Environment Agency

Evidence

Bioaccumulation of chemicals in fish:

Investigation of the dependence of depuration rate constant on lipid content of fish

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Evidence at the Environment Agency

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Miranda Kavanagh

Director of Evidence

Executive summary

A method for determining the accumulation of chemicals in fish following dietary exposure has recently been agreed and incorporated into the OECD 305 test guideline. The new test method underwent a ring test during 2011 and 2012 and the results were analysed by the Environment Agency resulting in a validation report.

This analysis highlighted a number of technical considerations for the interpretation of data from dietary bioaccumulation tests. In this report we investigate further the following aspects using the ring test and other relevant data:

- The potential relationship between fish lipid content and the (growth-corrected) depuration rate constant.
- Selection of the most appropriate depuration rate constant for bioconcentration factor (BCF) estimation.
- Experimental uncertainty in key parameters derived from the study.

Our analysis of depuration data has shown that the growth-corrected depuration rate constant shows a dependence on the lipid content of the fish, in accordance with bioaccumulation theory. This confirms that the (growth-corrected) depuration rate constant should be normalised to a 'standard' lipid content to allow comparisons for this parameter to be made between different studies (as shown in the report, this normalisation is actually the theoretical basis for the commonly used lipid normalisation of BCF as recommended in the OECD 305 test guideline). This lipid normalisation is an important consideration when using the growth-corrected depuration rate constant from an OECD 305 feeding study to calculate a BCF value using an estimate for the uptake rate constant (it will not influence the calculation of biomagnification factor (BMF), which is corrected for the lipid content of both fish and food). Our analysis suggests that either the mean lipid content at the end of the depuration phase, the arithmetic mean concentration of the two sampling points over the depuration phase, or the time-weighted average lipid over the depuration phase are appropriate measures for carrying out this normalisation.

Selection of the most appropriate depuration rate constant for BCF estimation is difficult as most of the existing experimental BCF data have not been growth corrected and it is not always clear whether they have been normalised to a standard lipid content. However, based on our analysis, the most appropriate depuration rate constant to use is the growth-corrected and lipid-normalised depuration rate constant. This is in keeping with the basis for bioconcentration and biomagnification factors recommended in the OECD 305 test guideline.

Equations for approximating the propagation of errors in the bioaccumulation parameters derived from an OECD 305 dietary study are presented. However, these are subject to a number of assumptions which may not be valid in all cases. We recommend that these equations are used cautiously and other methods, such as those based on Monte Carlo analysis, are explored for comparative purposes (e.g. distributions other than normal distributions can theoretically be considered).

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1 Introduction

1.1 Background

A method for determining the accumulation of chemicals in fish following dietary exposure has recently been agreed and incorporated into the OECD 305 test guideline (OECD 2012a). The new test method underwent a ring test during 2011 and 2012 and the results (tests carried out using a 3% of body weight feeding rate) were analysed by the Environment Agency and a validation report was produced (OECD 2012b). Further analysis of data obtained in the ring test at a lower feeding rate (1.5% of body weight) was also undertaken and is reported in OECD (2013).

This analysis highlighted a number of technical considerations for the interpretation of data from dietary bioaccumulation tests. In this report we investigate further the following aspects using the ring test and other relevant data:

- The potential relationship between fish lipid content and the (growth-corrected) depuration rate constant.
- Selection of the most appropriate depuration rate constant for bioconcentration factor (BCF) estimation.
- Experimental uncertainty in key parameters derived from the study.

1.2 Relationship between fish lipid content and the (growth-corrected) depuration rate constant

It is widely accepted that changes in fish lipid content will affect the rate at which an accumulated lipophilic substance is depurated. This has been discussed briefly in previous reports (OECD 2012b, 2013, among others), where it was recognised that each of the terms that are accepted to contribute to the overall depuration rate constant may be differently affected by changes in the lipid content. The co-variance of the fish lipid and depuration rate constant was addressed briefly in the previous ring test reports, and is explored further here.

The data covered in the ring test report (test runs with a feeding rate of 3%; OECD 2012b) suggested that there was a slight decreasing trend in the overall depuration rate constant with increasing fish lipid content for four of the five test substances. The opposite appeared to be the case for the remaining substance; however, depuration for this substance was so rapid that it is entirely plausible that this was simply an artefact of limited data. Similar analysis for the growth-corrected depuration rate constant against fish lipid content showed even less evidence for such a trend (no significant differences at the $p>0.05$ level). The report noted that analysis of this kind is not easy, because the limited size of the datasets available makes it difficult to draw definitive conclusions on whether the observed trends are real or not.

