Environment Agency

Evidence

Estimation of fish bioconcentration factor (BCF) from depuration data

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Miranda Kavanagh

Director of Evidence

Executive summary

Bioaccumulation – the accumulation of chemicals and other pollutants in living organisms - is an important information requirement for chemicals risk assessment and for regulatory regimes such as REACH, which regulates chemicals in the EU. The most widely used test guideline for measuring bioaccumulation in fish is the Organisation for Economic Cooperation and Development (OECD) 305 Test Guideline. This test guideline is currently being revised and should, in the future, include a method recommended for poorly water soluble chemicals which cannot be tested by aqueous exposure. This new method involves exposing organisms to the test chemical via the diet (in a dietary study) and so results in a biomagnification factor (BMF) rather than a bioconcentration factor (BCF, as is derived from the aqueous exposure method).

In several regulatory regimes, including REACH in the EU, the criteria for a chemical being categorised as bioaccumulative (B) or very bioaccumulative (vB) are based on BCF and not BMF. In addition, risk assessment requires a BCF (and in some cases also a BMF) to estimate concentrations in prey for the investigation of risks from secondary poisoning. BMFs obtained from the new dietary study could be used to demonstrate qualitatively that a chemical is not taken up (and so does not meet the criteria for B or vB) or, in other cases, indicate that a chemical would be likely to meet the B or vB criteria. However, as many of the chemicals that will be tested with the new method are likely to be B or vB candidates, being able to estimate a BCF from the data generated in the dietary study would be a great advantage and meet an accepted regulatory need.

The information that is generated by the dietary study includes the depuration rate constant (both growth-corrected and non-corrected), that is, the rate at which the organism rids itself of the chemical. In order to use these data to estimate a BCF an estimate of the uptake rate constant is needed, which would then allow the kinetic BCF (the ratio of the uptake rate constant to the depuration rate constant) to be calculated.

A mathematical relationship has been derived in the literature relating fish size (weight) to the uptake rate constant at the gill for hydrophobic (poorly water soluble) chemicals and this forms the basis of the approach currently proposed in the new draft OECD 305 test guideline. This report considers further the allometric equation/method that may be proposed in the draft OECD 305 Test Guideline and reviews alternative approaches that are available. The various approaches are tested in this report using a database of valid bioconcentration data and the following conclusions and recommendations are drawn from this analysis.

The analysis identifies a number of approaches that could be used to estimate the uptake rate constant, and these are listed below.

- Hayton and Barron (1990).
- Erickson and McKim (1990a).
- Barber *et al.* (1991).
- Barber (2003).
- Barber (2001).
- Streit and Sire (1993).
- Erickson and McKim (1990b).
- Hendriks *et al*. (2001).

- Tolls and Sijm (1995).
- Sijm *et al*. (1995).
- Spacie and Hamelink (1982).
- Thomann (1989).

These methods were tested over the approximate log K_{ow} range between 3.5 and 8.2. When using these methods, there will be uncertainty in the resulting prediction for any given substance as described in this report, and this uncertainty in the predicted k_1 (rate constant for uptake into fish) should be taken into account when considering the use of any method(s), for example within the draft updated OECD 305 Test Guideline.

For some applications the uncertainty in the predictions may be acceptable, for example if an estimate of a k_1 and hence BCF is needed for modelling purposes or to show that the BCF is well below or well above a regulatory criteria value. However, in other cases the uncertainty in the predicted k_1 and BCF may be more problematic, for example where the prediction leads to a BCF value that is close to (either above or below) a regulatory criteria value.

Suggested areas for further work to reduce uncertainty in the predicted values are given in the report.

This work also identifies a number of issues around analysis of actual BCF data:

- For some substances no uptake is seen in bioconcentration studies. It is not always clear whether this results from an actual very low k_1 value or from methodological limitations in the study.

- A fish weight needs to be assumed for many of the methods used to estimate k_1. The choice of weight needs to be considered carefully, in particular if the resulting BCF value is to be compared with regulatory criteria.

- The lipid normalisation of the BCF value also needs to be considered carefully, particularly for substances that are rapidly metabolised or where depuration by growth dilution is significant.

- For the examples considered here, the uptake rate did not appear to follow strict first-order kinetics. This has been considered in relation to the growth of the fish and other possible factors, and a tentative approach for analysing such data is outlined. However, this has been tested so far with only a limited amount of data, and the mechanistic interpretation of the approach is currently unclear. Further work is recommended to clarify the general applicability of the approach. If such work demonstrates that this is a real effect rather than an experimental artefact, consideration should be given to including such an approach to the analysis of bioconcentration data in the appropriate test guideline (OECD 305).

In the meantime it would be prudent to check for deviations from first-order behaviour when analysing bioconcentration data. A suggested approach for this is included in this report.

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Contents

1 Introduction

Bioaccumulation – the accumulation of chemicals and other pollutants in living organisms - is an important information requirement for chemicals risk assessment and for regulatory regimes such as REACH[1]. Under REACH, bioaccumulation testing may in theory be triggered for more than 3,000 chemicals based on supply tonnages and partition coefficient cut-off values. In addition, specific *in vivo* bioaccumulation testing may be triggered for substances which meet the PBT (persistent, bioaccumulative and toxic) or vPvB (very persistent and very bioaccumulative) screening criteria.

The most widely used test guideline for measuring bioaccumulation in fish is the Organisation for Economic Cooperation and Development (OECD) 305 test guideline (OECD 1996). This test guideline is currently being revised and should, in the future, include a method recommended for poorly water soluble chemicals which cannot be tested by aqueous exposure. This new method involves exposing organisms to the test chemical via the diet (in a dietary study) and so results in a biomagnification factor (BMF) rather than a bioconcentration factor (BCF, as is derived from the aqueous exposure method). The dietary study also gives a depuration rate constant (rate at which the organism rids itself of the chemical) and assimilation efficiency (efficiency with which the chemical is taken up from food). In several regulatory regimes, including REACH in the EU, the criteria for a chemical being categorised as bioaccumulative (B) or very bioaccumulative (vB) are based on BCF and not BMF. In addition, risk assessment requires a BCF (and in some cases also a BMF) to estimate concentrations in prey for the investigation of risks from secondary poisoning. BMFs obtained from the new dietary study could be used to demonstrate qualitatively that a chemical is not taken up (and so does not meet the criteria for B or vB) or, in other cases, indicate that a chemical would be likely to meet the B or vB criteria. However, as many of the chemicals that will be tested with the new method are likely to be B or vB candidates, being able to estimate a BCF from the data generated in the dietary study would be a great advantage and meet an accepted regulatory need.

The information that is generated by the dietary study includes the following:

- depuration rate constant (both growth-corrected and non-corrected);
- assimilation efficiency;
- fish growth data.

In order to use these data to estimate a BCF an estimate of the uptake rate constant is needed. This can then be used in conjunction with the depuration rate constant to estimate the kinetic BCF (ratio of uptake rate constant to depuration rate constant).

A mathematical relationship has been derived in the literature relating fish size (weight) to the uptake rate constant at the gill for hydrophobic (poorly water soluble) chemicals (Sijm *et al*. 1993, 1994 and 1995) and this forms the basis of the approach proposed in the current iteration of the draft revised OECD 305 Test Guideline[2]. This allometric relationship was used here to estimate BCFs from studies conducted according to the new dietary exposure method. Weight data collected at the end of the uptake

[1] Regulation (EC) No 1907/2006 of the European parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.

[2] Broadly the same methodology is also in the REACH Guidance Document (ECHA 2008).

phase/start of the depuration phase was used to estimate an uptake rate constant according to the literature equation. This, together with the chemical's growth-corrected depuration rate constant obtained from the dietary accumulation study, was used to estimate a kinetic BCF. However, other methods are available to estimate bioconcentration data from the depuration data generated in the dietary study (such as fugacity models and octanol-water partition coefficient driven mass balance models).

This report considers further the allometric equation/method proposed in the current version of the draft OECD 305 Test Guideline and reviews alternative approaches that are available. The various approaches are tested here using a database of valid bioconcentration data. Finally, recommendations are given on the most appropriate methods to estimate a BCF value from the data generated in the dietary study, as proposed in the new OECD 305 Test Guideline.

2 Background to bioconcentration and bioaccumulation

2.1 Definitions

The various factors that are determined in laboratory and field studies are defined in Table 2.1. The definitions are based on the recent publication by Gobas *et al*. (2009).

Table 2.1 Definitions of accumulation factors

Factor	Definition
Bioconcentration factor (BCF)	Ratio of the steady-state chemical concentrations in an aquatic water-respiring organism and the water determined in a controlled laboratory experiment in which the test organisms are exposed to a chemical in the water (but not in the diet).
Bioaccumulation factor (BAF)	Ratio of the steady-state chemical concentrations in an aquatic water-respiring organism and the water determined from field data in which sampled organisms are exposed to a chemical in the water and in their diet.
Biomagnification factor – laboratory based (BMF_{food})	Ratio of the steady-state chemical concentrations in a water- or air-respiring organism and in the diet of the organism determined in a controlled laboratory experiment in which the test organisms are exposed to chemical in the diet (but not the water or air).
Biomagnification factor – field based (BMF)	Ratio of the steady-state chemical concentrations in a water- or air-respiring organism and in the diet of the organism determined from field data in which sampled organisms are exposed to chemical in air, water and diet.
Trophic magnification factor (TMF) or food web magnification factor (FWMF)	The average factor by which the normalized chemical concentration in biota or a food web increases per trophic level. The TMF is determined from the slope derived by linear regression of logarithmically transformed normalised chemical concentration in biota and trophic position of the sample biota.

A BMF determined from a laboratory feeding study (where exposure is via the diet alone) is NOT the same as a BMF determined in a field study (where, for aquatic organisms, exposure will be via both diet and water). For this reason, the BMF derived from a feeding study will be designated as BMF_{food} in this report. It is important to bear in mind this distinction when using such BMF values.

2.2 Theoretical considerations

2.2.1 Kinetic approach

In laboratory studies it is possible to determine both a BCF and BMF_{food} for fish on a kinetic basis.

2.2.1.1 BCF

The BCF is usually determined at steady state as follows:

Equation 1

$$BCF_{fish} = \frac{\text{Concentration in fish at steady state (mg kg}^{-1}\text{ wet fish)}}{\text{Concentration in water at steady state (mg l}^{-1}\text{)}}$$

In this case the BCF will have units of l kg^{-1} wet weight. A lipid normalised BCF can similarly be defined if the concentrations in fish are determined on a mg kg^{-1} lipid basis.

The BCF can also be determined on the basis of the rates of uptake and depuration (assuming that both processes are first order).

Equation 2

$$\text{Rate of uptake} = k_1 \times [C_{water}]$$

Equation 3

$$\text{Rate of depuration} = k_2 \times [C_{fish}]$$

Where k_1 = First-order rate constant for uptake into fish (day^{-1}).

k_2 = First-order rate constant for depuration/elimination from fish (day^{-1}).

$[C_{water}]$ = Concentration in water (mg l^{-1}).

$[C_{fish}]$ = Concentration in fish (mg kg^{-1} wet weight).

At steady state the rate of uptake = the rate of depuration, and so combining gives the following relationship:

Equation 4

$$BCF = \frac{[C_{fish}] \text{ at steady state (mg kg}^{-1}\text{ wet weight)}}{[C_{water}] \text{ at steady state (mg l}^{-1}\text{)}} = \frac{k_1}{k_2}$$

The ratio of k_1/k_2 is known as the kinetic BCF and should be equal to the steady state BCF obtained by the ratio of the steady-state concentration in fish to that in water if first-order kinetics are followed.

2.2.1.2 BMF_{food}

Similar to the case with the BCF above, a BMF_{food} can be defined as follows:

Equation 5

$$BMF_{food} = \frac{[C_{fish}] \text{ at steady state}}{[C_{food}] \text{ at steady state}}$$

Where BMF_{food} = Biomagnification factor.

$[C_{fish}]$ = Concentration in fish (mg kg^{-1} wet weight).

$[C_{food}]$ = Concentration in food (usually mg kg^{-1} dry weight).

Again, the BMF_{food} can also be determined on a lipid normalised basis using the lipid normalised concentrations in fish and food.

Similar to the BCF, the BMF_{food} can equally be determined on the basis of the rates of uptake and depuration (assuming that both processes are first order).

Equation 6 $$\text{Rate of uptake} = FR \times \alpha \times [C_{food}]$$

Equation 7 $$\text{Rate of depuration} = k_2 \times [C_{fish}]$$

Where FR = Daily feeding rate (g food g^{-1} body weight).

α = Assimilation efficiency expressed as a fraction - this is effectively the fraction of the dose that is absorbed through the gut.

k_2 = First-order rate constant for depuration/elimination from fish (day^{-1}).

$[C_{fish}]$ = Concentration in fish (mg kg^{-1} wet weight).

$[C_{food}]$ = Concentration in food (usually mg kg^{-1} dry weight).

At steady state, the rate of uptake = the rate of depuration giving the following relationship:

Equation 8 $$BMF_{food} = \frac{[C_{fish}] \text{ at steady state}}{[C_{food}] \text{ at steady state}} = \frac{FR \times \alpha}{k_2}$$

In this case the kinetic BMF is represented by the term $FR \times \alpha / k_2$. It is also possible to determine the BMF_{food} on a lipid normalised basis[3].

Equation 8 implies that the BMF_{food} obtained in a fish feeding study is proportional to the feeding rate used. This may have important consequences when comparing BMF_{food} data from studies where different feeding rates have been used. The significance of the feeding rate to the BMF obtained is considered further in 2.2.2.

2.2.2 Important variables in determining the BMF_{food}

Although the focus of this report is on the determination of a BCF from the data obtained in a fish feeding study, it is relevant to consider some of the variables that may affect the BMF_{food} obtained in a feeding study. These are considered below.

Using a conceptualised model of a fish, the equilibrium between a fish and food and water has been expressed in terms of fugacity[4] as follows (Mackay 1991, Clarke and Mackay 1991):

Equation 9 $$V_F Z_F \frac{df_F}{dt} = D_V(f_W - f_F) - D_M f_F - D_G f_F + E_o D_A f_A - \frac{E_o D_A f_F}{Q}$$

Where V_F = Volume of fish (m^3).

Z_F = Fugacity capacity of fish (mol m^{-3} Pa^{-1}).

f_W = Fugacity of chemical in water phase (Pa).

f_F = Fugacity of chemical in fish (Pa).

f_A = Fugacity of chemical in food (Pa).

[3] In this case the concentrations in fish and food are determined in units of mg kg^{-1} lipid and the feeding rate (FR) is determined in terms of g lipid food g^{-1} lipid fish.

[4] Here fugacity is the leaving tendency of a substance from a compartment, see Glossary.

D_V = D-Value (transport parameter) for gill ventilation (mol Pa^{-1} h^{-1}).

D_M = D-Value for metabolism (mol Pa^{-1} h^{-1}).

D_G = D-value for growth dilution (mol Pa^{-1} h^{-1}).

D_A = D-value for food consumption (mol Pa^{-1} h^{-1}) = food ingestion rate. (m^3 h^{-1}) × fugacity capacity (Z_A) for food (mol m^{-3} Pa^{-1}).

E_o = Uptake efficiency (or assimilation efficiency – α) from food.

Q = a factor equivalent to the maximum BMF.

In Equation 9 the term $E_oD_Af_F/Q$ represents the egestion (excretion in faeces) of chemical in the undigested food. At steady state the following equation applies:

Equation 10

$$f_F = \frac{(D_V f_W + E_o D_A f_A)}{(D_V + D_M + D_G + \frac{E_o D_A}{Q})}$$

If it is assumed that both the fish and food are in equilibrium with the water ($f_A = f_W$), the value of f_F can only approach that of f_W when the D_v term dominates both the numerator and denominator of Equation 10. When the value of the log K_{ow} is large (for example, around 6 and above) the D_A term will tend to dominate over the D_v term and so the uptake from food becomes important, and the fish fugacity approaches a value of $Q \times f_A$ (or $Q \times f_W$), that is, the fugacity in the fish is greater than that in either the food or water and hence biomagnification can be said to be occurring (Mackay 1991). In this case Q represents the maximum biomagnification factor.

It has been hypothesised (Connolly and Pedersen 1988; Gobas and Morrison 2002; Gobas *et al*. 1988) that the increase in fugacity in the fish over that in the water (or food) results from a fugacity gradient in the gut of the organism as the food is digested.

Clarke and Mackay (1991) found that, although the assimilation efficiency at a constant feeding rate was approximately constant for different concentrations in food, an increase in feeding rate led to a less than proportional increase in uptake (or an apparent decrease in the absorption/assimilation efficiency) of four organochlorine chemicals in guppies (*Poecilia reticulata*) (Equation 9 predicts that the uptake should be directly proportional to the feeding rate). Further, experiments were carried out to show that when the same total amount of chemical was fed to the fish but at two different feeding rates (one group received food containing twice the concentration of the substance, but at half the feeding rate, compared to the other group) the fugacity of the chemical in the fish was higher in the fish fed at the lower feeding rate (higher concentration in food) than at the higher feeding rate. This apparent decrease in the assimilation efficiency was explained in terms of an increased rate of loss of the substance from the system by egestion: as the feeding rate (amount of food consumed) increased, a larger proportion of the food remained undigested and was eliminated from the fish (see Section 2.2.3). This can be seen from Equation 10.

For example, if two fish are fed the same concentration in food (f_A is the same for both fish) but at different rates (D_A differs), then the concentration/fugacity in the fish (f_F) will tend to be higher in the fish fed at the higher feeding rate, but the increase will be less than linear. Similarly, if two fish are fed the same total daily quantity of a chemical (f_AD_A is the same for both fish) but using different combinations of concentration and feeding rate, the fish fed at the higher feeding rate D_A (using a corresponding lower concentration in food f_A) will achieve a lower overall fugacity/concentration in the fish (f_F). In both cases the fish appear to be absorbing less substance (an apparent lower assimilation efficiency) at the higher feeding rate, but in actual fact this is due to an increased depuration rate in faeces. Clarke and Mackay (1991) concluded that the

uptake of chemical by fish from food is controlled not only by the amount of chemical the fish ingests (as is predicted in kinetic models), but also by the amount of food it digests (the passage of food through the gastrointestinal tract was considered to be an important elimination process that can be increased by the ingestion of more food which in turn increases the faecal egestion rate).

Gobas *et al*. (1993) studied the uptake by fish (*Carassius auratus*) from food of several organochlorine compounds. The study used foods of various lipid contents to investigate the processes involved in uptake from the gut. The authors concluded that intestinal absorption of the chemicals was predominantly controlled by chemical diffusion rather than by co-transport with the lipids and indicated that the important factors that determined if a chemical biomagnified were the following:

- the feeding and faecal egestion rates of the organisms;
- the chemical's partitioning between the gastrointestinal contents and the organism;
- the rate of chemical elimination through routes other than faecal egestion (such as via gills and metabolic transformation), relative to the rate of chemical elimination in the faeces.

The Gobas *et al*. (1993) study found that the assimilation efficiency appeared to decrease with increasing lipid content of the food and thought that this was related to the decreasing food digestibility (as evidenced by the increasing faecal egestion rate) as the lipid content of the food increased (food digestibility was found to decrease from 76 per cent in the food below 0.2 per cent lipid, to 70 per cent in the food with 6.3 per cent lipid, to 60 per cent in food with 13.5 per cent lipid, based on the faecal egestion rates of 3.1, 3.9 and 5.2 mg feces $fish^{-1}$ for the three foods respectively).

Gobas *et al*. (1988) suggested that in fish in general, food is reduced in the gastro-intestinal tract to around one-third of its initial volume. This process is thought to cause the chemical fugacity and concentration within the gastro-intestinal contents to increase over that in the original food, which provides the driving force for subsequent uptake into the organism. Gobas *et al*. (1988) also speculated that a further increase in the chemical's fugacity in the gastro-intestinal tract may occur as a result of the digestion processes (for example hydrolysis of lipids), which may lower the affinity of the gastro-intestinal contents for the chemical.

A later study by Gobas *et al*. (1999) confirmed that food digestibility and food absorption are critical factors that control the assimilation efficiencies and food accumulation factors under both laboratory and field conditions, and found indications that components other than lipids may contribute significantly to the fugacity capacity of the food (such as carbohydrates, proteins, fibres and other non-lipid organic matter).

The importance of feeding rate was also studied by Fisher *et al*. (1986). In this study spot (*Leiostomus xanthurus*) were fed grass shrimp containing ^{14}C-labelled kepone at a rate of four, eight or 20 per cent based on the average weight of the fish. In contrast to the work of Clarke and Mackay (1991), the authors found that a doubling of the feeding rate resulted in an approximate doubling of the whole body concentration of kepone in the fish. The authors also indicated that some studies investigating the importance of dietary exposure may have been unconsciously biased by the use of small rations to feed the test organisms. One other difference between the results of Fisher *et al*. (1986) and those of Clarke and Mackay (1991) and Gobas *et al*. (1993) is that the first used natural food (grass shrimp) while the others used proprietary fish foods, and it is possible that the digestibility (and hence faecal excretion rate) of natural food does not depend as much on the feeding rate as proprietary fish food appears to do.

The effect of food concentration on the assimilation efficiency of two polychlorinated biphenyls (2,2',3,3',5,5'-hexachlorobiphenyl and 2,2',3,3',4,4',6,6'-octachlorobiphenyl) in fish (*Poecilia reticulata*) was studied by Opperhuizen and Schrap (1988) over periods up to 250 days. The study used five dietary concentrations ranging from 7.1 μg g^{-1} to 1,400 μg g^{-1} at a feeding rate of 0.02 g g^{-1} day^{-1}. The assimilation efficiency was found to be relatively constant for both substances for food concentrations in the range 7.1 to 150 μg g^{-1} (values around 50 per cent for both substances), but was found to reduce to around 25 per cent at the two higher food concentrations used. However, toxic effects (both sublethal and lethal) were also seen at the two highest concentrations tested and so this could have adversely affected the feeding rate in these experiments (and hence affected the calculated assimilation efficiency). Based on the experiments at the lower concentrations, it is apparent that, at least at low concentrations, the assimilation efficiency is relatively independent of the food concentration.

Similarly, Muir *et al*. (1990) found the assimilation efficiency of 2,3,4,7,8-pentachlorodibenzofuran in juvenile fish (*Oncorhynchus mykiss*) was independent of the food concentration over an eleven-fold range. These findings are consistent with the uptake from food being a kinetically first-order process.

In conclusion, the assimilation efficiency (and hence BMF_{food}) appears to be independent of the chemical's concentration in food. However, high concentrations of some substances in the feed used in laboratory studies could result in reduced feeding or food avoidance in the exposed organism due to the toxic or taste effects of the chemical, reducing the actual exposure during the test.

With regard to the feeding rate, there is both theoretical and experimental evidence that the feeding rate (at least when using proprietary fish food) can affect the overall digestibility of the food, and this is an important factor to consider in feeding studies as this can affect the overall accumulation seen. This therefore means that the BMF_{food} obtained from such studies may be dependent on the experimental conditions used and thus may present problems in extrapolating the BMF_{food} obtained under laboratory conditions to a BMF for organisms in the wild.

2.2.3 Elimination processes and growth dilution

In Section 2.2.1 the elimination of the chemical from the fish is considered to be a single process following first-order kinetics. In reality, a number of elimination processes can occur. Bioconcentration and biomagnification models generally consider the following elimination processes (Gobas and Morrison 2002):

- respiratory elimination via the gills - k_r;
- metabolic transformation - k_m;
- growth dilution - k_g;
- elimination via faeces – k_e.

Although growth dilution does not result in loss of mass of the substance from the fish, it can lead to lowering of the actual concentration present in the fish and so is often considered alongside removal mechanisms for the purpose of the model development.

Most experimental uptake studies (be they from water or food) would usually just determine the overall elimination rate constant from the fish (usually designated k_2), but the value k_2 includes contributions from all of the above processes as follows (all of the processes are usually assumed to follow first-order kinetics in relation to the concentration in the fish, hence the rate constants are additive).

Equation 11 $$k_2 = k_r + k_m + k_g + k_e$$

Where k_2 = Overall elimination/depuration rate constant (day^{-1}).

k_r = Rate constant for respiratory elimination via the gills (day^{-1}).

k_m= Rate constant for metabolic transformation.

k_g = Rate constant for growth dilution (day^{-1}).

k_e = Rate constant for elimination via faeces (day^{-1}).

When estimating the BCF from fish feeding data, an assumption inherent in the approach is that the rate of elimination of the substance from fish is independent of the exposure route, that is, the k_2 value obtained from a fish feeding study would be equally applicable to fish exposed via water only. This assumption is also inherent in many bioaccumulation models. Some studies (such as Fisher *et al*. 1986) have shown small differences between the rates of elimination estimated for uptake from water compared with uptake from food, but these differences may be due to experimental variability rather than a true difference. However, it is important to be aware of this assumption, and that it may not always be applicable.

It is not always possible to distinguish between individual elimination processes in a bioconcentration study or fish feeding study (generally it is possible to account for growth dilution only; see Section 5.3). However, distinguishing between these processes becomes important when considering whether a substance has the potential to biomagnify (increasing concentrations with increasing trophic level) through the food chain.

The rate of egestion of the unabsorbed chemical in the undigested food in faeces (rate constant k_e) is thought to be an important consideration in determining if a chemical biomagnifies. Gobas and Morrison (2002) indicate that the concentration in the organism exceeds that in the food (a BMF_{food} above one) in cases where the combined rate constants for elimination via the respiratory surface area (gills), metabolic transformation and growth dilution are small compared with the rate constant for faecal egestion ($k_r + k_m + k_g < k_e$). The authors state that this usually occurs for substances with a log K_{ow} between 5 and 7.5 (unless they are being metabolized at a significant rate) and those with log K_{ow} above 7.5 (which are assumed to be metabolized slowly).

Another important consideration is that in laboratory feeding studies, it is not usually possible to determine the assimilation efficiency independently of the elimination rate constant (Sijm *et al*. 1992). One direct consequence of this is that lower estimates of the elimination rate constant also result in a lower estimate of the uptake efficiency. This has consequences for the applicability of assimilation efficiency data between fish of different ages, as the elimination (by growth dilution or metabolism) may change with age (for example Sijm *et al*. (1992) found that elimination rate constants were generally higher in small fish (under five grams) than in large fish (over five grams). Sijm *et al*. (1992) also reported that as growth dilution is usually higher in fish early lifestages, and uptake may be lower, accumulation from food is usually less in early lifestages than in older fish; they also indicated that the BMF_{food} for growing fish is highly dependent on the growth rate.

Growth dilution, and its possible consequences, is considered further in Section 5.

2.2.4 Species and lifestage differences

Species-specific factors are likely to be important in determining the uptake of chemicals from food and water, and also for estimating a BCF from the data obtained

in a feeding study. For example fish of different species and lifestages will grow at different rates, may have different metabolic capacities, may feed at different rates and on different food sources in the environment, may digest food at different rates, may respire at different rates and so on.

Opperhuizen (1990) postulated that the rate constant for the uptake of a substance from water (bioconcentration) would depend on the volume of water passing over the gills (V_w), the efficiency of uptake of the chemical via gills from water and the concentration in water, and indicated that a typical value for V_w would be 2,000 ml g^{-1} day^{-1} for a small fish, whereas the volume would be considerably lower for larger fish, possibly by a factor of 10, resulting in a lower rate of uptake from water. In contrast, the feeding rates for small and large fish do not appear to vary by such a large amount. Therefore, if the overall rate of elimination of a substance is similar in small fish and large fish, the contribution from uptake via food may become more important relative to that from water as the size of the fish increases. It is also important to remember that elimination by respiration from the fish can also occur and this may be different in larger fish than for smaller fish.

Overall, many species-related factors can affect the size of the BMF_{food} obtained in a feeding study and the estimated BCF. This means that a BMF_{food} (and associated kinetic data) obtained with one species/lifestage of fish (for example a relatively fast growing early lifestage) may not be applicable to another species/lifestage (such as a slower growing mature fish). This is particularly important when considering growth correction of data as discussed in Section 5.

2.3 Considerations for estimating a BCF from a fish feeding study

The fish feeding study will generate a number of parameters that represent the accumulation of the substance by the organism. These include the following:

- BMF_{food};
- assimilation efficiency (α);
- overall depuration rate constant (k_2).

Many species and experimental factors can affect the values of BMF_{food} and the assimilation efficiency and this makes these factors less appropriate as a basis for estimating the BCF than the k_2 value obtained.

The k_2 value obtained can, theoretically, be used directly to estimate a BCF provided a value for the uptake rate constant (k_1) can be estimated. Methods for estimating k_1 are considered below in this report. The main assumption inherent in this read-across approach is that the k_2 value obtained in the dietary study is also applicable to fish exposed via water alone (the same elimination processes are occurring). For some substances, faecal elimination may be an important elimination process and this may not necessarily be the same in fish exposed via water alone compared with fish exposed via food.

3 Description of methods to predict BCF using data from a dietary study

The models and methods described here were identified from the following sources:

- A previous review of bioaccumulation models for use in environmental standards carried out for the Environment Agency (Brooke and Crookes 2007).
- A search of the more recently published literature using PubMed[5].

The focus of the search was on methods for estimating the uptake rate constant (k_1) from water exposure alone.

3.1 Sijm *et al*. (1995) method

This method is described in Sijm *et al*. (1993, 1994 and 1995) and is included in the draft OECD 305 test method. The approach uses the following allometric equation to estimate the uptake rate constant for hydrophobic chemicals from the weight of the fish:

Equation 12 $$k_1 = 520 \times W^{-0.32}$$

Where k_1 = Uptake rate constant (l kg^{-1} day^{-1}).

W = Fish weight (g).

This equation was derived using a total of 29 data points and the allometric equation had a correlation coefficient (r^2) of 0.85.

The chemicals used to derive Equation 12 are summarised in

Table 3.1. The rate constant data used in the equation were derived from gill perfusion studies using rainbow trout (*Oncorhynchus mykiss*) or from *in vivo* studies in guppy (*Poecilia reticulate*).

The studies with isolated perfused gills were carried out at 12ºC using fish of average weight 54 g or 109 g. The gill perfusate rate of artificial blood (pH 7.8) through the gills used in the study was two ml per 100 g fish per minute (equivalent to 28.8 l kg^{-1} day^{-1}; a value similar to the normal gill perfusate rate in fish). Water (pH 6.8-7.0) containing mixtures of the substances tested was passed over the gills at a rate of 1.0 l min^{-1}.

The test solutions were prepared (with the exception of phenol and tetrachloroveratrole) using a generator column. Phenol was dissolved directly in the water and tetrachloroveratrole was added to the glass wall of a flask (as a solution in ethanol) prior to collection of the water from the generator column. In all, four series of experiments were carried out.

[5] See http://www.ncbi.nlm.nih.gov/sites/entrez/

Table 3.1 Substances used to derive allometric equation for uptake rate constant (Sijm *et al*. 1995)

Substance	Purity	Log K_{ow}[1]	Uptake rate constant (l kg^{-1} day^{-1})	
			Perfused gills – rainbow trout[2, 3]	Guppy – *in vivo*[4]
Phenol (^{14}C-uniformly ring labelled)	>99%	1.8	49±5 [a]	-
Anthracene	>99%	4.7	134±40 [d]	1,841
1,2,3,4-Tetrachlorobenzene	>99%	4.6	130±20 [b]	810
Pentachlorobenzene	>99%	5.2	147±25 [b]	1,200
			62±12 [c]	1,490
			75±49 [d]	1,000
Hexachlorobenzene	>99%	5.7	271±105 [a]	540
			142±26 [b]	
Hexabromobenzene	>99%	7.8	300±156 [a]	<1
2,2',5,5'-Tetrachlorobiphenyl	98.2%	6.1	208±63 [a]	1,100
			102±19 [b]	
Decachlorobiphenyl	95.4%	8.3	nd [a]	600
2,3,5-Trichloroanisole	>99%	3.9	57±12 [c]	1,480
2,3,6-Trichloroanisole	>99%	3.6	49±13 [c]	1,610
2,3,4,5-Tetrachloroanisole	>99%	4.5	134±33 [c]	940
Pentachloroanisole	>99%	5.5	93±6 [c]	1,710
Octachloronaphthalene	>99%	8.5	nd [a]	<1
Octachlorodibenzo-*p*-dioxin	>99%	8.5	nd [a]	<1
Tetrachloroveratrole (^{13}H-labelled on the methyl group)	>99%	4.7	295±71 [a]	-

[1]Log K_{ow} values are those reported in the Sijm *et al*. (1995) paper.
[2]Values are given as mean ± standard deviation. nd = not determined (no uptake was evident).
[3]Four series were carried out with the perfused gills. [a] – First series of experiments with 54 g fish. [b] – Second series of experiments with109 g fish. [c] – Third series of experiments with 109 g fish. [d] – Fourth series of experiments with 109 g fish.
[4]Guppy weight was 0.1 g. The Guppy data are taken from Opperhuizen (1986), Opperhuizen and Voors (1987) and de Voogt (1990).

At intervals during the test (the test was carried out for between 60 and 90 minutes) samples of perfusate and water were taken and analysed for each substance. The uptake rate constant was then estimated from these data using Equation 13.

Equation 13 $$k_1 = \frac{C_p}{C_w} \times F$$

Where k_1 = Uptake rate constant (l kg^{-1} day^{-1}).

C_P = Concentration in perfusate (µg l^{-1}).

C_w = Concentration in water (µg l^{-1}).

F = Perfusate flow-rate through the gills (l kg^{-1} day^{-1}).

The uptake rate constants obtained in the gill perfusion experiments are summarised in

Table 3.1.

The uptake rate constants obtained *in vivo* in guppy were taken from experiments carried out by Opperhuizen (1986), Opperhuizen and Voors (1987) and de Voogt (1990). The weight of the fish used in these experiments was around 0.1 g[6].

The Opperhuizen (1986) study used one-year-old male guppy with a mean lipid content of 5.1 per cent. The method used was a flow-through test system whereby two groups of nine fish were exposed to water containing the test substances (2,5-dichlorobiphenyl, 2,2',5,5'-tetrachlorobiphenyl, 2,2',4,4',5,5'-hexachlorobiphenyl, 2,2',3,3',4,4',5,5'-octachlorobiphenyl, decachlorobiphenyl, hexabromobenzene, octachloronaphthalene and octachlorodibenzo-*p*-dioxin were used) for either six or sixteen days at 22°C. At day six, and again day sixteen, three samples of fish (each sample being a pooled sample of three individual fish) were analysed for the presence of the test substance. The concentration of each substance in the water phase was also measured daily during the test (a total of fourteen samples were analysed). The concentration of each substance was found to be stable during the test. However the concentration of decachlorobiphenyl was found to be more variable than the other substances and the mean measured concentration (45 ng l^{-1}) was higher than the reported solubility (around 20 ng l^{-1}). Opperhuizen (1986) suggested that the water in the aquarium could have been supersaturated with this substance. The uptake rate constant determined in this study for the substances considered by Sijm *et al*. (1995) are shown in

Table 3.1. No measurable uptake of octachloronaphthalene, octachlorodibenzo-*p*-dioxin or hexabromobenzene was seen in the study. Opperhuizen (1986) also gives uptake rate constants for 2,5-dichlorobiphenyl (k_1 = 1,200 l kg^{-1} day^{-1}), 2,2',4,4',5,5'-hexachlorobiphenyl (k_1 = 1,100 l kg^{-1} day^{-1}) and 2,2',3,3',4,4',5,5'-octachlorobiphenyl (k_1 = 1,100 l kg^{-1} day^{-1}), these substances were not considered in the Sijm *et al*. (1995) evaluation but are similar to the values obtained for the other substances considered.

The Opperhuizen and Voors (1987) study determined the uptake rate constants for ten chloroanisoles (covering a range of log K_{ow} values from 3.64 to 5.45; four of the substances were considered in the Sijm *et al*. (1995) study). The fish used in the study were one-year-old male guppy with a mean lipid content of five per cent. A continuous flow system was used using water saturated with the test substances. However, contamination of the water was stopped prior to addition of the fish (and so the test was carried out in essentially a static system). The fish were exposed under these conditions for seven days, followed by a depuration period. Samples of water and fish were analysed at regular intervals during this period. The concentration of each chloroanisole in water was found to decrease throughout the uptake period. Uptake rate constants were estimated based on the ratios of the concentration in fish to the concentration in water and the depuration rate constant. Uptake rate constants were in the range 940 to 1,710 l kg^{-1} day^{-1} for all of the substances tested. The uptake rate constants for the four substances considered in the Sijm *et al*. (1995) study are shown in

Table 3.1.

[6] Mean weight was 0.085 g in the Opperhuizen (1986) study and 0.098 g in the Opperhuizen and Voors (1987) study. The fish weight used in the de Voogt (1990) study is unclear as this study is unpublished.

The de Voogt study is an unpublished Ph.D thesis and details of this study are not considered further here.

Sijm *et al*. (1995) combined the data from the above *in vivo* studies with guppy with the data from the studies with perfused gills to derive Equation 12. This equation implies that smaller fish have higher uptake rate constants than larger fish (see Figure 3.1) and this was explained in terms of the higher relative ventilation rates in smaller fish compared with larger fish and/or a larger gill area to body weight ratio for smaller fish compared with larger fish. The rate-limiting step in the uptake process was thought to be diffusion, either through the aqueous diffusion layer or through the lipid membrane (Sijm *et al*. 1993).

The data for phenol, octachloronaphthalene and octachlorodibenzo-*p*-dioxin were not used in the derivation of the equation. The uptake rate constant for phenol was lower than would be predicted using this equation. In addition, the apparent lack of uptake of octachloronaphthalene and octachlorodibenzo-*p*-dioxin was thought to result from the large molecular size of these substances which may limit their diffusion across gill membranes.

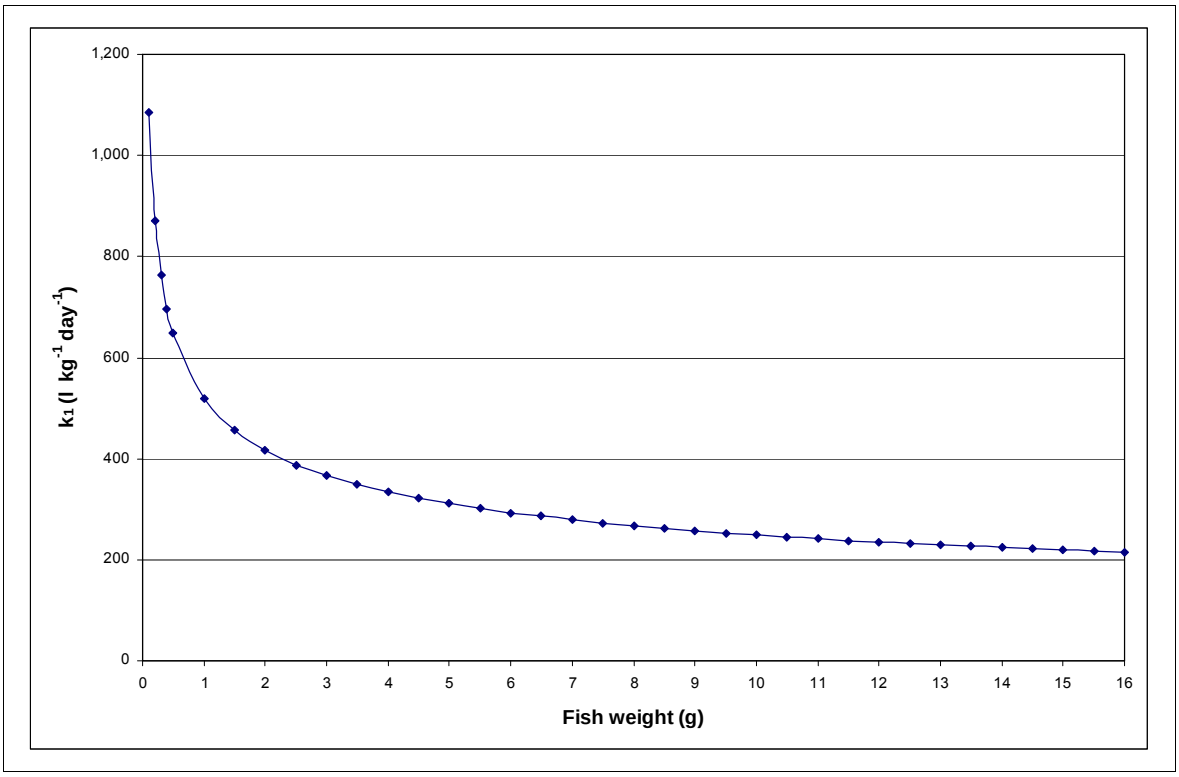

Figure 3.1 Relationship between uptake rate constant (k_1) and fish body weight predicted by the allometric equation of Sijm *et al*. (1995)

3.2 Opperhuizen (1986)

Opperhuizen (1986) discussed uptake of chemicals into fish in terms of a two-compartment model where biomembranes at the fish/water interface act as barriers to the transfer from water to fish. The study considered the influence of membrane permeation rates on the bioconcentration process. The following general equation was suggested for the membrane permeation rate:

Equation 14

$$J = \Delta_c \left(\frac{1}{R_{aq} + R_m / K_m + R_{cav}} \right) + \left(\frac{1}{R_{pore}} \right)$$

Where J = Steady-state net flux of the solute across the whole membrane. The net flux between fish and ambient water is considered to be equal to the rate of change of concentration in the fish multiplied by the total fish weight.

Δ_c = Molar concentration difference.

R_{aq} = Transfer resistance of the solute in the aqueous phase of the membrane.

R_m = Transfer resistance of the solute in the lipid phase of the membrane.

K_m = Lipid/water partition coefficient.

R_{cav} = Transfer resistance of hydrophobic chemicals across polar layers of bilipid membranes.

R_{pore} = Transfer resistance for transport through hydrophilic channels in the membrane.

Based on this equation, Opperhuizen (1986) came to the following general conclusions regarding the uptake rate constant.

- The resistance factor R_{cav} results mainly from repulsive forces between a solute and the polar groups in the bilipid layers. It is thought that these forces will be small (R_{cav} will be close to zero) for chemicals of small molecular size. In addition, for hydrophobic substances K_m and R_{pore} will be large (and hence the terms R_m/K_m and $1/R_{pore}$ will be close to zero). In these cases, the uptake rate constant can be expressed as follows:

  $k_1 = \frac{1}{R_{aq} \times F}$, where F denotes the fish weight.

  This shows that the uptake rate constant is predicted to be independent of the hydrophobicity of the substance, and depends only on the diffusion through the aqueous phase of the membrane and the fish weight.

  For larger hydrophobic substances, the value of R_{cav} may not be negligible and this may influence the steady-state flux of solutes across the membrane. Opperhuizen (1986) considered that for substances with effective cross sections above 0.95 nm or with chain lengths above 4.3 n, the value of R_{cav} would be very large resulting in k_1 values close to zero.

- For substance of low hydrophobicity, the value of R_m/K_m will not be negligible. In this case Opperhuizen (1986) predicted that the uptake rate constant would be proportional to the lipid/water partition coefficient as follows. In addition, R_{pore} may also be important for hydrophilic substances.

  $k_1 = \frac{K_m}{R_m \times F}$

Opperhuizen (1986) compared these theoretical findings against the available uptake rate constants of a number of substances and found that the uptake rate constant did increase with increasing log K_{ow} for substances of relatively low hydrophobicity and became independent of the log K_{ow} at a log K_{ow} between four and 10.

It is not possible to use these equations directly to estimate an uptake rate constant, as the values of R_{aq} and R_m are not known. However the findings of this theoretical study appear to agree with the available experimental data, and provide some useful background on the applicability of other approaches to estimate a k_1 value. An equation with a similar form to Equation 3 is given in the following section discussing the Hendriks *et al*. (2001) paper.

3.3 Hendriks *et al*. (2001) – OMEGA model

This study developed an equation for estimating the uptake rate constant based on the fugacity concept. Some of the parameters needed for the model (for example, resistances for diffusion through water and permeation through lipid layers) were obtained through fitting rate constants on literature data. The equation for the rate constant for uptake from water developed is given below.

Equation 15

$$k_1 = \frac{W^{-\kappa}}{\rho_{H_2O} + \frac{\rho_{CH_2}}{Kow} + \frac{1}{\gamma}}$$

Where k_1 = Uptake rate constant (l kg^{-1} day^{-1}).

W = Fish weight in kg.

κ = Rate exponent = 0.25.

ρ_{H2O} = Water layer diffusion resistance = 2.8×10^{-3} day kg^{-1}.

ρ_{CH2} = Lipid layer permeation resistance = 68 day kg^{-1}.

K_{ow} = Octanol-water partition coefficient.

γ = Water absorption – excretion coefficient = 200 kg day^{-1}.

Using this equation it is possible to investigate the variation of the uptake rate constant with both fish weight and octanol-water partition coefficient. Example plots are shown below in Figure 3.2 for the variation with fish weight at a constant K_{ow} (a value of 10^5 (log K_{ow} of five) was used here) and in Figure 3.3 for the variation with log K_{ow} (a fish weight of 0.005 kg (or five grams) was assumed here).

The overall model given in Hendriks *et al*. (2001) also includes methods for estimating the depuration rate constant and is known as OMEGA (optimal modelling for ecotoxicological assessment) and has been used in several studies to investigate the bioaccumulation potential of organic chemicals (such as Veltman *et al*. (2005) and de Vos *et al*. (2008)). The same equation appears in Traas *et al*. (2004).

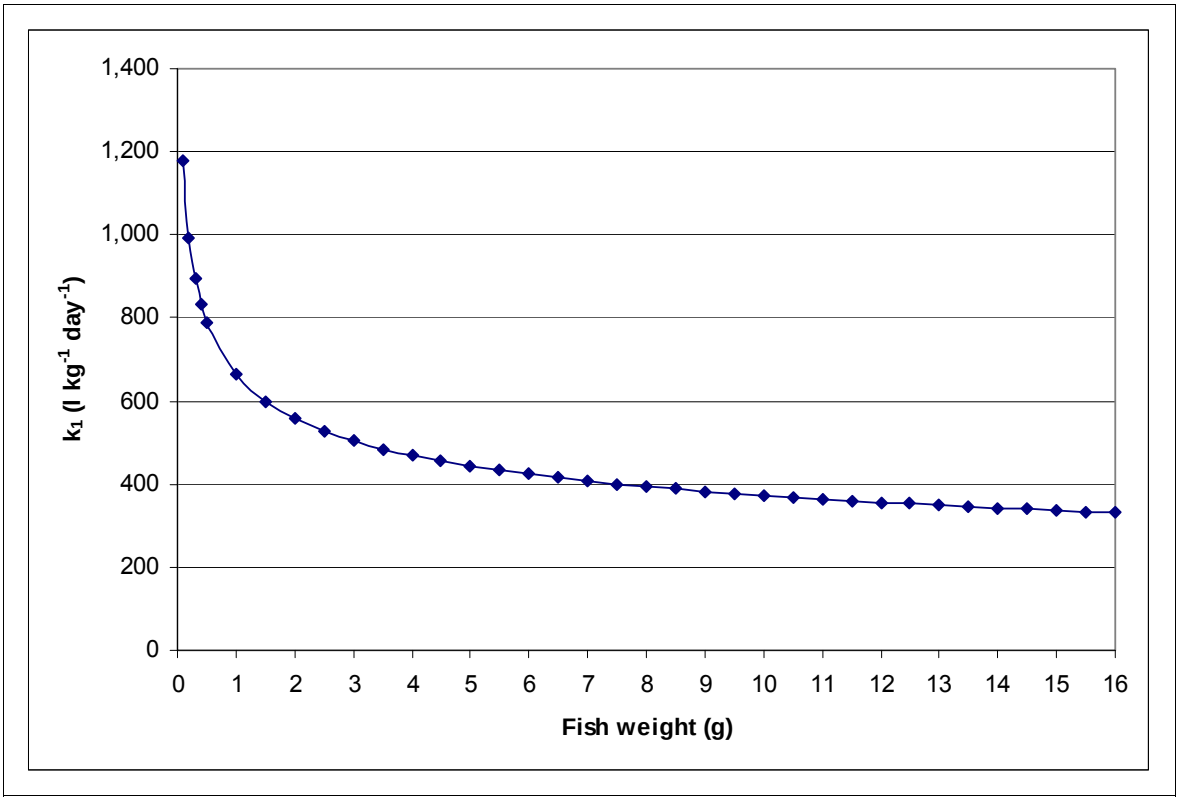

Figure 3.2 Variation of uptake rate constant with fish weight as predicted using the method in Hendriks *et al*. (2001)

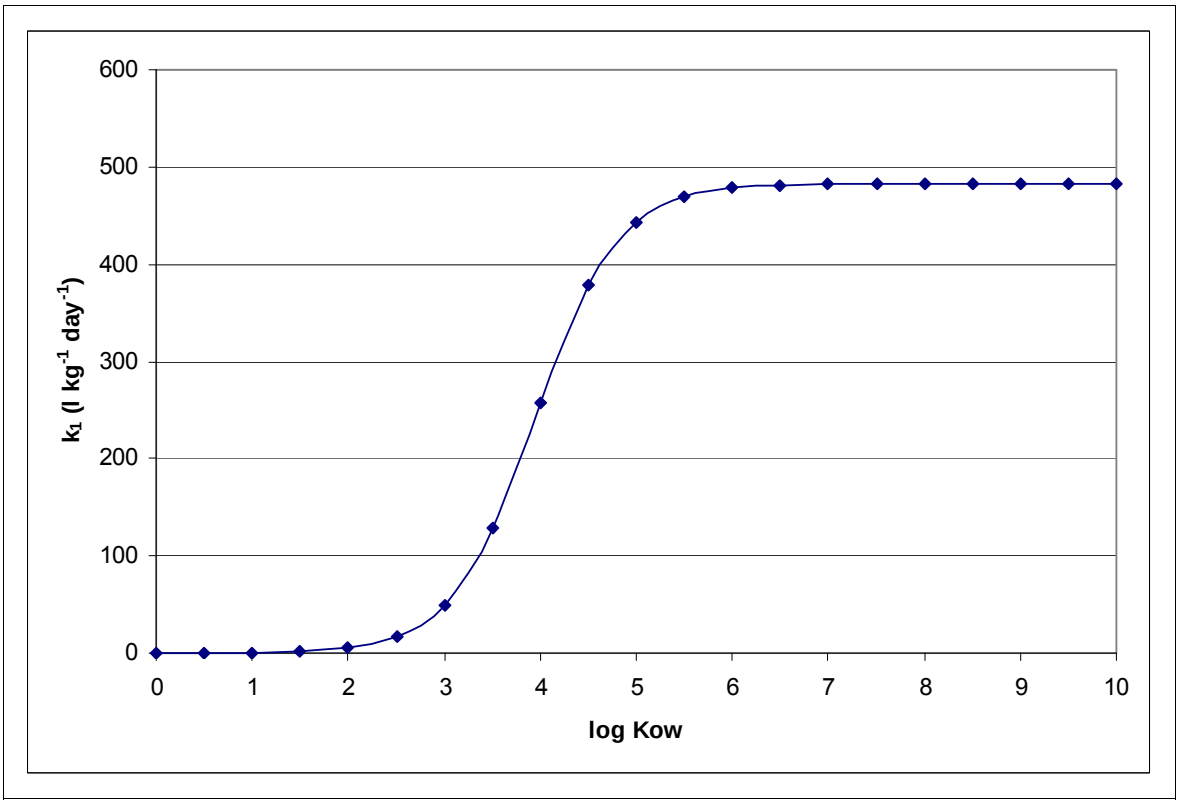

Figure 3.3 Variation of uptake rate constant with log K_{ow} as predicted using the method in Hendriks *et al*. (2001)

3.4 Campfens and Mackay (1997) – Foodweb model

The Foodweb model is available from the Canadian Environmental Modelling Centre at Trent University[7] and the theory behind the model is presented in Campfens and Mackay (1997).

The model is a fugacity-based mass balance model where uptake into an organism occurs via diffusion from water and from diet and depuration occurs via respiration, egestion and metabolism. The model also takes into account growth dilution. The model allows food webs to be developed consisting of any number of organisms, each with its own feeding preference. In its simplest form the model can be run to simulate uptake from water into a single organism (to simulate the conditions in a BCF test). The basis behind the model is that at steady state, the following equation holds:

Equation 16 $$f_W D_W + f_A D_A = f_F (D_W + D_E + D_M + D_G)$$

Where f_W = Fugacity in water.

f_A = Fugacity in food.

f_f = Fugacity in fish.

D_W = D-value for exchange with water.

D_A = D-value for food uptake.

D_E = D-value for egestion.

D_M = D-value for metabolism.

D_G = D-value for growth dilution.

The rate constants for uptake via the gill (k_1) and elimination rate constants (k_2) are estimated in the model using the following correlation equation derived by Gobas and Mackay (1987) and Gobas (1993):

Equation 17 $$\frac{1}{k_1} = \frac{V_F}{Q_W} + \frac{\left(V_F / Q_L\right)}{Kow} = \frac{1}{(L \times Kow \times k_2)}$$

Where k_1 = Uptake rate constant from water (l kg^{-1} day^{-1}).

k_2 = Elimination (depuration) rate constant (day^{-1}).

V_F = Fish volume (l).

L = Fish lipid content (as a fraction).

K_{ow} = Octanol-water partition coefficient.

Q_W = Transport parameter that expresses water phase conductivity (l day^{-1}). $Q_W = 88.3 \times V_F^{0.6}$

Q_L = Transport parameter that expresses lipid phase conductivity l day^{-1}). $Q_L = 0.001 \times Q_W$.

This equation therefore provides a direct estimate of the k_1 value from the k_2 value when the octanol-water partition coefficient and fish lipid contents are known. A plot showing how the value of k_1 varies with log K_{ow} (assuming a fish lipid content of five per cent and an overall depuration rate constant (k_2) of 0.02 day^{-1}) is shown in Figure 3.4.

[7] See http://www.trentu.ca/academic/aminss/envmodel/models/models.html.

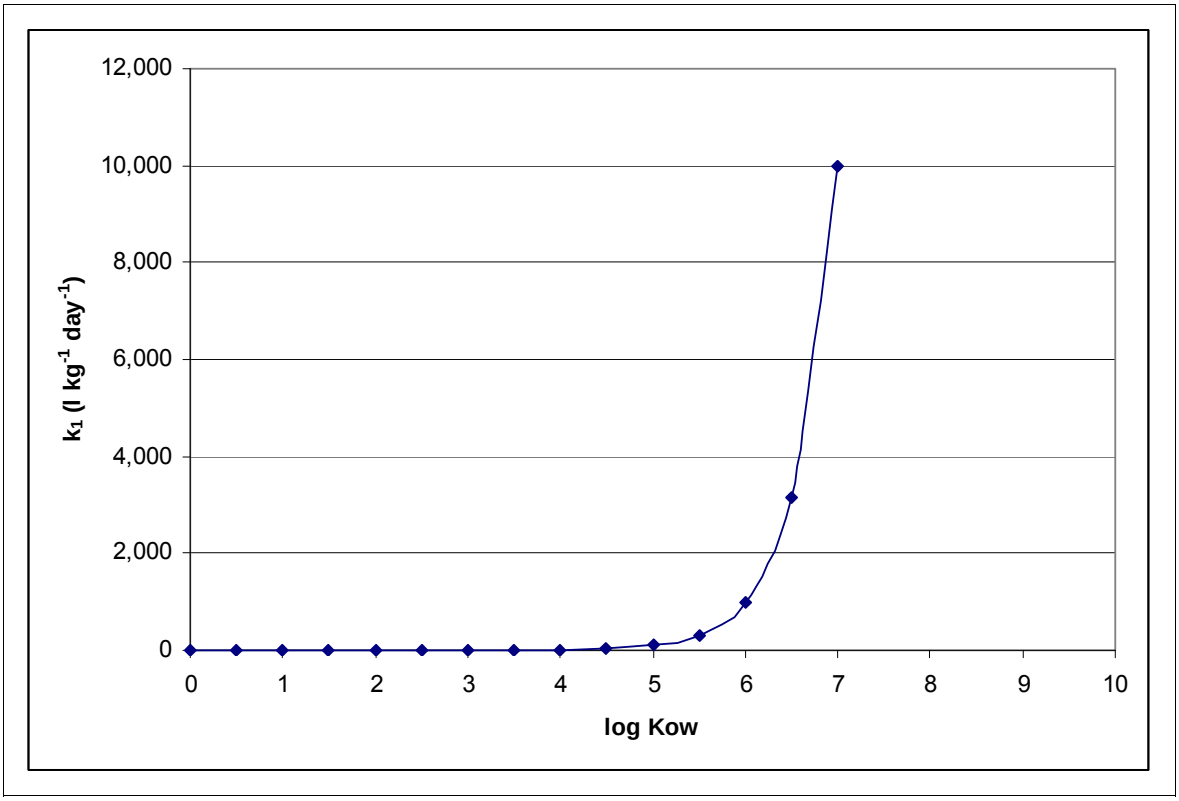

Figure 3.4 Variation in uptake rate constant with octanol-water partition coefficient predicted in the Foodweb model

The methodology outlined in Gobas (1993) is also used in several other models, for example the ECOFATE model and the Gobas 1993 model (see Section 3.7).

3.5 Arnot and Gobas (2003)

This paper described a generalised equation for predicting a bioaccumulation factor in aquatic food webs. The method was derived based on a non-steady state mass balance approach. The relevant equations are given below.

Equation 18

$$BAF = \frac{C_B}{C_W} = (1 - L_B) + \left(\frac{k_1 \times \phi + (k_D \times \beta \times \tau \times \phi \times L_D \times Kow)}{k_2 + k_E + k_G + k_M} \right)$$

Equation 19

$$k_1 = \frac{1}{(0.01 + 1/Kow) \times W^{0.4}}$$

Where BAF = Bioaccumulation factor (l kg^{-1}).

C_B = Concentration in biota (fish) (mg kg^{-1}).

C_W = Concentration in water (mg l^{-1}).

Φ = Fraction of total chemical concentration in water that is freely dissolved. This can be taken to be one for a laboratory BCF test.

k_1 = Rate constant for chemical uptake via gills (l kg^{-1} day^{-1}).

K_{ow} = Octanol-water partition coefficient.

W = Weight of fish (kg).

k_D = Rate constant for chemical uptake via diet (kg kg^{-1} day^{-1}). To consider accumulation from water alone (bioconcentration) k_D can be set to zero.

k_2 = Rate constant for elimination of chemical via respiratory surfaces (day^{-1}) = $k_1/(L_B \times K_{ow})$.

k_E = Rate constant for elimination of the chemical via faecal egestion (day^{-1}) = $0.125 \times k_D$.

k_G = Rate constant for growth dilution (day^{-1}) = $0.0005 \times W^{-0.2}$.

k_M = Rate constant for elimination of chemical by metabolism (day^{-1}).

L_B = Fraction lipid content of fish.

L_D = Fraction lipid content of diet for the lowest trophic level organism.

β = Empirical factor derived from calibrating the model to measured (field) data. This factor is highly dependent on the species of interest, food web structure and ecosystem characteristics. A value of 130 was used in the example food web in the Arnot and Gobas (2003) paper. However, if the equation is used to consider accumulation from water alone (setting k_D to zero) this factor is not important.

τ = A factor representing the degree of trophic dilution for substances that are metabolised at a significant rate in organisms in the food web = $(0.0065/(k_M+0.0065))^{n-1}$, where n = number of trophic interactions (levels) in the food web.

Although this equation is used to estimate a BAF, it could easily be adapted to estimate a BCF by assuming the rate constant for chemical uptake via diet (k_D) is zero and that the total fraction of chemical in water that is freely dissolved (Φ) is one. Further the uptake rate constant from water can be estimated directly from Equation 19. Equation 19 depends on both the fish weight and log K_{ow}. Plots showing the variation in the predicted uptake rate constant with fish weight (assuming a constant K_{ow} of 10^5 (a log K_{ow} of five)) and octanol-water partition coefficient (assuming a constant fish weight of 0.005 kg (five grams)) are shown in Figure 3.5 and Figure 3.6 respectively.

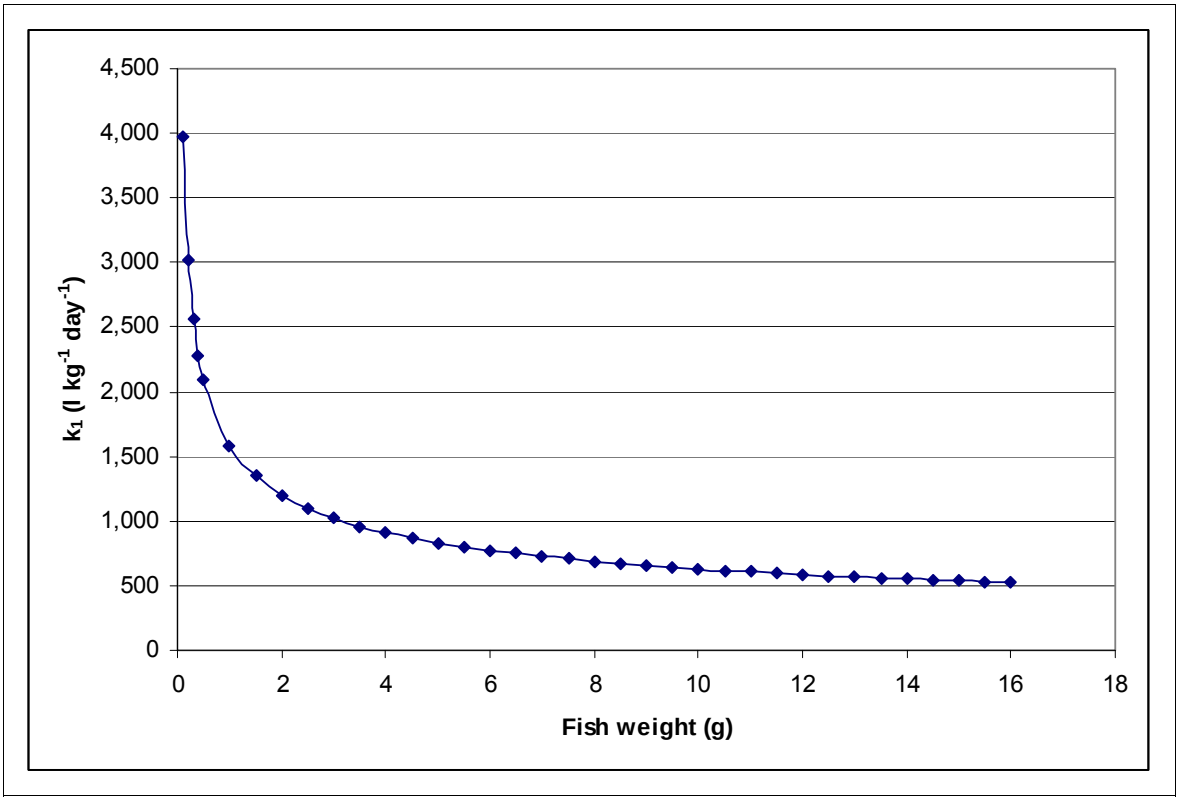

Figure 3.5 Variation of predicted uptake rate constant with fish weight using the equation developed by Arnot and Gobas (2003)

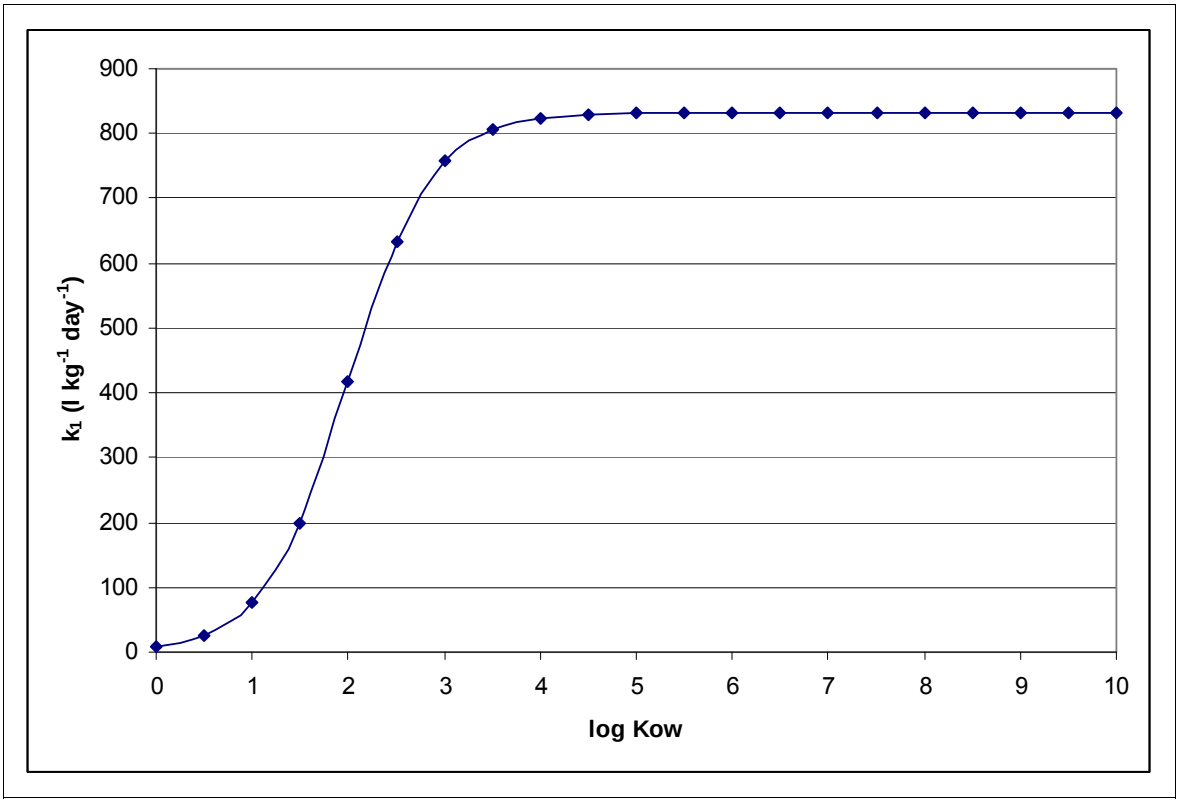

Figure 3.6 Variation of predicted uptake rate constant with log K_{ow} using the equation developed by Arnot and Gobas (2003)

3.6 Arnot and Gobas (2004)

This model is essentially an update to the Gobas (1993) model and incorporates more recent insights into the mechanism of bioaccumulation as well as improved model parameterisation.

In this approach, the rate constant for uptake via gills is assumed to be a function of the ventilation rate and the diffusion rate of the chemical across the respiratory surface. The following equations were developed:

Equation 20 $$k_1 = \frac{E_W \times G_V}{W}$$

Equation 21 $$E_W = \frac{1}{\left(1.85 + \left(155 / Kow\right)\right)}$$

Equation 22 $$G_V = \frac{1{,}400 \times W^{0.65}}{C_{OX}}$$

Where k_1 = Uptake rate constant (l kg^{-1} day^{-1}).

E_W = Gill uptake efficiency – assumed to be a function of K_{ow}.

G_V = Gill ventilation rate (l day^{-1}).

W = Weight of the organisms (kg).

K_{ow} = Octanol-water partition coefficient.

C_{OX} = Dissolved oxygen concentration (mg O_2 l^{-1}). This can be estimated as C_{OX}=(-0.24×T +14.04)×S, where T is the temperature in °C and S is the degree of oxygen saturation in water. For water at 12°C and a minimum 60 per cent oxygen saturation (as may typically be found in a laboratory BCF test with rainbow trout), the C_{OX} would be 6.7 mg O_2 l^{-1}.

The uptake rate constant can thus be calculated directly from the above equations. Plots showing the variation of the predicted k_1 with fish weight (assuming a constant K_{ow} of 10^5 (log K_{ow} of five)) and log K_{ow} (assuming a constant fish weight of 0.005 kg (five grams)) are shown in Figure 3.7 and Figure 3.8 respectively. For these example calculations the dissolved oxygen concentration is assumed to be 6.7 mg O_2 l^{-1} throughout.

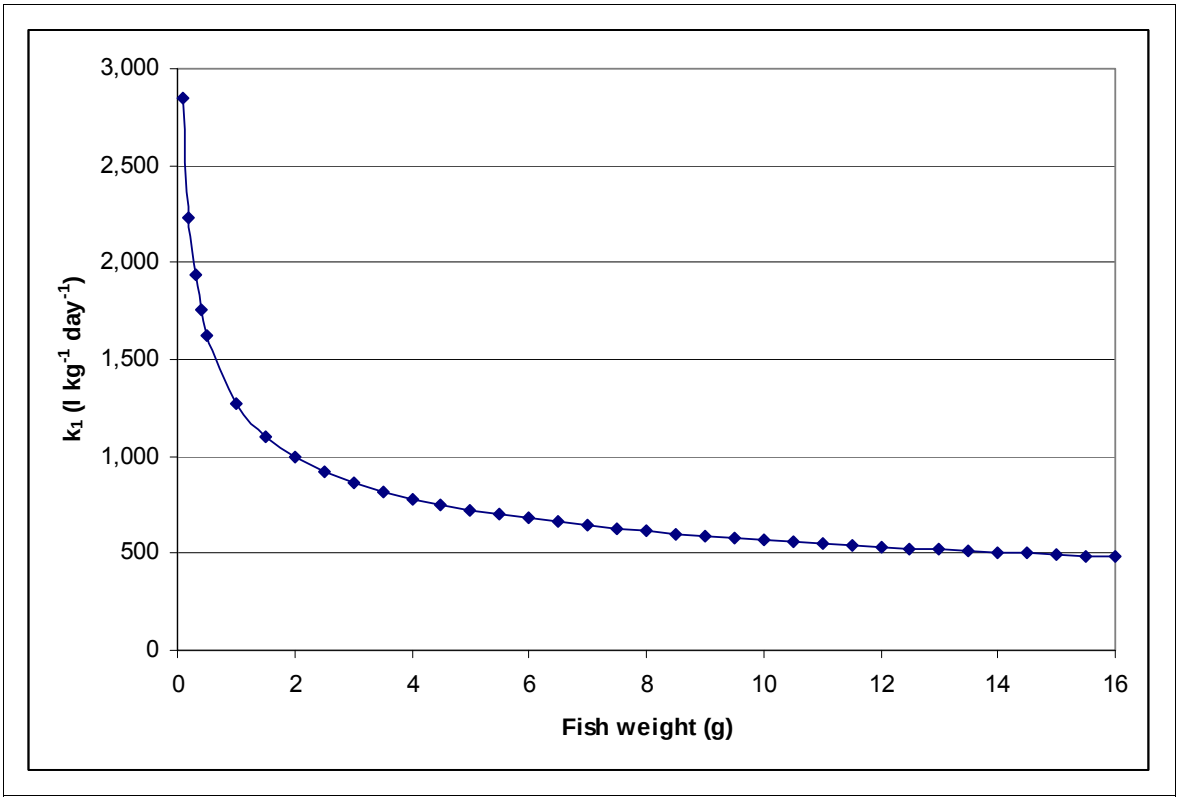

Figure 3.7 Variation of predicted uptake rate constant with fish weight using the method developed by Arnot and Gobas (2004)

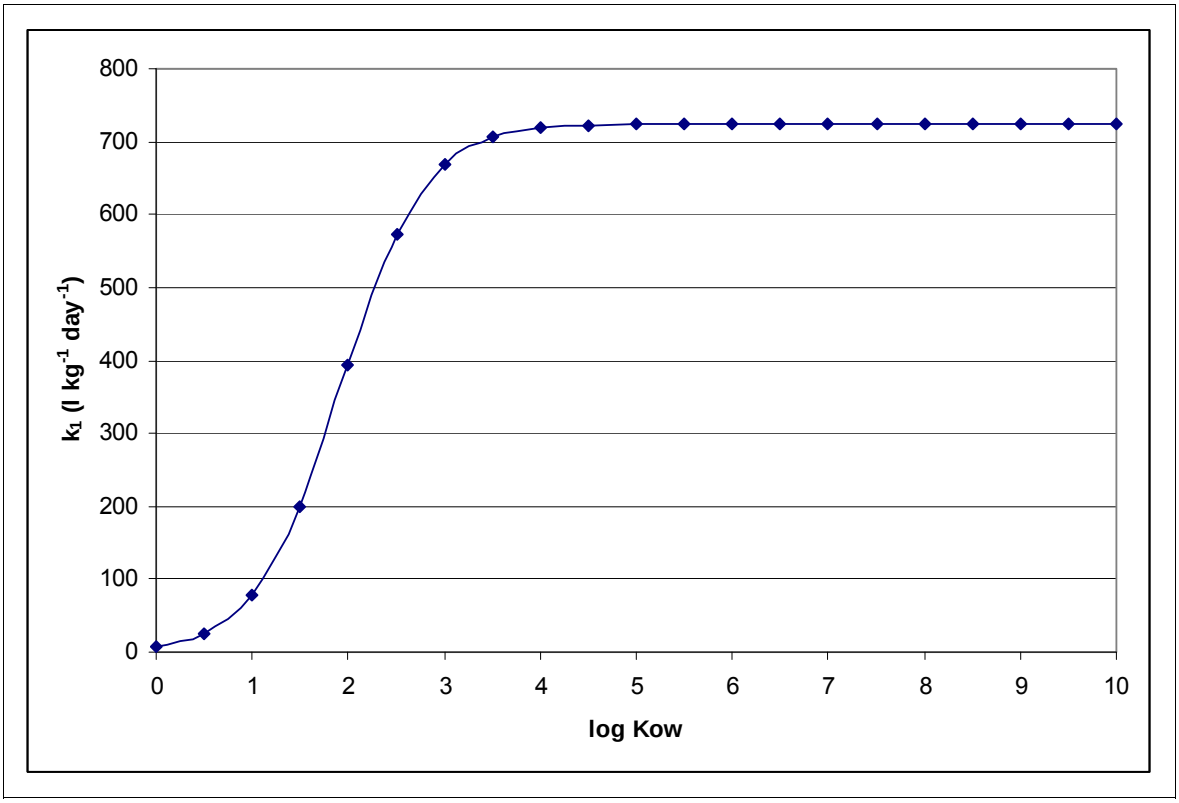

Figure 3.8 Variation of predicted uptake rate constant with log K_{ow} using the equation developed by Arnot and Gobas (2004)

Arnot and Gobas (2004) indicate that this model is applicable to non-ionising organic chemicals with a log K_{ow} in the approximate range one to nine.

3.7 Thomann (1989)

This paper describes a model for calculating the concentration of a chemical in a generic aquatic food chain and the general approach is incorporated into the QEAFDCHN model (see Section 3.9.2). For uptake from water into an organism, the uptake rate constant is related to the ventilation volume using the following equations:

Equation 23 $$k_1' = \frac{V \times E}{W_{lipid}}$$

Equation 24 $$V = \frac{r' \times W_{lipid}}{C_O}$$

Equation 25 $$r' = \frac{(a_{OC} \times a_C)}{(a_{wd} \times \rho)} \times r$$

Equation 26 $$r = \phi \times W^{-\gamma}$$

Combining Equation 24, Equation 25 and Equation 26 with Equation 23, gives the following equation to estimate the uptake rate constant (a similar equation is derived in other papers by the same group, for example Thomann *et al*. 1992):

Equation 27 $$k_1' = \frac{(a_{oc} \times a_c \times \phi \times W^{-\gamma} \times E)}{(a \times a_{wd} \times \rho \times C_0)}$$

Where k_1' = Uptake rate constant (l kg lipid^{-1} day^{-1}).

