written as a specification of the general model:

$$BCF = \frac{F_{water} + F_{fat} \cdot Kow}{\rho_{fish}}$$

With:

BCF	bioconcentration factor for fish	$[m_{water}^3.kg_{wwt}^{-1}]$
F_{water}	volume fraction water in fish	$[m_{water}^3.m_{wwt}^{-3}]$
F_{fat}	volume fraction fat in fish	$[m_{fat}^3.m_{fish}^{-3}]$
Kow	octanol-water partition coefficient	$[m_{water}^3.m_{oct}^{-3}]$
ρ_{fish}	bulk density of fish	$[kg_{wwt}.m_{fish}^{-3}]$

This BCF is the concentration ratio due to diffusive exchange with the water phase only. Other uptake and elimination processes are not considered. For very hydrophobic chemicals, elimination through diffusion tends to be slow and other processes like growth dilution and metabolism can become important even if their rate constants are small (see e.g. Thomann, 1989; Sijm *et al.*, 1992). The BCF equation can then be rewritten to:

$$BCF(+growth) = \frac{k_1}{k_2 + k_g + k_m}$$

It should be noted that in this steady-state BCF equation, in contrast with the previous equation, the kinetic rate constants are required.

4.3. Parameters

The steady-state formulation of BCF for fish requires the volume fractions fat and water of a fish. Data presented by Hendriks & Pieters (1993) are recalculated to fresh weight in Table 1. For the fat compartment, volume fractions are calculated, assuming a density of fat equal to octanol (827 kg/m^3).

Table 1 *Fractions water and fat in several aquatic species. Data in first two results columns are taken from Hendriks & Pieters (1993). Minimum and maximum values are shown between parentheses.*

Taxon	dwt / fwt (kg/kg)	fat / dwt (kg/kg)	F_{water} (kg/kg) (m^3/m^3)	WF_{fat} (kg/kg)	F_{fat} (m^3/m^3)
Crustacea	9 (3-15)	15 (10-27)	0.91	0.014	0.017
Bivalvia	12 (8-13)	14 (6-28)	0.88	0.017	0.021
Chironomidae	5 (2-7)	13 (9-42)	0.95	0.0065	0.0079
O. eperlanus	18 (16-19)	14 (9-21)	0.82	0.025	0.030
R. rutilus	24 (19-29)	12 (6-23)	0.76	0.029	0.035
A. anguilla	38 (31-45)	53 (48-68)	0.62	0.20	0.24
S. lucioperca	23 (19-23)	4 (3-9)	0.77	0.009	0.011

[1] Other constituents of fish are assumed to have no affinity for the chemical.

From this table, parameters for the general model are selected and shown in Table 2 which are appropriate for risk assessment purposes. It should be noted that although these properties are highly variable among different species of fish, modelling one generic fish (which could be an average or a "reasonable-worst case") is sufficient. Since risk assessment is generally concerned with chronic exposure, predators and humans will consume more than one fish species and in fact *many* individual fish. Therefore, the inter- and intra-species differences will show a tendency to average out in the exposure assessment (unless the diet of a predator is very specific).

***Table 2** Parameterisation of a hypothetical, generic fish for risk assessment purposes.*

	Value	Unit	Remarks
Weight of fish (W)	0.20	kg	Representative value for risk assessment purposes [a]
F_{fat}	0.03	m^3/m^3	Hendriks & Pieters (1993), excluding eel [b]
F_{water}	0.80	m^3/m^3	Hendriks & Pieters (1993), excluding eel
k_g	0.0025	$m^3.m^{-3}.d^{-1}$	Thomann (1989)
k_m	0	d^{-1}	Due to lack of general knowledge [c]
ρ_{fish}	1000	kg/m^3	Set equal to water [d]

[a] The value given here is a value which we consider "not unreasonable" for the fish consumed by humans and predating birds or mammals. Note that this weight is much higher than that of the fish generally used for deriving experimental BCFs.
[b] Since this species is much fatter than the other species, it is advisable to consider eel (*Anguilla anguilla*) separately in risk assessment, or adjust the generic fat fraction to accommodate a diet including eel.
[c] The rate of metabolism is highly dependent on the species of fish and the compound under consideration. The rate constant cannot simply be related to physico-chemical properties. For more discussion on metabolism for specific groups of compound, the reader is referred to ECETOC (1995).
[d] this is an initial estimate as fish generally "float around" in water (although this is caused by gas in the swim bladder). What is required here is the bulk density of the fish tissue excluding any gasses. The volume fractions are derived from the weight fraction and since the weight fractions of gasses is very low, this fraction is ignored. Since the largest fraction is water, and since the lower density of fat may be counteracted by heavier "other" fractions, this seems a reasonable estimate.

4.4. Rate constants

For fish, Sijm & Van der Linde (1995) developed an allometric model to estimate the diffusive rate constants k_1 and k_2. The model is based on a diffusive mass-transfer model presented by Gobas *et al.* (1986) and chemical transport is modelled according to the classical two-film resistance model (see e.g. Mackay *et al.*, 1992). Chemicals are assumed to be transported through aqueous and lipid diffusion layers in series. The uptake and elimination rates depend on diffusion coefficients in both layers, on the lipid-water partition coefficient of the compound, and on the exchange area and the size of the fish. The diffusion flux depends on diffusion path lengths which, in principle, depend on the size of the fish. The exchange area depends also on the size of the fish. Within the model, fish weight is used as a measure for its size. The rate constants k_1 and k_2 are described as (Sijm & Van der Linde, 1995):

$$k_1 = \left(\frac{\delta_w}{D_w} + \frac{\delta_m}{Kow \cdot D_m}\right)^{-1}\left(\frac{A}{W}\right) \text{ and } k_2 = \left(\frac{\delta_w}{D_w} + \frac{\delta_m}{Kow \cdot D_m}\right)^{-1}\left(\frac{A}{W}\right)BCF^{-1}$$

with

k_1	uptake rate constant	[$m^3.kg_{wwt}^{-1}.d^{-1}$]
k_2	elimination rate constant	[d^{-1}]
δ_w	diffusion length of the aqueous diffusion layer	[m]
D_w	diffusion coefficient in the aqueous diffusion layer	[$m^2.d^{-1}$]
δ_m	diffusion length of the lipid diffusion layer	[m]
D_m	diffusion coefficient in the lipid diffusion layer	[$m^2.d^{-1}$]
Kow	octanol/water partition coefficient on volume basis	[$m_{water}^3.m_{octanol}^{-3}$]
A	gill surface area	[m^2]
W	fish weight	[kg_{wwt}]

Parameterisation of the theoretical model with allometric relationships[1], based on the weight of fish (Sijm & Van der Linde, 1995):

$$A = 0.114 \cdot W^{0.77}$$

$$\delta_w = 4.51 \cdot 10^{-6} \cdot W^{0.114} \qquad \delta_m = 0.47 \cdot 10^{-3}$$

$$D_w = 9.3 \cdot 10^{-5} \cdot M^{-0.71} \qquad D_m = 0.3 \cdot D_w$$

With:

M	molecular weight	[$g.mol^{-1}$]

The quotient of a diffusion coefficient and a diffusion path length (D_w/δ_w) is also known in environmental chemistry as a "partial mass-transfer coefficient". The inverse of this coefficient for the aqueous diffusion layer is given by:

$$\frac{\delta_w}{D_w} = 0.0483 \cdot W^{0.114} \cdot M^{0.71} \quad \text{[d/m]}$$

The inverse of the partial mass-transfer coefficient for the lipid diffusion layer is given by:

$$\frac{\delta_m}{D_m} = 16.8 \cdot M^{0.71} \quad \text{[d/m]}$$

From these parameter calculations, the following over-all equations can be derived:

$$k_1 = \left[M^{0.71}\left(0.424 \cdot W^{0.344} + \frac{147 \cdot W^{0.23}}{Kow}\right)\right]^{-1}$$

$$k_2 = \frac{k_1}{BCF}$$

[1] Allometric relationships should be used with care as they are theoretically suspect due to inconsistency of the units. E.g., the calculation $W^{0.114}$ is not dimensionless but must result in the strange unit $kg^{0.114}$. Nevertheless, this type of equation is generally applied because of its descriptive value. Care must be taken when expressing parameters in different units (e.g. when changing [W] from kg to g)!

where BCF is estimated from *Kow* as outlined in Section 4.2. The behaviour of these equations as a function of *Kow* is shown in Figure 6 for hypothetical compounds (constant molecular weight[1] of 300 g/mol) in two hypothetical fish (weight 0.1 g and 1 kg, fraction fat 6 vol%).

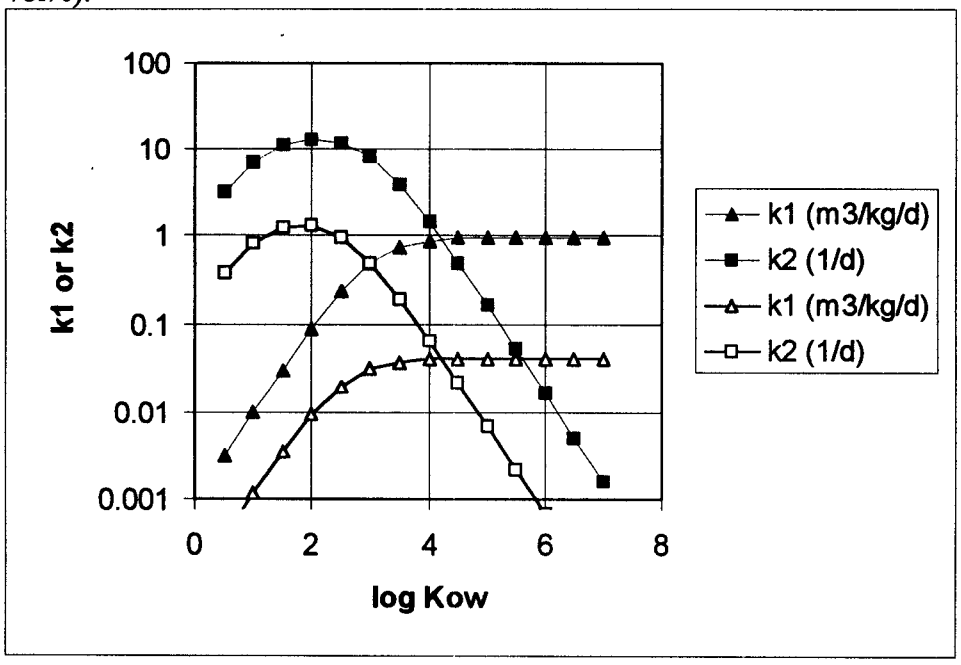

***Figure 6** Behaviour of the model of Sijm & Van der Linde (1995) for estimating* k_1 *and* k_2 *in a small and a large hypothetical fish: filled and open symbols respectively (weight 0.1 g and 1 kg and 6 vol% fat). A constant molecular weight (300 g/mol) is assumed.*

As shown in Figure 6, k_1 is expected to increase with increasing log *Kow* until a log *Kow* of about 4. The elimination rate constant k_2 will first be relatively constant but above log *Kow*=3 decreases log-linearly. The slope of decrease in k_2 is governed by the theoretical relationship for BCF. For larger fish, both k_1 and k_2 are expected to be lower than for smaller fish. The maximum BCF (ratio of k_1 and k_2) remains the same but as k_2 for the larger fish is lower, growth and metabolism will be more important in larger fish (i.e. significantly affect BCF at lower *Kow* values).

4.5. Comparison to measured data

To test the theoretical BCF approach as described in the previous sections, the results with and without growth dilution are compared to the BCF data sets as collected by Nendza (1991) and Veith & Kosian (1983) in Figure 7 and Figure 8, respectively. In Figure 9, the data from Opperhuizen & Sijm (1990) are shown for PCDD congeners which show a deviating pattern of bioconcentration. For the comparison with experimental data in Figure 7 to Figure 11, the theoretical model is parameterised as follows: weight of fish 2 g, fraction fat 6 vol%, growth rate 0.0025 g/g/d, molecular weight 300 g/mol. The parameters for the fish are different from the parameters in Table 2 because we think these values are more representative for the fish species used in the BCF experiments. The theoretical BCF approach is shown with and

[1] Generally, the molecular weight of compounds increases with increasing hydrophobicity. It is therefore likely that, when plotting experimental data, k_1 will decrease slowly with higher *Kow* values.

without growth dilution. Next to the theoretical BCF and the measured data, the approach described in the TGD (EC, 1996a) is shown. This approach consists of a log-linear regression for log *Kow* 1-6 (Veith *et al.*, 1979) and a parabolic equation for log *Kow* 6-10 (recalculated on data of Connell & Hawker, 1988). The original data of Connell & Hawker are shown in Figure 10.

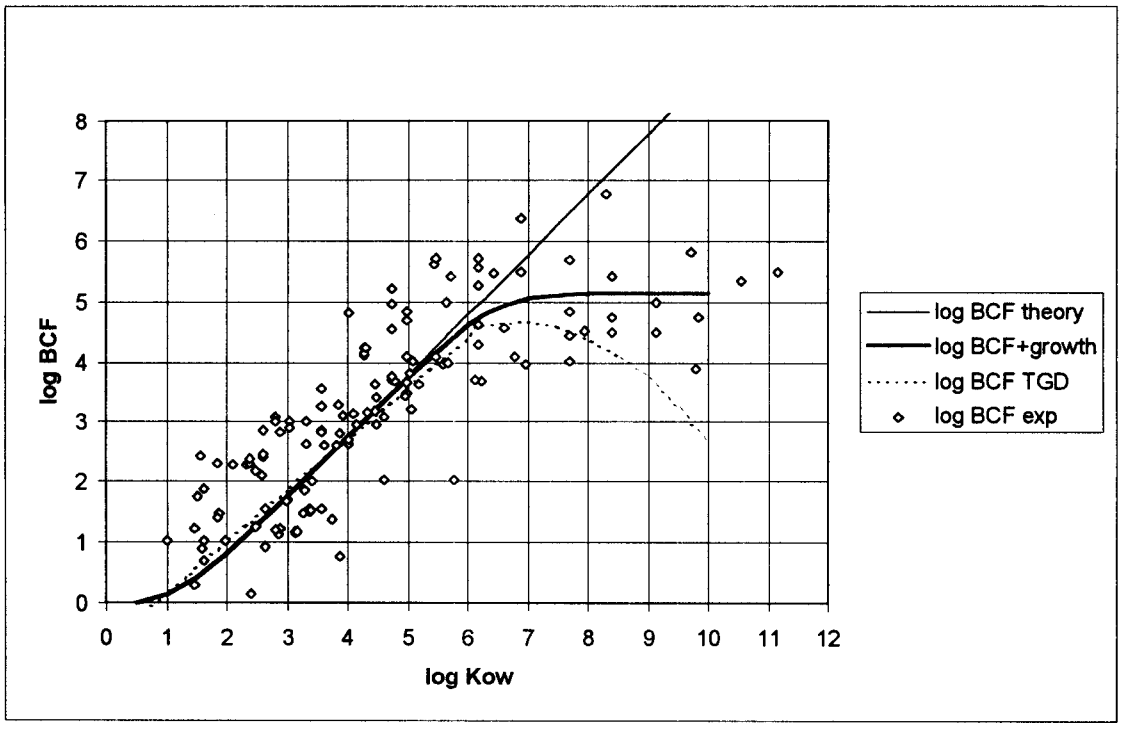

***Figure** 7 Comparison of the theoretical BCF approach to measured data and the approach laid down in the TGD. Data set from Nendza (1991).*

In Figure 7 and Figure 8, the theoretical model with growth dilution gives an acceptable description of the experimental data. Furthermore, in Figure 7, the polynomial approach of the TGD seems to underestimate the BCFs at high *Kow* significantly. The theoretical approach with growth dilution is sufficient to explain the lack of linearity for superlipophilic compounds observed in the data of Nendza (1991). Of course, this is no proof for the validity of this assumption since many other processes may also explain this deviation from linearity (see Section 2.2). The theoretical model seems to fail in predicting the BCFs for PCDD congeners (9th). Opperhuizen & Sijm (1990) found that the uptake rates of these compounds were comparable to other hydrophobic aromatic hydrocarbons. The much lower BCFs for PCDD congeners were caused by a much higher rate of excretion than expected, probably due to biotransformation. For octa-chlorodibenzo-*p*-dioxin, lack of uptake was found which was explained by a lack of membrane permeation due to a large cross-sectional diameter[1]. Other hydrophobic chemicals with comparable *Kows* were significantly accumulated as expected.

[1] The data set of Nendza (1991) also includes dioxins. This author reports a log BCF value for octachlorodibenzo-*p*-dioxin of 5.35 whereas Connell & Hawker (1988) report 1.92-3.35 and Opperhuizen & Sijm (1990) "no uptake"-2.15. Furthermore, there are large deviations in reported *Kow* for these super-lipophilic compounds (Shiu *et al.*, 1988, report for this chemical log *Kow* values from 7.53-12.72). Clearly, data evaluation is extremely critical for these compounds. Due to the uncertainty in *Kow* for very hydrophobic compounds, QSARs in this range must be handled with care!

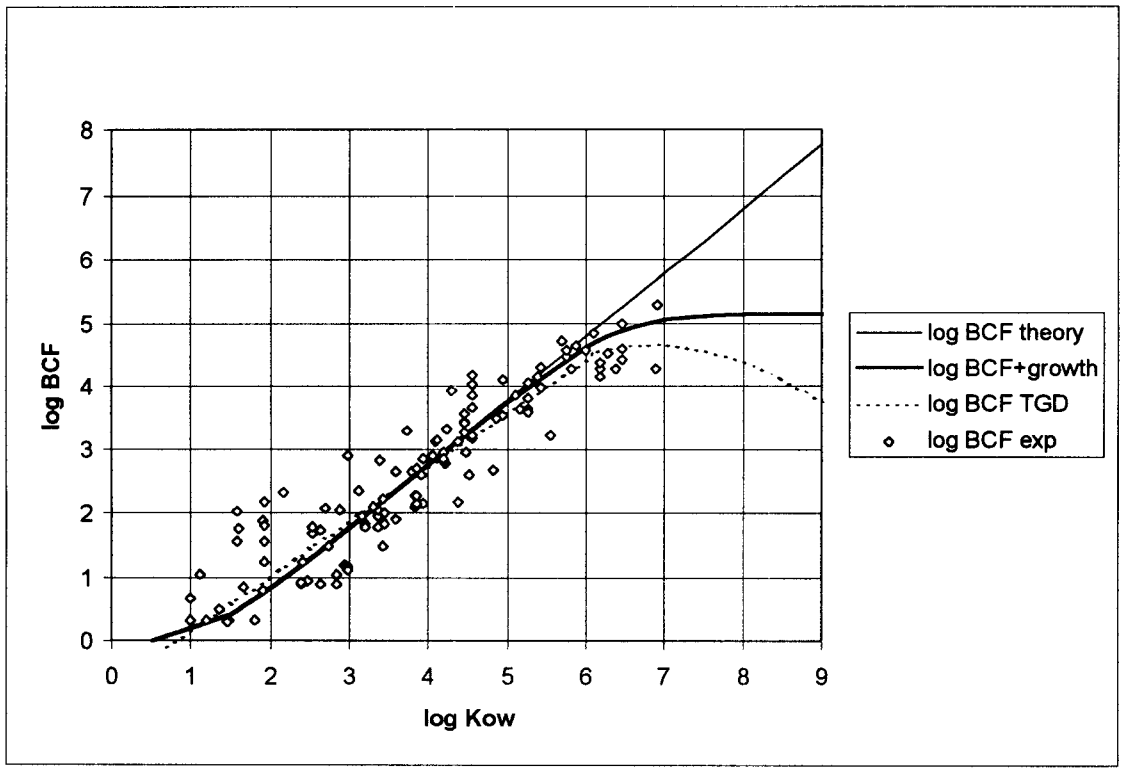

Figure 8 *Comparison of the theoretical BCF approach to measured data and the approach laid down in the TGD. Data set from Veith & Kosian (1983).*