However, the comparison between studies with differing feeding rates (1.5% versus 3% of body weight per day) showed a clearer trend (OECD 2013). For three of the five substances, overall depuration rate constants between the two sets of studies differed little (comparisons were not possible for the other two substances due to lower quality data). Comparison of growth-corrected depuration rate constants (k_{2g}) showed that, for the three studies with better quality data in the lower feeding rate studies, the k_{2g} values were higher in the 1.5% feeding rate studies, appearing to co-vary with the average lipid content difference. Plots of fish lipid contents for each study versus k_{2g}

showed a dependence, seemingly more marked for the faster depurating substances. This fact will not influence the calculation of biomagnification factor (BMF; which is lipid corrected for fish and food), but may have an important effect on the estimation of a BCF using kinetic data from the study.

In this project, we have investigated the effect of fish lipid content on depuration rate constant further using more available data. This has also included an investigation of which (average) lipid content is the 'best' to use for normalisation, as lipid contents are only measured at a few time points in experiments and lipid contents generally increase over the course of an experiment.

1.3 Selection of the most appropriate depuration rate constant for BCF estimation

The two reports which discussed the dietary ring test results (OECD 2012b, 2013) estimated indicative BCFs using the overall depuration and the growth-corrected depuration rate constants (the latter being appended to the reports). Uptake rate constants were estimated using the 'best' performing 13 models from a previous Environment Agency report (Crookes and Brooke 2011, Brooke *et al*., 2012). The estimates were made for the purpose of comparing them against measured BCF data for the ring test substances in the literature. The overall depuration rate constants were used because 'historical' measured values were likely not to have been corrected for growth.

Taking into account our findings for the relationship between fish lipid and depuration rate constants described above, in this report we have recalculated indicative BCFs according to several measures of depuration rate constant and we make recommendations on the most suitable of these for estimating BCFs.

1.4 Dealing with experimental uncertainty

In the OECD 305 dietary accumulation test a number of experimental values are derived from the raw data and used in the subsequent calculation of various bioaccumulation parameters. It is recognised that errors in bioaccumulation parameters propagate through calculation so that their effect in the final estimation of a bioaccumulation metric can be marked. However, only limited guidance is given in the OECD 305 test guideline on how to assess these errors in the final results.

Here, we have investigated to what extent it is possible to use measures of error from the various measured and derived parameters in a dietary study to derive BMFs with confidence intervals. This includes investigation of the error around the depuration rate constant (k_2) estimation and its use in the estimation of a BCF.

2 Investigation of the dependence of the depuration rate constant on fish lipid content

2.1 Existing OECD 305 ring test data

This part of the project builds on the analysis carried out previously for the OECD 305 dietary ring test. The OECD 305 ring test data have been evaluated previously in two reports. One of these reports, covering the data obtained using a feeding rate of 3% of body weight was published on the OECD website (OECD 2012b). The second report covering the data obtained using a feeding rate of 1.5% of body weight is now also available (OECD 2013).

For the reanalysis of the data, the values for the various depuration rate constants are taken from the above two reports. However, in order to investigate the effects of fish lipid on the derived results, a more detailed analysis of the lipid data reported in OECD (2012b, 2013) has been undertaken.

2.1.1 Depuration rate constants

The overall depuration rate constants (k_2) and the growth-corrected depuration rate constants (k_{2g}) from the OECD 305 ring test are summarised in Tables 2.1 to 2.5 (notes for all five tables are placed after Table 2.5). In all cases the k_2 value was obtained from the slope of a plot of the natural logarithm (ln) of concentration in fish (µg g^{-1}) against time for the depuration period. The intercept of this plot is ln [C_0], where C_0 is the concentration in fish at the start of the depuration period. The experiments were carried out using a feeding rate of 3% of body weight (Labs 1 to 8) or 1.5% of body weight (Labs 9 and 10) in rainbow trout (unless stated otherwise in the tables).

The rate constants for growth dilution (k_g) obtained previously are summarised in Table 2.6. These were obtained from the slope of a plot of ln [1/fish weight (g)] against time for the uptake phase, the depuration phase and the combined uptake and depuration phase. The preferred values are shown in **bold** (see OECD 2012b, 2013 for a discussion of how these preferred values were selected).