V = Ventilation volume (l day^{-1}).

E = Transfer efficiency of the chemical.

W_{lipid} = Lipid weight of the organism (kg lipid).

r' = Respiration rate on an oxygen basis (g O_2 day^{-1} kg lipid^{-1}).

C_O = Dissolved oxygen concentration in the water phase (kg l^{-1}).

a_{oc} = Oxygen to carbon ratio (of the fish).

a_c = Carbon to dry weight ratio (of the fish).

a_{wd} = Wet to dry weight ratio (of the fish).

ρ = Lipid fraction of the fish.

r = Respiration rate of the fish (day^{-1}).

W = Wet weight of the organisms (g).

Φ = The value is a function of the specific organism and ecosystem function. Values vary between 0.014 and 0.05 for routine metabolism.

γ = The value is a function of the specific organism and ecosystem function. Recommended values vary between 0.2 and 0.3 for routine metabolism.

Assuming a_{oc} = 2.67, a_c = 0.45, a_{wd} = 5, C_0 = 8.5 mg l^{-1} and Φ = 0.036, Thomann (1989) simplified Equation 27 to give Equation 28.

Equation 28 $$k_1' \approx \frac{10^3 \times W^{-\gamma} \times E}{\rho}$$

Thomann (1989) considered that the transfer efficiency (E) across gill membranes would depend on chemical properties such as the lipid partition coefficient (or octanol-water partition coefficient), steric properties and molecular weight. At low log K_{ow} values rapid diffusive transfer across the lipoprotein gill membrane would be expected, but this transfer would be hindered by the lipid membrane owing to limited fat solubility. As the log K_{ow} increased this resistance to transfer would be expected to reduce, and the transfer efficiency would be expected to increase proportionally to the log K_{ow}. This increase in transport efficiency would be expected to eventually reach a plateau where the transfer was controlled mainly by the aqueous diffusion layer. At very high log K_{ow}, the water solubility of the chemical might limit the transport and so the transfer efficiency would be expected to decrease with increasing log K_{ow}. Thomann (1989) considered the available experimental data on the variation of uptake efficiency with log K_{ow} for a range of fish weights and derived the following equations from the data.

For organisms weighing in the order of less than 10 to 100 g wet weight:

Equation 29 $\log E = -2.6 + 0.5 \times \log Kow$ for log K_{ow} in the range 2 to 5.

Equation 30 $E = 0.8$ for log K_{ow} in the range 5 to 6.

Equation 31 $\log E = 2.9 - 0.5 \times \log Kow$ for log K_{ow} in the range 6 to 10.

For organisms weighing in the order of more than 10 to 100 g wet weight:

Equation 32 $\log E = -1.5 + 0.4 \times \log Kow$ for log K_{ow} in the range 2 to 3.

Equation 33 $E = 0.5$ for log K_{ow} in the range 3 to 6.

Equation 34 $\log E = 1.2 - 0.25 \times \log Kow$ for log K_{ow} in the range 6 to 10.

Combining these equations with Equation 28 allows the uptake rate constant to be estimated for a range of fish weights and chemical (log K_{ow}) properties. Plots showing the predicted variation of the uptake rate constant with fish weight (at a constant K_{ow} of 10^5 (log K_{ow} of five)) and log K_{ow} (at a constant fish weight of five grams) are shown in Figure 3.9 and Figure 3.10. For this analysis the value of γ is assumed to be 0.25 (the middle of the range recommended by Thomann (1989)), the lipid fraction of the fish (ρ) is assumed to be 0.05 (five per cent; the default lipid content of fish recommended in the guidance for REACH) and Equation 29, Equation 30 and Equation 31 are used to estimate the value of E as appropriate. The variation of the predicted k_1 with the value of γ (in the range 0.20-0.30 recommended by Thomann (1989)) is shown in Figure 3.11 (here a constant log K_{ow} of five, a lipid fraction of 0.05 and a constant fish weight of five grams are assumed in the calculations). As can be seen, the value of γ chosen within this range has only a relatively minor impact on the k_1 predicted.

The units of k_1' estimated using this approach are l kg^{-1} $lipid^{-1}$ day^{-1}. To convert these to the more normal units of l kg $(wet\ weight)^{-1}$ day^{-1} the value of k_1' should be multiplied by the lipid fraction: $k_1 = k_1' \times \rho$. This conversion is done here for the plots below. This also means that although the k_1' value is inversely proportional to the lipid content, the final k_1 value estimated is independent of the lipid content.

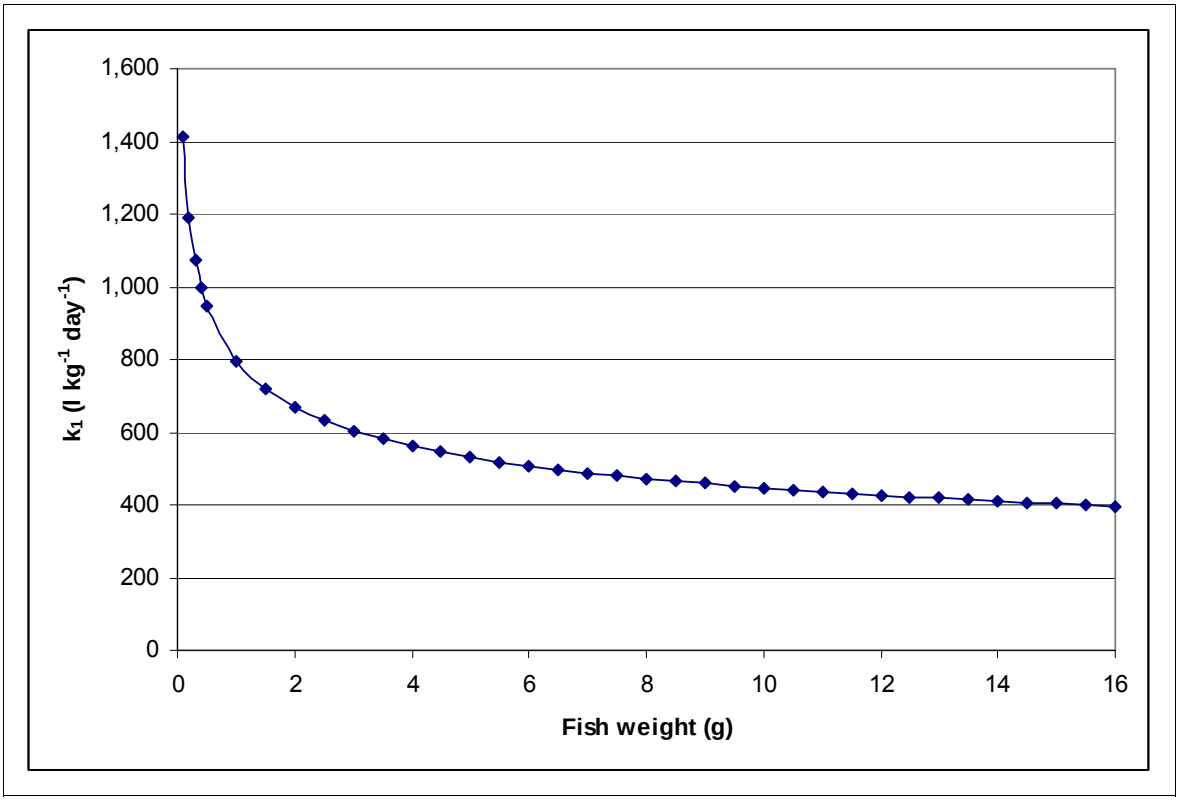

Figure 3.9 Variation of predicted uptake rate constant with fish weight based on Thomann (1989)

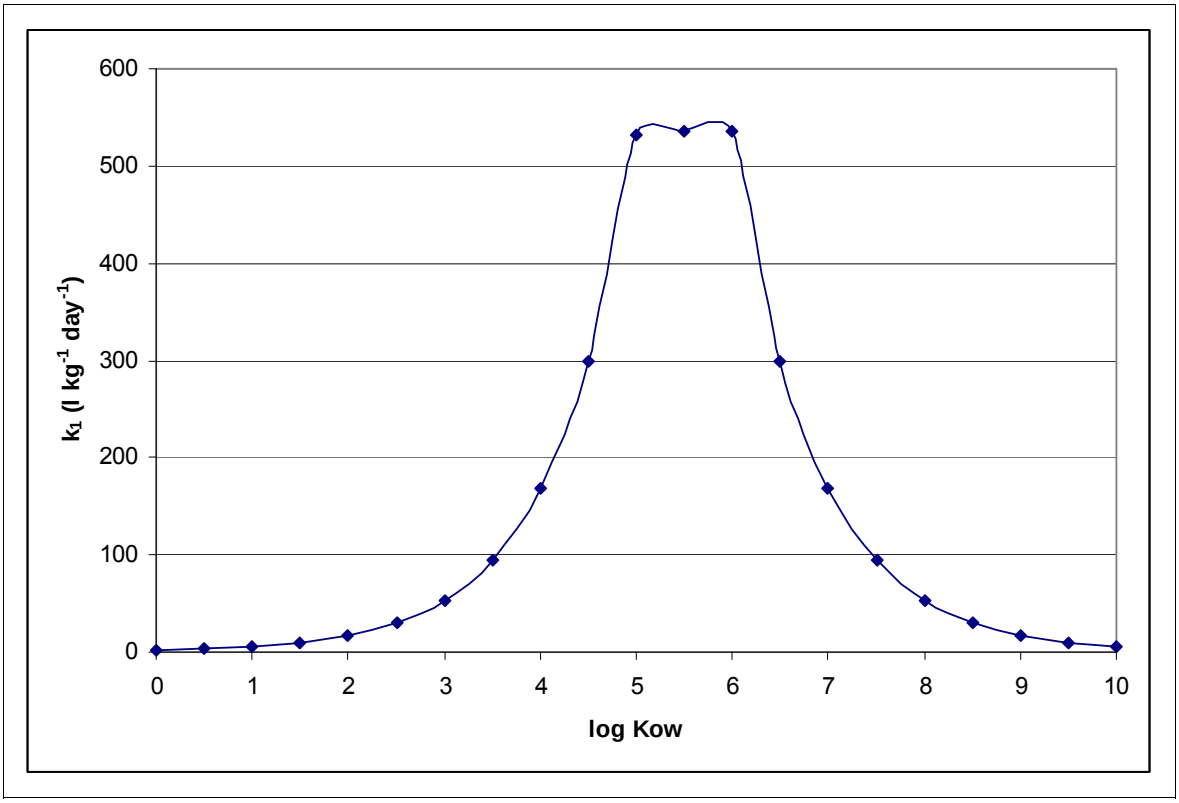

Figure 3.10 Variation of predicted uptake rate constant with log K_{ow} based on Thomann (1989)

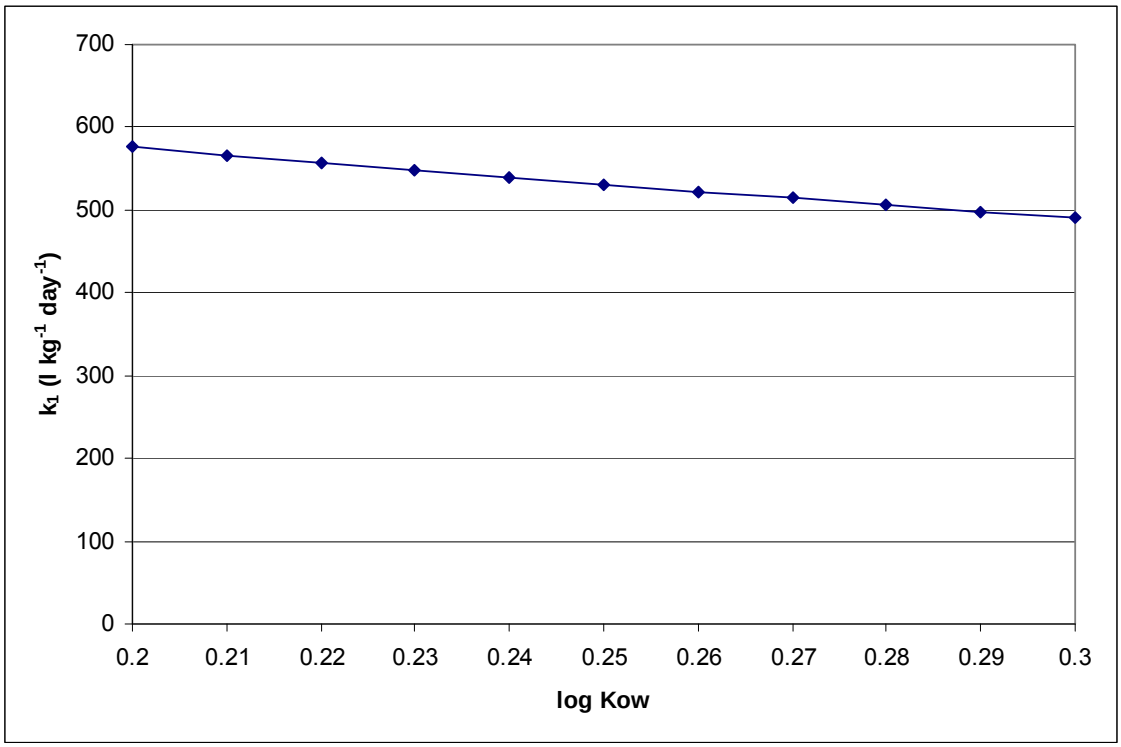

Figure 3.11 Variation of predicted uptake rate constant with γ based on Thomann (1989)

3.8 Barber (2003)

Barber (2003) carried out a detailed comparison and review of the available models and methods for predicting bioconcentration in fish. This review focused on the following ten models and considered methods for estimating both the uptake rate constant and the depuration rate constant.

- Barber (2001). This is essentially the approach used in the BASS model (see Section 3.9.3).
- Barber *et al*. (1991). This is similar to the approach used in the FGETs model (see Section 3.9.3).
- Erickson and McKim (1990a).
- Erickson and McKim (1990b).
- Gobas and Mackay (1987). See Section 3.4.
- Gobas *et al*. (1986) and Sijm and van der Linde (1995).
- Hayton and Barron (1990).
- Norstrom *et al*. (1976), Neely (1979), Thomann (1989) (see Section 3.7) and Connolly (1991).
- Streit and Siré (1993).
- Thomann and Connolly (1984) (see Section 3.9.2).

Barber (2003) compared the predictability of these models using a set of experimental bioconcentration data. This data set consisted of uptake and depuration rate constants obtained from the published literature for a wide range of freshwater species. The chemicals contained in the data set were either neutral organic chemicals or weakly ionisable organic chemicals for which a pKa value was available that indicated that the substance could be treated as an effectively neutral substance at the pH of the test and at physiological pHs. In all, the data set covered 284 substances and 22 species of fish. The fish size ranged from 0.015 g to 1,060 g. The identities and properties (log K_{ow}, uptake rate constant and so on) of the full data set were not given in the Barber (2003) paper but the substance covered included brominated benzenes, brominated toluenes, chlorinated anisoles, chlorinated anilines, chlorinated benzenes, hexachlorocyclohexanes, isopropyl polychlorinated biphenyls, nitrobenzenes, nitrotoluenes, organochlorine pesticides, organophosphorus pesticides, polyaromatic hydrocarbons, polyaromatic heterocyclic hydrocarbons, polybrominated biphenyls, polychlorinated alkanes, polychlorinated biphenyls, polychlorinated dibenzo-*p*-dioxins and furans, triaryl phosphates and alcohol ethoxylates amongst others.

Using this data set, Barber (2003) derived the following allometric relationship relating the uptake rate constant to the fish weight (this is referred to as Barber (2003) – observed in Section 4). This equation was derived using 517 datapoints and had a correlation coefficient (r^2) of 0.11.

Equation 35 $$k_1 = 445 \times W^{-0.197}$$

Where W = fish weight in g.

k_1 = Uptake rate constant (l kg^{-1} day^{-1}).

This equation is similar to that derived by Sijm *et al.* (1995; see Section 3.1) and although the r^2 value is low, the regression is highly significant. A plot showing the variation of the uptake rate constant with fish weight predicted by this equation is given below in Figure 3.12. The diagram also shows the predicted uptake rate constant obtained using the Sijm *et al*. (1995) method as a comparison.

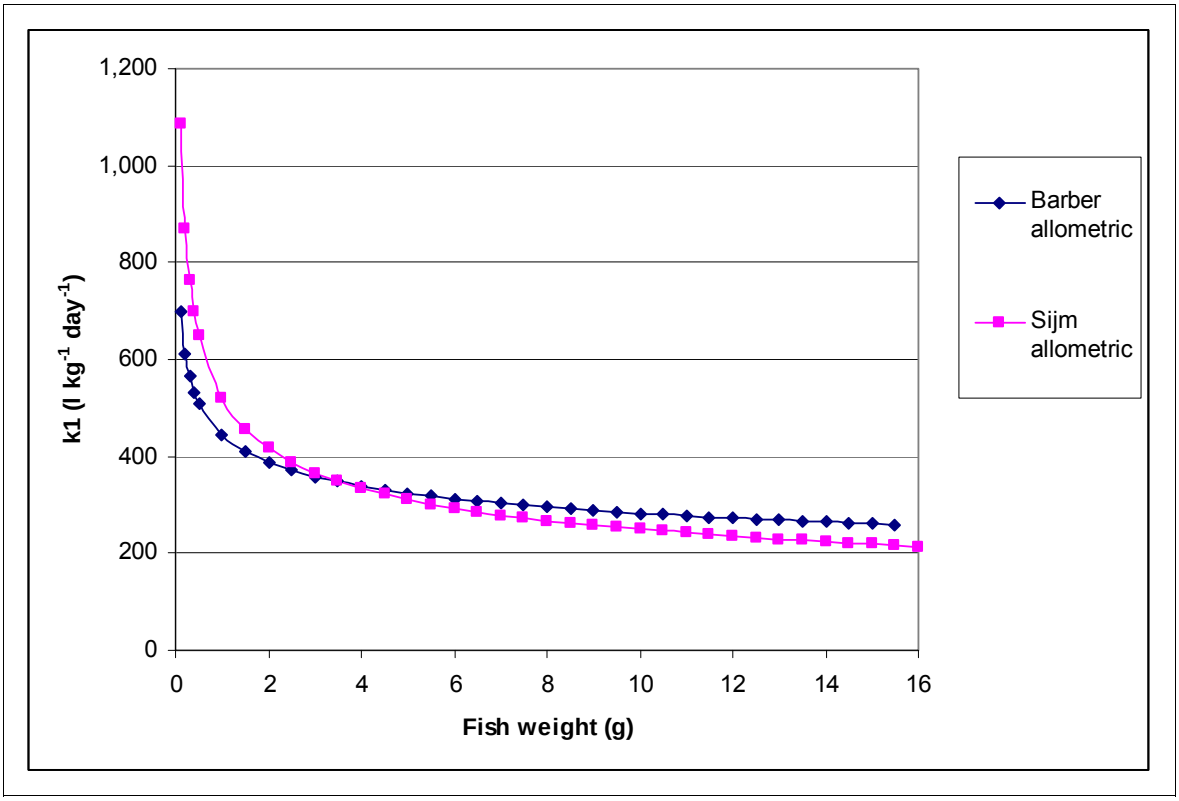

Figure 3.12 Variation of uptake rate constant with fish weight obtained using the allometric equation derived by Barber (2003)

For the ten models considered, Barber (2003) analysed the relationship between the predicted uptake rate constant and the fish weight assuming routine and standard respiratory demands[8]. The predictions were made for the same data set as indicated above and the models were parameterised for the fish species included in the data set. The allometric regression equations derived based on these predictions are summarised in Table 3.2. Also shown is the regression equation derived from the experimental data set (this is the same as Equation 24 above). As the log K_{ow} range covered by the data set is unknown, the range of applicability of these regression equations (in terms of log K_{ow} value) is unknown.

Table 3.2 Allometric regression equations derived by Barber (2003) based on various model predictions

Model	Regression equation[1]	Correlation coefficient (r^2)
Model 1: Barber (2001)	$\ln k_1 = -0.192 \times \ln W + 7.343$ - routine	0.733
	$\ln k_1 = -0.161 \times \ln W + 6.541$ - standard	0.512
Model 2: Barber *et al*. (1991)	$\ln k_1 = -0.241 \times \ln W + 7.279$ - routine	0.843
	$\ln k_1 = -0.182 \times \ln W + 6.523$ - standard	0.591
Model 3: Erickson and McKim (1990a)	$\ln k_1 = -0.183 \times \ln W + 7.259$ - routine	0.594
	$\ln k_1 = -0.157 \times \ln W + 6.511$ - standard	0.480

[8] Barber (2001) considered that under laboratory conditions (limited swimming space and scheduled feedings) the fish's actual respiratory demands may be more accurately reflected by its standard respiratory demand than its routine respiratory demand. In most cases, standard respiration was assumed to be half of routine respiration.

Model	Regression equation[1]	Correlation coefficient (r^2)
Model 4: Erickson and McKim (1990b)	$\ln k_1 = -0.274 \times \ln W + 6.795$ - routine	0.854
	$\ln k_1 = -0.228 \times \ln W + 6.345$ - standard	0.736
Model 5: Gobas and Mackay (1987)	$\ln k_1 = -0.394 \times \ln W + 7.135$ - routine	0.912
	$\ln k_1 = -0.394 \times \ln W + 7.135$ - standard	0.912
Model 6: Gobas *et al*. (1986) and Sijm and van der Linde (1995)	$\ln k_1 = -0.317 \times \ln W + 8.003$ - routine	0.904
	$\ln k_1 = -0.317 \times \ln W + 8.003$ - standard	0.904
Model 7: Hayton and Barron (1990)	$\ln k_1 = -0.234 \times \ln W + 6.769$ - routine	0.759
	$\ln k_1 = -0.196 \times \ln W + 6.222$ - standard	0.636
Model 8: Norstrom *et al*. (1976), Neely (1979), Thomann (1989) and Connolly (1991)	$\ln k_1 = -0.157 \times \ln W + 5.873$ - routine	0.065
	$\ln k_1 = -0.126 \times \ln W + 5.071$ - standard	0.041
Model 9: Streit and Siré (1993)	$\ln k_1 = -0.185 \times \ln W + 6.771$ - routine	0.638
	$\ln k_1 = -0.158 \times \ln W + 6.011$ - standard	0.494
Model 10: Thomann and Connolly (1984)	$\ln k_1 = -0.196 \times \ln W + 5.682$ - routine	0.649
	$\ln k_1 = -0.165 \times \ln W + 4.880$ - standard	0.449
Observed from experimental data: Barber (2003)	$\ln k_1 = -0.197 \times \ln W + 6.098$	0.105

[1]W = fish weight in g. k_1 = uptake rate constant (l kg^{-1} day^{-1}). Regression equations are given for the assumption of both routine and standard respiratory demands.

A plot showing how the predicted uptake rate constant varies with fish weight for the allometric equations in Table 3.2 is given below for the regression equations developed assuming standard respiratory demand (Figure 3.13).

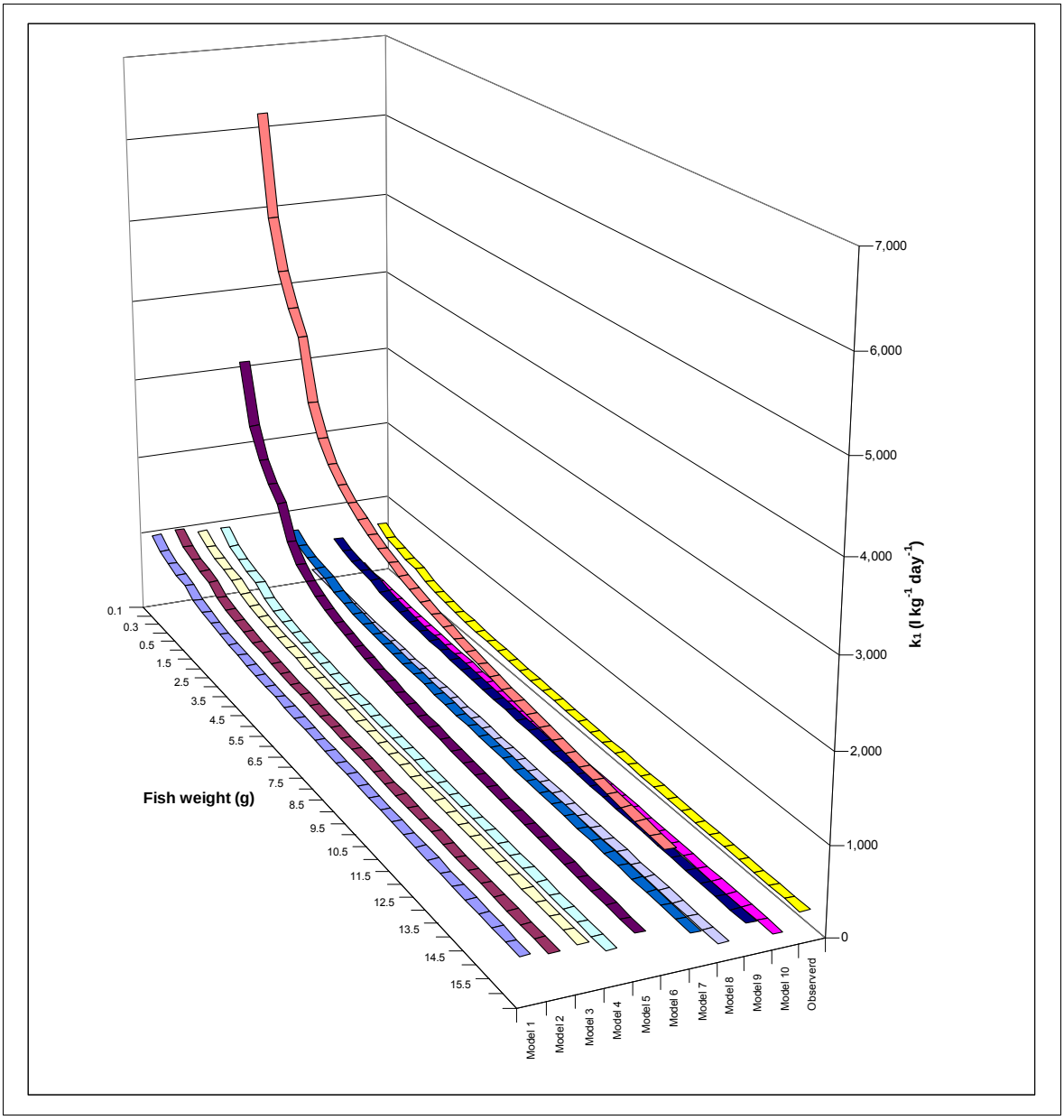

Figure 3.13 Variation of predicted uptake rate constant with fish weight for the allometric equations in Table 3.2 derived assuming standard respiratory demand

Barber (2003) used the available experimental database to calibrate the Gobas and Mackay (1987) model (Model 5 above) predictions (this is referred to as the Barber (2003) – calibrated method in Section 4). The equations derived are given below for the calibrated model assuming routine and standard respiratory demand.

Equation 36 $$k_1 = 0.343 \times \left(\frac{1{,}400 \times W^{-0.4} \times Kow}{100 + Kow} \right)^{1.048}$$ - routine respiratory demand

Equation 37 $$k_1 = 0.401 \times \left(\frac{1{,}400 \times W^{-0.4} \times Kow}{100 + Kow} \right)^{1.025}$$ - standard respiratory demand

Where k_1 = uptake rate constant (l kg^{-1} day^{-1}).

K_{ow} = octanol-water partition coefficient.

Plots showing the variation of the predicted k_1 with fish weight (assuming a constant K_{ow} of 10^5 (log K_{ow} of five) and log K_{ow} (assuming a constant fish weight of 0.005 kg (five grams) are shown in Figure 3.14 and Figure 3.15 respectively.

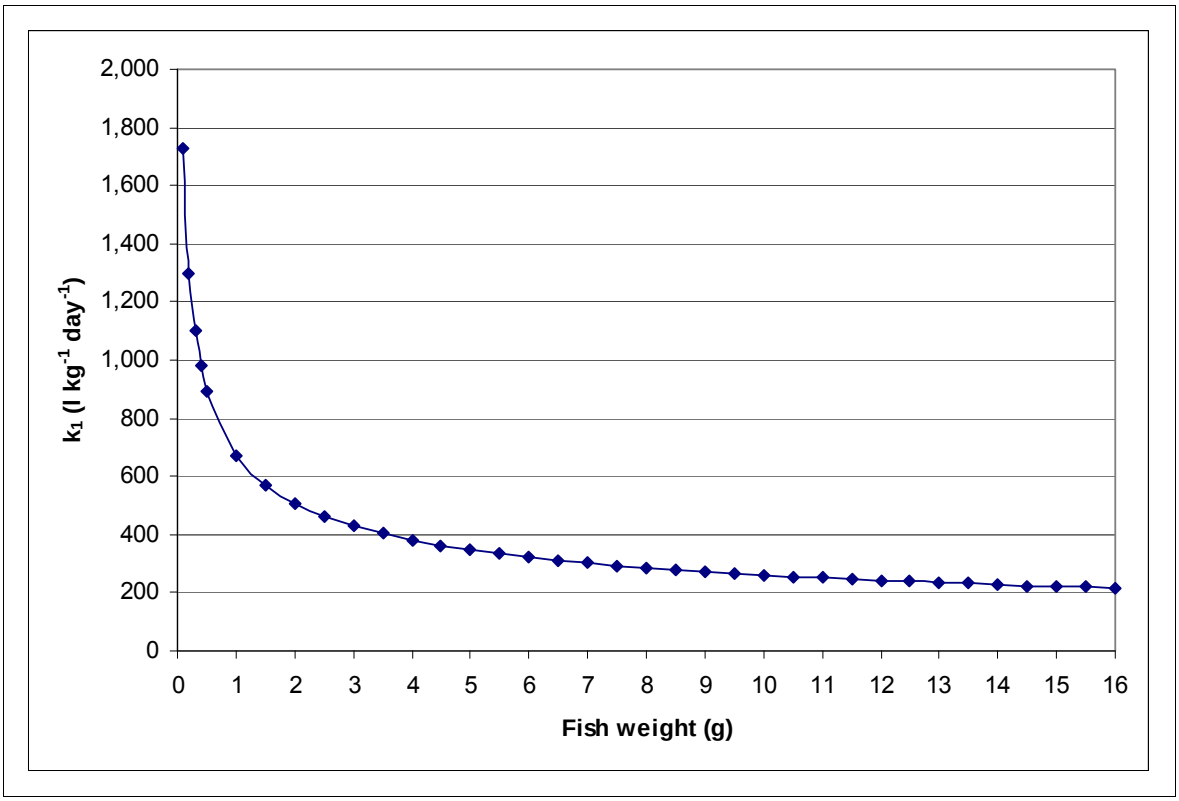

Figure 3.14 Variation of predicted uptake rate constant with fish weight using the calibrated Gobas and Mackay (1987)/Barber (2003) method

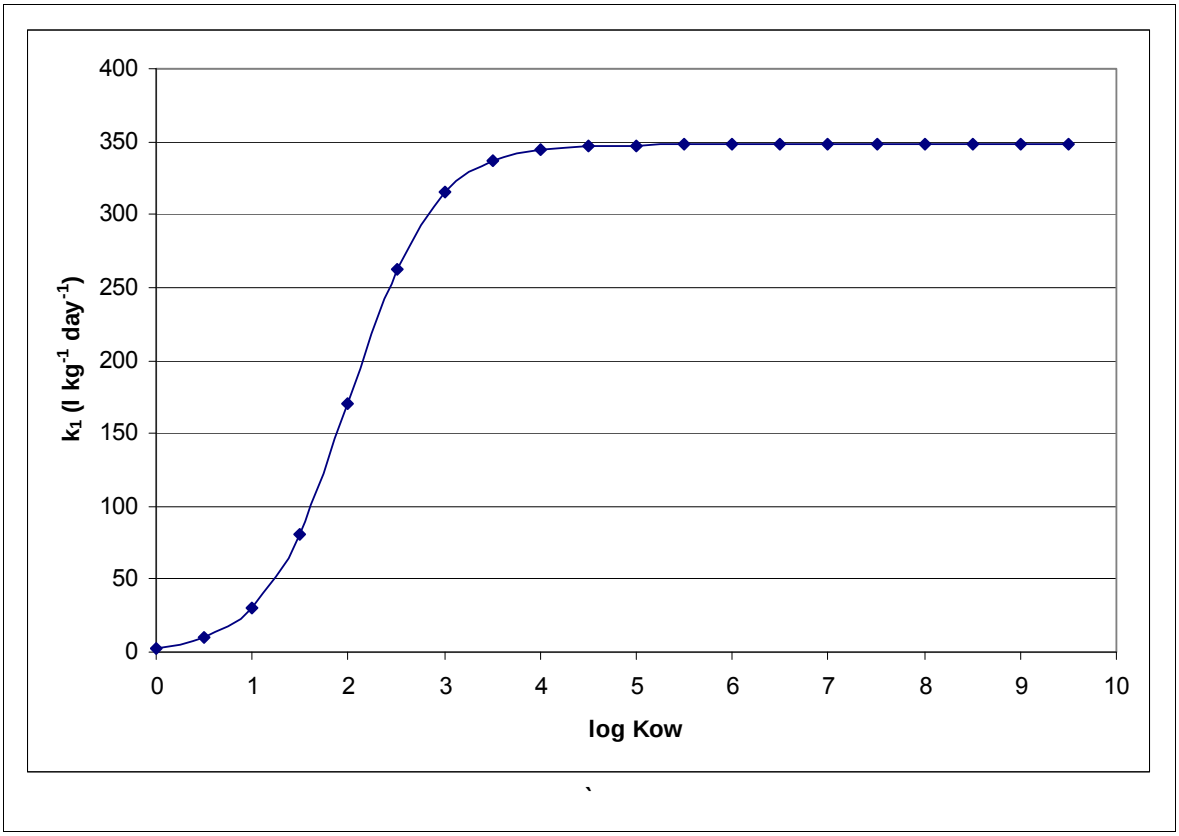

Figure 3.15 Variation of predicted uptake rate constant with log Kow using the calibrated Gobas and Mackay (1987)/Barber (2003) method

In addition to the analysis of the bioaccumulation models, the Barber (2003) review summarised the available quantitative structure activity relationships (QSARs) for estimating the uptake rate constant (k_1) in fish. These QSARs are summarised in Table 3.3 below.

The variation of the predicted uptake rate constant with log K_{ow} obtained using these QSARs is shown in Figure 3.16 (Hawker and Connell 1985), Figure 3.17 (Hawker and Connell 1988), Figure 3.18 (Spacie and Hamelink 1982) and Figure 3.19 (Tolls and Sijm 1985). Barber (2003) commented that the utility of these equations for prediction needs to be carefully evaluated, as they are based on very limited databases, and they implicitly assume that biological determinants of uptake are either insignificant or constant across species or body sizes.

Table 3.3 QSARs for predicting uptake rate constant in fish

Equation	Comment	Reference
$\log k_1 = 0.337 \times \log Kow - 0.373$	Based on an equation relating the fish BCF to log K_{ow} derived by Mackay and a regression equation developed related the depuration rate constant to log K_{ow}. Data covered chlorinated benzenes, chlorinated biphenyls, tetrachloroethane, carbon tetrachloride, diphenyl ether and biphenyl. Fish species included guppy (*Poecilia reticulata*), goldfish (*Carassius auratus*) and rainbow trout (*Oncorhynchus mykiss*). Log K_{ow} range: 2.60-6.23.	Hawker and Connell, 1985
$k_1 = \frac{0.048 \times Kow}{(0.00142 \times Kow + 12.01)}$	Re-analysis of the above data.	Hawker and Connell, 1988
$\log k_1 = 0.147 \times \log Kow + 1.98$	Fish species included guppy (*Poecilia reticulata*) and rainbow trout (*Oncorhynchus mykiss*)	Spacie and Hamelink, 1982
$\log k_1 = 0.122 \times \log Kow + 2.192$	Substances included polychlorinated biphenyls and chlorobenzenes.	Tolls and Sijm, 1995

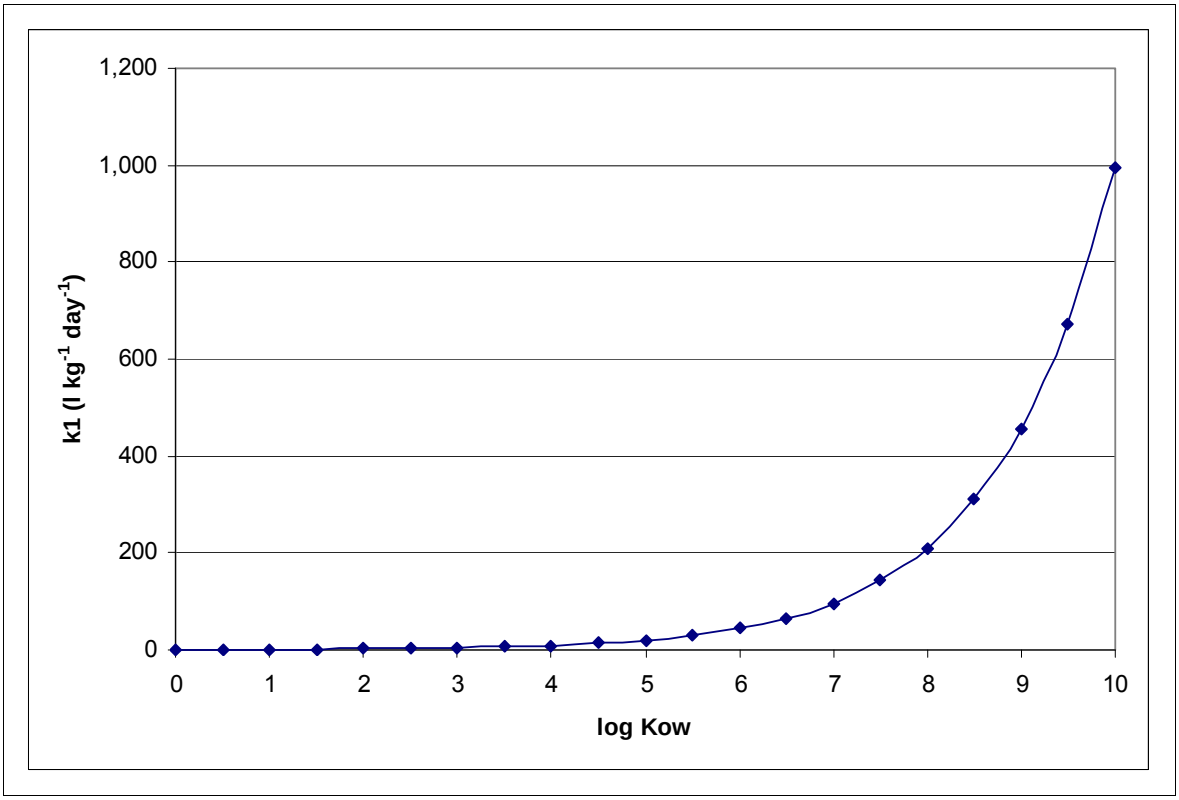

Figure 3.16 Variation of predicted uptake rate constant with log K_{ow} using the QSAR of Hawker and Connell (1985)

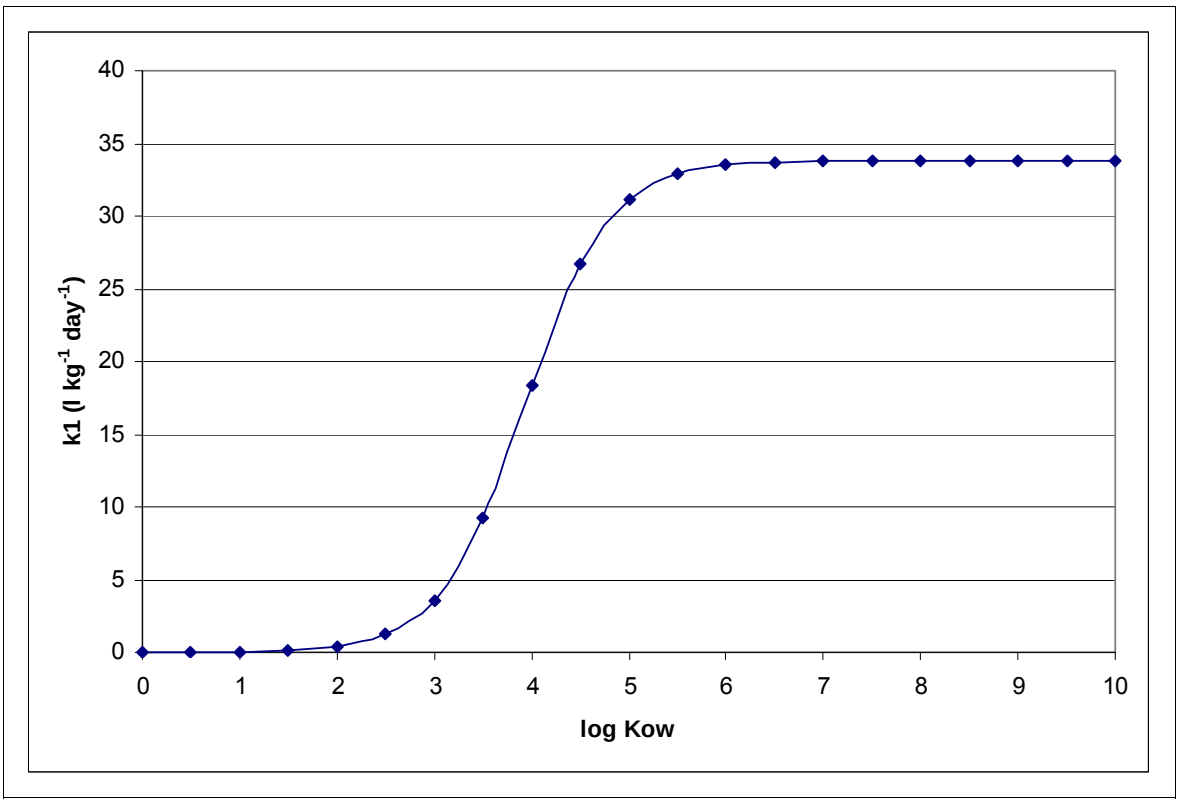

Figure 3.17 Variation of predicted uptake rate constant with log K_{ow} using the QSAR of Hawker and Connell (1988)

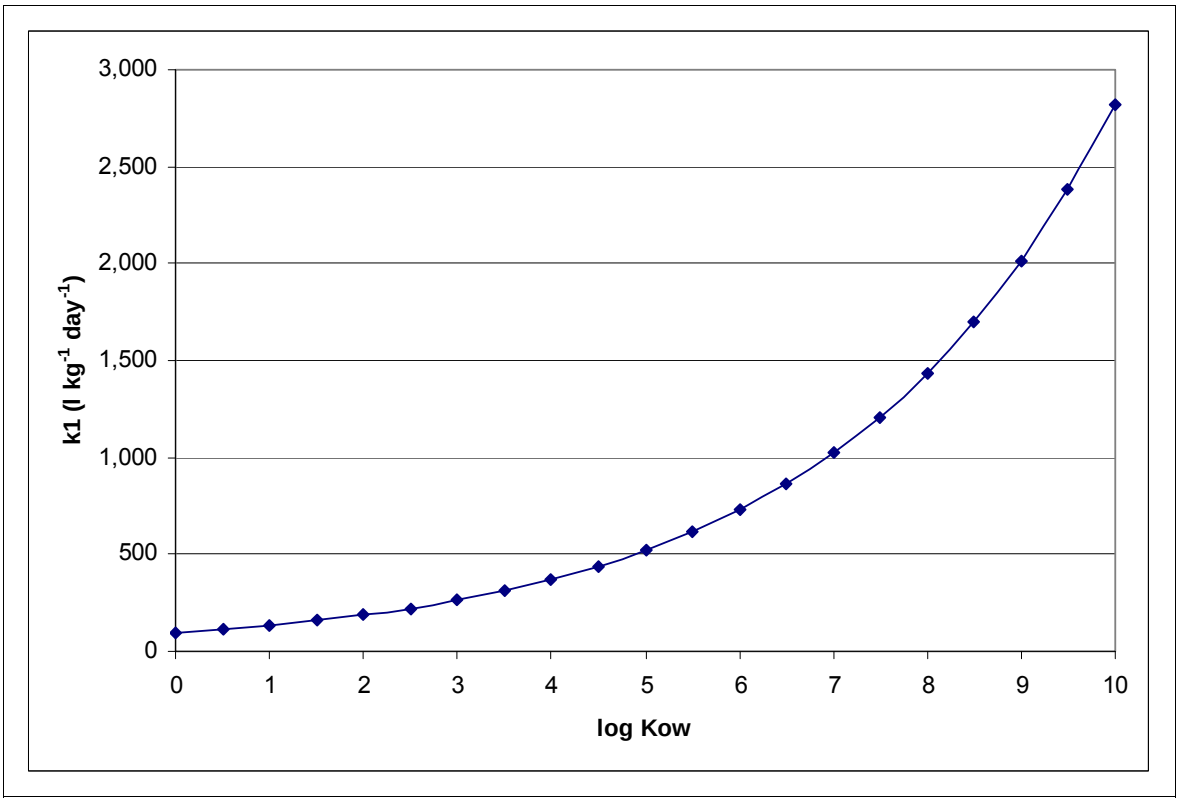

Figure 3.18 Variation of predicted uptake rate constant with log K_{ow} using the QSAR of Spacie and Hamelink (1982)

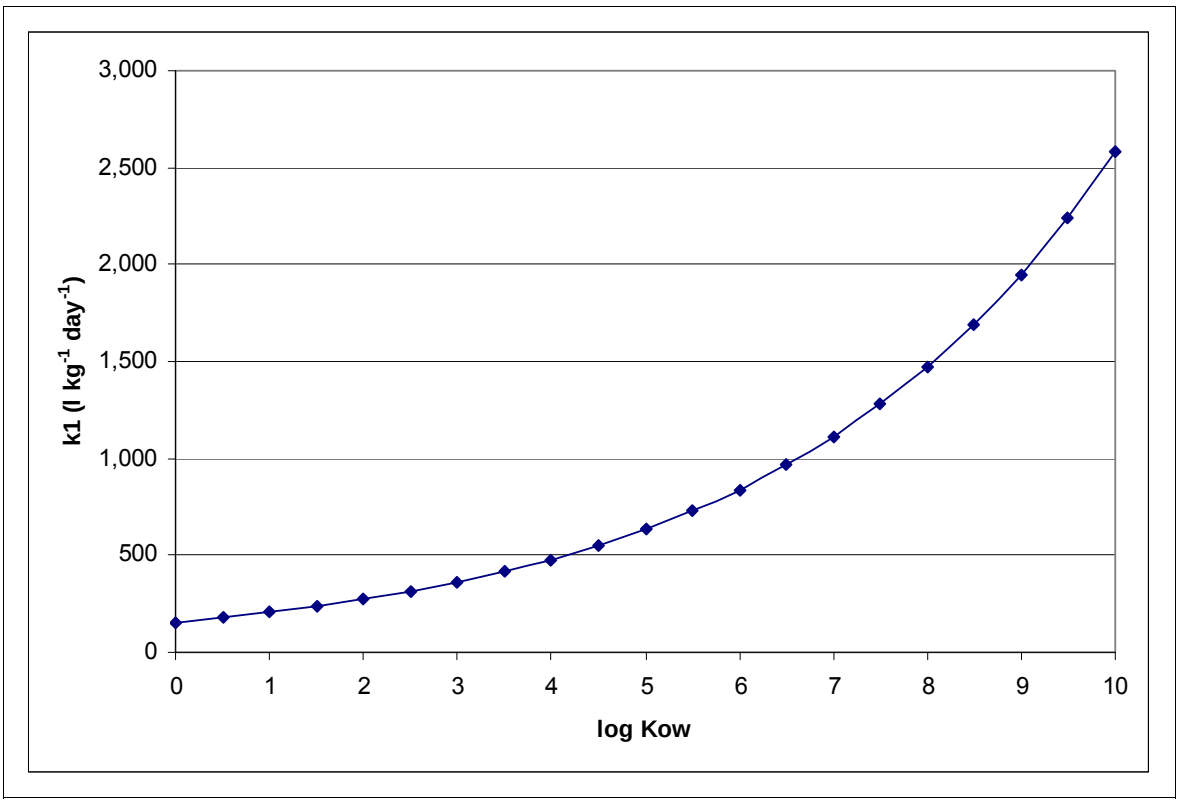

Figure 3.19 Variation of predicted uptake rate constant with log K_{ow} using the QSAR of Tolls and Sijm (1995)

For the analysis carried out in Section 4 the equations obtained assuming the standard respiratory demand (where appropriate) were used[8].

3.9 More complex food web and food chain models

The previous review of bioaccumulation models identified a number of more complex computer models that could be used to estimate bioaccumulation of substances within a food chain. The more relevant models are summarised below (for a more detailed description and evaluation see Brooke and Crookes 2007). Although these models are generally set up to model the bioaccumulation in a food chain (and hence take account of uptake into fish from both water (bioconcentration) and food), it is possible to adapt these models or run them in such a way that uptake into fish occurs via water (dissolved phase) only and so they can provide an estimate of the BCF.

On the face of it, these models are potentially useful in estimating a BCF from the data generated in a fish feeding study, as a feeding study will provide a depuration (elimination) rate constant that can potentially be used as an input into the model. However, this needs to be done carefully as some of the models are quite complex. In particular, it must be borne in mind that the depuration rate constant obtained in a fish feeding study includes some of the processes already assumed within the model. This is illustrated below.

Many of the more complex models assume that four elimination/depuration processes occur in the fish. These are respiration, elimination via faeces, metabolism and growth dilution. The overall depuration rate constant is assumed to be a combination of these processes.

3.9.1 Gobas (1993) – ECOFATE and GOBAS 1993 model

These models are non-steady state mass balance models where the bioaccumulation in fish is modelled based on the Gobas (1993) paper. These models were developed by workers at Simon Fraser University in Canada and are available on their website[9].

3.9.2 QEAFDCHN

This model was developed by Quantitative Environmental Analysis and is based on work by Connolly, Thomann and co-workers (for example Thomann 1981, Thomann 1989, Thomann and Connolly 1984, Thomann *et al*. 1992, Connolly 1991, Connolly *et al*. 2000, Connolly and Glaser 2002, Glaser and Connolly 2002 and QEA 1999 and 2001). The model is based on conservation of mass and energy. Chemicals are assumed to be taken up into an organism during respiration and ingestion of food (or sediment) and are depurated by diffusion across respiratory surfaces, metabolism excretion and growth dilution. The model is flexible and can be adapted to many different food chains.

3.9.3 Barber (2001) and Barber *et al*. (1988 and 1991) – FGETS/BASS models

The FGETS (Food and Gill Exchange of Toxic Substances)[10] is available as a stand-alone model or is incorporated into the BASS (Bioaccumulation and Aquatic System Simulator)[11] model (Barber 2001). Both models have been developed by the United States Environmental Protection Agency (USEPA). Details of the FGETS model are given in, for example, Barber *et al*. (1988 and 1991). The model is a mass-balance model based on diffusion kinetics coupled to a fish-growth model. The model considers uptake from water occurs as a result of diffusive exchange across gill membranes and this is modelled by Fick's first law of diffusion. Growth of fish is simulated using a mass balance bioenergetic model. Although the model could probably be used to estimate a BCF from the data generated in a fish feeding study the structure of the model is quite complex and requires some familiarity with the system. This means that the model is probably not so useful for routine estimation of a BCF from such data.

3.9.4 Czub and McLachlan (2004) – ACC-Human model

The ACC-Human model is a food chain model for predicting the accumulation of lipophilic organic chemicals in humans. The model is available for download from the Stockholm University website[12].

The model is a fugacity-based, non-steady state, mechanistic model that considers the accumulation of substances by humans through air, water, soil and food. The model contains representative food chains for an agricultural soil system and a marine water system; the latter food chain is most relevant to this current project.

The marine water food chain in the model consists of zooplankton, planktivorous fish and piscivorous fish. The model considers the main fish species harvested for human

[9] The models are available from http://www.rem.sfu.ca/toxicology/models/models.htm.
[10] See http://www.epa.gov/ceampubl/fchain/fgets/index.htm.
[11] See http://www.epa.gov/ceampubl/fchain/bass/index.html.
[12] See http://www.itm.su.se/research/model.php.

consumption, for example herring and cod, and that these species do not feed on benthic organisms.

The bioaccumulation in fish is estimated using a non-steady state model developed by Gobas *et al*. (1988)[13] and assumes uptake into the fish occurs via both water and food, and that metabolism of the substance occurs in the fish.

3.10 Summary of approaches

A number of approaches to estimate the uptake rate constant (k_1) have been identified. The methods vary in terms of the input parameters needed, the variation of k_1 with these parameters and the range of k_1 values predicted for a hypothetical, but as far as possible standardised, set of assumptions. The main points are summarised below.

- Thirteen of the methods considered are dependent solely on the fish weight. These are the approaches by Sijm *et al.* (1995), Barber (2003), the ten allometric regression equations derived by Barber (2003) and the calibrated Gobas and Mackay (1987)/Barber (2003) method. These methods vary in the magnitude of the k_1 value predicted for a given fish weight, although all of these methods predict that the k_1 value should decrease with increasing fish weight. For example the k_1 values predicted for a fish weight of 0.1 g (the lowest fish weight assumed in the various plots) are in the approximate range 190 to 6,200 l kg^{-1} day^{-1}. Similarly the k_1 values predicted for a fish weight of 16 g (the highest fish weight assumed in the various plots) are in the approximate range 80 to 1,240 l kg^{-1} day^{-1}. Therefore, the various estimates cover a range of a factor of around 16 (at the higher fish weight) and a factor of around 33 (at the lower fish weight) depending on the method used.

- Four of the methods considered are dependent on both the fish weight and the log K_{ow} of the substance. These are the approaches by Hendriks *et al.* (2001), Arnot and Gobas (2003), Arnot and Gobas (2004) and Thomann (1989). For a log K_{ow} of five, these methods predict a k_1 value in the approximate range 1,180 to 3,980 l kg^{-1} day^{-1} for a fish weight of 0.1 g and 330 to 520 l kg^{-1} day^{-1} for a fish weight of 16 g. Three of the methods (Hendriks *et al.* (2001), Arnot and Gobas (2003), Arnot and Gobas (2004)) predict that k_1 should increase with increasing log K_{ow} up to a limit, after which the k_1 becomes independent of log K_{ow}, with the log K_{ow} value at which this occurs being approximately log K_{ow} six and above (Hendriks *et al.* (2001) or log K_{ow} four and above (Arnot and Gobas (2003). The Thomann (1989) approach predicts a different dependence of k_1 on log K_{ow}, with an increase in k_1 with increasing log K_{ow} being predicted up to around a log K_{ow} of five, the predicted k_1 being independent of log K_{ow} in between approximately log K_{ow} of 5 and 6.5, and the predicted k_1 valued decreasing with increasing log K_{ow} above a log K_{ow} of around 6.5.

- Four of the methods are dependent on the log K_{ow} of the substance only. These are the approaches by Hawker and Connell (1985), Hawker and Connell (1988), Spacie and Hamelink (1982) and Tolls and Sijm (1995). Three of these approaches predict that k_1 should increase exponentially with increasing log K_{ow}, whereas the Hawker and Connell (1988) predicts that the k_1 value would reach a constant maximum of around 35 l kg^{-1} day^{-1} at log K_{ow} values around six and above. The three other methods predict that for a log K_{ow} value of 10, the k_1 would be in the approximate range 1,000-2,700 l kg^{-1} day^{-1}.

[13] This model is similar in principle to the Foodweb model (see Section 3.4) but this later model is solved at steady state.

- The Campfens and Mackay (1997) method is different from the other methods in that it depends on the elimination rate constant as well as the lipid content of the fish and the log K_{ow} of the substance. This method predicts that the k_1 value should increase markedly with log K_{ow} above a log K_{ow} of around five.
- The k_1 is predicted to show a lipid dependence in only one of the methods, the Campfens and Mackay (1997) method[14]. When considering lipid normalisation of the resulting BCF estimated from the k_1, the potential dependence of k_1 on the lipid content of the fish is clearly important.

Clearly, a wide range of k_1 values can be estimated for a given chemical/fish size depending on the method(s) used. The various methods are tested against actual uptake rate constants in Section 4.

[14] Although the Thomann (1989) method predicts that the k_1' value is inversely related to the lipid, the final k_1 value is estimated as the product of k_1' and the lipid content and so becomes independent of the lipid content.

4 Testing of approaches

4.1 Data

Three data sets were used in the analysis here, and are summarised in Appendix A.

The first data set was kindly provided by Jon Arnot of Trent University, Canada. This data set contained 87 data points for which a k_1 value was available. The data set was collated for use in validation of bioaccumulation models by Trent University. This data set is referred to as the Arnot data set in Appendix A.

The second data set was data on uptake rate constants for 18 pesticides kindly provided by Caren Rauert of the Umweltbundesamt (UBA) in Germany. Many of the details of these studies are confidential but the data have been accepted for regulatory purposes and so are valid for use in this study. This data set is referred to as the UBA data set in Appendix A.

The final data set considered is the EURAS Gold Standard database[15]. As many of the data in the Arnot data set also appeared in the Gold Standard database, the duplicate data were omitted here. This resulted in a further 23 data points. This data set is referred to as the Gold Standard data set in Appendix A.

The three data sets were combined in order to test the various approaches.

None of the data sets were re-evaluated for quality and reliability as part of this study. Issues that may arise from the potential uncertainties in these data sets are discussed in Section 7.

All calculations and statistical analysis were carried out in Microsoft Excel 2003 SP2.

4.2 Prediction of k_1 values

The approaches outlined in Section 3 were used to predict the k_1 values for each data point using the data in Appendix A. As for most of the data points only the initial fish weight is available; comparison of the predicted k_1 value with the experimental k_1 value is based on this initial weight (Section 5 considers the consequences of fish growth further). Experimental and predicted k_1 values for each data point are summarised in Appendix A. The equations used for the predictions are summarised below.

- Sijm *et al*. (1995) - Equation 12.
- Hendriks *et al*. (2001) - Equation 15.
- Campfens and Mackay (1997) - Equation 17, using the depuration rate constants measured in the test.
- Arnot and Gobas (2003) - Equation 19.
- Arnot and Gobas (2004) - Equation 20 to Equation 22, using the actual dissolved oxygen concentration reported in the test.

[15] See: http://ambit.sourceforge.net/euras/.

- Thomann (1989) - Equation 28 (using Equation 29 to Equation 31 to estimate the transfer efficiency), assuming a γ of 0.25 and using the actual dissolved oxygen concentration reported in the test.
- Barber (2001) – The equation for standard respiratory demand from Table 3.2.
- Barber (1991) – The equation for standard respiratory demand from Table 3.2.
- Erickson and McKim (1990a) – The equation for standard respiratory demand from Table 3.2.
- Erickson and McKim (1990b) – The equation for standard respiratory demand from Table 3.2.
- Gobas and Mackay (1987) – The equation for standard respiratory demand from Table 3.2.
- Gobas *et al*. (1986) – The equation for standard respiratory demand from Table 3.2.
- Hayton and Barron (1990) – The equation for standard respiratory demand from Table 3.2.
- Norstrom *et al*. (1976) – The equation for standard respiratory demand from Table 3.2.
- Streit and Siré (1993) – The equation for standard respiratory demand from Table 3.2.
- Thomann and Connolly - The equation for standard respiratory demand from Table 3.2.
- Barber (2003) observed - Equation 35.
- Barber (2003) calibrated - Equation 37.
- Hawker and Connell (1985) – Equation from Table 3.3.
- Hawker and Connell (1988) – Equation from Table 3.3.
- Spacie and Hameling (1982) – Equation from Table 3.3.
- Tolls and Sijm (1995) – Equation from Table 3.3.

The data obtained are summarised graphically in the plots below. The first series of plots (Figure 4.1 to Figure 4.22) show the experimental k_1 against predicted k_1 for the entire data set. If the method results in an ideal prediction, such plots should realise a straight line with a slope of one. As can be seen for most, if not all, of the plots, the data show a large amount of scatter and only relatively poor correspondence of the predicted k_1 with the experimental k_1.

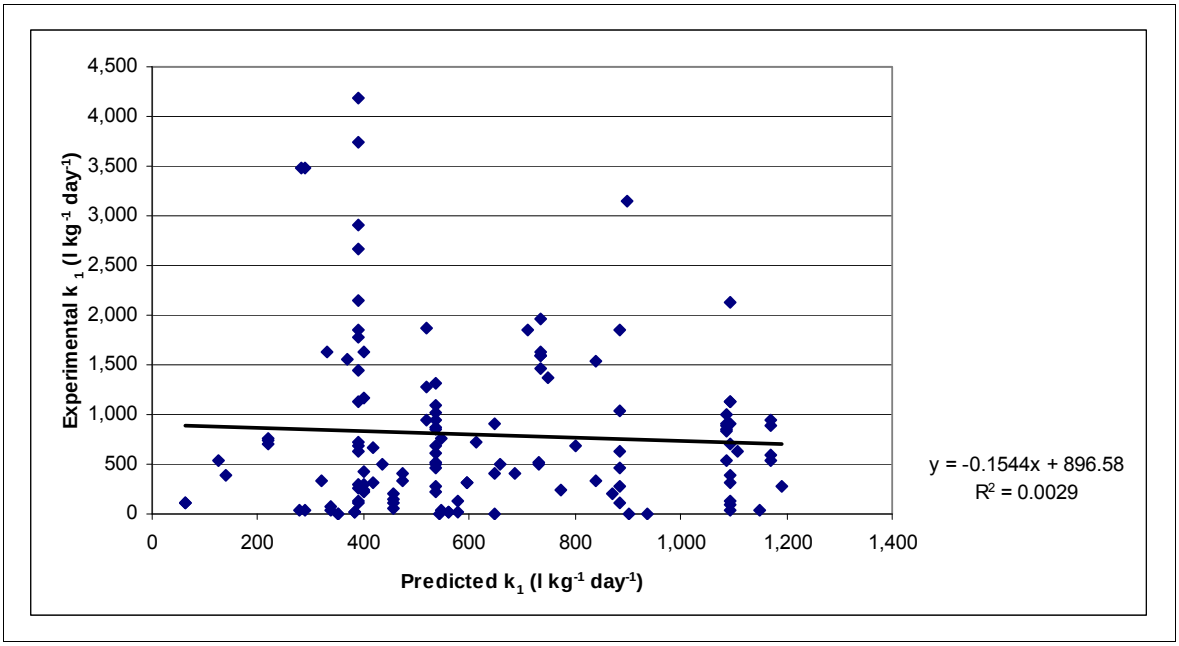

Figure 4.1 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Sijm *et al*. (1995) equation

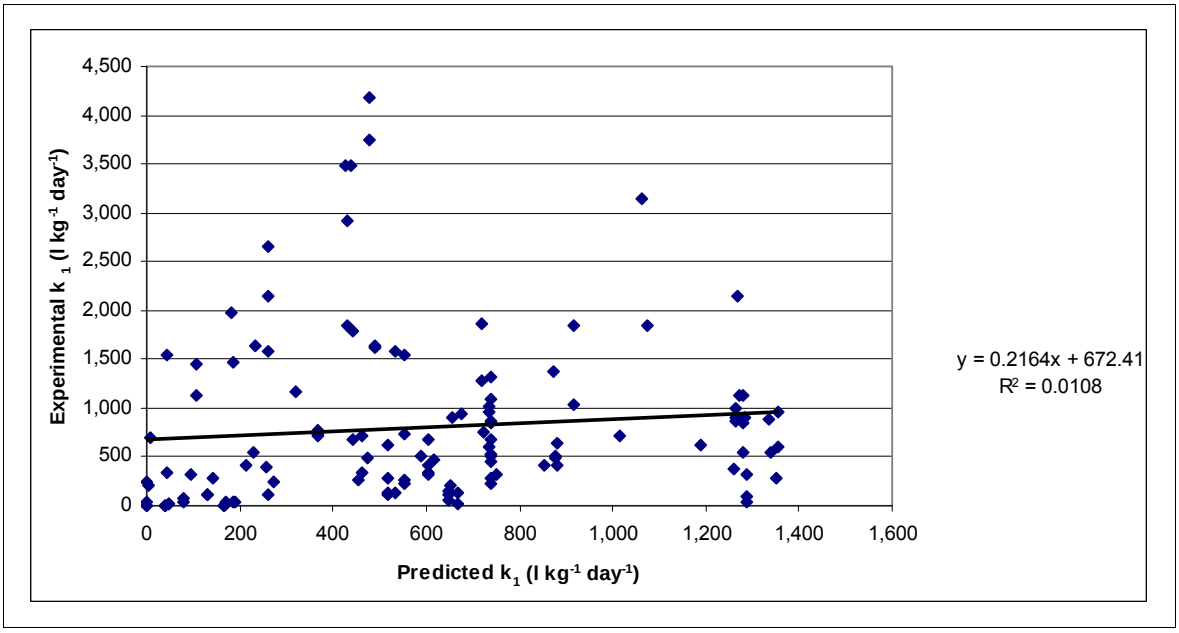

Figure 4.2 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Hendriks *et al*. (2001) equation

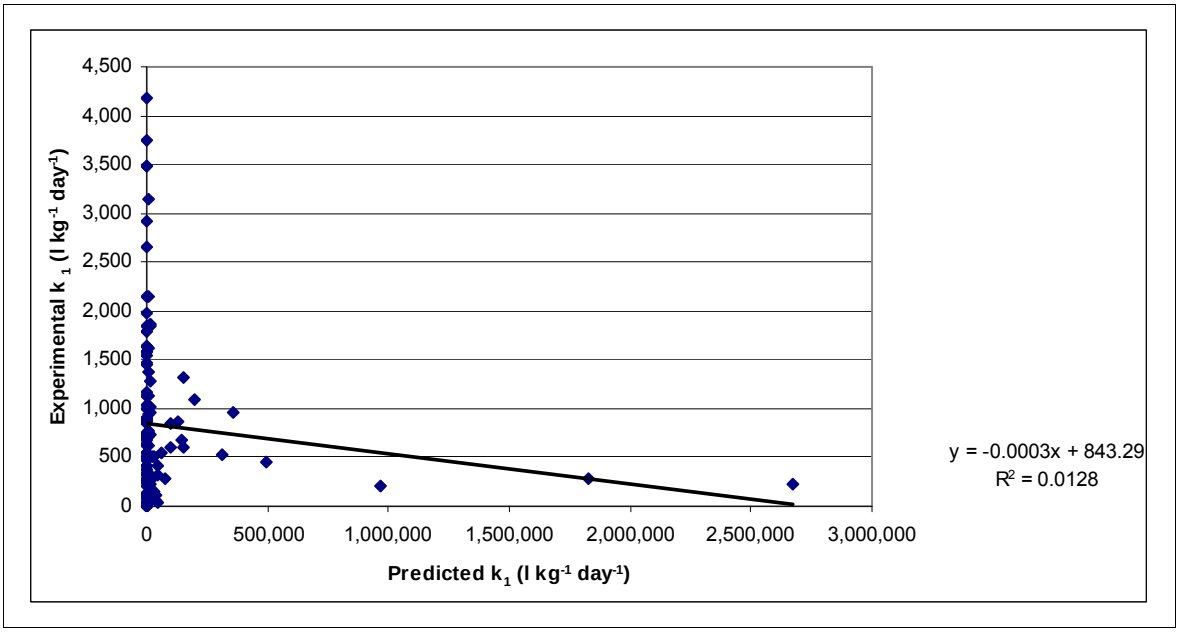

Figure 4.3 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Campfens and Mackay (1997) equation

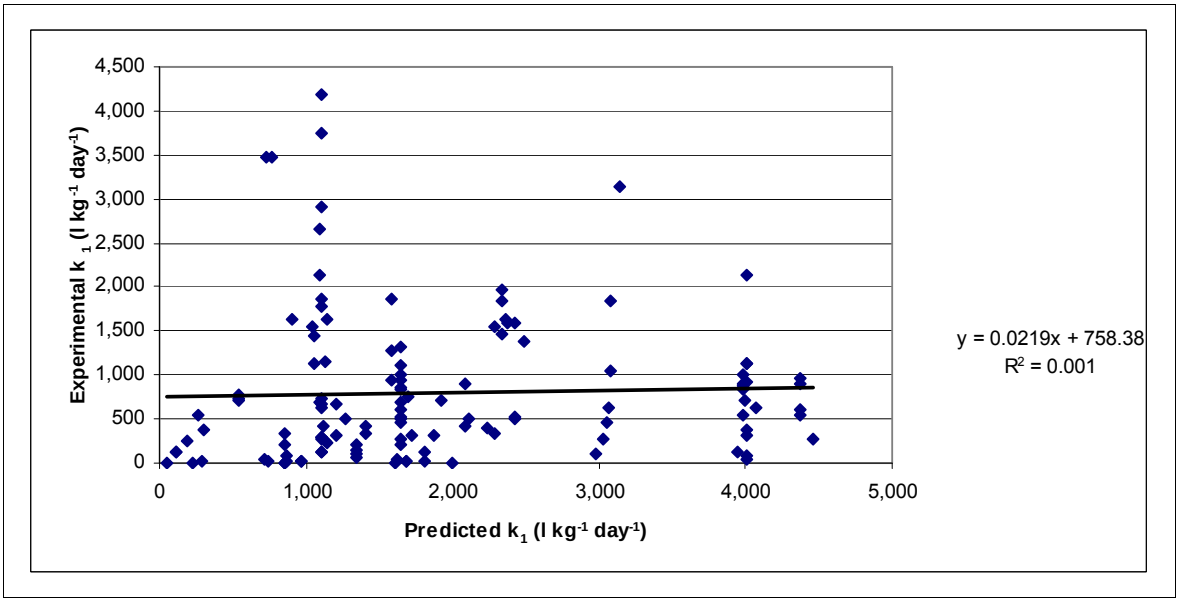

Figure 4.4 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Arnot and Gobas (2003) equation

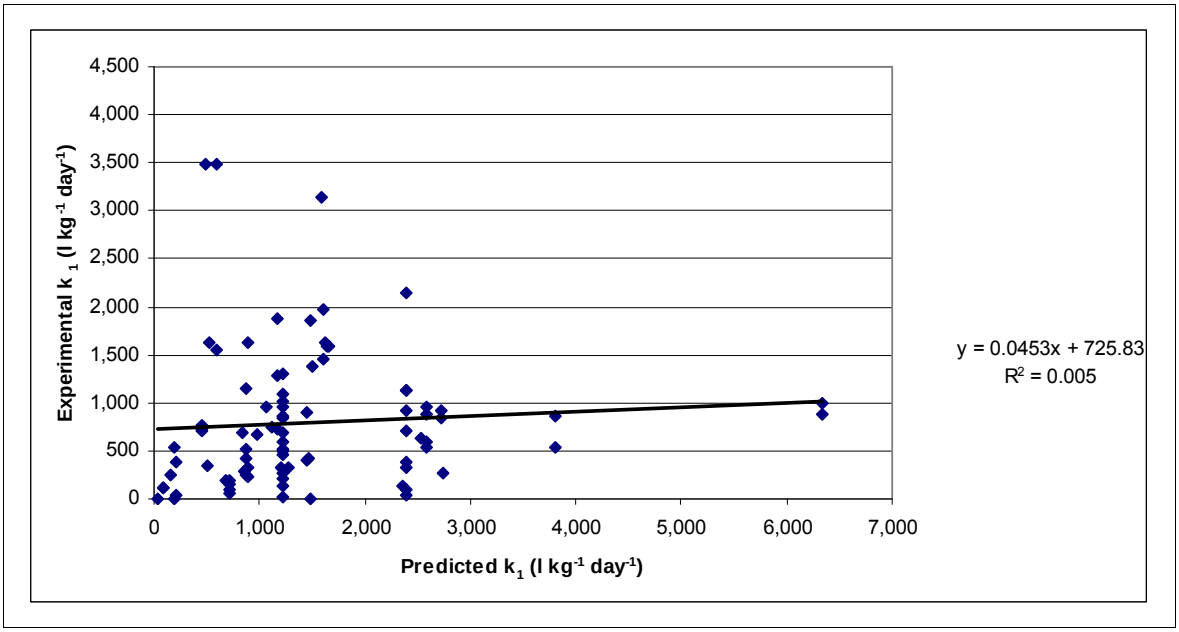

Figure 4.5 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Arnot and Gobas (2004) equation

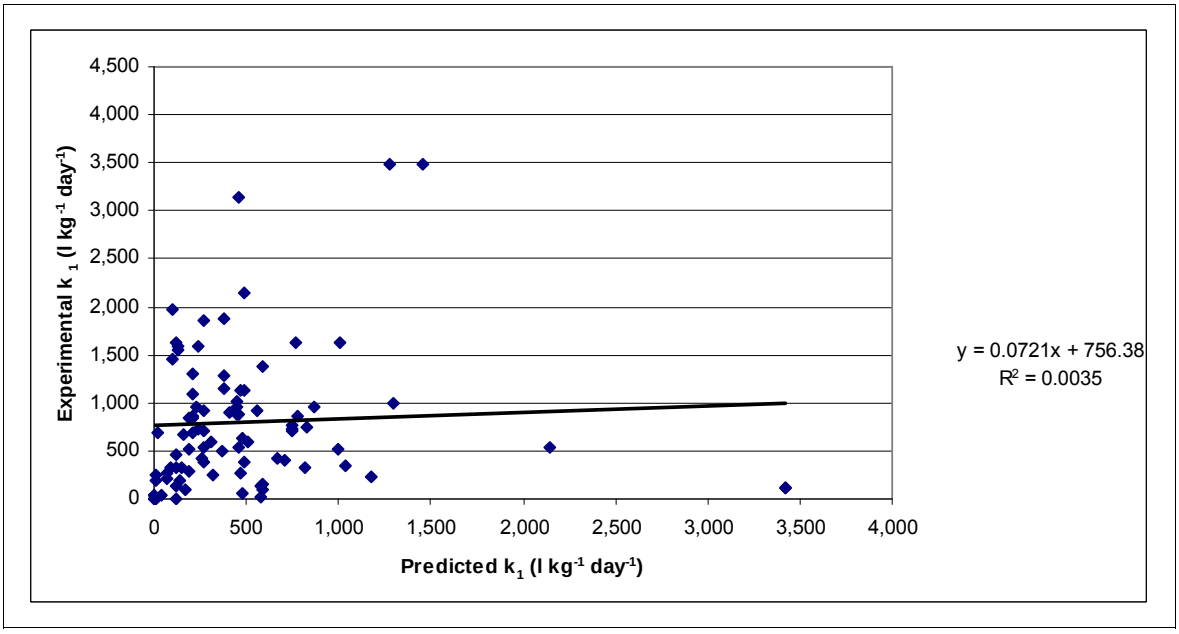

Figure 4.6 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Thomann (1989) equation