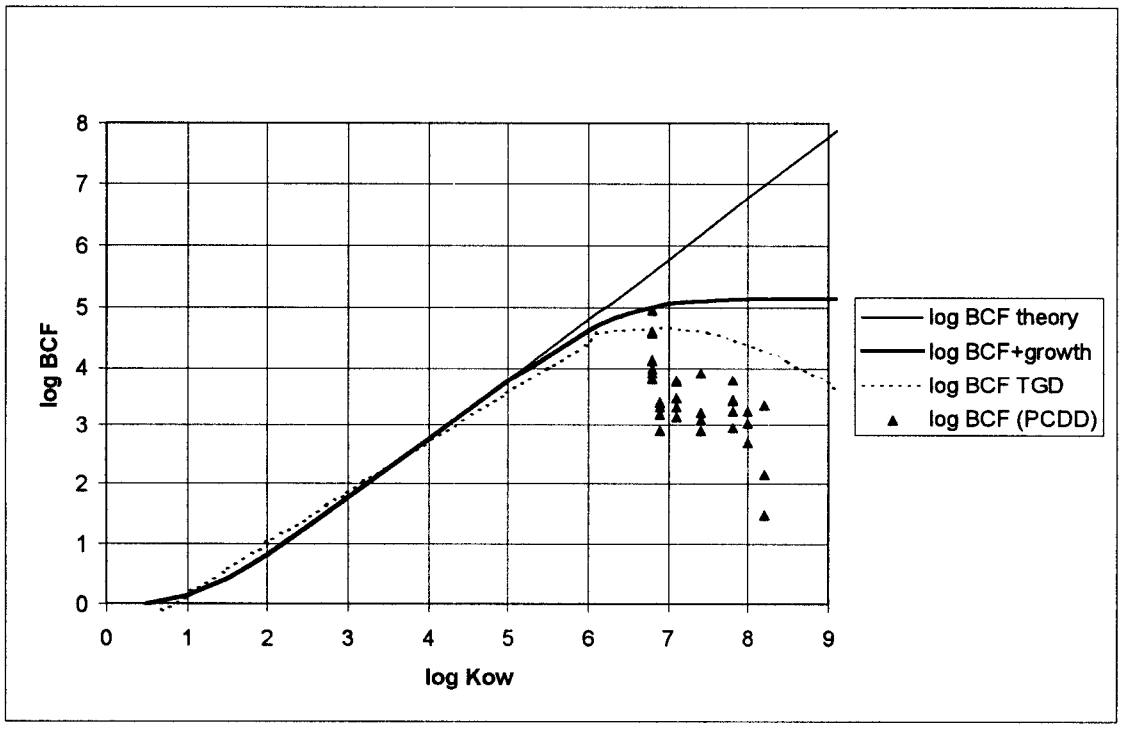

Figure 9 *Comparison of the theoretical BCF approach to measured data for PCDD congeners and the approach laid down in the TGD. Data set from Opperhuizen & Sijm (1990).*

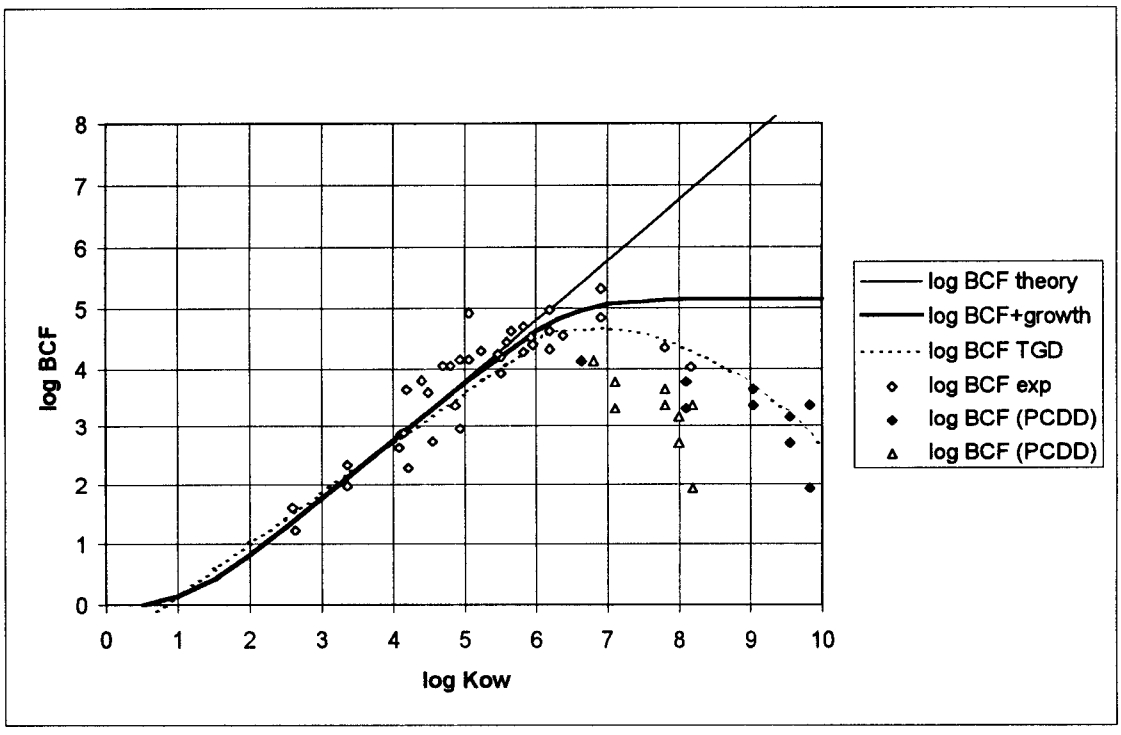

Figure 10 *Comparison of the theoretical BCF approach to measured data, collected by Connell & Hawker (1988). The PCDD congeners from the data set are indicated by filled symbols. The same PCDD data are also shown with corrected Kows (according to Shiu et al., 1988) as triangles.*

Figure 10 shows the data set of Connell & Hawker (1988) which was used to calculate the parabolic equation, advised in the TGD. Also, the BCF data are plotted using the evaluated *Kow* values for PCDDs as reported by Shiu *et al.* (1988). Clearly, the decrease of the polynomial relationship at high *Kow* is caused by few data on PCDD congeners only. Figure 11 shows the extensive data collected recently by Devillers *et al.* (1996). For this data set, several remarks can be made. Firstly, below log *Kow* of 6, the scatter is much larger than for the other data sets. Secondly, at log *Kow* around 8, both the theoretical model and the TGD approach seem to overestimate the experimental BCFs.

Concluding, the theoretical approach to bioconcentration, accounting for growth dilution, seems to predict BCFs satisfactorily for most chemicals. For extremely hydrophobic chemicals, the situation is less clear. For these compounds, diminished uptake may be caused by molecular size and biotransformation but the reliability of the experimental data (BCF as well as *Kow*) is also questionable. It therefore seems inappropriate to base a generic QSAR for risk assessment purposes on the results for these kinds of chemicals. The validity of the parabolic equation as advised in the TGD is questionable and may result in serious underestimation of BCF for hydrophobic compounds which are not metabolised and for which uptake is not limited due to size or shape. In our opinion, the theoretical model with growth dilution is the best option for risk assessment. Neglecting biotransformation and the effect of molecular size leads to a "reasonable-worst case" estimation.

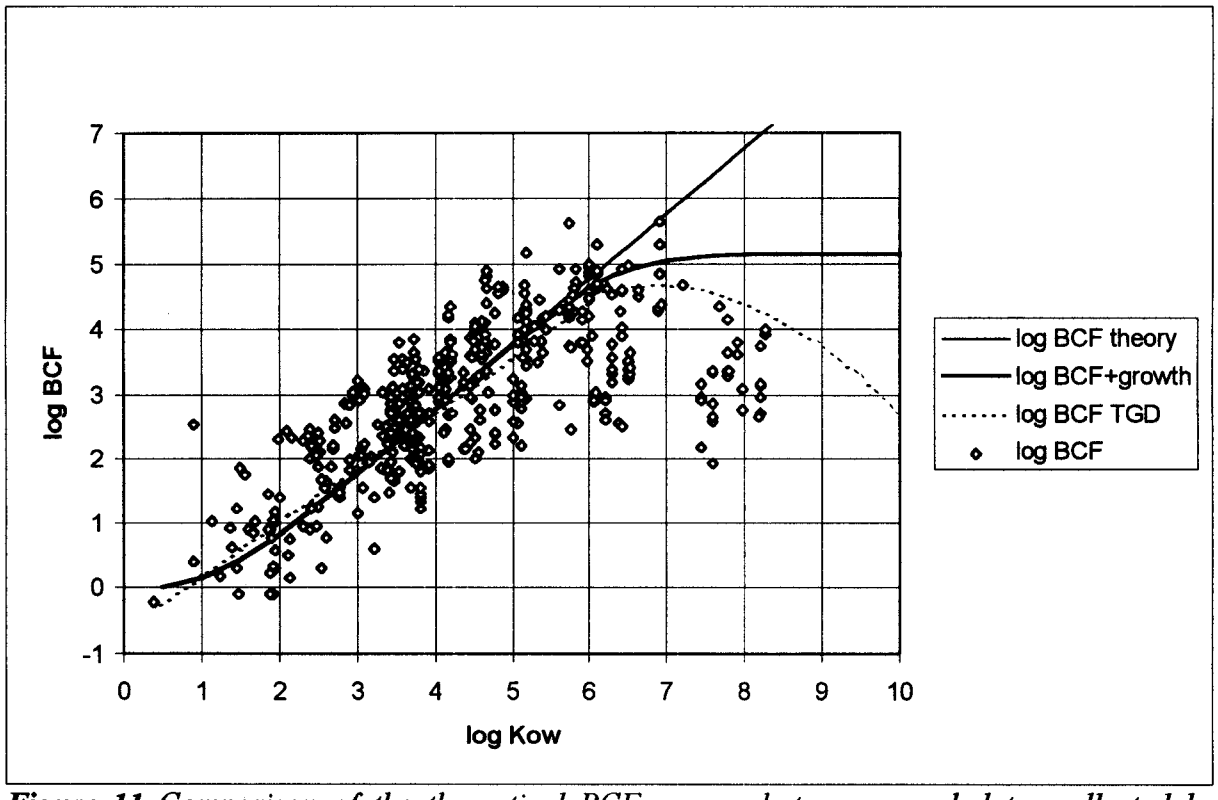

Figure 11 *Comparison of the theoretical BCF approach to measured data, collected by Devillers et al. (1996).*

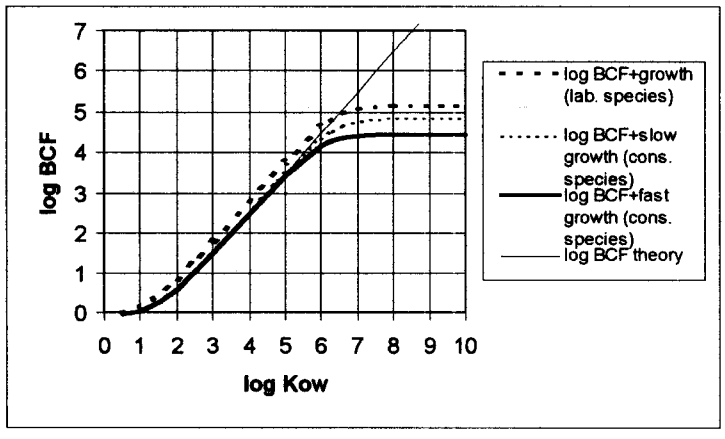

Figure 12 *Differences between the BCF+growth for laboratory species and with field relevant parameters. Two growth rates are shown (0.0025 and 0.001 d^{-1}).*

In the previous figures, the theoretical BCF models with and without growth were parameterised for laboratory species to facilitate comparison with experimental data. In Figure 12, the parameterisation for laboratory species is compared to the more "consumption-relevant" parameterisation of Table 2 and two different growth rates (a slower growth rate may be more representative for larger fish in the field situation). For the purpose of initial risk assessment, the BCF+growth model can be simplified to the theoretical straight line, with a maximum value reached at log *Kow*=6.

A large scatter around the estimate still remains, indicating that *Kow* is not a perfect descriptor for the behaviour of organic chemicals in fish. It should also be noted that the estimated BCF is a ***median*** estimate. This means that the true BCF can easily be a factor of 10 higher than this median. The estimation routines can hardly be seen as "worst case" but should be regarded as "most likely case". From a scientific point of view, it is advisable to take this uncertainty into account in the decision-making process. From analysis of the data of Devillers *et al.* (1996),

the uncertainty is a factor[1] of 27 for the log *Kow* range 1 to 6. For log *Kow* in the range 6-12, the estimation becomes very uncertain and most of the experimental data seem to be lower than expected by the model.

In Figure 13 and Figure 14, the estimated rate constants from the model of Sijm & Van der Linde (1995) are compared to measured data. Data were used from Fox *et al.* (1994) for PCBs, Butte *et al.* (1991) for HCH isomers, De Wolf *et al.* (1994) for chlorinated anilines, Bruggeman *et al.* (1981) for PCBs, Connell & Hawker (1988) (excluding the data already taken from Bruggeman *et al.*), Sijm *et al.* (1992) for PCBs (only elimination and excluding IUPAC no. 209), and Sijm *et al.* (1993). The plot for k_2 includes the minimum value when assuming a growth rate of 0.0039 taken from Sijm *et al.* (1992) for Guppy. Estimated lines assume 0.1 g body weight, 6 vol% fat, and a constant molecular weight of 300 g/mol. The estimate of k_2 is calculated with the estimated BCF from Section 4.2 without dilution by growth.

As shown in Figure 13, the estimated uptake rate constant is more or less consistent with the experimental data. The scatter is large, thereby making it difficult to draw definite conclusions on the validity of the model. The data from Fox *et al.* (1994) for PCBs are shown with grey symbols as their values for k_1 are consistently higher than the model. The reasons for this remain unclear. At high *Kow* values, mainly for dioxins, the uptake rate constants seem to decrease. According to Opperhuizen & Sijm (1990), the uptake rate constants for PCDDs are similar to other hydrophobic chemicals. Only octachlorodibenzo-*p*-dioxin (also included in Figure 13) was not taken up, probably due to its molecular size. Opperhuizen & Sijm also state that low uptake of extremely hydrophobic chemicals may be related to experimental difficulties rather than caused by decreasing uptake rate constants.

Figure 14 shows a close correlation between estimated and measured elimination rate constants for most compounds. The experimental data seem to suggest a slightly lower slope than unity. The same trend can be observed for BCF in Figure 8 and Figure 11. Molecular weight increases with hydrophobicity and as a result, structural differences between octanol and fish lipids may be responsible for different solvent characteristics (see also Section 2.2). For extremely hydrophobic chemicals, higher rate constants were found than expected. Apart from experimental difficulties, these deviations may be caused by metabolism of the parent compound to more polar metabolites. The data for these compounds are derived by Muir *et al.* (1985) as reported by Connell & Hawker (1988) for dioxins. According to Opperhuizen & Sijm (1990), elimination rate constants of most PCDD and PCDFs are high, relative to those of other chlorinated aromatic hydrocarbons, probably as a result of metabolism.

Concluding, the model of Sijm & Van der Linde (1995) is generally consistent with the experimental data. For extremely hydrophobic chemicals, deviations occur which may be caused by molecular size, metabolism or experimental difficulties (e.g. the use of cosolvents). On the other hand, a shortcoming of the theory behind the model cannot be ruled out. For more hydrophilic chemicals (i.e. log *Kow*<3), the model could not be tested as experimental data in this *Kow* range could not be found. A more extensive validation of this model can be performed by including parameters like fish weight and molecular weight from the experimental data sets.

[1] This factor is estimated from the standard deviation of the residuals using the method of Slob (1994). The use of this factor implies that 95% of the data can be found within this factor from the median estimate.

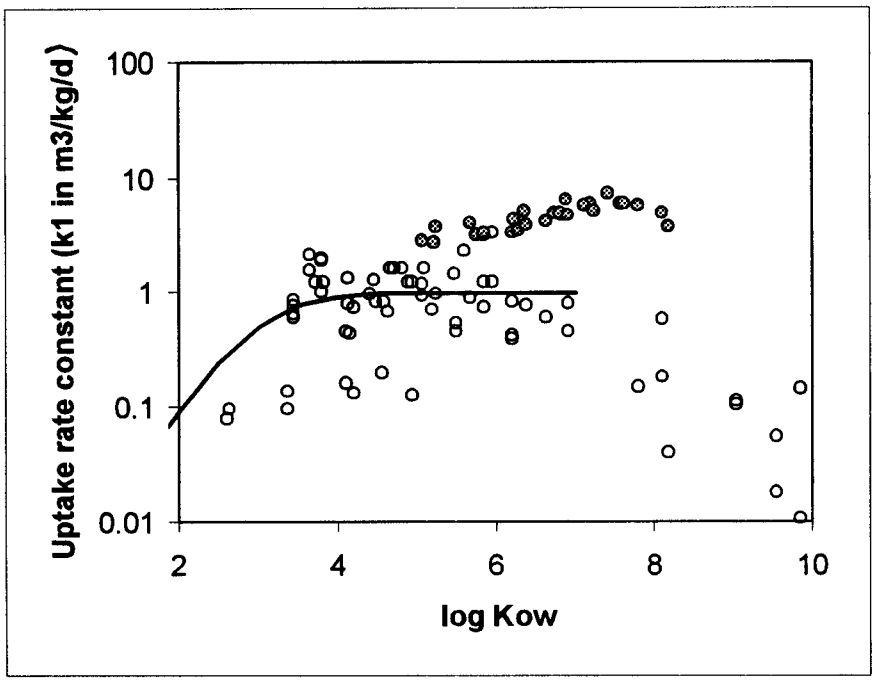

Figure 13 *Comparison of experimental and estimated uptake rate constants. Data from Fox et al. (1994) shown as filled symbols.*

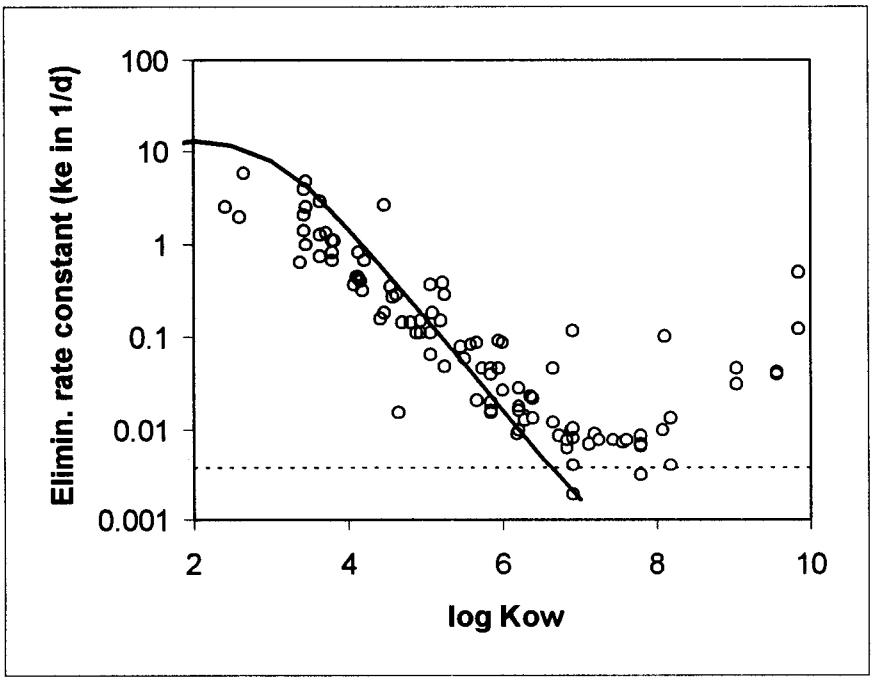

Figure 14 *Comparison of experimental and estimated elimination rate constants (including growth and metabolism). The dashed line represents the growth rate found by Sijm et al. (1992) for guppy.*

4.6. Sensitivity

The results of a limited sensitivity analysis on the BCF+growth are shown in Table 3 below. Each parameter was given a uniform distribution with a variation of + and -10%. Simulations were performed in Microsoft Excel with the simulation package Crystal Ball (4000 trials, Latin Hypercube sampling). Sensitivities are given as percentage of total variance in BCF+growth. At low *Kow*, uncertainty in *Kow* and fraction fat dominate. At high *Kow*, main sources of uncertainty/variation are molecular weight, fish weight and growth rate. At high *Kow*, the absolute value of *Kow* is not important anymore which is convenient since measurements of *Kow* in this range encounter numerous experimental difficulties (as indicated in Figure 10). The maximum BCF is governed mainly by the growth rate and also by the molecular weight of the chemical.