The growth-corrected depuration rate constants (k_{2g}) from OECD (2012b, 2013) are summarised in Tables 2.7 to 2.11. These were obtained by subtracting the k_g values from the k_2 values, assuming that both overall depuration and growth dilution were first-order kinetic processes in the concentration in fish (indicated as the OECD 305 method in the tables). In addition, the k_{2g} values were also estimated using an alternative method based on Brooke and Crookes (2012) (these are indicated as alternative method in the tables).

Differences and similarities in the k_2, k_g and k_{2g} values resulting from these two different feeding rates were considered in OECD (2013). In particular this analysis found that the growth-corrected depuration rate constants (k_{2g}) obtained using the rate constant subtraction method were statistically significantly lower at the 3% feeding rate than at the 1.5% feeding rate for hexachlorobenzene, musk xylene and o-terphenyl (no meaningful comparison could be carried out for methoxychlor and benzo[a]pyrene). We hypothesised that this difference could result from the higher lipid contents in the fish fed at the 3% feeding rate compared with the 1.5% feeding rate. Some evidence for the

dependence of k_{2g} on the fish lipid content was apparent, particularly for hexachlorobenzene and musk xylene, when plots of k_{2g} against lipid content were constructed (see OECD 2013 for a discussion). This is investigated further in the following sections.

Table 2.1 Summary of overall depuration rate constants and C_0 values for hexachlorobenzene

Laboratory	k_2 (day^{-1}) from slope	Intercept		R^2 value of regression	Standard error in slope (k_2)	Standard error in intercept (ln [C_0])	95% Confidence interval – k_2	95% Confidence interval – ln [C_0]
		ln [C_0]	[C_0] ($\mu g\ g^{-1}$)					
Lab 1	0.0502	1.275	3.58	0.78	0.005	0.079	0.040 to 0.061	1.112 to 1.437
Lab 2a – trout	0.0399	1.736	5.68	0.88	0.003	0.044	0.034 to 0.046	1.646 to 1.827
Lab 2b – carp (level 1)[a]	0.0603	3.170	23.81	0.90	0.011	0.123	0.024 to 0.097	2.779 to 3.561
Lab 2b – carp (level 2)[a]	0.0561	2.529	12.54	0.99	0.004	0.039	0.045 to 0.068	2.405 to 2.653
Lab 2b – carp (level 3)[a]	0.0486	0.839	2.31	0.71	0.018	0.193	-0.008 to 0.105	0.226 to 1.452
Lab 3	0.0537	1.533	4.63	0.85	0.004	0.066	0.045 to 0.062	1.398 to 1.669
Lab 4	0.0517	1.535	4.64	0.85	0.004	0.063	0.043 to 0.060	1.406 to 1.665
Lab 5	0.0407	2.282	9.80	0.36	0.010	0.160	0.020 to 0.062	1.954 to 2.610
Lab 6	0.0625	1.509	4.52	0.87	0.005	0.078	0.053 to 0.072	1.350 to 1.668
Lab 7	0.0491	0.701	2.02	0.72	0.006	0.090	0.037 to 0.061	0.517 to 0.885
Lab 8	0.0579	1.332	3.79	0.90	0.004	0.060	0.050 to 0.065	1.210 to 1.455
Lab 9	0.0589	1.219	3.38	0.92	0.003	0.049	0.053 to 0.065	1.118 to 1.320
Lab 10	0.0485	0.600	1.82	0.59	0.008	0.119	0.033 to 0.064	0.357 to 0.844

Table 2.2 Summary of overall depuration rate constants and C_0 values for musk xylene