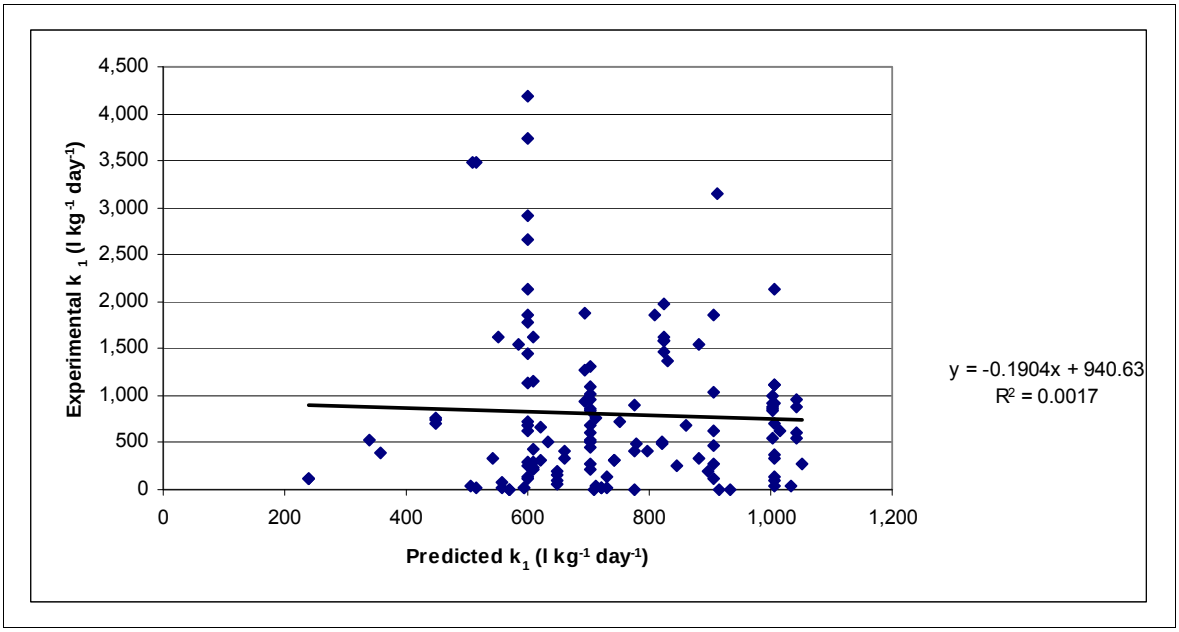

Figure 4.7 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Barber (2001) equation

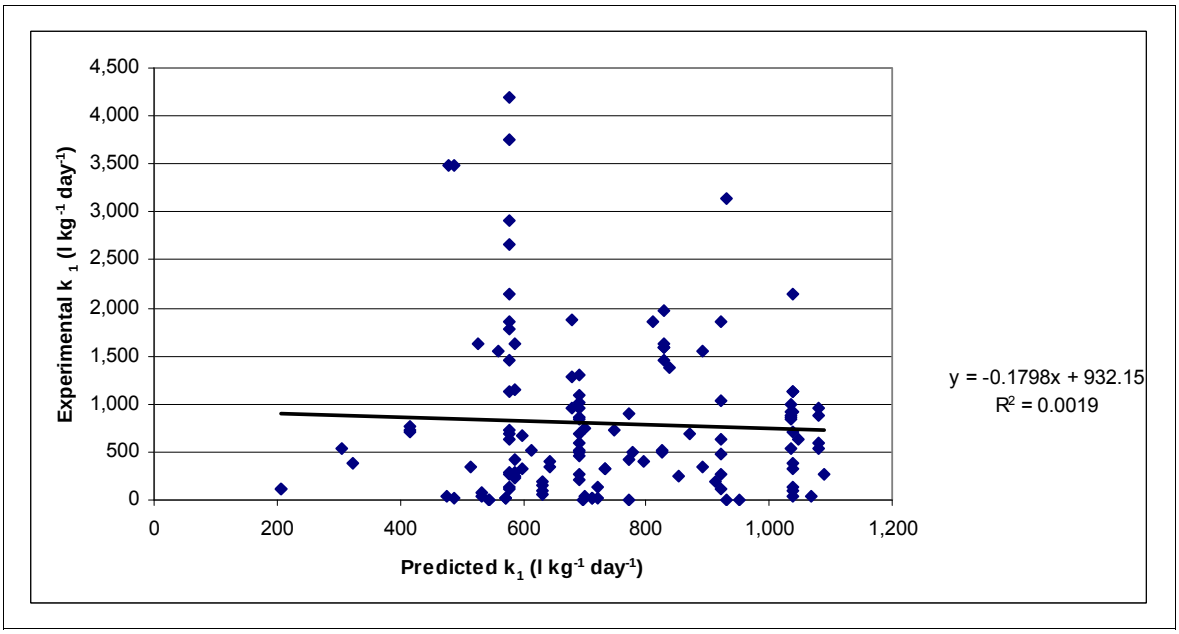

Figure 4.8 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Barber *et al*. (1991) equation

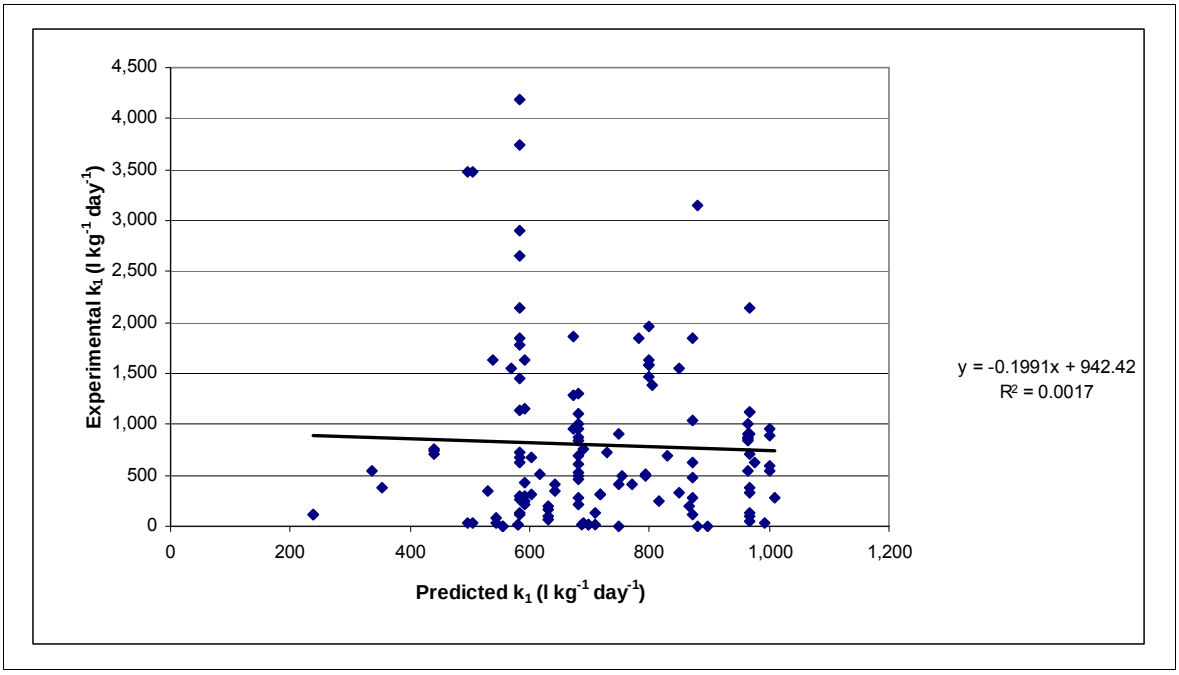

Figure 4.9 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Erickson and McKim *et al*. (1991a) equation

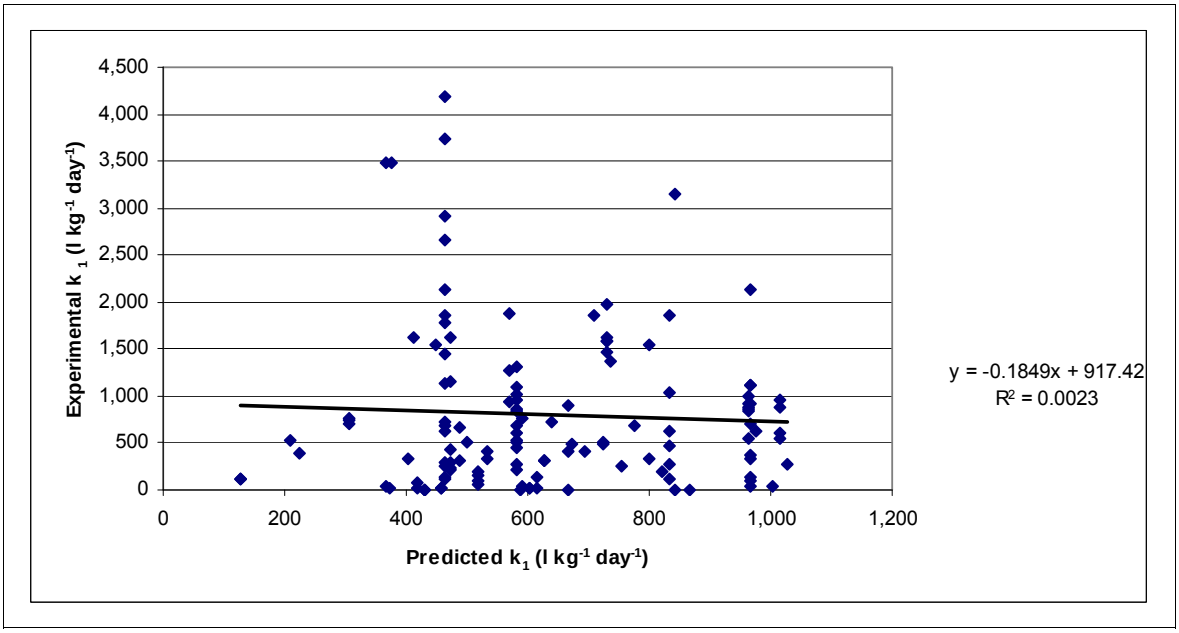

Figure 4.10 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Erickson and McKim *et al*. (1991b) equation

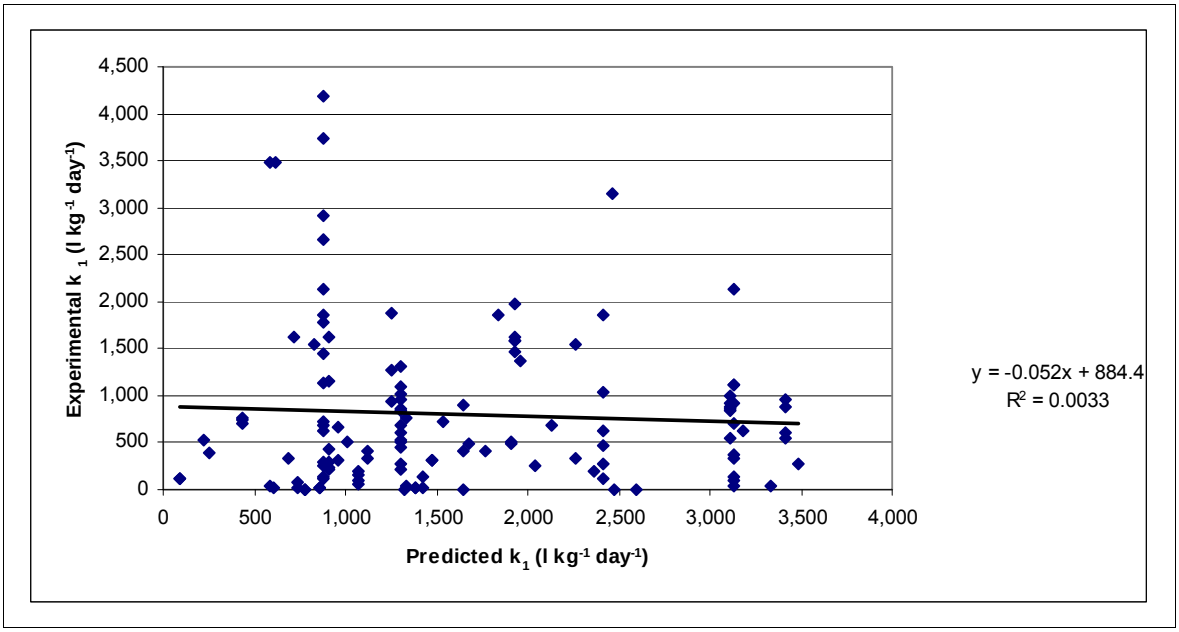

Figure 4.11 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Gobas and Mackay (1987) equation

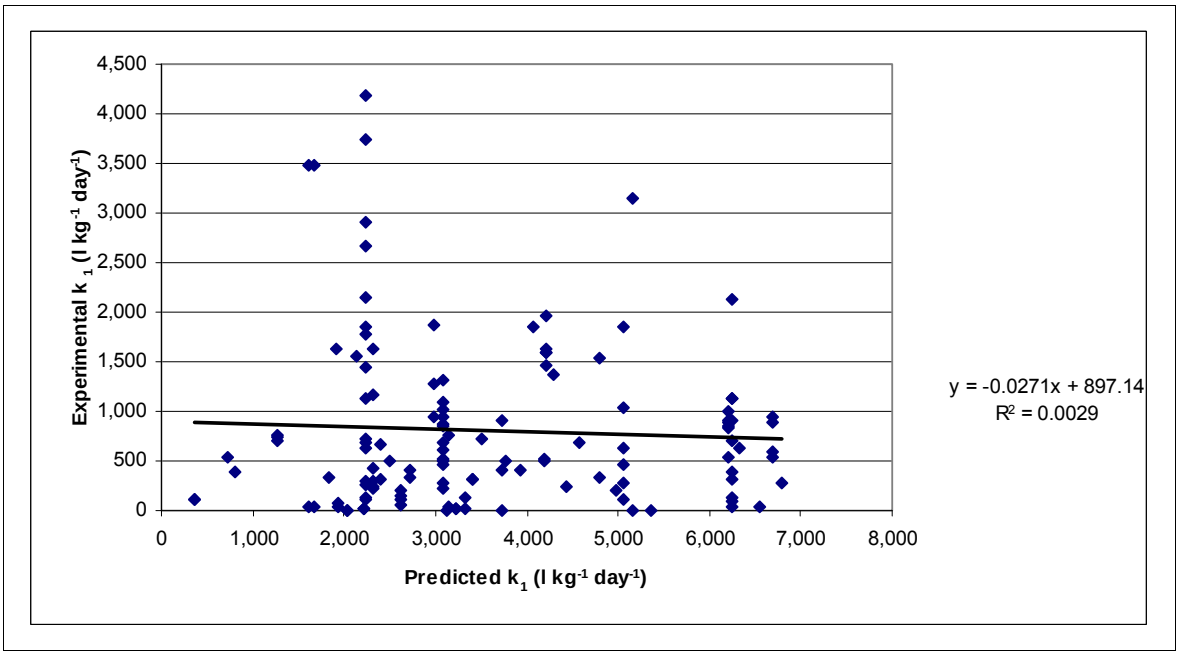

Figure 4.12 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Gobas *et al*. (1986) equation

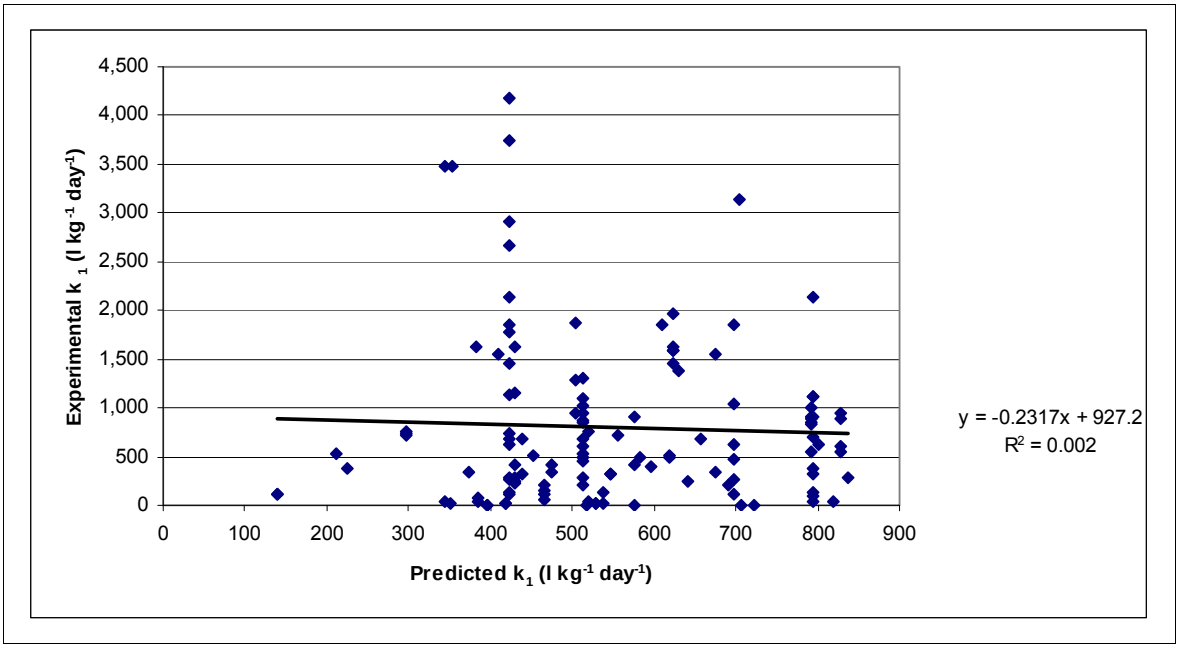

Figure 4.13 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Hayton and Barron (1990) equation

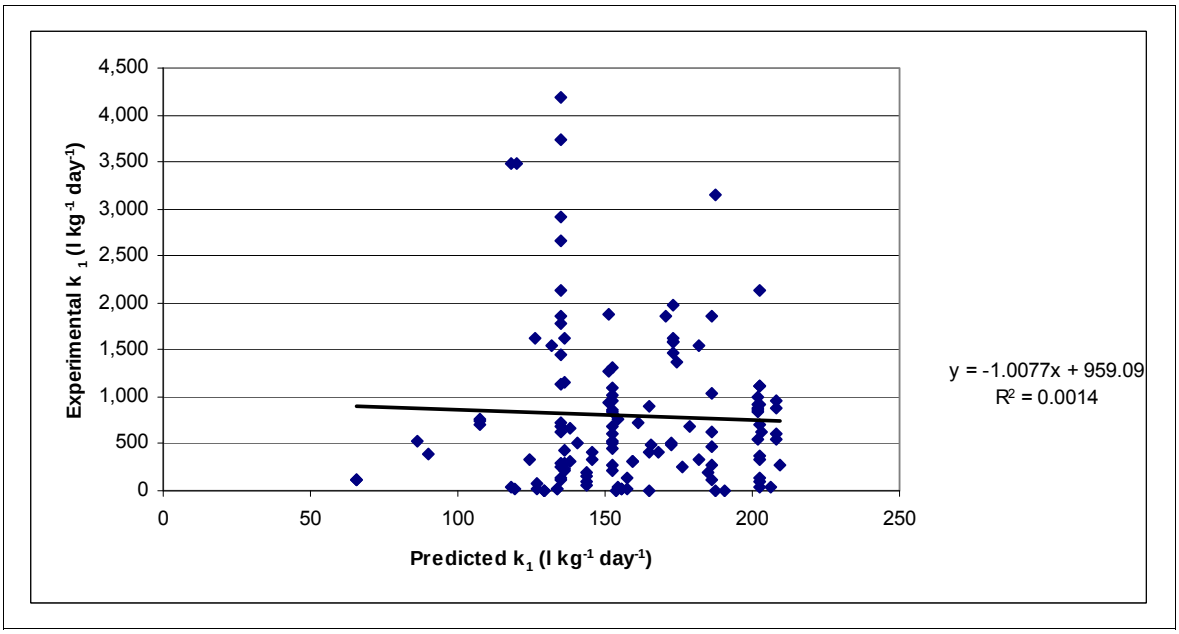

Figure 4.14 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Norstrom *et al*. (1976) equation

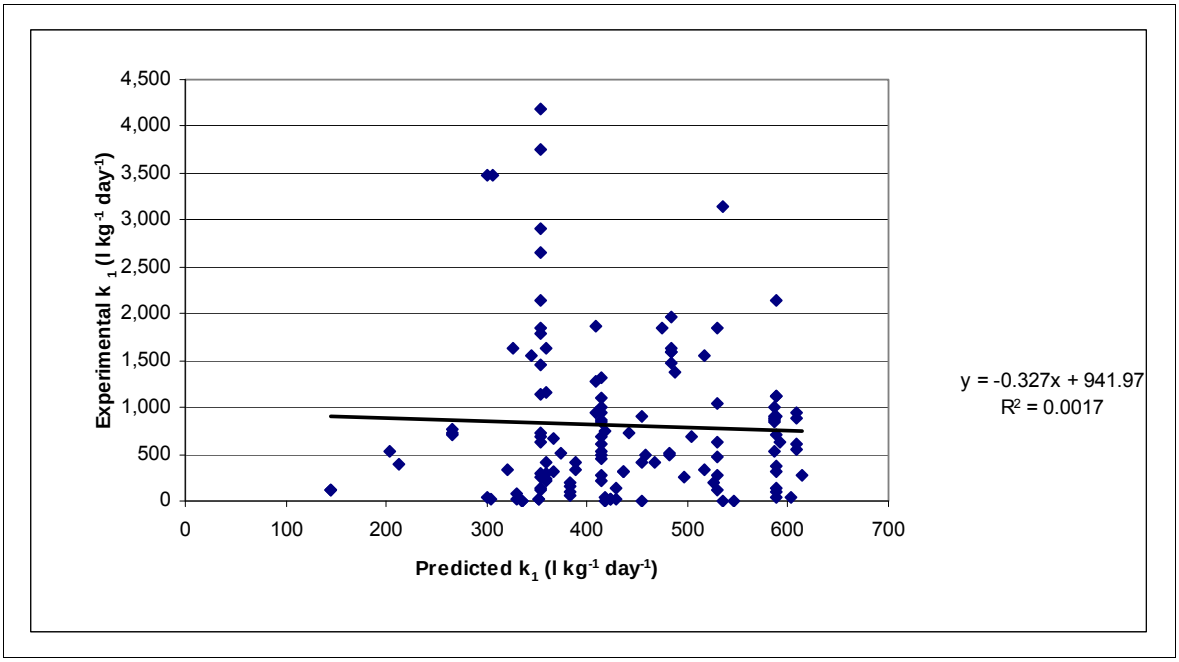

Figure 4.15 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Streit and Sire (1993) equation

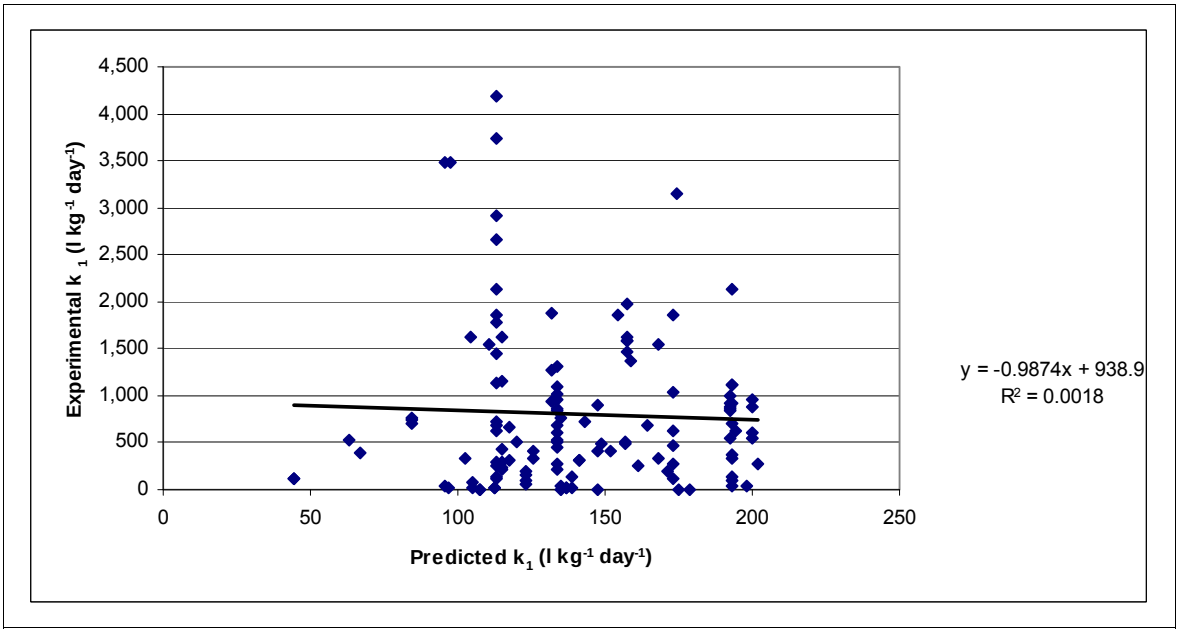

Figure 4.16 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Thomann and Connolly (1984) equation

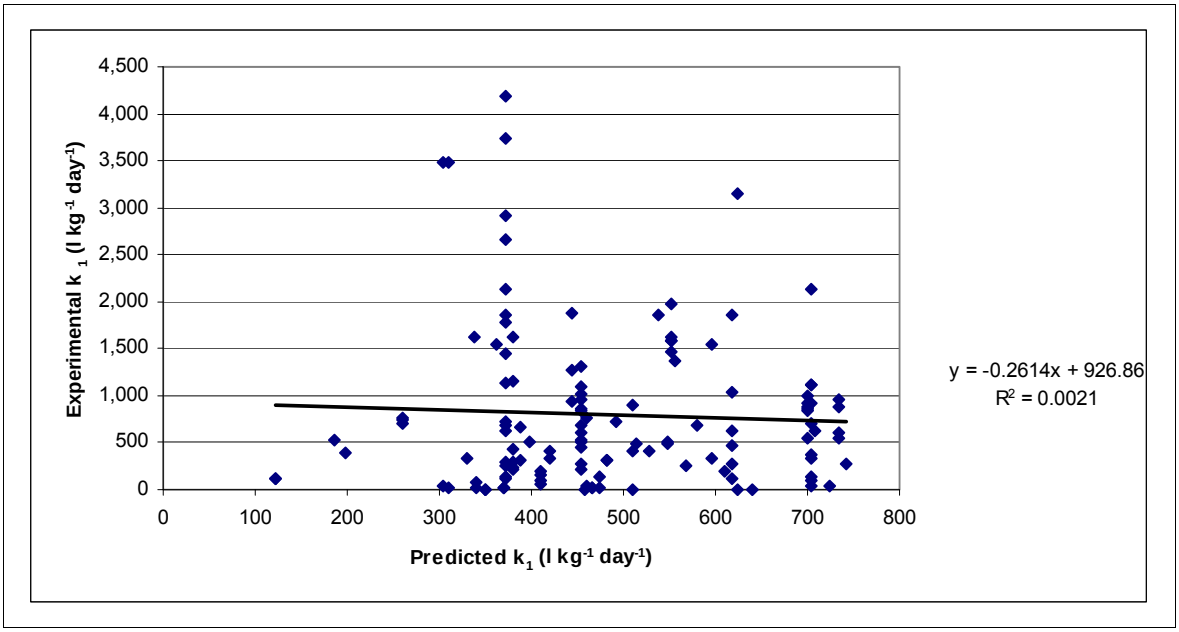

Figure 4.17 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Barber (2003) observed equation

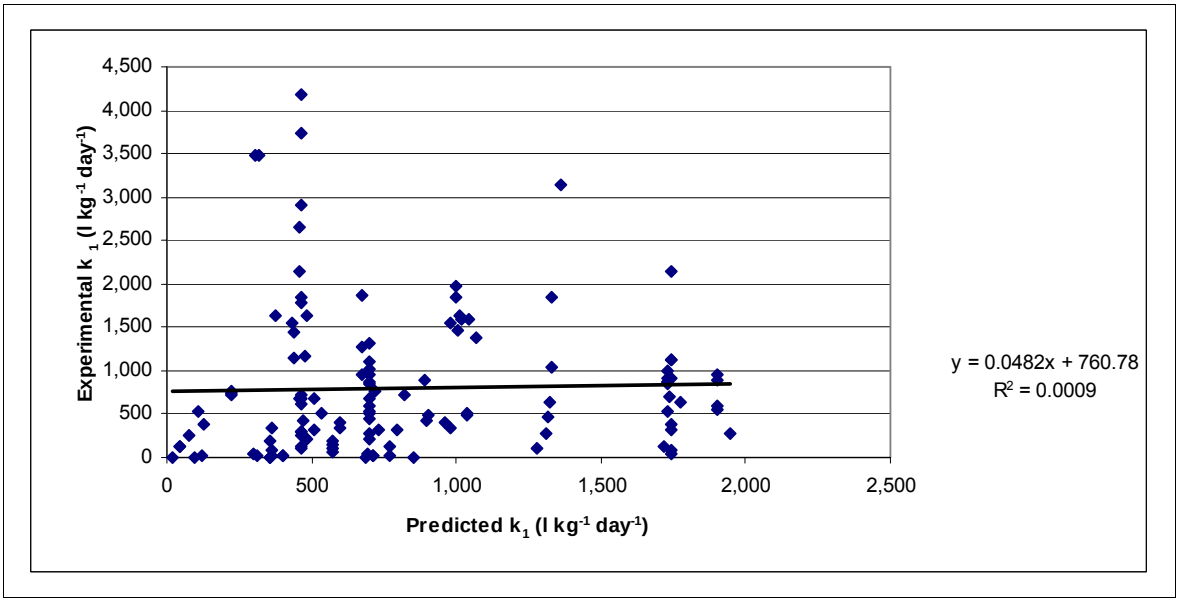

Figure 4.18 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Barber (2003) calibrated equation

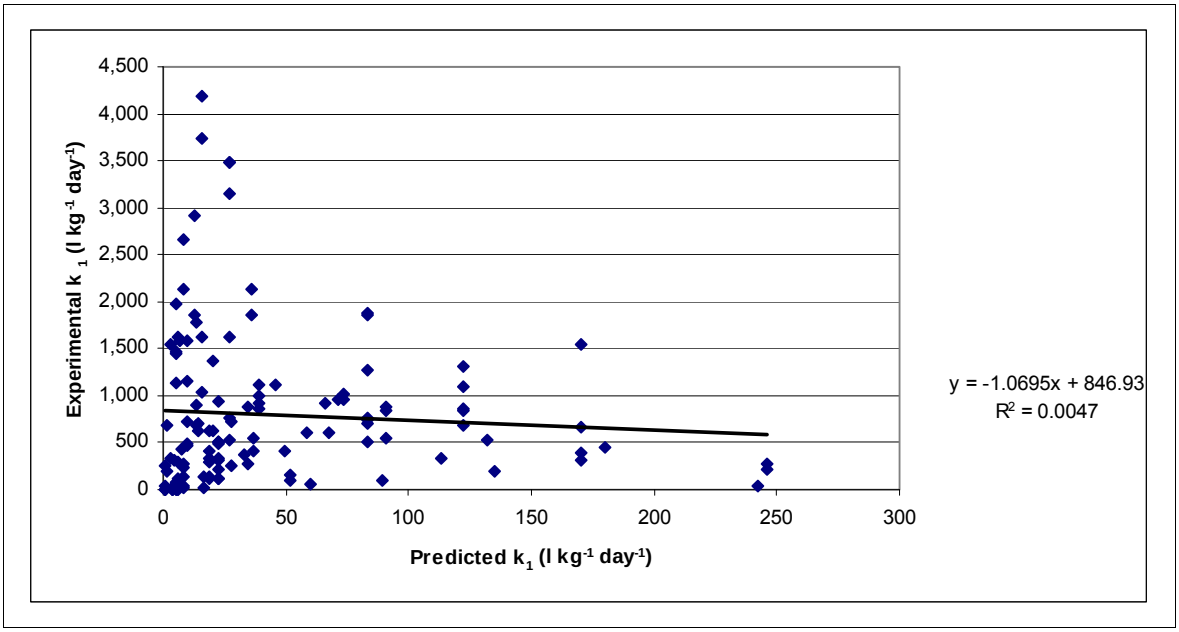

Figure 4.19 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Hawker and Connolly (1985) equation

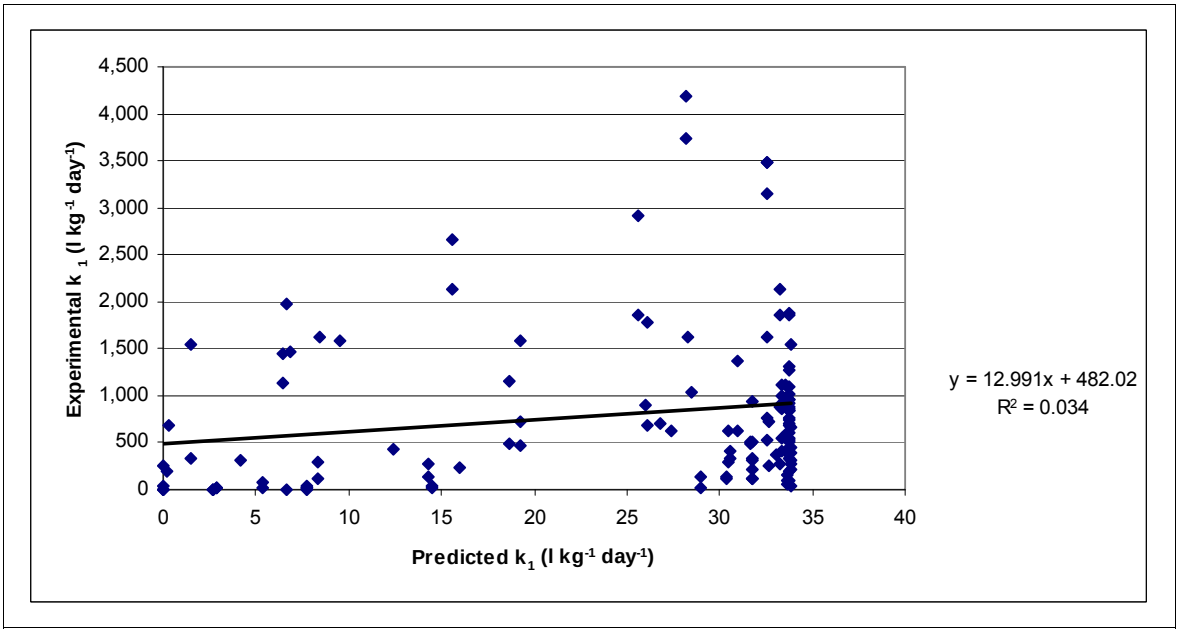

Figure 4.20 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Hawker and Connolly (1988) equation

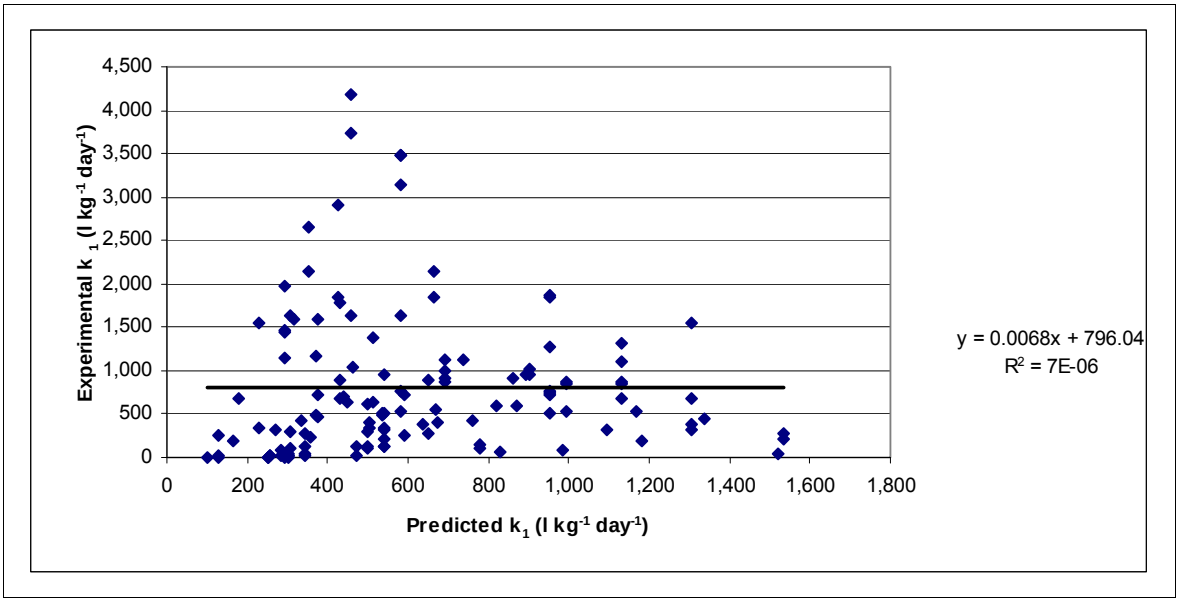

Figure 4.21 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Spacie and Hamelink (1982) equation

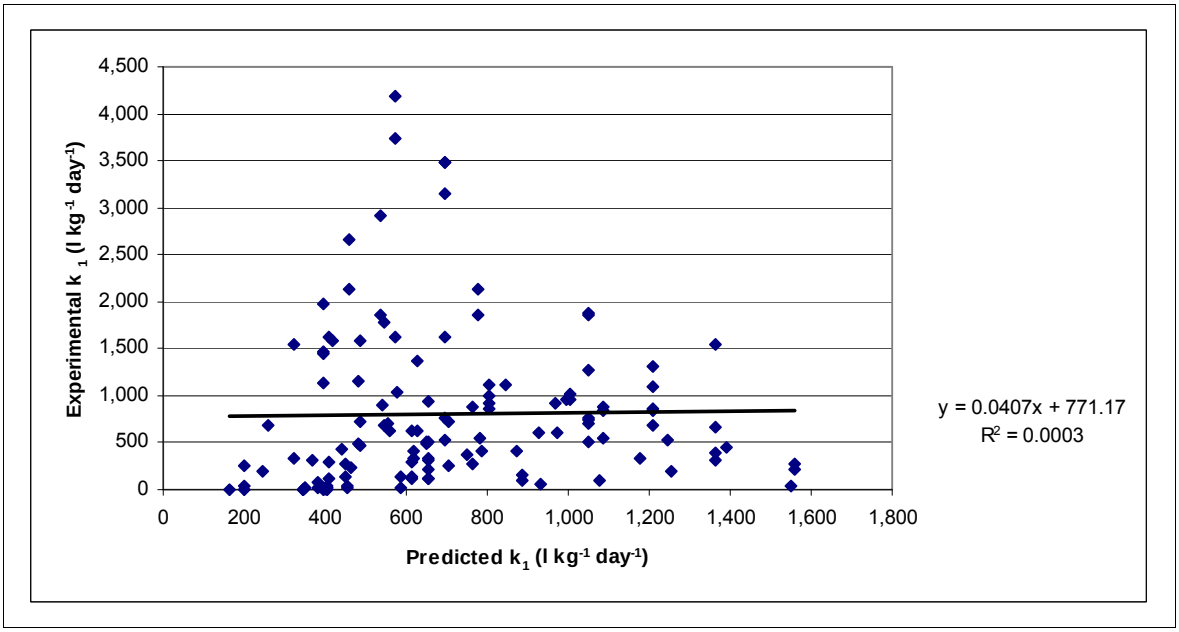

Figure 4.22 Comparison of experimental k_1 with predicted k_1 based on initial fish weight for the whole data set using the Tolls and Sijm (1995) equation

To compare further the predictive ability of each of the approaches, the ratio of the predicted k_1 to the experimental k_1 value was determined (data given in Appendix A). A ratio greater than one indicates that the method overestimates k_1, and a ratio below one indicates that the method underestimates k_1. The variation of these ratios with log K_{ow} are shown in Figure 4.23 to Figure 4.44 below (horizontal lines indicate the region where the predicted k_1 value is within a factor of two of the experimental k_1 value).

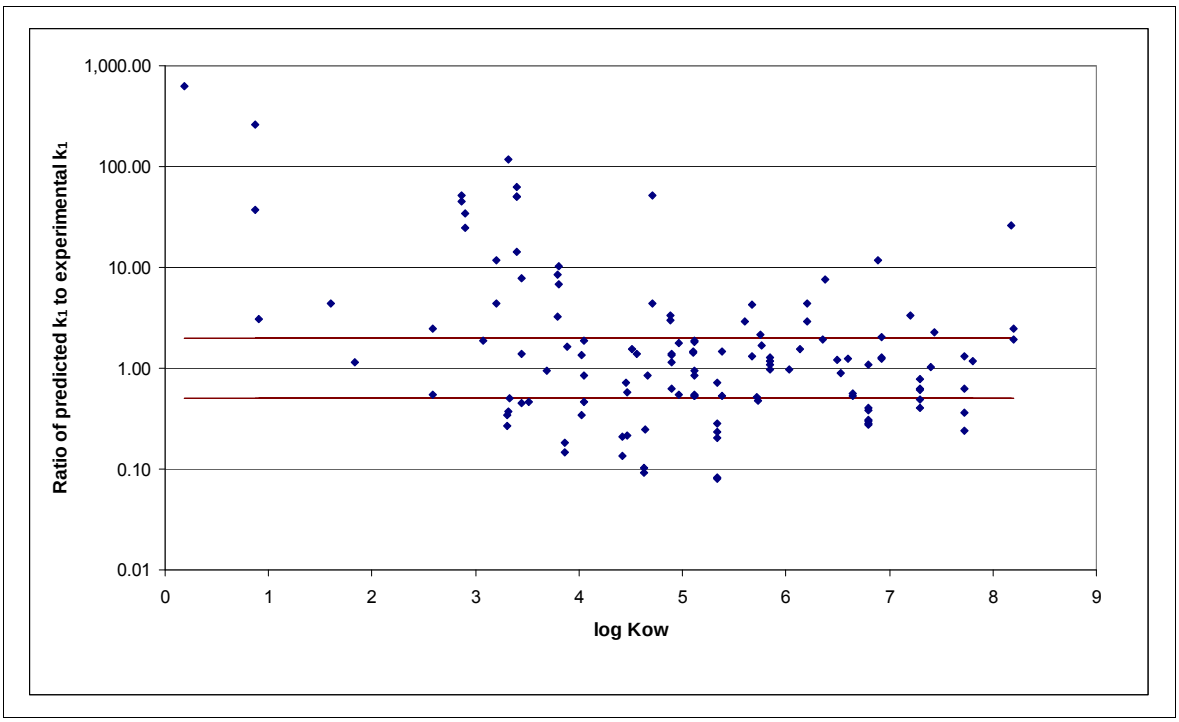

Figure 4.23 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Sijm *et al*. (1995) equation based on initial fish weights

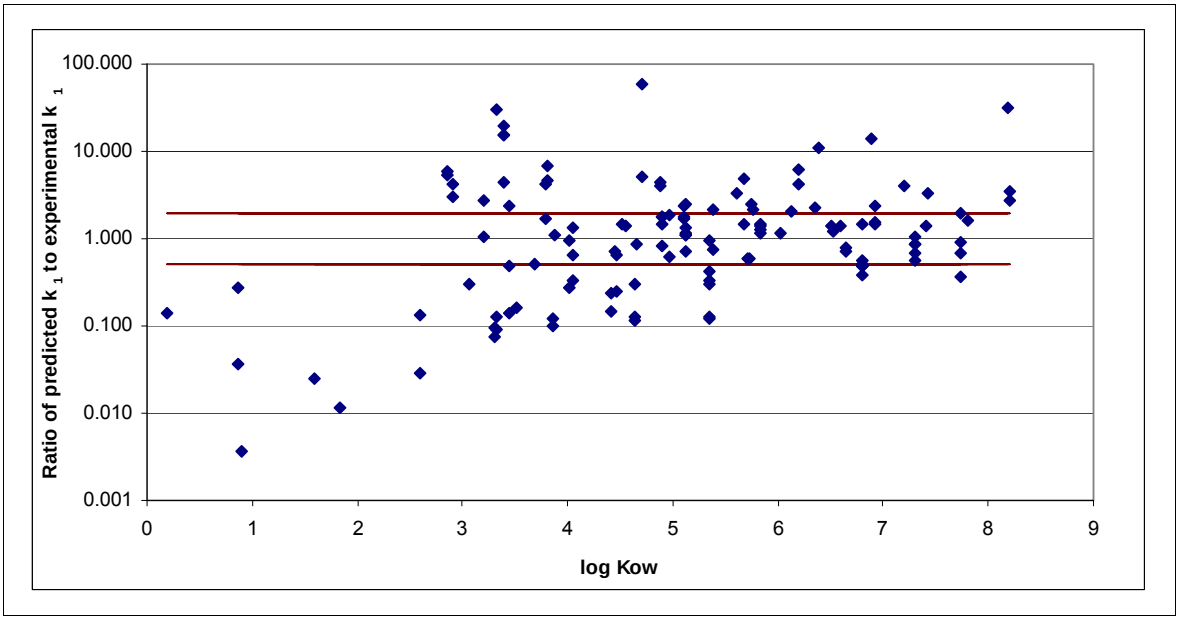

Figure 4.24 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Hendriks *et al*. (2001) equation based on initial fish weights

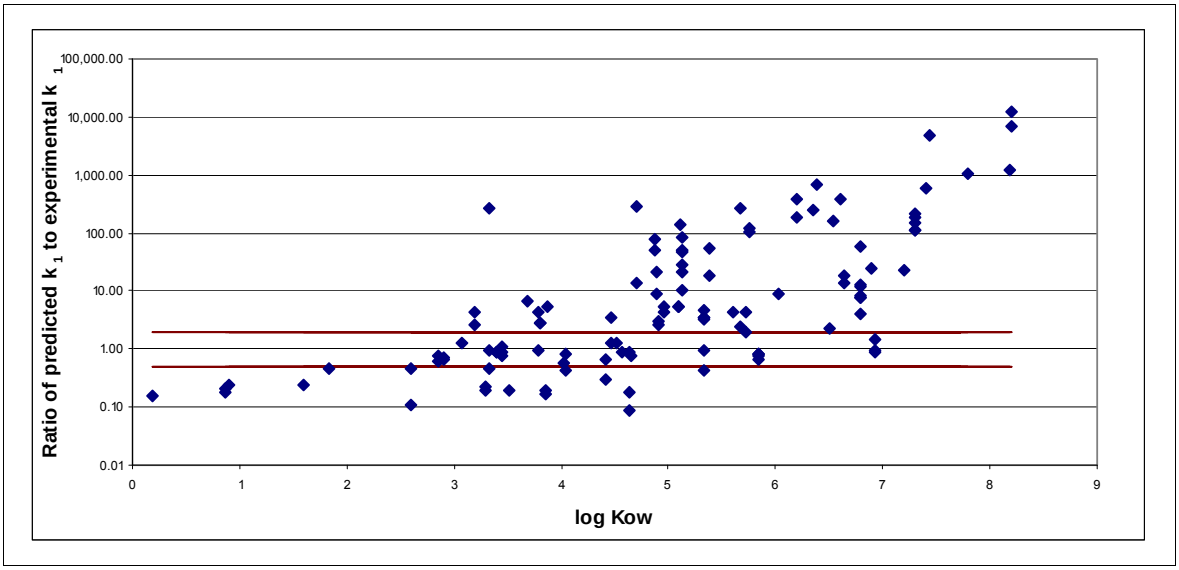

Figure 4.25 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Campfens and Mackay (1997) equation based on initial fish weights

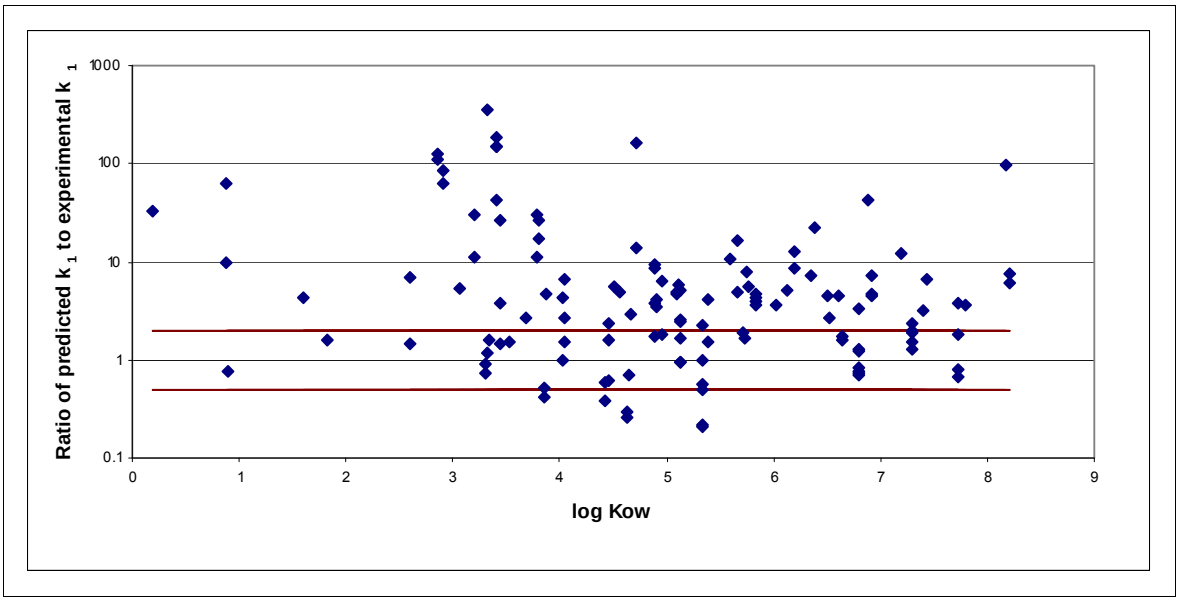

Figure 4.26 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Arnot and Gobas (2003) equation based on initial fish weights

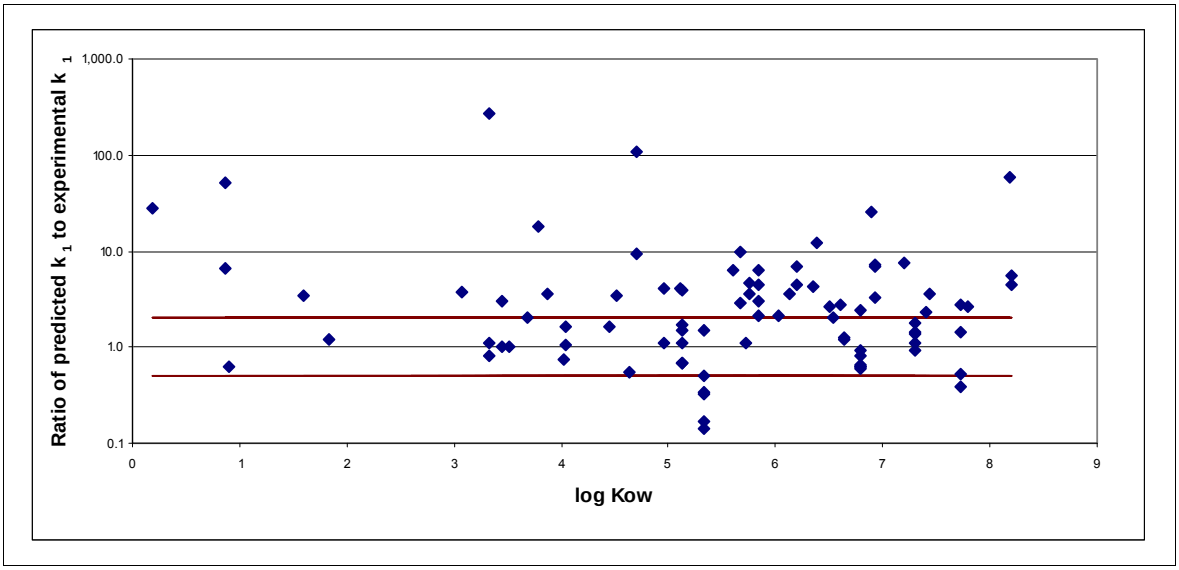

Figure 4.27 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Arnot and Gobas (2004) equation based on initial fish weights

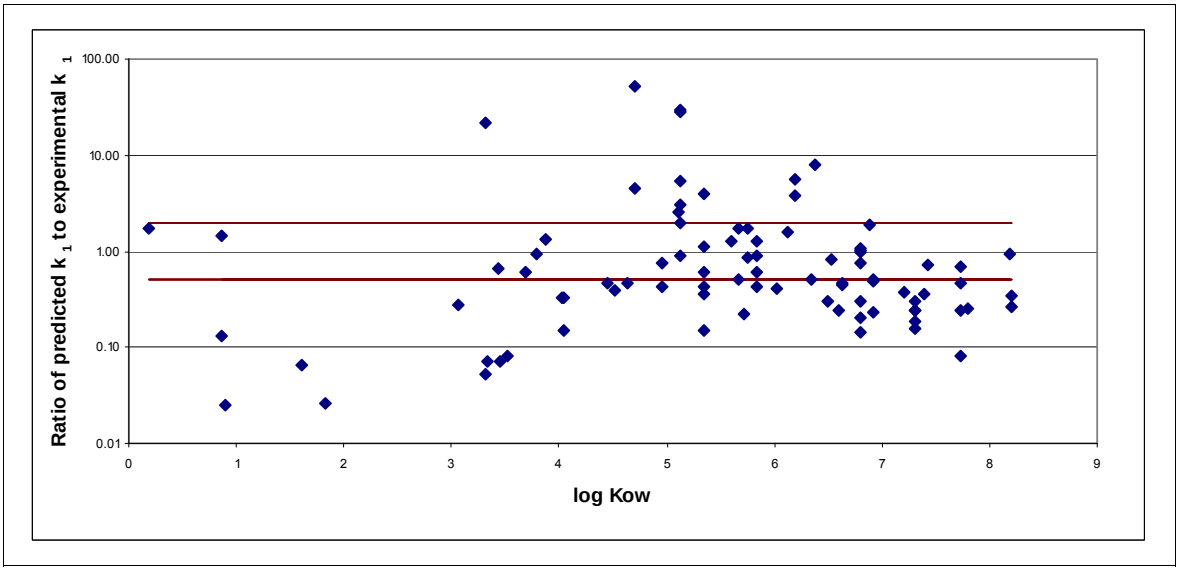

Figure 4.28 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Thomann (1989) equation based on initial fish weights

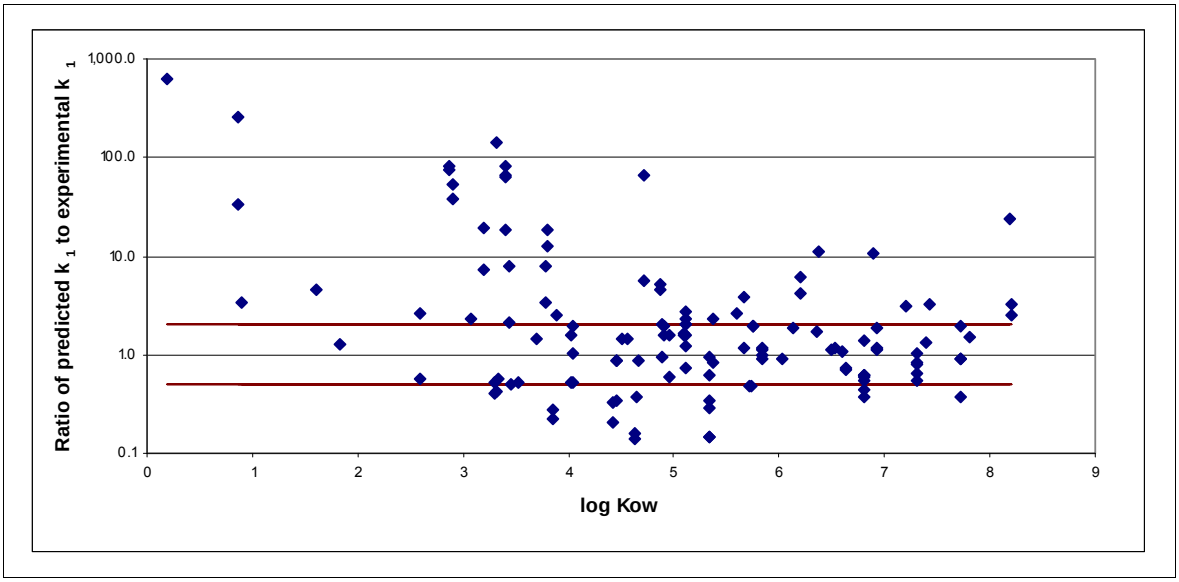

Figure 4.29 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Barber (2001) equation based on initial fish weights

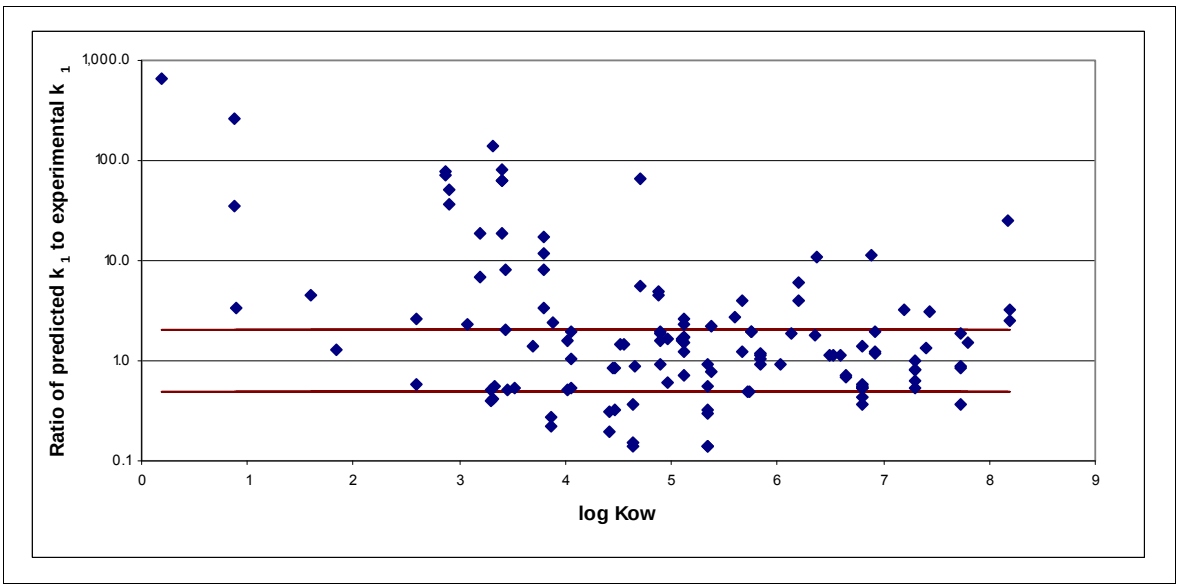

Figure 4.30 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Barber *et al*. (1991) equation based on initial fish weights

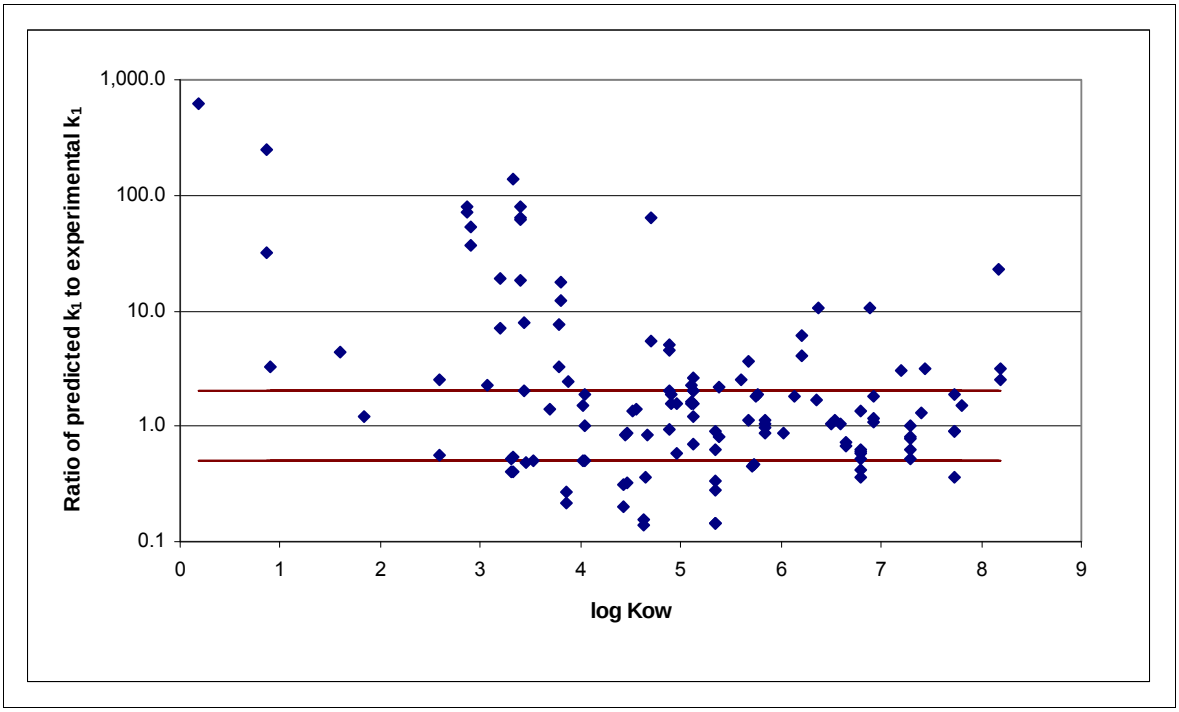

Figure 4.31 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Erickson and McKim (1991a) equation based on initial fish weights

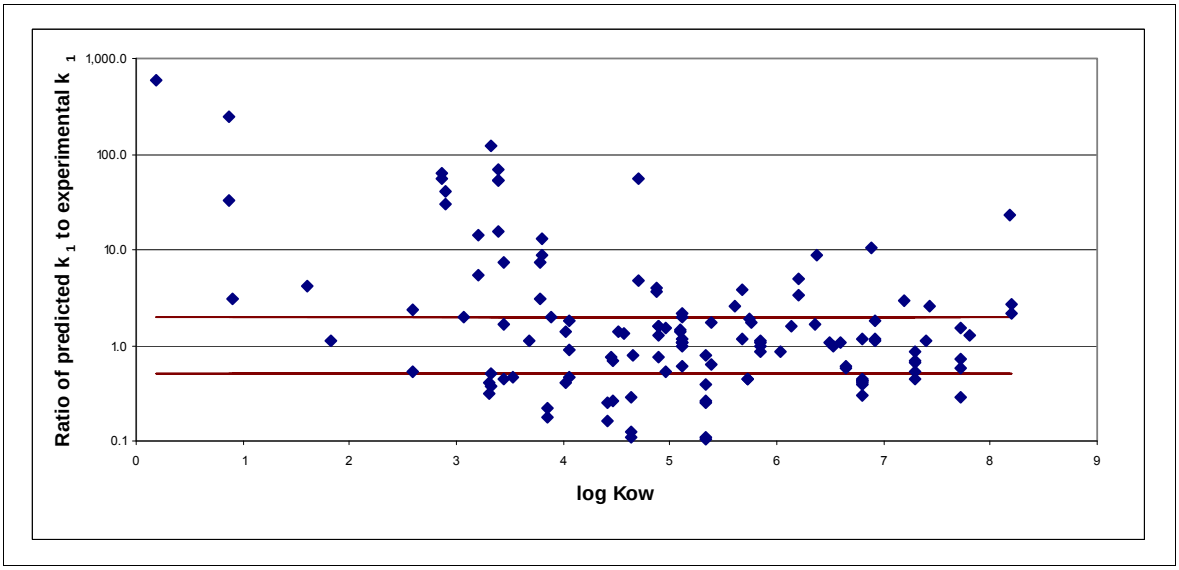

Figure 4.32 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Erickson and McKim (1991b) equation based on initial fish weights

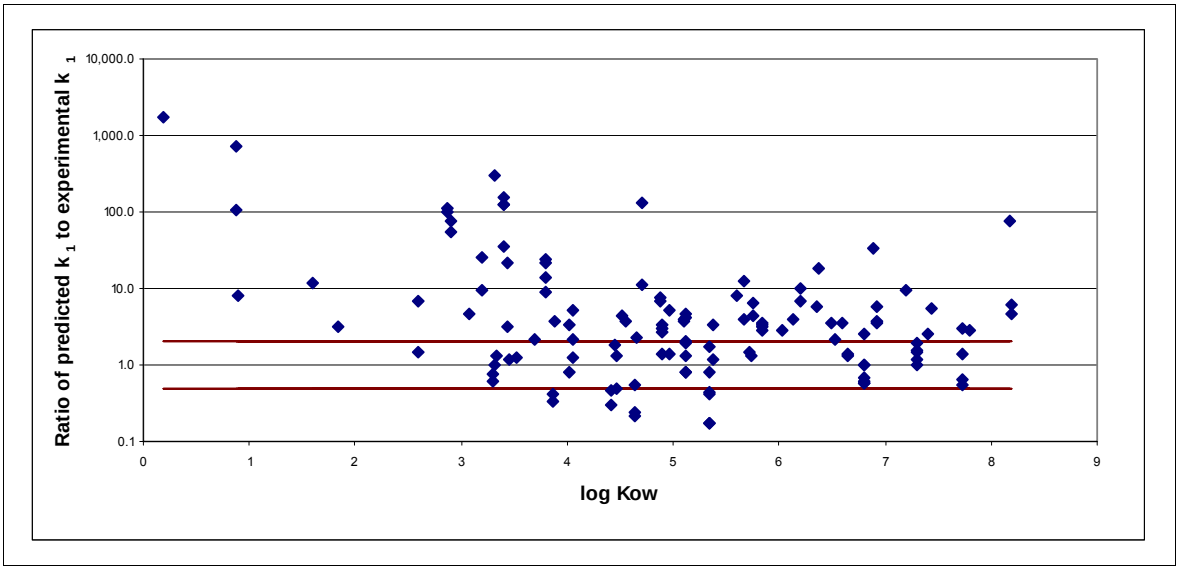

Figure 4.33 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Gobas and Mackay (1987) equation based on initial fish weights

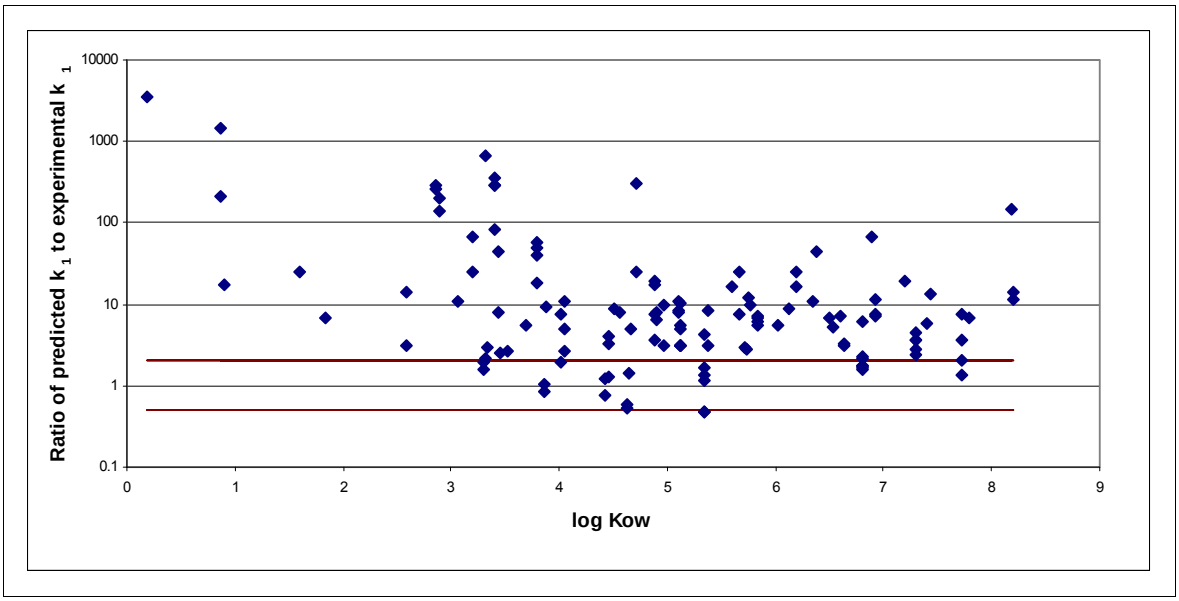

Figure 4.34 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Gobas and Mackay (1987) equation based on initial fish weights

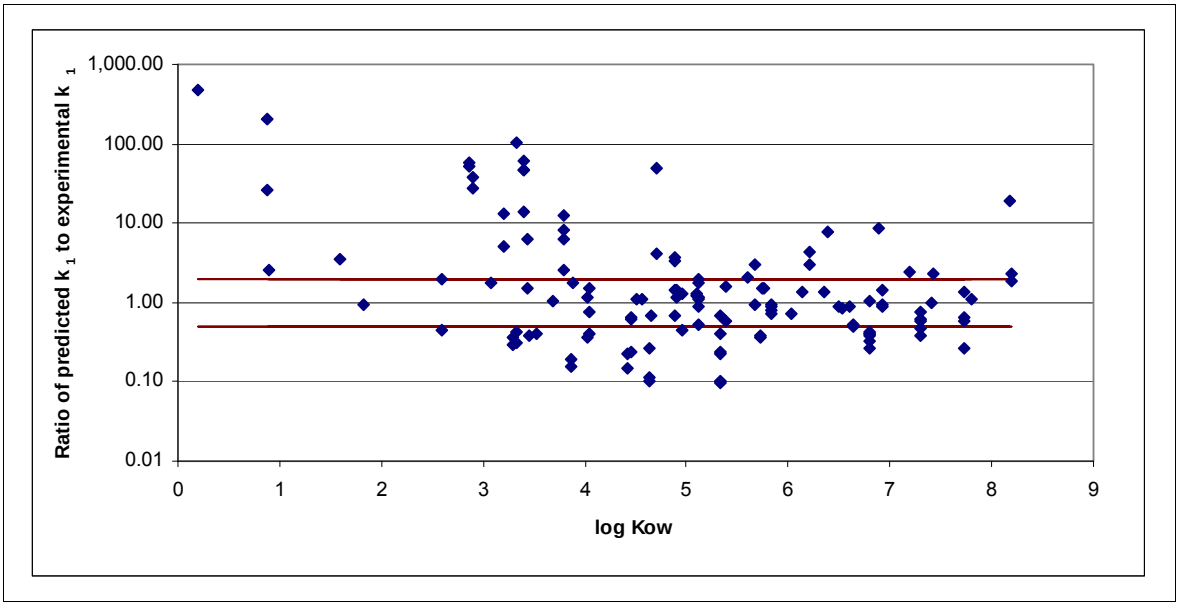

Figure 4.35 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Hayton and Barron (1990) equation based on initial fish weights

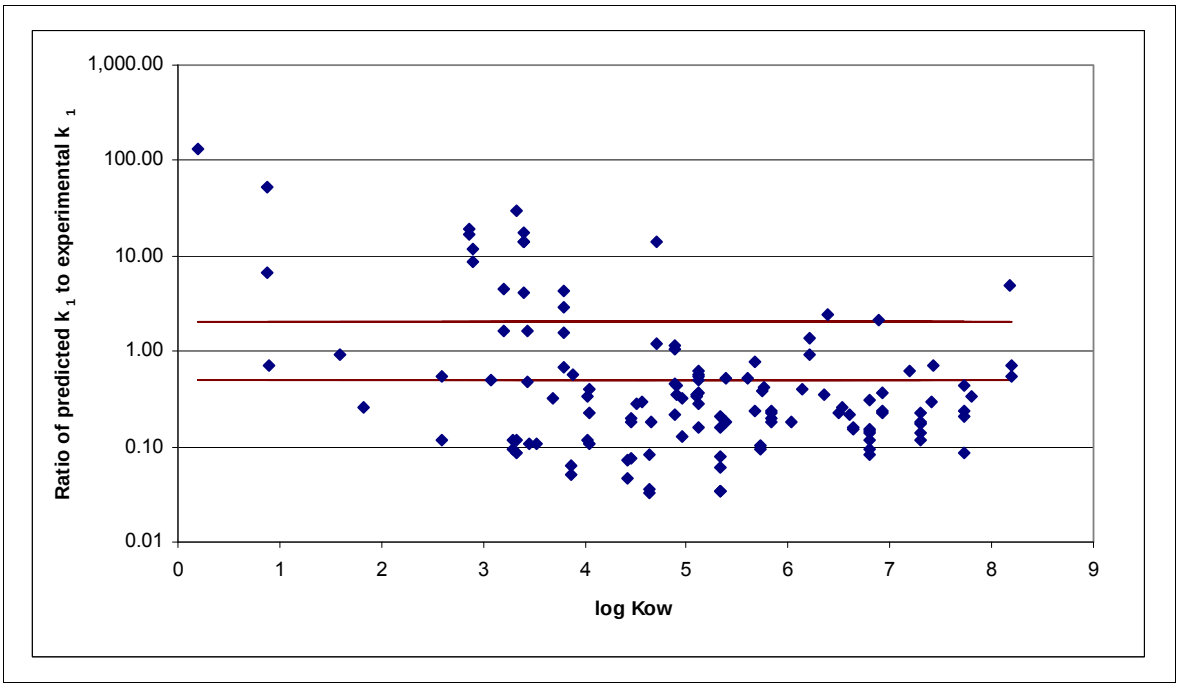

Figure 4.36 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Norstrom *et al*. (1976) equation based on initial fish weights

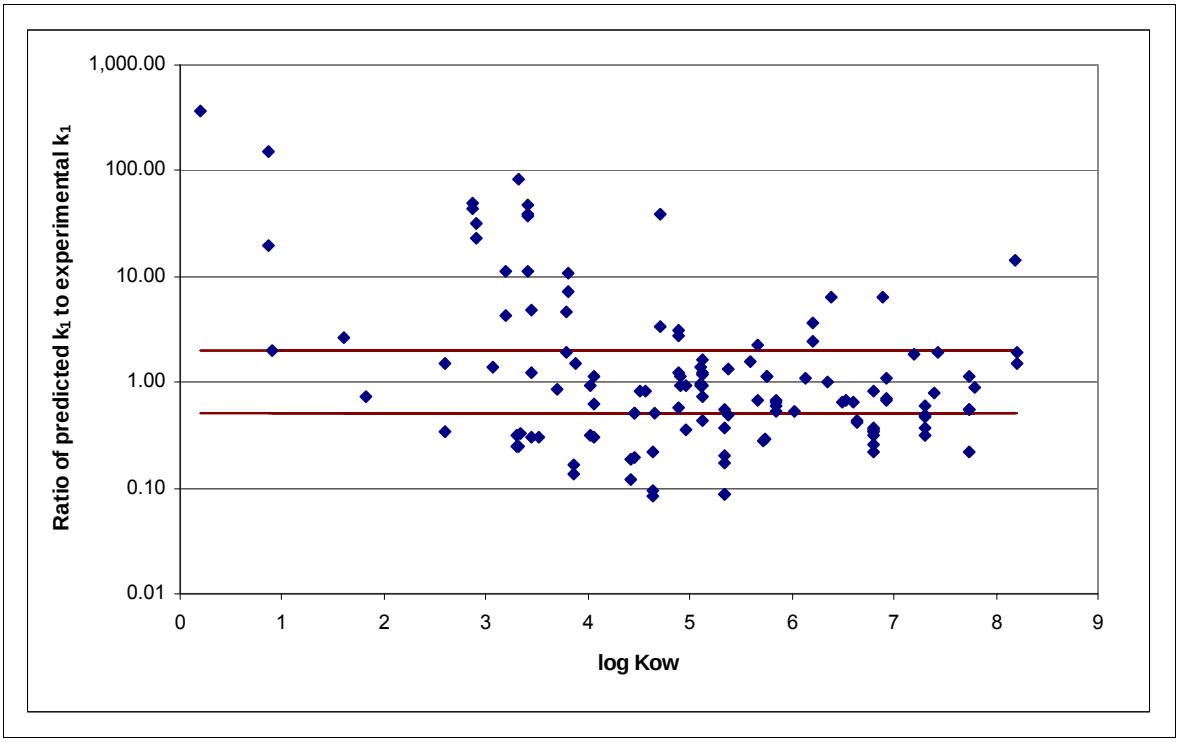

Figure 4.37 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Streit and Sire (1993) equation based on initial fish weights