Table 3 *Results of a limited sensitivity analysis for estimation of BCF for fish (including growth).*

Parameter	**likeliest**		**Sens log Kow=2**	**Sens log Kow=8**
Kow	100 10^8	m^3/m^3	49	 0
M	300	g/mol	0	28
F_{fat}	0.03	m^3/m^3	48	0
F_{water}	0.80	m^3/m^3	3	0
W	0.2	kg	0	7
k_g	0.0025	g/g/d	0	65

5. THE EARTHWORM MODEL

5.1. Dynamic model

The general model for bioaccumulation can be specified for the earthworm as follows (as done by Belfroid *et al.*, 1995a):

$$\frac{dC_{worm}}{dt} = k_1 \cdot C_{porew} + k_f \cdot C_{solids} - (k_2 + k_g + k_m) \cdot C_{worm}$$

With:

C_{worm}	concentration in earthworm	$[mg.kg_{wwt}^{-1}]$
C_{porew}	concentration in porewater	$[mg.m^{-3}]$
C_{solids}	concentration in soil solids	$[mg.kg_{solids}^{-1}]$
k_1	diffusive uptake	$[(mg.kg_{wwt}^{-1})/(mg.m_{porew}^{-3}).d^{-1}]$
k_2	diffusive losses	$[d^{-1}]$
k_f	uptake through food	$[(mg.kg_{wwt}^{-1})/(mg.kg_{food}^{-1}).d^{-1}]$
k_g	dilution by growth	$[d^{-1}]$
k_m	losses due to metabolism	$[d^{-1}]$

In contrast with fish, it is relatively easy to quantify the uptake through food for the earthworm since these organisms consume soil particles[1]. The chemical concentration in porewater and sorbed to solids are related according to the solids-water partition coefficient of the chemical:

$$C_{solids} = Kp \cdot C_{porew}$$

With:

Kp	solids-water partitioning coefficient in soil	$[m^3.kg_{solids}^{-1}]$

For organic chemicals, *Kp* is generally found to be related to the organic carbon content of the soil. This parameter can therefore be adjusted to different soils using the "organic-carbon normalised" partition coefficient (*Koc*):

$$Kp = Foc \cdot Koc$$

With

Foc	fraction organic carbon in soil	$[kg_{oc}.kg_{solids}^{-1}]$
Koc	organic carbon-water partition coefficient	$[m_{water}^3.kg_{oc}^{-1}]$

For non-polar organic compounds, *Koc* is generally related to *Kow*. In the TGD, the relationships derived by Sabljic *et al.* (1995) are advised. The QSAR for the group "predominantly hydrophobics" is used as default:

$$Koc = \frac{1.26 \cdot Kow^{0.81}}{1000}$$

[1] This is not true for all earthworm species. For example, *Lumbricus terrestris* burrows through the soil but mainly consumes organic litter from the soil surface. For the sake of simplicity, soil is taken as the sole food source in this study.

5.2. Steady state

According to the general model, the steady-state BCF in m^3/kg_{wwt} can be written as:

$$BCF = \frac{F_{water} + F_{fat} \cdot Kow}{\rho_{worm}}$$

With:

BCF	bioconcentration factor for worm	$[m_{water}^3.kg_{wwt}^{-1}]$
F_{water}	volume fraction water in fish	$[m_{water}^3.m_{worm}^{-3}]$
F_{fat}	volume fraction fat in worm	$[m_{fat}^3.m_{worm}^{-3}]$
Kow	octanol-water partition coefficient	$[m_{water}^3.m_{oct}^{-3}]$
ρ_{worm}	bulk density of worm	$[kg_{wwt}.m_{worm}^{-3}]$

From the dynamic model, a relationship for bioaccumulation, including food uptake and growth, can be derived:

$$BAF = \frac{k_1 + k_f \cdot Kp}{k_2 + k_g + k_m} \qquad [m_{porew}^3.kg_{wwt}^{-1}]$$

An expression for bioaccumulation thus requires the kinetic rate constants. On a soil dry-weight basis, the BAF can be expressed as:

$$BAF = \frac{\frac{k_1}{Kp} + k_f}{k_2 + k_g + k_m} \qquad [kg_{solids}.kg_{wwt}^{-1}]$$

5.3. Parameters

For earthworms, the parameter values that were selected for a typical earthworm are shown in Table 4.

***Table 4** Parameters for earthworms, representative for risk assessment purposes.*

Symbol	Value	Unit	Reference
F_{fat}	0.01	$m_{fat}^3.m_{worm}^{-3}$	Belfroid *et al.* (1993)
F_{water}	0.84	$m_{water}^3.m_{worm}^{-3}$	From data gathered by Romijn *et al.* (1994)
k_g	0.0058	$kg_{worm}.kg_{worm}^{-1}.d^{-1}$	Belfroid *et al.* (1994a)
k_m	0	d^{-1}	Due to lack of general knowledge[1]
k_f	0.034	$kg_{food}.kg_{worm}^{-1}.d^{-1}$	Belfroid *et al.* (1995a)
ρ_{worm}	1000	$kg.m^{-3}$	Initial estimate (see also discussion in Section 4.3, Table 2)

[1] The rate of metabolism is highly dependent on the species of worm and the compound under consideration. The rate constant cannot simply be related to physico-chemical properties.

5.4. Rate constants

The kinetics of uptake and elimination in earthworms are much less studied than in aquatic species. Quantifying appropriate rate constants is therefore difficult. As for fish, we may expect the rate constant for passive, diffusive uptake (k_1) to be constant in the log *Kow* range 4 to 8. As shown in Figure 15, experiments with earthworms in water seem to support this hypothesis (Belfroid *et al.*, 1993). The BCFs found for uptake from water are close to the values found for fish (see Section 4.5). The geometric average of the experimental k_1 values is 350 l/kg/d. This value is therefore initially selected as representative for compounds with a log *Kow*>4.

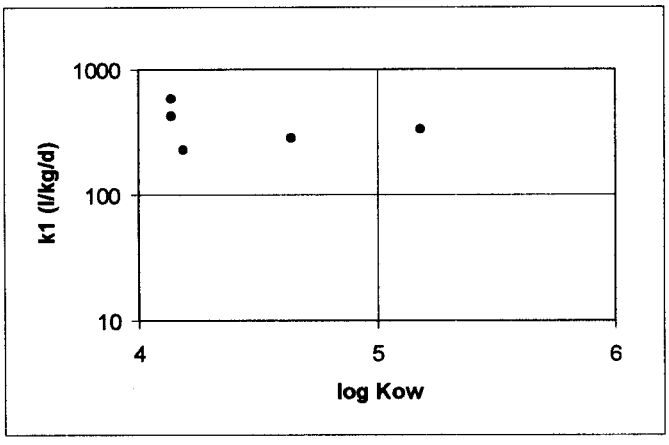

***Figure 15** Experimental uptake rate constants for earthworms (k_1). data from Belfroid et al. (1993).*

When the theoretical uptake model is correct, and when k_1 is indeed constant above a log *Kow* of around 4, the slope of k_2 is fixed. Only the intercept of the k_2 function remains to be defined. For the purpose of this study, the rate constants for passive uptake and elimination (k_1 and k_2) can be defined as follows for log *Kow* > 4:

$$k_1 = 0.35 \qquad [m^3.kg^{-1}.d^{-1}]$$

$$k_2 = \frac{0.35}{BCF} \qquad [d^{-1}]$$

Below log *Kow* of 4 we could not find any kinetic data but this range is usually less interesting for risk assessment purposes as these chemicals are unlikely to accumulate appreciably in organisms. Of course, the field relevance of these parameters is questionable as the exposure situation in water is radically different from exposure through porewater of soil. The soil compartment is a much less homogeneous environment where many processes may modify exposure. According to Posthuma (RIVM/ECO, pers. comm.), uptake and elimination kinetics can be radically different in different soil types for the same chemical and the same earthworm species. Furthermore, the kinetics will depend on the burial behaviour of the worm. In general, the time required to reach equilibrium for earthworms in soil experiments is relatively short: up to one or two months for extremely hydrophobic chemicals (Posthuma, pers. comm.). The time required to reach steady state[1] depends solely on the total elimination rate:

$$t(95\%) = \frac{\ln 20}{k_e}$$

For chlorobenzenes, the experimental elimination rate constants are consistent with the rapid equilibration observed (Belfroid *et al.*, 1994b; 1995b). For PCBs, Belfroid *et al.* (1995b) found a discrepancy between fast attainment of steady state and slow elimination rates. These authors report steady state within 10 days for chemicals with a log *Kow* up to 8 whereas the elimination rate constants would suggest an equilibration time of up to 100 days. Several reasons may explain this phenomenon:

[1] Theoretically, steady state will never be achieved as the steady-state concentration will be approached asymptotically. Steady state may be defined more pragmatically as 95% of the concentration after infinite exposure.

1. Rapid adsorption to the gut wall prior to diffusion over the gut membrane into the worm was proposed by the authors (Belfroid *et al.*, 1995b). If this is the case, one would not expect true steady state in a short timespan, but a continuing increase of worm body burdens until steady state is achieved. In a linear two-compartment model, rapid steady state in a compartment will be accompanied with rapid elimination from that compartment. Therefore, this explanation seems questionable.
2. Non-linear uptake kinetics. Some process resulting in rapid uptake but with a limited capacity. At some concentration, saturation occurs and steady-state is reached. After exposure is removed, this "compartment" slowly releases the chemical. This mechanism is not impossible but requires a different model concept.
3. No steady state was achieved in the experiments. Belfroid *et al.* (1995b) plotted the concentrations in earthworms during accumulation on a logarithmic scale. As discussed in Section 3.4 (shown in Figure 4), log transformation of data tends to clarify first-order elimination, but hides first order uptake. It is therefore possible that steady-state was assumed erroneously.

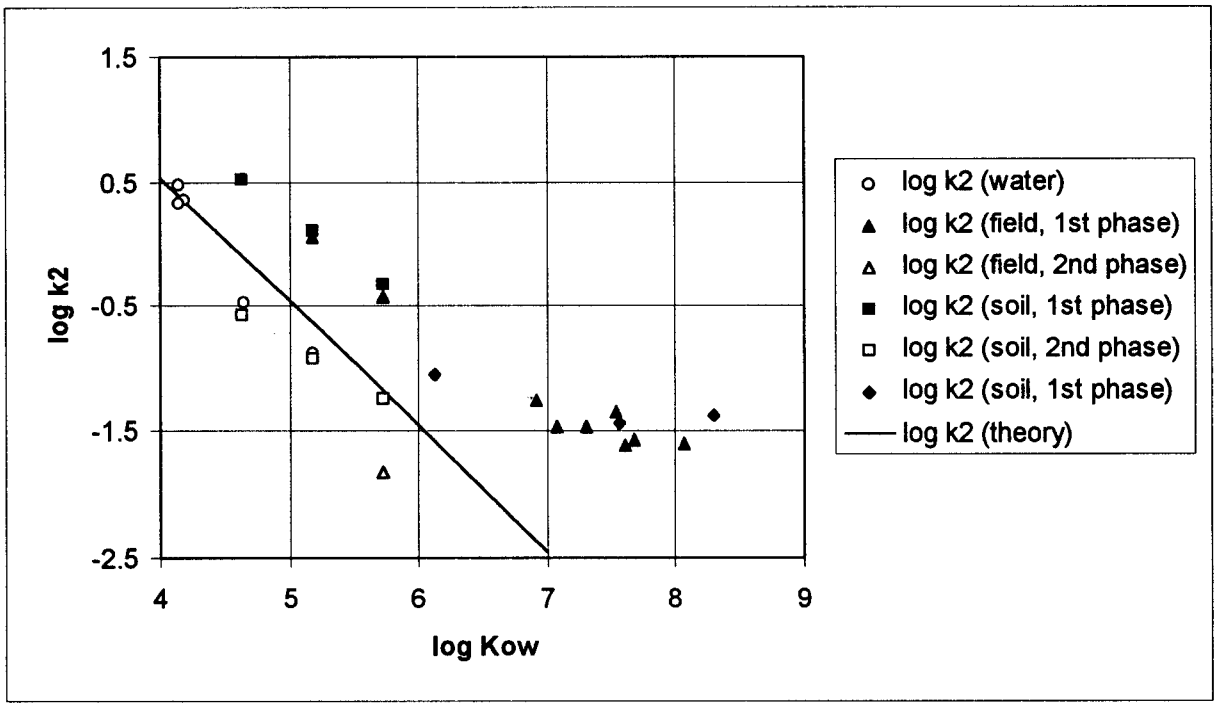

Figure 16 *Comparison of theoretical relationship for* k_2 *(d^{-1}) to measured data in water and field soils (Belfroid et al., 1993; 1994b; 1995b; 1995c).*

Figure 16 shows values of k_2 taken from four data sets of Belfroid *et al.* (1993, 1995b, 1994b, and 1995c). The four data sets are derived from different types of experiment:

1. Open circles: experiments with worms in water (1993). Chlorobenzenes. Only monophasic elimination found, indicating a one-compartment definition is appropriate.
2. Squares: experiments in artificial soil (1994b). Chlorobenzenes. Two-phase elimination kinetics observed for all compounds, both shown (2nd phase open symbols, 1st phase closed symbols), indicating a two-compartment definition is appropriate. Equilibrium was achieved in 2 days (7 days for hexachlorobenzene). Worms remained at the same weight during the experiments.
3. Triangles: experiments in field-contaminated soil (1995b). Chlorobenzenes and PCBs. Two-phase kinetics only shown for hexachlorobenzene, for the others only 1st phase

reported. The k_2 values were corrected for weight changes of worms. Metabolism was not likely in view of the PCB pattern in the worms and soil. Equilibrium was achieved within 10 days.

4. Diamonds: laboratory experiments in artificial soil with presumably only dietary uptake (1995c). Three superlipophilic compounds: hexabromobenzene, PCB 153 and octachloronaphthalene. Monophasic elimination found, in view of the findings of Belfroid *et al.* (1994b), this is assumed first phase.

For data set 3 (triangles), Belfroid *et al.* (1995b) state that the monophasic elimination of the PCBs may be only apparent. In fact, duration of the experiment may be too short to reveal the slower second phase. Experiment 2 showed that the duration of the first phase increased with increasing hydrophobicity of the chemical. In fact, not only the monophasic behaviour may be artificial, also the rapid attainment of steady state (see the discussion in Section 3.4). From the experimental results, it can be concluded that the kinetics of uptake and elimination in contaminated soil is best described by a two-compartment model. Nevertheless, since the first phase is responsible for the bulk of the body burden, the model may be simplified to one compartment (Belfroid *et al.*, 1995a). The results for chlorobenzenes in water and soil suggest that the first and rapid phase is related to soil exposure and could therefore be caused by some property of the gut.

Belfroid and co-workers use a QSAR regression for the elimination rate constant, based on the data from PCBs and chlorobenzenes as shown in Figure 16. The uptake rate constant can then be derived from k_2 and the estimate of BCF. The problem with the QSAR regression for k_2 is that the slope of the regression is less than unity, therefore, k_1 is expected to increase with increasing *Kow*[1]. In analogy with the approach for fish, we propose to keep k_1 constant in this *Kow* range. This would require a k_1 of approximately 5000 l/kg/d (as shown in Figure 17)[2]. Of course, the background of this assumption is extremely limited and more in-depth investigation of this subject is advisable. Nevertheless, for the descriptive purpose of this study, this assumption is appropriate.

Summarising, the model used for comparison with the steady-state situation is the standard one-compartment model with the rate constants:

$$k_1 = 5 \quad [m^3.kg^{-1}.d^{-1}] \quad k_2 = \frac{5}{BCF} \quad [d^{-1}] \quad k_g = 0.0025 \quad [kg.kg^{-1}.d^{-1}]$$

Judging from this estimate of k_2, dilution by growth will not become important until log *Kow* is above 8.

[1] For sediment worms, Connell *et al.* (1988) found k_1 to increase with *Kow* up to a log *Kow* of approx. 7. Furthermore, they k_2 to have little relation with *Kow*. Further investigation is needed, but it is possible that the uptake and elimination process of earthworms in soil is essentially different from fish.

[2] Note that this value is much higher than generally found for fish (up to about 1000 l/kg/d, see Figure 6).

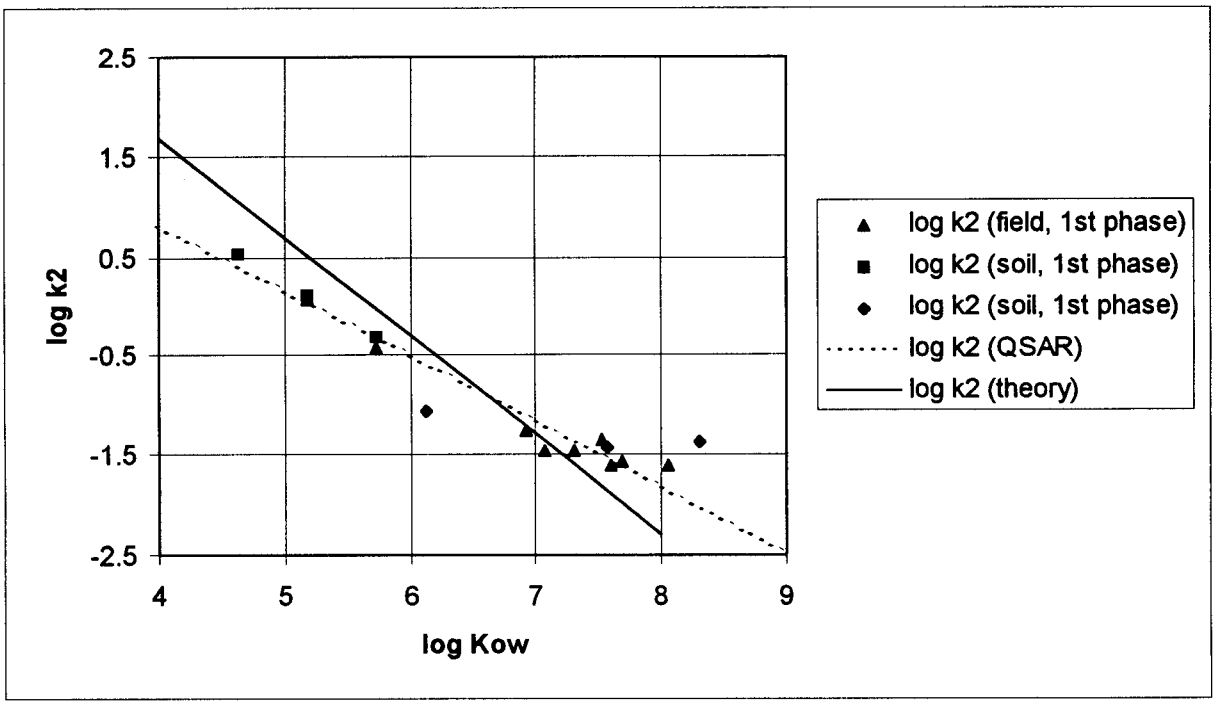

***Figure 17** Experimental data for elimination rate constant (first phase only) in worms. Solid line taken from Belfroid et al. (1995a), the broken line indicates the proposed estimator, assuming constant uptake rate constant in this* Kow *range.*

5.5. Comparison to measured data

To test the validity of the theoretical approach, the estimates for BCF are compared to the data collected by Connell & Markwell (1990) in Figure 18. Additionally, the QSAR derived by Belfroid *et al.* (1993) for earthworms exposed to water is shown.

The data of Connell & Markwell are corrected to worm wet weight by applying a weight fraction water of 0.84 (as proposed in Section 5.3). For most of the range, the predictions from the theoretical model are consistent with the QSAR reported by Belfroid *et al.* (1993). However, the theoretical line is on the lower end of the experimental data as gathered by Connell & Markwell (1990). The data evaluation of Connell & Markwell can however, be criticised. The authors used data from, among others, Van Gestel & Ma (1988). Van Gestel & Ma reported BCF values based on dry soil and on soil solution basis (calculated from measured sorption isotherms). Connell & Markwell used the BCFs on dry soil basis and applied estimation routines for soil sorption. This not only introduces an additional source of uncertainty but they subsequently used the fraction organic *matter* reported by the original authors where they should have used fraction organic *carbon*. The same mistake was made in the evaluation of the data of Gish & Hughes (1982). Furthermore, several data sets were recalculated assuming a worm dry weight/wet weight ratio of 0.25 whereas 16% seems more appropriate (see Section 5.3).