Laboratory	k_2 (day^{-1}) from slope	Intercept		R^2 value of regression	Standard error in slope (k_2)	Standard error in intercept (ln [C_0])	95% Confidence interval – k_2	95% Confidence interval – ln [C_0]
		ln [C_0]	[C_0] ($\mu g\ g^{-1}$)					
Lab 1	0.0904	1.460	4.30	0.88	0.006	0.097	0.078 to 0.103	1.261 to 1.658
Lab 2a – trout	0.0734	2.340	10.38	0.93	0.004	0.059	0.066 to 0.081	2.218 to 2.462
Lab 2b – carp (level 1)[a]	0.140	3.654	38.63	0.98	0.012	0.133	0.100 to 0.179	3.231 to 4.077
Lab 2b – carp (level 2)[a]	0.131	2.994	19.97	0.99	0.006	0.068	0.110 to 0.151	2.777 to 3.211
Lab 2b – carp (level 3)[a]	0.111	1.124	3.08	0.96	0.013	0.136	0.071 to 0.151	0.690 to 1.558
Lab 3	0.083	2.178	8.83	0.90	0.005	0.082	0.072 to 0.094	2.010 to 2.347
Lab 4	0.067	1.706	5.51	0.75	0.007	0.116	0.052 to 0.083	1.467 to 1.944
Lab 5	0.647	-1.705	0.182	0.86	0.073	0.326	0.488 to 0.805	-2.409 to -1.001
Lab 6	0.105	1.609	5.00	0.92	0.006	0.095	0.093 to 0.117	1.413 to 1.805
Lab 7	0.105	0.766	2.15	0.82	0.009	0.148	0.085 to 0.124	0.464 to 1.069
Lab 8	0.0948	1.761	5.82	0.95	0.004	0.065	0.086 to 0.103	1.627 to 1.895
Lab 9	0.110	1.563	4.77	0.95	0.005	0.074	0.101 to 0.120	1.411 to 1.714
Lab 10	0.105	0.805	2.24	0.70	0.013	0.207	0.078 to 0.132	0.380 to 1.229

Table 2.3 Summary of overall depuration rate constants and C_0 values for o-terphenyl

Laboratory	k_2 (day^{-1}) from slope	Intercept		R^2 value of regression	Standard error in slope (k_2)	Standard error in intercept (ln [C_0])	95% Confidence interval – k_2	95% Confidence interval – ln [C_0]
		ln [C_0]	[C_0] ($\mu g\ g^{-1}$)					
Lab 1	0.0872	1.224	3.40	0.82	0.008	0.121	0.071 to 0.103	0.976 to 1.471
Lab 2a – trout	0.0691	1.786	5.97	0.68	0.009	0.143	0.051 to 0.088	1.493 to 2.079
Lab 2b – carp (level 1)[a]	0.290	3.444	31.32	0.99	0.010	0.107	0.259 to 0322	3.105 to 3.783
Lab 2b – carp (level 2)[a]	0.351	2.443	11.50	0.98	0.033	0.194	0.209 to 0.492	1.607 to 3.279
Lab 2b – carp (level 3)[a]	0.297	-0.217	0.81	0.99	0.016	0.094	0.229 to 0.365	-0.619 to 0.186
Lab 3	0.104	1.614	5.02	0.79	0.011	0.152	0.082 to 0.125	1.302 to 1.925
Lab 4	0.0770	1.365	3.92	0.43	0.017	0.266	0.042 to 0.112	0.821 to 1.909
Lab 5	0.0445	2.402	11.04	0.40	0.010	0.161	0.023 to 0.066	2.071 to 2.732
Lab 6	0.133	1.086	2.96	0.73	0.016	0.256	0.101 to 0.166	0.559 to 1.613
Lab 7	0.0775	0.875	2.40	0.78	0.008	0.123	0.062 to 0.093	0.624 to 1.126
Lab 8	0.113	1.473	4.36	0.75	0.012	0.199	0.088 to 0.139	1.065 to 1.881
Lab 9	0.109	1.304	3.68	0.90	0.007	0.099	0.093 to 0.124	1.100 to 1.508
Lab 10	0.126	0.780	2.18	0.74	0.014	0.224	0.097 to 0.156	0.319 to 1.239

Table 2.4 Summary of overall depuration rate constants and C_0 values for methoxychlor

Laboratory	k_2 (day^{-1}) from slope	Intercept		R^2 value of regression	Standard error in slope (k_2)	Standard error in intercept (ln [C_0])	95% Confidence interval – k_2	95% Confidence interval – ln [C_0]
		ln [C_0]	[C_0] ($\mu g\ g^{-1}$)					
Lab 1	0.150	0.762	2.14	0.66	0.025	0.259	0.098 to 0.202	0.219 to 1.304
Lab 2a – trout	0.116	2.412	11.16	0.74	0.013	0.203	0.089 to 0.142	1.997 to 2.827
Lab 2b – carp (level 1)[a]	0.310	2.249	9.48	0.97	0.041	0.245	0.132 to 0.488	1.197 to 3.301
Lab 2b – carp (level 2)[a]	0.294	1.456	4.29	0.97	0.037	0.216	0.137 to 0.451	0.528 to 2.385
Lab 2b – carp (level 3)[a]	0.264	-0.466	0.63	0.89	0.095	0.422	-0.945 to 1.473	-5.827 to 4.895
Lab 3	0.102	1.176	3.24	0.56	0.018	0.271	0.065 to 0.140	0.618 to 1.735
Lab 4	0.0922	0.813	2.25	0.38	0.024	0.813	0.043 to 0.141	0.079 to 1.546
Lab 5	0.0046	1.531	4.62	0.003	0.017	0.262	-0.030 to 0.039	0.995 to 2.066
Lab 6	0.225	0.572	1.77	0.87	0.020	0.241	0.183 to 0.268	0.066 to 1.077
Lab 7	0.148	1.389	4.01	0.90	0.009	0.145	0.129 to 0.167	1.091 to 1.687
Lab 8	0.110	0.988	2.69	0.51	0.021	0.329	0.068 to 0.153	0.313 to 1.663
Lab 9	No data	No data	No data	No data	No data	No data	No data	No data
Lab 10	0.199	-0.722	0.49	0.66	0.027	0.425	0.144 to 0.255	-1.592 to 0.148