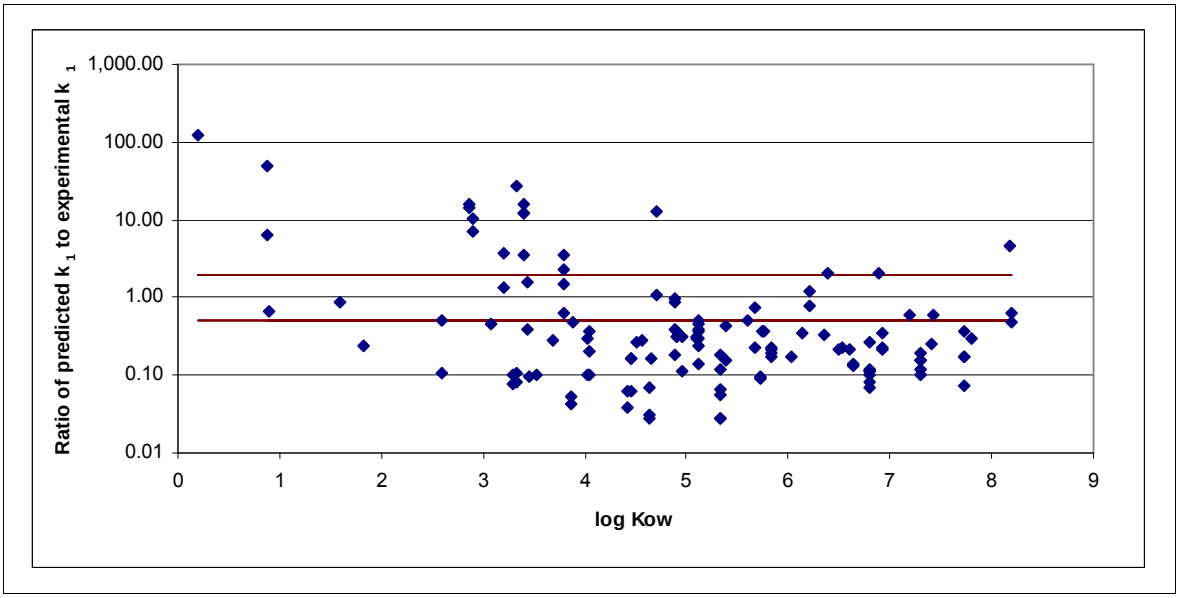

Figure 4.38 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Thomann and Connolly (1984) equation based on initial fish weights

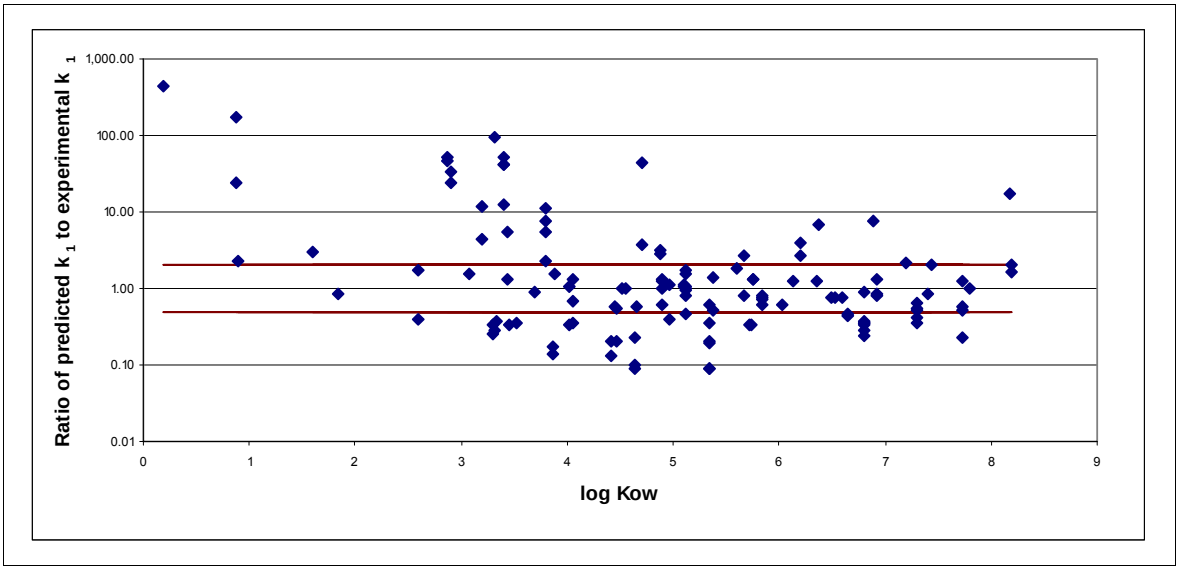

Figure 4.39 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Barber (2003) observed equation based on initial fish weights

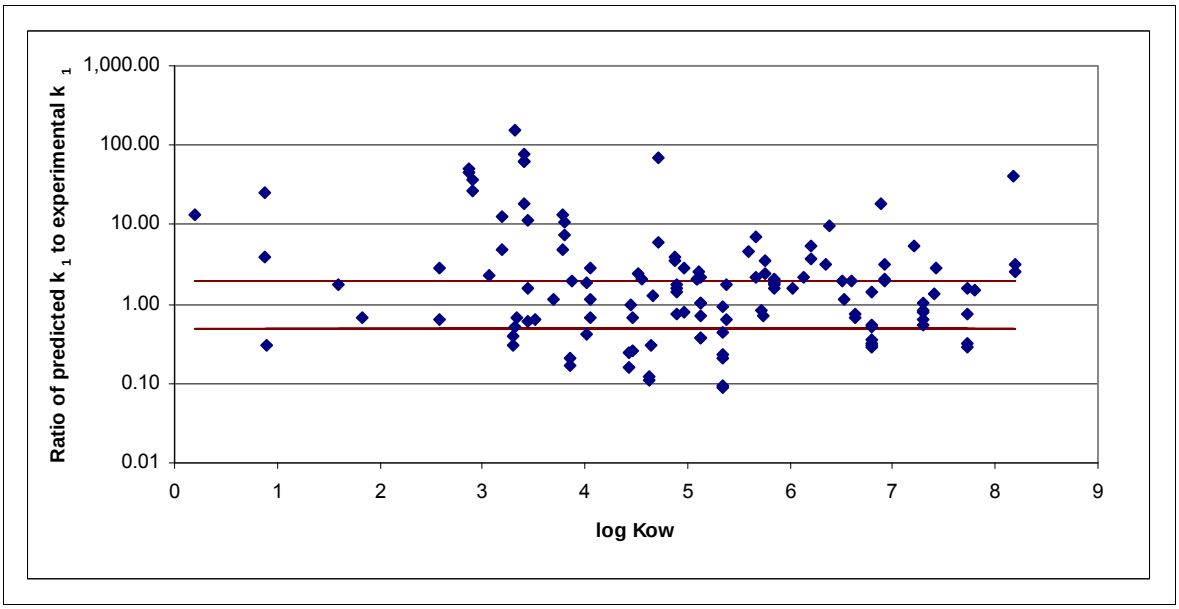

Figure 4.40 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Barber (2003) calibrated equation based on initial fish weights

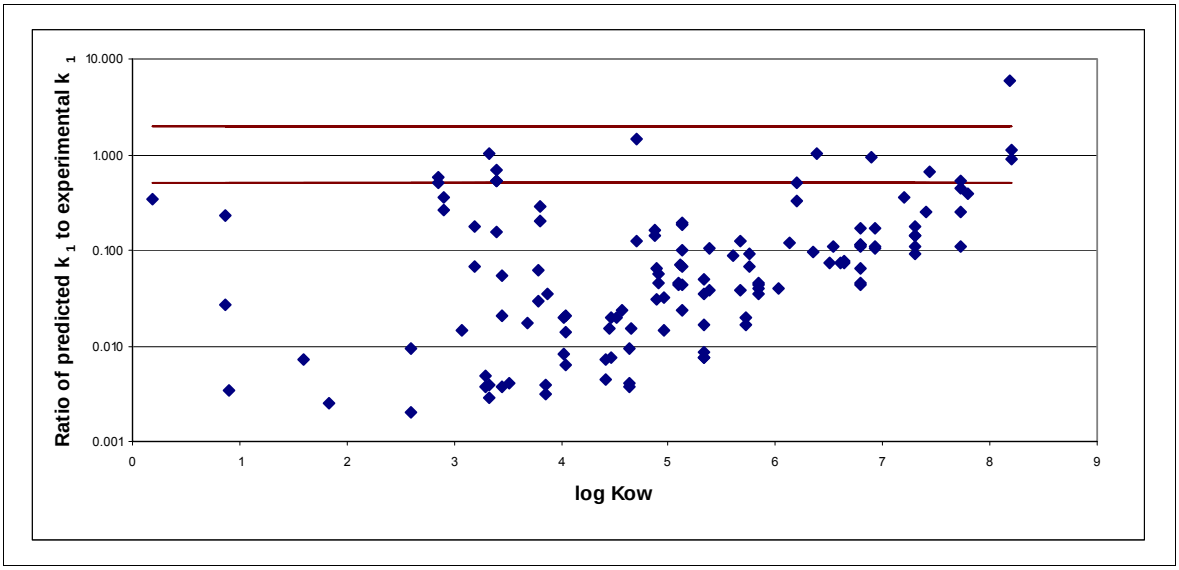

Figure 4.41 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Hawker and Connell (1985) equation based on initial fish weights

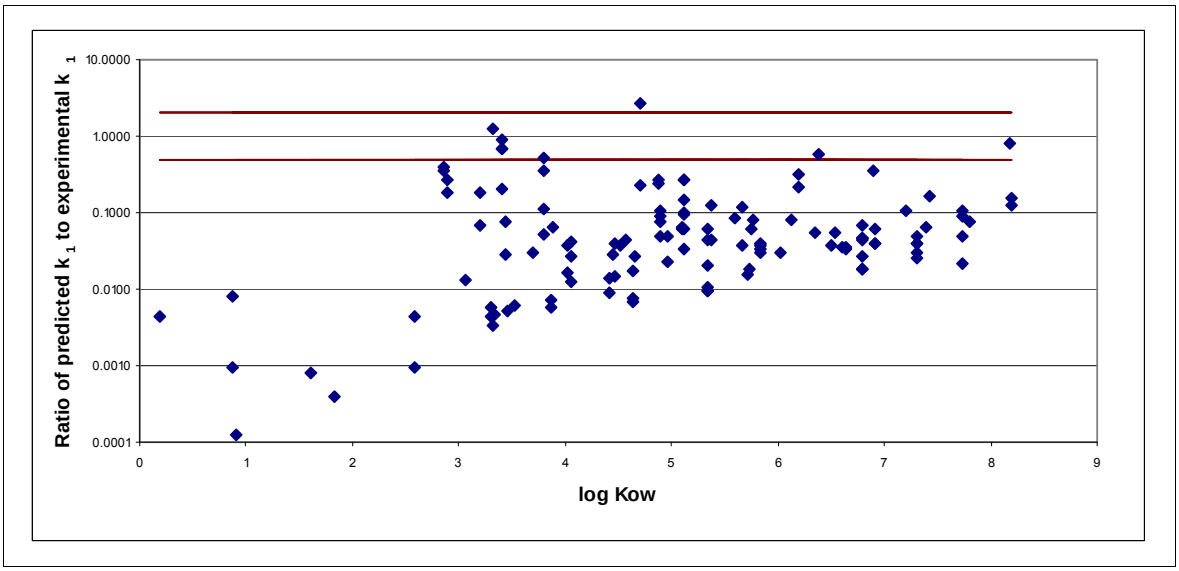

Figure 4.42 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Hawker and Connell (1988) equation based on initial fish weights

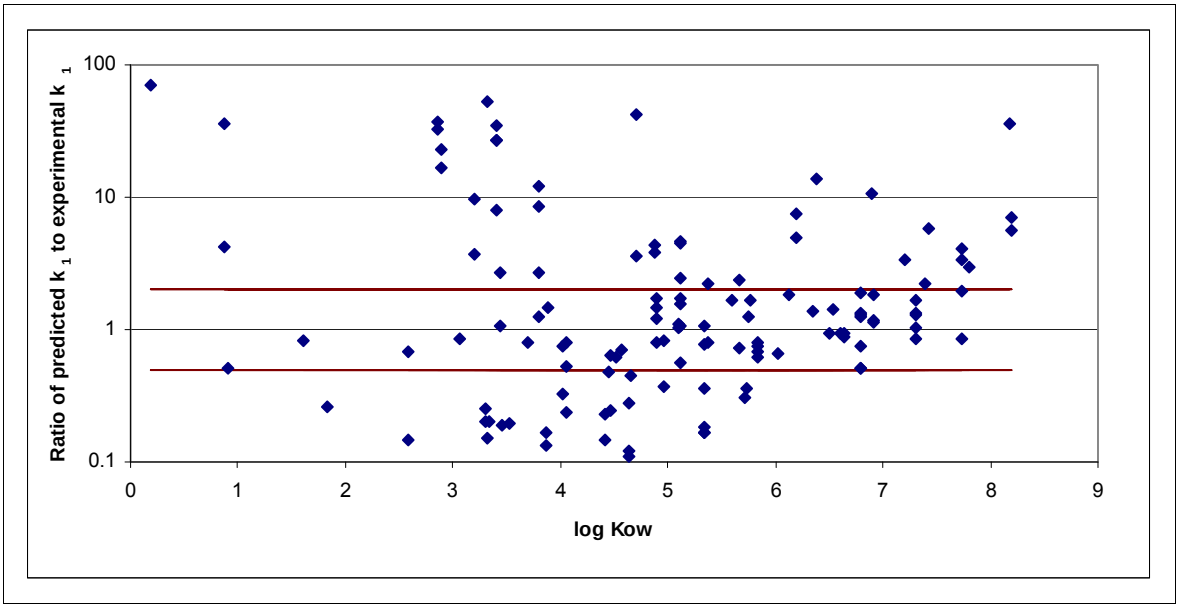

Figure 4.43 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Spacie and Hamelink (1982) equation based on initial fish weights

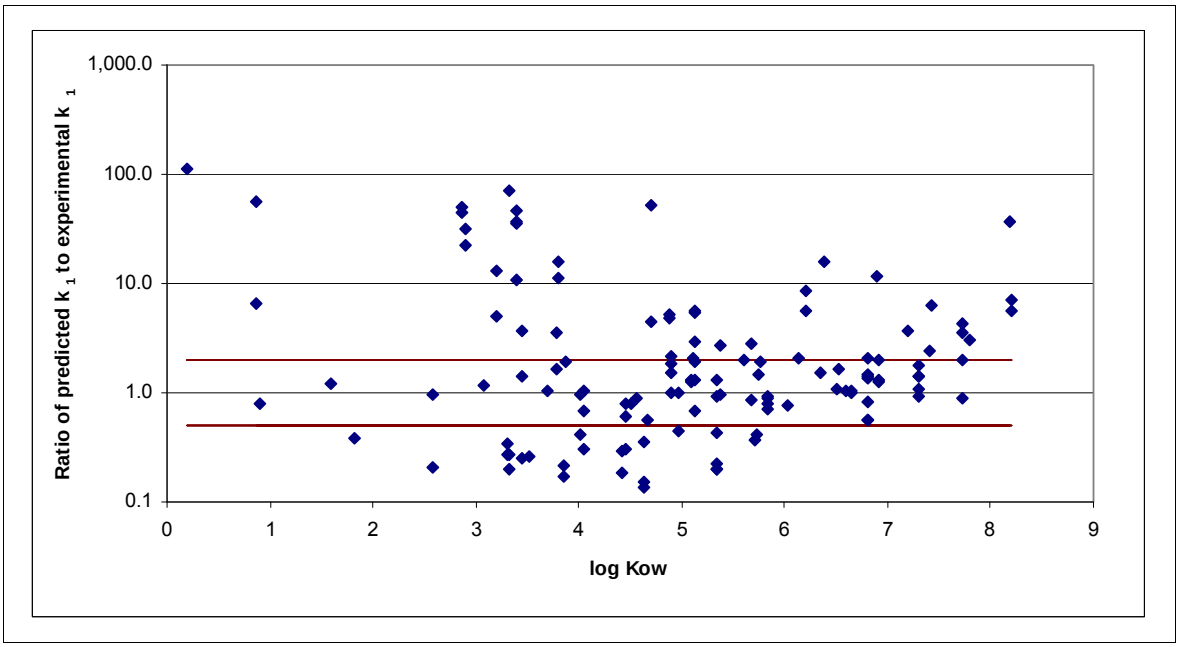

Figure 4.44 Variation of the predicted k_1 to the experimental k_1 with log K_{ow} obtained using the Tolls and Sijm (1995) equation based on initial fish weights

These data were also evaluated statistically. To carry out this analysis it was first assumed that the data were log normally distributed. The $\log_{10}$ of the ratio of k_1/k_2 was analysed statistically to determine the mean, median and standard deviation of the $\log_{10}$ ratio. The statistical data are summarised in Table 4.1. In this analysis a mean $\log_{10}$ ratio of zero indicates that the mean observed ratio is one, a negative (–ve) mean $\log_{10}$ ratio indicates that the method tends to underestimate the actual k_1 and a positive (+ve) mean $\log_{10}$ ratio indicates that the method tends to overestimate the actual k_1. Also relevant is the standard deviation, which provides a measure of the "scatter" of the ratios around the mean. Ideally, the "best" method would have a mean $\log_{10}$ ratio of zero and a small standard deviation.

As is evident from the plots and statistics, the following methods performed poorly in this exercise:

- Campfens and Mackay (1997).
- Hawker and Connell (1985).
- Hawker and Connell (1988).

The Campfens and Mackay (1997) method is dependent on estimating the uptake rate constant from the depuration rate constant. For this method to work properly the depuration rate constant should ideally be based on the loss from respiration. However, only the overall depuration rate constant is available in the data set used and this will include contributions from loss by metabolism and growth dilution amongst other processes. Therefore, the relatively poor performance of this method most probably results from deficiencies in the test data set rather than with the approach itself.

It is also evident from Figure 4.23 to Figure 4.44 that most, if not all, of the models perform relatively poorly for substances with low log K_{ow} values. In terms of the potential use in estimating the BCF from the data obtained in a feeding study, this is not so important as it is envisaged that feeding studies will be mainly carried out for substances with relatively high log K_{ow}.

In order to determine the performance of the methods at higher log K_{ow} values, the statistical analysis was carried out using a reduced data set consisting of only the substances with a log K_{ow} of 3.5 or above (log K_{ow} range of 3.5 to 8.2). The results of this analysis are summarised in Table 4.1.

This analysis was carried out using the initial fish weight. However, as fish growth during the uptake phase will have taken place in many studies, particularly those using small trout, the predictions were also generated using a fish weight of twice the initial weight to investigate the sensitivity of the analysis to the fish weight assumed. The results of this analysis are summarised in Table 4.1.

Table 4.1 Statistical data based on the log [ratio of the predicted k_1 to the experimental k_1]

Method	Whole data set using estimates based on initial fish weight	Substances with log K_{ow} of 3.5 and above based on initial fish weight	Substances with log K_{ow} of 3.5 and above based on twice the initial fish weight
Sijm *et al*. (1995)	Mean log ratio = 0.17 95% confidence interval = ±0.13 Standard deviation = ±0.73 Median = 0.08 Number of data points = 128 [Mean ratio = 1.48; 95% C.I. 1.1-1.98][1]	Mean log ratio = -0.02 95% confidence interval = ±0.10 Standard deviation = ±0.51 Median = -0.01 Number of data points = 101 [Mean ratio = 0.96; 95% C.I. 0.76-1.02][1]	Mean log ratio = -0.12 95% confidence interval = ±0.11 Standard deviation = ±0.51 Median = -0.11 Number of data points = 101 [Mean ratio = 0.77; 95% C.I. 0.61-0.96][1]
Hendriks *et al*. (2001)	Mean log ratio = -0.02 95% confidence interval = ±0.12 Standard deviation = ±0.68 Median = 0.05 Number of data points = 128 [Mean ratio = 0.95; 95% C.I. 0.73-1.25][1]	Mean log ratio = 0.05 95% confidence interval = ±0.10 Standard deviation = ±0.50 Median = 0.06 Number of data points = 101 [Mean ratio = 1.12; 95% C.I. 0.90-1.41][1]	Mean log ratio = -0.02 95% confidence interval = ±0.10 Standard deviation = ±0.50 Median = -0.01 Number of data points = 101 [Mean ratio = 0.95; 95% C.I. 0.75-1.18][1]
Campfens and Mackay (1997)	Mean log ratio = 0.77 95% confidence interval = ±0.21 Standard deviation = ±1.15 Median = 0.57 Number of data points = 118 [Mean ratio = 5.93; 95% C.I. 3.68-9.55][1]	Mean log ratio = 1.03 95% confidence interval = ±0.23 Standard deviation = ±1.12 Median = 0.84 Number of data points = 93 [Mean ratio = 10.61; 95% C.I. 6.28-17.91][1]	Mean log ratio = 1.03 95% confidence interval = ±0.23 Standard deviation = ±1.19 Median = 0.84 Number of data points = 93 [Mean ratio = 10.61; 95% C.I. 6.28-17.91][1]
Arnot and Gobas (2003)	Mean log ratio = 0.61 95% confidence interval = ±0.12 Standard deviation = ±0.67 Median = 0.58 Number of data points = 128 [Mean ratio = 4.03; 95% C.I. 3.08-5.27][1]	Mean log ratio = 0.47 95% confidence interval = ±0.10 Standard deviation = ±0.54 Median = 0.52 Number of data points = 101 [Mean ratio = 2.94; 95% C.I. 2.31-3.74][1]	Mean log ratio = 0.35 95% confidence interval = ±0.10 Standard deviation = ±0.54 Median = 0.40 Number of data points = 101 [Mean ratio = 2.23; 95% C.I. 1.75-2.83][1]

Method	Whole data set using estimates based on initial fish weight	Substances with log K_{ow} of 3.5 and above based on initial fish weight	Substances with log K_{ow} of 3.5 and above based on twice the initial fish weight
Arnot and Gobas (2004)	Mean log ratio = 0.38 95% confidence interval = ±0.12 Standard deviation = ±0.57 Median = 0.33 Number of data points = 87 [Mean ratio = 2.39; 95% C.I. 1.81-3.16][1]	Mean log ratio = 0.34 95% confidence interval = ±0.12 Standard deviation = ±0.52 Median = 0.33 Number of data points = 74 [Mean ratio = 2.18; 95% C.I. 1.66-2.87][1]	Mean log ratio = 0.23 95% confidence interval = ±0.12 Standard deviation = ±0.52 Median = 0.22 Number of data points = 74 [Mean ratio = 1.71; 95% C.I. 1.30-2.25][1]
Thomann (1989)	Mean log ratio = -0.22 95% confidence interval = ±0.13 Standard deviation = ±0.63 Median = -0.30 Number of data points = 87 [Mean ratio = 0.61; 95% C.I. 0.45-0.82][1]	Mean log ratio = -0.15 95% confidence interval = ±0.12 Standard deviation = ±0.55 Median = -0.29 Number of data points = 75 [Mean ratio = 0.71; 95% C.I. 0.53-0.95][1]	Mean log ratio = -0.07 95% confidence interval = ±0.12 Standard deviation = ±0.55 Median = -0.22 Number of data points = 75 [Mean ratio = 0.85; 95% C.I. 0.64-1.13][1]
Barber (2001)	Mean log ratio = 0.28 95% confidence interval = ±0.12 Standard deviation = ±0.71 Median = 0.15 Number of data points = 128 [Mean ratio = 1.92; 95% C.I. 1.45-2.54][1]	Mean log ratio = 0.10 95% confidence interval = ±0.09 Standard deviation = ±0.48 Median = 0.07 Number of data points = 101 [Mean ratio = 1.25; 95% C.I. 1.01-1.55][1]	Mean log ratio = 0.05 95% confidence interval = ±0.09 Standard deviation = ±0.48 Median = 0.02 Number of data points = 101 [Mean ratio = 1.12; 95% C.I. 0.90-1.39][1]
Barber *et al*. (1991)	Mean log ratio = 0.28 95% confidence interval = ±0.12 Standard deviation = ±0.71 Median = 0.14 Number of data points = 128 [Mean ratio = 1.89; 95% C.I. 1.43-2.51][1]	Mean log ratio = 0.09 95% confidence interval = ±0.09 Standard deviation = ±0.48 Median = 0.07 Number of data points = 101 [Mean ratio = 1.23; 95% C.I. 0.99-1.53][1]	Mean log ratio = 0.04 95% confidence interval = ±0.09 Standard deviation = ±0.48 Median = 0.02 Number of data points = 101 [Mean ratio = 1.09; 95% C.I. 0.88-1.35][1]
Erickson and McKim (1990a)	Mean log ratio = 0.27 95% confidence interval = ±0.12 Standard deviation = ±0.71 Median = 0.14 Number of data points = 128 [Mean ratio = 1.86; 95% C.I. 1.40-2.47][1]	Mean log ratio = 0.08 95% confidence interval = ±0.09 Standard deviation = ±0.48 Median = 0.05 Number of data points = 101 [Mean ratio = 1.21; 95% C.I. 0.98-1.50][1]	Mean log ratio = 0.04 95% confidence interval = ±0.09 Standard deviation = ±0.48 Median = 0.00 Number of data points = 101 [Mean ratio = 1.09; 95% C.I. 0.88-1.35][1]

Method	Whole data set using estimates based on initial fish weight	Substances with log K_{ow} of 3.5 and above based on initial fish weight	Substances with log K_{ow} of 3.5 and above based on twice the initial fish weight
Erickson and McKim (1990b)	Mean log ratio = 0.20 95% confidence interval = ±0.12 Standard deviation = ±0.71 Median = 0.05 Number of data points = 128 [Mean ratio = 1.60; 95% C.I. 1.20-2.12][1]	Mean log ratio = 0.02 95% confidence interval = ±0.10 Standard deviation = ±0.49 Median = 0.03 Number of data points = 101 [Mean ratio = 1.04; 95% C.I. 0.83-1.29][1]	Mean log ratio = -0.05 95% confidence interval = ±0.10 Standard deviation = ±0.49 Median = -0.04 Number of data points = 101 [Mean ratio = 0.89; 95% C.I. 0.71-1.10][1]
Gobas and Mackay (1987)	Mean log ratio = 0.56 95% confidence interval = ±0.13 Standard deviation = ±0.74 Median = 0.49 Number of data points = 128 [Mean ratio = 3.62; 95% C.I. 2.69-4.87][1]	Mean log ratio = 0.37 95% confidence interval = ±0.10 Standard deviation = ±0.54 Median = 0.42 Number of data points = 101 [Mean ratio = 2.33; 95% C.I. 1.83-2.96][1]	Mean log ratio = 0.25 95% confidence interval = ±0.10 Standard deviation = ±0.54 Median = 0.30 Number of data points = 101 [Mean ratio = 1.75; 95% C.I. 1.39-2.26][1]
Gobas *et al*. (1986)	Mean log ratio = 0.93 95% confidence interval = ±0.13 Standard deviation = ±0.73 Median = 0.83 Number of data points = 128 [Mean ratio = 8.51; 95% C.I. 6.37-11.38][1]	Mean log ratio = 0.74 95% confidence interval = ±0.10 Standard deviation = ±0.51 Median = 0.75 Number of data points = 101 [Mean ratio = 5.50; 95% C.I. 4.38-6.92][1]	Mean log ratio = 0.65 95% confidence interval = ±0.10 Standard deviation = ±0.51 Median = 0.65 Number of data points = 101 [Mean ratio = 4.42; 95% C.I. 3.51-5.55][1]
Hayton and Barron (1990)	Mean log ratio = 0.15 95% confidence interval = ±0.12 Standard deviation = ±0.71 Median = 0.01 Number of data points = 128 [Mean ratio = 1.40; 95% C.I. 1.06-1.86][1]	Mean log ratio = -0.04 95% confidence interval = ±0.09 Standard deviation = ±0.48 Median = -0.05 Number of data points = 101 [Mean ratio = 0.91; 95% C.I. 0.74-1.13][1]	Mean log ratio = -0.10 95% confidence interval = ±0.09 Standard deviation = ±0.48 Median = -0.11 Number of data points = 101 [Mean ratio = 0.80; 95% C.I. 0.64-0.99][1]
Norstrom *et al*. (1976)	Mean log ratio = -0.38 95% confidence interval = ±0.12 Standard deviation = ±0.70 Median = -0.53 Number of data points = 128 [Mean ratio = 0.42; 95% C.I. 0.31-0.55][1]	Mean log ratio = -0.57 95% confidence interval = ±0.09 Standard deviation = ±0.47 Median = -0.63 Number of data points = 101 [Mean ratio = 0.27; 95% C.I. 0.22-0.34][1]	Mean log ratio = -0.60 95% confidence interval = ±0.09 Standard deviation = ±0.47 Median = -0.67 Number of data points = 101 [Mean ratio = 0.25; 95% C.I. 0.20-0.31][1]

Method	Whole data set using estimates based on initial fish weight	Substances with log K_{ow} of 3.5 and above based on initial fish weight	Substances with log K_{ow} of 3.5 and above based on twice the initial fish weight
Streit and Sire (1993)	Mean log ratio = 0.05 95% confidence interval = ±0.12 Standard deviation = ±0.71 Median = -0.08 Number of data points = 128 [Mean ratio = 1.13; 95% C.I. 0.85-1.50][1]	Mean log ratio = -0.13 95% confidence interval = ±0.09 Standard deviation = ±0.48 Median = -0.17 Number of data points = 101 [Mean ratio = 0.74; 95% C.I. 0.59-0.91][1]	Mean log ratio = -0.18 95% confidence interval = ±0.09 Standard deviation = ±0.48 Median = -0.21 Number of data points = 101 [Mean ratio = 0.66; 95% C.I. 0.53-0.82][1]
Thomann and Connolly (1984)	Mean log ratio = -0.44 95% confidence interval = ±0.12 Standard deviation = ±0.71 Median = 0.27 Number of data points = 128 [Mean ratio = 0.36; 95% C.I. 0.28-0.48][1]	Mean log ratio = -0.62 95% confidence interval = ±0.09 Standard deviation = ±0.48 Median = -0.65 Number of data points = 101 [Mean ratio = 0.24; 95% C.I. 0.19-0.29][1]	Mean log ratio = -0.67 95% confidence interval = ±0.09 Standard deviation = ±0.48 Median = -0.70 Number of data points = 101 [Mean ratio = 0.21; 95% C.I. 0.17-0.26][1]
Barber (2003) observed	Mean log ratio = 0.09 95% confidence interval = ±0.12 Standard deviation = ±0.71 Median = -0.04 Number of data points = 128 [Mean ratio = 1.24; 95% C.I. 0.93-1.65][1]	Mean log ratio = -0.09 95% confidence interval = ±0.09 Standard deviation = ±0.48 Median = -0.11 Number of data points = 101 [Mean ratio = 0.81; 95% C.I. 0.65-1.00][1]	Mean log ratio = -0.15 95% confidence interval = ±0.09 Standard deviation = ±0.48 Median = -0.17 Number of data points = 101 [Mean ratio = 0.70; 95% C.I. 0.57-0.87][1]
Barber (2003) calibrated	Mean log ratio = 0.23 95% confidence interval = ±0.12 Standard deviation = ±0.67 Median = 0.20 Number of data points = 128 [Mean ratio = 1.71; 95% C.I. 1.31-2.24][1]	Mean log ratio = 0.10 95% confidence interval = ±0.11 Standard deviation = ±0.54 Median = 0.15 Number of data points = 101 [Mean ratio = 1.25; 95% C.I. 0.98-1.59][1]	Mean log ratio = -0.03 95% confidence interval = ±0.11 Standard deviation = ±0.54 Median = 0.02 Number of data points = 101 [Mean ratio = 0.94; 95% C.I. 0.74-1.20][1]
Hawker and Connell (1985)	Mean log ratio = -1.24 95% confidence interval = ±0.12 Standard deviation = ±0.71 Median = -1.19 Number of data points = 128 [Mean ratio = 0.06; 95% C.I. 0.04-0.08][1]	Mean log ratio = -1.22 95% confidence interval = ±0.12 Standard deviation = ±0.64 Median = -1.19 Number of data points = 101 [Mean ratio = 0.06; 95% C.I. 0.05-0.08][1]	Mean log ratio = -1.22 95% confidence interval = ±0.12 Standard deviation = ±0.64 Median = -1.19 Number of data points = 101 [Mean ratio = 0.06; 95% C.I. 0.05-0.08][1]

Method	Whole data set using estimates based on initial fish weight	Substances with log K_{ow} of 3.5 and above based on initial fish weight	Substances with log K_{ow} of 3.5 and above based on twice the initial fish weight
Hawker and Connolly (1988)	Mean log ratio = -1.37 95% confidence interval = ±0.12 Standard deviation = ±0.69 Median = -1.36 Number of data points = 128 [Mean ratio = 0.04; 95% C.I. 0.03-0.06][1]	Mean log ratio = -1.29 95% confidence interval = ±0.09 Standard deviation = ±0.48 Median = -1.35 Number of data points = 101 [Mean ratio = 0.05; 95% C.I. 0.04-0.06][1]	Mean log ratio = -1.29 95% confidence interval = ±0.09 Standard deviation = ±0.48 Median = -1.35 Number of data points = 101 [Mean ratio = 0.05; 95% C.I. 0.04-0.06][1]
Spacie and Hamelink (1982)	Mean log ratio = 0.15 95% confidence interval = ±0.11 Standard deviation = ±0.65 Median = 0.05 Number of data points = 128 [Mean ratio = 1.41; 95% C.I. 1.09-1.83][1]	Mean log ratio = 0.06 95% confidence interval = ±0.10 Standard deviation = ±0.51 Median = 0.03 Number of data points = 101 [Mean ratio = 1.15; 95% C.I. 0.91-1.45][1]	Mean log ratio = 0.06 95% confidence interval = ±0.10 Standard deviation = ±0.51 Median = 0.03 Number of data points = 101 [Mean ratio = 1.15; 95% C.I. 0.91-1.45][1]
Tolls and Sijm (1995)	Mean log ratio = 0.24 95% confidence interval = ±0.11 Standard deviation = ±0.65 Median = 0.12 Number of data points = 128 [Mean ratio = 8.51; 95% C.I. 6.37-11.38][1]	Mean log ratio = 0.13 95% confidence interval = ±0.10 Standard deviation = ±0.50 Median = 0.11 Number of data points = 101 [Mean ratio = 1.35; 95% C.I. 1.08-1.69][1]	Mean log ratio = 0.13 95% confidence interval = ±0.10 Standard deviation = ±0.50 Median = 0.11 Number of data points = 101 [Mean ratio = 1.35; 95% C.I. 1.08-1.69][1]

[1]The statistics were determined based on the $\log_{10}(k_1$ predicted/k_1 experimental). The equivalent mean (and 95% confidence interval) were estimated from the mean value obtained from the $\log_{10}$-transformed data.

To put the derived statistical data into context, it is useful to consider what would happen based on a purely random estimate of the k_1 value. The mean $\log_{10}$ ratio of the predicted k_1 to the experimental k_1 provides an estimate of the bias in the method and two independent randomly selected sets of numbers would be expected to tend to a mean $\log_{10}$ ratio of zero. In order to test this, the equivalent statistics were generated assuming two random variables from a uniform distribution ranging between 1.4 and 4,188 l kg^{-1} (the range of k_1 values in the test data set). The results from five independent trials are summarised in Table 4.2. As can be seen, the predictions based on two random variables tend to a mean $\log_{10}$ ratio of zero.

As well as bias in the predictions, precision in the predictions is an important consideration, and in particular, whether the precision is better than would be obtained from a purely random estimate of the k_1 value. As can be seen from the very low r^2 values in the correlation plots presented earlier, the precision of most methods is generally low. In the analysis here, random predictions of k_1 values were made and compared with the available experimental data set in a similar way as before. This was done both on the full data set (assuming a purely random estimate of the k_1 value in the range 1,4 to 4,188 l kg^{-1}) and the reduced data set for substances with log K_{ow} values of 3.5 and above (assuming a purely random estimate of the k_1 value in the range 11 to 4,188 l kg^{-1}, the range of k_1 values in this reduced data set). Again, five independent trials for each scenario were carried out and the results are summarised in Table 4.2. These show that random predictions within the range of test data led to a systematic overprediction of the k_1 value for this data set. In addition, the standard deviation (a measure of scatter around the mean) was larger than for many of the predictive methods considered.

Overall, although the available predictive methods show a generally low precision, many of the methods appear to perform better than purely random predictions within the two ranges of k_1 values.

Table 4.2 Statistical data based on random predictions

Scenario	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Random k_1 versus random k_1 in the range 1.4 to 4,188	Mean log ratio = -0.04 95% confidence interval = ±0.11 Standard deviation = ±0.61 Median = -0.04 Number of data points = 128	Mean log ratio = 0.02 95% confidence interval = ±0.11 Standard deviation = ±0.63 Median = 0.03 Number of data points = 128	Mean log ratio = -0.08 95% confidence interval = ±0.10 Standard deviation = ±0.61 Median = -0.05 Number of data points = 128	Mean log ratio = -0.04 95% confidence interval = ±0.10 Standard deviation = ±0.60 Median = -0.04 Number of data points = 128	Mean log ratio = 0.04 95% confidence interval = ±0.10 Standard deviation = ±0.58 Median = -0.02 Number of data points = 128
Experimental k_1 versus random k_1 in the range 1.4 to 4,188 for the whole data set	Mean log ratio = 0.64 95% confidence interval = ±0.14 Standard deviation = ±0.82 Median = 0.48 Number of data points = 128	Mean log ratio = 0.66 95% confidence interval = ±0.14 Standard deviation = ±0.78 Median = 0.55 Number of data points = 128	Mean log ratio = 0.60 95% confidence interval = ±0.14 Standard deviation = ±0.81 Median = 0.58 Number of data points = 128	Mean log ratio = 0.59 95% confidence interval = ±0.15 Standard deviation = ±0.88 Median = 0.52 Number of data points = 128	Mean log ratio = 0.60 95% confidence interval = ±0.14 Standard deviation = ±0.79 Median = 0.60 Number of data points = 128
Experimental k_1 versus random k_1 in the range 11 to 4,188 for the substances with log K_{ow} of 3.5 and above	Mean log ratio = 0.39 95% confidence interval = ±0.13 Standard deviation = ±0.68 Median = 0.47 Number of data points = 101	Mean log ratio = 0.46 95% confidence interval = ±0.13 Standard deviation = ±0.67 Median = 0.53 Number of data points = 101	Mean log ratio = 0.48 95% confidence interval = ±0.11 Standard deviation = ±0.56 Median = 0.46 Number of data points = 101	Mean log ratio = 0.46 95% confidence interval = ±0.12 Standard deviation = ±0.62 Median = 0.45 Number of data points = 101	Mean log ratio = 0.39 95% confidence interval = ±0.13 Standard deviation = ±0.66 Median = 0.44 Number of data points = 101

4.3 Discussion of results

In terms of the "best" method for predicting the k_1 value, this should ideally have a mean $\log_{10}$ ratio of the predicted to experimental k_1 value of zero and as small a standard deviation as possible. To help identify the best methods, the following pragmatic approach was used here to rank the methods[16]:

- Acceptable methods are taken to have a mean $\log_{10}$ ratio of the predicted to experimental k_1 value of between -0.15 and 0.15 (this corresponds to actual ratios of 0.70 to 1.41).
- The highest ranked method will have the lowest standard deviation around the mean $\log_{10}$ ratio.

The calculations using the whole data set and initial fish weight results in only five log ratios within the acceptable range. The number increases to thirteen when the set is restricted to substances with log K_{ow} values of 3.5 or over, and decreases to twelve when using twice the initial fish weight. The standard deviation is reduced for all methods when the data set is restricted by log K_{ow} value, with virtually no further changes when the calculations are based on twice the initial weight.

Using the criteria above, the following methods rank most highly (listed in increasing order of the standard deviation followed by closeness of the mean $\log_{10}$ ratio to zero) for the data set of substances with log K_{ow} of 3.5 to around 8.2.

Analysis based on initial weight	**Analysis based on twice initial weight**
Hayton and Barron (1990) [Mean log ratio = -0.04; standard deviation ±0.48]	Erickson and McKim (1990a) [Mean log ratio = 0.04; standard deviation ±0.48]
Erickson and McKim (1990a) [Mean log ratio = 0.08; standard deviation ±0.48]	Barber *et al.* (1991) [Mean log ratio 0.04; standard deviation ±0.48]
Barber *et al.* (1991) [Mean log ratio = 0.09; standard deviation ±0.48]	Barber (2001) [Mean log ratio = 0.05; standard deviation ±0.48]
Barber (2003) - observed [Mean log ratio = -0.09; standard deviation ±0.48]	Hayton and Barron (1990) [Mean log ratio = -0.10; standard deviation ±0.48]
Barber (2001) [Mean log ratio = 0.10; standard deviation ±0.48]	Barber (2003) - observed [Mean log ratio = -0.15; standard deviation ±0.48]
Streit and Sire (1993) [Mean log ratio - 0.13; standard deviation ±0.48]	Erickson and McKim (1990b) [Mean log ratio = -0.05; standard deviation ±0.49]
Erickson and McKim (1990b) [Mean log ratio = 0.02; standard deviation ±0.49]	Hendriks *et al*. (2001) [Mean log ratio = -0.02; standard deviation ±0.50]
Hendriks *et al*. (2001) [Mean log ratio = 0.05; standard deviation ±0.50]	Tolls and Sijm (1995) [Mean log ratio = 0.13; standard deviation ±0.50]
Tolls and Sijm (1995) [Mean log ratio = 0.13; standard deviation ±0.50]	Spacie and Hamelink (1982) [Mean log ratio 0.06; standard deviation ±0.51]

[16] These values are used here as an arbitrary pragmatic approach to distinguishing between the performance of the various methods. The meaning of these criteria, in terms of whether a prediction using the method is scientifically acceptable, is not clear.

Sijm *et al.* (1995) [Mean log ratio = -0.02; standard deviation ±0.51]

Spacie and Hamelink (1982) [Mean log ratio 0.06; standard deviation ±0.51]

Barber (2003) - calibrated [Mean log ratio = 0.10; standard deviation ±0.54]

Thomann (1989) [Mean log ratio = -0.15; standard deviation ±0.55]

Sijm *et al.* (1995) [Mean log ratio = -0.12; standard deviation ±0.51]

Barber (2003) - calibrated [Mean log ratio = -0.03; standard deviation ±0.54]

Thomann (1989) [Mean log ratio = -0.07; standard deviation ±0.55]

All of these methods appear to give a similar predictive performance (similar mean log ratios and standard deviations) for predicting a k_1 value. This is not entirely surprising as many of the methods have similar formulations based on the fish weight. However, it is interesting that two of the methods (Spacie and Hamelink 1982 and Tolls and Sijm 1995) are not dependent on the fish weight but rather the properties of the substance (log K_{ow}) and these seem to perform equally well as some of the other methods.

The standard deviations of the predictions are rather high (for example a mean $\log_{10}$ ratio of zero with a standard deviation of 0.5 log units is equivalent to a range of the actual ratio ± one standard deviation of 0.32 to 3.2, that is, under- or overestimation by a factor of three). This means that although the mean ratio from these methods is close to one, for any one specific substance there will be a large uncertainty in the predicted k_1 obtained. This uncertainty in the predicted k_1 should be taken into account when considering the use of any method(s), for example within the draft updated OECD 305 Test Guideline.

4.4 Considerations on available experimental data

Approaches for estimating the uptake rate constant were tested here using uptake rate constants determined in actual bioaccumulation studies. Most of the approaches available indicate that the uptake rate constant will vary with fish weight. This has consequences for the derivation of the uptake rate constant in a standard bioconcentration experiment. Up to now, most of the available bioconcentration data have been derived assuming that the uptake rate constant (k_1) is actually constant during the uptake portion of the experiment (this was assumed to be the case in the data sets used here). However, current theories on bioconcentration in fish suggest that this is not the case for fish growing during the experiment[17]. Thus this may have introduced, inadvertently, errors into much of the experimental kinetic data for uptake into fish during bioconcentration experiments. The implications of this for the analysis of bioconcentration data are considered further in Section 5, and this introduces a further source of uncertainty in the data analysis carried out here. The sources of uncertainty in the underlying data set are considered further in Section 7.

[17] The discussion here should not be confused with correction for growth dilution. Growth dilution results in dilution of the substance within the fish as the fish grows. This is effectively a depuration process and a correction for this can be applied to the overall depuration rate constant if required.

5 Consequences of fish growth for analysis of bioconcentration data

As noted in Section 4.4, many of the methods for predicting k_1 values suggest a dependence on the fish size. This may introduce complications into the analysis of experimental data using fish that grow significantly during the test. This section considers this issue further.

5.1 Dependence of uptake rate constant on fish size

The relevant equations normally assumed in the analysis of fish bioconcentration data for an experiment where the concentration in the water phase is held constant during the uptake portion of the experiment are given below.

During the uptake phase, the concentration in fish can be described in terms of first-order kinetics.

Equation 38

$$\frac{d[C_{fish}]}{dt} = k_1 \times [C_{water}] - k_2 \times [C_{fish}]$$

Where $[C_{fish}]$ = Concentration in fish (mg kg^{-1}).

$[C_{water}]$ = Concentration in water (mg l^{-1}).

k_1 = Uptake rate constant (l kg^{-1} day^{-1}).

k_2 = Overall depuration rate constant (day^{-1}).

$d[C_{fish}]/dt$ = Rate of change in the concentration in fish (mg kg^{-1} day^{-1}).

The integrated form of Equation 38 is shown in Equation 39 and Equation 40 and these form the basis of the current approach in the OECD Test Guideline 305.

Equation 39

$$[C_{fish}] = [C_{water}] \times \frac{k_1}{k_2} \times \left(1 - e^{-k_2 t}\right) \text{ when } 0 < t < t_c$$

Equation 40

$$[C_{fish}] = [C_{water}] \times \frac{k_1}{k_2} \times (e^{-k_2 (t - t_c)} - e^{-k_2 t}) \text{ when } t > t_c$$

Where $[C_{fish}]$ = Concentration in fish at time t (mg kg^{-1}).

$[C_{water}]$ = Concentration in water during the uptake phase (mg l^{-1}).

k_1 = Uptake rate constant (l kg^{-1} day^{-1}).

k_2 = Depuration rate constant (day^{-1}).

t = Time (days).

t_c = Time at the end of the uptake phase (days).

In addition, during the depuration phase, the concentration in water is effectively zero and hence the concentration in fish can be described by the following:

Equation 41 $$\frac{d[C_{fish}]}{dt} = -k_2 \times [C_{fish}]$$

At steady state, the rate of change of concentration in the fish is zero, hence the following applies:

Equation 42 $$k_1 \times [C_{water}] = k_2 \times [C_{fish}] \text{ or } \frac{k_1}{k_2} = \frac{[C_{fish}]}{[C_{water}]} = BCF$$

This method for the analysis of bioconcentration data assumes that the uptake of the substance is a first-order process in relation to the concentration in water and the depuration of the substance is a first-order process in relation to the concentration in fish. Further, the method depends on the value of the uptake rate constant (k_1) and the overall depuration rate constant (k_2) being constant over the experimental period. Clearly, if the fish are growing, and the value of k_1 (or k_2) is dependent on the fish weight (size or age)[18], the methods currently used may not be adequate.

There are essentially two main methods (one with two main variants) whereby the value of k_1 and k_2 can be obtained from the concentration data measured during a fish bioconcentration study. These are termed here sequential and simultaneous methods.

In the sequential method, the k_2 value is first determined from the depuration data. Equation 41 represents a simple first-order decay process, and the integrated form of this rate equation yields the following equation:

Equation 43 $$\ln[C_{fish}] = -k_2 \times t + Constant$$

where $\ln[C_{fish}]$ = The natural logarithm of the concentration in fish.

t = Time (days).

Constant = A constant (in this case it is the natural logarithm of the concentration in fish at the start of the depuration phase).

The value of k_2 can then be determined directly from the slope of a plot of $\ln[C_{fish}]$ against time.

Once the value of k_2 is determined, this is used to determine the value of k_1 during the uptake phase data. Equation 38 cannot be solved using a linear plot and so curve fitting methods have to be used. These are based on Equation 39 and assume that the concentration in water, k_1 and k_2 are constant during the uptake phase. The curve fitting method adjusts the value of k_1 to give the best fit (usually by minimising the squares of the residuals[19]) to the observed concentration – time plot.

In the simultaneous method, the values of k_1 and k_2 are obtained directly from curve fitting. This can be carried out over the uptake period only using Equation 39 or over the entire uptake and depuration period (using Equation 40).

In both the sequential and simultaneous methods, the value of k_1 (and k_2) is assumed to be constant. If k_1 decreases as the fish grow this means that the fitted curve may poorly reflect the observed data. In particular, the sequential method may be expected to give a poorer fit to the uptake data than the simultaneous method, purely because the former uses one variable (k_1) and the latter two (k_1 and k_2). This does not mean that the simultaneous method should always be used, but rather may explain why the

[18] This should not be confused with growth dilution (see Section 5.3). What is being considered here may result from changes, for example, in the ventilation rates, uptake efficiency and metabolic capacity and so on, as the fish grow or age.

[19] The residuals are the difference between the observed concentration in the fish and the predicted concentration in the fish from fitting either Equation 39 or Equation 40 to the data.

simultaneous method may sometimes appear, at first glance, to give a better fit to the uptake data. In particular, if the sequential method is used on the uptake data only, a good apparent fit to the data may be obtained but this may result from an erroneous value for k_2.

The assumption that k_1 (and k_2) is constant during the uptake phase is inherent in the interpretation of many (if not the majority) of the existing experimental BCF data (including most if not all of the data used in this study). This may therefore present a problem in terms of measurement of the experimental k_1 value if the fish are actively growing. The potential effect of this is demonstrated in Section 5.2.

If it is accepted that fish size does affect the k_1 value, the methods for estimating the k_1 value over the uptake period of a study may not be appropriate, particularly for small fish that are rapidly growing, and alternative approaches may need to be considered. A recommended approach is considered below and is tested in Section 5.5.

- In order to simplify the data, it would be preferable if the k_2 value were obtained from the depuration data directly (a plot of $\ln[C_{fish}]$ against time). As such a plot should yield a straight line, this is a good check to ensure that at a) the k_2 value is constant during the experiment and b) the depuration follows first-order kinetics (see Section 5.6 for a discussion of factors that could affect the depuration kinetics).
- Once the k_2 value has been determined, the value of k_1 should be calculated for each individual time point of the uptake phase using Equation 39[20]. The advantage of estimating k_1 this way rather than by curve fitting is that any trends in the value of k_1 can be seen (particularly if the k_1 decreases with increasing exposure time as might be expected if the fish are growing).
- If the value of k_1 is found not to show an increasing or decreasing trend at each time point during the uptake phase, the normal curve-fitting approach can be used to estimate the k_1 value, or the k_1 obtained at each individual time point could be averaged. The kinetic BCF can then be estimated as k_1/k_2.
- If the value of k_1 is found to decrease (or increase) at each time point, this indicates that k_1 may not be constant during the uptake phase and presents a problem for which value to use in determining the BCF. If an apparent steady state appears to be reached, it would appear to be preferable to use the k_1 (or average k_1) value determined for those time points at which steady state was indicated (see also Section 5.2). Alternatively, the range of k_1 values could be considered to determine a range of BCF values associated with fish of different sizes. In this latter case, growth correction would need to be applied carefully (see Section 5.3 and Section 5.8).

5.2 Apparent steady state

The fact that k_1 may decrease with fish size has some important implications for when steady state is reached. As the fish grow, the uptake rate (which is the product of the concentration in water and the uptake rate constant k_1) will continually decrease and an apparent steady state will be reached when the uptake rate equals the depuration rate. However, after this period the uptake rate will continue to decrease, resulting in a

[20] This equation can easily be rearranged to give $k_1 = \frac{[C_{fish}] \times k_2}{[C_{water}] \times \left(1 - e^{-k2 \times t}\right)}$.

decline in concentration in the fish with continued growth (assuming that k_2 remains constant[21]).

This is shown below for a hypothetical BCF experiment. For this example, the fish are assumed to start at a weight of around one gram and grow during the experiments with growth rate constants of 0.01 day^{-1}, 0.015 day^{-1} and 0.020 day^{-1} (as may be typical for experiments with rainbow trout[22]). For the analysis, a constant total depuration rate constant (including the growth dilution component) of 0.05 day^{-1} (a relatively slowly depurating substance) or 0.5 day^{-1} (a relatively rapidly depurating substance) was assumed over the entire period, and the uptake rate constant was calculated from the fish weight using the Sijm *et al*. (1995) method. A constant exposure concentration of 0.001 mg l^{-1} was assumed over a 250-day uptake period[23]. The resulting concentrations estimated in the fish are shown in Figure 5.1 and Figure 5.2. The underlying data and uptake rate constants obtained using the Sijm *et al*. (1995) equation for Figure 5.1 and Figure 5.2 are shown in Table 5.1 and Table 5.2 along with the calculated concentrations in the fish. Also shown in the plots are the idealised plots assuming that k_1 is constant during the simulation (effectively zero growth is assumed).

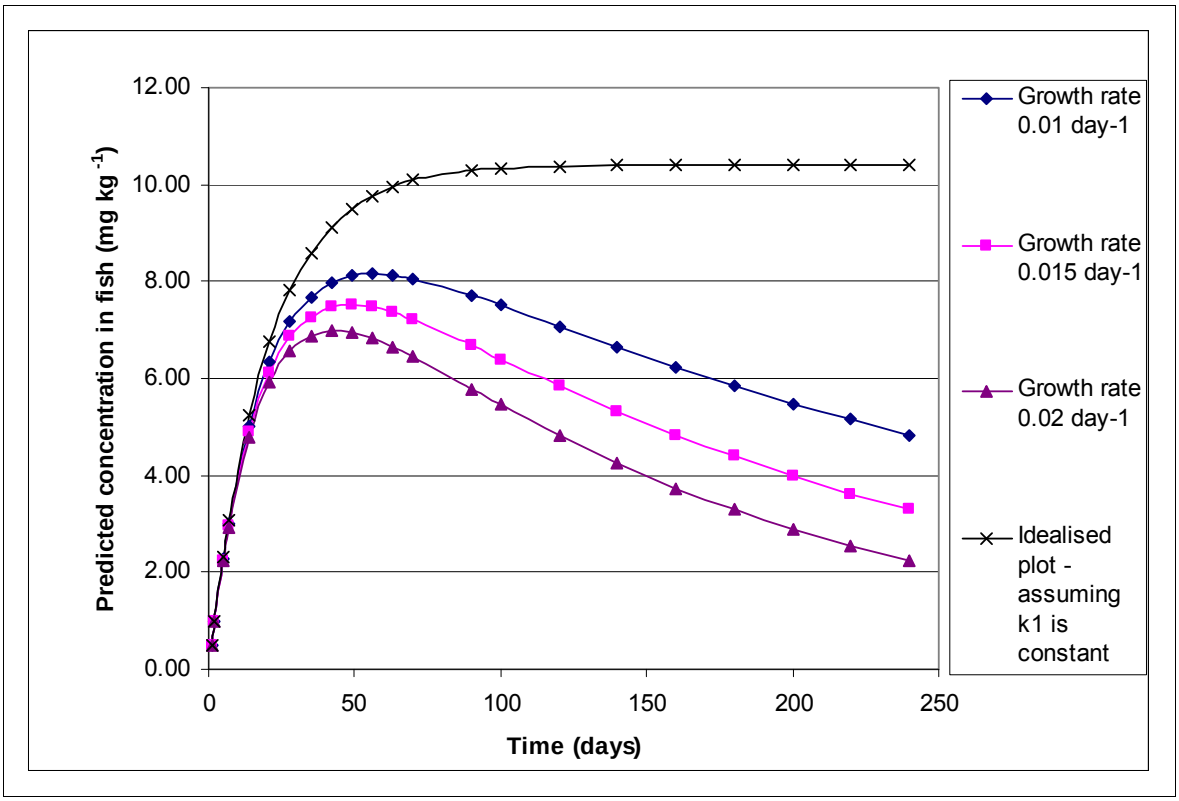

Figure 5.1 Hypothetical uptake curves showing the effect of reducing uptake rate constant as fish grow for a relatively slowly depurating substance (k_2 = 0.05 day^{-1})

[21] The possible variation of k_2 with growth is considered further in Section 5.6. If k_2 also declines with growth then this may reduce or even cancel out the effect described here.
[22] These rate constants are equivalent to the fish reaching a weight of around 2.0, 2.9 and 4.1 g after 70 days.
[23] Duration for calculations extended over usual study duration to show effects clearly.

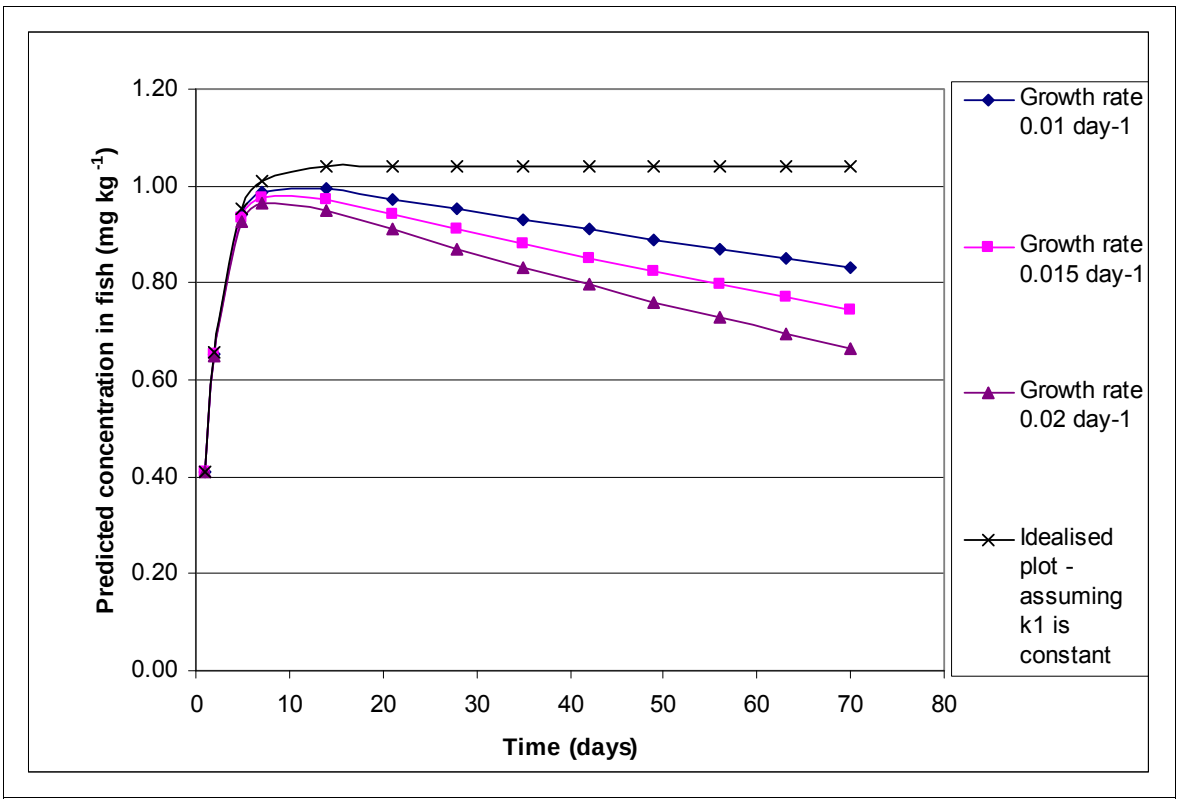

Figure 5.2 Hypothetical uptake curves showing the effect of reducing uptake rate constant as fish grow for a relatively rapidly depurating substance (k_2 = 0.5 day^{-1})

Table 5.1 Hypothetical uptake data for a relatively slowly depurating substance

Time (days)	Water concen-tration (mg l^{-1})	Fish weight (g)[1]			Predicted k_1[1, 2] (l kg^{-1} day^{-1})			k_2[1, 3] assumed (day^{-1})	Predicted concentration in fish (mg kg^{-1})[1]		
		k_G = 0.01 day^{-1}	k_G = 0.015 day^{-1}	k_G = 0.02 day^{-1}	k_G = 0.01 day^{-1}	k_G = 0.015 day^{-1}	k_G = 0.02 day^{-1}		k_G = 0.01 day^{-1}	k_G = 0.015 day^{-1}	k_G = 0.02 day^{-1}
1	0.001	1.00	1.00	1.00	520	520	520	0.05	0.51	0.51	0.51
2	0.001	1.02	1.03	1.04	517	516	514	0.05	0.98	0.98	0.98
5	0.001	1.05	1.07	1.10	512	508	504	0.05	2.27	2.25	2.23
7	0.001	1.07	1.11	1.15	509	503	498	0.05	3.01	2.97	2.94
14	0.001	1.15	1.23	1.32	498	487	476	0.05	5.01	4.90	4.79
21	0.001	1.23	1.37	1.52	487	471	455	0.05	6.33	6.12	5.92
28	0.001	1.32	1.52	1.74	476	455	435	0.05	7.17	6.86	6.56
35	0.001	1.42	1.69	2.01	465	440	416	0.05	7.69	7.27	6.88
42	0.001	1.52	1.87	2.31	455	425	398	0.05	7.98	7.47	6.98
49	0.001	1.63	2.08	2.65	445	411	381	0.05	8.13	7.52	6.95
56	0.001	1.75	2.31	3.05	435	398	364	0.05	8.17	7.47	6.83
63	0.001	1.87	2.57	3.51	425	385	348	0.05	8.14	7.36	6.66
70	0.001	2.01	2.85	4.04	416	372	333	0.05	8.07	7.21	6.45
90	0.001	2.45	3.85	6.03	390	338	293	0.05	7.72	6.68	5.79
100	0.001	2.71	4.47	7.36	378	322	275	0.05	7.51	6.40	5.45
120	0.001	3.31	6.03	10.98	354	293	242	0.05	7.07	5.84	4.82
140	0.001	4.05	8.14	16.38	332	266	213	0.05	6.64	5.31	4.25
160	0.001	4.94	10.99	24.43	312	241	187	0.05	6.23	4.83	3.74
180	0.001	6.04	14.84	36.45	293	219	165	0.05	5.85	4.39	3.29
200	0.001	7.37	20.03	54.38	274	199	145	0.05	5.49	3.99	2.90
220	0.001	9.01	27.03	81.13	257	181	127	0.05	5.15	3.62	2.55
240	0.001	11.00	36.49	121.03	241	164	112	0.05	4.83	3.29	2.24

[1]Estimated assuming the given growth rate constant (k_G).
[2]Uptake rate constant, estimated from the fish weight using the Sijm *et al*. (1995) method.
[3]Total depuration rate constant.

Table 5.2 Hypothetical uptake data for a relatively rapidly depurating substance

Time (days)	Water concen-tration (mg l^{-1})	Fish weight (g)[1]			Predicted k_1[1, 2] (l kg^{-1} day^{-1})			k_2[1, 3] assumed (day^{-1})	Predicted concentration in fish (mg kg^{-1})[1]		
		k_G = 0.01 day^{-1}	k_G = 0.015 day^{-1}	k_G = 0.02 day^{-1}	k_G = 0.01 day^{-1}	k_G = 0.015 day^{-1}	k_G = 0.02 day^{-1}		k_G = 0.01 day^{-1}	k_G = 0.015 day^{-1}	k_G = 0.02 day^{-1}
1	0.001	1.00	1.00	1.00	520	520	520	0.5	0.41	0.41	0.41
2	0.001	1.02	1.03	1.04	517	516	514	0.5	0.65	0.65	0.65
5	0.001	1.05	1.07	1.10	512	508	504	0.5	0.94	0.93	0.93
7	0.001	1.07	1.11	1.15	509	503	498	0.5	0.99	0.98	0.97
14	0.001	1.15	1.23	1.32	498	487	476	0.5	0.99	0.97	0.95
21	0.001	1.23	1.37	1.52	487	471	455	0.5	0.97	0.94	0.91
28	0.001	1.32	1.52	1.74	476	455	435	0.5	0.95	0.91	0.87
35	0.001	1.42	1.69	2.01	465	440	416	0.5	0.93	0.88	0.83
42	0.001	1.52	1.87	2.31	455	425	398	0.5	0.91	0.85	0.80
49	0.001	1.63	2.08	2.65	445	411	381	0.5	0.89	0.82	0.76
56	0.001	1.75	2.31	3.05	435	398	364	0.5	0.87	0.80	0.73
63	0.001	1.87	2.57	3.51	425	385	348	0.5	0.85	0.77	0.70
70	0.001	2.01	2.85	4.04	416	372	333	0.5	0.83	0.74	0.67
90	0.001	2.45	3.85	6.03	390	338	293	0.5	0.78	0.68	0.59
100	0.001	2.71	4.47	7.36	378	322	275	0.5	0.76	0.64	0.55
120	0.001	3.31	6.03	10.98	354	293	242	0.5	0.71	0.59	0.48
140	0.001	4.05	8.14	16.38	332	266	213	0.5	0.66	0.53	0.43
160	0.001	4.94	10.99	24.43	312	241	187	0.5	0.62	0.48	0.37
180	0.001	6.04	14.84	36.45	293	219	165	0.5	0.59	0.44	0.33
200	0.001	7.37	20.03	54.38	274	199	145	0.5	0.55	0.40	0.29
220	0.001	9.01	27.03	81.13	257	181	127	0.5	0.51	0.36	0.25
240	0.001	11.00	36.49	121.03	241	164	112	0.5	0.48	0.33	0.22

[1]Estimated assuming the given growth rate constant (k_G).
[2]Uptake rate constant, estimated from the fish weight using the Sijm *et al*. (1995) method.
[3]Total depuration rate constant.

As can be seen from Figure 5.1 and Figure 5.2, the curves show a reducing concentration in the fish at longer exposure times (note that this is despite a constant exposure concentration, where no depuration phase is assumed in the plots). Such a reduction in concentration towards the end of the uptake phase is seen in some actual bioconcentration experiments and it is often explained in terms of induction of enzymes leading to enhanced metabolism (with th effect of increasing k_2). However, the analysis here indicates that it could also result from a reduction in k_1 as the fish grow. The effect of this will depend on the relative values of k_1 and k_2 (and how they change with the size of the fish).

A summary of the main points apparent from these plots is given below.

- An apparent steady state is reached between 10 to 20 days (in the rapidly depurated example) or 40 to 60 days (in the more slowly depurated example) followed by a steady decline in concentration in the fish.
- The apparent steady state is reached more quickly in the more rapidly growing fish.
- The apparent steady state concentration (BCF) is lower in faster growing fish than in slower growing fish. This difference becomes more marked the slower the overall depuration of the substance in the fish.
- The apparent steady state is always lower than the steady state assuming a constant k_1 value.

This analysis implies that the reduction in k_1 as a result of the growth of the fish[18] can affect the apparent steady-state level reached in the fish. Further, with increased exposure the level in the fish would be expected to decline below the apparent steady-state level (resulting in a lower BCF). This is an important point as the current OECD 305 Test Guideline considers that steady state has been reached when the curve of the plot of the test substance in fish (C_{fish}) against time becomes parallel to the time axis and three successive analyses of C_{fish} made on samples at intervals of at least two days are within ±20 per cent of each other, and there are no significant differences among the three sampling periods. Thus, normally the uptake phase of a BCF study would be stopped when the apparent steady state was reached, and a BCF based on this level would normally be reported.

The time to the apparent steady state in growing fish will be shorter than given by the method outlined in the OECD 305 Test Guideline. The equations used are given below, but these equations only hold if the value for the uptake rate constant (k_1) is constant during the test. If the value of k_1 decreases during the test, the apparent steady state will be reached in a shorter period of time than predicted using these equations.

Equation 44 $$t_{80} = \frac{1.6}{k_2}$$

Equation 45 $$t_{95} = \frac{3.0}{k_2}$$

Where t_{80} = Time to reach 80 per cent of steady state (days).

t_{95} = Time to reach 95 per cent of steady state (days).

k_2 = Overall depuration rate constant (days).

The importance of the apparent steady state in the comparison of steady state BCF against regulatory criteria, such as the REACH[24] Annex XIII for bioaccumulative (B) and very bioaccumulative (vB) substances is considered in Section 5.8.

5.3 Growth dilution and the kinetic BCF

Growth dilution is a process whereby the concentration in a fish drops as the fish grows (in effect the same amount of substance is "diluted" within a larger mass of fish). It is usually considered a depuration process (even though it does not result in a loss of mass of substance from the fish) as it contributes to the decline in concentration in the fish during the depuration phase of a BCF or a BMF_{food} study. The contribution from growth dilution is already included in the overall depuration rate constant (k_2) determined from such studies (and this overall k_2 value should be used when fitting the experimental data from such studies). When the amount of substance (as opposed to concentration) in fish is similar at the start and end of the depuration phase, the main depuration process is likely to be growth. In such cases, depuration is likely to be slow when the fish ceases growth or is only growing slowly. Some regulatory regimes (for example REACH) require the overall depuration rate constant from such studies to be growth-corrected and a growth-corrected BCF (or BMF_{food}) estimated from the data. The background and method for carrying out this correction is given below.

The correction relies on the depuration process following first-order kinetics in relation to the concentration in the fish, with each contribution to the overall depuration rate constant also following first-order kinetics with relation to the concentration in the fish. In this case, the overall depuration rate constant can be written in terms of its constituent first-order processes as follows:

Equation 46 $$k_2 = k_r + k_m + k_g + k_e$$

Where k_2 = Overall depuration rate constant obtained from the experimental depuration curve (day^{-1}).

k_r = Rate constant for elimination via respiratory surfaces (day^{-1}).

k_m = Rate constant for metabolism (day^{-1}).

k_g = Rate constant for growth dilution (day^{-1}).

k_e = Rate constant for elimination via faeces (day^{-1}).

Different models are available for estimating the growth rate constants of fish, for example exponential models and linear models (see Domoradzki (2008) in Brooke *et al*. (2009) for examples). However in terms of the correction for growth dilution here, it is not the actual fish growth rate constant that is important, rather the rate constant for growth dilution (these are not necessarily the same parameter). One way to visualise this is to consider a hypothetical fish in which the only process that reduces the concentration is growth dilution (based on Brooke *et al*. 2009). In this case the rate of depuration can be written in terms of a simple first-order process as follows:

Equation 47 $$\frac{d[C_{fish}]}{dt} = -k_{growth-dilution}[C_{fish}]$$

[24] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.

Where $d[C_{fish}]/dt$ = Rate of change in the concentration in fish (mg kg^{-1} day^{-1}).

$[C_{fish}]$ = Concentration in fish (mg kg^{-1}).

$k_{growth\text{-}dilution}$ = Rate constant for growth dilution (day^{-1}).

The integrated form of this rate equation leads to the following solution:

Equation 48 $$\ln[C_{fish}] = -k_{growth-dilution} \times t + constant$$

Where $\ln[C_{fish}]$ = Natural logarithm of the fish concentration.

t = Time (days).

Constant = A constant.

If it is then assumed that the amount of chemical in the fish is x mg, the concentration in fish at any time (t) can be estimated as $[C_{fish}]$ = x/fish weight (kg). Substituting this into Equation 48 leads to the following:

Equation 49 $$\ln\left(\frac{x}{\text{Fish weight}}\right) = -k_{growth-dilution} \times t + constant$$

As only growth dilution is considered here, and as growth dilution itself does not lead to a reduction in the mass (x) in the fish, this will hold at all time points during the depuration (x is effectively constant). This allows the above equation to be re-written as follows (where the constant now also includes the term –ln (x)):

Equation 50 $$\ln\left(\frac{1}{\text{Fish weight}}\right) = -k_{growth-dilution} \times t + constant$$

Thus a plot of 1/fish weight against time should give a straight line with the slope representing the first-order rate constant for growth dilution.

Once the first-order rate constant for growth dilution is obtained ($k_{growth\text{-}dilution}$) it is then straightforward to estimate the growth-corrected depuration rate constant from the overall depuration rate constant as follows:

Equation 51 $$k_{2-growth-corrected} = k_2 - k_{growth-dilution}$$

Where $k_{2\text{-}growth\text{-}corrected}$ = Growth-corrected depuration rate constant (day^{-1}).

k_2 = Overall depuration rate constant obtained from the experimental depuration curve (day^{-1}).

$k_{growth\text{-}dilution}$ = Rate constant for growth dilution (day^{-1}).

The growth-corrected depuration rate constant ($k_{2\text{-}growth\text{-}corrected}$) is in effect the rate constant that would be expected in a fish that was not growing. It still includes contributions from all of the other depuration processes occurring in the fish (such as elimination via respiratory surfaces, elimination via faeces and metabolism).

The growth-corrected $BCF_{growth\text{-}corrected}$ can then be estimated from the ratio of the uptake rate constant (k_1) and the growth-corrected depuration rate constant ($k_{2\text{-}growth\text{-}corrected}$). This BCF is effectively for a fish where no growth dilution occurs. There is an important assumption inherent in the growth-corrected BCF: the uptake rate constant (k_1) is the same in growing fish as it would be in fish that are not growing[25]. Further considerations on how these growth-corrected BCFs should be compared against regulatory criteria are discussed in Section 5.8.

[25] The growth-corrected BCF also assumes that the $k_{2\text{-}growth\text{-}corrected}$ obtained in this way is appropriate for larger, slowly growing fish (see Section 5.6).

As can be seen, the importance of growth dilution depends on how large the $k_{growth\text{-}dilution}$ value is compared with the overall depuration rate constant. This means that growth dilution can still be important even for fish that appear to be growing only at a slow rate if the overall depuration rate constant is small. Conversely, if fish are growing at a very fast rate it could be that growth dilution is not important if the overall depuration rate constant is also very high.

A further point to note is that if the rate constant for growth dilution ($k_{growth\text{-}dilution}$) is large relative to the overall depuration rate constant (k_2), that is, the overall depuration is dominated by growth, there will be a large uncertainty in the growth-corrected rate constant ($k_{2\text{-}growth\text{-}corrected}$) as this is the difference between two similar numbers, each with its own uncertainty. This is demonstrated in Example 1 in Section 5.7.1.