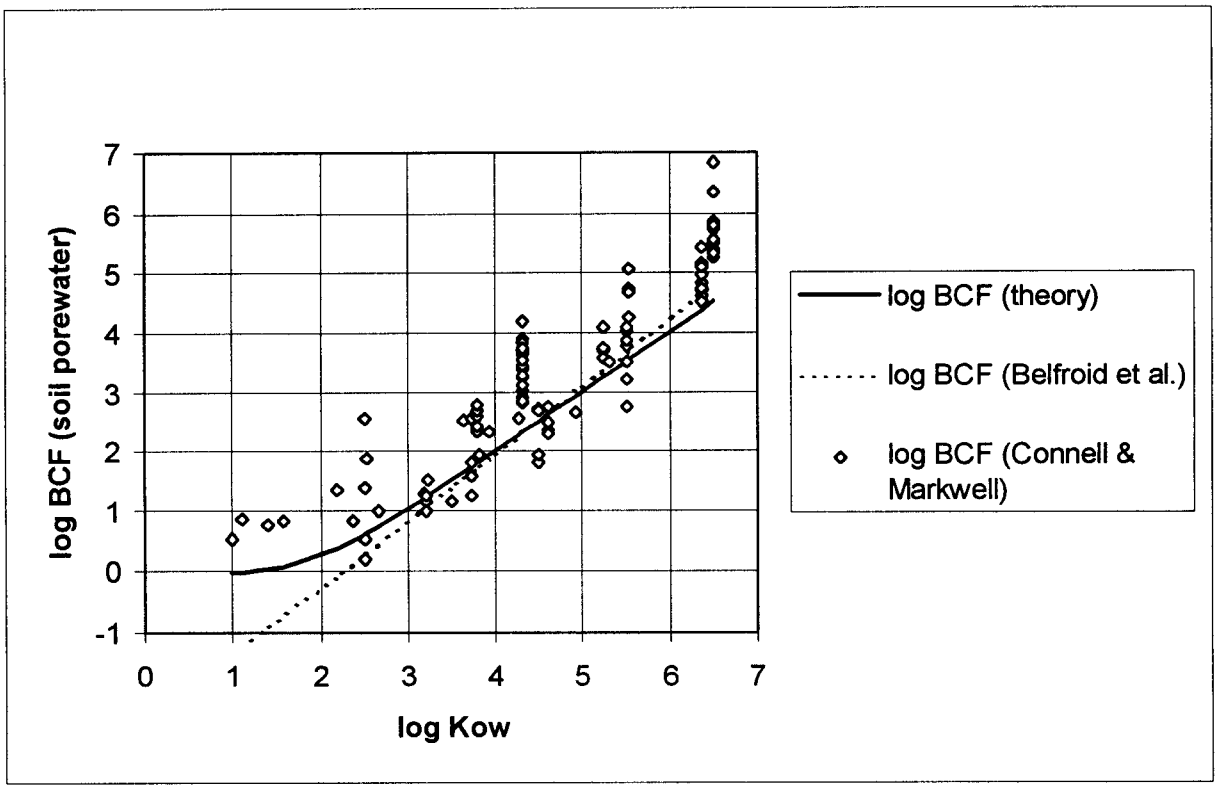

Figure 18 *Comparison of the theoretical BCF approach to measured data and the QSAR calculated by Belfroid et al. (1993). Data set collected by Connell & Markwell (1990).*

Clearly, there is sufficient reason to re-examine the data. Going back to the original literature used by Connell & Markwell and using additional sources, the following data sets are selected for a preliminary comparison (for these data sets, reliable water or soil water concentrations were available):

1. Van Gestel & Ma (1988), worms exposed to soil with porewater concentrations calculated from experimental sorption isotherms. Data for chlorophenols. Data recalculated to worm wet weight assuming 16% dwt/fwt ratio.
2. Lord *et al.* (1980), macerated worms in water (according to the author, this procedure provides the same results as whole worms in water, but shows faster equilibration). Data for several pesticides. Data recalculated to worm wet weight assuming 16% dwt/fwt ratio.
3. Belfroid et al. (1993), whole worms in water. Data for chlorobenzenes.

The data sets from Wheatley & Hardman (1968) and Gish & Hughes (1982), which were included in the data of Connell & Markwell (1990), were not used here because too many uncertainties are attached to these field studies (Wheatley & Hardman did not report organic matter content of the soils, the BAFs of Gish & Hughes were highly variable in time).

Figure 19 shows the theoretical model with the re-evaluated data. The re-evaluated data are more consistent with the theoretical model but still show considerable scatter. The data from Belfroid *et al.* (1993) are very close to the estimate as is to be expected from the experimental set-up: whole worms in water. The data from Lord *et al.* (1980) are systematically underestimated, especially at lower *Kow* values. The authors used macerated worms but in preliminary tests, whole worms gave the same BCFs (although equilibration was slower). For experiments with worms is soil, the authors found that the concentrations in worms were

about one-fifth of those calculated from equilibrium relationships. This finding was accounted to slow diffusion in soil. The data from Van Gestel & Ma (1988) are generally overestimated. These data come from experiments with worms in soil and may therefore be caused by limited diffusion in soil. It is interesting to note that Lord and co-workers did preliminary tests with two earthworm and two slug species, all showing the same results when exposed to aqueous solution. Applying ligatures on both ends of the worm to prevent uptake and excretion through the gut had no effect on accumulation, indicating that uptake through the outer skin is the dominant route in water exposure.

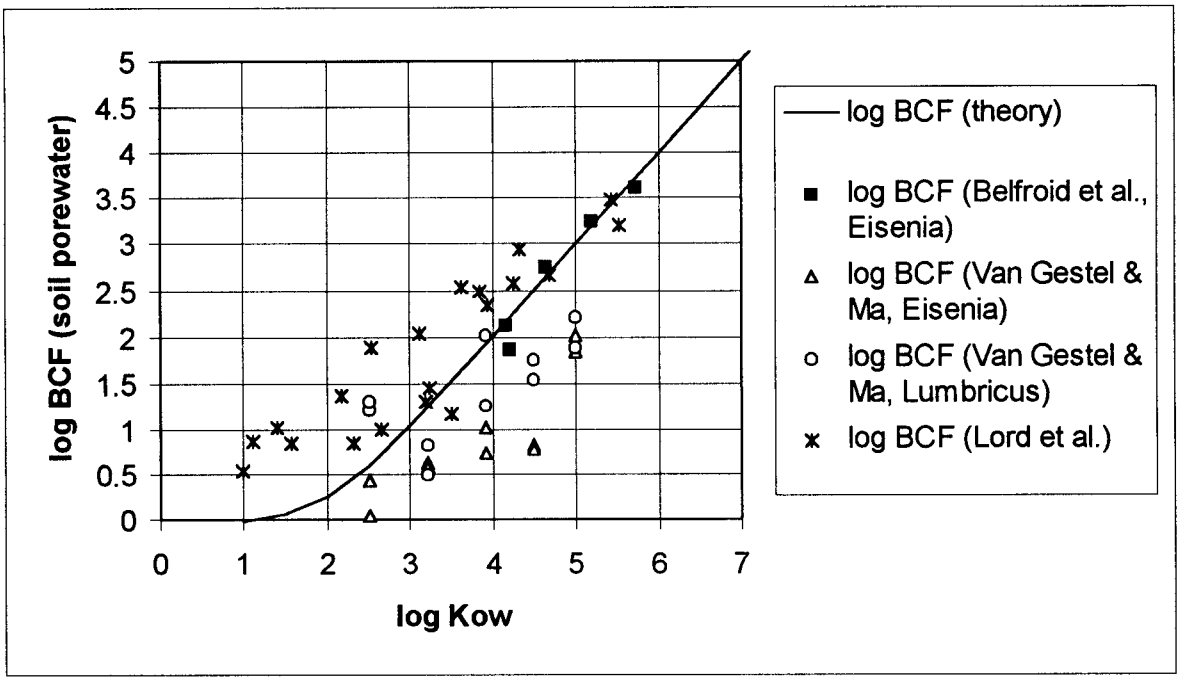

***Figure 19** Comparison of the theoretical approach to evaluated BCF data.*

On the basis of these data, the theoretical model need not be rejected. Especially the consistency with the data of Belfroid *et al.* (1993) is supporting this approach. From the available data, it seems that this approach gives a maximum value for earthworms exposed to soil. Depending on their burial behaviour, and since diffusion in soil is slow, earthworms may be exposed to lower concentrations than the average. Especially for species like *Lumbricus terrestris* which lives in well-defined burrows and feeds on litter, exposure may be much lower than expected from equilibrium relations (Lord *et al.*, 1980; Wheatley & Hardman, 1968). In view of the errors and uncertainties in this estimation routine, a more detailed evaluation of the available literature is currently in preparation (Jager, in prep.). As uptake is generally connected to porewater concentrations, experiments are preferred where these concentrations are measured or calculated from experimental sorption isotherms.

Figure 20 shows the behaviour of the model for BCF and BAF with and without growth dilution. Above log *Kow* of 7, the approaches start to deviate. The line "BAF+growth" will be most realistic for the field exposure situation and thus for risk assessment purposes, but differs little from the straight BCF approach. For risk assessment, the latter may therefore be preferred for its simplicity. The estimates including food uptake and growth are more uncertain as they require kinetic uptake and elimination rate constants which were shown to be very uncertain. As a side note, Figure 20 indicates that the effects assessment of the TGD for

the terrestrial ecosystem is questionable. In this approach, a safety factor of 10 is added for lipophilic chemicals (log *Kow*>5) when equilibrium partitioning is applied to derive a PNEC. This additional factor of 10 is added to account for exposure through ingestion. The model calculations indicate that for the standard soil, ingestion may play a role for chemicals with a log *Kow*>7, but the effect is countered by growth dilution. Therefore, no net effect of ingestion on the body burden is expected. Of course, the aquatic organisms will also have grown during their chronic toxicity tests, so growth dilution may be irrelevant in this case. Nevertheless, based on this limited evidence, a factor of 10 for log *Kow*>5 seems to be an unrealistic worst case.

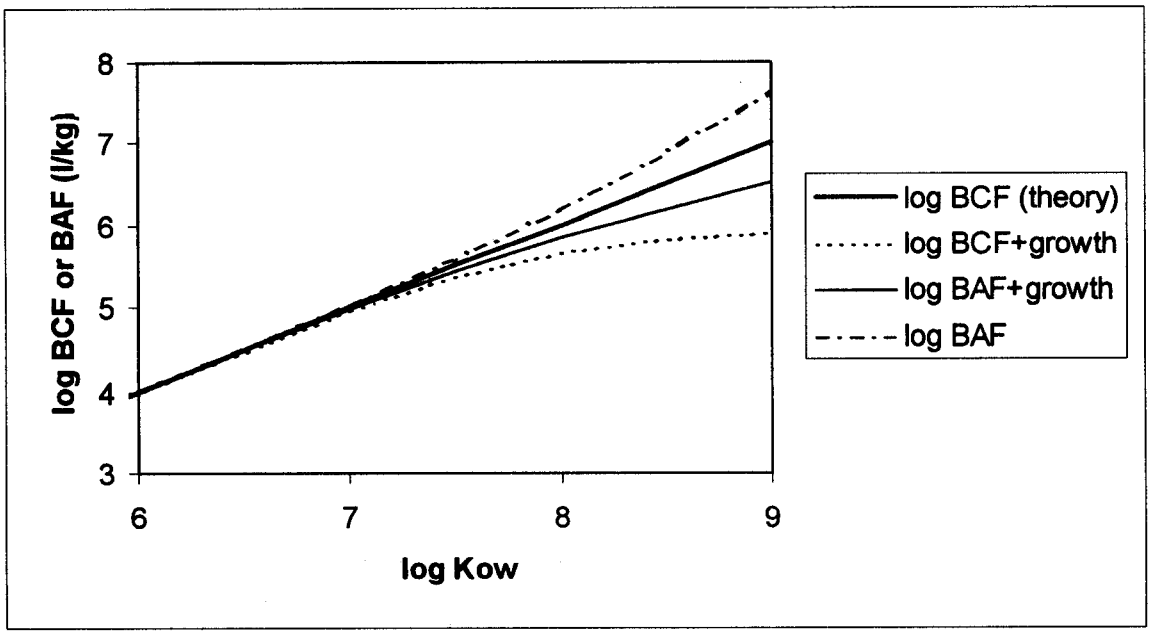

***Figure 20** Differences between BCF and BAF, with and without growth dilution.*

5.6. Sensitivity

In view of the simplicity of the selected model, a sensitivity analysis will not give much information. *Kow* and F_{fat} will be equally important along the entire range. At very low log *Kow* values (around 1 or 2), F_{water} will have some influence. As shown in Figure 20, it can be anticipated that growth and food uptake will become important at very high *Kow* values (log *Kow*>8).

6. PLANT MODEL

6.1. Dynamic model for leaves

The modelling approach proposed by Trapp and Matthies (1995) is rewritten to fit the general model proposed in Chapter 3. The sink term in the model is formed by diffusive transfer from leaf to air, elimination in the plant tissue, and dilution by growth. The source term is formed by uptake and translocation from soil pore water and gaseous uptake from air. Deposition is not considered in the model[1]. In contrast with fish and earthworms, were diffusive uptake was from the water phase, for leaves diffusive uptake is referenced to the air compartment. The uptake from porewater for the leaf is more comparable to food uptake: one-way due to translocation from root to shoot. The concentration in the leaf can be described by the following differential equation:

$$\frac{dC_{leaf}}{dt} = k_1 \cdot C_{air} + k_p \cdot C_{porew} - (k_2 + k_g + k_m) \cdot C_{leaf}$$

With:

C_{leaf}	concentration in leaf tissue	$[mg.kg_{wwt}^{-1}]$
C_{porew}	concentration in porewater of soil	$[mg.m_{porew}^{-3}]$
C_{air}	gaseous concentration in air	$[mg.m_{air}^{-3}]$
k_p	rate constant for uptake from porewater	$[m_{porew}^{3}.kg_{wwt}^{-1}.d^{-1}]$
k_1	rate constant for diffusive uptake from air	$[m_{air}^{3}.kg_{wwt}^{-1}.d^{-1}]$
k_2	rate constant for diffusive loss to air	$[d^{-1}]$
k_g	rate constant for dilution due to growth	$[d^{-1}]$
k_m	rate constant for biotransformation	$[d^{-1}]$

For the root, the dynamics of uptake and elimination are not considered since equilibration is rapid (see Tam *et al.*, 1996). Tam and co-workers placed plant tissue from soybean in water to equilibrate. Depletion half lives were usually below one hour, for the most hydrophobic compound 2.2 hours (HCB, log *Kow*=5.5). These authors, however, used small portions of root tissue of 1-1.2 g. The sorption kinetics may very well be different for larger roots, or especially, root crops like potatoes. For the purpose of risk assessment, and due to a lack of kinetic data, instantaneous steady state for the root compartment is assumed.

6.2. Steady state

Root

The plant-water partition coefficient is described as a thermodynamical partitioning of the chemical with the fraction water and lipids of the plant tissue. The octanol-water partition coefficient is corrected slightly for the differences between octanol and water[2]:

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[1] Trapp & Matthies argue that dry particulate and wet deposition are unlikely to contribute significantly to the concentration in leaf. Even for (super-)lipophilic chloro-organics, gaseous deposition is usually the dominant route. Recent work by Chrostowski & Foster (1996) indicates that this route may be very important for dioxins and similar compounds. More investigation in the importance of this route is appropriate.

[2] In contrast with fish and earthworms, the correction exponent is introduced for plant tissue. This was done because Trapp & Matthies suggest a value for this parameter (0.95) and for the, admittedly subjective, reasoning that it improves the fit of measured data displayed in Figure 21.

$$K_{root-water} = F_{water} + F_{fat} \cdot Kow^{b}$$

$$BCF_{root} = \frac{K_{root-water}}{\rho_{root}}$$

With:

$K_{root-water}$	root-water partition coefficient	$[m^3.m^{-3}]$
BCF_{root}	bioconcentration factor for plant roots	$[m_{water}^3.kg_{wwt}^{-1}]$
F_{fat}	volume fraction lipids in plant tissue	$[m^3.m^{-3}]$
F_{water}	volume fraction water in plant tissue	$[m^3.m^{-3}]$
Kow	octanol-water partition coefficient	$[m^3.m^{-3}]$
b	correction exponent for difference fat and octanol	[-]
ρ_{root}	bulk density of root tissue	$[kg_{wwt}.m^{-3}]$

Leaves

The partitioning between air and plant is again viewed as a thermodynamical partitioning, taking also into account the air fraction of the plant. Firstly, as done for roots, the tissue-water partition coefficient is estimated:

$$K_{leaf-water} = F_{water} + F_{fat} \cdot Kow^{b}$$

The leaf-air partition coefficient is calculated as the ratio of the leaf-water and air-water partition coefficients:

$$K_{leaf-air} = F_{air} + \frac{K_{leaf-water}}{Kaw} = F_{air} + F_{water} \cdot \frac{1}{Kaw} + F_{fat} \cdot \frac{Kow^{b}}{Kaw}$$

$$BCF_{leaf} = \frac{K_{leaf-air}}{\rho_{leaf}}$$

With:

$K_{leaf-water}$	leaf-water partition coefficient	$[m^3.m^{-3}]$
$K_{leaf-air}$	leaf-air partition coefficient	$[m^3.m^{-3}]$
Kaw	air-water partition coefficient	$[m^3.m^{-3}]$
Kow	octanol-water partition coefficient	$[m^3.m^{-3}]$
b	correction exponent for difference fat and octanol	[-]
F_{fat}	volume fraction lipids in plant tissue	$[m^3.m^{-3}]$
F_{water}	volume fraction water in plant tissue	$[m^3.m^{-3}]$
F_{air}	volume fraction air in plant tissue	$[m^3.m^{-3}]$
BCF_{leaf}	bioconcentration factor for plant leafs from air	$[m_{air}^3.kg_{wwt}^{-1}]$
ρ_{leaf}	bulk density of leaf tissue	$[kg_{wwt}.m^{-3}]$

The air-water partition coefficient, also known as the "dimensionless" Henry's law constant, is estimated from the ratio of vapour pressure and water solubility:

$$Kaw = \frac{VP \cdot M}{SOL} \cdot \frac{1}{R \cdot TEMP}$$

With:

VP	vapour pressure	[Pa]
M	molecular weight	$[mg.mol^{-1}]$
SOL	water solubility	$[mg.m^{-3}]$
R	gas constant	$[Pa.m^3.mol^{-1}.K^{-1}]$
TEMP	absolute temperature	[K]

6.3. Parameters for plants

Several publications were found, reporting plant-specific parameters like volume fractions water, fat and air, bulk densities and area/volume ratios. These data are shown in Table 6 to Table 8. Weight fractions (WF) fat were converted to volume fractions by the following equation:

$$F_{fat} = WF_{fat} \cdot \frac{\rho_{tissue}}{\rho_{fat}} \quad \text{with } \rho_{fat} = 827 \text{ kg/m}^3 \text{ (octanol)}$$

The values proposed in the TGD are shown in Table 5. Roots and leaves share the same fractions and bulk densities. In the same table, a proposal is made to distinguish between the parameter values for roots and leaves, based on the data found. For grass, there does not seem to be reason to select other fractions than for other crops.

Appropriate parameter values for the area to volume ratio are more difficult to select. Trapp & Matthies (1995) advice to use an initial value for risk assessment purposes of 2500 m^2/m^3, but use a value of 854 m^2/m^3 for soybean. The data of McGrady (1994) in Table 7 suggest much larger values for several plant species. It is possible that Trapp & Matthies included the stem in the calculations, resulting in lower area per volume of plant. It is unclear if stem must be included in this ratio since the shoot may not be well represented by one homogeneous compartment (significant transfer of xenobiotics from leaf to stem with the phloem is not likely). Nevertheless, the value proposed by Trapp & Matthies is maintained as this value is related to other plant-species specific properties like transpiration and growth. It should be noted that the search for parameters in the framework of this study was not very extensive. For agricultural crops much more data must be available and a more extensive search may be appropriate, possibly even selecting different values for different crops. Especially grass seems to have a much higher leaf to volume ratio which could result in more rapid uptake and elimination kinetics (see e.g. McGrady, 1994).