Table 2.5 Summary of overall depuration rate constants and C_0 values for benzo[a]pyrene

Laboratory	k_2 (day^{-1}) from slope	Intercept		R^2 value of regression	Standard error in slope (k_2)	Standard error in intercept (ln [C_0])	95% Confidence interval – k_2	95% Confidence interval – ln [C_0]
		ln [C_0]	[C_0] ($\mu g\ g^{-1}$)					
Lab 1	0.986	-0.666	0.51	0.81	0.236	0.360	0.331 to 1.641	-1.666 to 0.334
Lab 2a – trout	2.094	1.568	4.80	0.93	0.208	0.464	1.615 to 2.572	0.497 to 2.639
Lab 2b – carp (levels 1, 2 and 3)[a]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]
Lab 3	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]
Lab 4	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]
Lab 5	2.066	2.402	11.05	0.77	0.398	0.889	1.149 to 2.983	0.353 to 4.452
Lab 6	1.684	1.840	6.29	0.92	0.225	0.530	1.107 to 2.261	0.477 to 3.202
Lab 7	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]
Lab 8	1.179	0.541	1.72	0.92	0.120	0.268	0.902 to 1.456	-0.077 to 1.160
Lab 9	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]
Lab 10	0.077[c]	-3.740	0.024	0.17	0.032	0.502	0.011 to 0.143	-4.769 to -2.711
	0.298[d]	-2.945	0.053	0.07	0.38	0.85	-1.175 to 0.578	-4.904 to -0.985

Notes:
[a] Levels 1, 2 and 3 refer to different concentrations of the test substances in the food, with level 1 having the highest concentrations.
[b] Owing to rapid depuration, there were insufficient data points available to derive the depuration curve.
[c] Derived using all reported data during the depuration period.
[d] Derived using only the data reported for depuration days 1 and 3.

Table 2.6 Summary of growth rate constants

Laboratory	Time frame	Control group			Test group			Combined group		
		k_{growth} (day^{-1})	R^2 value of regression	Standard error in slope (k_{growth})	k_{growth} (day^{-1})	R^2 value of regression	Standard error in slope (k_{growth})	k_{growth} (day^{-1})	R^2 value of regression	Standard error in slope (k_{growth})
Lab 1	Uptake	0.0277	0.49	0.0079	0.0428	0.58	0.0101	0.0352	0.52	0.0063
	Depuration	0.0373	0.69	0.0043	0.0347	0.72	0.0038	0.0360	0.70	0.0028
	Overall	0.0357	0.81	0.0025	0.0375	0.84	0.0023	**0.0366**	0.83	0.0017
Lab 2a – trout	Uptake	0.0110	0.55	0.0035	0.0119	0.43	0.0049	0.0114	0.42	0.0037
	Depuration	0.0199	0.71	0.0024	0.0152	0.55	0.0026	0.0175	0.61	0.0018
	Overall	0.0162	0.74	0.0016	0.0159	0.71	0.0017	**0.0165**	0.68	0.0013
Lab 2b – carp (level 1)[a]	Uptake	0.0225*	0.80	0.0040	0.0217	0.83	0.0035	0.0204*	0.50	0.0042
	Depuration	0.0379*	0.84	0.0035	0.0302	0.83	0.0029	**0.0334***	0.79	0.0017
	Overall	0.0299	0.85	0.0022	0.0245	0.85	0.0018	0.0298	0.79	0.0014
Lab 2b – carp (level 2)[a]	Uptake	As above[a]			0.0204	0.62	0.0056	As above[a]		
	Depuration				0.0320	0.75	0.0038			
	Overall				0.0259	0.78	0.0024			
Lab 2b – carp (level 3)[a]	Uptake	As above[a]			0.0170*	0.60	0.0049	As above[a]		
	Depuration				0.0334*	0.80	0.0034			
	Overall				0.0278	0.83	0.0022			
Lab 3	Uptake	0.0448	0.75	0.0071	0.0441	0.80	0.0061	0.0444	0.78	0.0045
	Depuration	0.0431	0.90	0.0025	0.0441	0.88	0.0029	0.0436	0.89	0.0019
	Overall	0.0442	0.94	0.0015	0.0438	0.93	0.0017	**0.0440**	0.94	0.0011
Lab 4	Uptake	0.0466	0.81	0.0062	0.0402	0.64	0.0084	0.0406	0.67	0.0059
	Depuration	0.0394	0.79	0.0035	0.0278	0.65	0.0035	0.0336	0.72	0.0025