In such cases an alternative approach could be considered to estimate the growth-corrected depuration rate constant. This possible alternative approach results from the fact that a) growth dilution, although considered a depuration process, does not actually result in loss of substance from the fish (the substance is simply diluted in a greater mass of fish as the fish grows) and b) for a first-order process there is no reason why units other than mass of substance/mass of fish should be used for the ln [C_{fish}] versus time plot (Equation 43). For example, if the concentration in fish is expressed in terms of mg substance/fish, this will effectively factor out the effect of growth dilution (for a given mg kg^{-1} concentration, larger fish will have more substance present than smaller fish) and so a plot of ln [amount/fish] should give the growth-corrected k_2 directly (as the slope of the plot) without having to separately subtract out the growth rate constant. The amount of substance per fish can be readily obtained from the measured concentration and the fish weight at each time point. The effect of this analysis of depuration data is shown in Example 1 in Section 5.7.1.

This alternative method has not yet been validated and should be used with caution until it has been tested further.

5.4 Is growth dilution a relevant consideration for a steady-state BCF?

At first sight, growth dilution may not be relevant to a steady-state BCF. However if the underlying processes are considered, the apparent steady state is reached as a consequence of all of the depuration processes acting in the fish at the same time. Thus if growth of the fish is occurring, growth dilution would be one of the contributory factors to the final concentration reached in the fish and hence the steady-state BCF. Therefore in regulatory regimes that require growth-corrected BCF to be used, growth correction of a steady-state BCF may be equally as relevant as that for a kinetic BCF.

Unfortunately, the only way to reliably correct a BCF for growth dilution is via the kinetic approach outlined in Section 5.3.

One possible alternative approach for growth correction of a steady-state BCF not requiring knowledge of the underlying rate constants is outlined below.

Assume that the steady-state concentration reached in the fish is 5 mg kg^{-1} following exposure to 0.001 mg l^{-1} for 28 days. The steady-state BCF would therefore be 5,000 l kg^{-1}. Over the same 28-day period the fish have grown from an initial weight of X kg to a final weight of Y kg. The amount of substance present in the fish at steady state is given by the following equation:

Equation 52

$$\text{Amount}_{fish-steady-state} = C_{fish-steady-state} \times Y$$

Where $Amount_{fish\text{-}steady\text{-}state}$ = amount of substance in the fish at apparent steady state (mg).

$C_{fish\text{-}steady\text{-}state}$ = concentration of substance in fish at apparent steady state (mg kg^{-1}).

Y = final weight of fish at apparent steady state (kg).

This is 5Y mg in this example.

If growth was not occurring, the steady-state amount of substance in the fish would be present in the initial weight of the fish. Thus, the growth-corrected steady-state concentration in the fish could be estimated as follows:

Equation 53

$$C_{fish-steady-state-growth-corrected} = \frac{Amount_{fish-steady-state}}{X}$$

Where $C_{fish\text{-}steady\text{-}state\text{-}growth\text{-}corrected}$ = estimated steady-state concentration in fish assuming no growth was occurring.

X = initial weight of fish (kg).

In this example the growth-corrected concentration is 5Y/X mg kg^{-1}.

The growth-corrected steady-state BCF could then be estimated as the ratio of the $C_{fish\text{-}steady\text{-}state\text{-}growth\text{-}corrected}$ to the water concentration, or (5Y/X)/0.001 = 5000Y/X in this example. The correction is therefore the ratio of the final to the initial weight of the fish.

However, this approach is not considered appropriate as it is not the actual growth of the fish itself that is important, but the relative magnitude of the rate constant for growth dilution compared with the overall rate constant for depuration (as explained in Section 5.3 and shown by comparison of Example 1 and Example 2 in Sections 5.7.1 and 5.7.2 where, although the rate constant for growth dilution was similar in both examples, the effects of correcting for growth dilution were significant in Example 1 but not Example 2). For example, it could be that even though the fish double in size over the uptake phase, this could still translate to a growth dilution rate constant that is insignificant compared with the overall depuration rate constant (and hence this approach would underestimate the growth-corrected steady-state BCF). Conversely, if the fish only slowly increase in size this could still be important if the growth dilution rate constant is significant compared with the overall depuration rate constant (in this case the above approach would underestimate the growth-corrected steady-state BCF).

5.5 Lipid normalisation

Some regulatory regimes require the BCF value to be normalised to a standard lipid content (for example the REACH guidance suggests that BCFs should be normalised to a lipid content of five per cent). In principle the lipid normalisation/standardisation procedure is straightforward as shown in Equation 54:

Equation 54

$$BCF_{norm} = BCF_{exp} \times \frac{Lipid_{stand}}{Lipid_{fish}}$$

Where BCF_{norm} = BCF value normalised to the standard lipid content (l kg^{-1}).

BCF_{exp} = BCF determined experimentally (l kg^{-1}).

$Lipid_{stand}$ = standard percentage of lipid assumed in the fish (%).

$Lipid_{fish}$ = actual percentage lipid in the fish used in the experiment (%).

When considered kinetically in relation to the use of predicted k_1 values and a k_2 value obtained from a dietary study, the issue may not be so straightforward. All but one of the methods considered for predicting k_1 show no direct dependence on the lipid content in the fish. This implies that in most cases, the dependence on lipid will be related to the k_2 value (and hence the lipid content of the fish used in the feeding study will be important). However, the k_2 value obtained in a fish feeding study is the overall depuration rate constant and, as explained in Section 5.3, will consist of contributions from all depuration processes including metabolism, excretion via respiration, excretion via faeces and growth dilution. It is not clear if all of these processes will show the (same) dependence on the lipid content of the fish (for example, it is not immediately obvious whether processes such as metabolism or growth dilution will depend on the lipid content of the fish, or whether dependence will be the same for each parameter). Therefore, lipid normalisation of data for substances that are rapidly metabolised or where growth dilution makes up a significant proportion of the overall depuration seen, may need to be done carefully (or may not be appropriate)[26].

5.6 Indirect effects of growth on depuration

Although the main intention of this report is to concentrate on methods for predicting an uptake rate constant, k_1, that could be used in conjunction with a depuration rate constant, k_2, from a feeding study in order to estimate a (growth-corrected) BCF, consider that growth may also have an indirect effect on the depuration rate. An assumption inherent in the estimation of BCF in this way is that the growth-corrected k_2 value obtained in the feeding study is appropriate for larger, slowly growing fish.

Equation 46 in Section 5.3 shows that the overall depuration rate constant (k_2) consists of four main components. Of these, the rate constant for growth dilution (k_g) can be calculated separately and subtracted to give the growth-corrected depuration rate constant (Equation 51). Therefore, the growth-corrected depuration rate constant consists of contributions from elimination via respiratory surfaces, metabolism and elimination via faeces.

Equation 55 $$k_{2-\text{growth}-\text{corrected}} = k_r + k_m + k_e$$

Where $k_{2\text{-growth-corrected}}$ = Growth-corrected depuration rate constant (day^{-1}).

k_r = Rate constant for elimination via respiratory surfaces (day^{-1}).

k_m = Rate constant for metabolism (day^{-1}).

k_e = Rate constant for elimination via faeces (day^{-1}).

Growth, or the size of the fish, may have an effect on these individual rate constants, and hence $k_{2\text{-growth-corrected}}$. This is discussed here qualitatively as a detailed analysis of growth-corrected depuration rate constants is beyond the scope of this report.

Elimination via respiratory surfaces is thought of as the transfer from the fish to the water phase via the gills. The rate of this transfer would be expected to depend, to some extent, on the gill ventilation rate of the fish. The gill ventilation rate of fish is known to be dependent on fish size, with smaller fish generally having a higher ventilation rate relative to their size and/or larger gill area to body weight ratios than larger fish (Sijm *et al.* (1995), Gobas and Mackay (1987), Opperhuizen (1991) and Barber *et al.* (1988)). Therefore, a size dependence on the rate constant for elimination via respiratory surfaces (k_r) could be expected with the k_r decreasing with increasing size of the fish.

[26] Further investigation of this is beyond the scope of the current project.

It has been suggested that the metabolic capacity of fish may depend on their size (Arnot, personal communication). For example, Hendriks *et al.* (2001) assumed that the metabolic rate constant was proportional to the species weight to the power of -0.25 to -0.33 for a range of different species based on allometric regression equations. If this is the case for a specific chemical a size dependence of k_m would be expected, with the metabolic capacity and hence k_m decreasing as the weight of the fish increased.

Faecal elimination of a chemical would be expected to depend on the faecal production rate within the organism, which is likely to depend on the feeding rate used (see Section 2.2.2). As most BCF tests and, in particular, dietary BMF_{food} tests are carried out at constant feeding rates, the size dependence of k_e during any given BCF or BMF_{food} study could be expected to be small. However, this may not necessarily be the case when considering extrapolation of the results from laboratory tests to fish in the wild. For example, as the ventilation rate and metabolic rate of fish both appear to decrease with fish size, the food ingestion per unit body mass could also follow a similar pattern (McLachlan, personal communication).

If these individual rate constants do vary with the size of the fish, this would be shown by the non-linearity of a plot of ln $[C_{fish}]$ against time for the data in the depuration phase. However, it may not always be possible to detect such non-linearities as the depuration phase can often be dominated by the growth rate constant (k_g).

One final consideration on the feeding rate is that, although most BCF tests and dietary BMF_{food} tests are carried out at constant feeding rates during the test, the actual feeding rate may be different in one study compared with another. For substances that depurate significantly by faecal elimination, this may result in complications in comparing depuration rate constants from different study designs. For example the current proposal for the OECD 305 feeding study Test Guideline is to use a feeding rate of three per cent body weight whereas the OECD 305 BCF study Test Guideline currently recommends a lower feeding rate of one to two per cent body weight.

5.7 Examples

To illustrate some of the issues and potential solutions raised in Sections 5.1 to 5.6, a number of real examples are considered. These are substances for which a reliable BCF test has been carried out according to the OECD 305 Test Guideline, and a full laboratory test report is available outlining all of the experimental details. However, in order to maintain confidentiality, the identities of the substances are not given.

5.7.1 Example 1

Substance (log K_{ow} ~7) was tested with rainbow trout (*Oncorhynchus mykiss*) over a 35-day uptake period followed by a 42-day depuration period. Mean measured exposure concentration was 0.34 µg l^{-1}. The concentrations measured in the fish at various time points are summarised in

Table 5.3.

Table 5.3 Fish concentration data for Example 1

Time (day)[1]	Mean concentration in fish (mg kg^{-1})	Mean fish weight (g)
Uptake phase		
0		1.21
7	1.04	1.96
14	1.56	2.49
21	1.92	3.01
28	2.14	3.85
35	2.26	5.08
Depuration phase		
7 (42)	1.65	5.97
14 (49)	1.40	7.72
21 (56)	0.94	9.00
28 (63)	0.87	9.35
35 (70)	0.65	10.61
42 (77)	0.44	14.85

[1]The days given in brackets reflect the overall study day.

Uptake and depuration rate constants were determined using a commercial program employing first-order kinetics (presumably using the simultaneous method fitted over uptake and depuration curves). These were k_1 = 395 l kg^{-1} day^{-1} and k_2 = 0.0432 day^{-1}. Comparing these kinetic parameters to the experimental data shows reasonable agreement (see Figure 5.3). The kinetic BCF derived from these data is 9,100 l kg^{-1}.

The data were reanalysed here using the approach outlined in Section 5.1. Firstly the value of k_2 was obtained from of plot of ln [C_{fish}] against time for the depuration phase alone. This is shown in Figure 5.4. From this the overall depuration rate constant k_2 can be determined to be 0.0382 day^{-1}. The linearity of the plot, and r^2 value confirmed that the depuration follows first-order kinetics in this case.

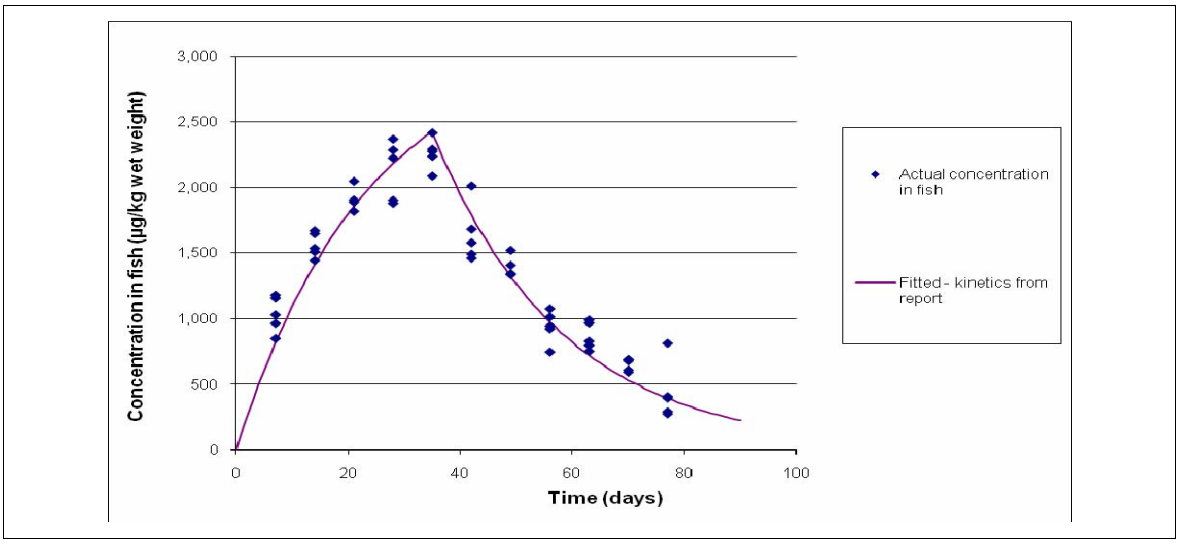

Figure 5.3 Comparison of derived kinetics with the actual uptake and depuration curve for Example 1

Next, the value for the uptake rate constant was determined at each time point[27] using Equation 39 and assuming the k_2 value was 0.0382 day^{-1}. The results of this analysis are shown in Table 5.4. These data clearly show that the value of k_1 decreases with increasing exposure time. The equivalent kinetic BCF is also shown at each time point and these values show a gradual decrease in the BCF with time. A plot showing the fit of these kinetic data to the experimental data over the entire experimental period is given in Figure 5.5. As can be seen, the revised kinetics explain the observed uptake and depuration curves well.

The k_1 values estimated in Table 5.4 are generally very close to values that would be predicted using the Sijm *et al.* (1995) method. For example, assuming the initial fish weight is 1.21 g, the Sijm *et al.* (1995) method would predict a k_1 value of 489 l kg^{-1} day^{-1} compared with the value of 497 l kg^{-1} day^{-1} estimated in Table 5.4 at Day 7, and the k_1 estimated at Day 35 of the uptake using the Sijm *et al.* (1995) method (fish weight 5.08 g) would be 309 l kg^{-1} day^{-1} compared with the value of 345 l kg^{-1} day^{-1} estimated in Table 5.4.

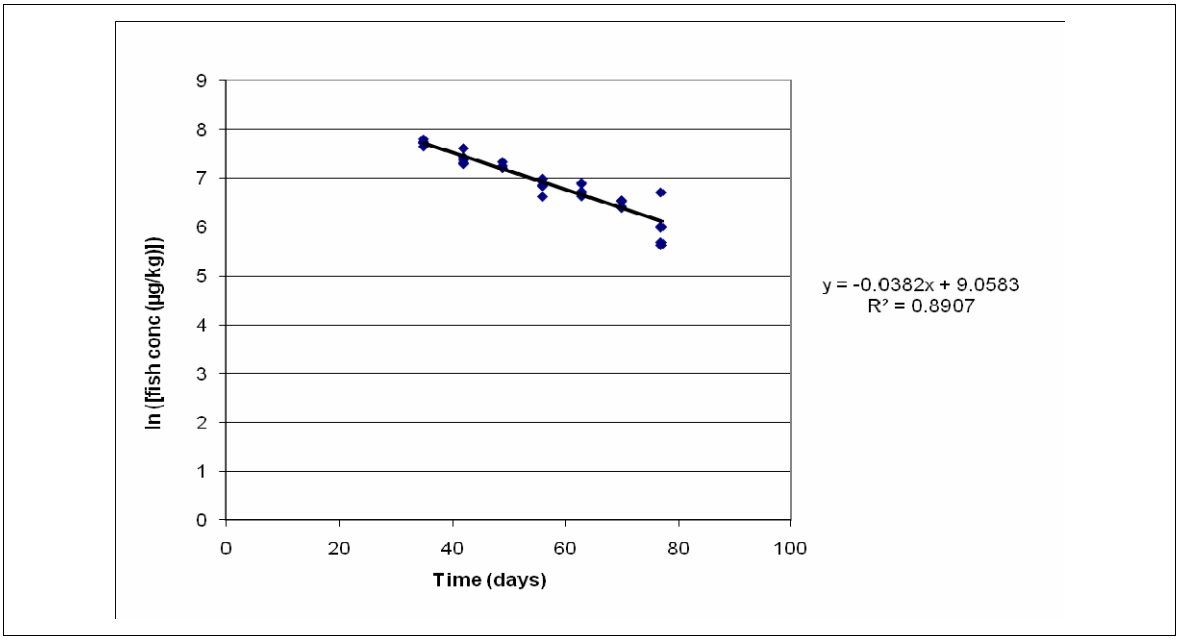

Figure 5.4 Plot of ln [C_{fish}] against time for the depuration phase in Example 1

Table 5.4 Calculation of uptake rate constant (k_1) for each time point for Example 1

Time (days)	Calculated k_1 (l kg^{-1} day^{-1})[1]	Kinetic BCF (l kg^{-1})	Growth corrected kinetic BCF (l kg^{-1})
7	497	13,000	60,600
14	424	11,100	51,700
21	390	10,200	47,600
28	365	9,600	44,500
35	345	9,000	42,000

[27] Individual k_1 values calculated at each time point using Equation 39 are not direct estimates of k_1 at each respective time point but rather they represent an "average" or "integrated" value over the exposure time period up to that time point. Therefore a single elevated k_1 value at the beginning of the experiment will automatically result in a continuous decrease in this "average" k_1 over time, even if actual k_1 at each time point is constant for the remainder of the experiment.

In this study, the rate constant for growth dilution ($k_{growth\text{-}dilution}$) can be estimated as 0.030 day^{-1} from a plot of ln (1/fish weight (kg)) against time (see Figure 5.6). Using this, the growth-corrected depuration rate constant ($k_{2\text{-}growth\text{-}corrected}$) can be estimated as approximately 0.0082 day^{-1}. The growth-corrected BCF obtained at each time point can then be estimated. The values are shown in Table 5.4. In this case, as the value for the $k_{growth\text{-}dilution}$ is close to the value for the overall depuration rate constant (k_2) growth-correction has a large effect on the overall BCF obtained, even though the fish only increase in size from around 1.2 g to around 5.1 g over the uptake period.

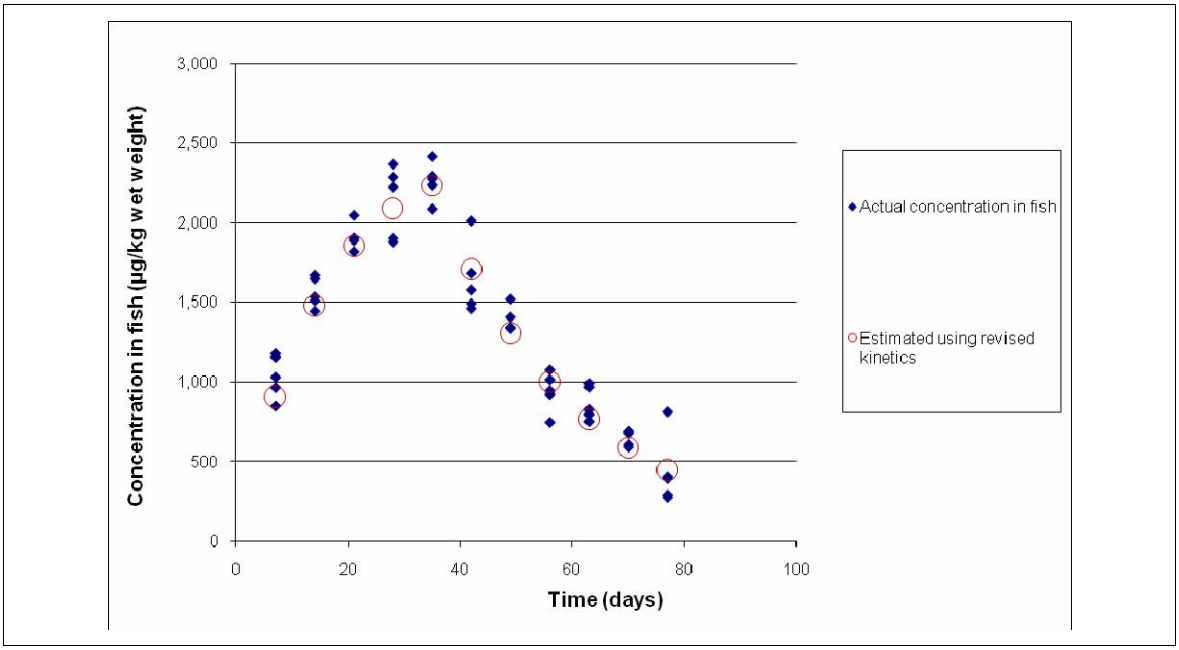

Figure 5.5 Comparison of re-analysed kinetic data with the experimental data for Example 1

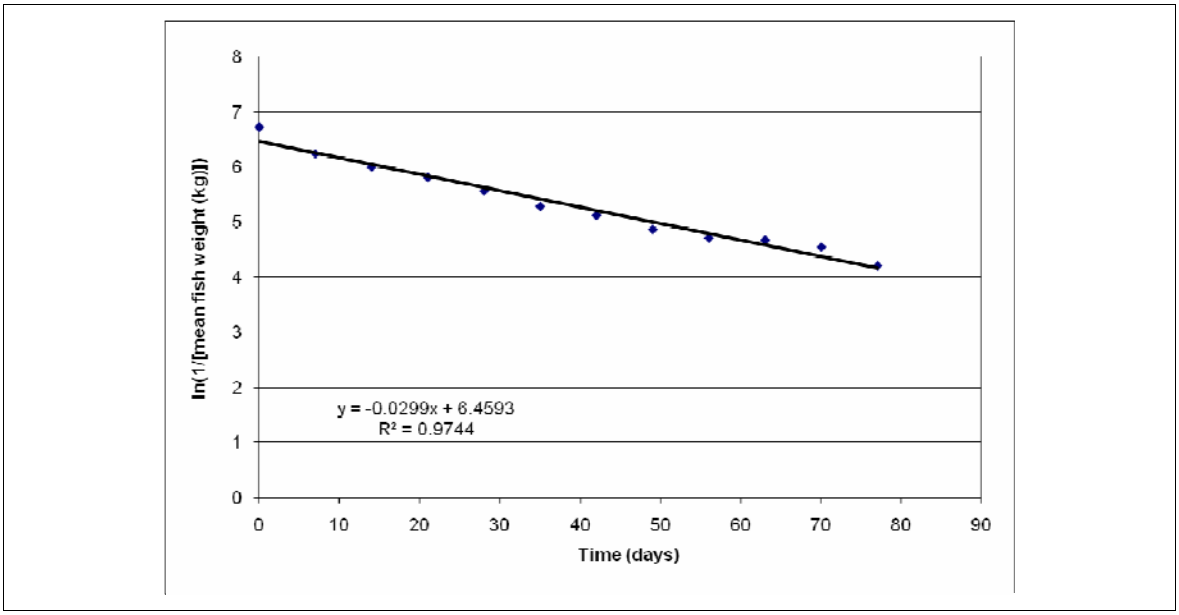

Figure 5.6 Rate constant for growth dilution for Example 1

In this example, the growth-corrected depuration rate constant of 0.0082 day^{-1} will have considerable uncertainty attached to it as it is obtained by difference between two large, and similar, numbers, each with their own uncertainty (in this case the overall

depuration rate constant is 0.0382 day^{-1} and the rate constant for growth dilution is 0.030 day^{-1}). The results should be considered with this in mind.

As discussed in Section 5.3, a plot of ln [amount of substance/fish] against time should provide the growth-corrected depuration rate constant directly. Such a plot is shown in Figure 5.7 for this substance. In this case, the amount of substance/fish was estimated from the mean measured concentration (in mg kg^{-1}) at each time point and the mean fish weight at each time point.

As can be seen, the slope of this plot would suggest that the growth-corrected depuration rate constant would be around 0.014 day^{-1}, which is of a similar order to that obtained above by difference. However, as the value of 0.014 day^{-1} is obtained by regression analysis of several data points, it is likely to have a lower overall uncertainty than the value obtained by difference in this case. In addition, the linearity of this plot and the r^2 value of 0.92 suggests that the remaining depuration processes (excluding the growth dilution) follow first-order kinetics (no indirect effects of growth on the growth-corrected k_2 value are evident in this case).

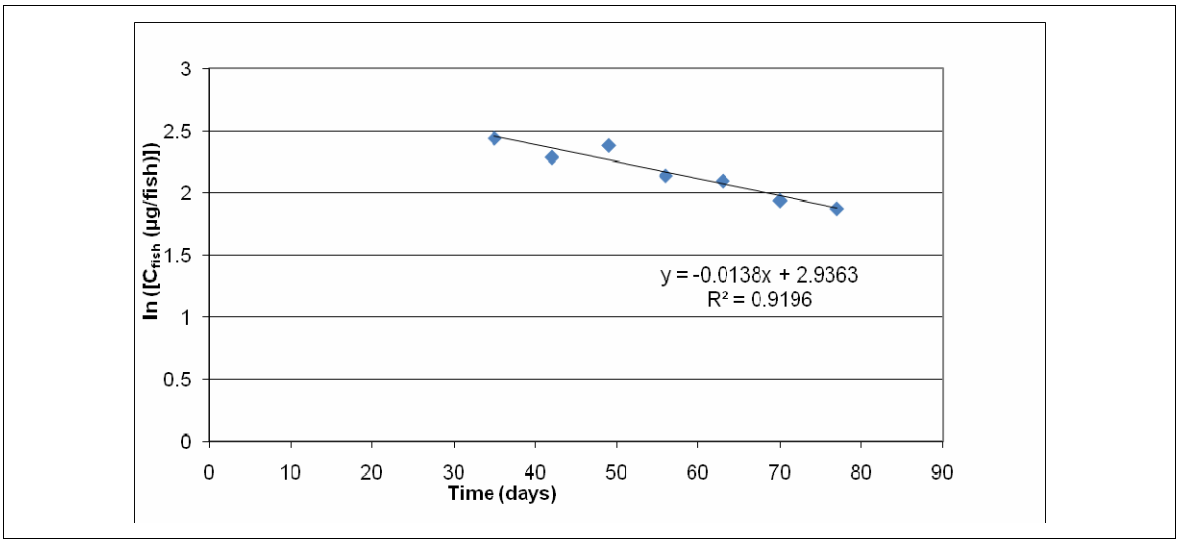

Figure 5.7 Growth-corrected depuration rate constant determined directly from the mass of substance per fish for Example 1

5.7.2 Example 2

The substance was tested with rainbow trout over a 27-day uptake period and 11-day depuration period. Two exposure concentrations were used (mean concentration 0.10 µg/l and 1.2 µg/l). The concentrations measured in the fish at various time points are summarised in

Table 5.5.

Table 5.5 Fish concentration data for Example 2

Time (day)[1]	Mean concentration in fish (µg kg^{-1})		Mean fish weight (g)
	Low exposure group	High exposure group	
Uptake phase			
0	0	0	2.36
0.167 (4 hours)	8.3	123	
1	24.1	297	2.31
3	36.4	430	2.25
7	44.8	511	2.52
11	42.4	489	2.90
14	40.4	542	2.80
21	43.9	477	3.18
24	47.9	600	2.82
Depuration phase			
1 (28)	35.6	463	3.86
3 (30)	24.3	348	3.59
8 (35)	10.4	103	3.57
11 (38)	No data	No data	4.16

[1]The days given in brackets reflect the overall study day.

Apparent steady state was reached rapidly in this study (from around Day 3 onwards) and the steady-state BCF was determined to be 426 l kg^{-1} at the low exposure concentration and 423 l kg^{-1} at the high exposure concentration (based on the mean measured concentration in the fish between Day 3 and Day 24).

The uptake and depuration rate constants estimated in the test report using these data were k_1 = 91.5 l kg^{-1} day^{-1} and k_2 = 0.180 day^{-1} (giving a kinetic BCF of 508 l kg^{-1}) for the 0.10 µg l^{-1} treatment and k_1 = 109 l kg^{-1} day^{-1} and k_2 = 0.224 day^{-1} (giving a kinetic BCF of 487 l kg^{-1}) for the 1.2 µg l^{-1} treatment[28]. These data were not corrected for growth of the fish. A plot showing the fit of these kinetic parameters with the experimental data is shown in Figure 5.8 for the lower exposure group.

[28] The report is not clear which method was used to estimate the kinetic parameters.

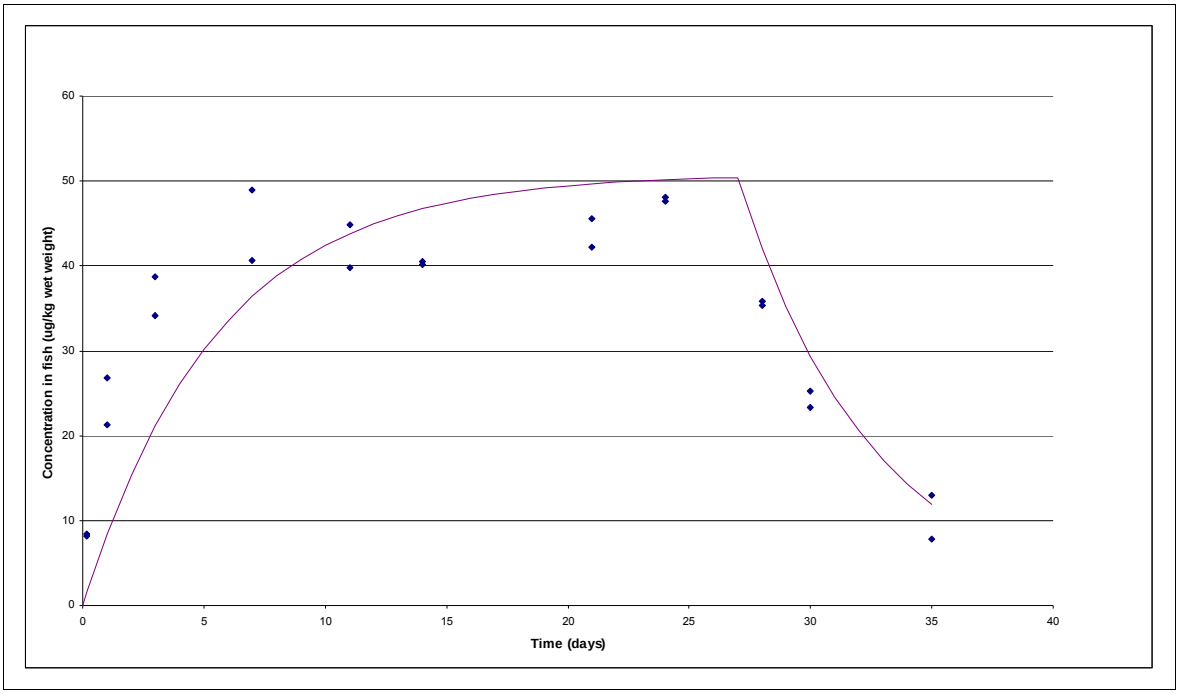

Figure 5.8 Comparison of derived kinetics with the actual uptake and depuration curve for Example 2 (low exposure concentration)

The data for low and high exposures were reanalysed here using the approach outlined in Section 5.1. Firstly, the value of k_2 was obtained from the plot of ln [C_{fish}] against time for the depuration phase alone. This is shown in Figure 5.9. The overall depuration rate constants k_2 determined were 0.18 day^{-1} for the low exposure group and 0.224 day^{-1} for the high exposure group. The linearity of the plots and the r^2 value confirmed that the depuration followed first-order kinetics.

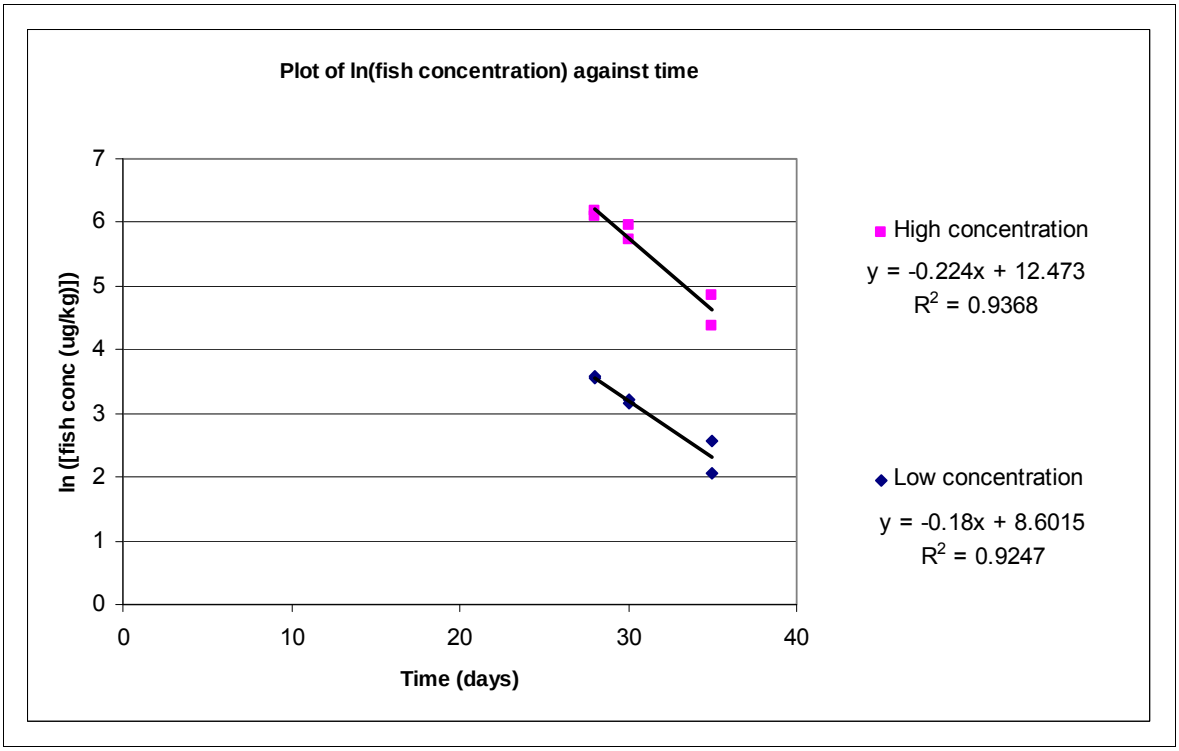

Figure 5.9 Plot of ln [C_{fish}] against time for the depuration phase in Example 2

Next, the value of the uptake rate constant was determined at each time point using Equation 39 and the overall depuration rate constant (0.18 day^{-1} for the low exposure group and 0.224 day^{-1} for the high exposure group). The results of this analysis are shown in Table 5.6. Again, these data clearly show that the value of k_1 decreases with increasing exposure time but it appears to level off to a value of around 90-100 l kg^{-1} as the apparent steady state is reached. The fit of these kinetic data to the experimental data for the low exposure concentration is given in Figure 5.10.

Table 5.6 Calculation of uptake rate constant (k_1) for each time point for Example 2

Time (days)	Calculated k_1 (l kg^{-1} day^{-1})		Kinetic BCF (l kg^{-1})		Growth corrected kinetic BCF (l kg^{-1})	
	Low exposure	High exposure	Low exposure	High exposure	Low exposure	High exposure
0.167	505	625	2,800	2,800	3,100	3,000
1	263	270	1,500	1,200	1,600	1,300
3	157	164	870	730	960	790
7	103	110	570	490	630	530
11	88	100	490	450	540	480
14	79	106	440	470	480	510
21	81	90	450	400	490	430
24	87	113	480	500	530	540

In this example, k_1 values estimated in Table 5.6 are generally similar to, but higher than, what would be predicted using the Sijm *et al.* (1995) method at the start of the experiment (assuming the initial fish weight is 2.36 g, the Sijm *et al.* method would predict a k_1 value of 395 l kg^{-1} day^{-1} compared with the value of 505-625 l kg^{-1} day^{-1} estimated in Table 5.4) but the k_1 estimated at the end of the uptake phase using the Sijm *et al.* (1995) method (fish weight 2.82 g) would be higher (375 l kg^{-1} day^{-1}) than the value of 87-113 l kg^{-1} day^{-1} estimated in Table 5.6.

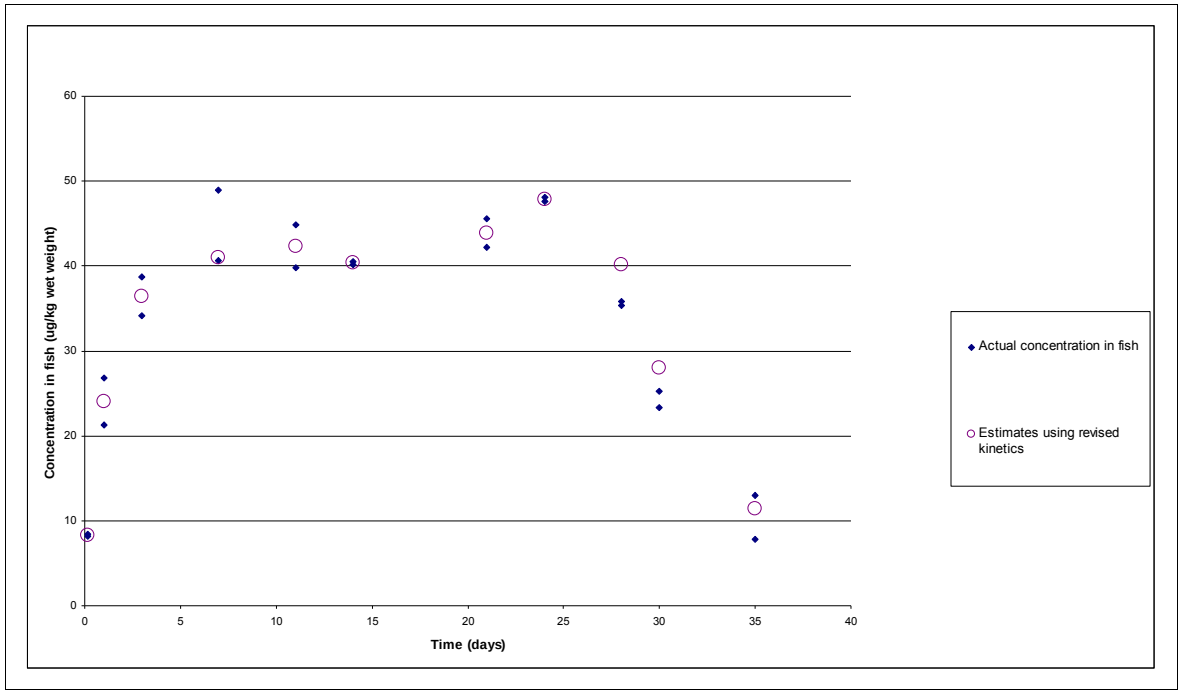

Figure 5.10 Comparison of re-analysed kinetic data with the experimental data for Example 2

The rate constant for growth dilution ($k_{growth\text{-}dilution}$) in the study is estimated to be 0.016 day^{-1} from a plot of ln (1/fish weight (kg) against time (see Figure 5.11)). The growth-corrected BCFs obtained using this value at each time point are shown in Table 5.6. As can be seen here, growth correction has little impact on the estimated BCF.

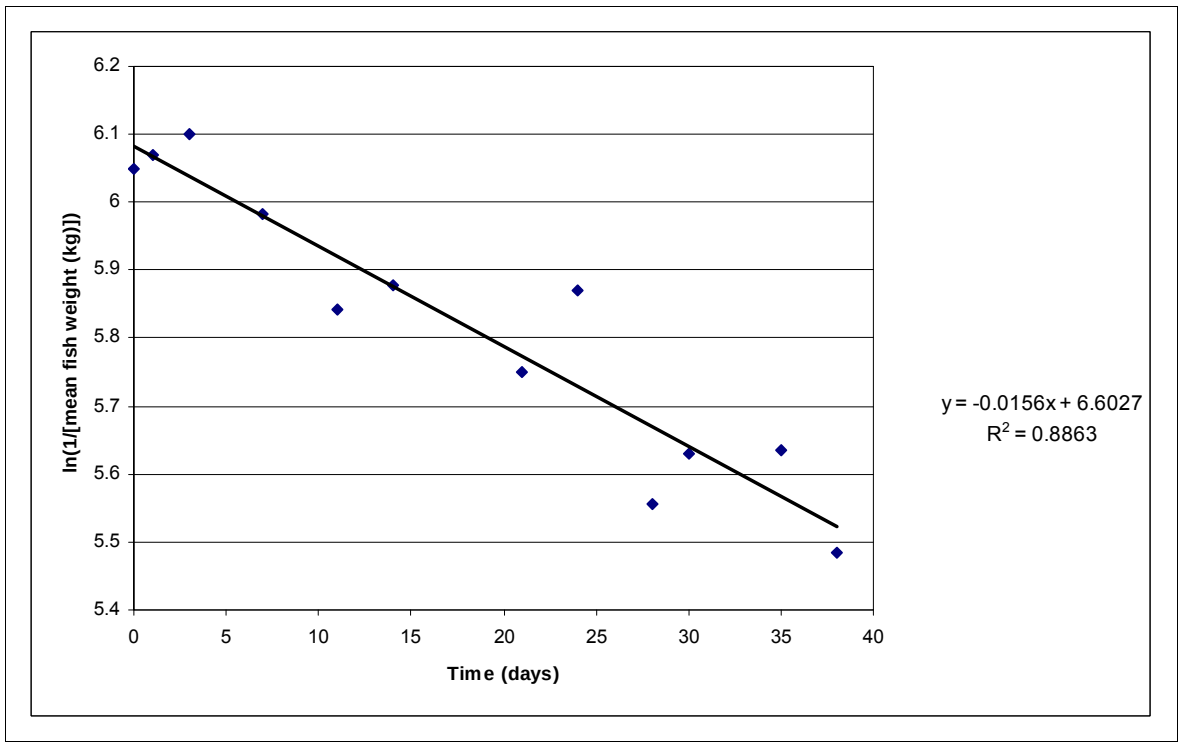

Figure 5.11 Estimation of rate constant for growth dilution ($k_{growth\text{-}dilution}$) for Example 2

5.7.3 Example 3

The substance (log K_{ow} 9.06) was tested with fathead minnows (*Pimephales promelas*) over a 49-day uptake period and a 98-day depuration period. Two exposure concentrations were used (mean measured concentrations of 0.41 and 4.4 µg l^{-1}). The concentrations measured in the fish at various timepoints are summarised in Table 5.7.

Apparent steady state was reached from around Day 35 onwards and the steady-state BCF was determined to be 1,160 l kg^{-1} at the low exposure concentration and 240 l kg^{-1} at the high exposure concentration. The uptake and depuration rate constants were estimated using the BIOFAC Computer software[29]. These were k_1 = 38.8 l kg^{-1} day^{-1} and k_2 = 0.0233 day^{-1} (giving a kinetic BCF of 1,660 l kg^{-1}) for the 0.41 µg l^{-1} treatment and k_1 = 8.29 l kg^{-1} day^{-1} and k_2 = 0.0260 day^{-1} (giving a kinetic BCF of 319 l kg^{-1}) for the 4.4 µg l^{-1} treatment. These data were not corrected for the growth of the fish. A plot showing the fit of these kinetic parameters with the experimental data is shown in Figure 5.12 for the lower exposure group.

Table 5.7 Fish concentration data for Example 3

Time (day)[1]	Mean concentration in fish (µg kg^{-1})		Mean fish weight (g)
	Low exposure group	High exposure group	
Uptake phase			
0	0	0	1.67
3	122	225	
7	223	355	
14	249	553	
21	339	658	
28	375	785	
35	513	1,010	
42	507	1,079	
49	404	1,079	1.29
Depuration phase			
1 (50)	327	932	
3 (52)	254	1,147	
7 (56)	454	369	
10 (59)	226	495	
14 (63)	215	516	
21 (70)	219	444	
28 (77)	203	472	
42 (91)	136	300	
56 (105)	197	259	
60 (119)	86.5	275	
84 (133)	169	196	
98 (147)	89	259	1.97

[1]The days given in brackets reflect the overall study day.

The data were reanalysed here using the approach outlined in Section 5.1. Firstly, the value of k_2 was obtained from the plot of ln [C_{fish}] against time for the depuration phase

[29] This is referenced as Blau and Agin (1978) BIOFAC. The Dow Chemical Company, Midland, Michigan.

alone. This is shown in Figure 5.13. The overall depuration rate constants k_2 determined were 0.0117 day^{-1} for the low exposure group and 0.0133 day^{-1} for the high exposure group. The r^2 value for the plots is relatively low, possibly reflecting a large amount of scatter in the data and/or potential non-linearity of the plot. As discussed in Section 5.6, a non-linear plot of the depuration data could suggest that the overall depuration is not strictly first order in this case; however, a more detailed evaluation of the depuration data is beyond the scope of the current report. For the following discussion, it is assumed that the depuration approximates first-order kinetics[30].

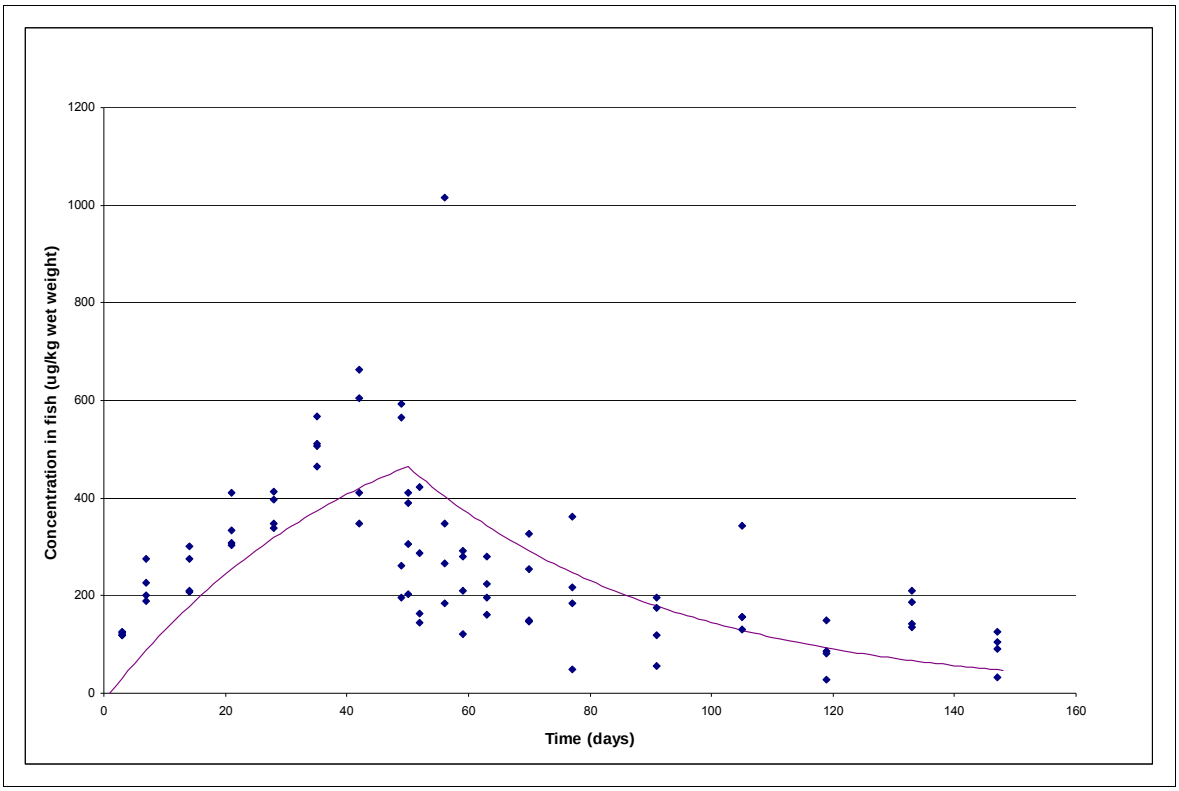

Figure 5.12 Comparison of derived kinetics with the actual uptake and depuration curve for Example 3 (low exposure concentration)

[30] Non-first order kinetics during the depuration phase would also have implications for the kinetics of the uptake phase as well as the depuration phase.

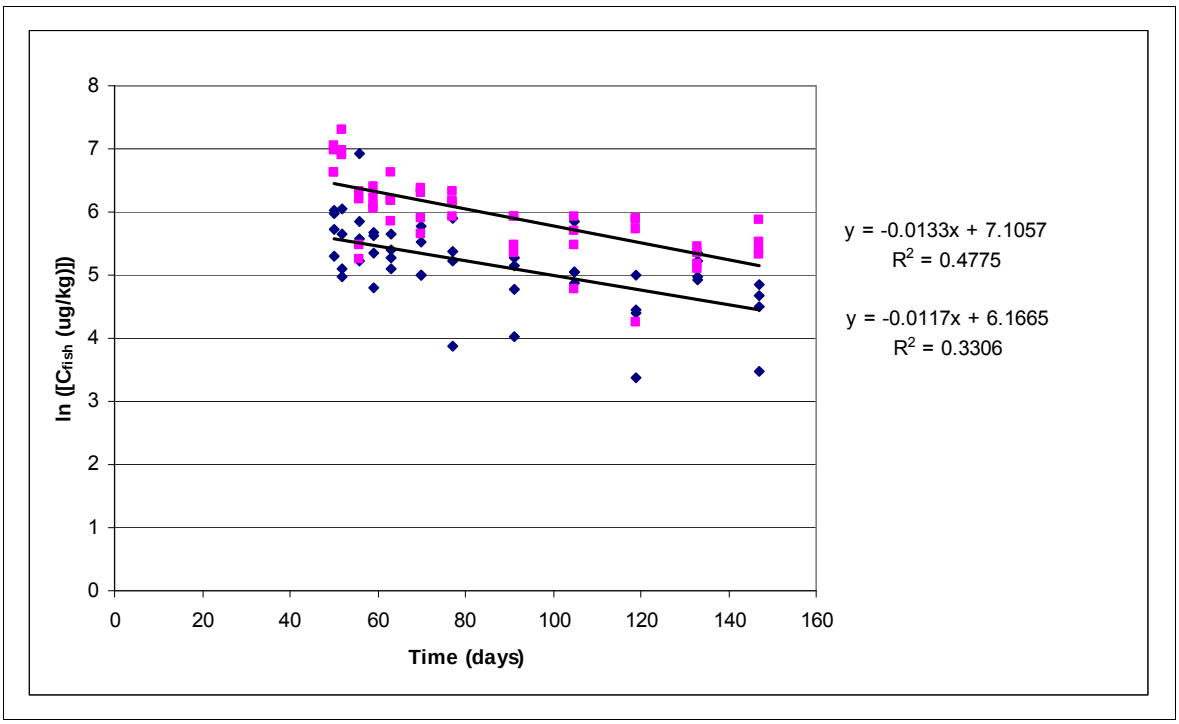

Figure 5.13 Plot of ln [C_{fish}] against time for the depuration phase in Example 3

Next, the value of the uptake rate constant was determined at each time point using Equation 39 and the appropriate overall depuration rate constant. The results of this analysis are shown in Table 5.8. Similar to Examples 1 and 2, the value of k_1 appeared to decrease during the study but tended to level off as steady state was reached. The fit of these kinetic data to the experimental data for the high exposure concentration is given in Figure 5.14. In this case, the revised kinetic data appear to fit well with data from the uptake phase of the study but less well with that from the depuration phase.

Table 5.8 Calculation of uptake rate constant (k_1) for each time point for Example 3

Time (days)	Calculated k_1 (l kg^{-1} day^{-1})		Kinetic BCF (l kg^{-1})	
	Low exposure	High exposure	Low exposure	High exposure
3	101	17	8,600	1,300
7	81	12	6,900	900
14	47	9.8	4,000	740
21	44	8.2	3,800	620
28	38	7.6	3,200	570
35	44	8.2	3,800	620
42	37	7.6	3,200	570
49	26	6.8	2,200	510

In this example, k_1 values estimated in Table 5.8 are much lower than what would be predicted using the Sijm *et al.* (1995) method. For example, assuming the initial fish weight is 1.67 g, the Sijm *et al.* method would predict a k_1 value of 441 l kg^{-1} day^{-1} compared with the value of 17-101 l kg^{-1} day^{-1} estimated in Table 5.8 and similarly the k_1 estimated at the end of the uptake phase using the Sijm *et al.* method (fish weight 1.29 g) would be much higher (479 l kg^{-1} day^{-1}) than the value of 6.8-26 l kg^{-1} day^{-1} estimated in Table 5.8.

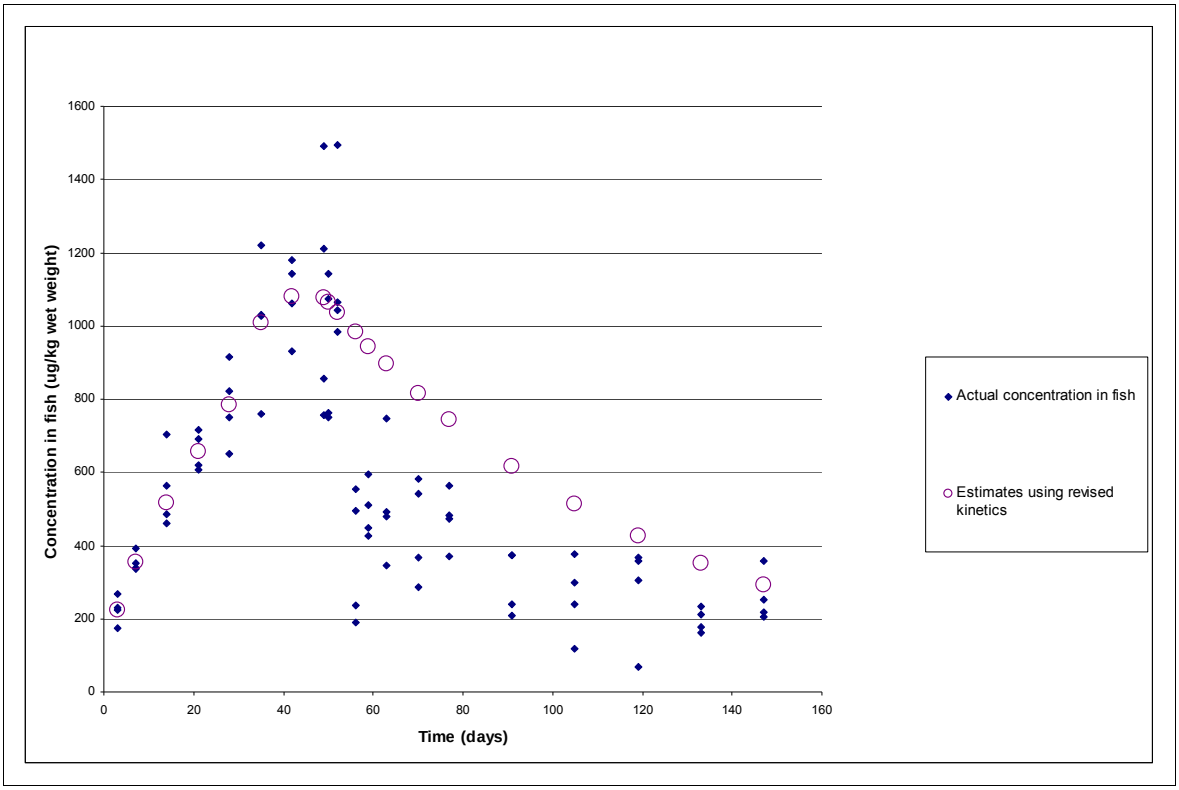

Figure 5.14 Comparison of re-analysed kinetic data with the experimental data for Example 3

In this case, it is not possible or necessary to correct the resulting BCF for growth dilution as the fish did not grow significantly during the study (the mean weight at the end of the study was only 18 per cent higher than at the start).

Analysis here, as elsewhere, assumes that the depuration phase follows first-order kinetics but, as noted above, deviations from first-order depuration kinetics may have occurred in this example. In this case, deviations from first-order kinetics during depuration are unlikely to be related to the growth of the fish (see Section 5.6) as essentially little or no growth of the fish occurred during the study.

5.7.4 Example 4

The substance (log K_{ow} around seven) was tested with rainbow trout (*Oncorhynchus mykiss*) over a 35-day uptake period and a 42-day depuration period. Two exposure concentrations were used (mean measured concentrations of 0.93 and 4.9 µg l^{-1}). The concentrations measured in the fish at various timepoints are summarised in Table 5 .9.

Apparent steady state was reached from around Day 21 onwards and the steady state BCF was determined to be 860 l kg^{-1} at the low exposure concentration and 265 l kg^{-1} at the high exposure concentration. The uptake and depuration rate constants were estimated using a variant of the sequential method. Firstly, the depuration rate constant was estimated from a plot of the natural logarithm of the tissue concentration against time using the concentration data from Day 35 to Day 77 (and forcing the linear regression through the measured tissue concentration at Day 35). This gave the depuration rate constant as 0.0448 day^{-1} for the low exposure concentration and 0.0407 day^{-1} for the high exposure concentration. The uptake rate constant was then obtained by fitting the uptake curve using the above values for the depuration rate constant (again forcing the curve fitting through the measured concentration for Day

35). The uptake rate constants estimated were k_1 = 48.7 l kg^{-1} day^{-1} for the low exposure concentration and k_1 = 14.2 l kg^{-1} day^{-1} for the high exposure concentration. The derived kinetic BCFs were 1,087 l kg^{-1} at the high exposure concentration and 349 l kg^{-1} for the low exposure concentration. These data were not corrected for the growth of the fish. A plot showing the fit of these kinetic parameters with the experimental data is shown in Figure 5.15 for the lower exposure group.

Table 5 .9 Fish concentration data for Example 4

Time (day)[1]	Mean concentration in fish (µg kg^{-1})		Mean fish weight (g)
	Low exposure group	High exposure group	
Uptake phase			
0	0	0	2.09
3	360	710	1.81
7	430	520	2.26
14	540	970	2.44
21	570	1,000	2.76
28	550	1,100	3.53
35	800	1,300	3.74
Depuration phase			
3 (38)	690	1,400	4.58
7 (42)	450	740	4.55
14 (49)	380	620	5.92
21 (56)	220	430	6.85
28 (63)	320	380	5.59
35 (70)	160	260	8.53
42 (77)	130	360	10.4

[1]The days given in brackets reflect the overall study day.

The data were reanalysed here using the approach outlined in Section 5.1. Firstly, the value of k_2 was obtained from the plot of ln [C_{fish}] against time for the depuration phase alone. This is shown in Figure 5.16. The overall depuration rate constants k_2 determined were 0.0416 day^{-1} for the low exposure group and 0.0381 day^{-1} for the high exposure group. The plots appear to be linear and consistent with first-order kinetics for the depuration phase.

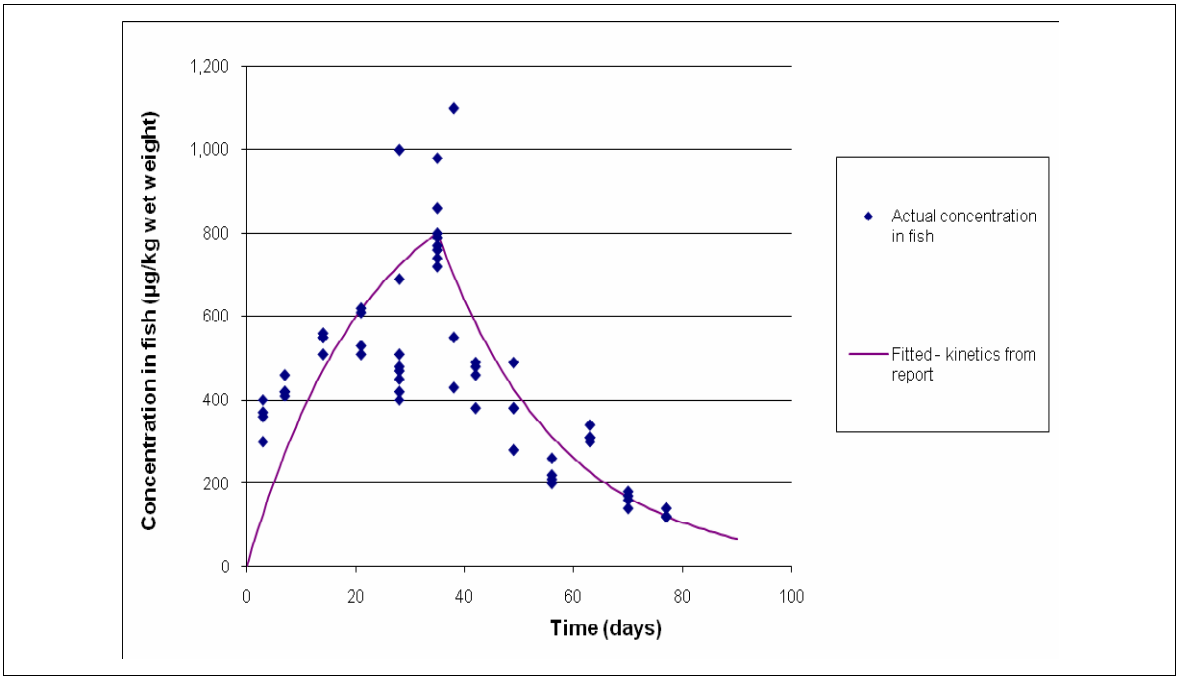

Figure 5.15 Comparison of derived kinetics with the actual uptake and depuration curve for Example 4 (low exposure concentration)

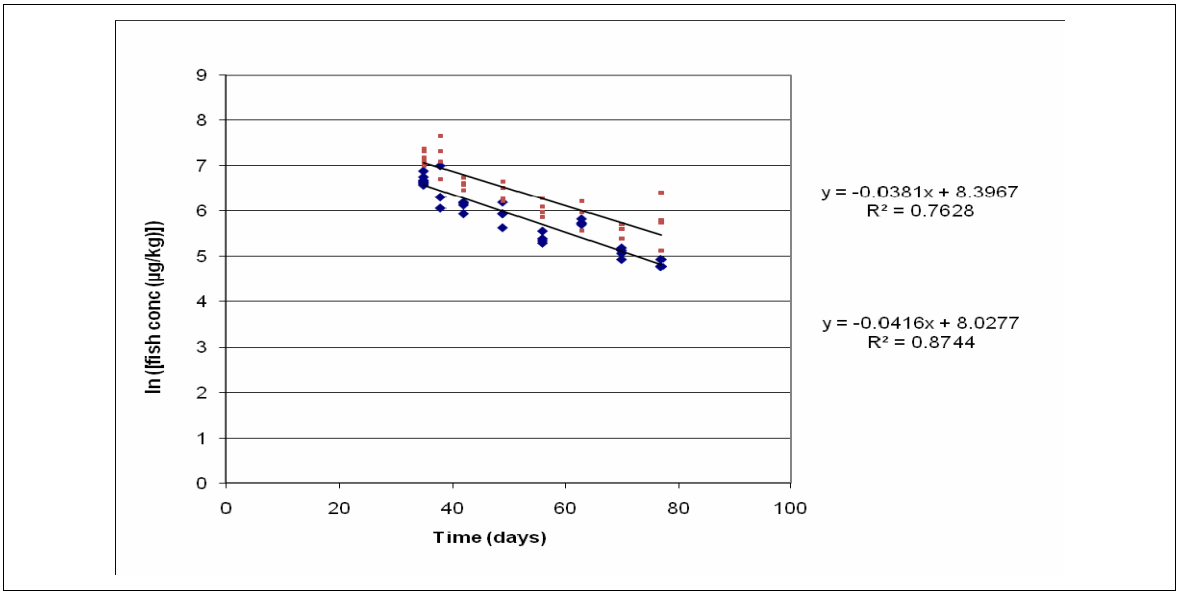

Figure 5.16 Plot of ln [C_{fish}] against time for the depuration phase in Example 4

Next, the value of the uptake rate constant was determined at each time point using Equation 39 and the appropriate overall depuration rate constant. The results of this analysis are shown in

Table 5.10. Similar to the previous examples, the value of k_1 appeared to decrease during the study but tended to level off as steady state was reached. The fit of these kinetic data to the experimental data for the high exposure concentration is given in Figure 5.17. The revised kinetic data appear to fit well with the experimental data. Note that in this re-analysis, the curves have not been forced through the Day 35 value.

Table 5.10 Calculation of uptake rate constant (k_1) for each time point for Example 4

Time (days)	Calculated k_1 (l kg^{-1} day^{-1})		Kinetic BCF (l kg^{-1})		Kinetic BCF – growth-corrected (l kg^{-1})	
	Low exposure	High exposure	Low exposure	High exposure	Low exposure	High exposure
3	136	51	3,300	1,300	6,600	3,000
7	76	17	1,800	450	3,700	990
14	55	18	1,300	470	2,700	1,100
21	44	14	1,100	370	2,100	820
28	36	13	870	341	1,700	760
35	47	14	1,100	370	2,300	820

In this example, the k_1 values estimated in Table 5.8 are much lower than would be predicted using the Sijm *et al.* (1995) method. For example, assuming the initial fish weight is 2.09 g, the Sijm *et al.* method would predict a k_1 value of 410 l kg^{-1} day^{-1} compared with the value of 51-136 l kg^{-1} day^{-1} estimated in

Table 5.10 and similarly the k_1 estimated at the end of the uptake phase using the Sijm *et al.* method (fish weight 3.74 g) would be higher (341 l kg^{-1} day^{-1}) than the value of 14-47 l kg^{-1} day^{-1} estimated in Table 5.8.

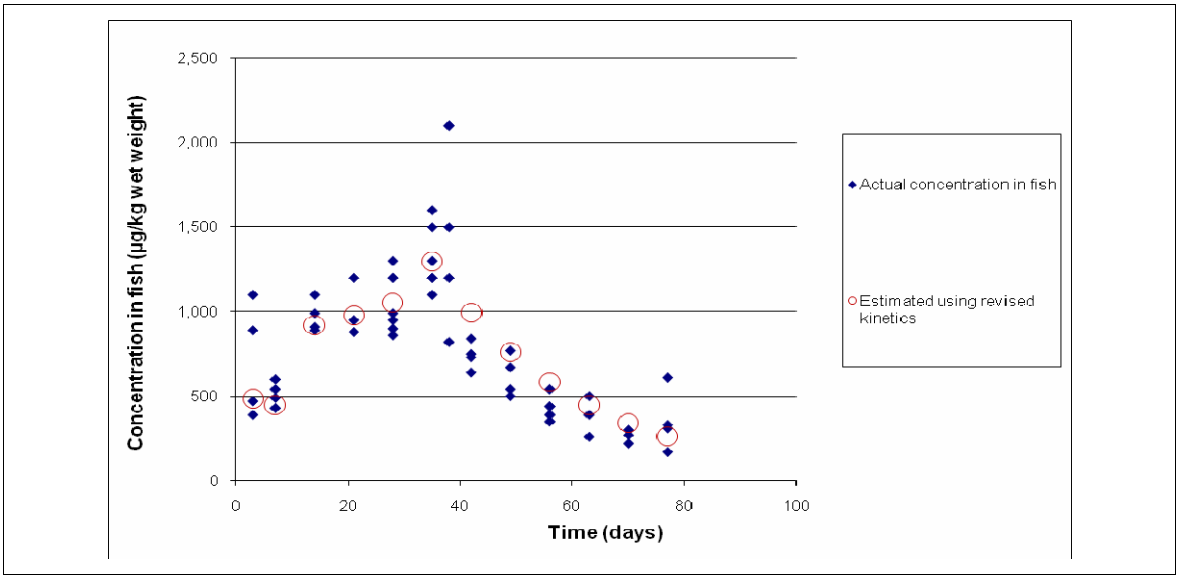

Figure 5.17 Comparison of re-analysed kinetic data with the experimental data for Example 4 (high exposure concentration)

The rate constant for growth dilution ($k_{growth\text{-}dilution}$) in the study is estimated to be 0.021 day^{-1} from a plot of ln (1/fish weight (kg) against time (see Figure 5.18)). The growth-corrected BCFs obtained using this value at each time point are shown in Table 5.6.

As can be seen in this example, growth correction leads to estimated BCFs around a factor of two times higher than would be obtained without considering growth.

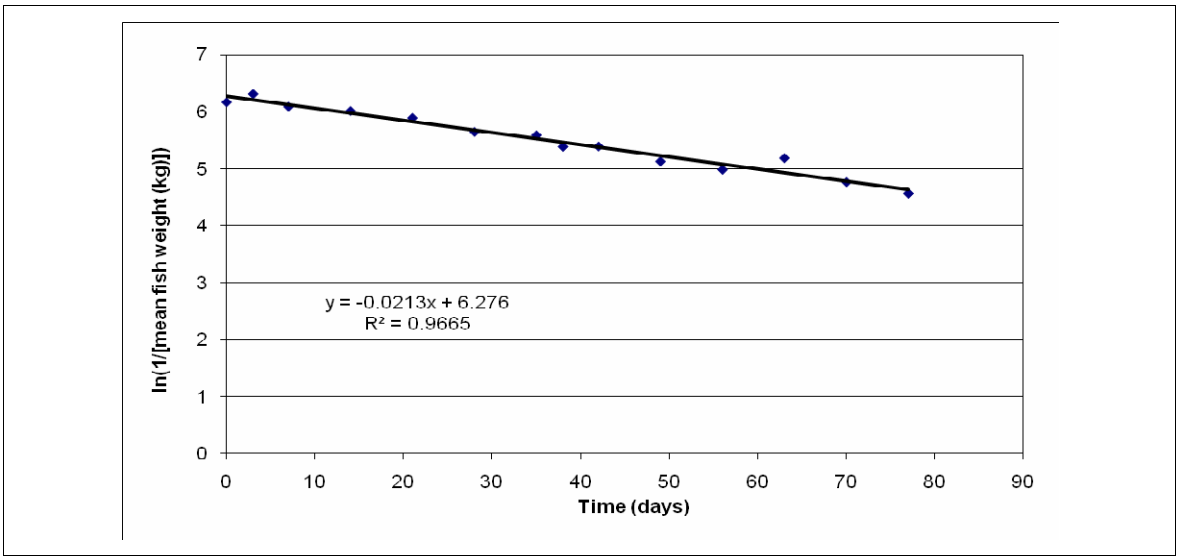

Figure 5.18 Estimation of rate constant for growth dilution ($k_{growth\text{-}dilution}$) for Example 4

5.7.5 Discussion of the findings from the four examples

In all four cases, it is evident that the uptake part of the experiment did not follow purely first-order kinetics, as evidenced by the apparent decline in the k_1 value as the uptake period progressed, and the relatively poor fit of Equation 39 to the data[31]. This can also be seen in the plots comparing the fitted kinetics with the experimental data (in most of the cases the fitted curve tends to predict the concentration in fish in the early stages of the uptake phase better than in the later stages.)

A possible explanation for this is that the k_1 value decreases as the fish grow, as would be predicted by the Sijm *et al*. (1995) equation and several other approaches described in Section 3. In general, these approaches suggest that the k_1 value should be related to the weight raised to the power around -0.2 to -0.35.

When the data used in the examples above are considered, although the weight of the fish increases significantly over the uptake period in three of the four examples, the increase in weight does not appear to be sufficient on its own to explain the observed decrease in the apparent k_1 value in all cases (with the exception of Example 1). In Example 3 the fish did not appear to grow significantly over the uptake period; however, a decline in the apparent k_1 was still evident.

This can also be seen from a plot of log (apparent k_1 value) against log (fish weight in g). If the trend in the apparent k_1 value was explained by the Sijm *et al*. (1995) approach alone, the slope of such a plot should equal the weight exponent, at around -0.32. As can be seen from such plots (see Figure 5.19), for Examples 2 and 4 the slope is different than would be expected based on growth alone (slopes of around -4.1 for Example 2 and around -1.5 for Example 4) but similar to what would be expected

[31] In one case, deviation from first-order kinetics may also have occurred in the depuration phase.

for Example 1 (slope around -0.37). This implies that the k_1 value was decreasing more markedly than would be expected from growth alone in three of the examples[32].

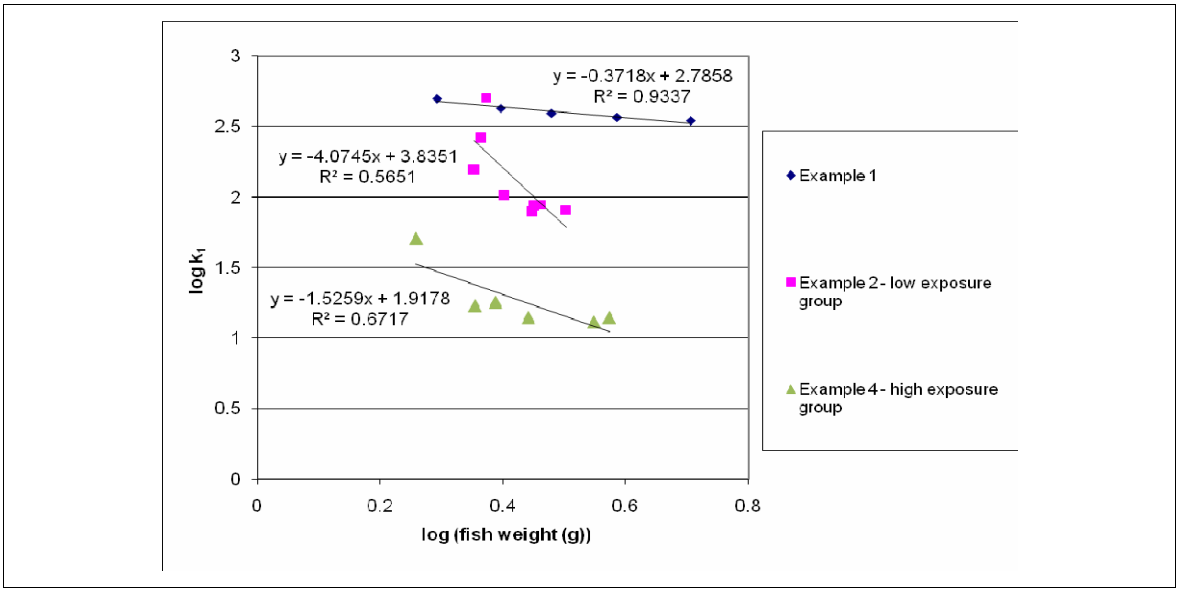

Figure 5.19 Plot of log (apparent k_1) against log (fish weight in g)

Based on this analysis, it appears other factors may also be important in the interpretation of the bioconcentration data. A possible explanation is outlined below. This is based on the analysis of a limited number of bioconcentration uptake curves for lipophilic chemicals and the actual mechanistic interpretation and general applicability is currently unknown.