***Table 5** Volume fractions for the constituents of roots and leaves as advised in the TGD (Riederer, 1990, for leaves of Brassica oleracea, A/V from Trapp & Matthies, 1995) and proposed new values.*

Parameter	Symbol	TGD values			Proposed new values	
		Roots	Leaves		Roots	Leaves
Fraction fat	F_{fat}	0.01	0.01		0.005	0.01
Fraction water	F_{water}	0.65	0.65		0.93	0.65
Fraction air	F_{air}	n.a.	0.30		n.a.	0.30
Bulk density	$\rho_{root/leaf}$	700	700		1000	800
Area/volume	A/V	n.a.	2500		n.a.	2500

Table 6 *Data from Tam et al. (1996) for soybean tissue. Data in italics are recalculated from the original data.*

	Lipid fraction (kg/kg fwt)	**Density (kg/m^3)**	**Water fraction (kg/kg fwt)**	**Lipid fraction (m^3/m^3)**	**Water fraction (m^3/m^3)**
Leaves	0.0192	750	0.826	*0.0174*	*0.620*
Petioles	0.0047	790	0.859	*0.0045*	*0.679*
Stems	0.004	980	0.840	*0.0047*	*0.823*
Roots	0.0038	1004	0.921	*0.0046*	*0.925*

Table 7 *Data from McCrady (1994). Data in italics are recalculated from the original data.*

	dwt/fwt	**A/fwt (cm^2/g)**	**Density (kg/m^3)**	**Lipid fraction (g/g fwt)**	**A/V (m^2/m^3)**	**Lipid fraction (m^3/m^3)**
Kale	0.28	58	830	0.012	*4814*	*0.012*
Pepper	0.28	92	870	0.01	*8004*	*0.011*
Azalea	0.34	90	910	0.019	*8190*	*0.021*
Grass	0.25	216	810	0.012	*17496*	*0.012*

Table 8 *Data from Müller et al. (1994).*

	Water (m^3/m^3)	**Cellular lipids (m^3/m^3)**	**Cuticular Membrane (m^3/m^3)**	**Carbo-hydrates (m^3/m^3)**	**Proteins (m^3/m^3)**
Spruce needles	0.58	0.037	0.027	0.216	0.024
Azalea leaves	0.62	0.013	0.019	0.09	0.042
Grass leaves	0.65	0.003	0.004	0.078	0.039

6.4. Rate constants

Trapp & Matthies (1995) present a simple, generic set of equations to estimate the rate constants of the differential equation. For leaves, the uptake from porewater is not diffusive but controlled by the transpiration stream of the plant (one-way transport). The rate constant k_p is based on the transpiration-stream concentration factor (TSCF) and the transpiration stream of the plant:

$$W = V \cdot \rho_{leaf}$$

$$k_p = TSCF \cdot \frac{Q}{W} \qquad [m^3/kg/d]$$

With:

W	weight of shoot	[kg]
V	volume of the shoot	[m^3]
ρ_{leaf}	bulk density of leaf tissue	[$kg_{wwt}.m^{-3}$]
k_p	rate constant for uptake from porewater	[$m_{porew}^3.kg_{wwt}^{-1}.d^{-1}$]
Q	transpiration stream	[$m^3.d^{-1}$]
TSCF	transpiration-stream concentration factor	[-]

The transpiration-stream concentration factor is estimated according to Briggs *et al.* (1982):

$$TSCF = 0.784 \cdot \exp\left[\frac{-(\log Kow - 1.78)^2}{2.44}\right]$$

Rate constants k_1 and k_2 for leaf regard diffusive exchange with air. Rate constant k_1 is estimated by the leaf's conductance and the area of leaf:

$$k_1 = g \cdot \frac{A}{W} \qquad [m^3/kg/d]$$

$$k_2 = \frac{k_1}{BCF_{leaf}} \qquad [d^{-1}]$$

With:

k_1	rate constant for diffusive uptake from air	[$m_{air}^3.kg_{wwt}^{-1}.d^{-1}$]
k_2	rate constant for diffusive loss to air	[d^{-1}]
BCF_{leaf}	bioconcentration factor from air to leaf	[$m_{air}^3.kg_{wwt}^{-1}$]
g	conductance of the leaf (≡partial mass-transfer coeff.)	[$m.d^{-1}$]

***Table 9** Parameter values for the model as proposed by Trapp & Matthies (1995). Values referenced to 1 m^2 of soil.*

Parameter	Symbol	Value	Unit
Leaf area	A	5	m^2
Leaf volume	V	0.002	m^3
Transpiration rate	Q	0.001	m^3/d
Growth rate constant	k_g	0.035	d^{-1}
Metabolism rate constant	k_m	0	d^{-1}
Leaf conductance	g	86.4	m/d
Correction exponent	b	0.95	[-]

6.5. Comparison to measured data

In Figure 21, the theoretical relationship for bioconcentration in roots is compared to the relationship of Briggs *et al.* (1982) and the measured data as reported by Polder *et al.* (1995) and Tam *et al.* (1996). The differences between the theoretical approach and the QSAR of Briggs and co-workers is small and both approaches can sufficiently describe the experimental data. In Figure 22, the regression for TSCF as derived by Briggs *et al.* (1982) is shown with measured data (from Briggs and co-workers and data collected by Polder *et al.*, 1995). From this comparison, it can be concluded that, in general, there seems to be little relation between TSCF on *Kow*. The regression goes through the experimental points but the deviations are large. Figure 23 compares the estimated leaf-air partition coefficient to measured data collected by Polder *et al.* (1996). The estimated $K_{leaf\text{-}air}$ takes growth into account and is estimated as:

$$K_{leaf-air} = \frac{k_1 \cdot \rho_{leaf}}{k_2 + k_g} \quad \text{(expressed as } m^3.m^{-3}\text{)}$$

From Figure 23, it is clear that the amount of collected data is too small to make a proper evaluation. Nevertheless, the estimated data are very consistent with the measured data even though the data were derived for different plant species and with different experimental designs.

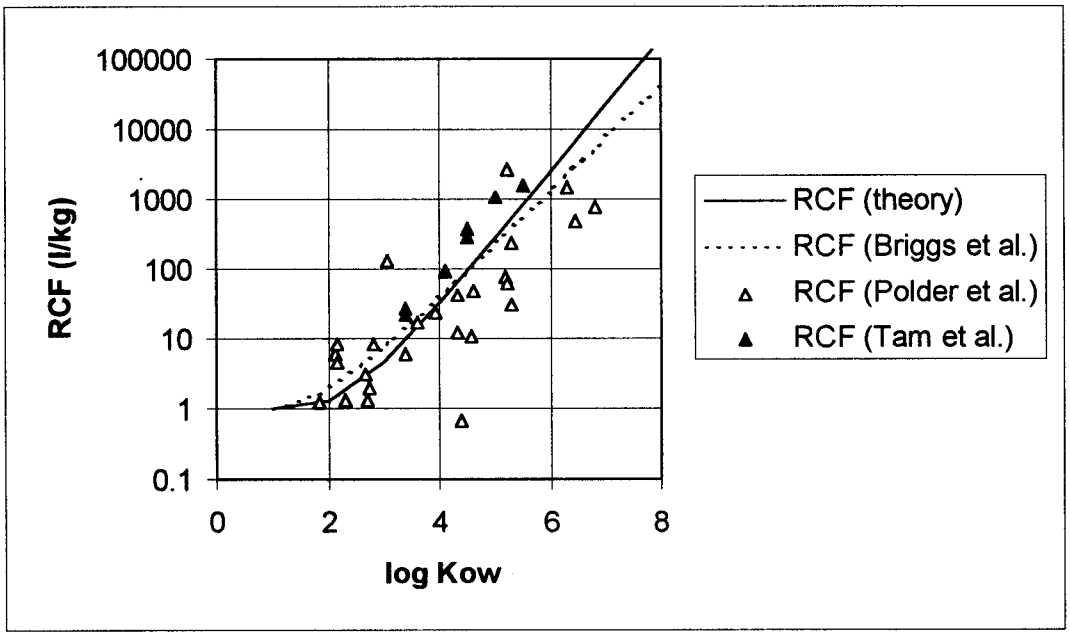

Figure 21 *Comparison of the theoretical root concentration factor with the relationship reported by Briggs et al. (1982) and measured data reported by Polder et al. (1995) and Tam et al. (1996).*

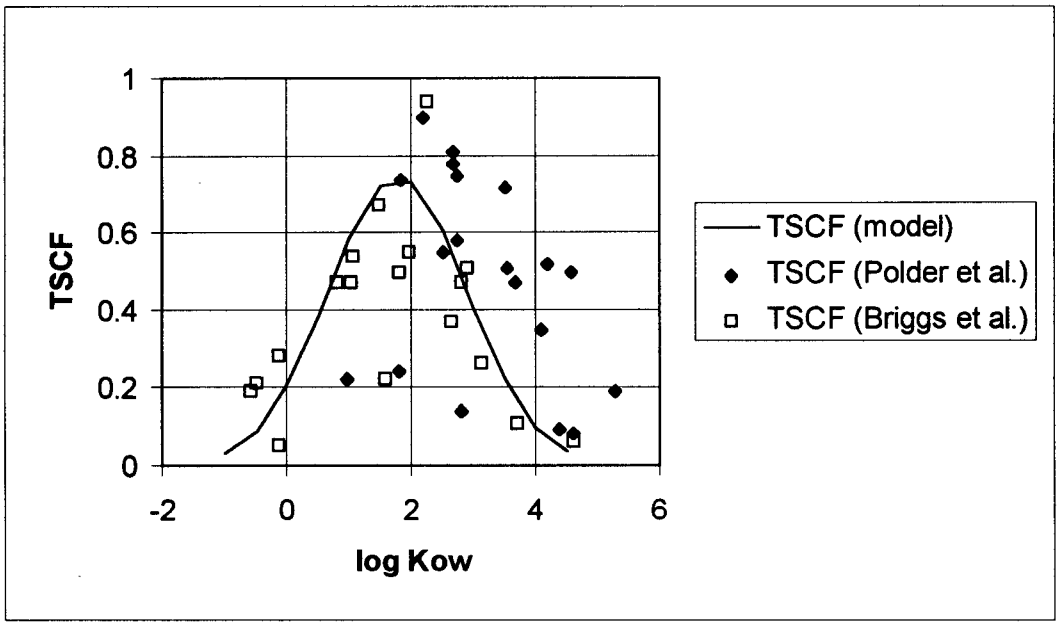

Figure 22 *Comparison of the regression of Briggs et al. (1982) with measured data which were used to derive the regression and additional data collected by Polder et al. (1995).*

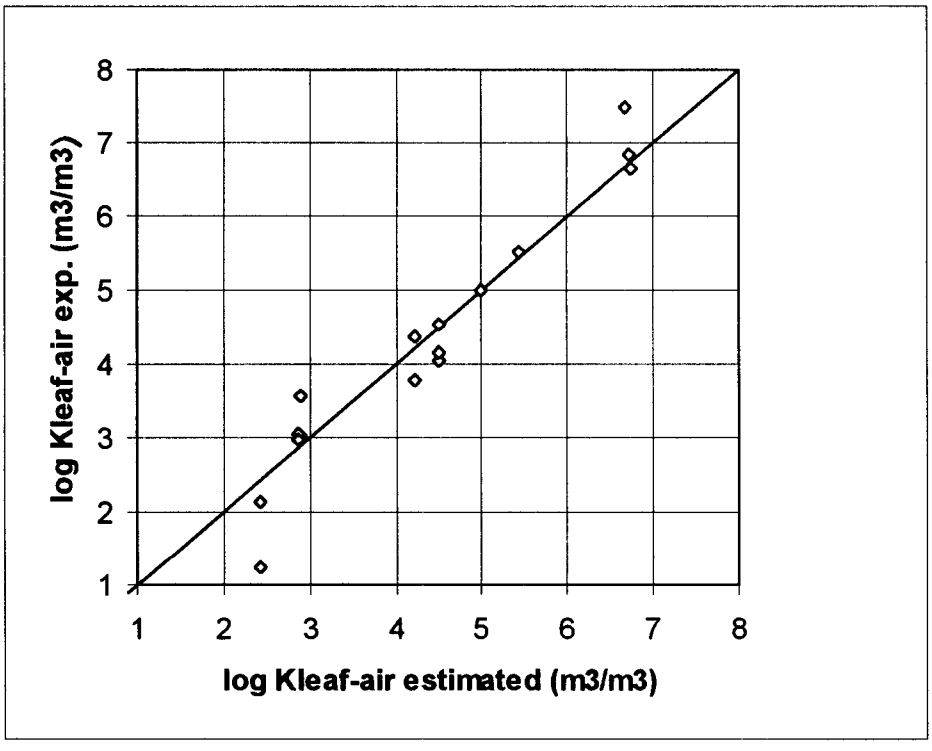

Figure 23 *Comparison of estimated leaf-air partition coefficient with measured data (data collected by Polder et al., 1996). The 1:1 line is shown.*

6.6. Sensitivity analysis

The results of a limited sensitivity analysis are shown in Table 10 (air-leaf concentration ratio) and Table 11 (porewater-leaf concentration ratio) below. Each parameter was given a uniform distribution with a variation of + and -10%. Simulations were performed in Microsoft Excel with the simulation package Crystal Ball (4000 trials, Latin Hypercube sampling). Sensitivities are given as percentage of total variance. The analysis was performed for different combinations of physico-chemical properties (*Kaw* and *Kow*).

Air-leaf ratios

The parameters A, V, k_g and g are only important when $K_{leaf\text{-}air}$ is extremely high. This is fortunate since these parameters are very crop specific and uncertain. Unfortunately, the correction exponent b is important, especially for hydrophobic compounds[1]. F_{fat} is only important when *Kow* is high and *Kaw* high (otherwise, F_{water} is more important). F_{water} is important when *Kow* low because water will be the dominant dissolving medium in the leaf. F_{air} is only important when $K_{leaf\text{-}air}$ is very low (which is not very interesting for risk assessment). The transpiration rate Q is obviously not important since there is no uptake from porewater. Summarising: F_{fat} and b are especially important for hydrophobic compounds, and F_{water} is important for less hydrophobic chemicals. Only for extreme chemical properties leading to very high $K_{leaf\text{-}air}$, the other parameters can become important. In that case, growth becomes the dominant removal process and therefore, the parameters influencing the rate constants k_1 and k_2 become sensitive (see equation on Page 50). When $K_{leaf\text{-}air}$ is small, the equation simplifies to the steady-state partitioning form given in Section 6.2.

Table 10 *Sensitivity of leaf-air partition coefficient to variation in parameters is shown below. Values in italics are set values or calculated values, others are sensitivities expressed as percentage of total variance in* $K_{leaf\text{-}air}$.

	Value	**log Kaw=-6 log Kow=4**	**log Kaw=0 log Kow=1**	**log Kaw=-6 log Kow=1**	**log Kaw=0 log Kow=7**	**log Kaw=0 log Kow=7 min b**
Value of log $K_{leaf\text{-}air}$ with growth (m^3/m^3)		***6.8***	***0.017***	***5.8***	***4.6***	***4.6***
b	***0.95***	20	5	6	99	n.a.
F_{air}	***0.30***	0	13	0	0	0
F_{water}	***0.65***	0	80	88	0	0
F_{fat}	***0.01***	0	2	2	1	100
A	***5***	19	0	1	0	0
V	***0.002***	23	0	2	0	0
g	***86.4***	19	0	1	0	0
k_g	***0.035***	19	0	1	0	0
Q	***0.001***	0	0	0	0	0

[1] This is unfortunate since the mechanistic background of this parameter value is limited. Trapp & Matthies report experimental values for b in the literature ranging from 0.75 to 0.97.

Table 11 *Concentration ratio between concentration in leaf and porewater. Values in italics are set values or calculated values, others are sensitivities expressed as percentage of total variance in* $K_{leaf\text{-}porewater}$.

	Value	log Kaw=-6 log Kow=4	log Kaw=0 log Kow=1	log Kaw=-6 log Kow=1	log Kaw=0 log Kow=7	log Kaw=0 log Kow=7 min b
Value of log $K_{leaf\text{-}porewater}$ with growth (m^3/m^3)		*0.13*	*-5.8*	*-0.030*	*-5.9*	*-5.9*
b	*0.95*	17	1	1	99	n.a.
F_{air}	*0.30*	0	1	1	0	0
F_{water}	*0.65*	0	7	13	0	0
F_{fat}	*0.01*	0	0	0	0	26
A	*5*	1	29	24	0	24
V	*0.002*	27	0	1	0	0
g	*86.4*	0	31	26	0	24
k_g	*0.035*	24	0	0	0	0
Q	*0.001*	31	31	34	0	26

Porewater-leaf ratios

When *Kaw* is high, the plant seems to lose the chemical to air after uptake from soil. The leaf-porewater concentration ratio is highest when *Kaw* is low. The parameter F_{air} seems not important at all. Again, *b* is important for chemicals with a high *Kow*. F_{fat} is only important when *Kow* and *Kaw* are both high (at low *Kaw*, the water phase is more important). The leaf volume is only important for the highest value of the leaf-porewater ratio, leaf area for the other scenarios. The transpiration rate *Q* seems to be important for each scenario. Growth is only important for the highest value of the ratio leaf-porewater. This is fortunate since the assumption of continuing exponential growth is unlikely for most crops.

General conclusion on sensitivity

It is difficult to draw general conclusions from this limited sensitivity analysis. Because there are so many routes in the model, each parameter seems to be important in a certain situation for a type of certain chemical. Only the volume fraction air (F_{air}) seems of limited importance. Strikingly, the correction exponent (*b*) is important for hydrophobic chemicals and extremely important for very hydrophobic chemicals (log *Kow*=7). Generally, growth is only relevant for very high air-leaf and porewater-leaf ratios.

6.7. Scenario

Apart from the plant en compound-specific data, the exposure scenario of the TGD is important. Especially the so-called "averaging time": the concentration in soil is averaged over 180 days and the concentration in crops is calculated as if they were in steady state with this concentration. The data in Table 12 may be useful in evaluating the scenario of the TGD. In view of these data, the currently applied vegetation period of 180 days seems too long. It is more realistic to select a vegetation period of 50-120 days for leaf crops and 140-150 days for

root crops. Trapp & Matthies (1995) suggest a value of 60 days for a typical short-growing crop.

Table 12 *Vegetation period and harvested weight for several crops. Data from Kliment & Wagnerová (1995).*

		Vegetation period (d)	**Yield of harvested weight ($kg.m^{-2}$)**
Wheat		100	0.51
Barley		100	0.46
Potato		140	1.8
Maize for silage		95	3.4
Sugar beet		180	3.5
Perennial fodder:			
Clover, Alfalfa	1 cutt.	50-55	2.2 (60%)
	2 cutt.	35-40	1.1 (30%)
	3 cutt.	65-75	0.4 (10%)
Pasture grass	1 cutt.	55-60	0.9 (60%)
	2 cutt.	65-75	0.6 (40%)
Vegetable fruit (cucumber)		90	2.3
Vegetable root (carrot)		150	3.4
Vegetable leafy spring (lettuce)		50	1.4
Vegetable leafy autumn (cabbage)		120	3.2
Fruit (apple)		150	0.9

7. DYNAMICS OF THE LOCAL FATE MODELS

7.1. Introduction

First step in an environmental exposure assessment is an assessment of the chemical's emission and fate in the environment. For the emission estimations, the TGD contains an appendix with default release fractions, depending on the type of use of the chemical (EC, 1996a, Appendix I of Chapter 3). Furthermore, tables are included for local situations to estimate the fraction emitted by a main point source and the number of emission days per year. The environmental fate models described in the TGD require a definition of the environment for which they need to predict chemical behaviour. The TGD aims at one risk characterisation for a substance at EU level. However, the characteristics of the environment in the EU Member States is highly variable in time and space. The flow of a river will e.g., be different at different locations, but may also be influenced by seasonal variability. To address this problem, concentrations are not calculated for a real location but for a hypothetical site, the so-called "standard environment". This standard environment combines a relatively worst-case exposure scenario with an average or typical compartment definition.

The chemical's fate in the environment is assessed on two spatial scales: locally in the vicinity of a large point and regionally, considering all sources in a larger area. The local distribution routes that are accounted for are shown in Figure 24. The regional model approach assumes emission to be a continuous diffuse flux into the environment and calculates the resulting steady-state concentration levels. The assumption that organisms are in steady state with this environment is therefore appropriate for this model concept. In the local standard environment, however, the direct emissions[1] to the environment are not necessarily continuous (e.g. in the case of batch processes). The indirect emission to soil is *strictly* discontinuous as it is caused by applying sewage sludge as fertiliser once, or few times, a year. The assumption of steady state between the prey organism and its environment is therefore not necessarily valid.

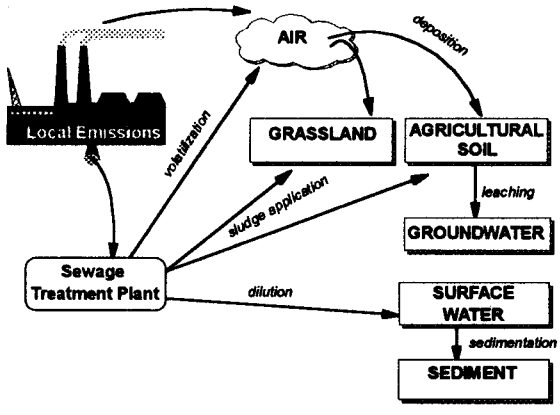

Figure 24 Local distribution routes of the chemical.

For this study, we will focus on the local exposure situation as this is the most relevant spatial scale to investigate the influence of uptake and elimination kinetics in prey organisms. The scenario applied in the TGD is summarised in the tables below.

[1] Direct emissions, in this framework, are those emissions resulting from industrial processes or use of the chemical. Indirect emissions are defined as the emissions from sewage treatment plants.

Table 13 Local exposure scenario: environmental concentrations.

Environmental concentrations	For exposure of:	Local scenario
Surface water	Fish and drinking water	Surface water after complete mixing of STP effluent and sorption to suspended matter
Agricultural soil	Root and leaf crops, earthworms	The soil after 10 years of sludge application (once a year) and deposition (continuously, annual average flux)
Grassland soil	Grass	The soil after 10 years of sludge application (once a year) and deposition (continuously, annual average flux)
Groundwater	Drinking water	Steady-state concentration in porewater of agricultural soil (180-day average)
Air	Inhalation by cattle and humans directly, uptake by leaf crops	Annual average concentration in air at 100 m from source (point source or STP)
Deposition flux	Input for soil model	Annual average flux over a circular area with diameter of 1000 m around the source (STP and point source added up)

Table 14 Local exposure scenario: intake media.

Exposure medium	For exposure of:	Local scenario
Fish	Humans	Concentration in fish is in steady-state with the concentration in surface water
Fish	Predators	Concentration in fish are in steady-state with the concentration in surface water (for predators the choice is made to derive exposure 50% from the regional, and 50% from the local spatial scale)
Earthworms	Predators	Concentration in earthworms are in steady-state with the concentration in agricultural soil (50% regional, 50% local)
Leaf crops	Humans	Concentration in leaf crops reach a steady-state with input from air and pore water (agricultural soil) and output through growth dilution and diffusion to air
Root crops	Humans	Concentration in roots are in equilibrium with the pore water concentration in agricultural soil
Grass	Cattle	Concentration in grass reaches a steady-state with input from air and pore water (agricultural soil) and output through growth dilution and diffusion to air
Air	Humans and cattle	See previous table
Drinking water	Humans and cattle	Produced from groundwater or purified surface water
Cattle meat and milk	Humans	The concentrations in cattle meat and milk are in steady-state with the daily dose of the chemical

In the following sections, the methods for local environmental distribution are discussed. The discussion focuses on the time aspects of the calculations. For the detailed description of these models, including the model formulas and default settings, the reader is referred to the TGD (EC, 1996a) or the EUSES documentation (EC, 1996b)

7.2. Direct emission to air and wastewater

The exposure assessment starts with estimation of the emissions to the standard environment. Emission patterns vary widely from well defined point sources (single or multiple) to diffuse releases from large numbers of small point sources (like households) or line sources (like a motorway with traffic emissions), and from continuous to discontinuous releases. The text block gives some definitions on these time aspects (taken from the EUSES documentation, EC, 1996b). The discontinuous emissions are the most abundant as most chemicals are produced and used in batch processes (Van der Poel, pers. comm.). Especially at the stage of production there are very few examples of continuous emission. Clearly, the dynamics of emission patterns is a relevant issue.

Types of emissions

Continuous emissions are characterised by an almost constant emission rate over a prolonged period (e.g. the emission of a substance from a continuous production process such as an oil refinery).

Discontinuous emissions can be peak emissions or block emissions.
Peak emissions are characterised by a relatively large amount discharged in a short time where the time intervals between peaks and the peak height can vary greatly (e.g. the discharge of spent liquid - reaction mixture - after isolation of the synthesised substance in a batch process).
Block emissions are characterised by a flow rate which is reasonably constant over certain time periods with regular intervals with a low or even zero background emission (e.g. the emissions from traffic during the day; during rush hours emissions are high in particular).

On the local spatial scale, the emissions from one, large point source are considered. When measured releases are not available, general estimates are applied which depend on the use pattern of the substance. These general release estimates do not specify the emissions as a function of time but only give the number of days per year during which emission takes place. In the subsequent fate calculations, only the direct-emission flux during an emission episode is used but for indirect exposure purposes, the concentrations in the environment are averaged over a year using the number of emission days per year.

By defining local emissions with an annual release and a number of emission days per year, the emissions can be characterised as a block emission[1], as shown in the examples of Figure 25. The following important points however, remain unspecified in the general emission estimation of the TGD:

- At what point in time does the emission take place?
- Is the emission episode one continuous block or does emission take place during several peaks a year?

At this point, we assume emission as a one-block function of time for the dynamic simulations. During the emission episode, the emission is assumed constant.

[1] Several other function might be possible like normal or triangular functions of time. Because a block function is the simplest form, and since more information on the time dependence of emissions is scarce, the block function is assumed in this study.

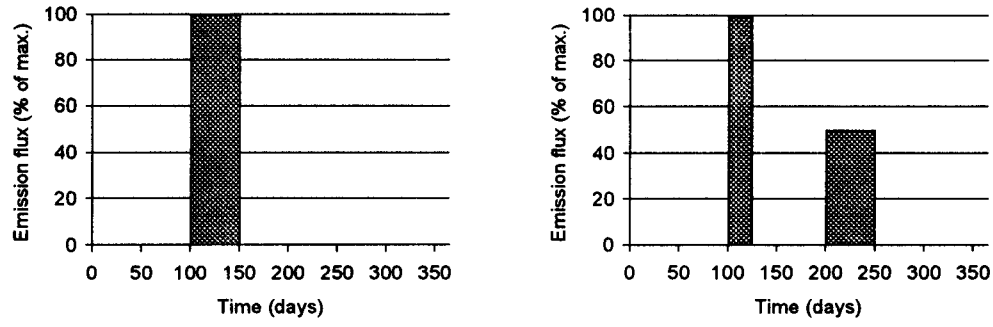

***Figure 25** Examples of possible emission patterns as block-function of time.*

7.3. Sewage treatment plant

Emissions to water are treated in a sewage treatment plant (STP). In the STP, the chemical may be removed through biodegradation, sorption to sludge or volatilisation to air. The remaining amount of chemical is passed on to the effluent. For the estimation of the chemical's fate in sewage treatment, the TGD advises to use the model SimpleTreat (Struijs, 1996). This model estimates the steady-state distribution between sludge, effluent and air and the amount of chemical degraded. For the purpose of this study, the dynamics of the sewage treatment plant are ignored. This implies that when the emission to wastewater is a block function of time, the effluent concentration will also be a block function of time with the same number of days per year. The same argumentation is used for indirect emission to air. For the concentration in sludge, a time-dependence is not strictly needed. Sludge is only applied once a year on soil so the relevant concentration in sludge is one constant concentration which is estimated with the emission during an episode[1]. Nevertheless, the dynamics of the STP remains an interesting point. Since the micro-organisms in the STP require some time to adapt to a chemical, it is conceivable that just after the start of the emission episode, the effluent concentration is higher than in steady state. After emission has ceased, the effluent concentration will not drop abruptly, but may decrease with a certain time lag (especially for hydrophobic chemicals). The dynamics of the STP is certainly not a trivial issue but this subject is beyond the scope of this study.

7.4. Concentration in air and deposition fluxes

The concentration of the chemical in air is estimated at a distance of 100 m from the source assuming standard source and meteorological characteristics. Results from the air distribution model OPS (Van Jaarsveld, 1990) were used to calculate a simple linear relation between source strength and concentration in air. Because of the small spatial scale considered, chemical reactions and degradation are ignored. Aerial deposition is calculated as a flux, averaged over a circular area with a radius of 1000 m. The deposition flux is estimated in the same manner as the air concentration with a linear relationship derived from the OPS model. Deposition of gaseous and aerosol-bound substance are treated separately. Because of the small spatial scale and the rapid dynamics of the fate processes in air, the concentration in air

[1] This is of course a worst case since the chemical's concentration in sludge can be extremely low outside of the emission episode. Nevertheless, this assumption is defensible as a "reasonable worst case".

and the deposition onto soil surfaces can be assumed to follow the emission pattern closely. In other words, no additional time dependence needs to be assumed: the air concentration and deposition are assumed to respond instantaneously to changes in emission rates.

7.5. Concentration in surface water

The concentration in surface water is calculated from the STP-effluent concentration by accounting for the two most dominant fate processes: dilution in the receiving surface water and sorption to suspended matter. Other removal processes as degradation and volatilisation are neglected at this small spatial scale. Since the dynamics of the fate processes in water are relatively fast, the concentration changes in water can be assumed to closely follow the changes in emission (as was done for the air compartment). Of course, this assumption is only valid for rivers, not for lakes or estuaries where accumulation of chemicals may occur. The TGD also advises to perform a risk characterisation for sediment-dwelling organisms. However, as no bioaccumulation through sediment is considered, this will also not be addressed in this study. Nevertheless, the detailed risk assessment for sediments dwellers, including organisms feeding on them, may need to be addressed in the future.

7.6. Concentration in agricultural soil and grassland

Sludge from the STP is applied on agricultural soils once a year at the beginning of the growing season (pulse-type input). Furthermore, the soil receives input from aerial deposition (block-type input). Soil is a much less dynamic environment than air and water, therefore, accumulation of the chemical can occur. The concentration in soil is calculated with a simple one-compartment model accounting for input through sludge and aerial deposition and removal by degradation, leaching and volatilisation. The removal processes are described as first-order processes. The initial concentration in the soil compartment is governed by application of sewage sludge at the beginning of the growing season. Each year, this initial concentration is added to the concentration remaining from the year before. Because sludge is applied over consecutive years, accumulation will occur when a substance does not fully disappear from the top-soil layer in one year. In the TGD, the choice is made to account for 9 years of accumulation and assess the concentration in the 10th year of sludge application. For indirect exposure purposes, the concentration in soil is averaged over 180 days in the 10th year. Two soil types are considered: agricultural soil for production of root crops and leafy vegetables, and grassland with grazing cattle. These soils differ in the amount of sludge applied and the mixing depth of the top-soil layer.

7.7. Summary of time dependence in local environmental concentrations

Summarising, the concentrations in water and air and the deposition flux to soil can be assumed to follow the time-dependence of the emission flux. The dynamics of the soil concentration are radically different. Since soil is modelled as one compartment with first-order removal, the concentration will be high just after sludge application and will decrease exponentially afterwards. However, since the soil also receives input through aerial deposition, this exponential decay will be disturbed during the emission episode. This effect is illustrated in Figure 26.

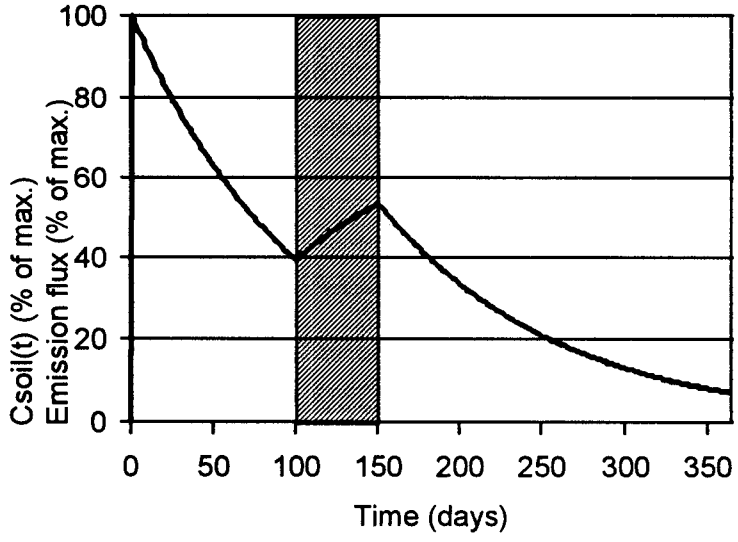

Figure 26 *Example of the influence of a block-type emission to air (indicated by the hatched block) on concentration in soil (thick line).*

Below, the local models are described generally as functions of time (*t*) and chemical properties.

Emission

The emission can be characterised by the following parameters:

$E_{air}(t)$	emission to air	[$kg.d^{-1}$]
$E_{water}(t)$	emission to water	[$kg.d^{-1}$]
T_e	length of the emission episode	[d]
T_s	start of emission episode	[d]

STP

$$C_{sludge} = f(E_{water}(\max), Kow, VP, SOL, MOLW)$$

C_{sludge}	concentration in sewage sludge	[$mg.kg_{dwt}^{-1}$]
$E_{water}(max)$	emission to waste water during emission episode	[$mg.d^{-1}$]
Kow	octanol-water partition coefficient	[$m^3.m^{-3}$]
VP	vapour pressure	[Pa]
SOL	water solubility	[$mg.m^{-3}$]

MOLW	molecular weight	[g.mol^{-1}]

Air

$$C_{air}(t) = f(E_{air}(t), VP)$$

$C_{air}(t)$	concentration in gas phase of air	[mg.m$_{air}^{-3}$]
$E_{air}(t)$	emission to air	[kg.d^{-1}]
VP	vapour pressure	[Pa]

Deposition

$$D(t) = f(E_{air}(t), VP, MOLW, SOL)$$

D(t)	deposition flux	[mg.m^{-2}.d^{-1}]
$E_{air}(t)$	emission to air	[mg.d^{-1}]
VP	vapour pressure	[Pa]
MOLW	molecular weight	[g.mol-1]
SOL	water solubility	[mg.m-3]

Water

$$C_{water}(t) = f(E_{water}(t), Kow)$$

$C_{water}(t)$	dissolved concentration in surface water	[mg.m$_{water}^{-3}$]
$E_{water}(t)$	emission to waste water	[mg.d^{-1}]
Kow	octanol-water partition coefficient	[m^3.m^{-3}]

Soil

$$\frac{dC_{soil}(t)}{dt} = D(t) - (k_{deg} + k_{leaching} + k_{volat}) \cdot C_{soil}(t)$$

$C_{soil}(t)$	concentration in soil	[mg.kg$_{soil}^{-1}$]
D(t)	aerial deposition (referenced to kg of soil)	[mg.kg$_{soil}^{-1}$.d^{-1}]
k_{deg}	rate constant for degradation in soil	[d^{-1}]
$k_{leaching}$	rate constant for leaching from top-soil layer	[d^{-1}]
k_{volat}	rate constant for volatilisation from soil	[d^{-1}]

8. COMPARISON OF APPROACHES

In this chapter, the steady-state approach as described in the TGD (EC, 1996a) is compared to the results of dynamic simulation. Dynamic calculations were performed with the simulation software STELLA®. The approaches are summarised in Table 15.

Table 15 Summary of the approaches which are compared in this chapter.

	Steady-state approach	Dynamic approach
Fish	$C_{fish} = BCF \cdot C_{water} \cdot \frac{T_e}{365}$	$C_{fish} = \frac{1}{365}\int_0^{365} C_{fish}(t)$
Earthworms	$C_{worm} = BCF \cdot \frac{1}{180}\int_0^{180} C_{porewater}(t)$ (soil receives sludge and annual average deposition)	$C_{worm} = \frac{1}{180}\int_0^{180} C_{worm}(t)$
Root crops	$C_{root} = BCF \cdot \frac{1}{180}\int_0^{180} C_{porew}(t)$ (soil receives sludge and annual average deposition)	No data on dynamics of uptake process. Assume instantaneous steady-state. $C_{root} = C_{root}(T_{harvest})$
Leaf crops	$C_{leaf} = \frac{k_p \cdot \frac{1}{180}\int_0^{180} C_{porew}(t) + k_1 \cdot C_{air} \cdot \frac{T_e}{365}}{k_2 + k_g + k_m}$ (soil receives sludge and annual average deposition, air concentration is annual average, gas-phase)	$C_{leaf} = C_{leaf}(T_{harvest})$
Grass	$C_{grass} = \frac{k_p \cdot \frac{1}{180}\int_0^{180} C_{porew}(t) + k_1 \cdot C_{air} \cdot \frac{T_e}{365}}{k_2 + k_g + k_m}$ (soil receives sludge and annual average deposition, air concentration is annual average, gas-phase)	$C_{grass} = \frac{1}{180}\int_0^{180} C_{grass}(t)$

8.1. Fish

First, the kinetic model for fish as specified in Chapter 4 was tested for its sensitivity to changes in model parameters (assuming an emission period of 50 days). As shown in Figure 27, hydrophobicity of the chemical has a major influence on the dynamics of chemical uptake and depuration. At log *Kow* = 4, the concentration in the fish follows the changes in the water concentration almost instantaneously. At higher values of *Kow*, the fish will not reach equilibrium anymore and retains significant concentrations over a longer period of time. Increasing molecular weight and fish body weight also slow down the dynamics of uptake and depuration.

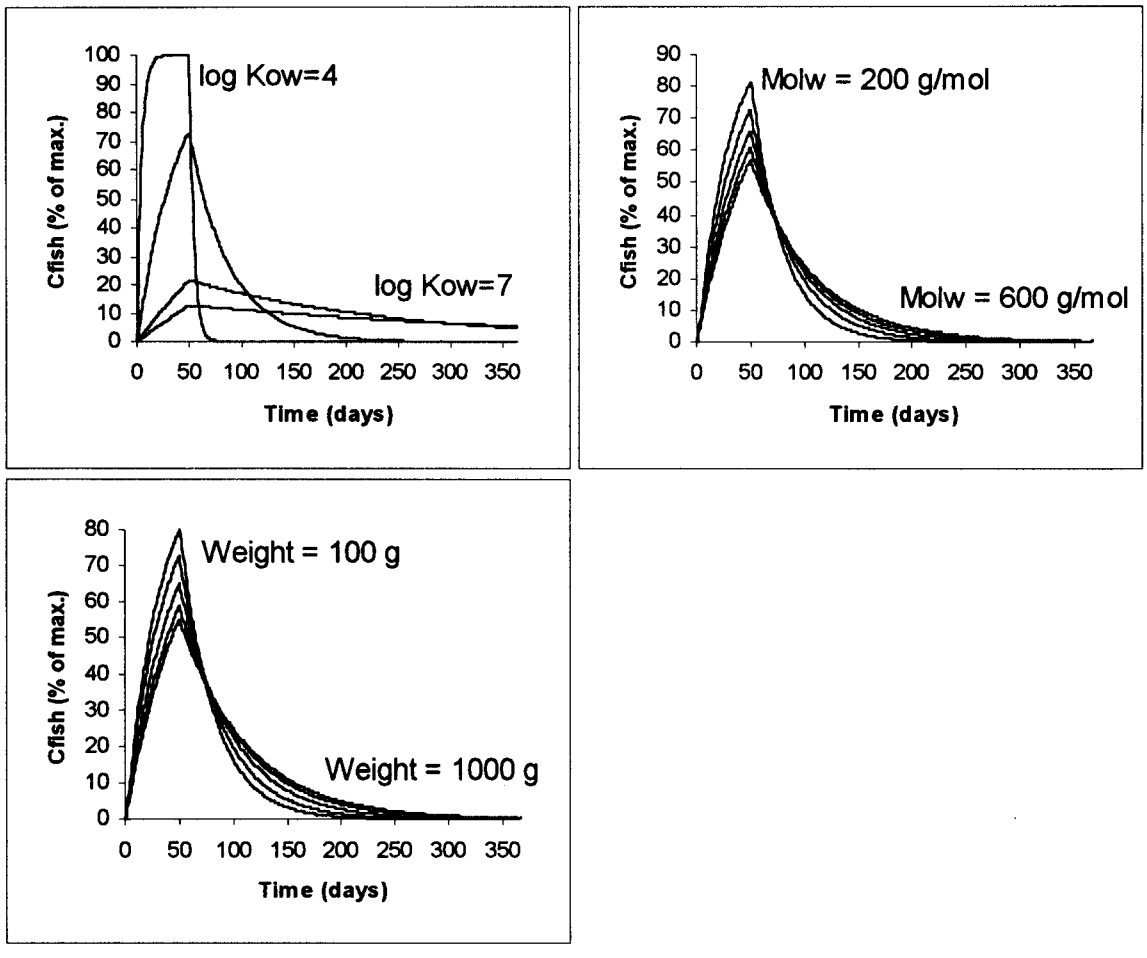

***Figure 27** Model sensitivity for the model parameters log* Kow *(4,5,6,7), molecular weight (200, 300, 400, 500, 600 g/mol) and fish weight (100, 200, 400, 700, 1000 g). Apart from the parameter that is varied, the other parameters are kept at the starting values: log* Kow*=5, mol. weight=300 g/mol, fish weight=200 g.*

Next, the outcome of the kinetic model is compared to the result of the TGD approach. The difference between the approaches is expressed as the ratio of the results of the kinetic approach to the steady-state approach:

$$Difference = \frac{\frac{1}{365}\int_{0}^{365} C_{fish}(t)}{BCF \cdot C_{water} \cdot \frac{T_e}{365}}$$

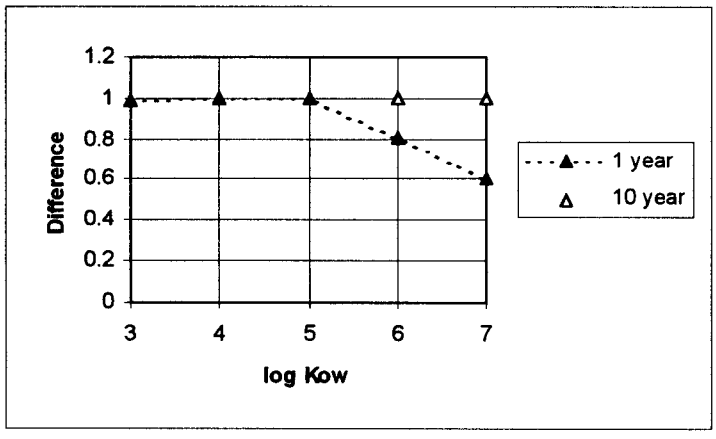

Figure 28 *Difference between kinetic and steady-state approach as function of log* Kow. *For the kinetic approach, 1 year and 10-year averages shown separately. An emission episode of 50 days is assumed.*

This difference ratio is plotted in Figure 28 for a range of log *Kow* values. For log *Kow*<5 the ratio between kinetic and steady-state approach is very close to one. Above this *Kow* value the difference ratio starts to fall below one. The highest log *Kow* shown in this figure is 7 but no further decrease above this value is expected since the maximum elimination rate is controlled by growth dilution which is independent of *Kow* (see Section 4.5).

For these high *Kow* values, the concentration in fish at the end of the year was not negligible. As a result, chemicals can accumulate in fish: concentrations in the second year were higher than in the first year. After several years, this pattern became stable (the concentration at the end of the year is the same each year). Therefore, in Figure 28 also the average concentrations in the 10th year (sufficient to reach this stable pattern) are shown. Remarkably, using these longer-term average concentrations again yields a difference ratio of one[1].

It can thus be concluded that for predicting annual-average concentrations in fish it is valid to assume a steady state with the annual-average surface water concentration as done in the TGD. This steady state, however, may take several years to achieve. It should also be kept in mind that, although not relevant for average concentrations, the *pattern* of exposure is very different for chemicals with low hydrophobicity and high hydrophobicity.