Uptake of a chemical from water into a fish is generally considered to result from passive transport across the gill surfaces into the blood stream[33]. The Sijm *et al*. (1995) study considers this to be a diffusion process related solely to the fish weight. From visual observation of the data for Examples 1, 2, 3 and 4, it appears that the value of k_1 may decrease as the concentration in the fish increases towards the steady-state value. If it is assumed for the moment that the uptake rate constant is inversely proportional to the concentration in fish[34], the following equations can be written:

Equation 56 $$k_1 \propto \frac{1}{[C_{fish}]} \text{ or } k_1 = \frac{B}{[C_{fish}]}$$

Where k_1 = rate constant for uptake across gills (l kg^{-1} day^{-1}).

B = constant with units of l mg^{-1} day^{-1}.

$[C_{fish}]$ = concentration in fish (mg kg^{-1}).

This equation suggests that the value of k_1 could decline with increasing concentration in fish but as steady state is reached, the concentration in fish will be constant and so

[32] A further confidential example (not shown) showed a similar trend in the k_1 value, decreasing more markedly than would expected from growth alone.
[33] Clearly, if other mechanisms occur for specific substances (such as active transport) such considerations will not be valid.
[34] The mechanistic interpretation of this is unclear. A similar formulation can be derived assuming an inverse relationship of k_1 with the concentration in blood.

k_1 will also be constant. This is a similar pattern to that found in the examples above, particularly Examples 2 and 4. Substituting this into Equation 39 gives the following:

Equation 57 $$[C_{fish}] = [C_{water}] \times \frac{B}{k_2 \times [C_{fish}]} \times \left(1 - e^{-k_2 t}\right)$$

This can be rewritten as follows:

Equation 58 $$[C_{fish}] = \sqrt{[C_{water}] \times \frac{B}{k_2} \times \left(1 - e^{-k_2 t}\right)}$$

If this holds true, Equation 58 should describe the uptake curve better than Equation 39 that is normally used.

The uptake data for the four examples considered before were refitted to Equation 58 using the simultaneous method[35]. The results of this analysis are shown below. As can be seen from the plots, the visual fit to the experimental data is reasonably good.

The above formulation is not entirely satisfactory as Equation 56 implies that at the start of the uptake phase (when the concentration in fish is zero) the uptake rate constant will be infinite. Therefore, although this formulation appears to fit with the experimental data in the examples, this may be fortuitous and it is possible that some other, as yet unidentified, process or mechanism may better explain the uptake curves seen.

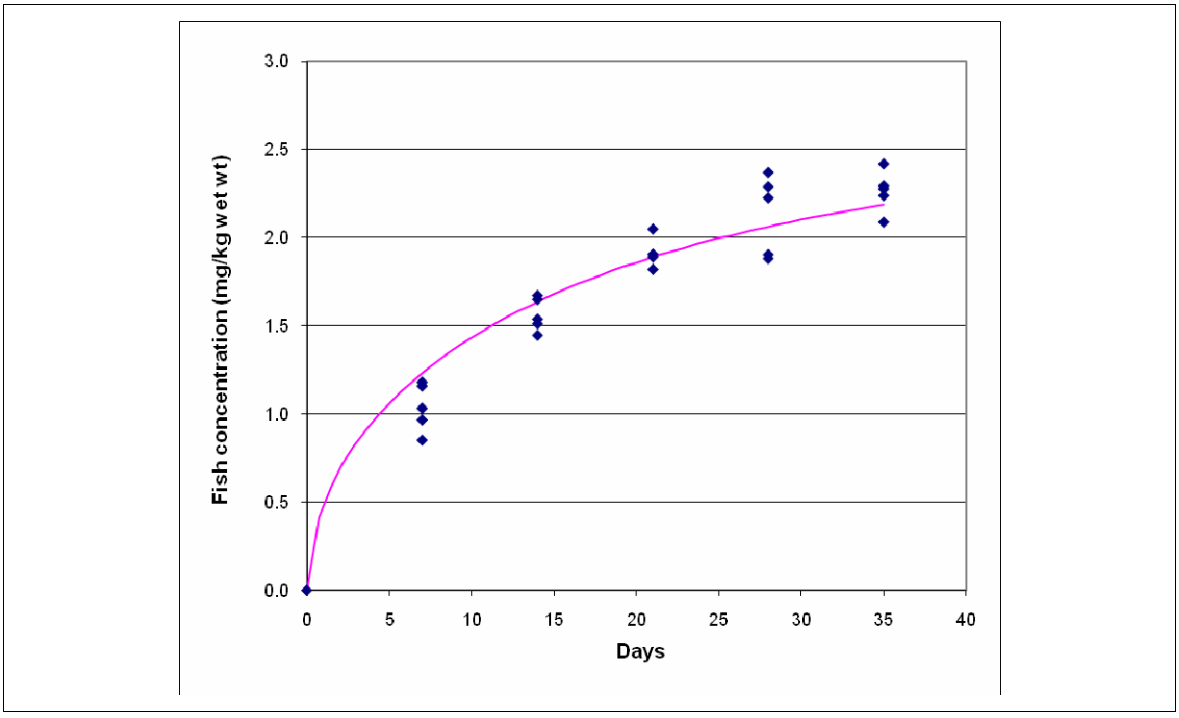

Figure 5.20 Fitting of Equation 58 to the experimental data for Example 1 (assuming a k_2 value of 0.0382 day^{-1})

[35] The fitting was carried out by minimising the squares of the residuals using the Solver add-in for Microsoft Excel 2003 SP2.

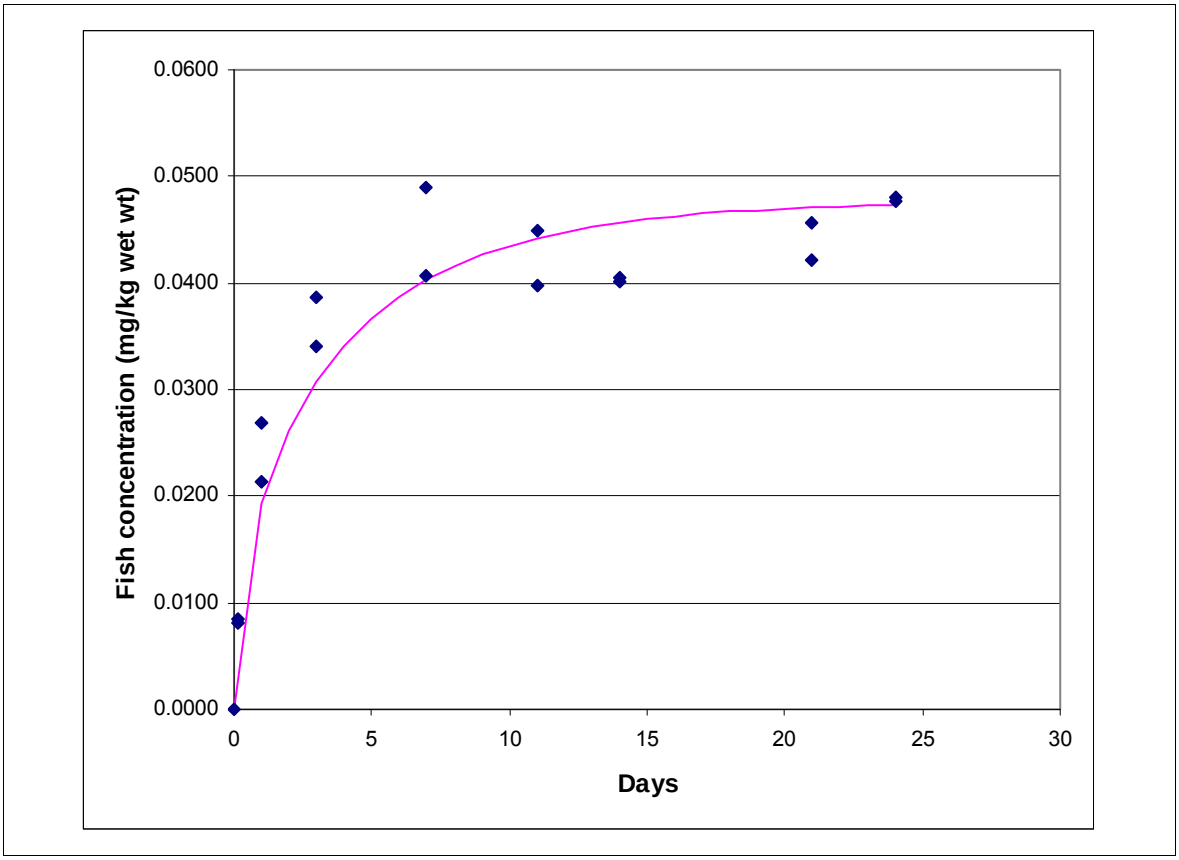

Figure 5.21 Fitting of Equation 58 to the experimental data for Example 2 – low exposure concentration (assuming a k_2 value of 0.18 day^{-1})

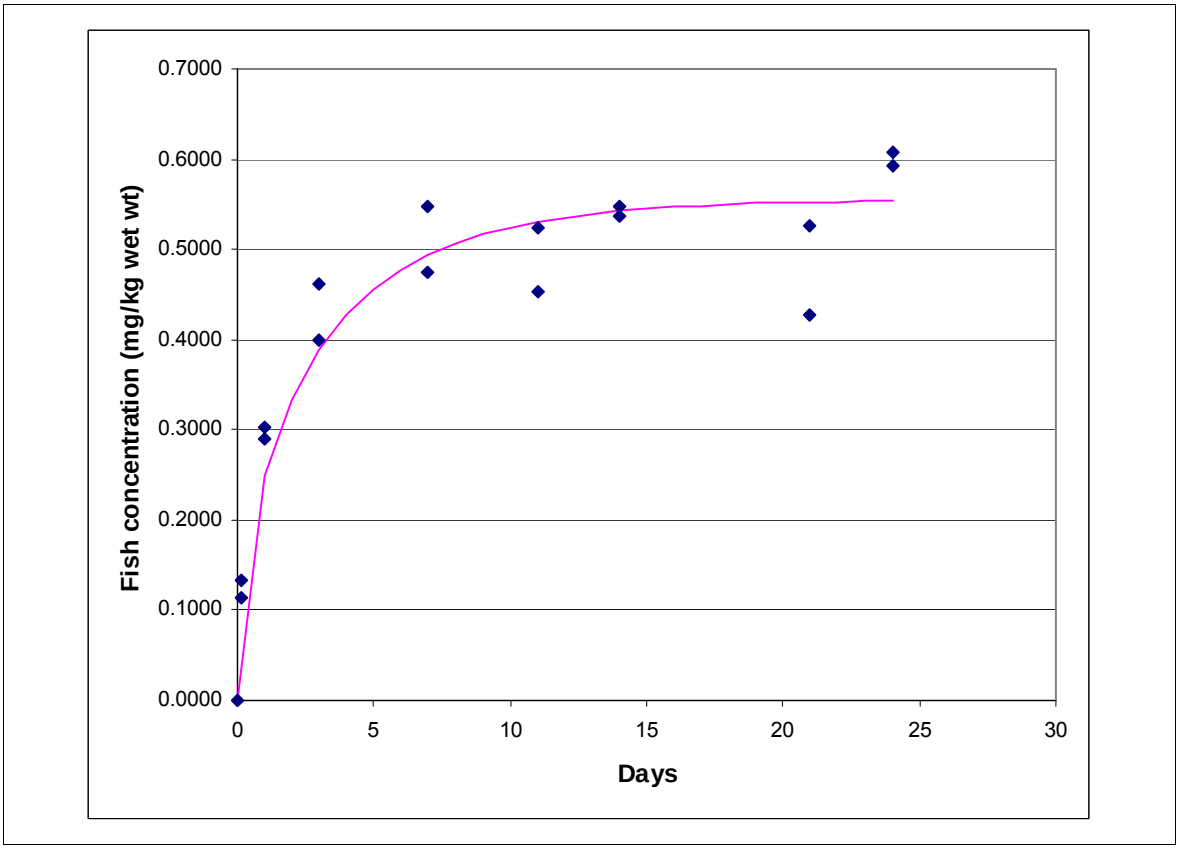

Figure 5.22 Fitting of Equation 58 to the experimental data for Example 2 – high exposure concentration (assuming a k_2 value of 0.224 day^{-1})

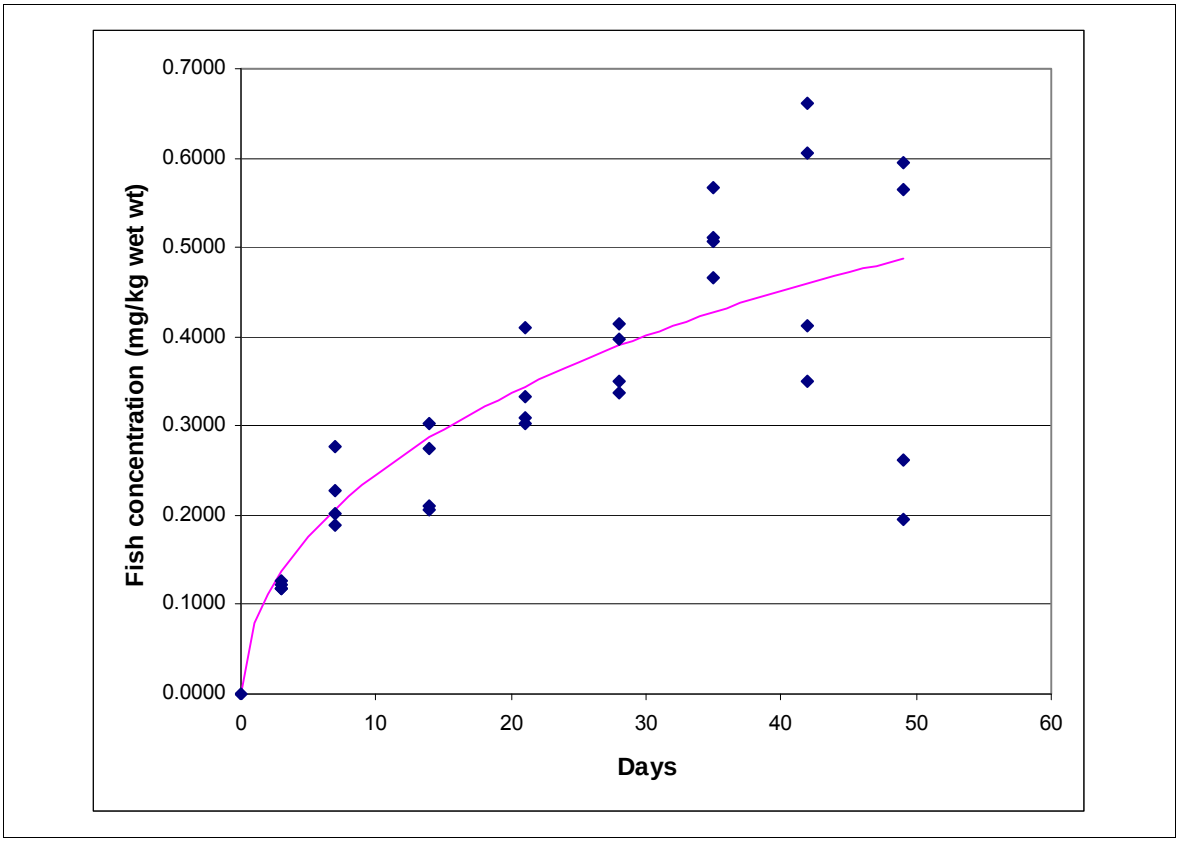

Figure 5.23 Fitting of Equation 58 to the experimental data for Example 3 – low exposure concentration (assuming a k_2 value of 0.0117 day^{-1})

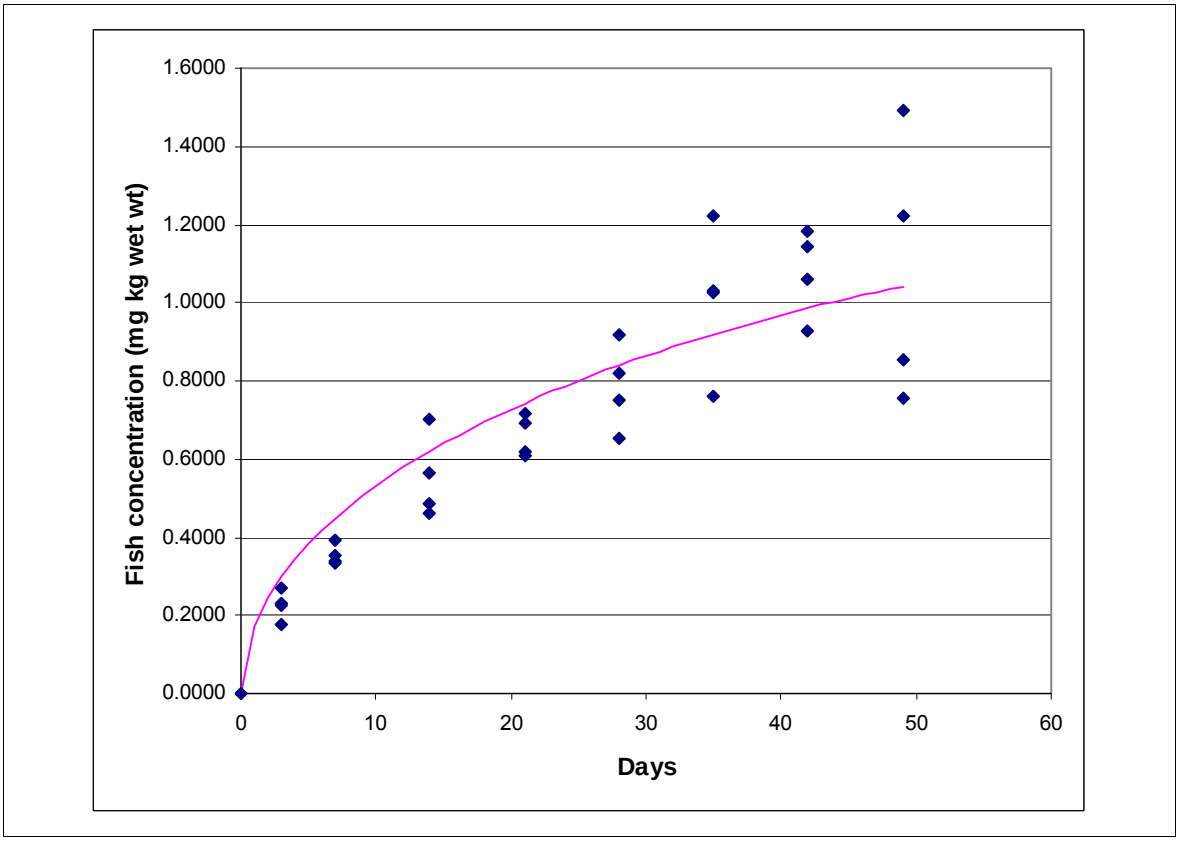

Figure 5.24 Fitting of Equation 58 to the experimental data for Example 3 – high exposure concentration (assuming a k_2 value of 0.0133 day^{-1})

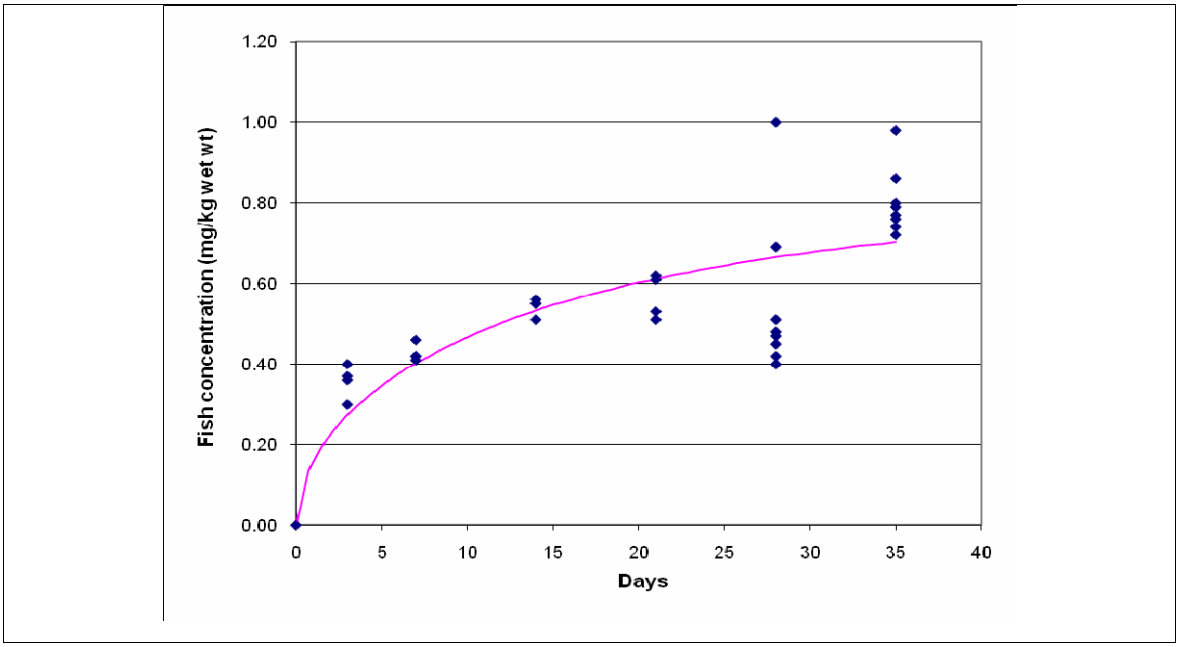

Figure 5.25 Fitting of Equation 58 to the experimental data for Example 4 – low exposure concentration (assuming a k_2 value of 0.0416 day^{-1})

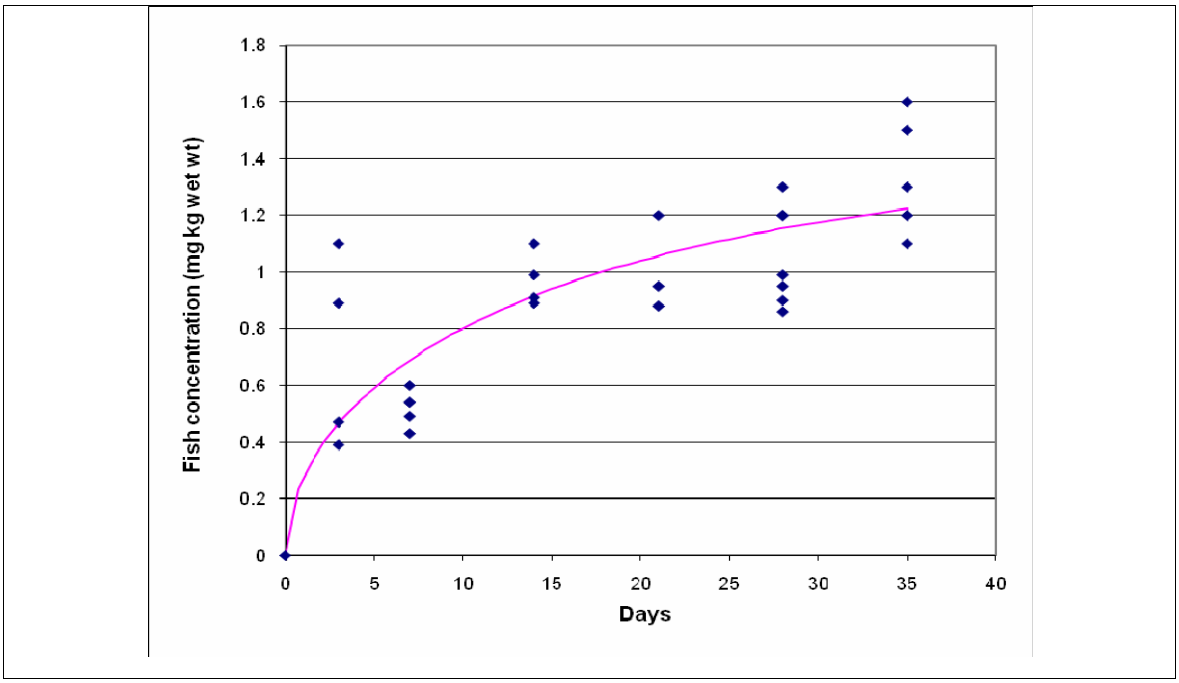

Figure 5.26 Fitting of Equation 58 to the experimental data for Example 3 – high exposure concentration (assuming a k_2 value of 0.0381 day^{-1})

Based on Equation 56 the value of B is related to the uptake rate constant by the concentration in fish. This implies that the kinetic BCF equivalent to steady state can be estimated from the value of B if the steady state concentration in fish is known. This is shown below for the four examples.

For Example 1, the value of B obtained from curve fitting to Equation 58 was 728 l mg^{-1} day^{-1}. The concentration in the fish at Day 35 was around 2.3 mg kg^{-1} (it is not clear if steady state was reached by this point). Using Equation 56, the value of k_1 at steady state can be estimated as 317 l kg^{-1} day^{-1} and so the kinetic BCF can be estimated as k_1/k_2 = 317/0.0382 = 8,300 l kg^{-1}.

For the low exposure concentration in Example 2 the value of B obtained from curve fitting was 4.08 l mg^{-1} day^{-1}. The apparent steady state concentration in the fish was around 0.043 mg kg^{-1}. Using Equation 56, the value of k_1 at steady state can be estimated as 94 l kg^{-1} day^{-1} and so the kinetic BCF can be estimated as k_1/k_2 = 94/0.18 = 520 l kg^{-1}. Similarly, for the high exposure concentration in Example 2 the B value obtained was 57 l mg^{-1} day^{-1}. In this case the apparent steady-state concentration in the fish was around 0.5 mg kg^{-1} giving the equivalent k_1 value as 114 l kg^{-1} day^{-1}. The kinetic BCF can then be estimated as k_1/k_2 = 114/0.224 = 509 l kg^{-1}.

For the low exposure concentration in Example 3, the value of B obtained from curve fitting was 15.5 l mg^{-1} day^{-1}. The apparent steady state concentration in the fish was around 0.48 mg kg^{-1} (although it is possible the concentration in the fish was still increasing). Using Equation 56, the value of k_1 at steady state can be estimated as 32 l kg^{-1} day^{-1} and so the kinetic BCF can be estimated as k_1/k_2 = 32/0.0117 = 2,735 l kg^{-1}. Similarly, for the high exposure concentration in Example 3 the B value obtained was 6.9 l mg^{-1} day^{-1}. In this case, the apparent steady state concentration in the fish was around 1 mg kg^{-1}, giving the equivalent k_1 value as 6.9 l kg^{-1} day^{-1}. The kinetic BCF can then be estimated as k_1/k_2 = 6.9/0.0133 = 520 l kg^{-1}.

For the low exposure concentration in Example 4, the value of B obtained from curve fitting was 28.8 l mg^{-1} day^{-1}. The apparent steady state concentration in the fish was around 0.8 mg kg^{-1} (although it is possible the concentration in the fish was still increasing). Using Equation 56, the value of k_1 at steady state can be estimated as 36 l kg^{-1} day^{-1} and so the kinetic BCF can be estimated as k_1/k_2 = 36/0.0416 = 865 l kg^{-1}. Similarly, for the high exposure concentration in Example 3 the B value obtained was 15.8 l mg^{-1} day^{-1}. In this case, the apparent steady state concentration in the fish was around 1.3 mg kg^{-1}, giving the equivalent k_1 value as 12 l kg^{-1} day^{-1}. The kinetic BCF can then be estimated as k_1/k_2 = 12/0.0381 = 315 l kg^{-1}.

In the examples above, the kinetic BCF now uses the steady state concentration in fish in its calculation and if this value is uncertain (as in Examples 1 and 3) the resulting kinetic BCF will also be uncertain.

Rearranging Equation 57 allows the B value to be calculated for each time point during the uptake phase. When this is done (in an analogous way as was done in the Examples for k_1) it is apparent that the B value is reasonably constant throughout the uptake phase and there is no obvious systematic trend. This is shown in Table 5.11.

Table 5.11 Calculation of B value at each time point

Example 1		Example 2			Example 3			Example 4		
Time (days)	**B value (l mg^{-1} day^{-1})**	**Time (days)**	**B value (l mg^{-1} day^{-1}) – low conc.**	**B value (l mg^{-1} day^{-1}) – high conc.**	**Time (days)**	**B value (l mg^{-1} day^{-1}) – low conc.**	**B value (l mg^{-1} day^{-1}) – high conc.**	**Time (days)**	**B value (l mg^{-1} day^{-1}) – low conc.**	**B value (l mg^{-1} day^{-1}) – high conc.**
7	523	0.167	4.2	76	3	12	4.0	3	49	43
14	666	1	6.4	82	7	18	4.3	7	32	9
21	750	3	5.7	71	14	12	5.6	14	30	18
28	787	7	5.1	62	21	15	5.4	21	25	15
35	783	11	3.8	49	28	14	6.1	28	22	14
		14	3.2	57	35	22	8.5	35	28	19
		21	3.6	43	42	20	8.3			
		24	4.2	68	49	13	7.9			

Although this apparent decrease in k_1 (over and above that expected from growth alone) has been tested only with relatively few data sets, and the mechanistic interpretation and general applicability is not yet clear, it has potentially important consequences for the interpretation of bioconcentration study data. It leads to a number of questions which are currently difficult to answer.

- Does the k_1 value depend on the lipid content of the fish, as this would affect the concentration in the fish?
- Does the k_1 value depend on the chemical properties? Several of the other methods reviewed here assume that it does not.
- If growth-correcting the kinetic BCF, which value of k_1 should be chosen?
- What implications does this have for predicting a k_1 value?
- It is recognised that k_1 will also decrease as the weight of the fish increases (as shown in Section 5.2). The current analysis indicates that this alone is not sufficient to explain the trends seen, but it could contribute to the trend (or be an extra factor on top of that observed).
- The current method given in the OECD 305 Test Guideline assumes that the k_1 value is constant during the uptake phase. If k_1 actually decreases, the current method will effectively estimate an "average" value over the uptake phase. Further, if the simultaneous method is used, although this may result in a better overall fit to the data than the sequential method (as it is effectively fitting two variables to the data rather than one), this may result in erroneous estimates for the overall depuration rate constant (k_2).

Although this analysis assumes that k_1 may vary with concentration in the fish, there may be other factors which co-vary with the concentration in the fish and may better explain the uptake curves.

Possible alternative explanations for the decrease in k_1 during the experiment have been suggested (McLachlan, personal communication). The first relates to respiration of the fish. If the fish respire more rapidly at the start of the test, for example as a result of stress caused by the initial exposure to the chemical, then, as k_1 would be expected to be related to respiration rate, the k_1 may be elevated at the start of the test compared with later on in the test (where the fish become acclimatised to the presence of test substance). Another possible explanation is variability of the freely dissolved concentration in the test water, for example, if the dissolved concentration reduces following introduction of the fish (this is considered unlikely here as the measured concentrations in water were reasonably constant throughout the test[36]).

Readers should note that the individual k_1 values calculated at each time point using Equation 39 are not direct estimates of the k_1 at each respective time point, but rather they represent an "average" or "integrated" value over the exposure time period up to that time point. Therefore, a single elevated k_1 value at the beginning of the experiment will automatically result in a continous decrease in this "average" k_1 over time, even if the actual k_1 at each time point is constant throughout the remainder of the experiment.

[36] For instance, in Example 1 the mean measured concentration over Days 0 to 35 (measurements on Day 0, 4, 7, 14, 21, 28 and 35) was 0.34 µg l^{-1} with a standard deviation of 0.06 µg l^{-1}. Actual measurements at each time point range between 0.26 µg l^{-1} and 0.44 µg l^{-1} (lowest value measured on Day 0 and highest on Day 7) and no systematic decrease in concentration with time was evident.

5.8 Considerations for comparison of experimental BCF data against regulatory criteria

The current standard test guidelines for bioconcentration assume that the uptake phase follows first-order kinetics (first order in the concentration in water). However, the analysis carried out in this section has highlighted a number of issues when analysing bioconcentration data that indicate that the uptake phase may not always follow strict first-order kinetics[37]. In particular, the apparent decrease in the k_1 value during the uptake phase of the experiment is generally not considered (or checked) in the current OECD 305 Test Guideline method. If this is found to be a true phenomena (for whatever reason), this has important consequences for comparison of BCF data against regulatory criteria, particularly if growth-corrected data are recommended to be used, as is the case with the REACH Annex XIII criteria for a bioaccumulative (B) or very bioaccumulative (vB) substance. In this context, it is important to remember that correction for growth dilution is effectively a hypothetical calculation whereby the effect of growth of the fish is factored out of the kinetic BCF. There are two assumptions inherent in this. The first is that depuration processes other than growth (such as respiration, metabolism and fecal elimination) occur at the same rate in the hypothetical fish that is not growing compared with the growing fish. Secondly, it is assumed that the uptake rate constant k_1 is the same in the growing and non-growing fish.

The analysis carried out here suggests that the assumption that the k_1 value may be the same in growing and non-growing fish may not always hold true as, at least in the examples considered, a) the k_1 value appears to decline during the uptake phase until steady state is reached and b) the time to steady state in the hypothetical non-growing fish will be longer than in growing fish (as the growth-corrected k_2 value will always be lower than the overall depuration rate constant). Therefore, the value of k_1 at apparent steady state seen in growing fish may not necessarily correspond to the steady state k_1 in the non-growing fish. Growth may have other indirect effects on k_2 that may mean that the growth-corrected k_2 value obtained in small, fast growing fish may not always be representative of the k_2 value in larger, slowly growing fish (see Section 5.6).

REACH Annex XIII criteria were developed before growth correction of BCF data was generally practiced. Therefore, the data used to define the criteria were most probably not growth-corrected. As growth correction can, in some cases, make a very large difference to the final BCF obtained (see Example 1), the practice of comparing the growth-corrected BCF against these criteria can be questioned.

To minimise the uncertainties in interpretation of the data that are introduced by rapidly growing fish in BCF (and BMF_{food} studies), particularly where the overall depuration rate constant can be dominated by the growth component, it is preferable to carry out the tests under conditions where the growth of the fish is minimized. This appears to be particularly relevant when there is a need to compare the resulting BCF value against regulatory criteria. This could potentially be achieved by careful choice of test species and/or feeding rate used. Of course there will also be practical limitations to carrying out such a test (for example, the health of the fish will need to be maintained during the test and the fish needs to be of sufficient size to allow for analysis of the test substance concentration). These aspects need to be taken into account in the study design.

As well as its effect on growth, the feeding rate appears to be an important variable for the rate of uptake in BMF_{food} studies, and the rate of depuration by faecal egestion for both BMF_{food} and BCF studies. Standardisation of the feeding rate between these two types of study may reduce uncertainty in the read-across of data from one to another.

[37] It is recognised that when taken in the context of experimental error, short uptake durations and other confounding factors inherent in laboratory procedures, the assumption of first-order kinetics may an appropriate approximation for reasons of simplicity.

6 Worked examples of estimating BCFs from the results of feeding studies

The following two examples are based on hypothetical, but realistic, data that may be generated by feeding studies. The examples show how the results of such studies can be combined with an estimate for the uptake rate constant from water to estimate BCF values. Given the relatively large uncertainties associatied with the prediction of the uptake rate constant by any one method, it is suggested that a weight of evidence approach is taken whereby several methods are used to estimate the uptake rate constant and a judgement made, based on the range of predicted values, as to the acceptability of the prediction. The acceptability of the prediction will, in part, be driven by the intended use of the final BCF. For example, for some environmental modelling purposes a rough, order of magnitude, estimate of the BCF value may be all that is required. However, in other cases, such as comparison against regulatory criteria under the EU REACH regulation, a more precise estimate, or more certainty that a BCF is above or below a given value, may be needed.

6.1 Example A

In this example, the substance tested had a log K_{ow} of 5.1. The study was carried out using a method similar to the draft OECD 305 Test Guideline. Briefly, groups of fish (*Oncorhynchus mykiss*; lipid content 4.8 per cent) were exposed to the test substance via food (concentration of 200 mg kg^{-1}) for 10 days, followed by a 28-day depuration period. The concentrations measured in the fish at various time points during the test are summarised in Table 6.1, along with the mean fish weights determined.

Table 6.1 Fish concentration data for Example A

Time (day)[1]	Mean concentration in fish (µg kg^{-1})	Mean fish weight (g)
Uptake phase		
0	0	2.53
10	4,026	3.81
Depuration phase		
1 (11)	3,041	3.76
3 (13)	1,090	4.03
7 (17)	397	4.95
14 (24)	100	6.07
21 (31)	<50	7.64
28 (38)	<50	10.28

[1]The days given in brackets reflect the overall study day.

To estimate a BCF from these data, the depuration rate constant and rate constant for growth dilution are determined from plots of the natural logarthim of the concentration in fish (during the depuration phase) against time and natural logarithm of 1/mean fish weight against time. These are shown in Figure 6.1 and Figure 6.2.

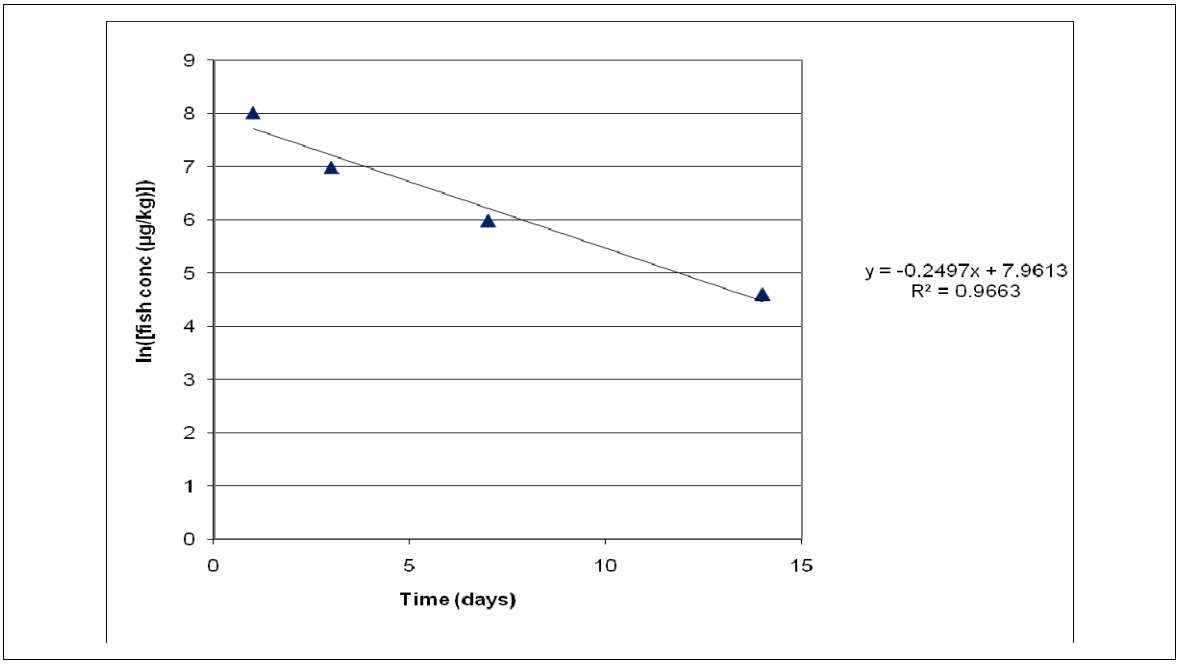

Figure 6.1 Estimation of the depuration rate constant for Example A

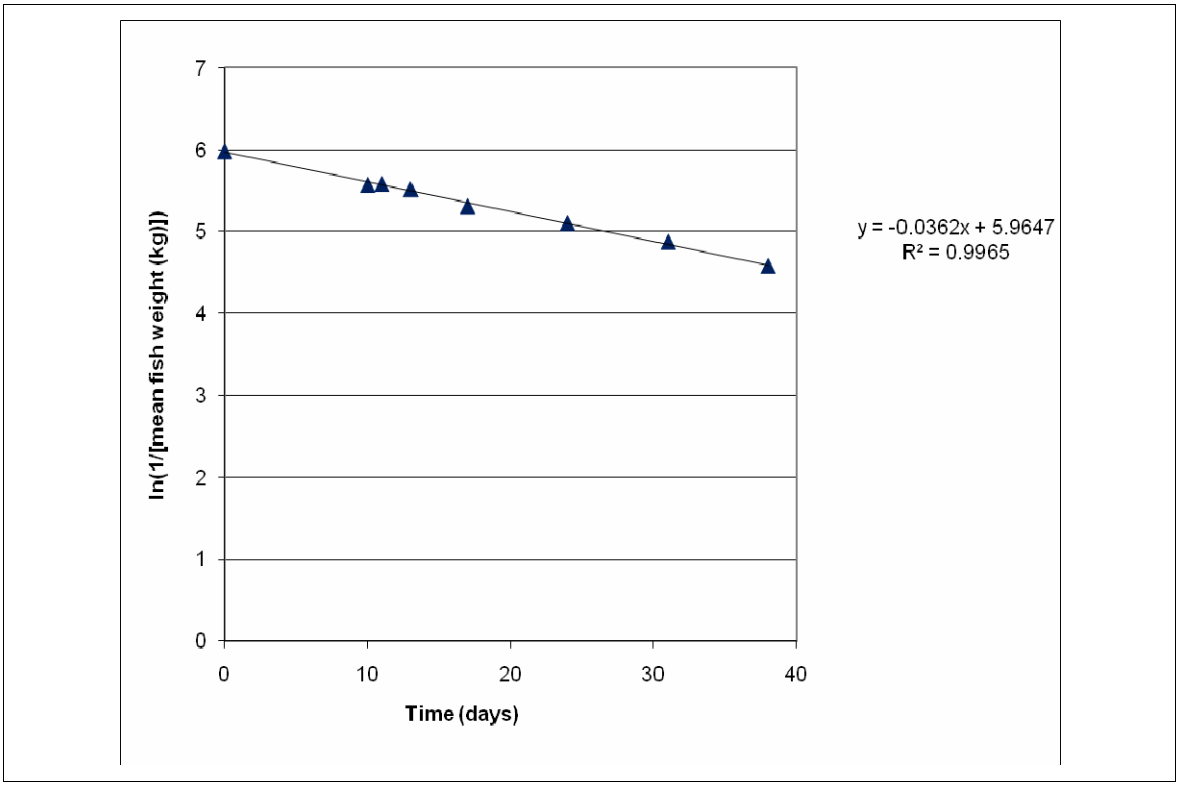

Figure 6.2 Estimation of the rate constant for growth dilution for Example A

From these plots, the depuration rate constant (k_2) is 0.250 day^{-1} and the rate constant for growth dilution ($k_{growth\text{-}dilution}$) is around 0.036 day^{-1}. Thus, the growth-corrected depuration rate constant ($k_{2\text{-}growth\text{-}corrected}$) is 0.250-0.036 = 0.214 day^{-1}.

The draft OECD 305 Test Guideline currently recommends that the equivalent uptake rate constant from water (k_1) is estimated using the Sijm *et al*. (1995) method based on the fish weight at the end of the uptake phase (3.81 g in this case). The predicted k_1

value obtained using this method is shown in Table 6.2 along with the resulting BCF estimates (the ratio of k_1 to k_2 or $k_{2\text{-growth-corrected}}$). The estimated growth-corrected BCF has also been normalised to a "standard" lipid content of five per cent using Equation 54. As discussed in Section 4.3, a number of other methods could be used to estimate k_1, and the resulting values estimated with these methods are also shown in Table 6.2.

Table 6.2 Calculation of BCFs for Example A

Method for estimating k_1	Estimated k_1 (l kg^{-1} day^{-1})	Estimated BCF (l kg^{-1})[a]		
		Not growth-corrected	Growth-corrected	Growth-corrected and lipid-normalised[c]
Sijm *et al*. (1995)	339	1,356	1,581	1,647
Hayton and Barron (1990)	388	1,552	1,813	1,889
Erickson and McKim (1990a)	545	2,180	2,547	2,653
Barber *et al*. (1991)	534	2,136	2,495	2,599
Barber (2003) - observed	342	1,368	1,598	1,665
Barber (2003) - calibrated	389	1,556	1,818	1,894
Barber (2001)	559	2,236	2,612	2,721
Streit and Sire (1993)	330	1,320	1,542	1,606
Erickson and McKim (1990b)	420	1,680	1,963	2,045
Hendriks *et al*. (2001)	483	1,932	2,257	2,351
Tolls and Sijm (1995)	652	2,608	3,047	3,174
Spacie and Hamelink (1982)	537	2,148	2,509	2,614
Thomann (1989)[a]	1,303	5,212	6,089	6,342

a) Estimates are based on fish weight of 3.81 g at end of uptake phase. Where a log K_{ow} was needed, a value of 5.1 was used.
b) This method also requires the dissolved oxygen concentration (DOC). A DOC of 7.42 mg l^{-1} was assumed in the calculation.
c) Lipid content of fish used in feeding study was 4.8 per cent. BCF was normalised here to a "standard" lipid content of five per cent using Equation 54.

Estimates for the growth-corrected and lipid-normalised value of k_1, and hence BCF, cover a factor of around four, however most estimates are within a range of a factor of two (for example 1,606-3,174 l kg^{-1} for the growth-corrected BCF). These estimates are likely to be sufficiently reliable for use in environmental models and/or for the prediction of environmental concentrations. However, in this case the estimated BCF values straddle the regulatory cut-off point of 2,000 l kg^{-1} that categorises substances as being bioaccumulative under REACH and so the choice of estimation method may have important implications in this context.

It is also worth noting that in this example, growth correction only has a limited impact on the estimated BCF despite a four-fold increase in the weight of the fish over the course of the study.

6.2 Example B

The substance tested in this example had a log K_{ow} of 6.7. The study was carried out using a method similar to the draft OECD 305 Test Guideline. Briefly, groups of fish (*Oncorhynchus mykiss*; lipid content 5.2 per cent) were exposed to the test substance via food (concentration of 50 mg kg^{-1}) for 10 days followed by a 28-day depuration period. The concentrations measured in the fish at various time points during the test are summarised in Table 6.3, along with the mean fish weights determined.

Table 6.3 Fish concentration data for Example B

Time (day)[1]	Mean concentration in fish (µg kg^{-1})	Mean fish weight (g)
Uptake phase		
0	0	1.218
10	16,720	1.254
Depuration phase		
1 (11)	15,210	1.310
3 (13)	15,420	1.647
7 (17)	12,670	2.021
14 (24)	8,748	2.758
21 (31)	6,487	2.947
28 (38)	5,675	3.918
42 (52)	3,014	6.258

[1]The days given in brackets reflect the overall study day.

To estimate a BCF from these data, the depuration rate constant and rate constant for growth dilution are determined from plots of the natural logarthim of the concentration in fish (during the depuration phase) against time and natural logarithm of 1/mean fish weight against time. These are shown in Figure 6.3 and Figure 6.4.

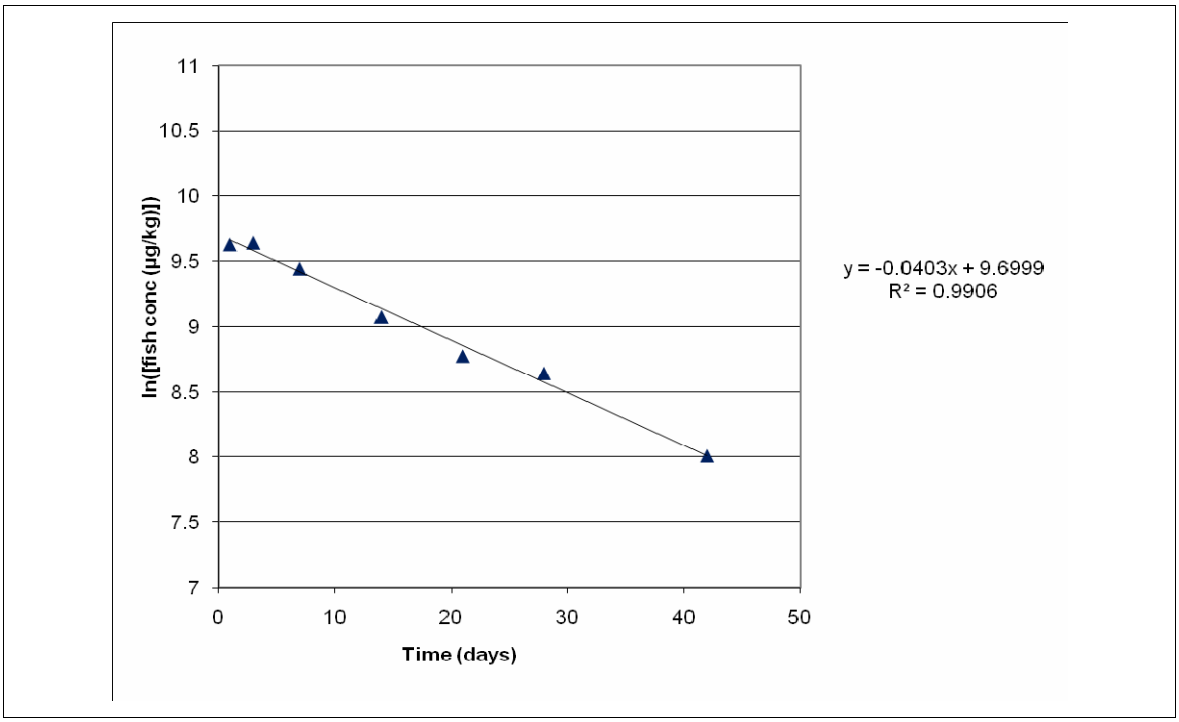

Figure 6.3 Estimation of the depuration rate constant for Example B

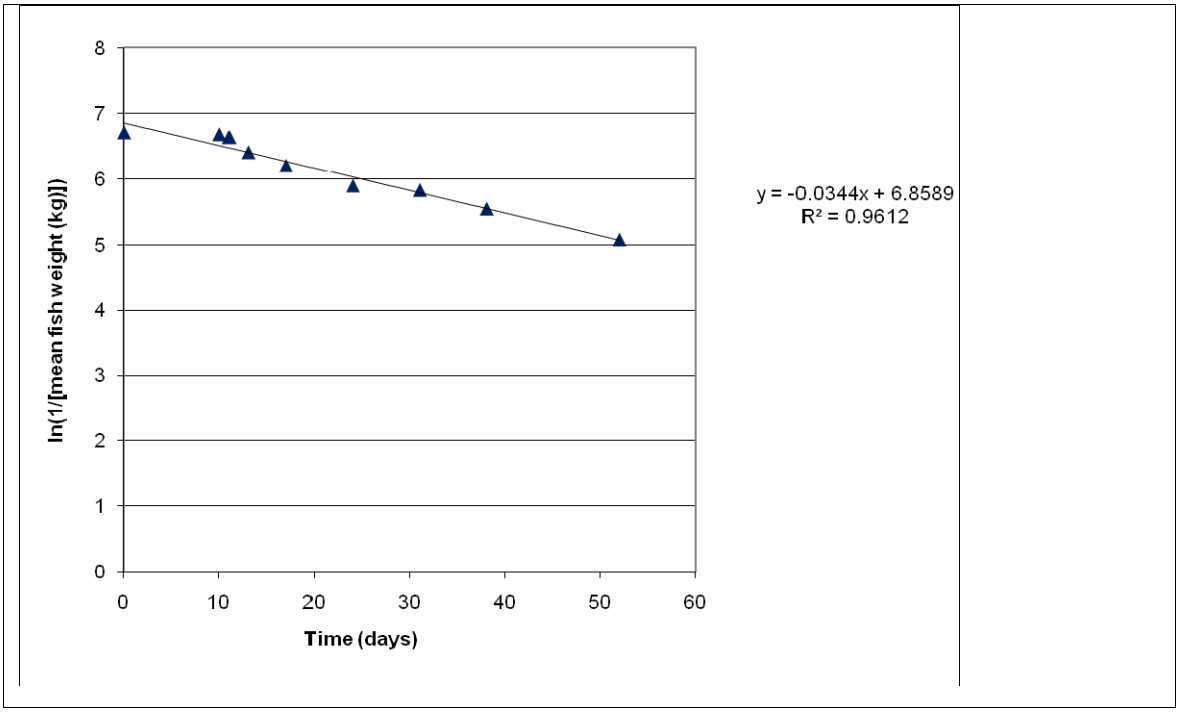

Figure 6.4 Estimation of the rate constant for growth dilution for Example B

From these plots, the depuration rate constant (k_2) is 0.0403 day^{-1} and the rate constant for growth dilution ($k_{growth\text{-}dilution}$) is around 0.0344 day^{-1}. Thus, the growth-corrected depuration rate constant ($k_{2\text{-}growth\text{-}corrected}$) is 0.0403-0.0344 = 0.006 day^{-1}.

Table 6.4 Calculation of BCFs for Example B

Method for estimating k_1	Estimated k_1 (l kg^{-1} day^{-1})	Estimated BCF (l kg^{-1})		
		Not growth-corrected	Growth-corrected	Growth-corrected and lipid-normalised[c]
Sijm *et al*. (1995)	484	12,010	80,670	77,570
Hayton and Barron (1990)	482	11,960	80,330	77,240
Erickson & McKim (1990a)	649	16,100	108,170	104,010
Barber *et al*. (1991)	653	16,200	108,830	104,640
Barber (2003) - observed	426	10,570	71,000	68,270
Barber (2003) - calibrated	613	15,210	102,170	98,240
Barber (2001)	668	17,070	111,330	107,050
Streit and Sire (1993)	394	9,780	65,670	63,140
Erickson & McKim (1990b)	541	13,420	90,170	86,700
Hendriks *et al*. (2001)	670	16,630	111,670	107,380
Tolls and Sijm (1995)	1,022	23,360	170,330	163,780
Spacie & Hamelink (1982)	922	22,880	153,670	147,760
Thomann (1989)[b]	1,061	26,330	176,830	170,030

a) Estimates based on fish weight of 1.254 g at end of uptake phase. Where a log K_{ow} was needed, a value of 6.7 was used.
b) This method also requires the dissolved oxygen concentration. A DOC of 6.9 mg l^{-1} was assumed in the calculation.
c) Lipid content of fish used in feeding study was 5.2 per cent. BCF was normalised here to a "standard" lipid content of five per cent using Equation 54.

The predicted k_1 value estimated using the Sijm *et al*. (1995) method based on the fish weight at the end of the uptake phase (1.254 g in this case) is shown in Table 6.4 along with the resulting BCF estimates. The resulting values estimated using other methods are also shown in Table 6.4.

As can be seen, estimates for the value of k_1, and hence BCF, cover a factor of around 2.7. The estimated BCF values are all well above the regulatory cut-off points of 2,000 l kg^{-1} and 5,000 l kg^{-1} re used to identify substances as being bioaccumulative or very bioaccumulative under REACH. This implies that the BCF estimates in this example are likely to be adequate to allow regulatory decisions to be made.

In this case, correcting the BCF for growth dilution has a large impact on the BCF.

7 Sources of uncertainty

When using predictive methods to estimate a k_1 value, as well as uncertainty in the estimate, there is also uncertainty associated with experimental k_1 values. Therefore the uncertainty in the predicted k_1 value needs to be set into this context.

For this report, the data set of experimental k_1 values was taken from three main sources (see Section 4.1) and none of these data were re-evaluated for quality or reliability (the aim of the study was to test the methods against existing data sets).

Since the main analysis has been completed, some information on the likely validity of the k_1 values in the Arnot data set has been provided (Arnot, personal communication) and the main points are summarised below. As far as is known, no data quality criteria have been explicitly developed to evaluate measured bioaccumulation rate constants, and so the data were only evaluated in a preliminary fashion by the following approach.

- Quality criteria have been proposed for reducing uncertainty in BCF data (Arnot and Gobas, 2006) and these six data quality criteria were considered in the preliminary evaluation of k_1 values.
- We also took into account whether the substance could be appreciably ionised in water as the degree of ionisation can affect the k_1 value, and most of the methods reviewed in this project have not been specifically developed to include ionised forms of organic chemicals.
- A further criterion considered was whether the measured data followed first-order kinetics, as most of the predictive methods assume this.

The preliminary evaluation showed that the information necessary to evaluate the data based on these criteria was not always available. For example, the published peer-reviewed papers on BCF studies rarely include the necessary raw data to allow the assumption of first-order kinetics to be investigated (Arnot, personal communication). Therefore, the rate constant data were categorised by Arnot (personal communication) as “acceptable” for use if they were obtained from studies in agreement with the quality criteria outlined in Arnot and Gobas (2006) and the data were given the categorisation “uncertain” if they did not meet at least one of the quality criteria outlined in Arnot and Gobas (2006). For ionisable substances that met the quality criteria outlined in Arnot and Gobas (2006), or for substances where the rate constants did not follow first-order kinetics, the categorisation “acceptable with restrictions” was used. The preliminary categorisations given for each data point are summarised in Table A.1 of Appendix A (Arnot, personal communication). Even though measured data may be considered to be “acceptable”, there will still be uncertainty associated with it.

Based on the analysis carried out by Arnot (personal communication), six of the 87 datapoints were categorised as “uncertain” and 17 of the data points were categorised as “acceptable with restrictions”. All of the substances categorised as “acceptable with restrictions” were ionisable substances.

When considering the ionisable substances in the database, a key aspect is whether the substance is in an ionised or neutral form at the pH under which the BCF test was conducted. This pH range will typically be in the range pH 5.5-8. As all of the ionisable substances in this data set were acidic (in that they lose a hydrogen ion in the ionised form) or basic (in that they gain a hydrogen ion in the ionised form), the acid dissociation constant (or pKa value) is an important factor here.

- Entries 1 and 4 are linear alkylbenzene sulphonate compounds. These are likely to have pKa values below one and so be ionised in the BCF test.

- Entries 158, 159, 160, 161, 162 and 163 all relate to pentachlorophenol. The pKa for this substance is around 4.74; thus, it is likely to be mainly present as the ionised form in the conditions of a BCF test. However, it is debatable whether the accumulation behaviour of this substance is dominated by its ionisation properties or its lipophilic properties.
- Entry 680 relates to 2,3,5,6-tetrachlorophenol. The pKa for this substance is around 5.14 and so it will be present, at least in part, as the ionised form under the conditions of a BCF test.
- Entry 164 relates to 2,4,6-trichlorophenol. The pKa for this is around 6.2. This is within the typical range of pHs found in lab tests and so could be present in the ionised form, depending on the actual pH used (at pHs lower than 6.2 it will predominantly be in the neutral form). As the test for this substance was carried out at pH 6.95, it would have been predominantly in the ionised form.
- Entry 54 relates to benzenamine (aniline). The pKa is around 4.6, so at pHs of five or above the substance will be present predominantly as the neutral form.
- Entry 111 relates to bisphenol-A. The pKa values for this substance are around 9.59 and 11.3. This means that within the pH range 5-8 the substance will be present predominantly as the neutral form.
- Entry 326 relates to 4-chlorobenzeneamine (4-chloroaniline). The pKa for this substance is around 3.98. Similar to aniline, this substance will be present predominantly as the neutral form under the conditions of the BCF test.
- Entry 996 relates to haloxyfop-methyl. No pKa value for this substance could be readily located. Although the substance contains a nitrogen (pyridine) group the evidence suggests that ionisation of this substance under the conditions of a BCF test may not be an issue (the pKa of pyridine is 5.21 and the presence of electron-withdrawing groups on the ring (a chlorine atom and a trifluoromethyl group) would be expected to reduce this pKa). Thus, this substance would be expected to be predominantly in the neutral form at the pHs used in BCF tests.
- Entries 653, 652, 659 and 654 all relate to trichloroaniline derivatives. The pKa values of these substances are all low (below one) and so these would be expected to be present predominantly in the neutral form during the BCF tests.

Based on this rough analysis, it would appear that only the linear alkybenzene sulphonate compounds and the chlorophenols would be ionised at the pHs typically used in BCF tests. In addition, some of these substances (the aniline derivatives except for one trichloroaniline) have log K_{ow} values below 3.5 and so would not have been included in the analysis of the subset of chemicals with log K_{ow} of 3.5 or above.

Similar to the Arnot data set, the UBA data set was not reviewed for quality and reliability as it was understood that the data had already undergone some screening. It was not possible to evaluate the quality of these data in detail as some study-specific parameters were not available. However, it is possible that some of the data did not follow first-order depuration kinetics (Arnot, personal communication). In addition, the identies of the substances were not available when our analysis was carried out. Information has since been provided that indicates that around half of the chemicals in the UBA data set are potentially ionisable. However, a large proportion of the identified ionisable substances also have log K_{ow} values below 3.5 and so would not be included in the analysis carried out on the subset of chemicals with log K_{ow} of 3.5 and above. Of the remaining substances that are potentially ionisable, several of these are amines/pyridines and although the pKa is unknown for most of them, our analysis suggests that at least some of these will be present in the neutral form at pHs typically used in BCF tests. The confidentiality of this data precludes a more detailed analysis.

The Gold Standard data set was reviewed previously by others and the validity of these data were not re-evaluated at the start of the test. However, few details are publically available on exactly how these data were evaluated and it is not possible to determine from the database if strict first-order kinetics were followed in each case.

To investigate the possible uncertainity introduced into the analysis by the inclusion of ionisable substances, a limited re-analysis for the first three methods (Sijm *et al*., Hendriks *et al*. (2001) and Campfens and Mackay (1997)) was done using substances with a log K_{ow} of 3.5 but removing all substances marked as “acceptable with restrictions” or “uncertain” from the Arnot data set and all substances from the UBA dataset. This reduced dataset was analysed as before. The statistics obtained are shown below in

Table 7.1, along with the original statistics from Table 4.1 of this report (for the data set without these substances removed). The statistics are similar when the ionogenic substances are removed to when they are included.

Arnot (personal communication) also suggested that certain chemicals may be subject to biotransformation in the gill compartment and this may result in a lower k_1 value than may be expected. If such biotransformation is significant for any of the chemicals included in the test set, this may introduce uncertainty as some, or all, of the methods may not be appropriate for such chemicals. However, without a detailed investigation of the mechanism of metabolism, it is currently difficult to know in advance which chemicals will be subject to significant transformation in the gill compartment[38].

Another possible source of uncertainty is that k_1 values used here to test the methods will have been obtained using a range of experimental test methods and this may have introduced some internal inconsistencies into the database. Some of the predictive methods, such as the Sijm *et al*. (1995) method, were developed based on data sets that were largely assembled by the authors themselves and may have a higher likelihood of being internally consistent (for example the data set used by Sijm *et al*. was developed using essentially two different methods and most of the chemicals included were non-ionisable chlorinated aromatic and polycyclic aromatic compounds). Thus, these methods may have a much higher predictive power for some types of chemical or test system than may be suggested by the analysis here, but their domain of applicability may be somewhat limited. A possible way forward here would be to identify those chemicals, organisms or test characteristics/conditions that result in the experimental k_1 value deviating substantially from the predictions of the models and methods which have a reasonably strong empirical and theoretical basis. This would help to better define the domain of applicability of the predictive methods.

Uncertainties will also be introduced from uncertainties in the test set from variables such as log K_{ow} for the substances and fish weight (in many instances only the the initial fish weight is available).

[38] In kinetic terms, it is also unclear if biotransformation in the gill compartment would reduce the k_1 value or increase the k_2 one, in effect which overall process would capture this phenomenon.

Table 7.1 Comparison of statistics obtained with and without inclusion of ionogenic substances

Method	Substances with log K_{ow} of 3.5 and above based on initial fish weight	
	Including ionogenic substances	**Data set minus substances categorised as "acceptable with restrictions" and "uncertain" from Arnot data set and substances from UBA data set**
Sijm *et al*. (1995)	Mean log ratio = -0.02	Mean log ratio = -0.06
	95% confidence interval = ±0.10	95% confidence interval = ±0.11
	Standard deviation = ±0.51	Standard deviation = ±0.50
	Median = -0.01	Median = -0.01
	Number of data points = 101	Number of data points = 78
	[Mean ratio = 0.96; 95% C.I. 0.76-1.02]	[Mean ratio = 0.87; 95% C.I. 0.67-1.12]
Hendriks *et al*. (2001)	Mean log ratio = 0.05	Mean log ratio = 0.02
	95% confidence interval = ±0.10	95% confidence interval = ±0.11
	Standard deviation = ±0.50	Standard deviation = ±0.49
	Median = 0.06	Median = 0.06
	Number of data points = 101	Number of data points = 78
	[Mean ratio = 1.12; 95% C.I. 0.90-1.41]	[Mean ratio = 1.04; 95% C.I. 0.81-1.34]
Campfens and Mackay (1997)	Mean log ratio = 1.03	Mean log ratio = 0.98
	95% confidence interval = ±0.23	95% confidence interval = ±0.27
	Standard deviation = ±1.12	Standard deviation = ±1.16
	Median = 0.84	Median = 0.66
	Number of data points = 93	Number of data points = 73
	[Mean ratio = 10.61; 95% C.I. 6.28-17.91]	[Mean ratio = 9.50; 95% C.I. 5.16-17.49]

8 Conclusions

Although the main purpose of this study was to identify methods for estimating k_1 values so that a kinetic BCF could be determined from the data generated in a fish feeding test, our study identified a number of wider issues with the analysis of, and interpretation of, bioaccumulation data in general.

In terms of methods that could be used for estimating a k_1 value, our study found the following methods to be potentially suitable:

- Hayton and Barron (1990).
- Erickson and McKim (1990a).
- Barber *et al.* (1991).
- Barber (2003) – observed.
- Barber (2001).
- Streit and Sire (1993).
- Erickson and McKim (1990b).
- Hendriks *et al*. (2001).
- Tolls and Sijm (1995).
- Sijm *et al*. (1995).
- Spacie and Hamelink (1982).
- Barber (2003) – calibrated.
- Thomann (1989).

These methods were tested over the approximate log K_{ow} range of 3.5 to 8.2. When using these methods, there will be a large uncertainty in the resulting prediction for any given substance, and this uncertainty in the predicted k_1 must be taken into account when considering the use of any method(s), for example within the draft updated OECD 305 Test Guideline.

For some applications the uncertainty in the predictions may be acceptable, for example if an estimate of a k_1 and hence BCF is needed for modelling purposes or to show that the BCF is well below or well above a regulatory criteria value. However, in other cases the uncertainty in the predicted k_1 and BCF may be more problematic, for example where the prediction leads to a BCF value that is close to a regulatory limit.

Suggested areas for further work to reduce the uncertainty in the predicted values are given in the report.

Most of these methods depend only on the size of the fish and not the properties of the substance. The following points are relevant when using these methods in conjunction with a depuration rate constant obtained in a fish feeding study to estimate a BCF:

- For some substances no uptake is seen in bioconcentration studies (for example in the data set used to develop the Sijm *et al*. (1995) method, no uptake was seen for octachloronaphthalene and octachlorodibenzo-*p*-dioxin; although BCF data for octachlorodibenzo-*p*-dioxin is included in the test set used here). It is not always clear if the lack of uptake seen results from an

actual very low k_1 value or results from methodological limitations (low bioavailability in the exposure media of experimental system used).

- When estimating the k_1 value a fish weight needs to be assumed for many of the methods. The current approach is usually to use the weight of the fish at the start of the depuration phase in the fish feeding study. Whilst this approach would appear to be appropriate to derive a non-growth corrected BCF, the choice of fish weight is not so clear cut if a growth-corrected BCF is to be derived. These are important considerations if the BCF is to be compared against fixed regulatory criteria as the fish weight chosen can theoretically have an impact on whether the BCF is above or below a given regulatory value.
- Most, but not all, of the methods investigated assume no dependence of the uptake rate constant on the lipid content of the fish. Therefore if lipid normalisation of the resulting BCF is needed, using the lipid content of the fish from the fish feeding study[39] should be appropriate. However, the overall depuration constant is made up of several processes, including metabolism and growth dilution, and not all of these may show the (same) dependence on the lipid content of the fish. Therefore lipid normalisation of data for substances that are rapidly metabolised or where growth dilution makes up a significant proportion of the overall depuration seen, may need to be done carefully (or may not be appropriate).

This study identified a number of issues that may need to be considered in the analysis of actual BCF data. For the examples considered here, the uptake rate did not appear to follow strict first-order kinetics. This has been considered in relation to the growth of the fish and other possible factors, and a tentative approach for analysing such data is outlined. Although this was tested with a limited number of data sets, and the mechanistic interpretation and general applicability of the approach is not yet clear, this has potentially important consequences for predicting the uptake rate constant and for interpreting bioconcentration study data in general. It leads to a number of questions which are currently difficult to answer.

- Does the k_1 value depend on the lipid content of the fish, as this would affect the concentration in the fish?
- Does the k_1 value depend on the chemical properties? Several of the methods reviewed here assume that it does not.
- If growth-correcting the kinetic BCF, which value of k_1 should be chosen?
- What implications does this have for predicting a k_1 value?
- It is recognised that k_1 will decrease as the weight of the fish increases (as shown in Section 5.2). The current analysis indicates that this alone is not sufficient to explain the trends seen but it contributes to the trend (or is an extra factor on top of that observed).
- The current method given in the OECD 305 Test Guideline assumes that the k_1 value is constant during the uptake phase. If k_1 actually decreases the method will effectively estimate an “average” value over the uptake phase. Further, if the simultaneous method is used, although this may result in a better overall fit to the data than the sequential method (as it is effectively fitting two variables to the data rather than one), this may result in erroneous estimates for the overall depuration rate constant (k_2).

[39] In effect, the depuration rate constant is lipid-normalised.

Although this analysis assumes that k_1 may vary with the concentration in the fish, other factors may co-vary with the concentration in the fish and may better explain the uptake curves.

Further work is recommended in this area to clarify the general applicability of the approach. If this demonstrates that this is a real effect rather than an experimental artefact, consideration should be given for including such an approach in the test guideline (OECD 305). This further work could include the following aspects.

- Re-evaluate the available database of k_1 values to check that the results are fully valid, and that the experiments followed first-order uptake and depuration kinetics. However, for many published studies insufficient data will be available to allow this to be checked.
- Develop further approaches to determing k_1 values. This could include empirical, regression-type analysis and theoretical modelling studies. It could also include the use of more complex fish accumulation models in conjunction with data generated in dietary accumulation studies to estimate BCF directly.
- Attempt to identify factors, such as chemical-related, organism-related or test characteristics/conditions that result in the k_1 value deviating substantially from predictions of models which have a reasonably strong empirical and theoretical basis. This will be useful in further elucidating the domain of applicability of the methods and perhaps in the development of new methods.
- Carry out a calibration exercise to estimate BCF from BMF data using information from substances with known bioaccumulative properties by comparing estimated BCF against REACH Annex XIII criteria for B and vB.
- Consider the use of metrics other than the BCF that are readily accessible from the dietary study, for example the BMF_{food} or (growth-corrected) depuration half-life, in relation to the REACH Annex XIII criteria for B- and vB.

In the meantime, it would be prudent to routinely check for deviations from first-order kinetics when analysing bioconcentration data. A suggested approach is given below.

- To simplify the data, it would be preferable if the k_2 value was obtained from the depuration data directly (a plot of $\ln[C_{fish}]$ against time). As such a plot should yield a straight line, this is a good check to ensure that a) the k_2 value is constant during the experiment and b) depuration follows first-order kinetics.
- Once the k_2 value has been determined, the value of k_1 should be calculated for each individual time point of the uptake phase using Equation 39[40]. The advantage of estimating k_1 this way rather than by curve fitting is that any trends in the value of k_1 can be seen.
- If the value of k_1 is found to be reasonably constant at each time point during the uptake phase, the normal curve-fitting approach can then be used to estimate the k_1 value, or the k_1 obtained at each individual time point could be averaged. The kinetic BCF can then be estimated as k_1/k_2.
- If the value of k_1 is found to decrease (or increase) at each time point, this indicates that k_1 is not constant during the uptake phase and presents a problem for which value to use in determining the BCF. If an apparent steady state appears to be reached, it is preferable to use the k_1 (or average k_1) value determined for those time points at steady state (see also Section 5.2).

[40] This equation can easily be rearranged to give $k_1 = \frac{[C_{fish}] \times k_2}{[C_{water}] \times \left(1 - e^{-k2 \times t}\right)}$.

Alternatively the k_1 values could be estimated using the approach outlined in Section 5.7.5 using Equation 58.

Growth correction of the BCF needs to be carried out carefully. In particular, the relevance of the growth-corrected BCF for comparison against the current regulatory criteria (which are likely to have been developed using non-growth corrected data) is unclear. This area warrants further discussion and agreement as there are important consequences in terms of regulatory control of substances.

In relation to the revision of the OECD 305 Test Guideline, given that growth of the fish can complicate the interpretation of BCF (and BMF_{food}) studies, it is preferable for such studies to be carried out in a way that minimises the growth of the fish during the study. This may need for example, consideration of the test species (a slow-growing fish species versus a rapidly growing fish species of a suitable size) and the feeding rate used in the test. It would also be useful to consider standardising the feeding rate in both the BCF and BMF_{food} study guidelines as, for substances where faecal elimination is important, the feeding rate may affect the rate of depuration seen by this route.

The use of feeding data to determine if the REACH Annex XIII B or vB criteria are met is potentially problematic owing to the wide range of factors that can affect the accumulation seen in the studies.