[1] For a mathematician, this result may be less remarkable as it is possible to predict this result analytically without the need for dynamic simulations. The mathematical proof is not given in this report but may provide an intellectual challenge for the interested reader!

8.2. Earthworm

It should be noted that the scientific support of the kinetic earthworm model is extremely limited, the results must therefore be interpreted with care (see Chapter 5).

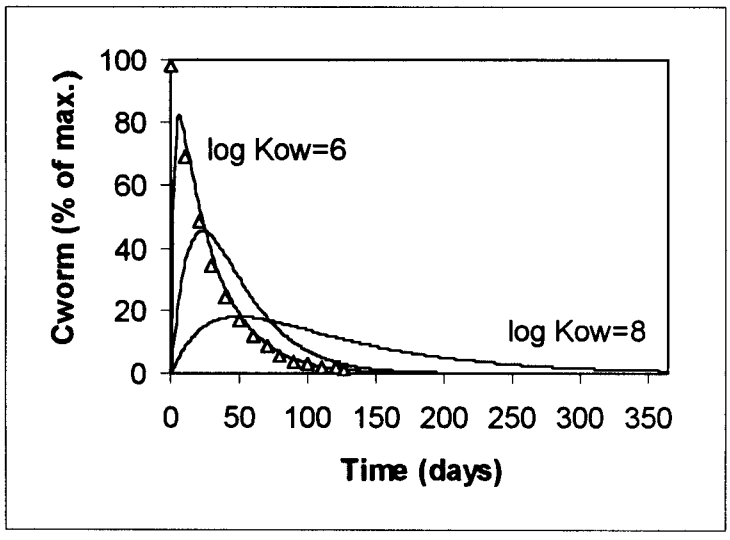

***Figure 29** Sensitivity of the kinetic earthworm model to changes in* Kow *(log* Kow*=6, 7, 8). The concentration in porewater is shown with triangles. The chemical's half life in soil is set to 20 days.*

Figure 29 shows the influence of hydrophobicity on the kinetic earthworm model. At log *Kow*=6, the concentration in the earthworm still follows the porewater concentration closely. At higher values of *Kow*, the earthworm response is slower, resulting in lower concentrations over a prolonged period of time. Since the concentration on day 365 is not zero, accumulation over the years will occur in the model. Accumulation over the years is, however, not very relevant as for most species of earthworm, an age of more than a year is unlikely to be achieved in the field.

The outcome of the kinetic model can now be compared to the result of the TGD approach. The difference between the approaches is calculated as the ratio of the kinetic approach to the steady-state approach[1]:

$$Difference = \frac{\frac{1}{180}\int_0^{180} C_{worm}(t)}{BCF \cdot \frac{1}{180}\int_0^{180} C_{porewater}(t)}$$

The 180 days average in this scenario is different from the 365 days used for fish. The reason for this deviation is not entirely clear but may be just pragmatic: a 180 days averaging period is also used for crops (reflecting the growing season). Using the same period for earthworms means that the same soil concentration can be used for both endpoints. In moderate climates as in the Netherlands, consumption of fish and worms by predators may decrease in the winter[2]. Harmonisation of these averaging times is advisable and can be done on the basis of a relevant time scale for chronic exposure in the predator organism.

[1] In the TGD and EUSES, a dynamic soil model is included which is solved analytically to give an average concentration over a certain period of time. The earthworms are assumed to be in equilibrium with this average.

[2] This may be due to a lack of "availability" of the prey or due to behaviour on the part of the predator: hibernation, change of food source or migration.

Figure 30 gives the difference ratios for the first year of sludge application and for half lifes in soil of 20 and 300 days (longer half lifes only deviate slightly more). Two time periods are shown to average the concentrations: 180 days according to the TGD and 365 days (consistent with the approach for fish). As was observed for fish, the difference ratio only starts to deviate at high values of *Kow* (above log *Kow* of 7) because the dynamics of bioconcentration become slow. This deviation is smaller for the 365-day average and would have disappeared when accumulation over several years was possible for earthworms (as demonstrated for fish). When a shorter averaging time would have been used (like 28 or 90 days, as discussed in the previous paragraph), the difference ratios may decrease even further.

The difference ratio decreases with increasing half life in soil from 20 to 300 days (for larger half lifes the difference ratio does not decrease further).

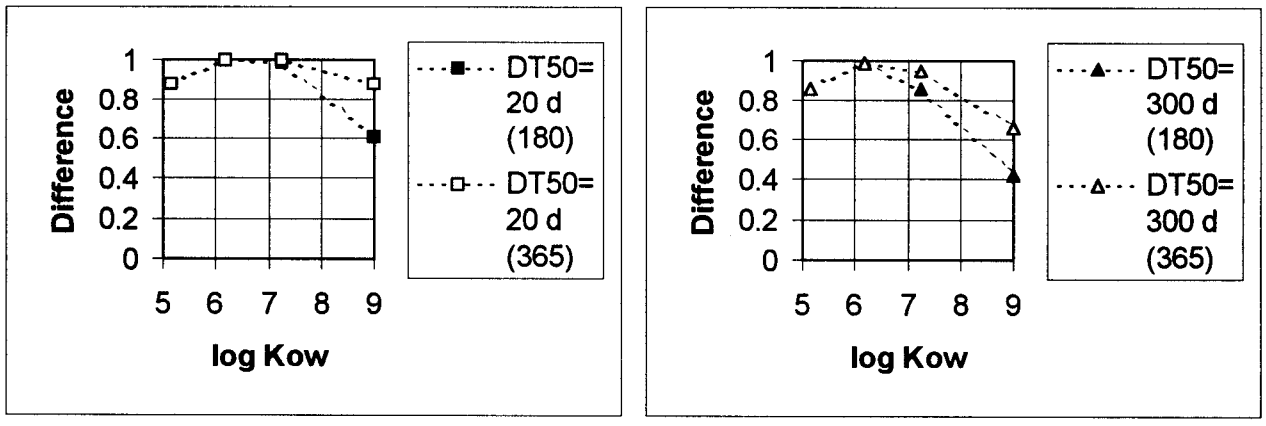

Figure 30 *Difference ratios for two half lifes in soil (20 and 300 days) and two averaging periods (180 and 365 days). Only the first year of sludge application shown.*

In conclusion, internal concentrations in earthworms can be overestimated for extremely hydrophobic chemicals (log *Kow*>7) up to a factor of 2. The theoretical support for this conclusion is limited. Shorter averaging periods may be more appropriate. However, changing this period for fish will also change the conclusions in Section 8.1. For shorter averaging times then a year, the overestimation of concentrations with the steady-state approach will increase.

8.3. Plants

Plants can be exposed through soil and through uptake from the air. These scenarios are handled separately in the following sections.

Exposure through soil only

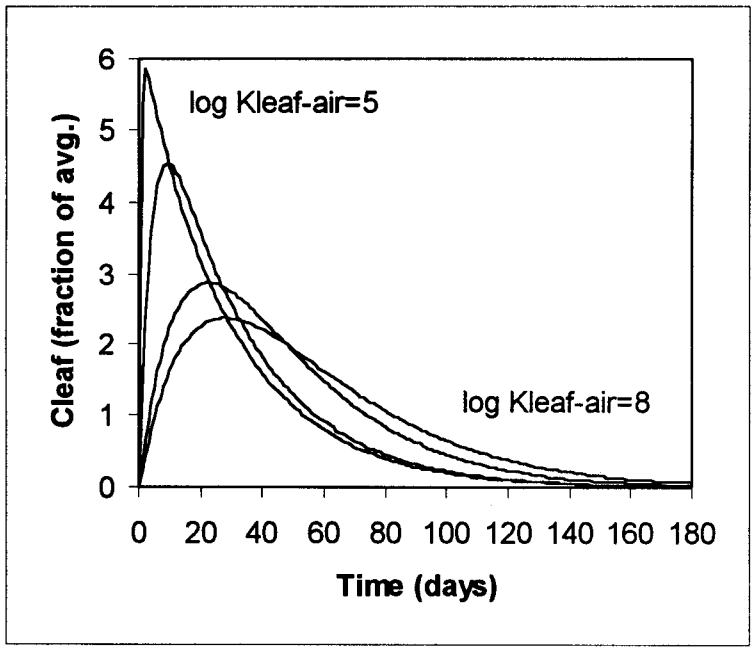

***Figure 31** Behaviour of the plant model for different values of the leaf-air partition coefficient (log $K_{leaf-air}$=5,6,7,8). Only uptake from soil, half life in soil set to 20 days.*

The behaviour of the dynamic model for leaves is shown in Figure 31. In this figure, the concentration in the leaf is plotted in time as fraction of the "average concentration". This average is calculated assuming steady state with the 180-day average concentration in porewater (the TGD approach).

The leaf-air partition coefficient is the key "chemical" property governing the dynamics of uptake and elimination. This coefficient is made up of plant-specific parameters and the physico-chemical properties *Kow*, vapour pressure, water solubility and molecular weight. At low values of $K_{leaf-air}$ (log $K_{leaf-air}$<6) the leaf concentration closely follows the kinetics of the donating compartment (the soil). At higher values of $K_{leaf-air}$, a slower response can be observed.

Two difference ratios can be calculated for the leaf concentrations, using two alternative approaches. In the first, the 180-day average concentration in leaf is compared to the steady-state approach. This approach is most appropriate to calculate a concentration is grass as feed for cattle since these leafs are continuously grazed. In the second difference ratio, the leaf concentration at time of harvest ($T_{harvest}$) is compared to the steady-state approach. Both approaches can be written down as:

$$Difference = \frac{\frac{1}{180}\int_0^{180} C_{leaf}(t)}{\frac{k_p}{k_2+k_g}\cdot\frac{1}{180}\int_0^{180} C_{porewater}(t)} \quad \text{or} \quad Difference = \frac{C_{leaf}(T_{harvest})}{\frac{k_p}{k_2+k_g}\cdot\frac{1}{180}\int_0^{180} C_{porewater}(t)}$$

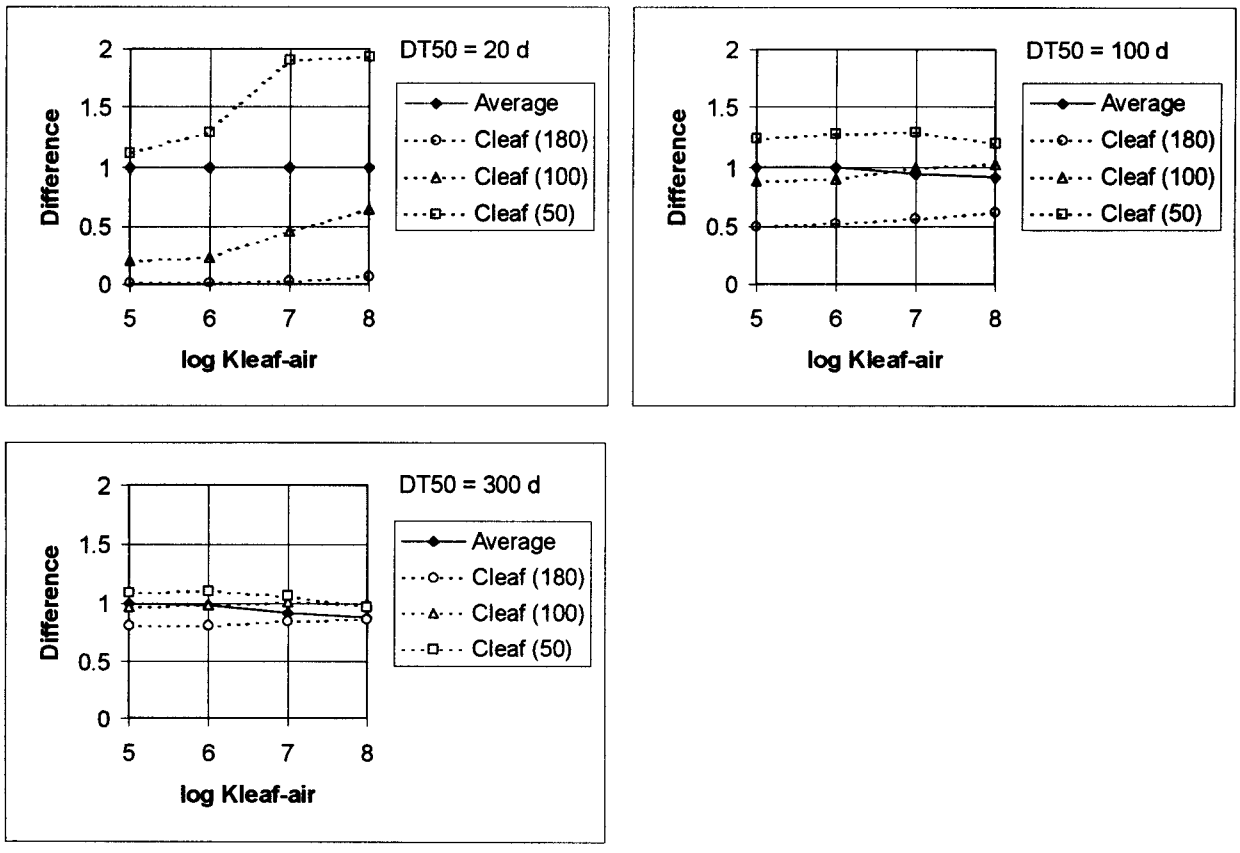

Figure 32 *Difference ratios for three half lifes in soil (20, 100 and 300 days) and different alternative approaches (average concentration in leaf over 180 days and concentration at 180, 100 and 50 days).*

In Figure 32, the difference ratios are plotted against the leaf-air partition coefficient. Again, as seen for fish and earthworms, averaging the leaf concentration over 180 days produces similar results as assuming a steady state with the average exposure concentration. The decrease of the difference ratio for "slow chemicals" (as observed for fish and earthworms) does not occur for leaves as the slowest chemicals possible (log $K_{leaf\text{-}air}$=8 is an appropriate maximum) is still sufficiently rapid to ensure a difference ratio close to 1. Therefore, for estimating concentrations in grass, the TGD scenario is appropriate.

As shown in Figure 32, deviations between the scenarios may occur, but only for short half lifes in soil. For larger half lifes, the 180-day average and the different harvest times yield remarkably similar results. At a half life in soil of around 20 days, the TGD approach will underestimate levels in short-growing crops like lettuce by a factor of 2 for chemicals with a log $K_{leaf\text{-}air}$ >6. At the same half life, longer-growing crops (like cabbages or root crops) will be seriously overestimated (factor 20 to 100) over most of the $K_{leaf\text{-}air}$ range. As discussed in Section 6.1, the dynamic behaviour of root crops is unknown (or at least, could not be found in the literature). When rapid steady state can be assumed, the difference ratio for roots will behave like the difference ratio of leaves at low values of $K_{leaf\text{-}air}$.

The dynamic model as used in these calculations assumes continuous exponential growth over the entire growing period. For crops like lettuce with a short growing period, and grass which is grazed, this assumption seems reasonable. For most other crops, however, this assumption is definitely false as growth rates generally decline when the plant matures and/or starts

flowering and setting seed. To test the influence of the growth rate, a time-dependent growth rate is assumed like plotted in Figure 33. Please note that this actual time-dependence is just a quick test and may very well disagree with the actual situation.

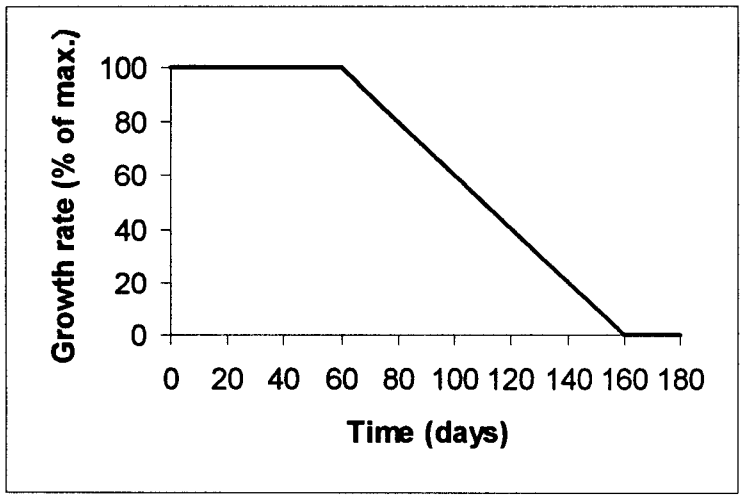

Figure 33 Growth rate as a function of time.

With this growth rate, the dynamic model was run and the results are shown in Figure 34. For short half lifes in soil, the difference ratio looks very similar to the previous results. This is the case because at the moment when the growth rate starts to decrease, the exposure concentration is already low and the plant has already lost most of its chemical content. For longer half lifes in soil and for longer harvest times, a decreasing growth rate can seriously increase the concentrations for log $K_{leaf\text{-}air}$ > 6 (in this example up to a factor of 2.7). It can therefore be concluded that when the growth rate decreases well before harvest, an underestimation of the leaf concentration may occur. This underestimation will, however, only be profound when the half life in soil is not too low (larger than about 100 days) and when log $K_{leaf\text{-}air}$ is larger than 6.

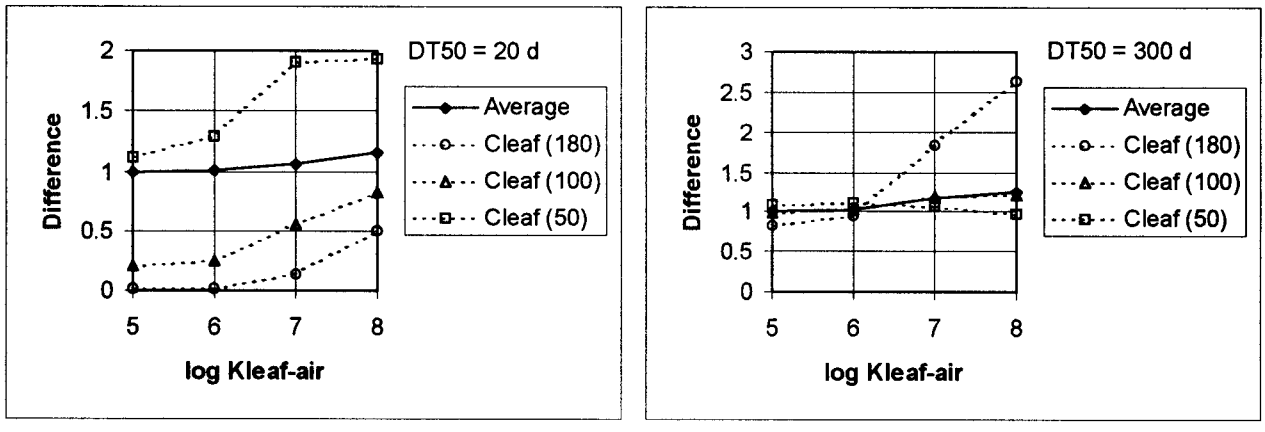

***Figure 34** Difference ratios for two half lifes in soil (20 and 300 days, figure left and right respectively) and different alternative approaches (average concentration in leaf over 180 days and concentration at 180, 100 and 50 days). Growth rate as in 33rd.*

Exposure through air (gas phase) only

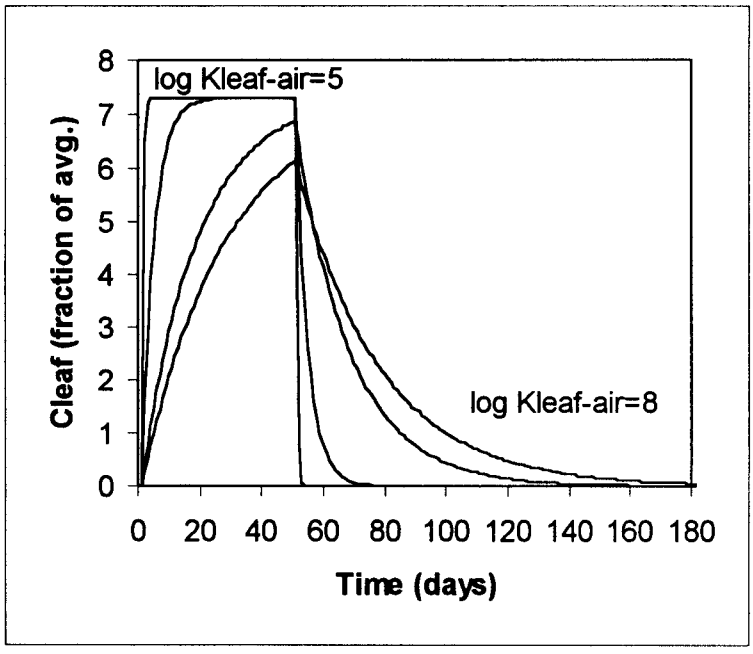

***Figure 35** Behaviour of the plant model for different values of the leaf-air partition coefficient (log $K_{leaf-air}$=5,6,7,8). Only uptake through air, emission episode of 50 days.*

In Figure 35, the behaviour of the dynamic model is shown for a block-type emission to air. The concentration in the leaf is plotted in time as fraction of the "average concentration". This average is calculated assuming steady state with the annual-average air concentration (as done in the TGD).

For values of log $K_{leaf-air}$ below 6, the leaf concentration closely follows the changes in the air concentration (as was also observed for uptake from soil). For higher values, the response becomes slower. The maximum concentration of 7.3 times the average is not arbitrary, but equals 365 divided by 50 (the length of the emission period). This maximum will increase with decreasing length of the emission period.

The additional problem with airborne exposure in comparison with exposure through soil is that the moment at which the exposure starts is generally unknown. Assuming an emission episode of 50 days, the concentration at time of harvest may thus, in reality, be a factor of 7.3 higher than the TGD approach or plain zero when the emission episode is outside of the growing season. The shorter the emission episode, the larger the possible variation. Assuming that the emission episode can start at each time in the year with the same probability, the TGD scenario will provide a kind of average approach, independent of harvest time or averaging period. The variation around this average increases with decreasing length of the emission period. It can be anticipated that a decreasing growth rate for longer-growing crops will also have a profound influence on leaf concentrations when the emission episode is just before harvest (for chemicals with a log $K_{leaf-air} > 6$, as demonstrated earlier in this section).

The route deposition→soil→plant is of less interest than the other routes discussed. When there is only emission to air, gas-phase uptake tends to be the dominant exposure route. Chemicals for which $K_{leaf-air}$ is low may be taken up from soil in significant amounts compared to uptake from air, but will also be rapidly lost to air again from the leaf.

9. DISCUSSION

In this study, the estimation methods are examined which are used to assess the impact of bioaccumulation in risk assessment for organic compounds. Three types of organism were examined: fish, earthworms and plants (root crops, leaf crops and grass). As described in Chapter 2, two approaches can be distinguished for estimating BCFs and BAFs: QSAR regressions based on physico-chemical properties (usually *Kow*) and mechanistic approaches which try to incorporate parameters which have physiological meaning (e.g. body weight and fat content). The use of QSAR regressions, although widely applied in risk assessment, has several weak points:

1. QSARs are, in principle, only valid for the type of chemicals for which they were derived. This training set must be a representative sample from all possible chemicals and organism species for which the QSAR can be used. A different or extended data set will yield a different regression.
2. Extrapolation beyond the range of the training set could lead to unacceptable results. This is especially true for polynomial equations. Polynomial equations in itself are suspect since there is absolutely no mechanistic background to assume a polynomial equation. The only reason for this type of regression is an improved fit (in general, the statement holds that the more terms are added to the equation, the better the fit).
3. Regressions provide little insight in the mechanism of bioconcentration and the role of organism and environmental properties in this process.

We prefer to use mechanistic approaches since they do not have these weaknesses, provided that the theoretical support is sound and that they can deliver similar or better descriptive power. In this study, a general steady-state partition model for BCF and a general dynamic one-compartment model are described and parameterised for the three types of organisms selected. Subsequently, these approaches are compared to the approach laid down in the Technical Guidance Documents for new and existing substances (EC, 1996a) and to selected measured data. The performance of the mechanistic approaches are discussed in the next sections.

9.1. Evaluation of the models for fish

Estimation of BCF fish

The simple partition model describes the data very well. The log-linear regression of the TGD for the range log *Kow* 1 to 6 is in principle equally valid, but not necessary to describe the data. Furthermore, this simple model makes a discussion on the data set to be used for the regression redundant (each new data set will provide a different regression). Nevertheless, the experimental BCFs shows considerable deviations from the model in this *Kow* range, indicating that *Kow* is not a perfect predictor for the bioconcentration behaviour of fish fat. For specific groups of compounds it may be possible to construct different model formulations starting from the basic partition model. This may be done by modifying *Kow* to express a compound-specific difference of octanol with fish lipids. In fact, this could be achieved by fitting the coefficients a and b in the parameter group $a.Kow^b$ as given in Section 3.1. Modifying *Kow* in this manner should be handled with care as the theoretical background is limited and a procedure like this decreases the mechanistic advantages of the model. Perhaps it

is possible to establish fish lipid-water partition coefficients in the laboratory and compare these to *Kow*s.

After critical evaluation of the experimental data, the validity of the polynomial equation for the log *Kow* range 6 to 10 is questionable. Several explanations for deviating behaviour above log *Kow* of 6 are put forward in Section 2.2. These explanations range from a truly deviating behaviour due to molecular size to poorly conducted experiments. It therefore seems inappropriate to base a general QSAR for risk assessment on the basis of these values. The theoretical model with growth dilution predicts a constant BCF above log *Kow* of around 6. Furthermore, there is a discrepancy between the different data sets in this *Kow* range. The data of Nendza (1991), as shown in Figure 7, seem to suggest a constant BCF above log *Kow* of 6 and therefore supports the "dilution by growth" model (see Section 4.5). Other data sets include data for extremely hydrophobic chemicals which show lower BCFs than expected. In view of the high uncertainty in the BCFs as well as the *Kow* values in this range, it is advisable to assume a constant BCF above log *Kow* of 6 for risk assessment purposes. In the absence of better data, this provides an initial "reasonable-worst case" estimate unless sufficient proof for truly deviating behaviour is available, e.g. from structurally similar compounds.

Especially at high values of Kow, uptake from food may become an important exposure route for fish. (see Thomann, 1989). To investigate whether the TGD food chains are sufficiently protective, this route may be examined further in the future.

Parameters
For the general fish as consumed by humans and predators, a fat percentage of 3 vol% is selected. This is a representative value for most freshwater species. The fat percentage for eel, however, is a factor of 8 higher. Therefore, we propose to consider this species separately in risk assessment. Since fat is the main dissolving tissue for hydrophobic chemicals, it may be more appropriate to express BCFs on a lipid basis. This is especially appropriate since laboratory experiments are usually performed with relatively fat fish (although not as fat as eel).

Dynamic model
For fish, the allometric model of Sijm & Van der Linde (1995) was selected to estimate the parameters of the dynamic model, k_1 and k_2. The model of Sijm & Van der Linde (1995) was generally consistent with the experimental data which could be found. For extremely hydrophobic chemicals, deviations occur which may be caused by molecular size, metabolism or experimental difficulties.

9.2. Evaluation of the models for earthworms

Estimation of BCF earthworm
The relationship of Connell & Markwell (1990) as advised in the TGD has serious shortcomings as discussed in Section 5.5. Furthermore, the TGD contains an error while the QSAR for BCF gives a value on dry-weight basis whereas it is applied on wet-weight basis[1].

[1] This error arises from the fact that Connell & Markwell do not mention *any* unit in their publication. Going back to the original publications (Lord *et al.*, 1980 and Wheatley & Hardman, 1968) confirmed that they reported BCFs based on worm dry weight. This stresses again the importance of conscientiously reporting units. This error is corrected in the official release of the TGD.

Based on the evaluated data plotted in Figure 19, we advice the use of the simple partition approach to estimate BCF. This approach may overestimate field concentrations in worms but owing to a lack of reliable measured data, this safety must be appreciated. Uptake from food and dilution by growth seem to be of limited importance and need not be included in the risk assessment procedures at this moment. This also implies that the factor of 10 of the TGD is questionable. This factor is added to the PEC to account for uptake through food when equilibrium partitioning is applied to derive a PNEC.

Dynamic model
For earthworms, few kinetic bioaccumulation data are available. Generally, elimination in soil seems best described by a linear two-compartment model. It seems likely that also for uptake a two-compartment model is appropriate. It is, however, difficult to distinguish between one and two-phase uptake (especially on log scale). Since the first, rapid, phase is responsible for the main part of the body burden, a one-compartment simplification can be followed (Belfroid *et al.*, 1995a). At this moment, a fixed k_1 was defined which, in combination with the partition estimate for BCF, would yield a reasonable fit on the experimental k_2 data. This approach must be seen as provisional.

9.3. Evaluation of the models for crops and grass

The model advised by the TGD, the model of Trapp & Matthies (1995), was also selected as the most appropriate for this study.

Partition model
The root-water and air-leaf partition coefficient (including growth dilution) compare very well to measured data. The estimation routine for the transpiration-stream concentration factor (TSCF) is however questionable as there seems little correlation with *Kow*.

Parameters for the model
In the TGD, the same bulk density and volume fractions are given for roots and leaves. From the limited data gathered, it is clear that roots have different properties than leaves: less lipids, more water, and therefore a higher bulk density. The value for other parameters could not be evaluated: leaf area, leaf volume, conductance, transpiration, growth rate, correction exponent. Sufficient information for these parameters must be available in the agricultural literature and research institutes. These sources may be explored further, possibly making a distinction between different (types of) crops. Especially the properties of grass may be different from regular crops, specifically the leaf area in relation to volume and transpiration rates. Finally, the use of a growing period of 180 for leaf crops seems much too long. Based on the data of Kliment & Wagnerová (1995), a growing period for leafy crops between 50 days (lettuce) and 120 days (cabbage) is more appropriate. For root crops, a period of 140 days (potato) to 150 days (carrot) is more likely.

Dynamic model
The model settings of Trapp & Matthies (1995) were used. The route from porewater to leaves could not be tested against measured data.

9.4. Dynamics of the bioconcentration process

In Chapter 8, the behaviour of the dynamic models is tested to evaluate the potential consequences of assuming steady state in risk assessment. For most chemicals, the concentration in the organisms will closely follow the concentration in the environment. Only for larger values of *Kow* (for fish and earthworms) or $K_{leaf\text{-}air}$ (for plants) the bioaccumulation starts to lag behind the environmental concentrations: for fish log *Kow*>4, for earthworms log *Kow*>6 for plants log $K_{leaf\text{-}air}$>6. For these types of chemicals, the exposure pattern of the predators starts to differ from the emission pattern. This different pattern may also influence the toxic effect of the chemical but little general knowledge seems to be available on the effect of time-dependent exposure. Also, when interpreting measured data in fish, earthworms, and plants, the expected pattern of exposure in time must be taken into account.

Although affecting the pattern of exposure, the bioconcentration dynamics have little influence on the average concentrations in the organisms. Generally, the average concentration in a linear one-compartment model exposed to a varying external concentrations, is equal to the steady-state concentration in the organism when exposed to the average external concentration. In other words, the difference between a dynamic approach and the steady-state approach as proposed in the TGD is small. For earthworms, the difference is the largest since these animals are short-lived and the concentration in soil is averaged over 180 days instead of a whole year. These two causes make that the average concentration in earthworms can be lower than predicted from steady-state methods: up to a factor of two for chemicals with a log *Kow* of 8 or 9. Still, this error is small compared to the uncertainty in the estimate of BCF which is at least a factor of 10.

For fish and earthworms, different time periods are used to average the environmental concentrations: 365 and 180 days respectively. Since the type of predatory organisms is the same, these time periods should be harmonised to reflect a relevant time scale for chronic effects.

For grass, the scenario of using average concentrations in soil is appropriate as cattle can be expected to graze continuously. For crops which are harvested at a certain moment in time, however, this scenario is fundamentally wrong. The numerical consequences are, however, only evident when the dynamics of the soil concentration are rapid, i.e. at short half lifes of the chemical in soil (around 20 days). At a half life in soil of around 20 days, the TGD approach will underestimate levels in short-growing crops like lettuce by a factor of 2 for chemicals with a log $K_{leaf\text{-}air}$ >6. At the same half life in soil, longer-growing crops (like cabbages or root crops) will be seriously overestimated (factor 20 to 100) over most of the $K_{leaf\text{-}air}$ range since, at the time of harvest, the chemical has already disappeared from the soil and the crop. For chemicals with a short half life in soil (e.g. due to rapid biodegradation, volatilisation), results of the TGD approach must be interpreted with care. A better way would be to estimate the concentration in crops at each moment in time. It may be possible to solve this problem analytically since dynamic model simulation is in general too time consuming to be included in the TGD methodology.

Uptake from plants by air is a more difficult subject. Unlike sludge application, which starts in the beginning of the growing season, emission to air may take place at any time in the year. The TGD scenario will provide a kind of average approach, independent of harvest time or

averaging period. However, the shorter the emission episode, the larger the variation around this average.

Finally, the assumption of continuing exponential growth was examined. When the growth rate decreases well before harvest, an underestimation of the leaf concentration may occur. This underestimation will, however, only be profound when the half life in soil is not too low (larger than about 100 days) and when log $K_{leaf\text{-}air}$ is greater than 6.

In view of the difficulties with the TGD scenario for crops, care must be taken when this route is crucial and under the conditions given. It is possible to use the dynamic models to refine the risk assessment on a case-by-case basis. It may also be possible to make separate estimates for different types of crops. In that case, some literature research must be initiated to establish relevant model parameters en scenarios for different crops.

9.5. Further research

The scope of the indirect exposure assessment for humans and predators in the risk assessment procedure of the TGD is limited. Although important, worms are not the sole food source for terrestrial predators. Hard-bodied animals residing in the litter layer or on top of soil like beetles and woodlice have different routes of exposure. Animals may also feed on plant material, dead organic matter, or other organisms. Predators on the aquatic ecosystem do not only consume fish but may also prey on mussels, aquatic oligochaetes, etcetera. Although there is generally insufficient information on a chemical to assess al these possible endpoints, it may be possible to examine the importance semi-quantitatively. In this way, it may become clear if the selected food chains for secondary poisoning are sufficiently protective for the ecosystem. As an example, model calculation of Thomann (1989) indicate that exposure of top predators in a four-level food chain may be up to a factor of 10 higher than the first predator (log *Kow* around 6). Further literature research can be initiated to evaluate the safety of the two standard food chains.

For human indirect exposure assessment, although several food sources are taken into account, the assessment is still limited. Especially the role of the human exposure scenario is crucial in the assessment. In the TGD, a scenario is advised where all of the intake media are derived from the direct vicinity of the point source (local scale). A kind of worst-case EU consumption rate is assumed. This scenario is worst case, but is only used to indicate potential problems with a chemical. Whenever there are indications of a problem, more realistic scenarios can be applied. Several possible food sources are not taken into account in the TGD scenario but which may be investigated in detail:

1. Chicken meat and eggs. There is some information on the bioaccumulation in chicken which may be studied (e.g. in the CalTOX system).
2. Sea fish. The bulk of consumed fish is retrieved from the sea water environment and not from the fresh water. This may be accounted for in the exposure scenario.
3. Shellfish like mussels are not included. It is not investigated in this study if these organisms behave similar as fish.
4. Soil. Especially for small children, ingestion of soil can be major route. However, the TGD does not specifically perform an assessment for children.
5. The categories root and leaf crops may be specified further to different types of crops.

It should be kept in mind that the indirect exposure assessment is used to *indicate* potential problems with a chemical. There is no need for an in-depth assessment in the initial or screening stage of risk assessment. Therefore, it may not be necessary to include all possible sources of exposure in the assessment.

Several routes are proposed in the TGD but not discussed in this report:

1. Drinking water. The procedure for the purification of drinking water is not a bioaccumulation process and therefore not handled in this study. As the estimation method is rough and quite worst case, this is a candidate for future investigation (this route will, in several cases, turn out as very relevant for human exposure).
2. Inhalation of air. The estimation of concentrations in air is not a bioaccumulation process, but the result of an environmental distribution model.
3. Bioaccumulation by cattle. Previous study has indicated the considerable uncertainty of the regression for biotransfer to meat and milk (Jager & Slob, 1995). In view of its importance for very hydrophobic chemicals, this route is a prime candidate for further investigation. Since regressions or mechanistic models based on *Kow* do not seem to provide sufficient descriptive power, this route is not discussed in this study. For cattle, other approaches must be investigated. A physiologically-based pharmacokinetic (PB-PK) model would be the first choice (see e.g. Derks *et al.*, 1994). Other possibilities include the use of molecular-connectivity indices (an index derived from molecular structure) which looks promising (Dowdy *et al.*, 1996). This route will be further investigated in 1997.

Finally, it should be note that the estimation routines for BCFs or BAFs aim at a median estimate. This is seldom explicitly stated, but QSARs are usually taken as linear regressions on a log scale and therefore provide a median estimate. The scatter around this estimate can still be considerable. The best way to account for this scatter is to incorporate it into an uncertainty analysis. The possibilities to include uncertainty analysis in risk assessment will be studied in a project in 1997.

10. CONCLUSIONS AND RECOMMENDATIONS

Summarising, the following concrete recommendations can be made to update the methods as advised in the TGD:

1. For estimating BCF for fish, it is better to use the simple partition model with a maximum BCF at log *Kow*=6 (parameters given in Table *16*). Growth dilution is sufficient to describe this lack of linearity but this dynamic behaviour need not be included in the generic risk assessment methodology. The use of a polynomial QSAR is not recommended and may lead to serious underestimation of risk. Make a separate estimate for eel.
2. For estimating BCF for earthworms, the simple partition model performs much better than the QSAR advised in the TGD (this QSAR was based on poorly evaluated data and overestimates BCF by a factor of 4). The QSAR of Belfroid *et al.* (1993) would also provide a good estimate. Parameters for the theoretical model given in Table *16*.
3. The factor of 10 in the effects assessment for the terrestrial ecosystem in the TGD to account for ingestion needs to be reconsidered.
4. The model of Trapp & Matthies (1995) is appropriate for estimating concentrations in crops. Different properties for root and leaf tissue are advised in Table 16.
5. Care must be taken when log *Kow*>4, log *Kow*>6 and log $K_{leaf\text{-}air}$>6 because the concentration pattern in time of fish, earthworms and crops, respectively, starts to deviate from the external concentration pattern. This does not significantly affect average concentrations, but the different pattern can be important when more information on the toxicity mechanism of the chemical is known.
6. The 180-day average soil concentration is wrong for crops, but may be used when the following points are kept in mind:
   a) The TGD approach will overestimate concentration in root crops and long-growing leaf crops (e.g. cabbage) when the chemical's half life in soil is short (a factor of 1.5-2 when the half life is 100 days, a factor of 20-100 when the half life is 20 days).
   b) At short half lifes in soil (20 days) and log $K_{leaf\text{-}air}$>6, the TGD approach will underestimate levels in short-growing crops (e.g. lettuce) by a factor of 2.
   c) When the half life in soil is larger than 100 days and log $K_{leaf\text{-}air}$>6, the assumption of continuous growth becomes crucial for long-growing crops and it is better to use actual time-related growth information.

   In these cases, dynamic simulation can give more insight
7. Regarding uptake by plants from air: for crops which are harvested, the timing of the emission episode in relation to the time of harvest is crucial (the shorter the emission episode, the larger the variation). The TGD approach gives an average.
8. BCF estimates are median case. The uncertainty should be included in uncertainty analysis.

Proposals for further research:

1. Investigate the other parameter values for plants: area-volume ratios, transpiration stream, growth rate, leaf conductance, and possibly, their dependence on time. Maybe it is possible to differentiate between different (types of) crops and different harvest times. It may be investigated if behaviour of root crops is similar to roots. Grass can be specified separately.
2. Investigate if the current one-step food chain is sufficiently protective for the ecosystem. Maybe it is necessary to include different or more elaborate food chains.
3. Investigate more appropriate estimation routines for cattle meat and milk (addressed in 1997).
4. Investigate the appropriateness of the human exposure scenario.

5. Investigate the possibilities/necessity to include other human intake media for humans: soil ingestion, chicken meat and eggs, sea fish, shellfish.

Table 16 Proposed parameters of the partition model for estimating BCFs.

Parameter	Symbol	Value	Unit
Fish general			
Volume fraction fat	F_{fat}	0.03	$m^3.m^{-3}$
Volume fraction water	F_{water}	0.80	$m^3.m^{-3}$
Bulk density of fish	ρ_{fish}	1000	$kg.m^{-3}$
Remarks	No further increase in BCF expected for log *Kow*>6		
Eel			
Volume fraction fat	F_{fat}	0.24	$m^3.m^{-3}$
Volume fraction water	F_{water}	0.62	$m^3.m^{-3}$
Remarks	This species is much fatter than other species		
Earthworms			
Volume fraction fat	F_{fat}	0.01	$m^3.m^{-3}$
Volume fraction water	F_{water}	0.84	$m^3.m^{-3}$
Bulk density of earthworms	ρ_{worm}	1000	$kg.m^{-3}$
Remarks	This approach may be used up to a log *Kow* of 8, above that point, it is unclear what happens		
Plant leaves			
Volume fraction fat	F_{fat}	0.01	$m^3.m^{-3}$
Volume fraction water	F_{water}	0.65	$m^3.m^{-3}$
Volume fraction air	F_{air}	0.3	$m^3.m^{-3}$
Bulk density of tissue	ρ_{leaf}	800	$kg.m^{-3}$
Remarks	Growth dilution accounted for in TGD approach		
Plant roots			
Volume fraction fat	F_{fat}	0.005	$m^3.m^{-3}$
Volume fraction water	F_{water}	0.93	$m^3.m^{-3}$
Bulk density of tissue	ρ_{root}	1000	$kg.m^{-3}$

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