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10 Glossary

Assimilation efficiency	The efficiency with which a chemical is taken up from food
B	Bioaccumulative. Under REACH a substance is considered to be bioaccumulative if the fish BCF is above 2,000 l kg^{-1}.
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BMF	Biomagnification factor
D-values	D-vales are transport parameters (units of mol Pa^{-1} $hour^{-1}$) used within the fugacity approach. The rates of transport (or loss) are obtained by multiplying the D-value by the fugacity.
EU	European Union
Fugacity	Fugacity is the leaving tendency of a substance from a compartment. Fugacity is related to concentration (in units of mol m^{-3}) by the equation concentration = Z × fugacity, where Z is the fugacity capacity (units of mol m^{-3} Pa^{-1}) and fugacity has units of Pa.
In vivo	In the living organism
ln	Natural logarithm (base e)
log	Logarithm to base 10 (sometimes written as $\log_{10}$)
k_1	Uptake rate constant.
k_2	Depuration rate constant
PBT	Persistent, bioaccumulative and toxic
pH	The negative logarithm (base 10) of the hydrogen ion concentration
pKa	The negative logarithm (base 10) of the acid dissociation constant
QSAR	Quantitative structure activity relationship
r^2	Square of the correlation coefficient
Secondary poisoning	Effects occurring in a top predator as a result of accumulation of a substance in its diet
TMF	Trophic magnification factor
vB	Very bioaccumulative. Under REACH a substance is considered to be very bioaccumulative if the fish BCF is above 5,000 l kg^{-1}.
vPvB	Very persistent and very bioaccumulative

11 Appendix A – Data sets used

Table A.1 Arnot data set – substance identities

Ref#[1]	CAS No	Name	Log K_{ow}	Molecular weight	Smiles	Initial evaluation of data quality
432	120-12-7	Anthracene	4.45	178.24	c(c(ccc1)cc(c2ccc3)c3)(c1)c2	Acceptable
996	69806-40-2	Haloxyfop-methyl	4.05	375.73	Clc1cc(C(F)(F)F)cnc1Oc2ccc(OC(C)C(=O)OC)cc2	Acceptable with restrictions
16	50-32-8	Benzo[a]pyrene	6.13	252.32	c(c(c(cc1)ccc2)c2cc3)(c3cc(c4ccc5)c5)c14	Acceptable
710	1582-09-8	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	5.34	335.29	CCCN(CCC)c1c(cc(cc1N(=O)(=O))C(F)(F)F)N(=O)(=O)	Acceptable
711	1582-09-8	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	5.34	335.29	CCCN(CCC)c1c(cc(cc1N(=O)(=O))C(F)(F)F)N(=O)(=O)	Acceptable
458	121-82-4	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	0.87	222.12	N(=O)(=O)N(CN(N(=O)(=O))CN1N(=O)(=O))C1	Acceptable
726	1746-01-6	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	6.80	321.98	Clc3cc2Oc1cc(Cl)c(Cl)cc1Oc2cc3Cl	Acceptable
727	1746-01-6	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	6.80	321.98	Clc3cc2Oc1cc(Cl)c(Cl)cc1Oc2cc3Cl	Acceptable
725	1746-01-6	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	6.80	321.98	Clc3cc2Oc1cc(Cl)c(Cl)cc1Oc2cc3Cl	Acceptable
729	1746-01-6	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	6.80	321.98	Clc3cc2Oc1cc(Cl)c(Cl)cc1Oc2cc3Cl	Acceptable
728	1746-01-6	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	6.80	321.98	Clc3cc2Oc1cc(Cl)c(Cl)cc1Oc2cc3Cl	Acceptable
712	1582-09-8	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	5.34	335.29	CCCN(CCC)c1c(cc(cc1N(=O)(=O))C(F)(F)F)N(=O)(=O)	Acceptable
29	56-55-3	Benzo[a]anthracene	5.76	228.30	c(c(c(c(c1)ccc2)c2)cc(c3ccc4)c4)(c1)c3	Acceptable

Ref#[1]	CAS No	Name	Log K_{ow}	Molecular weight	Smiles	Initial evaluation of data quality
2		Octaethylene glycol monotridecyl ether	3.07	552.80	OCCOCCOCCOCCOCCOCCOCCOCCOCCCCCCCCCCCC	Acceptable
3		Octaethylene glycol monotridecyl ether	5.11	552.80	OCCOCCOCCOCCOCCOCCOCCOCCOCCCCCCCCCCCC	Acceptable
531	226-36-8	Dibenz(a,h)acridine	5.67	279.34	c1ccc4c(c1)ccc5nc2c(ccc3ccccc23)cc45	Acceptable
1		C-12-2-LAS	4.71	326.50	S(=O)(=O)(O)c1ccc(C(CCCCCCCCCC)C)cc1	Acceptable with restrictions
4		C-12-5-LAS	4.71	326.50	S(=O)(=O)(O)c1ccc(C(CCCCCCC)CCCC)cc1	Acceptable with restrictions
236	95-94-3	Benzene, 1,2,4,5-tetrachloro-	4.64	215.89	c(c(cc(c1Cl)Cl)Cl)(c1)Cl	Acceptable
441	120-82-1	Benzene, 1,2,4-trichloro-	4.02	181.45	c(ccc(c1Cl)Cl)(c1)Cl	Acceptable
164	88-06-2	2,4,6-Trichlorophenol	3.69	197.45	Oc(c(cc(c1)Cl)Cl)c1Cl	Acceptable with restrictions
321	106-46-7	Benzene, 1,4-dichloro-	3.44	147.00	c(ccc(c1)Cl)(c1)Cl	Acceptable
680	935-95-5	2,3,5,6-Tetrachlorophenol	3.88	231.89	Oc1c(Cl)c(Cl)cc(Cl)c1Cl	Acceptable with restrictions
160	87-86-5	Phenol, pentachloro-	5.12	266.34	Oc(c(c(c(c1Cl)Cl)Cl)Cl)c1Cl	Acceptable with restrictions
163	87-86-5	Phenol, pentachloro-	5.12	266.34	Oc(c(c(c(c1Cl)Cl)Cl)Cl)c1Cl	Acceptable with restrictions
162	87-86-5	Phenol, pentachloro-	5.12	266.34	Oc(c(c(c(c1Cl)Cl)Cl)Cl)c1Cl	Acceptable with restrictions
200	92-86-4	4,4'-dibromobiphenyl	5.72	312.01	c(ccc(c(ccc(c1)Br)c1)c2)(c2)Br	Acceptable
653	634-91-3	3,4,5-Trichloroaniline	3.32	196.46	Nc1cc(Cl)c(Cl)c(Cl)c1	Acceptable with restrictions
659	636-30-6	2,4,5-Trichloroaniline	3.45	196.46	Nc(c(cc(c1Cl)Cl)Cl)c1	Acceptable with restrictions
145	87-61-6	Benzene, 1,2,3-trichloro-	4.05	181.45	c(c(c(cc1)Cl)Cl)(c1)Cl	Acceptable
654	634-93-5	2,4,6-Trichloroaniline	3.52	196.46	Nc(c(cc(c1)Cl)Cl)c1Cl	Acceptable with restrictions
652	634-67-3	2,3,4-Trichloroaniline	3.33	196.46	Nc1ccc(Cl)c(Cl)c1Cl	Acceptable with restrictions
984	57117-44-9	1,2,3,6,7,8-Hexachlorodibenzofuran	7.30	374.87	Clc1c(Cl)c2c3cc(Cl)c(Cl)c(Cl)c3Oc2cc1Cl	Acceptable
940	35693-99-3	2,2',5,5'-Tetrachloro-1,1'-biphenyl	5.84	291.99	c1c(Cl)ccc(Cl)c1c2c(Cl)ccc(Cl)c2	Acceptable
986	59080-33-0	2,4,6-Tribromobiphenyl	6.03	390.90	Brc2c(c(cc(c2)Br)Br)c1ccccc1	Acceptable
991	60851-34-5	2,3,4,6,7,8-Hexachlorodibenzofuran	7.30	374.87	Clc1cc2c3cc(Cl)c(Cl)c(Cl)c3Oc2c(Cl)c1Cl	Acceptable
982	57117-31-4	2,3,4,7,8-Pentachlorodibenzofuran	6.64	340.42	c1(Cl)cc2c3cc(Cl)c(Cl)cc3oc2c(Cl)c1Cl	Acceptable

Ref#[1]	CAS No	Name	Log K_{ow}	Molecular weight	Smiles	Initial evaluation of data quality
943	35693-99-3	2,2',5,5'-Tetrachloro-1,1'-biphenyl	5.84	291.99	c1c(Cl)ccc(Cl)c1c2c(Cl)ccc(Cl)c2	Acceptable
929	30746-58-8	1,2,3,4-Tetrachlorodibenzo-p-dioxin	6.60	321.98	Clc1c(Cl)c(Cl)c2Oc3ccccc3Oc2c1Cl	Uncertain
961	40321-76-4	1,2,3,7,8-Pentachlorodibenzo-p-dioxin	6.64	356.42	ClC(C1=C2OC(C=C3Cl)=C(C=C3Cl)O1)=C(C(Cl)=C2)Cl	Acceptable
987	59080-37-4	2,2',5,5'-Tetrabromobiphenyl	6.50	469.80	Brc2c(c(ccc2)Br)c1c(cccc1Br)Br	Acceptable
941	35693-99-3	2,2',5,5'-Tetrachloro-1,1'-biphenyl	5.84	291.99	c1c(Cl)ccc(Cl)c1c2c(Cl)ccc(Cl)c2	Acceptable
876	16606-02-3	2,4',5-Trichloro-1,1'-biphenyl	5.67	257.55	Clc1ccc(cc1)c2cc(Cl)ccc2Cl	Acceptable
939	35065-27-1	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	6.92	360.88	Clc1cc(Cl)c(cc1Cl)c2cc(Cl)c(Cl)cc2Cl	Acceptable
959	39227-28-6	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	7.30	390.87	Clc1c(Cl)c(Cl)c2Oc3ccc(Cl)c(Cl)c3Oc2c1Cl	Acceptable
942	35693-99-3	2,2',5,5'-Tetrachloro-1,1'-biphenyl	5.84	291.99	c1c(Cl)ccc(Cl)c1c2c(Cl)ccc(Cl)c2	Acceptable
985	57653-85-7	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	7.30	390.87	Clc2cc1Oc3c(Oc1c(Cl)c2Cl)cc(Cl)c(Cl)c3Cl	Acceptable
938	35065-27-1	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	6.92	360.88	Clc1cc(Cl)c(cc1Cl)c2cc(Cl)c(Cl)cc2Cl	Acceptable
641	626-39-1	Benzene, 1,3,5-tribromo-	4.51	314.80	c(cc(cc1Br)Br)(c1)Br	Acceptable
888	19408-74-3	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	7.30	390.87	Clc(c1c3Oc2c(c(Cl)c(c(Cl)c2)Cl)O1)c(c(Cl)c3)Cl	Acceptable
790	2921-88-2	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	4.96	350.59	CCOP(=S)(OCC)Oc1nc(Cl)c(Cl)cc1Cl	Acceptable
968	51207-31-9	2,3,7,8-Tetrachlorodibenzofuran	6.53	305.98	Clc3cc2oc1cc(Cl)c(Cl)cc1c2cc3Cl	Acceptable
960	39227-58-2	1,2,4-Trichlorodibenzo[b,e][1,4]dioxin	6.35	287.53	Clc3cc(Cl)c2Oc1ccccc1Oc2c3Cl	Acceptable

Ref#[1]	CAS No	Name	Log K_{ow}	Molecular weight	Smiles	Initial evaluation of data quality
934	33857-26-0	2,7-Dichlorodibenzo[b,e][1,4]dioxin	5.75	253.09	Clc3ccc2Oc1cc(Cl)ccc1Oc2c3	Acceptable
937	35065-27-1	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	6.92	360.88	Clc1cc(Cl)c(cc1Cl)c2cc(Cl)c(Cl)cc2Cl	Acceptable
995	67562-39-4	1,2,3,4,6,7,8-Heptachlorodibenzofuran	7.40	409.31	c1(Cl)c(Cl)c2c3cc(Cl)c(Cl)c(Cl)c3oc2c(Cl)c1Cl	Acceptable
730	1746-01-6	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	6.80	321.98	Clc3cc2Oc1cc(Cl)c(Cl)cc1Oc2cc3Cl	Acceptable
945	35822-46-9	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	7.80	425.31	Clc1c(Cl)c(Cl)c2Oc3cc(Cl)c(Cl)c(Cl)c3Oc2c1Cl	Acceptable
871	15862-07-4	2,4,5-Trichloro-1,1'-biphenyl	5.60	257.55	Clc1cc(Cl)c(cc1Cl)c2ccccc2	Acceptable
988	59261-08-4	2,2',4,4',6,6'-Hexabromobiphenyl	7.20	627.59	Brc2c(c(cc(c2)Br)Br)c1c(cc(cc1Br)Br)Br	Acceptable
808	3268-87-9	1,2,3,4,5,6,7,8-Octachlorodibenzo-p-dioxin	8.20	459.76	Clc3c(Cl)c(Cl)c2Oc1c(Cl)c(Cl)c(Cl)c(Cl)c1Oc2c3Cl	Uncertain
958	39001-02-0	1,2,3,4,5,6,7,8-Octachlorodibenzofuran	8.20	443.76	Clc3c(Cl)c(Cl)c1c(oc2c(Cl)c(Cl)c(Cl)c(Cl)c12)c3Cl	Acceptable
316	106-37-6	Benzene, 1,4-dibromo-	3.79	235.91	c(ccc(c1)Br)(c1)Br	Acceptable
765	2385-85-5	Mirex	6.89	545.55	ClC2(Cl)C4(Cl)C1(Cl)C5(Cl)C(Cl)(Cl)C3(Cl)C1(Cl)C2(Cl)C3(Cl)C45Cl	Acceptable
746	2051-24-3	Decachlorobiphenyl	8.18	498.66	Clc1c(Cl)c(Cl)c(c(Cl)c1Cl)c2c(Cl)c(Cl)c(Cl)c(Cl)c2Cl	Acceptable
326	106-47-8	Benzenamine, 4-chloro-	1.83	127.57	Nc(ccc(c1)Cl)c1	Acceptable with restrictions
54	62-53-3	Benzenamine	0.90	93.13	Nc(cccc1)c1	Acceptable with restrictions
706	1582-09-8	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	5.34	335.29	CCCN(CCC)c1c(cc(cc1N(=O)(=O))C(F)(F)F)N(=O)(=O)	Acceptable
731	1746-01-6	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	6.80	321.98	Clc3cc2Oc1cc(Cl)c(Cl)cc1Oc2cc3Cl	Uncertain

Ref#[1]	CAS No	Name	Log K_{ow}	Molecular weight	Smiles	Initial evaluation of data quality
707	1582-09-8	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	5.34	335.29	CCCN(CCC)c1c(cc(cc1N(=O)(=O))C(F)(F)F)N(=O)(=O)	Acceptable
399	117-81-7	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	7.73	390.57	O=C(OCC(CCCC)CC)c(c(ccc1)C(=O)OCC(CCCC)CC)c1	Uncertain
708	1582-09-8	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	5.34	335.29	CCCN(CCC)c1c(cc(cc1N(=O)(=O))C(F)(F)F)N(=O)(=O)	Acceptable
398	117-81-7	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	7.73	390.57	O=C(OCC(CCCC)CC)c(c(ccc1)C(=O)OCC(CCCC)CC)c1	Uncertain
161	87-86-5	Phenol, pentachloro-	5.12	266.34	Oc(c(c(c(c1Cl)Cl)Cl)Cl)c1Cl	Uncertain
992	61949-76-6	cis-Permethrin	7.43	391.30	O=C(OCC2=CC=CC(OC3=CC=CC=C3)=C2)C1C(C)(C)C1C=C(Cl)Cl	Acceptable
970	51630-58-1	Cyano(3-phenoxyphenyl)methyl ester, 4-Chloro-alpha-(1-methylethyl)benzeneacetic acid	6.20	419.91	CC(C)C(C(=O)OC(C#N)c2cccc(Oc1ccccc1)c2)c3ccc(Cl)cc3	Acceptable
158	87-86-5	Phenol, pentachloro-	5.12	266.34	Oc(c(c(c(c1Cl)Cl)Cl)Cl)c1Cl	Acceptable with restrictions
159	87-86-5	Phenol, pentachloro-	5.12	266.34	Oc(c(c(c(c1Cl)Cl)Cl)Cl)c1Cl	Acceptable with restrictions
971	52918-63-5	[1R-[1 alpha(S*),3 alpha]]Cyano(3-phenoxyphenyl)methyl ester 3-(2,2-dibromoethenyl)-2,2-dimethyl cyclopropane carboxylic acid	6.20	505.21	CC1(C)C(C=C(Br)Br)C1C(=O)OC(C#N)c3cccc(Oc2ccccc2)c3	Acceptable
994	67375-30-8	[1 alpha(S*), 3 alpha]-(+-)-3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropane carboxylic acid cyano (3-phenoxyphenyl)methyl ester	6.38	416.31	ClC(Cl)=CC1C(C)(C)C1C(=O)OC(C#N)c2cccc(Oc3ccccc3)c2	Acceptable
393	117-81-7	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	7.73	390.57	O=C(OCC(CCCC)CC)c(c(ccc1)C(=O)OCC(CCCC)CC)c1	Acceptable
392	117-81-7	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	7.73	390.57	O=C(OCC(CCCC)CC)c(c(ccc1)C(=O)OCC(CCCC)CC)c1	Acceptable

Ref#[1]	CAS No	Name	Log K_{ow}	Molecular weight	Smiles	Initial evaluation of data quality
418	118-96-7	Benzene, 2-methyl-1,3,5-trinitro-	1.60	227.13	O=N(=O)c(cc(N(=O)=O)c(c1N(=O)=O)C)c1	Acceptable
459	121-82-4	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	0.87	222.12	N(=O)(=O)N(CN(N(=O)(=O))CN1N(=O)(=O))C1	Acceptable
788	2691-41-0	Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine (HMX)	0.19	296.16	O=N(=O)N(CN(N(=O)=O)CN(N(=O)=O)CN1N(=O)=O)C1	Acceptable
795	2921-88-2	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	4.96	350.59	CCOP(=S)(OCC)Oc1nc(Cl)c(Cl)cc1Cl	Acceptable
111	80-05-7	Phenol, 4,4 -(1-methylethylidene)bis-	3.32	228.29	Oc(ccc(c1)C(c(ccc(O)c2)c2)(C)C)c1	Acceptable with restrictions

[1]Reference number from the original data set

Table A.2 Arnot data set – bioconcentration data

Ref#[1]	Substance	Common name	Scientific name	Experimental data					Reference[3]
				k_1 (l kg^{-1} day^{-1})	Fish weight (kg)[2]	Lipid (kg kg^{-1})	Temperature (°C)	Dissolved oxygen (mg l^{-1})	
432	Anthracene	Bluegill sunfish	*Lepomis macrochirus*	900	5×10^{-4}	0.048	23.5	7.4	Spacie *et al*., 1983
996	Haloxyfop-methyl	Bluegill sunfish	*Lepomis macrochirus*	720	6×10^{-4}	0.048	17	8.6	Murphy and Lutenske, 1990
16	Benzo[a]pyrene	Bluegill sunfish	*Lepomis macrochirus*	416	5×10^{-4}	0.048	23.5	7.4	Spacie *et al*., 1983
710	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	Channel catfish	*Ictalurus punctatus*	3,480	6.2×10^{-3}	0.040	23	7.5	Schultz and Hayton, 1999

Ref#[1]	Substance	Common name	Scientific name	Experimental data					Reference[3]
				k_1 (l kg^{-1} day^{-1})	Fish weight (kg)[2]	Lipid (kg kg^{-1})	Temperature (°C)	Dissolved oxygen (mg l^{-1})	
711	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	Channel catfish	*Ictalurus punctatus*	3,480	6.89×10^{-3}	0.070	15	8.8	Schultz and Hayton, 1999
458	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	Channel catfish	*Ictalurus punctatus*	30.7	8.4×10^{-5}	0.048	25	8.0	Belden *et al*., 2005
726	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	Common carp	*Cyprinus carpio*	765	0.015	0.085	25	7.2	Cook *et al*., 1991
727	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	Common carp	*Cyprinus carpio*	736	0.015	0.055	25	7.2	Cook *et al*., 1991
725	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	Common carp	*Cyprinus carpio*	712	0.015	0.096	25	7.2	Cook *et al*., 1991
729	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	Fathead minnow	*Pimephales promelas*	1,870	1×10^{-3}	0.190	25	7.2	Cook *et al*., 1991
728	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	Fathead minnow	*Pimephales promelas*	1,280	1×10^{-3}	0.190	25	7.2	Cook *et al*., 1991
712	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	Fathead minnow	*Pimephales promelas*	756	8.5×10^{-4}	0.048	20	8.0	Spacie and Hamelink, 1979
29	Benzo[a]anthracene	Fathead minnow	*Pimephales promelas*	405	4.2×10^{-4}	0.048	20.5	7.9	de Maagd *et al*., 1998
2	Octaethylene glycol monotridecyl ether	Fathead minnow	*Pimephales promelas*	317	6.6×10^{-4}	0.033	22	7.7	Tolls and Sijm, 1999
3	Octaethylene glycol monotridecyl ether	Fathead minnow	*Pimephales promelas*	317	6.6×10^{-4}	0.033	22	7.7	Tolls and Sijm, 1999
531	Dibenz(a,h)acridine	Fathead minnow	*Pimephales promelas*	276	7.5×10^{-5}	0.048	22	7.7	Southworth *et al*., 1980
1	C-12-2-LAS	Fathead	*Pimephales*	130	7.2×10^{-4}	0.050	21	7.8	Tolls and

Ref#[1]	Substance	Common name	Scientific name	Experimental data					Reference[3]
				k_1 (l kg^{-1} day^{-1})	Fish weight (kg)[2]	Lipid (kg kg^{-1})	Temperature (°C)	Dissolved oxygen (mg l^{-1})	
		minnow	*promelas*						Sijm, 1999
4	C-12-5-LAS	Fathead minnow	*Pimephales promelas*	11.1	7.2×10^{-4}	0.050	21	7.8	Tolls, 1998
236	Benzene, 1,2,4,5-tetrachloro-	Flagfish	*Jordanella floridae*	1,630	2.25×10^{-3}	0.085	25	7.2	Smith *et al*., 1990
441	Benzene, 1,2,4-trichloro-	Flagfish	*Jordanella floridae*	1,160	2.25×10^{-3}	0.114	25	7.2	Smith *et al*., 1990
164	2,4,6-Trichlorophenol	Flagfish	*Jordanella floridae*	421	2.25×10^{-3}	0.124	25	7.2	Smith *et al*., 1990
321	Benzene, 1,4-dichloro-	Flagfish	*Jordanella floridae*	291	2.25×10^{-3}	0.085	25	7.2	Smith *et al*., 1990
680	2,3,5,6-Tetrachlorophenol	Flagfish	*Jordanella floridae*	243	2.25×10^{-3}	0.098	25	7.2	Smith *et al*., 1990
160	Phenol, pentachloro-	Flagfish	*Jordanella floridae*	222	2.25×10^{-3}	0.133	25	7.2	Smith *et al*., 1990
163	Phenol, pentachloro-	Goldfish	*Carassius auratus*	948	1×10^{-3}	0.048	20	8.0	Stehly and Hayton, 1990
162	Phenol, pentachloro-	Goldfish	*Carassius auratus*	509	1.75×10^{-3}	0.048	20	8.0	Stehly and Hayton, 1990
200	4,4'-Dibromobiphenyl	Guppy	*Poecilia reticulata*	2,140	9.8×10^{-5}	0.065	22	8.0	Gobas *et al*., 1989
653	3,4,5-Trichloroaniline	Guppy	*Poecilia reticulata*	1,970	3.37×10^{-4}	0.137	23.5	7.4	de Wolf *et al*., 1993
659	2,4,5-Trichloroaniline	Guppy	*Poecilia reticulata*	1,630	3.37×10^{-4}	0.137	23.5	7.4	de Wolf *et al*., 1993
145	Benzene, 1,2,3-trichloro-	Guppy	*Poecilia reticulata*	1,580	3.37×10^{-4}	0.137	23.5	7.4	de Wolf *et al*., 1993
654	2,4,6-Trichloroaniline	Guppy	*Poecilia reticulata*	1,580	3.37×10^{-4}	0.137	23.5	7.4	de Wolf *et al*., 1993
652	2,3,4-Trichloroaniline	Guppy	*Poecilia reticulata*	1,460	3.37×10^{-4}	0.137	23.5	7.4	de Wolf *et al*., 1993

Ref#[1]	Substance	Common name	Scientific name	Experimental data					Reference[3]
				k_1 (l kg^{-1} day^{-1})	Fish weight (kg)[2]	Lipid (kg kg^{-1})	Temperature (°C)	Dissolved oxygen (mg l^{-1})	
984	1,2,3,6,7,8-Hexachlorodibenzofuran	Guppy	*Poecilia reticulata*	1,310	9.1×10^{-4}	0.097	25	7.2	Loonen *et al*., 1994
940	2,2',5,5'-Tetrachloro-1,1'-biphenyl	Guppy	*Poecilia reticulata*	1,120	9.8×10^{-5}	0.065	22	8.0	Gobas *et al*., 1989
986	2,4,6-Tribromobiphenyl	Guppy	*Poecilia reticulata*	1,120	9.8×10^{-5}	0.065	22	8.0	Gobas *et al*., 1989
991	2,3,4,6,7,8-Hexachlorodibenzofuran	Guppy	*Poecilia reticulata*	1,100	9.1×10^{-4}	0.097	25	7.2	Loonen *et al*., 1994
982	2,3,4,7,8-Pentachlorodibenzofuran	Guppy	*Poecilia reticulata*	1,010	9.1×10^{-4}	0.097	25	7.2	Loonen *et al*., 1994
943	2,2',5,5'-Tetrachloro-1,1'-biphenyl	Guppy	*Poecilia reticulata*	1,000	1×10^{-4}	0.048	18	3.0	Opperhuizen and Schrap, 1987
929	1,2,3,4-Tetrachlorodibenzo-p-dioxin	Guppy	*Poecilia reticulata*	953	7.9×10^{-5}	0.075	22	8.0	Gobas and Schrap, 1990
961	1,2,3,7,8-Pentachlorodibenzo-p-dioxin	Guppy	*Poecilia reticulata*	952	9.1×10^{-4}	0.097	25	7.2	Loonen *et al*., 1994
987	2,2',5,5'-Tetrabromobiphenyl	Guppy	*Poecilia reticulata*	912	9.8×10^{-5}	0.065	22	8.0	Gobas *et al*., 1989
941	2,2',5,5'-Tetrachloro-1,1'-biphenyl	Guppy	*Poecilia reticulata*	910	1×10^{-4}	0.048	18	7.0	Opperhuizen and Schrap, 1987
876	2,4',5-Trichloro-1,1'-biphenyl	Guppy	*Poecilia reticulata*	890	7.9×10^{-5}	0.075	22	8.0	Gobas and Schrap, 1990
939	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	Guppy	*Poecilia reticulata*	880	1×10^{-4}	0.048	18	3.0	Opperhuizen and Schrap, 1987
959	1,2,3,4,7,8-	Guppy	*Poecilia*	868	9.1×10^{-4}	0.097	25	7.2	Loonen *et*

Ref#[1]	Substance	Common name	Scientific name	Experimental data					Reference[3]
				k_1 (l kg^{-1} day^{-1})	Fish weight (kg)[2]	Lipid (kg kg^{-1})	Temperature (°C)	Dissolved oxygen (mg l^{-1})	
	Hexachlorodibenzo-p-dioxin		*reticulata*						*al*., 1994
942	2,2',5,5'-Tetrachloro-1,1'-biphenyl	Guppy	*Poecilia reticulata*	860	1×10^{-4}	0.048	18	5.0	Opperhuizen and Schrap, 1987
985	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	Guppy	*Poecilia reticulata*	844	9.1×10^{-4}	0.097	25	7.2	Loonen *et al*., 1994
938	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	Guppy	*Poecilia reticulata*	840	1×10^{-4}	0.048	18	7.0	Opperhuizen and Schrap, 1987
641	Benzene, 1,3,5-tribromo-	Guppy	*Poecilia reticulata*	708	9.8×10^{-5}	0.065	22	8.0	Gobas *et al*., 1989
888	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	Guppy	*Poecilia reticulata*	687	9.1×10^{-4}	0.097	25	7.2	Loonen *et al*., 1994
790	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	Guppy	*Poecilia reticulata*	630	9.4×10^{-5}	0.090	22	7.7	Deneer, 1993
968	2,3,7,8-Tetrachlorodibenzofuran	Guppy	*Poecilia reticulata*	603	9.1×10^{-4}	0.097	25	7.2	Loonen *et al*., 1994
960	1,2,4-Trichlorodibenzo[b,e][1,4]dioxin	Guppy	*Poecilia reticulata*	601	7.9×10^{-5}	0.075	22	8.0	Gobas and Schrap, 1990
934	2,7-Dichlorodibenzo[b,e][1,4]dioxin	Guppy	*Poecilia reticulata*	543	7.9×10^{-5}	0.075	22	8.0	Gobas and Schrap, 1990
937	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	Guppy	*Poecilia reticulata*	540	1×10^{-4}	0.048	18	5.0	Opperhuizen and Schrap, 1987
995	1,2,3,4,6,7,8-Heptachlorodibenzofuran	Guppy	*Poecilia reticulata*	524	9.1×10^{-4}	0.097	25	7.2	Loonen *et al*., 1994

Ref#[1]	Substance	Common name	Scientific name	Experimental data					Reference[3]
				k_1 (l kg^{-1} day^{-1})	Fish weight (kg)[2]	Lipid (kg kg^{-1})	Temperature (°C)	Dissolved oxygen (mg l^{-1})	
730	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	Guppy	*Poecilia reticulata*	500	9.1×10^{-4}	0.097	25	7.2	Loonen *et al*., 1994
945	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	Guppy	*Poecilia reticulata*	456	9.1×10^{-4}	0.097	25	7.2	Loonen *et al*., 1994
871	2,4,5-Trichloro-1,1'-biphenyl	Guppy	*Poecilia reticulata*	380	9.8×10^{-5}	0.065	22	8.0	Gobas *et al*., 1989
988	2,2',4,4',6,6'-Hexabromobiphenyl	Guppy	*Poecilia reticulata*	324	9.8×10^{-5}	0.065	22	8.0	Gobas *et al*., 1989
808	1,2,3,4,5,6,7,8-Octachlorodibenzo-p-dioxin	Guppy	*Poecilia reticulata*	275	9.1×10^{-4}	0.097	25	7.2	Loonen *et al*., 1994
958	1,2,3,4,5,6,7,8-Octachlorodibenzofuran	Guppy	*Poecilia reticulata*	217	9.1×10^{-4}	0.097	25	7.2	Loonen *et al*., 1994
316	Benzene, 1,4-dibromo-	Guppy	*Poecilia reticulata*	129	9.8×10^{-5}	0.065	22	8.0	Gobas *et al*., 1989
765	Mirex	Guppy	*Poecilia reticulata*	93.3	9.8×10^{-5}	0.065	22	8.0	Gobas *et al*., 1989
746	Decachlorobiphenyl	Guppy	*Poecilia reticulata*	41.7	9.8×10^{-5}	0.065	22	8.0	Gobas *et al*., 1989
326	Benzenamine, 4-chloro-	Medaka, high-eyes	*Oryzias latipes*	689	2.6×10^{-4}	0.048	25	7.2	Bradbury *et al*., 1993
54	Benzenamine	Medaka, high-eyes	*Oryzias latipes*	250	2.9×10^{-4}	0.048	25	7.2	Bradbury *et al*., 1993
706	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	Rainbow trout	*Oncorhynchus mykiss*	3,140	1.8×10^{-4}	0.048	12	9.8	Schultz and Hayton, 1994
731	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	Rainbow trout	*Oncorhynchus mykiss*	1,850	3.8×10^{-4}	0.048	12	8.0	Mehrle *et al*., 1988
707	Benzenamine, 2,6-	Rainbow trout	*Oncorhynchus*	1,630	4.07×10^{-3}	0.074	12	9.8	Schultz and

Ref#[1]	Substance	Common name	Scientific name	Experimental data					Reference[3]
				k_1 (l kg^{-1} day^{-1})	Fish weight (kg)[2]	Lipid (kg kg^{-1})	Temperature (°C)	Dissolved oxygen (mg l^{-1})	
	dinitro-N,N-dipropyl-4-(trifluoromethyl)-		*mykiss*						Hayton, 1994
399	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	Rainbow trout	*Oncorhynchus mykiss*	1,550	2.89×10^{-3}	0.048	12	9.8	Tarr *et al*., 1990
708	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	Rainbow trout	*Oncorhynchus mykiss*	538	0.0836	0.076	12	9.8	Schultz and Hayton, 1994
398	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	Rainbow trout	*Oncorhynchus mykiss*	386	0.0613	0.048	12	9.8	Tarr *et al*., 1990
161	Phenol, pentachloro-	Rainbow trout	*Oncorhynchus mykiss*	341	4.6×10^{-3}	0.048	12	9.8	Stehly and Hayton, 1989
992	cis-Permethrin	Rainbow trout	*Oncorhynchus mykiss*	201	1.5×10^{-3}	0.080	10	10.3	Muir *et al*., 1994
970	Cyano(3-phenoxyphenyl)methyl ester, 4-Chloro-alpha-(1-methylethyl)benzeneacetic acid	Rainbow trout	*Oncorhynchus mykiss*	157	1.5×10^{-3}	0.080	10	10.3	Muir *et al*., 1994
158	Phenol, pentachloro-	Rainbow trout	*Oncorhynchus mykiss*	120	0.723	0.070	11	10.5	McKim *et al*., 1986
159	Phenol, pentachloro-	Rainbow trout	*Oncorhynchus mykiss*	118	0.723	0.070	11	10.5	McKim *et al*., 1986
971	[1R-[1 alpha(S*),3 alpha]]Cyano(3-phenoxyphenyl)methyl ester 3-(2,2-dibromoethenyl)-2,2-dimethyl cyclopropane	Rainbow trout	*Oncorhynchus mykiss*	105	1.5×10^{-3}	0.080	10	10.3	Muir *et al*., 1994

Ref#[1]	Substance	Common name	Scientific name	Experimental data					Reference[3]
				k_1 (l kg^{-1} day^{-1})	Fish weight (kg)[2]	Lipid (kg kg^{-1})	Temperature (°C)	Dissolved oxygen (mg l^{-1})	
	carboxylic acid								
994	[1 alpha(S*), 3 alpha]-(+-)-3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropane carboxylic acid cyano (3-phenoxyphenyl)methyl ester	Rainbow trout	*Oncorhynchus mykiss*	59.3	1.5×10^{-3}	0.080	10	10.3	Muir *et al*., 1994
393	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	Sheepshead minnow	*Cyprinodon variegatus*	672	2×10^{-3}	0.048	29	6.8	Karara and Hayton, 1989
392	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	Sheepshead minnow	*Cyprinodon variegatus*	317	2×10^{-3}	0.048	23	7.5	Karara and Hayton, 1989
418	Benzene, 2-methyl-1,3,5-trinitro-	Sheepshead minnow	*Cyprinodon variegatus*	200	2×10^{-4}	0.048	23	7.0	Lotufo and Lydy, 2005
459	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	Sheepshead minnow	*Cyprinodon variegatus*	3.6	1.58×10^{-4}	0.048	23	7.0	Lotufo and Lydy, 2005
788	Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine (HMX)	Sheepshead minnow	*Cyprinodon variegatus*	1.4	1.79×10^{-4}	0.048	23	7.0	Lotufo and Lydy, 2005
795	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	Threespine stickleback	*Gasterosteus aculeatus*	1,380	3.22×10^{-4}	0.053	21.5	8.4	Deneer, 1994
111	Phenol, 4,4 -(1-methylethylidene)bis-	Zebrafish	*Brachydanio rerio*	5.5	5×10^{-4}	0.048	27	7.0	Lindholst *et al*., 2003

[1]Reference number from the original data set.

[2]The initial fish weight (either reported or estimated where available/possible).

[3]References for the bioconcentration data are as follows.

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Table A.3 UBA set – substance identities

Ref#[1]	CAS No	Name	Log Kow	Molecular weight	Smiles
UBA 11	Confidential	Confidential	3.8	Confidential	Confidential
UBA 11	Confidential	Confidential	3.8	Confidential	Confidential
UBA 4	Confidential	Confidential	2.9	Confidential	Confidential
UBA 4	Confidential	Confidential	2.9	Confidential	Confidential
UBA 6	Confidential	Confidential	2.86	Confidential	Confidential
UBA 6	Confidential	Confidential	2.86	Confidential	Confidential
UBA 10	Confidential	Confidential	3.2	Confidential	Confidential
UBA 10	Confidential	Confidential	3.2	Confidential	Confidential
UBA 5	Confidential	Confidential	4.9	Confidential	Confidential
UBA 5	Confidential	Confidential	4.9	Confidential	Confidential
UBA 13	Confidential	Confidential	3.4	Confidential	Confidential
UBA 14	Confidential	Confidential	3.4	Confidential	Confidential
UBA 14	Confidential	Confidential	3.4	Confidential	Confidential
UBA 13	Confidential	Confidential	3.4	Confidential	Confidential
UBA 9	Confidential	Confidential	2.59	Confidential	Confidential
UBA 3	Confidential	Confidential	5.1	Confidential	Confidential
UBA 3	Confidential	Confidential	5.1	Confidential	Confidential
UBA 9	Confidential	Confidential	2.59	Confidential	Confidential

[1]Reference number from the original data set

Table A.4 UBA data set – bioconcentration data

Ref#	Substance	Common name	Scientific name	Experimental data					Reference[3]
				k_1 (l kg^{-1} day^{-1})	Fish weight (kg)[2]	Lipid (kg kg^{-1})	Temperature (°C)[4]	Dissolved oxygen (mg l^{-1})[4]	
UBA 11	Confidential	Bluegill sunfish	*Lepomis macrochirus*	40.9	7.03×10^{-3}	0.061	no data	no data	UBA
UBA 11	Confidential	Bluegill sunfish	*Lepomis macrochirus*	28.3	6.32×10^{-3}	0.061	no data	no data	UBA
UBA 4	Confidential	Bluegill sunfish	*Lepomis macrochirus*	15.5	2.58×10^{-3}	0.129	no data	no data	UBA
UBA 4	Confidential	Bluegill sunfish	*Lepomis macrochirus*	11.1	2.58×10^{-3}	0.13	no data	no data	UBA
UBA 6	Confidential	Bluegill sunfish	*Lepomis macrochirus*	7.73	3.40×10^{-3}	0.0629	no data	no data	UBA
UBA 6	Confidential	Bluegill sunfish	*Lepomis macrochirus*	6.88	3.40×10^{-3}	0.0629	no data	no data	UBA
UBA 10	Confidential	Fathead minnow	*Pimephales promelas*	76.7	3.90×10^{-3}	0.11	no data	no data	UBA
UBA 10	Confidential	Fathead minnow	*Pimephales promelas*	28.9	3.90×10^{-3}	0.11	no data	no data	UBA
UBA 5	Confidential	Rainbow trout	*Oncorhynchus mykiss*	411	1.35×10^{-3}	0.049	no data	no data	UBA
UBA 5	Confidential	Rainbow trout	*Oncorhynchus mykiss*	339	1.35×10^{-3}	0.049	no data	no data	UBA
UBA 13	Confidential	Rainbow trout	*Oncorhynchus mykiss*	37.9	8.50×10^{-4}	no data	no data	no data	UBA
UBA 14	Confidential	Rainbow trout	*Oncorhynchus mykiss*	11.2	7.87×10^{-4}	0.0323	no data	no data	UBA
UBA 14	Confidential	Rainbow trout	*Oncorhynchus mykiss*	11.1	7.87×10^{-4}	0.0323	no data	no data	UBA
UBA 13	Confidential	Rainbow trout	*Oncorhynchus mykiss*	8.66	8.70×10^{-4}	no data	no data	no data	UBA
UBA 9	Confidential	Zebrafish	*Brachydanio rerio*	1,543	2.25×10^{-4}	0.049	no data	no data	UBA
UBA 3	Confidential	Zebrafish	*Brachydanio rerio*	516	3.47×10^{-4}	0.123	no data	no data	UBA
UBA 3	Confidential	Zebrafish	*Brachydanio rerio*	492	3.47×10^{-4}	0.123	no data	no data	UBA
UBA 9	Confidential	Zebrafish	*Brachydanio rerio*	336	2.25×10^{-4}	0.049	no data	no data	UBA

[1]Reference number from the original data set.
[2]The initial fish weight (either reported or estimated where available/possible).
[3]All data provided by UBA. The test reports are confidential.
[4]Data on the temperature and dissolved oxygen concentration were not supplied but should be available in the confidential test report.

Table A.5 Gold standard data set – substance identities

Ref#	CAS No	Name	Log K_{ow}	Molecular weight	Smiles
GS32	120-82-1	1,2,4-Trichlorobenzene	4.02	181.45	c(ccc(c1Cl)Cl)(c1)Cl
GS45	118-74-1	Hexachlorobenzene	5.73	284.78	c(c(c(c(c1Cl)Cl)Cl)Cl)(c1Cl)Cl
GS44	615-54-3	1,2,4-Tribromobenzene	4.66	314.80	c(ccc(c1Br)Br)(c1)Br
GS43	634-90-2	1,2,3,5-Tetrachlorobenzene	4.56	215.89	c(cc(c(c1Cl)Cl)Cl)(c1)Cl
GS42	87-61-6	1,2,3-Trichlorobenzene	4.05	181.45	c(c(c(cc1)Cl)Cl)(c1)Cl
GS41	106-37-6	1,4-Dibromobenzene	3.79	235.91	c(ccc(c1)Br)(c1)Br
GS40	106-46-7	1,4-Dichlorobenzene	3.44	147.00	c(ccc(c1)Cl)(c1)Cl
GS7	2027-17-0	2-Isopropylnaphthalene	4.63	170.26	c(c(ccc1C(C)C)ccc2)(c2)c1
GS8	2027-17-0	2-Isopropylnaphthalene	4.63	170.26	c(c(ccc1C(C)C)ccc2)(c2)c1
GS5	575-41-7	1,3-Dimethylnaphthalene	4.42	156.23	Cc2cc(C)c1ccccc1c2
GS3	91-57-6	2-Methylnaphthalene	3.86	142.20	c(c(ccc1C)ccc2)(c2)c1
GS4	91-57-6	2-Methylnaphthalene	3.86	142.20	c(c(ccc1C)ccc2)(c2)c1
GS6	575-41-7	1,3-Dimethylnaphthalene	4.42	156.23	Cc2cc(C)c1ccccc1c2
GS9	85-01-8	Phenanthrene	4.46	178.24	c(c(c(c(c1)ccc2)c2)ccc3)(c1)c3
GS1	91-20-3	Naphthalene	3.3	128.18	c(c(ccc1)ccc2)(c1)c2
GS2	91-20-3	Naphthalene	3.3	128.18	c(c(ccc1)ccc2)(c1)c2
GS13	3674-75-7	9-Ethylphenanthrene	5.38	206.29	c(ccc1c(ccc2)c3c2)cc1cc3CC
GS10	85-01-8	Phenanthrene	4.46	178.24	c(c(c(c(c1)ccc2)c2)ccc3)(c1)c3
GS11	883-20-5	9-Methylphenanthrene	4.89	192.26	c(ccc1c(ccc2)c3c2)cc1cc3C
GS12	883-20-5	9-Methylphenanthrene	4.89	192.26	c(ccc1c(ccc2)c3c2)cc1cc3C
GS14	3674-75-7	9-Ethylphenanthrene	5.38	206.29	c(ccc1c(ccc2)c3c2)cc1cc3CC
GS16	129-00-0	Pyrene	4.88	202.26	c(c(c(cc1)ccc2)c2cc3)(c1ccc4)c34
GS15	129-00-0	Pyrene	4.88	202.26	c(c(c(cc1)ccc2)c2cc3)(c1ccc4)c34

[1]Reference number from the original data set

Table A.6 Gold standard data set – bioconcentration data

Ref#	Substance	Common name	Scientific name	Experimental data					Reference[3]
				k_1 (l kg^{-1} day^{-1})	Fish weight (kg)[2]	Lipid (kg kg^{-1})	Temperature (°C)	Dissolved oxygen (mg l^{-1})	
GS32	1,2,4-Trichlorobenzene	Guppy	Poecilia reticulata	492	4.8×10^{-4}	not determined	21	not reported in database	van Eck *et al*. (1997)
GS45	Hexachlorobenzene	Mosquito fish	Gambusia affinis	1,850	1.9×10^{-4}	0.031	23.1	not reported in database	Chaisuksant *et al*. (1997)
GS44	1,2,4-Tribromobenzene	Mosquito fish	Gambusia affinis	1,040	1.9×10^{-4}	0.031	23.1	not reported in database	Chaisuksant *et al*. (1997)
GS43	1,2,3,5-Tetrachlorobenzene	Mosquito fish	Gambusia affinis	631	1.9×10^{-4}	0.031	23.1	not reported in database	Chaisuksant *et al*. (1997)
GS42	1,2,3-Trichlorobenzene	Mosquito fish	Gambusia affinis	470	1.9×10^{-4}	0.031	23.1	not reported in database	Chaisuksant *et al*. (1997)
GS41	1,4-Dibromobenzene	Mosquito fish	Gambusia affinis	272	1.9×10^{-4}	0.031	23.1	not reported in database	Chaisuksant *et al*. (1997)
GS40	1,4-Dichlorobenzene	Mosquito fish	Gambusia affinis	112	1.9×10^{-4}	0.031	23.1	not reported in database	Chaisuksant *et al*. (1997)
GS7	2-Isopropylnaphthalene	Sheepshead minnow	Cyprinodon variegatus	4,188	2.47×10^{-3}	0.097	25	not reported in database	Jonsson *et al*. (2004)
GS8	2-Isopropylnaphthalene	Sheepshead minnow	Cyprinodon variegatus	3,746	2.47×10^{-3}	0.097	25	not reported in database	Jonsson *et al*. (2004)
GS5	1,3-Dimethylnaphthalene	Sheepshead minnow	Cyprinodon variegatus	2,909	2.47×10^{-3}	0.097	25	not reported in database	Jonsson *et al*. (2004)
GS3	2-Methylnaphthalene	Sheepshead minnow	Cyprinodon variegatus	2,659	2.47×10^{-3}	0.097	25	not reported in database	Jonsson *et al*. (2004)
GS4	2-Methylnaphthalene	Sheepshead minnow	Cyprinodon variegatus	2,142	2.47×10^{-3}	0.097	25	not reported in database	Jonsson *et al*. (2004)
GS6	1,3-Dimethylnaphthalene	Sheepshead minnow	Cyprinodon variegatus	1,854	2.47×10^{-3}	0.097	25	not reported in database	Jonsson *et al*. (2004)
GS9	Phenanthrene	Sheepshead minnow	Cyprinodon variegatus	1,783	2.47×10^{-3}	0.097	25	not reported in database	Jonsson *et al*. (2004)
GS1	Naphthalene	Sheepshead minnow	Cyprinodon variegatus	1,450	2.47×10^{-3}	0.097	25	not reported in database	Jonsson *et al*. (2004)

Ref#	Substance	Common name	Scientific name	Experimental data					Reference[3]
				k_1 (l kg^{-1} day^{-1})	Fish weight (kg)[2]	Lipid (kg kg^{-1})	Temperature (°C)	Dissolved oxygen (mg l^{-1})	
GS2	Naphthalene	Sheepshead minnow	Cyprinodon variegatus	1,137	2.47×10^{-3}	0.097	25	not reported in database	Jonsson *et al*. (2004)
GS13	9-Ethylphenanthrene	Sheepshead minnow	Cyprinodon variegatus	731	2.47×10^{-3}	0.097	25	not reported in database	Jonsson *et al*. (2004)
GS10	Phenanthrene	Sheepshead minnow	Cyprinodon variegatus	680	2.47×10^{-3}	0.097	25	not reported in database	Jonsson *et al*. (2004)
GS11	9-Methylphenanthrene	Sheepshead minnow	Cyprinodon variegatus	623	2.47×10^{-3}	0.097	25	not reported in database	Jonsson *et al*. (2004)
GS12	9-Methylphenanthrene	Sheepshead minnow	Cyprinodon variegatus	290	2.47×10^{-3}	0.097	25	not reported in database	Jonsson *et al*. (2004)
GS14	9-Ethylphenanthrene	Sheepshead minnow	Cyprinodon variegatus	263	2.47×10^{-3}	0.097	25	not reported in database	Jonsson *et al*. (2004)
GS16	Pyrene	Sheepshead minnow	Cyprinodon variegatus	129	2.47×10^{-3}	0.097	25	not reported in database	Jonsson *et al*. (2004)
GS15	Pyrene	Sheepshead minnow	Cyprinodon variegatus	116	2.47×10^{-3}	0.097	25	not reported in database	Jonsson *et al*. (2004)

[1]Reference number from the original data set.

[2]The initial fish weight.

[3]References for the bioconcentration data are as follows.

CHAISUKSANT Y, YU, Q. AND CONNELL, D.W., 1997. Bioconcentration of bromo- and chlorobenzenes by fish (*Gambusia affinis*). *Water Research*, **31**, 61-68.

JONSSON, G., BECHMANN, R.K., BAMBER, S.D. AND BAUSSANT, T, 2004. Bioconcentration, biotransformation, and elimination of polycyclic aromatic hydrocarbons in sheepshead minnows (*Cyprinodon variegatus*) exposed to contaminated seawater. *Environmental Toxicology and Chemistry*, **23**, 1538-1548.

VAN ECK, J.M.C., KOELMANS, A.A. AND DENEER, J.W., 1997. Uptake and elimination of 1,2,4-trichlorobenzene in the guppy (*Poecilia reticulata*) at sublethal and lethal aqueous concentrations. *Chemosphere*, **34**, 2259-2270.

Table A.7 Arnot data set – predictions (Part A)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Sijm *et al*. (1995)		Hendriks *et al*. (2001)		Campfens and Mackay (1997)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
432	Anthracene	900	649	0.72	655	0.73		
996	Haloxyfop-methyl	720	612	0.85	461	0.64		
16	Benzo[a]pyrene	416	649	1.56	852	2.05		
710	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,480	290	0.08	439	0.13	1,478	0.42
711	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,480	280	0.08	428	0.12	3,360	0.97
458	1,3,5-Triazine, hexahydro-1,3,5-trinitro-(RDX)	30.7	1,149	37.39	1	0.04	5	0.18
726	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	765	219	0.29	366	0.48	6,409	8.38
727	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	736	219	0.30	366	0.50	2,864	3.89
725	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	712	219	0.31	366	0.51	8,747	12.29
729	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,870	520	0.28	720	0.39	14,084	7.53
728	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,280	520	0.41	720	0.56	15,980	12.48
712	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	756	548	0.72	722	0.96	2,436	3.22
29	Benzo[a]anthracene	405	686	1.69	882	2.18	42,260	104.35
2	Octaethylene glycol monotridecyl ether	317	594	1.87	95	0.30	392	1.24

Ref#	Name	$k_{1\ (exp)}$[2]	Sijm *et al*. (1995)		Hendriks *et al*. (2001)		Campfens and Mackay (1997)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
3	Octaethylene glycol monotridecyl ether	317	594	1.87	749	2.36	42,937	135.41
531	Dibenz(a,h)acridine	276	1,191	4.32	1,352	4.91	75,436	273.80
1	C-12-2-LAS	130	578	4.44	669	5.15	1,795	13.81
4	C-12-5-LAS	11.1	578	52.04	669	60.27	3,077	277.22
236	Benzene, 1,2,4,5-tetrachloro-	1,630	401	0.25	491	0.30	1,484	0.91
441	Benzene, 1,2,4-trichloro-	1,160	401	0.35	321	0.28	681	0.59
164	2,4,6-Trichlorophenol	421	401	0.95	212	0.50	2,891	6.87
321	Benzene, 1,4-dichloro-	291	401	1.38	141	0.49	229	0.79
680	2,3,5,6-Tetrachlorophenol	243	401	1.65	274	1.13	1,266	5.21
160	Phenol, pentachloro-	222	401	1.81	552	2.49	17,991	81.04
163	Phenol, pentachloro-	948	520	0.55	676	0.71	9,882	10.42
162	Phenol, pentachloro-	509	435	0.85	588	1.16	24,957	49.05
200	4,4'-dibromobiphenyl	2,140	1,093	0.51	1,267	0.59	4,197	1.96
653	3,4,5-Trichloroaniline	1,970	736	0.37	183	0.09	893	0.45
659	2,4,5-Trichloroaniline	1,630	736	0.45	231	0.14	1,482	0.91
145	Benzene, 1,2,3-trichloro-	1,580	736	0.46	532	0.34	664	0.42
654	2,4,6-Trichloroaniline	1,580	736	0.46	260	0.16	316	0.20
652	2,3,4-Trichloroaniline	1,460	736	0.50	186	0.13	1,406	0.96
984	1,2,3,6,7,8-Hexachlorodibenzofuran	1,310	536	0.41	738	0.56	150,961	115.24
940	2,2',5,5'-Tetrachloro-1,1'-biphenyl	1,120	1,093	0.97	1,273	1.13	729	0.65
986	2,4,6-tribromobiphenyl	1,120	1,093	0.97	1,278	1.14	10,302	9.18
991	2,3,4,6,7,8-Hexachlorodibenzofuran	1,100	536	0.49	738	0.67	199,346	181.06
982	2,3,4,7,8-Pentachlorodibenzofuran	1,010	536	0.53	737	0.73	18,630	18.41
943	2,2',5,5'-Tetrachloro-1,1'-biphenyl	1,000	1,086	1.09	1,266	1.27	764	0.76

Ref#	Name	$k_{1\ (exp)}$ [2]	Sijm *et al*. (1995)		Hendriks *et al*. (2001)		Campfens and Mackay (1997)	
			$k_{1\ (pred)}$ [3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$ [3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$ [3]	$k_{1\ (pred)}/k_{1\ (exp)}$
929	1,2,3,4-Tetrachlorodibenzo-p-dioxin	953	1,172	1.23	1,357	1.42	358,296	375.97
961	1,2,3,7,8-Pentachlorodibenzo-p-dioxin	952	536	0.56	737	0.77	12,703	13.34
987	2,2',5,5'-tetrabromobiphenyl	912	1,093	1.20	1,285	1.41	2,009	2.20
941	2,2',5,5'-Tetrachloro-1,1'-biphenyl	910	1,086	1.19	1,266	1.39	764	0.84
876	2,4',5-Trichloro-1,1'-biphenyl	890	1,172	1.32	1,335	1.50	2,210	2.48
939	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	880	1,086	1.23	1,281	1.46	798	0.91
959	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	868	536	0.62	738	0.85	125,801	144.93
942	2,2',5,5'-Tetrachloro-1,1'-biphenyl	860	1,086	1.26	1,266	1.47	697	0.81
985	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	844	536	0.63	738	0.87	96,770	114.66
938	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	840	1,086	1.29	1,281	1.52	798	0.95
641	Benzene, 1,3,5-tribromo-	708	1,093	1.54	1,015	1.43	877	1.24
888	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	687	536	0.78	738	1.07	143,220	208.47
790	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	630	1,108	1.76	1,188	1.89	3,283	5.21
968	2,3,7,8-Tetrachlorodibenzofuran	603	536	0.89	736	1.22	95,974	159.16

Ref#	Name	$k_{1\ (exp)}$ [2]	Sijm *et al*. (1995)		Hendriks *et al*. (2001)		Campfens and Mackay (1997)	
			$k_{1\ (pred)}$ [3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$ [3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$ [3]	$k_{1\ (pred)}/k_{1\ (exp)}$
960	1,2,4-Trichlorodibenzo[b,e][1,4]dioxin	601	1,172	1.95	1,355	2.25	152,793	254.23
934	2,7-Dichlorodibenzo[b,e][1,4]dioxin	543	1,172	2.16	1,339	2.47	63,263	116.51
937	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	540	1,086	2.01	1,281	2.37	798	1.48
995	1,2,3,4,6,7,8-Heptachlorodibenzofuran	524	536	1.02	738	1.41	309,439	590.53
730	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	500	536	1.07	737	1.47	29,989	59.98
945	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	456	536	1.18	738	1.62	495,743	1,087.16
871	2,4,5-Trichloro-1,1'-biphenyl	380	1,093	2.88	1,261	3.32	1,633	4.29
988	2,2',4,4',6,6'-hexabromobiphenyl	324	1,093	3.38	1,288	3.98	7,293	22.54
808	1,2,3,4,5,6,7,8-Octachlorodibenzo-p-dioxin	275	536	1.95	738	2.68	1,829,442	6,652.52
958	1,2,3,4,5,6,7,8-Octachlorodibenzofuran	217	536	2.47	738	3.40	2,674,982	12,327.11
316	Benzene, 1,4-dibromo-	129	1,093	8.49	534	4.14	566	4.39
765	Mirex	93.3	1,093	11.72	1,287	13.79	2,306	24.71
746	Decachlorobiphenyl	41.7	1,093	26.23	1,288	30.91	49,308	1,182.81
326	Benzenamine, 4-chloro-	689	800	1.16	8	0.01	320	0.46
54	Benzenamine	250	773	3.10	1	0.004	59	0.24
706	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,140	900	0.29	1,064	0.34	11,268	3.58

Ref#	Name	$k_{1\ (exp)}$ [2]	Sijm *et al*. (1995)		Hendriks *et al*. (2001)		Campfens and Mackay (1997)	
			$k_{1\ (pred)}$ [3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$ [3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$ [3]	$k_{1\ (pred)}/k_{1\ (exp)}$
731	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,850	709	0.38	917	0.50	14,234	7.69
707	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	1,630	332	0.20	488	0.30	7,500	4.61
399	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	1,550	370	0.24	553	0.36		
708	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	538	126	0.23	229	0.43	1,920	3.57
398	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	386	139	0.36	258	0.67		
161	Phenol, pentachloro-	341	319	0.94	462	1.35	9,857	28.92
992	cis-Permethrin	201	457	2.27	651	3.24	966,369	4810.68
970	Cyano(3-phenoxyphenyl)methyl ester, 4-Chloro-alpha-(1-methylethyl)benzeneacetic acid	157	457	2.91	648	4.13	28,300	180.58
158	Phenol, pentachloro-	120	63	0.53	130	1.09	5,537	46.14
159	Phenol, pentachloro-	118	63	0.54	130	1.11	2,436	20.72
971	[1R-[1 alpha(S*),3 alpha]]Cyano(3-phenoxyphenyl)methyl ester 3-(2,2-dibromoethenyl)-2,2-dimethyl cyclopropane carboxylic acid	105	457	4.35	648	6.18	41,385	394.59

Ref#	Name	$k_{1\ (exp)}$[2]	Sijm *et al*. (1995)		Hendriks *et al*. (2001)		Campfens and Mackay (1997)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
994	[1 alpha(S*), 3 alpha]-(+-)-3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropane carboxylic acid cyano (3-phenoxyphenyl)methyl ester	59.3	457	7.70	649	10.95	41,452	699.25
393	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	672	417	0.62	606	0.90		
392	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	317	417	1.31	606	1.91		
418	Benzene, 2-methyl-1,3,5-trinitro-	200	869	4.34	5	0.02	47	0.23
459	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	3.6	938	260.69	1	0.27	1	0.21
788	Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine (HMX)	1.4	902	626.22	0.2	0.14	0	0.15
795	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	1,380	747	0.54	874	0.63	5,945	4.31
111	Phenol, 4,4 -(1-methylethylidene)bis-	5.5	649	117.60	166	30.02	1,516	274.66

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.

[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.

[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.8 Arnot data set – predictions (Part B)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Arnot and Gobas (2003)		Arnot and Gobas (2004)		Thomann (1989)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
432	Anthracene	900	2,084	2.32	1,454	1.62	413	0.46
996	Haloxyfop-methyl	720	1,927	2.68	1,175	1.63	236	0.33
16	Benzo[a]pyrene	416	2,091	5.03	1,458	3.51	670	1.61
710	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,480	764	0.22	598	0.17	1,456	0.42
711	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,480	732	0.21	491	0.14	1,274	0.37
458	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	30.7	295	9.59	205	6.68	4	0.13
726	2,3,7,8-Tetrachlorodibenzo [b,e][1,4]dioxin	765	536	0.70	456	0.60	746	0.97
727	2,3,7,8-Tetrachlorodibenzo[b,e] [1,4]dioxin	736	536	0.73	456	0.62	746	1.01
725	2,3,7,8-Tetrachlorodibenzo[b,e] [1,4]dioxin	712	536	0.75	456	0.64	746	1.05
729	2,3,7,8-Tetrachlorodibenzo[b,e] [1,4]dioxin	1,870	1,585	0.85	1,176	0.63	379	0.20
728	2,3,7,8-Tetrachlorodibenzo[b,e] [1,4]dioxin	1,280	1,585	1.24	1,176	0.92	379	0.30
712	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	756	1,691	2.24	1,124	1.49	832	1.10
29	Benzo[a]anthracene	405	2,242	5.54	1,456	3.59	705	1.74
2	Octaethylene glycol monotridecyl ether	317	1,725	5.44	1,198	3.78	88	0.28

Ref#	Name	$k_{1\ (exp)}$[2]	Arnot and Gobas (2003)		Arnot and Gobas (2004)		Thomann (1989)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
3	Octaethylene glycol monotridecyl ether	317	1,870	5.90	1,283	4.05	815	2.57
531	Dibenz(a,h)acridine	276	4,466	16.21	2,748	9.97	473	1.72
1	C-12-2-LAS	130	1,804	13.88	1,216	9.35	580	4.46
4	C-12-5-LAS	11.1	1,804	162.52	1,216	109.56	580	52.22
236	Benzene, 1,2,4,5-tetrachloro-	1,630	1,143	0.70	884	0.54	770	0.47
441	Benzene, 1,2,4-trichloro-	1,160	1,135	0.98	878	0.76	377	0.33
164	2,4,6-Trichlorophenol	421	1,123	2.67	871	2.07	258	0.61
321	Benzene, 1,4-dichloro-	291	1,106	3.80	859	2.95	193	0.66
680	2,3,5,6-Tetrachlorophenol	243	1,131	4.65	876	3.60	321	1.32
160	Phenol, pentachloro-	222	1,145	5.16	885	3.99	1,174	5.29
163	Phenol, pentachloro-	948	1,584	1.67	1,061	1.12	865	0.91
162	Phenol, pentachloro-	509	1,266	2.49	872	1.71	995	1.96
200	4,4'-dibromobiphenyl	2,140	4,013	1.88	2,393	1.12	484	0.23
653	3,4,5-Trichloroaniline	1,970	2,337	1.19	1,610	0.82	102	0.05
659	2,4,5-Trichloroaniline	1,630	2,365	1.45	1,626	1.00	118	0.07
145	Benzene, 1,2,3-trichloro-	1,580	2,427	1.53	1,662	1.05	236	0.15
654	2,4,6-Trichloroaniline	1,580	2,377	1.50	1,633	1.03	128	0.08
652	2,3,4-Trichloroaniline	1,460	2,339	1.60	1,611	1.10	103	0.07
984	1,2,3,6,7,8-Hexachlorodibenzofuran	1,310	1,646	1.26	1,215	0.93	208	0.16
940	2,2',5,5'-Tetrachloro-1,1'-biphenyl	1,120	4,013	3.58	2,393	2.13	484	0.43
986	2,4,6-tribromobiphenyl	1,120	4,013	3.58	2,393	2.13	464	0.41
991	2,3,4,6,7,8-Hexachlorodibenzofuran	1,100	1,646	1.49	1,215	1.10	208	0.19
982	2,3,4,7,8-Pentachlorodibenzofuran	1,010	1,646	1.63	1,215	1.20	445	0.44
943	2,2',5,5'-Tetrachloro-1,1'-biphenyl	1,000	3,980	3.98	6,336	6.34	1,297	1.30

Ref#	Name	$k_{1\ (exp)}$[2]	Arnot and Gobas (2003)		Arnot and Gobas (2004)		Thomann (1989)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
929	1,2,3,4-Tetrachlorodibenzo-p-dioxin	953	4,375	4.59	2,580	2.71	228	0.24
961	1,2,3,7,8-Pentachlorodibenzo-p-dioxin	952	1,646	1.73	1,215	1.28	445	0.47
987	2,2',5,5'-tetrabromobiphenyl	912	4,013	4.40	2,393	2.62	270	0.30
941	2,2',5,5'-Tetrachloro-1,1'-biphenyl	910	3,980	4.37	2,715	2.98	556	0.61
876	2,4',5-Trichloro-1,1'-biphenyl	890	4,374	4.91	2,580	2.90	459	0.52
939	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	880	3,981	4.52	6,336	7.20	447	0.51
959	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	868	1,646	1.90	1,215	1.40	208	0.24
942	2,2',5,5'-Tetrachloro-1,1'-biphenyl	860	3,980	4.63	3,801	4.42	778	0.91
985	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	844	1,646	1.95	1,215	1.44	208	0.25
938	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	840	3,981	4.74	2,716	3.23	191	0.23
641	Benzene, 1,3,5-tribromo-	708	4,001	5.65	2,387	3.37	273	0.39
888	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	687	1,646	2.40	1,215	1.77	208	0.30
790	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	630	4,076	6.47	2,537	4.03	475	0.75
968	2,3,7,8-Tetrachlorodibenzofuran	603	1,646	2.73	1,215	2.02	505	0.84

Ref#	Name	$k_{1\ (exp)}$[2]	Arnot and Gobas (2003)		Arnot and Gobas (2004)		Thomann (1989)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
960	1,2,4-Trichlorodibenzo[b,e][1,4]dioxin	601	4,375	7.28	2,580	4.29	304	0.51
934	2,7-Dichlorodibenzo[b,e][1,4]dioxin	543	4,374	8.06	2,580	4.75	459	0.84
937	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	540	3,981	7.37	3,802	7.04	268	0.50
995	1,2,3,4,6,7,8-Heptachlorodibenzofuran	524	1,646	3.14	1,215	2.32	185	0.35
730	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	500	1,646	3.29	1,215	2.43	370	0.74
945	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	456	1,646	3.61	1,215	2.67	117	0.26
871	2,4,5-Trichloro-1,1'-biphenyl	380	4,012	10.55	2,392	6.29	484	1.27
988	2,2',4,4',6,6'-hexabromobiphenyl	324	4,013	12.40	2,393	7.39	121	0.37
808	1,2,3,4,5,6,7,8-Octachlorodibenzo-p-dioxin	275	1,646	5.98	1,215	4.42	74	0.27
958	1,2,3,4,5,6,7,8-Octachlorodibenzofuran	217	1,646	7.58	1,215	5.60	74	0.34
316	Benzene, 1,4-dibromo-	129	3,949	30.66	2,361	18.33	119	0.93
765	Mirex	93.3	4,013	43.00	2,393	25.64	172	1.85
746	Decachlorobiphenyl	41.7	4,013	96.27	2,393	57.40	39	0.94
326	Benzenamine, 4-chloro-	689	1,096	1.59	842	1.22	18	0.03
54	Benzenamine	250	191	0.77	157	0.63	6	0.02
706	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,140	3,146	1.00	1,582	0.50	461	0.15

Ref#	Name	$k_{1\ (exp)}$[2]	Arnot and Gobas (2003)		Arnot and Gobas (2004)		Thomann (1989)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
731	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,850	2,334	1.26	1,489	0.80	268	0.14
707	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	1,630	904	0.56	531	0.33	1,005	0.62
399	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	1,550	1,037	0.67	599	0.39	125	0.08
708	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	538	270	0.50	184	0.34	2,140	3.98
398	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	386	306	0.79	206	0.53	268	0.69
161	Phenol, pentachloro-	341	860	2.52	508	1.49	1,034	3.03
992	cis-Permethrin	201	1,348	6.71	713	3.55	142	0.71
970	Cyano(3-phenoxyphenyl)methyl ester, 4-Chloro-alpha-(1-methylethyl)benzeneacetic acid	157	1,348	8.60	713	4.55	585	3.73
158	Phenol, pentachloro-	120	114	0.95	81	0.67	3,418	28.48
159	Phenol, pentachloro-	118	114	0.97	81	0.69	3,418	29.06
971	[1R-[1 alpha(S*),3 alpha]]Cyano(3-phenoxyphenyl)methyl ester 3-(2,2-dibromoethenyl)-2,2-dimethyl cyclopropane carboxylic acid	105	1,348	12.85	713	6.80	585	5.58

Ref#	Name	$k_{1\ (exp)}$[2]	Arnot and Gobas (2003)		Arnot and Gobas (2004)		Thomann (1989)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
994	[1 alpha(S*), 3 alpha]-(+-)-3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropane carboxylic acid cyano (3-phenoxyphenyl)methyl ester	59.3	1,348	22.73	713	12.03	475	8.02
393	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	672	1,201	1.79	981	1.46	164	0.24
392	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	317	1,201	3.79	888	2.80	149	0.47
418	Benzene, 2-methyl-1,3,5-trinitro-	200	857	4.28	685	3.42	13	0.07
459	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	3.6	229	63.56	188	52.24	5	1.48
788	Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine (HMX)	1.4	48	33.41	40	27.92	3	1.75
795	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	1,380	2,491	1.81	1,502	1.09	588	0.43
111	Phenol, 4,4 -(1-methylethylidene)bis-	5.5	1,996	361.55	1,491	270.05	120	21.68

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.9 Arnot data set – predictions (Part C)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Barber (2001)		Barber *et al*. (1991)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
432	Anthracene	900	775	0.86	772	0.86
996	Haloxyfop-methyl	720	752	1.04	747	1.04
16	Benzo[a]pyrene	416	775	1.86	772	1.86
710	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,480	517	0.15	488	0.14
711	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,480	508	0.15	479	0.14
458	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	30.7	1,033	33.61	1,068	34.77
726	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	765	448	0.59	416	0.54
727	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	736	448	0.61	416	0.56
725	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	712	448	0.63	416	0.58
729	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,870	693	0.37	681	0.36
728	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,280	693	0.54	681	0.53
712	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	756	711	0.94	701	0.93
29	Benzo[a]anthracene	405	797	1.97	797	1.97
2	Octaethylene glycol monotridecyl ether	317	741	2.34	734	2.31
3	Octaethylene glycol monotridecyl ether	317	741	2.34	734	2.31
531	Dibenz(a,h)acridine	276	1,052	3.82	1,091	3.96
1	C-12-2-LAS	130	731	5.62	723	5.56
4	C-12-5-LAS	11.1	731	65.82	723	65.09
236	Benzene, 1,2,4,5-tetrachloro-	1,630	608	0.37	587	0.36
441	Benzene, 1,2,4-trichloro-	1,160	608	0.53	587	0.51

Ref#	Name	$k_{1\ (exp)}$[2]	Barber (2001)		Barber *et al*. (1991)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
164	2,4,6-Trichlorophenol	421	608	1.44	587	1.39
321	Benzene, 1,4-dichloro-	291	608	2.09	587	2.02
680	2,3,5,6-Tetrachlorophenol	243	608	2.50	587	2.42
160	Phenol, pentachloro-	222	608	2.74	587	2.65
163	Phenol, pentachloro-	948	693	0.73	681	0.72
162	Phenol, pentachloro-	509	633	1.24	615	1.21
200	4,4'-dibromobiphenyl	2,140	1,007	0.47	1,039	0.49
653	3,4,5-Trichloroaniline	1,970	826	0.42	830	0.42
659	2,4,5-Trichloroaniline	1,630	826	0.51	830	0.51
145	Benzene, 1,2,3-trichloro-	1,580	826	0.52	830	0.52
654	2,4,6-Trichloroaniline	1,580	826	0.52	830	0.52
652	2,3,4-Trichloroaniline	1,460	826	0.56	830	0.57
984	1,2,3,6,7,8-Hexachlorodibenzofuran	1,310	704	0.54	692	0.53
940	2,2',5,5'-Tetrachloro-1,1'-biphenyl	1,120	1,007	0.90	1,039	0.93
986	2,4,6-tribromobiphenyl	1,120	1,007	0.90	1,039	0.93
991	2,3,4,6,7,8-Hexachlorodibenzofuran	1,100	704	0.64	692	0.63
982	2,3,4,7,8-Pentachlorodibenzofuran	1,010	704	0.70	692	0.68
943	2,2',5,5'-Tetrachloro-1,1'-biphenyl	1,000	1,004	1.00	1,035	1.03
929	1,2,3,4-Tetrachlorodibenzo-p-dioxin	953	1,043	1.09	1,080	1.13
961	1,2,3,7,8-Pentachlorodibenzo-p-dioxin	952	704	0.74	692	0.73
987	2,2',5,5'-tetrabromobiphenyl	912	1,007	1.10	1,039	1.14
941	2,2',5,5'-Tetrachloro-1,1'-biphenyl	910	1,004	1.10	1,035	1.14
876	2,4',5-Trichloro-1,1'-biphenyl	890	1,043	1.17	1,080	1.21
939	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	880	1,004	1.14	1,035	1.18
959	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	868	704	0.81	692	0.80

Ref#	Name	$k_{1\ (exp)}$[2]	Barber (2001)		Barber *et al*. (1991)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
942	2,2',5,5'-Tetrachloro-1,1'-biphenyl	860	1,004	1.17	1,035	1.20
985	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	844	704	0.83	692	0.82
938	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	840	1,004	1.20	1,035	1.23
641	Benzene, 1,3,5-tribromo-	708	1,007	1.42	1,039	1.47
888	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	687	704	1.02	692	1.01
790	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	630	1,014	1.61	1,047	1.66
968	2,3,7,8-Tetrachlorodibenzofuran	603	704	1.17	692	1.15
960	1,2,4-Trichlorodibenzo[b,e][1,4]dioxin	601	1,043	1.74	1,080	1.80
934	2,7-Dichlorodibenzo[b,e][1,4]dioxin	543	1,043	1.92	1,080	1.99
937	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	540	1,004	1.86	1,035	1.92
995	1,2,3,4,6,7,8-Heptachlorodibenzofuran	524	704	1.34	692	1.32
730	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	500	704	1.41	692	1.38
945	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	456	704	1.54	692	1.52
871	2,4,5-Trichloro-1,1'-biphenyl	380	1,007	2.65	1,039	2.73
988	2,2',4,4',6,6'-hexabromobiphenyl	324	1,007	3.11	1,039	3.21
808	1,2,3,4,5,6,7,8-Octachlorodibenzo-p-dioxin	275	704	2.56	692	2.52
958	1,2,3,4,5,6,7,8-Octachlorodibenzofuran	217	704	3.24	692	3.19
316	Benzene, 1,4-dibromo-	129	1,007	7.82	1,039	8.06
765	Mirex	93.3	1,007	10.79	1,039	11.13
746	Decachlorobiphenyl	41.7	1,007	24.16	1,039	24.92

Ref#	Name	$k_{1\ (exp)}$[2]	Barber (2001)		Barber *et al*. (1991)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
326	Benzenamine, 4-chloro-	689	861	1.25	870	1.26
54	Benzenamine	250	846	3.39	853	3.42
706	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,140	913	0.29	930	0.30
731	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,850	810	0.44	812	0.44
707	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	1,630	553	0.34	527	0.32
399	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	1,550	584	0.38	561	0.36
708	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	538	340	0.63	304	0.57
398	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	386	357	0.92	322	0.83
161	Phenol, pentachloro-	341	542	1.59	516	1.51
992	cis-Permethrin	201	649	3.23	632	3.15
970	Cyano(3-phenoxyphenyl)methyl ester, 4-Chloro-alpha-(1-methylethyl)benzeneacetic acid	157	649	4.14	632	4.03
158	Phenol, pentachloro-	120	240	2.00	205	1.71
159	Phenol, pentachloro-	118	240	2.04	205	1.75
971	[1R-[1 alpha(S*),3 alpha]]Cyano(3-phenoxyphenyl)methyl ester 3-(2,2-dibromoethenyl)-2,2-dimethyl cyclopropane carboxylic acid	105	649	6.19	632	6.03
994	[1 alpha(S*), 3 alpha]-(+-)-3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropane carboxylic acid cyano (3-phenoxyphenyl)methyl ester	59.3	649	10.95	632	10.66

Ref#	Name	$k_{1\ (exp)}$[2]	Barber (2001)		Barber *et al*. (1991)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
393	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	672	620	0.92	600	0.89
392	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	317	620	1.96	600	1.89
418	Benzene, 2-methyl-1,3,5-trinitro-	200	897	4.48	911	4.55
459	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	3.6	933	259.08	952	264.51
788	Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine (HMX)	1.4	914	634.82	931	646.43
795	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	1,380	832	0.60	837	0.61
111	Phenol, 4,4 -(1-methylethylidene)bis-	5.5	775	140.36	772	139.88

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.10 Arnot data set – predictions (Part D)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Erickson and McKim (1990a)		Erickson and McKim (1990b)		Gobas and Mackay (1987)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
432	Anthracene	900	750	0.83	667	0.74	1,649	1.83
996	Haloxyfop-methyl	720	729	1.01	640	0.89	1,535	2.13
16	Benzo[a]pyrene	416	750	1.80	667	1.61	1,649	3.97
710	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,480	505	0.15	376	0.11	612	0.18
711	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,480	497	0.14	367	0.11	587	0.17
458	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	30.7	992	32.30	1,002	32.62	3,331	108.42
726	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	765	440	0.57	307	0.40	432	0.56
727	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	736	440	0.60	307	0.42	432	0.59
725	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	712	440	0.62	307	0.43	432	0.61
729	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,870	672	0.36	570	0.30	1,255	0.67
728	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,280	672	0.53	570	0.45	1,255	0.98
712	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	756	690	0.91	591	0.78	1,338	1.77
29	Benzo[a]anthracene	405	771	1.90	694	1.71	1,767	4.36
2	Octaethylene glycol monotridecyl ether	317	718	2.26	626	1.97	1,478	4.66

Ref#	Name	$k_{1\ (exp)}$[2]	Erickson and McKim (1990a)		Erickson and McKim (1990b)		Gobas and Mackay (1987)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
3	Octaethylene glycol monotridecyl ether	317	718	2.26	626	1.97	1,478	4.66
531	Dibenz(a,h)acridine	276	1,010	3.67	1,028	3.73	3,483	12.64
1	C-12-2-LAS	130	708	5.45	614	4.72	1,429	10.99
4	C-12-5-LAS	11.1	708	63.79	614	55.31	1,429	128.70
236	Benzene, 1,2,4,5-tetrachloro-	1,630	592	0.36	473	0.29	912	0.56
441	Benzene, 1,2,4-trichloro-	1,160	592	0.51	473	0.41	912	0.79
164	2,4,6-Trichlorophenol	421	592	1.41	473	1.12	912	2.17
321	Benzene, 1,4-dichloro-	291	592	2.03	473	1.63	912	3.13
680	2,3,5,6-Tetrachlorophenol	243	592	2.44	473	1.95	912	3.75
160	Phenol, pentachloro-	222	592	2.67	473	2.13	912	4.11
163	Phenol, pentachloro-	948	672	0.71	570	0.60	1,255	1.32
162	Phenol, pentachloro-	509	616	1.21	501	0.99	1,007	1.98
200	4,4'-dibromobiphenyl	2,140	968	0.45	967	0.45	3,134	1.47
653	3,4,5-Trichloroaniline	1,970	798	0.41	730	0.37	1,927	0.98
659	2,4,5-Trichloroaniline	1,630	798	0.49	730	0.45	1,927	1.18
145	Benzene, 1,2,3-trichloro-	1,580	798	0.50	730	0.46	1,927	1.22
654	2,4,6-Trichloroaniline	1,580	798	0.50	730	0.46	1,927	1.22
652	2,3,4-Trichloroaniline	1,460	798	0.54	730	0.50	1,927	1.32
984	1,2,3,6,7,8-Hexachlorodibenzofuran	1,310	683	0.52	582	0.44	1,303	0.99
940	2,2',5,5'-Tetrachloro-1,1'-biphenyl	1,120	968	0.86	967	0.86	3,134	2.79
986	2,4,6-tribromobiphenyl	1,120	968	0.86	967	0.86	3,134	2.79
991	2,3,4,6,7,8-Hexachlorodibenzofuran	1,100	683	0.62	582	0.53	1,303	1.18
982	2,3,4,7,8-Pentachlorodibenzofuran	1,010	683	0.67	582	0.58	1,303	1.29
943	2,2',5,5'-Tetrachloro-1,1'-biphenyl	1,000	965	0.97	963	0.96	3,110	3.11

Ref#	Name	$k_{1\ (exp)}$[2]	Erickson and McKim (1990a)		Erickson and McKim (1990b)		Gobas and Mackay (1987)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
929	1,2,3,4-Tetrachlorodibenzo-p-dioxin	953	1,002	1.05	1,016	1.07	3,412	3.58
961	1,2,3,7,8-Pentachlorodibenzo-p-dioxin	952	683	0.72	582	0.61	1,303	1.37
987	2,2',5,5'-tetrabromobiphenyl	912	968	1.06	967	1.06	3,134	3.44
941	2,2',5,5'-Tetrachloro-1,1'-biphenyl	910	965	1.06	963	1.06	3,110	3.42
876	2,4',5-Trichloro-1,1'-biphenyl	890	1,002	1.13	1,016	1.14	3,412	3.83
939	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	880	965	1.10	963	1.09	3,110	3.53
959	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	868	683	0.79	582	0.67	1,303	1.50
942	2,2',5,5'-Tetrachloro-1,1'-biphenyl	860	965	1.12	963	1.12	3,110	3.62
985	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	844	683	0.81	582	0.69	1,303	1.54
938	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	840	965	1.15	963	1.15	3,110	3.70
641	Benzene, 1,3,5-tribromo-	708	968	1.37	967	1.37	3,134	4.43
888	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	687	683	0.99	582	0.85	1,303	1.90
790	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	630	975	1.55	977	1.55	3,186	5.06
968	2,3,7,8-Tetrachlorodibenzofuran	603	683	1.13	582	0.97	1,303	2.16

Ref#	Name	$k_{1\ (exp)}$[2]	Erickson and McKim (1990a)		Erickson and McKim (1990b)		Gobas and Mackay (1987)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
960	1,2,4-Trichlorodibenzo[b,e][1,4]dioxin	601	1,002	1.67	1,016	1.69	3,412	5.68
934	2,7-Dichlorodibenzo[b,e][1,4]dioxin	543	1,002	1.84	1,016	1.87	3,412	6.28
937	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	540	965	1.79	963	1.78	3,110	5.76
995	1,2,3,4,6,7,8-Heptachlorodibenzofuran	524	683	1.30	582	1.11	1,303	2.49
730	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	500	683	1.37	582	1.16	1,303	2.61
945	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	456	683	1.50	582	1.28	1,303	2.86
871	2,4,5-Trichloro-1,1'-biphenyl	380	968	2.55	967	2.54	3,134	8.24
988	2,2',4,4',6,6'-hexabromobiphenyl	324	968	2.99	967	2.99	3,134	9.69
808	1,2,3,4,5,6,7,8-Octachlorodibenzo-p-dioxin	275	683	2.48	582	2.12	1,303	4.74
958	1,2,3,4,5,6,7,8-Octachlorodibenzofuran	217	683	3.15	582	2.68	1,303	6.00
316	Benzene, 1,4-dibromo-	129	968	7.52	967	7.51	3,134	24.33
765	Mirex	93.3	968	10.38	967	10.37	3,134	33.59
746	Decachlorobiphenyl	41.7	968	23.23	967	23.21	3,134	75.19
326	Benzenamine, 4-chloro-	689	831	1.21	774	1.12	2,134	3.10
54	Benzenamine	250	817	3.27	755	3.03	2,044	8.19
706	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,140	880	0.28	842	0.27	2,467	0.78

Ref#	Name	$k_{1\ (exp)}$[2]	Erickson and McKim (1990a)		Erickson and McKim (1990b)		Gobas and Mackay (1987)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
731	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,850	783	0.42	710	0.38	1,838	0.99
707	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	1,630	539	0.33	414	0.25	722	0.44
399	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	1,550	569	0.37	447	0.29	826	0.53
708	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	538	336	0.62	208	0.39	219	0.41
398	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	386	352	0.91	223	0.58	248	0.64
161	Phenol, pentachloro-	341	529	1.55	402	1.18	688	2.02
992	cis-Permethrin	201	631	3.14	519	2.59	1,070	5.33
970	Cyano(3-phenoxyphenyl)methyl ester, 4-Chloro-alpha-(1-methylethyl)benzeneacetic acid	157	631	4.03	519	3.31	1,070	6.83
158	Phenol, pentachloro-	120	239	1.99	127	1.06	94	0.78
159	Phenol, pentachloro-	118	239	2.03	127	1.08	94	0.80
971	[1R-[1 alpha(S*),3 alpha]]Cyano(3-phenoxyphenyl)methyl ester 3-(2,2-dibromoethenyl)-2,2-dimethyl cyclopropane carboxylic acid	105	631	6.02	519	4.95	1,070	10.20

Ref#	Name	$k_{1\ (exp)}$[2]	Erickson and McKim (1990a)		Erickson and McKim (1990b)		Gobas and Mackay (1987)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
994	[1 alpha(S*), 3 alpha]-(+-)-3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropane carboxylic acid cyano (3-phenoxyphenyl)methyl ester	59.3	631	10.64	519	8.76	1,070	18.05
393	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	672	603	0.90	486	0.72	955	1.42
392	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	317	603	1.90	486	1.53	955	3.01
418	Benzene, 2-methyl-1,3,5-trinitro-	200	865	4.32	821	4.10	2,362	11.80
459	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	3.6	898	249.57	868	240.99	2,597	721.30
788	Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine (HMX)	1.4	881	611.83	843	585.58	2,472	1716.74
795	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	1,380	803	0.58	738	0.54	1,962	1.42
111	Phenol, 4,4 -(1-methylethylidene)bis-	5.5	750	135.84	667	120.86	1,649	298.78

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.11 Arnot data set – predictions (Part E)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Gobas *et al*. (1986)		Hayton and Barron (1990)		Norstrom *et al*. (1976)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
432	Anthracene	900	3,725	4.14	577	0.64	165	0.18
996	Haloxyfop-methyl	720	3,515	4.88	557	0.77	161	0.22
16	Benzo[a]pyrene	416	3,725	8.96	577	1.39	165	0.40
710	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,480	1,677	0.48	352	0.10	120	0.03
711	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,480	1,622	0.47	345	0.10	118	0.03
458	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	30.7	6,556	213.42	819	26.64	206	6.71
726	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	765	1,267	1.66	296	0.39	107	0.14
727	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	736	1,267	1.72	296	0.40	107	0.15
725	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	712	1,267	1.78	296	0.42	107	0.15
729	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,870	2,990	1.60	504	0.27	151	0.08
728	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,280	2,990	2.34	504	0.39	151	0.12
712	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	756	3,148	4.16	520	0.69	154	0.20
29	Benzo[a]anthracene	405	3,936	9.72	597	1.47	168	0.42
2	Octaethylene glycol monotridecyl ether	317	3,411	10.76	546	1.72	159	0.50

Ref#	Name	$k_{1\ (exp)}$[2]	Gobas *et al*. (1986)		Hayton and Barron (1990)		Norstrom *et al*. (1976)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
3	Octaethylene glycol monotridecyl ether	317	3,411	10.76	546	1.72	159	0.50
531	Dibenz(a,h)acridine	276	6,796	24.67	837	3.04	209	0.76
1	C-12-2-LAS	130	3,318	25.52	537	4.13	157	1.21
4	C-12-5-LAS	11.1	3,318	298.92	537	48.40	157	14.17
236	Benzene, 1,2,4,5-tetrachloro-	1,630	2,312	1.42	430	0.26	136	0.08
441	Benzene, 1,2,4-trichloro-	1,160	2,312	2.00	430	0.37	136	0.12
164	2,4,6-Trichlorophenol	421	2,312	5.49	430	1.02	136	0.32
321	Benzene, 1,4-dichloro-	291	2,312	7.95	430	1.48	136	0.47
680	2,3,5,6-Tetrachlorophenol	243	2,312	9.52	430	1.77	136	0.56
160	Phenol, pentachloro-	222	2,312	10.42	430	1.94	136	0.61
163	Phenol, pentachloro-	948	2,990	3.15	504	0.53	151	0.16
162	Phenol, pentachloro-	509	2,504	4.92	451	0.89	141	0.28
200	4,4'-dibromobiphenyl	2,140	6,244	2.92	794	0.37	202	0.09
653	3,4,5-Trichloroaniline	1,970	4,221	2.14	623	0.32	173	0.09
659	2,4,5-Trichloroaniline	1,630	4,221	2.59	623	0.38	173	0.11
145	Benzene, 1,2,3-trichloro-	1,580	4,221	2.66	623	0.39	173	0.11
654	2,4,6-Trichloroaniline	1,580	4,221	2.66	623	0.39	173	0.11
652	2,3,4-Trichloroaniline	1,460	4,221	2.88	623	0.43	173	0.12
984	1,2,3,6,7,8-Hexachlorodibenzofuran	1,310	3,081	2.35	513	0.39	153	0.12
940	2,2',5,5'-Tetrachloro-1,1'-biphenyl	1,120	6,244	5.56	794	0.71	202	0.18
986	2,4,6-tribromobiphenyl	1,120	6,244	5.56	794	0.71	202	0.18
991	2,3,4,6,7,8-Hexachlorodibenzofuran	1,100	3,081	2.80	513	0.47	153	0.14
982	2,3,4,7,8-Pentachlorodibenzofuran	1,010	3,081	3.04	513	0.51	153	0.15
943	2,2',5,5'-Tetrachloro-1,1'-biphenyl	1,000	6,204	6.20	791	0.79	202	0.20

Ref#	Name	$k_{1\ (exp)}$[2]	Gobas *et al*. (1986)		Hayton and Barron (1990)		Norstrom *et al*. (1976)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
929	1,2,3,4-Tetrachlorodibenzo-p-dioxin	953	6,685	7.01	828	0.87	208	0.22
961	1,2,3,7,8-Pentachlorodibenzo-p-dioxin	952	3,081	3.24	513	0.54	153	0.16
987	2,2',5,5'-tetrabromobiphenyl	912	6,244	6.85	794	0.87	202	0.22
941	2,2',5,5'-Tetrachloro-1,1'-biphenyl	910	6,204	6.82	791	0.87	202	0.22
876	2,4',5-Trichloro-1,1'-biphenyl	890	6,685	7.51	828	0.93	208	0.23
939	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	880	6,204	7.05	791	0.90	202	0.23
959	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	868	3,081	3.55	513	0.59	153	0.18
942	2,2',5,5'-Tetrachloro-1,1'-biphenyl	860	6,204	7.21	791	0.92	202	0.23
985	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	844	3,081	3.65	513	0.61	153	0.18
938	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	840	6,204	7.39	791	0.94	202	0.24
641	Benzene, 1,3,5-tribromo-	708	6,244	8.82	794	1.12	202	0.29
888	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	687	3,081	4.48	513	0.75	153	0.22
790	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	630	6,327	10.04	801	1.27	203	0.32
968	2,3,7,8-Tetrachlorodibenzofuran	603	3,081	5.11	513	0.85	153	0.25

Ref#	Name	$k_{1\ (exp)}$[2]	Gobas *et al*. (1986)		Hayton and Barron (1990)		Norstrom *et al*. (1976)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
960	1,2,4-Trichlorodibenzo[b,e][1,4]dioxin	601	6,685	11.12	828	1.38	208	0.35
934	2,7-Dichlorodibenzo[b,e][1,4]dioxin	543	6,685	12.31	828	1.53	208	0.38
937	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	540	6,204	11.49	791	1.46	202	0.37
995	1,2,3,4,6,7,8-Heptachlorodibenzofuran	524	3,081	5.88	513	0.98	153	0.29
730	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	500	3,081	6.16	513	1.03	153	0.31
945	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	456	3,081	6.76	513	1.13	153	0.34
871	2,4,5-Trichloro-1,1'-biphenyl	380	6,244	16.42	794	2.09	202	0.53
988	2,2',4,4',6,6'-hexabromobiphenyl	324	6,244	19.29	794	2.45	202	0.63
808	1,2,3,4,5,6,7,8-Octachlorodibenzo-p-dioxin	275	3,081	11.20	513	1.87	153	0.56
958	1,2,3,4,5,6,7,8-Octachlorodibenzofuran	217	3,081	14.20	513	2.36	153	0.70
316	Benzene, 1,4-dibromo-	129	6,244	48.47	794	6.16	202	1.57
765	Mirex	93.3	6,244	66.90	794	8.51	202	2.17
746	Decachlorobiphenyl	41.7	6,244	149.78	794	19.05	202	4.85
326	Benzenamine, 4-chloro-	689	4,583	6.65	656	0.95	179	0.26
54	Benzenamine	250	4,427	17.74	642	2.57	176	0.71
706	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,140	5,149	1.64	705	0.22	187	0.06

Ref#	Name	$k_{1\ (exp)}$[2]	Gobas *et al*. (1986)		Hayton and Barron (1990)		Norstrom *et al*. (1976)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
731	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,850	4,063	2.19	609	0.33	171	0.09
707	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	1,630	1,916	1.18	383	0.24	126	0.08
399	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	1,550	2,136	1.38	409	0.26	132	0.09
708	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	538	735	1.37	212	0.39	86	0.16
398	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	386	811	2.10	225	0.58	90	0.23
161	Phenol, pentachloro-	341	1,843	5.41	373	1.10	125	0.37
992	cis-Permethrin	201	2,629	13.09	465	2.32	143	0.71
970	Cyano(3-phenoxyphenyl)methyl ester, 4-Chloro-alpha-(1-methylethyl)benzeneacetic acid	157	2,629	16.78	465	2.97	143	0.92
158	Phenol, pentachloro-	120	371	3.09	139	1.16	66	0.55
159	Phenol, pentachloro-	118	371	3.15	139	1.18	66	0.56
971	[1R-[1 alpha(S*),3 alpha]]Cyano(3-phenoxyphenyl)methyl ester 3-(2,2-dibromoethenyl)-2,2-dimethyl cyclopropane carboxylic acid	105	2,629	25.07	465	4.44	143	1.37

Ref#	Name	$k_{1\ (exp)}$[2]	Gobas *et al*. (1986)		Hayton and Barron (1990)		Norstrom *et al*. (1976)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
994	[1 alpha(S*), 3 alpha]-(+-)-3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropane carboxylic acid cyano (3-phenoxyphenyl)methyl ester	59.3	2,629	44.35	465	7.85	143	2.42
393	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	672	2,400	3.57	440	0.65	138	0.21
392	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	317	2,400	7.57	440	1.39	138	0.44
418	Benzene, 2-methyl-1,3,5-trinitro-	200	4,972	24.84	690	3.45	185	0.92
459	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	3.6	5,366	1490.67	723	200.88	190	52.91
788	Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine (HMX)	1.4	5,158	3582.13	706	490.07	187	130.21
795	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	1,380	4,282	3.11	629	0.46	174	0.13
111	Phenol, 4,4 -(1-methylethylidene)bis-	5.5	3,725	674.76	577	104.53	165	29.84

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.12 Arnot data set – predictions (Part F)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Streit and Sire (1993)		Thomann and Connolly (1984)		Barber (2003) - observed	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
432	Anthracene	900	455	0.51	148	0.16	510	0.57
996	Haloxyfop-methyl	720	442	0.61	143	0.20	492	0.68
16	Benzo[a]pyrene	416	455	1.10	148	0.36	510	1.23
710	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,480	306	0.09	97	0.03	311	0.09
711	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,480	301	0.09	96	0.03	304	0.09
458	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	30.7	603	19.64	198	6.45	725	23.60
726	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	765	266	0.35	84	0.11	261	0.34
727	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	736	266	0.36	84	0.11	261	0.35
725	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	712	266	0.37	84	0.12	261	0.37
729	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,870	408	0.22	132	0.07	445	0.24
728	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,280	408	0.32	132	0.10	445	0.35
712	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	756	419	0.55	135	0.18	459	0.61
29	Benzo[a]anthracene	405	468	1.16	152	0.38	528	1.30
2	Octaethylene glycol monotridecyl ether	317	436	1.37	141	0.44	483	1.52

Ref#	Name	$k_{1\ (exp)}$[2]	Streit and Sire (1993)		Thomann and Connolly (1984)		Barber (2003) - observed	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
3	Octaethylene glycol monotridecyl ether	317	436	1.37	141	0.44	483	1.52
531	Dibenz(a,h)acridine	276	614	2.23	202	0.73	741	2.69
1	C-12-2-LAS	130	430	3.30	139	1.07	475	3.65
4	C-12-5-LAS	11.1	430	38.70	139	12.52	475	42.77
236	Benzene, 1,2,4,5-tetrachloro-	1,630	359	0.22	115	0.07	379	0.23
441	Benzene, 1,2,4-trichloro-	1,160	359	0.31	115	0.10	379	0.33
164	2,4,6-Trichlorophenol	421	359	0.85	115	0.27	379	0.90
321	Benzene, 1,4-dichloro-	291	359	1.23	115	0.40	379	1.30
680	2,3,5,6-Tetrachlorophenol	243	359	1.48	115	0.47	379	1.56
160	Phenol, pentachloro-	222	359	1.62	115	0.52	379	1.71
163	Phenol, pentachloro-	948	408	0.43	132	0.14	445	0.47
162	Phenol, pentachloro-	509	373	0.73	120	0.24	399	0.78
200	4,4'-dibromobiphenyl	2,140	589	0.28	193	0.09	703	0.33
653	3,4,5-Trichloroaniline	1,970	484	0.25	158	0.08	551	0.28
659	2,4,5-Trichloroaniline	1,630	484	0.30	158	0.10	551	0.34
145	Benzene, 1,2,3-trichloro-	1,580	484	0.31	158	0.10	551	0.35
654	2,4,6-Trichloroaniline	1,580	484	0.31	158	0.10	551	0.35
652	2,3,4-Trichloroaniline	1,460	484	0.33	158	0.11	551	0.38
984	1,2,3,6,7,8-Hexachlorodibenzofuran	1,310	414	0.32	134	0.10	453	0.35
940	2,2',5,5'-Tetrachloro-1,1'-biphenyl	1,120	589	0.52	193	0.17	703	0.63
986	2,4,6-tribromobiphenyl	1,120	589	0.52	193	0.17	703	0.63
991	2,3,4,6,7,8-Hexachlorodibenzofuran	1,100	414	0.38	134	0.12	453	0.41
982	2,3,4,7,8-Pentachlorodibenzofuran	1,010	414	0.41	134	0.13	453	0.45
943	2,2',5,5'-Tetrachloro-1,1'-biphenyl	1,000	587	0.59	192	0.19	700	0.70

Ref#	Name	$k_{1\ (exp)}$[2]	Streit and Sire (1993)		Thomann and Connolly (1984)		Barber (2003) - observed	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
929	1,2,3,4-Tetrachlorodibenzo-p-dioxin	953	609	0.64	200	0.21	734	0.77
961	1,2,3,7,8-Pentachlorodibenzo-p-dioxin	952	414	0.43	134	0.14	453	0.48
987	2,2',5,5'-tetrabromobiphenyl	912	589	0.65	193	0.21	703	0.77
941	2,2',5,5'-Tetrachloro-1,1'-biphenyl	910	587	0.64	192	0.21	700	0.77
876	2,4',5-Trichloro-1,1'-biphenyl	890	609	0.68	200	0.22	734	0.82
939	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	880	587	0.67	192	0.22	700	0.80
959	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	868	414	0.48	134	0.15	453	0.52
942	2,2',5,5'-Tetrachloro-1,1'-biphenyl	860	587	0.68	192	0.22	700	0.81
985	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	844	414	0.49	134	0.16	453	0.54
938	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	840	587	0.70	192	0.23	700	0.83
641	Benzene, 1,3,5-tribromo-	708	589	0.83	193	0.27	703	0.99
888	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	687	414	0.60	134	0.19	453	0.66
790	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	630	593	0.94	194	0.31	709	1.13
968	2,3,7,8-Tetrachlorodibenzofuran	603	414	0.69	134	0.22	453	0.75

Ref#	Name	$k_{1\ (exp)}$[2]	Streit and Sire (1993)		Thomann and Connolly (1984)		Barber (2003) - observed	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
960	1,2,4-Trichlorodibenzo[b,e][1,4]dioxin	601	609	1.01	200	0.33	734	1.22
934	2,7-Dichlorodibenzo[b,e][1,4]dioxin	543	609	1.12	200	0.37	734	1.35
937	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	540	587	1.09	192	0.36	700	1.30
995	1,2,3,4,6,7,8-Heptachlorodibenzofuran	524	414	0.79	134	0.26	453	0.87
730	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	500	414	0.83	134	0.27	453	0.91
945	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	456	414	0.91	134	0.29	453	0.99
871	2,4,5-Trichloro-1,1'-biphenyl	380	589	1.55	193	0.51	703	1.85
988	2,2',4,4',6,6'-hexabromobiphenyl	324	589	1.82	193	0.60	703	2.17
808	1,2,3,4,5,6,7,8-Octachlorodibenzo-p-dioxin	275	414	1.51	134	0.49	453	1.65
958	1,2,3,4,5,6,7,8-Octachlorodibenzofuran	217	414	1.91	134	0.62	453	2.09
316	Benzene, 1,4-dibromo-	129	589	4.57	193	1.50	703	5.46
765	Mirex	93.3	589	6.31	193	2.07	703	7.53
746	Decachlorobiphenyl	41.7	589	14.12	193	4.63	703	16.87
326	Benzenamine, 4-chloro-	689	505	0.73	164	0.24	580	0.84
54	Benzenamine	250	496	1.99	161	0.65	568	2.28
706	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,140	535	0.17	175	0.06	624	0.20

Ref#	Name	$k_{1\ (exp)}$[2]	Streit and Sire (1993)		Thomann and Connolly (1984)		Barber (2003) - observed	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
731	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,850	475	0.26	154	0.08	538	0.29
707	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	1,630	327	0.20	104	0.06	337	0.21
399	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	1,550	345	0.22	110	0.07	361	0.23
708	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	538	203	0.38	63	0.12	186	0.35
398	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	386	213	0.55	67	0.17	198	0.51
161	Phenol, pentachloro-	341	321	0.94	102	0.30	329	0.97
992	cis-Permethrin	201	383	1.90	123	0.61	411	2.05
970	Cyano(3-phenoxyphenyl)methyl ester, 4-Chloro-alpha-(1-methylethyl)benzeneacetic acid	157	383	2.44	123	0.79	411	2.62
158	Phenol, pentachloro-	120	144	1.20	44	0.37	122	1.01
159	Phenol, pentachloro-	118	144	1.23	44	0.38	122	1.03
971	[1R-[1 alpha(S*),3 alpha]]Cyano(3-phenoxyphenyl)methyl ester 3-(2,2-dibromoethenyl)-2,2-dimethyl cyclopropane carboxylic acid	105	383	3.65	123	1.17	411	3.92

Ref#	Name	$k_{1\ (exp)}$[2]	Streit and Sire (1993)		Thomann and Connolly (1984)		Barber (2003) - observed	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
994	[1 alpha(S*), 3 alpha]-(+-)-3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropane carboxylic acid cyano (3-phenoxyphenyl)methyl ester	59.3	383	6.45	123	2.08	411	6.93
393	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	672	366	0.54	117	0.17	388	0.58
392	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	317	366	1.15	117	0.37	388	1.22
418	Benzene, 2-methyl-1,3,5-trinitro-	200	526	2.63	172	0.86	610	3.05
459	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	3.6	546	151.65	178	49.58	640	177.78
788	Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine (HMX)	1.4	535	371.73	175	121.42	624	433.66
795	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	1,380	488	0.35	159	0.12	556	0.40
111	Phenol, 4,4 -(1-methylethylidene)bis-	5.5	455	82.45	148	26.74	510	92.40

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.13 Arnot data set – predictions (Part G)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Barber (2003) - calibrated		Hawker and Connell (1985)		Hawker and Connell (1988)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
432	Anthracene	900	891	0.99	13.39	0.015	26.00	0.029
996	Haloxyfop-methyl	720	822	1.14	9.81	0.014	19.27	0.027
16	Benzo[a]pyrene	416	894	2.15	49.30	0.119	33.59	0.081
710	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,480	318	0.09	26.70	0.008	32.54	0.009
711	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,480	305	0.09	26.70	0.008	32.54	0.009
458	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	30.7	120	3.90	0.83	0.027	0.03	0.001
726	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	765	222	0.29	82.91	0.108	33.76	0.044
727	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	736	222	0.30	82.91	0.113	33.76	0.046
725	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	712	222	0.31	82.91	0.116	33.76	0.047
729	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,870	673	0.36	82.91	0.044	33.76	0.018
728	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,280	673	0.53	82.91	0.065	33.76	0.026
712	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	756	719	0.95	26.70	0.035	32.54	0.043
29	Benzo[a]anthracene	405	960	2.37	36.99	0.091	33.31	0.082
2	Octaethylene glycol monotridecyl ether	317	734	2.31	4.59	0.014	4.12	0.013

Ref#	Name	$k_{1\ (exp)}$[2]	Barber (2003) - calibrated		Hawker and Connell (1985)		Hawker and Connell (1988)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
3	Octaethylene glycol monotridecyl ether	317	797	2.51	22.34	0.070	31.72	0.100
531	Dibenz(a,h)acridine	276	1,946	7.06	34.50	0.125	33.20	0.121
1	C-12-2-LAS	130	768	5.91	16.38	0.126	29.02	0.223
4	C-12-5-LAS	11.1	768	69.22	16.38	1.476	29.02	2.614
236	Benzene, 1,2,4,5-tetrachloro-	1,630	481	0.30	15.51	0.010	28.32	0.017
441	Benzene, 1,2,4-trichloro-	1,160	478	0.41	9.59	0.008	18.70	0.016
164	2,4,6-Trichlorophenol	421	473	1.12	7.42	0.018	12.40	0.029
321	Benzene, 1,4-dichloro-	291	465	1.60	6.11	0.021	8.30	0.029
680	2,3,5,6-Tetrachlorophenol	243	476	1.96	8.60	0.035	15.98	0.066
160	Phenol, pentachloro-	222	482	2.17	22.51	0.101	31.76	0.143
163	Phenol, pentachloro-	948	672	0.71	22.51	0.024	31.76	0.034
162	Phenol, pentachloro-	509	534	1.05	22.51	0.044	31.76	0.062
200	4,4'-dibromobiphenyl	2,140	1,744	0.82	35.86	0.017	33.27	0.016
653	3,4,5-Trichloroaniline	1,970	1,002	0.51	5.57	0.003	6.70	0.003
659	2,4,5-Trichloroaniline	1,630	1,014	0.62	6.16	0.004	8.45	0.005
145	Benzene, 1,2,3-trichloro-	1,580	1,041	0.66	9.81	0.006	19.27	0.012
654	2,4,6-Trichloroaniline	1,580	1,019	0.64	6.50	0.004	9.51	0.006
652	2,3,4-Trichloroaniline	1,460	1,003	0.69	5.61	0.004	6.82	0.005
984	1,2,3,6,7,8-Hexachlorodibenzofuran	1,310	699	0.53	122.21	0.093	33.79	0.026
940	2,2',5,5'-Tetrachloro-1,1'-biphenyl	1,120	1,744	1.55	39.36	0.035	33.39	0.030
986	2,4,6-tribromobiphenyl	1,120	1,744	1.55	45.62	0.041	33.54	0.030
991	2,3,4,6,7,8-Hexachlorodibenzofuran	1,100	699	0.64	122.21	0.111	33.79	0.031
982	2,3,4,7,8-Pentachlorodibenzofuran	1,010	699	0.69	73.23	0.072	33.74	0.033
943	2,2',5,5'-Tetrachloro-1,1'-biphenyl	1,000	1,729	1.73	39.36	0.039	33.39	0.033

Ref#	Name	$k_{1\ (exp)}$[2]	Barber (2003) - calibrated		Hawker and Connell (1985)		Hawker and Connell (1988)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
929	1,2,3,4-Tetrachlorodibenzo-p-dioxin	953	1,905	2.00	70.99	0.074	33.73	0.035
961	1,2,3,7,8-Pentachlorodibenzo-p-dioxin	952	699	0.73	73.23	0.077	33.74	0.035
987	2,2',5,5'-tetrabromobiphenyl	912	1,744	1.91	65.69	0.072	33.71	0.037
941	2,2',5,5'-Tetrachloro-1,1'-biphenyl	910	1,729	1.90	39.36	0.043	33.39	0.037
876	2,4',5-Trichloro-1,1'-biphenyl	890	1,905	2.14	34.50	0.039	33.20	0.037
939	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	880	1,729	1.97	91.00	0.103	33.77	0.038
959	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	868	699	0.81	122.21	0.141	33.79	0.039
942	2,2',5,5'-Tetrachloro-1,1'-biphenyl	860	1,729	2.01	39.36	0.046	33.39	0.039
985	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	844	699	0.83	122.21	0.145	33.79	0.040
938	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	840	1,729	2.06	91.00	0.108	33.77	0.040
641	Benzene, 1,3,5-tribromo-	708	1,738	2.46	14.02	0.020	26.80	0.038
888	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	687	699	1.02	122.21	0.178	33.79	0.049
790	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	630	1,772	2.81	19.88	0.032	30.93	0.049
968	2,3,7,8-Tetrachlorodibenzofuran	603	699	1.16	67.24	0.112	33.72	0.056

Ref#	Name	$k_{1\ (exp)}$[2]	Barber (2003) - calibrated		Hawker and Connell (1985)		Hawker and Connell (1988)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
960	1,2,4-Trichlorodibenzo[b,e][1,4]dioxin	601	1,905	3.17	58.47	0.097	33.68	0.056
934	2,7-Dichlorodibenzo[b,e][1,4]dioxin	543	1,905	3.51	36.71	0.068	33.30	0.061
937	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	540	1,729	3.20	91.00	0.169	33.77	0.063
995	1,2,3,4,6,7,8-Heptachlorodibenzofuran	524	699	1.33	132.07	0.252	33.79	0.064
730	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	500	699	1.40	82.91	0.166	33.76	0.068
945	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	456	699	1.53	180.14	0.395	33.80	0.074
871	2,4,5-Trichloro-1,1'-biphenyl	380	1,743	4.59	32.67	0.086	33.10	0.087
988	2,2',4,4',6,6'-hexabromobiphenyl	324	1,744	5.39	113.08	0.349	33.78	0.104
808	1,2,3,4,5,6,7,8-Octachlorodibenzo-p-dioxin	275	699	2.54	245.70	0.893	33.80	0.123
958	1,2,3,4,5,6,7,8-Octachlorodibenzofuran	217	699	3.22	245.70	1.132	33.80	0.156
316	Benzene, 1,4-dibromo-	129	1,715	13.32	8.02	0.062	14.25	0.111
765	Mirex	93.3	1,744	18.69	88.91	0.953	33.77	0.362
746	Decachlorobiphenyl	41.7	1,744	41.83	241.91	5.803	33.80	0.811
326	Benzenamine, 4-chloro-	689	461	0.67	1.75	0.003	0.27	0.0004
54	Benzenamine	250	77	0.31	0.85	0.003	0.03	0.0001
706	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,140	1,359	0.43	26.70	0.008	32.54	0.010

Ref#	Name	$k_{1\ (exp)}$[2]	Barber (2003) - calibrated		Hawker and Connell (1985)		Hawker and Connell (1988)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
731	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,850	1,000	0.54	82.91	0.045	33.76	0.018
707	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	1,630	378	0.23	26.70	0.016	32.54	0.020
399	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	1,550	435	0.28	170.61	0.110	33.80	0.022
708	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	538	110	0.20	26.70	0.050	32.54	0.061
398	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	386	124	0.32	170.61	0.442	33.80	0.087
161	Phenol, pentachloro-	341	360	1.06	22.51	0.066	31.76	0.093
992	cis-Permethrin	201	570	2.84	135.18	0.673	33.79	0.168
970	Cyano(3-phenoxyphenyl)methyl ester, 4-Chloro-alpha-(1-methylethyl)benzeneacetic acid	157	570	3.64	52.05	0.332	33.62	0.215
158	Phenol, pentachloro-	120	45	0.38	22.51	0.188	31.76	0.265
159	Phenol, pentachloro-	118	45	0.38	22.51	0.191	31.76	0.270
971	[1R-[1 alpha(S*),3 alpha]]Cyano(3-phenoxyphenyl)methyl ester 3-(2,2-dibromoethenyl)-2,2-dimethyl cyclopropane carboxylic acid	105	570	5.43	52.05	0.496	33.62	0.321
994	[1 alpha(S*), 3 alpha]-(+-)-3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropane carboxylic acid cyano (3-phenoxyphenyl)methyl ester	59.3	570	9.61	59.85	1.010	33.68	0.568

Ref#	Name	$k_{1\ (exp)}$[2]	Barber (2003) - calibrated		Hawker and Connell (1985)		Hawker and Connell (1988)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
393	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	672	506	0.75	170.61	0.254	33.80	0.050
392	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	317	506	1.60	170.61	0.538	33.80	0.107
418	Benzene, 2-methyl-1,3,5-trinitro-	200	358	1.79	1.47	0.007	0.16	0.001
459	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	3.6	93	25.71	0.83	0.231	0.03	0.008
788	Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine (HMX)	1.4	19	13.00	0.49	0.341	0.01	0.004
795	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	1,380	1,070	0.78	19.88	0.014	30.93	0.022
111	Phenol, 4,4 -(1-methylethylidene)bis-	5.5	852	154.38	5.57	1.009	6.70	1.213

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.14 Arnot data set – predictions (Part H)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Spacie and Hamelink (1982)		Tolls and Sijm (1995)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
432	Anthracene	900	431	0.48	543	0.60
996	Haloxyfop-methyl	720	376	0.52	485	0.67
16	Benzo[a]pyrene	416	761	1.83	871	2.10
710	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,480	582	0.17	697	0.20
711	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,480	582	0.17	697	0.20
458	1,3,5-Triazine, hexahydro-1,3,5-trinitro-(RDX)	30.7	128	4.17	199	6.47
726	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	765	954	1.25	1,051	1.37
727	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	736	954	1.30	1,051	1.43
725	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	712	954	1.34	1,051	1.48
729	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,870	954	0.51	1,051	0.56
728	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,280	954	0.75	1,051	0.82
712	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	756	582	0.77	697	0.92
29	Benzo[a]anthracene	405	671	1.66	785	1.94
2	Octaethylene glycol monotridecyl ether	317	270	0.85	369	1.16
3	Octaethylene glycol monotridecyl ether	317	538	1.70	654	2.06
531	Dibenz(a,h)acridine	276	651	2.36	765	2.78
1	C-12-2-LAS	130	470	3.62	584	4.49
4	C-12-5-LAS	11.1	470	42.37	584	52.64
236	Benzene, 1,2,4,5-tetrachloro-	1,630	459	0.28	573	0.35
441	Benzene, 1,2,4-trichloro-	1,160	372	0.32	481	0.42
164	2,4,6-Trichlorophenol	421	333	0.79	439	1.04
321	Benzene, 1,4-dichloro-	291	306	1.05	409	1.41
680	2,3,5,6-Tetrachlorophenol	243	355	1.46	463	1.90
160	Phenol, pentachloro-	222	540	2.43	656	2.95
163	Phenol, pentachloro-	948	540	0.57	656	0.69

Ref#	Name	$k_{1\ (exp)}$[2]	Spacie and Hamelink (1982)		Tolls and Sijm (1995)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
162	Phenol, pentachloro-	509	540	1.06	656	1.29
200	4,4'-dibromobiphenyl	2,140	662	0.31	776	0.36
653	3,4,5-Trichloroaniline	1,970	294	0.15	395	0.20
659	2,4,5-Trichloroaniline	1,630	307	0.19	410	0.25
145	Benzene, 1,2,3-trichloro-	1,580	376	0.24	485	0.31
654	2,4,6-Trichloroaniline	1,580	314	0.20	418	0.26
652	2,3,4-Trichloroaniline	1,460	295	0.20	397	0.27
984	1,2,3,6,7,8-Hexachlorodibenzofuran	1,310	1,130	0.86	1,209	0.92
940	2,2',5,5'-Tetrachloro-1,1'-biphenyl	1,120	689	0.61	803	0.72
986	2,4,6-tribromobiphenyl	1,120	735	0.66	847	0.75
991	2,3,4,6,7,8-Hexachlorodibenzofuran	1,100	1,130	1.03	1,209	1.10
982	2,3,4,7,8-Pentachlorodibenzofuran	1,010	904	0.89	1,005	0.99
943	2,2',5,5'-Tetrachloro-1,1'-biphenyl	1,000	689	0.69	803	0.80
929	1,2,3,4-Tetrachlorodibenzo-p-dioxin	953	892	0.94	994	1.04
961	1,2,3,7,8-Pentachlorodibenzo-p-dioxin	952	904	0.95	1,005	1.06
987	2,2',5,5'-tetrabromobiphenyl	912	862	0.95	966	1.06
941	2,2',5,5'-Tetrachloro-1,1'-biphenyl	910	689	0.76	803	0.88
876	2,4',5-Trichloro-1,1'-biphenyl	890	651	0.73	765	0.86
939	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	880	994	1.13	1,087	1.24
959	1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	868	1,130	1.30	1,209	1.39
942	2,2',5,5'-Tetrachloro-1,1'-biphenyl	860	689	0.80	803	0.93
985	1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	844	1,130	1.34	1,209	1.43
938	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	840	994	1.18	1,087	1.29
641	Benzene, 1,3,5-tribromo-	708	440	0.62	552	0.78
888	1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	687	1,130	1.64	1,209	1.76
790	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	630	512	0.81	627	0.99
968	2,3,7,8-Tetrachlorodibenzofuran	603	871	1.44	974	1.62
960	1,2,4-Trichlorodibenzo[b,e][1,4]dioxin	601	819	1.36	926	1.54
934	2,7-Dichlorodibenzo[b,e][1,4]dioxin	543	669	1.23	783	1.44

Ref#	Name	$k_{1\ (exp)}$[2]	Spacie and Hamelink (1982)		Tolls and Sijm (1995)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
937	2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl	540	994	1.84	1,087	2.01
995	1,2,3,4,6,7,8-Heptachlorodibenzofuran	524	1,169	2.23	1,244	2.37
730	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	500	954	1.91	1,051	2.10
945	1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	456	1,338	2.94	1,392	3.05
871	2,4,5-Trichloro-1,1'-biphenyl	380	636	1.67	750	1.97
988	2,2',4,4',6,6'-hexabromobiphenyl	324	1,092	3.38	1,176	3.63
808	1,2,3,4,5,6,7,8-Octachlorodibenzo-p-dioxin	275	1,532	5.57	1,557	5.66
958	1,2,3,4,5,6,7,8-Octachlorodibenzofuran	217	1,532	7.06	1,557	7.18
316	Benzene, 1,4-dibromo-	129	344	2.67	451	3.50
765	Mirex	93.3	984	10.54	1,078	11.55
746	Decachlorobiphenyl	41.7	1,522	36.51	1,549	37.15
326	Benzenamine, 4-chloro-	689	177	0.26	260	0.38
54	Benzenamine	250	130	0.52	200	0.80
706	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	3,140	582	0.19	697	0.22
731	2,3,7,8-Tetrachlorodibenzo[b,e][1,4]dioxin	1,850	954	0.52	1,051	0.57
707	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	1,630	582	0.36	697	0.43
399	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	1,550	1,307	0.84	1,365	0.88
708	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	538	582	1.08	697	1.30
398	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	386	1,307	3.38	1,365	3.53
161	Phenol, pentachloro-	341	540	1.59	656	1.92
992	cis-Permethrin	201	1,181	5.88	1,254	6.24
970	Cyano(3-phenoxyphenyl)methyl ester, 4-Chloro-alpha-(1-methylethyl)benzeneacetic acid	157	779	4.97	888	5.67
158	Phenol, pentachloro-	120	540	4.50	656	5.46
159	Phenol, pentachloro-	118	540	4.59	656	5.57

Ref#	Name	$k_{1\ (exp)}$[2]	Spacie and Hamelink (1982)		Tolls and Sijm (1995)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
971	[1R-[1 alpha(S*),3 alpha]]Cyano(3-phenoxyphenyl)methyl ester 3-(2,2-dibromoethenyl)-2,2-dimethyl cyclopropane carboxylic acid	105	779	7.43	888	8.47
994	[1 alpha(S*), 3 alpha]-(+-)-3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropane carboxylic acid cyano (3-phenoxyphenyl)methyl ester	59.3	828	13.96	934	15.76
393	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	672	1,307	1.95	1,365	2.03
392	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	317	1,307	4.12	1,365	4.31
418	Benzene, 2-methyl-1,3,5-trinitro-	200	164	0.82	244	1.22
459	1,3,5-Triazine, hexahydro-1,3,5-trinitro- (RDX)	3.6	128	35.61	199	55.19
788	Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine (HMX)	1.4	102	70.73	164	113.98
795	Phosphorothioic acid, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester	1,380	512	0.37	627	0.45
111	Phenol, 4,4 -(1-methylethylidene)bis-	5.5	294	53.22	395	71.63

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.15 UBA data set – predictions (Part A)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Sijm *et al*. (1995)		Hendriks *et al*. (2001)		Campfens and Mackay (1997)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
UBA 11	Confidential	40.9	279	6.81	186	4.54	117	2.87
UBA 11	Confidential	28.3	288	10.18	191	6.74	79	2.80
UBA 4	Confidential	15.5	384	24.73	47	3.06	10	0.64
UBA 4	Confidential	11.1	384	34.58	47	4.28	8	0.72
UBA 6	Confidential	7.73	352	45.47	41	5.27	5	0.60
UBA 6	Confidential	6.88	352	51.09	41	5.92	5	0.79
UBA 10	Confidential	76.7	336	4.39	79	1.03	328	4.27
UBA 10	Confidential	28.9	336	11.64	79	2.73	76	2.62
UBA 5	Confidential	411	473	1.15	603	1.47	1,041	2.53
UBA 5	Confidential	339	473	1.40	603	1.78	1,015	3.00
UBA 13	Confidential	37.9	548	14.47	168	4.44		
UBA 14	Confidential	11.2	561	49.99	171	15.25	10	0.90
UBA 14	Confidential	11.1	561	50.49	171	15.40	10	0.87
UBA 13	Confidential	8.66	544	62.78	167	19.28		
UBA 9	Confidential	1,543	838	0.54	45	0.03	168	0.11
UBA 3	Confidential	516	730	1.41	879	1.70	2,708	5.25
UBA 3	Confidential	492	730	1.48	879	1.78	2,603	5.29
UBA 9	Confidential	336	838	2.49	45	0.13	150	0.44

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.16 UBA data set – predictions (Part B)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Arnot and Gobas (2003)		Arnot and Gobas (2004)		Thomann (1989)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
UBA 11	Confidential	40.9	715	17.48	no estimate	no estimate	no estimate	no estimate
UBA 11	Confidential	28.3	746	26.36	no estimate	no estimate	no estimate	no estimate
UBA 4	Confidential	15.5	963	62.05	no estimate	no estimate	no estimate	no estimate
UBA 4	Confidential	11.1	963	86.76	no estimate	no estimate	no estimate	no estimate
UBA 6	Confidential	7.73	854	110.43	no estimate	no estimate	no estimate	no estimate
UBA 6	Confidential	6.88	854	124.07	no estimate	no estimate	no estimate	no estimate
UBA 10	Confidential	76.7	865	11.28	no estimate	no estimate	no estimate	no estimate
UBA 10	Confidential	28.9	865	29.93	no estimate	no estimate	no estimate	no estimate
UBA 5	Confidential	411	1,406	3.42	no estimate	no estimate	no estimate	no estimate
UBA 5	Confidential	339	1,406	4.15	no estimate	no estimate	no estimate	no estimate
UBA 13	Confidential	37.9	1,627	42.97	no estimate	no estimate	no estimate	no estimate
UBA 14	Confidential	11.2	1,677	149.37	no estimate	no estimate	no estimate	no estimate
UBA 14	Confidential	11.1	1,677	150.85	no estimate	no estimate	no estimate	no estimate
UBA 13	Confidential	8.66	1,612	186.09	no estimate	no estimate	no estimate	no estimate
UBA 9	Confidential	1,543	2,290	1.48	no estimate	no estimate	no estimate	no estimate
UBA 3	Confidential	516	2,418	4.69	no estimate	no estimate	no estimate	no estimate
UBA 3	Confidential	492	2,418	4.91	no estimate	no estimate	no estimate	no estimate
UBA 9	Confidential	336	2,290	6.81	no estimate	no estimate	no estimate	no estimate

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.17 UBA data set – predictions (Part C)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Barber (2001)		Barber *et al*. (1991)	
			$k_{1\ (pred)}$	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$	$k_{1\ (pred)}/k_{1\ (exp)}$
UBA 11	Confidential	40.9	506	12.37	477	11.66
UBA 11	Confidential	28.3	515	18.19	487	17.19
UBA 4	Confidential	15.5	595	38.32	573	36.90
UBA 4	Confidential	11.1	595	53.59	573	51.59
UBA 6	Confidential	7.73	569	73.62	545	70.47
UBA 6	Confidential	6.88	569	82.71	545	79.17
UBA 10	Confidential	76.7	557	7.26	531	6.93
UBA 10	Confidential	28.9	557	19.26	531	18.38
UBA 5	Confidential	411	661	1.61	645	1.57
UBA 5	Confidential	339	661	1.95	645	1.90
UBA 13	Confidential	37.9	711	18.79	701	18.52
UBA 14	Confidential	11.2	720	64.13	711	63.31
UBA 14	Confidential	11.1	720	64.77	711	63.93
UBA 13	Confidential	8.66	709	81.84	698	80.61
UBA 9	Confidential	1,543	881	0.57	893	0.58
UBA 3	Confidential	516	822	1.59	825	1.60
UBA 3	Confidential	492	822	1.67	825	1.68
UBA 9	Confidential	336	881	2.62	893	2.66

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.18 UBA data set – predictions (Part D)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Erickson and McKim (1990a)		Erickson and McKim (1990b)		Gobas and Mackay (1987)	
			$k_{1\ (pred)}$	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$	$k_{1\ (pred)}/k_{1\ (exp)}$
UBA 11	Confidential	40.9	495	12.10	365	8.92	582	14.23
UBA 11	Confidential	28.3	503	17.78	374	13.22	607	21.44
UBA 4	Confidential	15.5	579	37.33	459	29.56	864	55.64
UBA 4	Confidential	11.1	579	52.20	459	41.33	864	77.80
UBA 6	Confidential	7.73	555	71.79	431	55.75	775	100.26
UBA 6	Confidential	6.88	555	80.66	431	62.64	775	112.64
UBA 10	Confidential	76.7	543	7.08	418	5.45	734	9.58
UBA 10	Confidential	28.9	543	18.79	418	14.45	734	25.40
UBA 5	Confidential	411	642	1.56	532	1.30	1,116	2.72
UBA 5	Confidential	339	642	1.89	532	1.57	1,116	3.30
UBA 13	Confidential	37.9	690	18.23	591	15.62	1,338	35.35
UBA 14	Confidential	11.2	698	62.18	602	53.57	1,379	122.83
UBA 14	Confidential	11.1	698	62.79	602	54.10	1,379	124.04
UBA 13	Confidential	8.66	687	79.37	588	67.90	1,326	153.11
UBA 9	Confidential	1,543	850	0.55	800	0.52	2,259	1.46
UBA 3	Confidential	516	794	1.54	725	1.41	1,905	3.69
UBA 3	Confidential	492	794	1.61	725	1.47	1,905	3.87
UBA 9	Confidential	336	850	2.53	800	2.38	2,259	6.72

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.19 UBA data set – predictions (Part E)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Gobas *et al*. (1986)		Hayton and Barron (1990)		Norstrom *et al*. (1976)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
UBA 11	Confidential	40.9	1,611	39.38	344	8.40	118	2.89
UBA 11	Confidential	28.3	1,667	58.87	351	12.40	120	4.23
UBA 4	Confidential	15.5	2,213	142.60	418	26.95	134	8.63
UBA 4	Confidential	11.1	2,213	199.38	418	37.68	134	12.07
UBA 6	Confidential	7.73	2,029	262.42	396	51.27	129	16.74
UBA 6	Confidential	6.88	2,029	294.84	396	57.60	129	18.81
UBA 10	Confidential	76.7	1,942	25.34	386	5.03	127	1.66
UBA 10	Confidential	28.9	1,942	67.20	386	13.35	127	4.40
UBA 5	Confidential	411	2,721	6.62	475	1.16	145	0.35
UBA 5	Confidential	339	2,721	8.03	475	1.40	145	0.43
UBA 13	Confidential	37.9	3,148	83.17	520	13.74	154	4.07
UBA 14	Confidential	11.2	3,226	287.25	528	47.01	156	13.85
UBA 14	Confidential	11.1	3,226	290.09	528	47.47	156	13.99
UBA 13	Confidential	8.66	3,125	360.84	518	59.77	154	17.74
UBA 9	Confidential	1,543	4,798	3.11	675	0.44	182	0.12
UBA 3	Confidential	516	4,182	8.11	620	1.20	172	0.33
UBA 3	Confidential	492	4,182	8.49	620	1.26	172	0.35
UBA 9	Confidential	336	4,798	14.27	675	2.01	182	0.54

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.20 UBA data set – predictions (Part F)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Streit and Sire (1993)		Thomann and Connolly (1984)		Barber (2003) - observed	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
UBA 11	Confidential	40.9	300	7.32	95	2.33	303	7.41
UBA 11	Confidential	28.3	305	10.77	97	3.43	309	10.93
UBA 4	Confidential	15.5	351	22.62	113	7.25	369	23.78
UBA 4	Confidential	11.1	351	31.63	113	10.14	369	33.25
UBA 6	Confidential	7.73	336	43.49	108	13.92	350	45.23
UBA 6	Confidential	6.88	336	48.86	108	15.63	350	50.82
UBA 10	Confidential	76.7	329	4.29	105	1.37	340	4.44
UBA 10	Confidential	28.9	329	11.38	105	3.64	340	11.78
UBA 5	Confidential	411	389	0.95	125	0.31	420	1.02
UBA 5	Confidential	339	389	1.15	125	0.37	420	1.24
UBA 13	Confidential	37.9	419	11.06	135	3.57	459	12.14
UBA 14	Confidential	11.2	424	37.72	137	12.19	466	41.54
UBA 14	Confidential	11.1	424	38.10	137	12.31	466	41.95
UBA 13	Confidential	8.66	417	48.15	135	15.55	457	52.81
UBA 9	Confidential	1,543	516	0.33	168	0.11	597	0.39
UBA 3	Confidential	516	482	0.93	157	0.30	548	1.06
UBA 3	Confidential	492	482	0.98	157	0.32	548	1.11
UBA 9	Confidential	336	516	1.54	168	0.50	597	1.78

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.21 UBA data set – predictions (Part G)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Barber (2003) - calibrated		Hawker and Connell (1985)		Hawker and Connell (1988)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
UBA 11	Confidential	40.9	298	7.27	8.08	0.198	14.44	0.353
UBA 11	Confidential	28.3	311	10.98	8.08	0.286	14.44	0.510
UBA 4	Confidential	15.5	404	26.02	4.02	0.259	2.90	0.187
UBA 4	Confidential	11.1	404	36.38	4.02	0.362	2.90	0.261
UBA 6	Confidential	7.73	357	46.16	3.90	0.504	2.67	0.345
UBA 6	Confidential	6.88	357	51.86	3.90	0.567	2.67	0.388
UBA 10	Confidential	76.7	362	4.72	5.07	0.066	5.33	0.070
UBA 10	Confidential	28.9	362	12.52	5.07	0.176	5.33	0.185
UBA 5	Confidential	411	595	1.45	18.98	0.046	30.55	0.074
UBA 5	Confidential	339	595	1.76	18.98	0.056	30.55	0.090
UBA 13	Confidential	37.9	691	18.26	5.93	0.157	7.74	0.205
UBA 14	Confidential	11.2	713	63.51	5.93	0.528	7.74	0.689
UBA 14	Confidential	11.1	713	64.13	5.93	0.533	7.74	0.696
UBA 13	Confidential	8.66	684	79.04	5.93	0.684	7.74	0.894
UBA 9	Confidential	1,543	981	0.64	3.16	0.002	1.49	0.001
UBA 3	Confidential	516	1,038	2.01	22.17	0.043	31.67	0.061
UBA 3	Confidential	492	1,038	2.11	22.17	0.045	31.67	0.064
UBA 9	Confidential	336	981	2.92	3.16	0.009	1.49	0.004

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.22 UBA data set – predictions (Part H)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Spacie and Hamelink (1982)		Tolls and Sijm (1995)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
UBA 11	Confidential	40.9	346	8.45	452	11.06
UBA 11	Confidential	28.3	346	12.21	452	15.98
UBA 4	Confidential	15.5	255	16.42	351	22.64
UBA 4	Confidential	11.1	255	22.96	351	31.66
UBA 6	Confidential	7.73	251	32.53	347	44.95
UBA 6	Confidential	6.88	251	36.55	347	50.50
UBA 10	Confidential	76.7	282	3.68	382	4.99
UBA 10	Confidential	28.9	282	9.76	382	13.23
UBA 5	Confidential	411	502	1.22	616	1.50
UBA 5	Confidential	339	502	1.48	616	1.82
UBA 13	Confidential	37.9	302	7.98	404	10.68
UBA 14	Confidential	11.2	302	26.88	404	36.01
UBA 14	Confidential	11.1	302	27.15	404	36.37
UBA 13	Confidential	8.66	302	34.86	404	46.70
UBA 9	Confidential	1,543	229	0.15	322	0.21
UBA 3	Confidential	516	537	1.04	652	1.26
UBA 3	Confidential	492	537	1.09	652	1.32
UBA 9	Confidential	336	229	0.68	322	0.96

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.23 Gold standard data set – predictions (Part A)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Sijm *et al*. (1995)		Hendriks *et al*. (2001)		Campfens and Mackay (1997)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
GS32	1,2,4-Trichlorobenzene	492	658	1.34	473	0.96		
GS45	Hexachlorobenzene	1,850	885	0.48	1,075	0.58	8,158	4.41
GS44	1,2,4-Tribromobenzene	1,040	885	0.85	917	0.88	822	0.79
GS43	1,2,3,5-Tetrachlorobenzene	631	885	1.40	881	1.40	540	0.86
GS42	1,2,3-Trichlorobenzene	470	885	1.88	615	1.31	383	0.81
GS41	1,4-Dibromobenzene	272	885	3.25	452	1.66	252	0.93
GS40	1,4-Dichlorobenzene	112	885	7.90	262	2.34	123	1.10
GS7	2-Isopropylnaphthalene	4,188	389	0.09	477	0.11	372	0.09
GS8	2-Isopropylnaphthalene	3,746	389	0.10	477	0.13	662	0.18
GS5	1,3-Dimethylnaphthalene	2,909	389	0.13	432	0.15	893	0.31
GS3	2-Methylnaphthalene	2,659	389	0.15	261	0.10	436	0.16
GS4	2-Methylnaphthalene	2,142	389	0.18	261	0.12	422	0.20
GS6	1,3-Dimethylnaphthalene	1,854	389	0.21	432	0.23	1,199	0.65
GS9	Phenanthrene	1,783	389	0.22	442	0.25	2,238	1.26
GS1	Naphthalene	1,450	389	0.27	107	0.07	281	0.19
GS2	Naphthalene	1,137	389	0.34	107	0.09	246	0.22
GS13	9-Ethylphenanthrene	731	389	0.53	555	0.76	13,030	17.83
GS10	Phenanthrene	680	389	0.57	442	0.65	2,350	3.46
GS11	9-Methylphenanthrene	623	389	0.62	517	0.83	5,421	8.70
GS12	9-Methylphenanthrene	290	389	1.34	517	1.78	6,024	20.77
GS14	9-Ethylphenanthrene	263	389	1.48	555	2.11	14,194	53.97
GS16	Pyrene	129	389	3.02	516	4.00	6,549	50.77
GS15	Pyrene	116	389	3.36	516	4.45	8,756	75.49

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.24 Gold standar data set – predictions (Part B)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Arnot and Gobas (2003)		Arnot and Gobas (2004)		Thomann (1989)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
GS32	1,2,4-Trichlorobenzene	492	2,106	4.28	no estimate	no estimate	no estimate	no estimate
GS45	Hexachlorobenzene	1,850	3,079	1.66	no estimate	no estimate	no estimate	no estimate
GS44	1,2,4-Tribromobenzene	1,040	3,073	2.95	no estimate	no estimate	no estimate	no estimate
GS43	1,2,3,5-Tetrachlorobenzene	631	3,071	4.87	no estimate	no estimate	no estimate	no estimate
GS42	1,2,3-Trichlorobenzene	470	3,052	6.49	no estimate	no estimate	no estimate	no estimate
GS41	1,4-Dibromobenzene	272	3,030	11.14	no estimate	no estimate	no estimate	no estimate
GS40	1,4-Dichlorobenzene	112	2,972	26.53	no estimate	no estimate	no estimate	no estimate
GS7	2-Isopropylnaphthalene	4,188	1,101	0.26	no estimate	no estimate	no estimate	no estimate
GS8	2-Isopropylnaphthalene	3,746	1,101	0.29	no estimate	no estimate	no estimate	no estimate
GS5	1,3-Dimethylnaphthalene	2,909	1,100	0.38	no estimate	no estimate	no estimate	no estimate
GS3	2-Methylnaphthalene	2,659	1,089	0.41	no estimate	no estimate	no estimate	no estimate
GS4	2-Methylnaphthalene	2,142	1,089	0.51	no estimate	no estimate	no estimate	no estimate
GS6	1,3-Dimethylnaphthalene	1,854	1,100	0.59	no estimate	no estimate	no estimate	no estimate
GS9	Phenanthrene	1,783	1,100	0.62	no estimate	no estimate	no estimate	no estimate
GS1	Naphthalene	1,450	1,051	0.72	no estimate	no estimate	no estimate	no estimate
GS2	Naphthalene	1,137	1,051	0.92	no estimate	no estimate	no estimate	no estimate
GS13	9-Ethylphenanthrene	731	1,103	1.51	no estimate	no estimate	no estimate	no estimate
GS10	Phenanthrene	680	1,100	1.62	no estimate	no estimate	no estimate	no estimate
GS11	9-Methylphenanthrene	623	1,102	1.77	no estimate	no estimate	no estimate	no estimate
GS12	9-Methylphenanthrene	290	1,102	3.80	no estimate	no estimate	no estimate	no estimate
GS14	9-Ethylphenanthrene	263	1,103	4.20	no estimate	no estimate	no estimate	no estimate
GS16	Pyrene	129	1,102	8.55	no estimate	no estimate	no estimate	no estimate
GS15	Pyrene	116	1,102	9.50	no estimate	no estimate	no estimate	no estimate

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.25 Gold standard data set – predictions (Part C)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Barber (2001)		Barber *et al*. (1991)	
			$k_{1\ (pred)}$	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$	$k_{1\ (pred)}/k_{1\ (exp)}$
GS32	1,2,4-Trichlorobenzene	492	780	1.59	778	1.58
GS45	Hexachlorobenzene	1,850	905	0.49	921	0.50
GS44	1,2,4-Tribromobenzene	1,040	905	0.87	921	0.89
GS43	1,2,3,5-Tetrachlorobenzene	631	905	1.43	921	1.46
GS42	1,2,3-Trichlorobenzene	470	905	1.93	921	1.96
GS41	1,4-Dibromobenzene	272	905	3.33	921	3.39
GS40	1,4-Dichlorobenzene	112	905	8.08	921	8.22
GS7	2-Isopropylnaphthalene	4,188	599	0.14	577	0.14
GS8	2-Isopropylnaphthalene	3,746	599	0.16	577	0.15
GS5	1,3-Dimethylnaphthalene	2,909	599	0.21	577	0.20
GS3	2-Methylnaphthalene	2,659	599	0.23	577	0.22
GS4	2-Methylnaphthalene	2,142	599	0.28	577	0.27
GS6	1,3-Dimethylnaphthalene	1,854	599	0.32	577	0.31
GS9	Phenanthrene	1,783	599	0.34	577	0.32
GS1	Naphthalene	1,450	599	0.41	577	0.40
GS2	Naphthalene	1,137	599	0.53	577	0.51
GS13	9-Ethylphenanthrene	731	599	0.82	577	0.79
GS10	Phenanthrene	680	599	0.88	577	0.85
GS11	9-Methylphenanthrene	623	599	0.96	577	0.93
GS12	9-Methylphenanthrene	290	599	2.07	577	1.99
GS14	9-Ethylphenanthrene	263	599	2.28	577	2.20
GS16	Pyrene	129	599	4.64	577	4.48
GS15	Pyrene	116	599	5.16	577	4.98

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.26 Gold standard data set – predictions (Part D)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Erickson and McKim (1990a)		Erickson and McKim (1990b)		Gobas and Mackay (1987)	
			$k_{1\ (pred)}$	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$	$k_{1\ (pred)}/k_{1\ (exp)}$
GS32	1,2,4-Trichlorobenzene	492	755	1.53	673	1.37	1,676	3.41
GS45	Hexachlorobenzene	1,850	873	0.47	832	0.45	2,415	1.31
GS44	1,2,4-Tribromobenzene	1,040	873	0.84	832	0.80	2,415	2.32
GS43	1,2,3,5-Tetrachlorobenzene	631	873	1.38	832	1.32	2,415	3.83
GS42	1,2,3-Trichlorobenzene	470	873	1.86	832	1.77	2,415	5.14
GS41	1,4-Dibromobenzene	272	873	3.21	832	3.06	2,415	8.88
GS40	1,4-Dichlorobenzene	112	873	7.79	832	7.43	2,415	21.56
GS7	2-Isopropylnaphthalene	4,188	583	0.14	464	0.11	879	0.21
GS8	2-Isopropylnaphthalene	3,746	583	0.16	464	0.12	879	0.23
GS5	1,3-Dimethylnaphthalene	2,909	583	0.20	464	0.16	879	0.30
GS3	2-Methylnaphthalene	2,659	583	0.22	464	0.17	879	0.33
GS4	2-Methylnaphthalene	2,142	583	0.27	464	0.22	879	0.41
GS6	1,3-Dimethylnaphthalene	1,854	583	0.31	464	0.25	879	0.47
GS9	Phenanthrene	1,783	583	0.33	464	0.26	879	0.49
GS1	Naphthalene	1,450	583	0.40	464	0.32	879	0.61
GS2	Naphthalene	1,137	583	0.51	464	0.41	879	0.77
GS13	9-Ethylphenanthrene	731	583	0.80	464	0.63	879	1.20
GS10	Phenanthrene	680	583	0.86	464	0.68	879	1.29
GS11	9-Methylphenanthrene	623	583	0.94	464	0.74	879	1.41
GS12	9-Methylphenanthrene	290	583	2.01	464	1.60	879	3.03
GS14	9-Ethylphenanthrene	263	583	2.22	464	1.76	879	3.34
GS16	Pyrene	129	583	4.52	464	3.59	879	6.81
GS15	Pyrene	116	583	5.03	464	4.00	879	7.58

[1] All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2] $k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3] $k_{1\ (pred)}$ is the predicted k_1 value.

Table A.27 Gold standard data set – predictions (Part E)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Gobas *et al*. (1986)		Hayton and Barron (1990)		Norstrom *et al*. (1976)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
GS32	1,2,4-Trichlorobenzene	492	3,773	7.67	582	1.18	166	0.34
GS45	Hexachlorobenzene	1,850	5,062	2.74	698	0.38	186	0.10
GS44	1,2,4-Tribromobenzene	1,040	5,062	4.87	698	0.67	186	0.18
GS43	1,2,3,5-Tetrachlorobenzene	631	5,062	8.02	698	1.11	186	0.29
GS42	1,2,3-Trichlorobenzene	470	5,062	10.77	698	1.48	186	0.40
GS41	1,4-Dibromobenzene	272	5,062	18.61	698	2.56	186	0.68
GS40	1,4-Dichlorobenzene	112	5,062	45.19	698	6.23	186	1.66
GS7	2-Isopropylnaphthalene	4,188	2,245	0.54	422	0.10	135	0.03
GS8	2-Isopropylnaphthalene	3,746	2,245	0.60	422	0.11	135	0.04
GS5	1,3-Dimethylnaphthalene	2,909	2,245	0.77	422	0.15	135	0.05
GS3	2-Methylnaphthalene	2,659	2,245	0.84	422	0.16	135	0.05
GS4	2-Methylnaphthalene	2,142	2,245	1.05	422	0.20	135	0.06
GS6	1,3-Dimethylnaphthalene	1,854	2,245	1.21	422	0.23	135	0.07
GS9	Phenanthrene	1,783	2,245	1.26	422	0.24	135	0.08
GS1	Naphthalene	1,450	2,245	1.55	422	0.29	135	0.09
GS2	Naphthalene	1,137	2,245	1.97	422	0.37	135	0.12
GS13	9-Ethylphenanthrene	731	2,245	3.07	422	0.58	135	0.18
GS10	Phenanthrene	680	2,245	3.30	422	0.62	135	0.20
GS11	9-Methylphenanthrene	623	2,245	3.60	422	0.68	135	0.22
GS12	9-Methylphenanthrene	290	2,245	7.74	422	1.45	135	0.46
GS14	9-Ethylphenanthrene	263	2,245	8.54	422	1.60	135	0.51
GS16	Pyrene	129	2,245	17.40	422	3.27	135	1.04
GS15	Pyrene	116	2,245	19.35	422	3.64	135	1.16

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.28 Gold standard data set – predictions (Part F)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Streit and Sire (1993)		Thomann and Connolly (1984)		Barber (2003) - observed	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
GS32	1,2,4-Trichlorobenzene	492	458	0.93	149	0.30	514	1.05
GS45	Hexachlorobenzene	1,850	530	0.29	173	0.09	617	0.33
GS44	1,2,4-Tribromobenzene	1,040	530	0.51	173	0.17	617	0.59
GS43	1,2,3,5-Tetrachlorobenzene	631	530	0.84	173	0.27	617	0.98
GS42	1,2,3-Trichlorobenzene	470	530	1.13	173	0.37	617	1.31
GS41	1,4-Dibromobenzene	272	530	1.95	173	0.64	617	2.27
GS40	1,4-Dichlorobenzene	112	530	4.73	173	1.55	617	5.51
GS7	2-Isopropylnaphthalene	4,188	354	0.08	113	0.03	372	0.09
GS8	2-Isopropylnaphthalene	3,746	354	0.09	113	0.03	372	0.10
GS5	1,3-Dimethylnaphthalene	2,909	354	0.12	113	0.04	372	0.13
GS3	2-Methylnaphthalene	2,659	354	0.13	113	0.04	372	0.14
GS4	2-Methylnaphthalene	2,142	354	0.17	113	0.05	372	0.17
GS6	1,3-Dimethylnaphthalene	1,854	354	0.19	113	0.06	372	0.20
GS9	Phenanthrene	1,783	354	0.20	113	0.06	372	0.21
GS1	Naphthalene	1,450	354	0.24	113	0.08	372	0.26
GS2	Naphthalene	1,137	354	0.31	113	0.10	372	0.33
GS13	9-Ethylphenanthrene	731	354	0.48	113	0.16	372	0.51
GS10	Phenanthrene	680	354	0.52	113	0.17	372	0.55
GS11	9-Methylphenanthrene	623	354	0.57	113	0.18	372	0.60
GS12	9-Methylphenanthrene	290	354	1.22	113	0.39	372	1.28
GS14	9-Ethylphenanthrene	263	354	1.34	113	0.43	372	1.42
GS16	Pyrene	129	354	2.74	113	0.88	372	2.89
GS15	Pyrene	116	354	3.05	113	0.98	372	3.21

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.29 Gold standard data set – predictions (Part G)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Barber (2003) - calibrated		Hawker and Connell (1985)		Hawker and Connell (1988)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
GS32	1,2,4-Trichlorobenzene	492	900	1.83	9.59	0.019	18.70	0.038
GS45	Hexachlorobenzene	1,850	1,329	0.72	36.14	0.020	33.28	0.018
GS44	1,2,4-Tribromobenzene	1,040	1,326	1.28	15.76	0.015	28.52	0.027
GS43	1,2,3,5-Tetrachlorobenzene	631	1,326	2.10	14.58	0.023	27.42	0.043
GS42	1,2,3-Trichlorobenzene	470	1,317	2.80	9.81	0.021	19.27	0.041
GS41	1,4-Dibromobenzene	272	1,308	4.81	8.02	0.029	14.25	0.052
GS40	1,4-Dichlorobenzene	112	1,282	11.44	6.11	0.055	8.30	0.074
GS7	2-Isopropylnaphthalene	4,188	463	0.11	15.39	0.004	28.21	0.007
GS8	2-Isopropylnaphthalene	3,746	463	0.12	15.39	0.004	28.21	0.008
GS5	1,3-Dimethylnaphthalene	2,909	463	0.16	13.08	0.004	25.58	0.009
GS3	2-Methylnaphthalene	2,659	458	0.17	8.47	0.003	15.60	0.006
GS4	2-Methylnaphthalene	2,142	458	0.21	8.47	0.004	15.60	0.007
GS6	1,3-Dimethylnaphthalene	1,854	463	0.25	13.08	0.007	25.58	0.014
GS9	Phenanthrene	1,783	463	0.26	13.49	0.008	26.14	0.015
GS1	Naphthalene	1,450	442	0.30	5.48	0.004	6.45	0.004
GS2	Naphthalene	1,137	442	0.39	5.48	0.005	6.45	0.006
GS13	9-Ethylphenanthrene	731	464	0.64	27.55	0.038	32.65	0.045
GS10	Phenanthrene	680	463	0.68	13.49	0.020	26.14	0.038
GS11	9-Methylphenanthrene	623	464	0.74	18.83	0.030	30.48	0.049
GS12	9-Methylphenanthrene	290	464	1.60	18.83	0.065	30.48	0.105
GS14	9-Ethylphenanthrene	263	464	1.77	27.55	0.105	32.65	0.124
GS16	Pyrene	129	464	3.60	18.69	0.145	30.41	0.236
GS15	Pyrene	116	464	4.00	18.69	0.161	30.41	0.262

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

Table A.30 Gold standard data set – predictions (Part H)[1]

Ref#	Name	$k_{1\ (exp)}$[2]	Spacie and Hamelink (1982)		Tolls and Sijm (1995)	
			$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$	$k_{1\ (pred)}$[3]	$k_{1\ (pred)}/k_{1\ (exp)}$
GS32	1,2,4-Trichlorobenzene	492	372	0.76	481	0.98
GS45	Hexachlorobenzene	1,850	664	0.36	778	0.42
GS44	1,2,4-Tribromobenzene	1,040	462	0.44	576	0.55
GS43	1,2,3,5-Tetrachlorobenzene	631	447	0.71	560	0.89
GS42	1,2,3-Trichlorobenzene	470	376	0.80	485	1.03
GS41	1,4-Dibromobenzene	272	344	1.27	451	1.66
GS40	1,4-Dichlorobenzene	112	306	2.73	409	3.65
GS7	2-Isopropylnaphthalene	4,188	458	0.11	571	0.14
GS8	2-Isopropylnaphthalene	3,746	458	0.12	571	0.15
GS5	1,3-Dimethylnaphthalene	2,909	426	0.15	539	0.19
GS3	2-Methylnaphthalene	2,659	353	0.13	460	0.17
GS4	2-Methylnaphthalene	2,142	353	0.16	460	0.21
GS6	1,3-Dimethylnaphthalene	1,854	426	0.23	539	0.29
GS9	Phenanthrene	1,783	432	0.24	545	0.31
GS1	Naphthalene	1,450	292	0.20	393	0.27
GS2	Naphthalene	1,137	292	0.26	393	0.35
GS13	9-Ethylphenanthrene	731	590	0.81	705	0.96
GS10	Phenanthrene	680	432	0.64	545	0.80
GS11	9-Methylphenanthrene	623	500	0.80	615	0.99
GS12	9-Methylphenanthrene	290	500	1.72	615	2.12
GS14	9-Ethylphenanthrene	263	590	2.24	705	2.68
GS16	Pyrene	129	498	3.86	613	4.75
GS15	Pyrene	116	498	4.29	613	5.28

[1]All k_1 values are in units of l kg^{-1} day^{-1}. All predictions are based on the initial fish weight.
[2]$k_{1\ (exp)}$ is the experimentally determined k_1 value.
[3]$k_{1\ (pred)}$ is the predicted k_1 value.

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