Laboratory	Time frame	Control group			Test group			Combined group		
		k_{growth} (day^{-1})	R^2 value of regression	Standard error in slope (k_{growth})	k_{growth} (day^{-1})	R^2 value of regression	Standard error in slope (k_{growth})	k_{growth} (day^{-1})	R^2 value of regression	Standard error in slope (k_{growth})
	Overall	0.0419	0.89	0.0020	0.0374	0.83	0.0024	**0.0386**	0.85	0.0017
Lab 5	Uptake	0.0058	0.012	0.0184	0.0160	0.08	0.0192	0.0109	0.04	0.0157
	Depuration	0.0116	0.10	0.0065	0.0106	0.08	0.0064	0.0107	0.08	0.0045
	Overall	0.0187	0.29	0.0047	0.0151	0.21	0.0045	**0.0158**	0.20	0.0036
Lab 6	Uptake	0.0584*	0.81	0.0077	0.0631*	0.85	0.0074	0.0598*	0.82	0.0058
	Depuration	0.0388*	0.88	0.0025	0.0345*	0.79	0.0031	**0.0367***	0.83	0.0020
	Overall	0.0405	0.92	0.0017	0.0391	0.88	0.0021	0.0389	0.89	0.0014
Lab 7	Uptake	0.0273	0.59	0.0064	0.0329	0.51	0.0089	0.0315	0.54	0.0060
	Depuration	0.0367	0.84	0.0028	0.0302	0.68	0.0038	0.0336	0.77	0.0023
	Overall	0.0300	0.84	0.0018	0.0305	0.80	0.0023	**0.0307**	0.81	0.0015
Lab 8	Uptake	0.0249*	0.36	0.0092	0.0480	0.74	0.0078	0.0364	0.55	0.0062
	Depuration	0.0499*	0.83	0.0039	0.0445	0.92	0.0023	0.0471	0.87	0.0023
	Overall	0.0496	0.89	0.0025	0.0470	0.95	0.0015	**0.0483**	0.92	0.0014
Lab 9	Uptake	0.024	0.48	0.006	0.033	0.55	0.007	0.029	0.51	0.005
	Depuration	0.020	0.71	0.002	0.023	0.73	0.002	0.022	0.72	0.002
	Overall	0.020	0.81	0.001	0.021	0.76	0.002	**0.020**	0.78	0.001
Lab 10	Uptake	0.030	0.13	0.034	0.037	0.13	0.025	0.033	0.12	0.021
	Depuration	0.020	0.31	0.010	0.015	0.46	0.006	0.017	0.49	0.005
	Overall	0.012	0.17	0.007	0.019	0.29	0.004	**0.017**	0.25	0.004

Notes: * Denotes a statistically significant difference between the k_{growth} determined during uptake phase and during the depuration phase (tested using the t-test with alpha =0.05).
[a] Levels 1, 2 and 3 refer to different concentrations of the test substances in the food, with level 1 having the highest concentrations.
Values in **bold** are the preferred values.

Table 2.7 Summary of growth-corrected depuration rate constants (k_{2g}) for hexachlorobenzene

Laboratory	OECD 305 method			Alternative method				
	k_2 (day^{-1})	k_{growth} (day^{-1})	k_{2g} (day^{-1})	k_{2g} (day^{-1}) from slope	R^2 value of regression	Standard error in slope (k_{2g})	Relative standard error (%)	Statistical significance of slope (p)
Lab 1	0.0502	0.0366	0.0136	0.018	0.23	0.006	35.1	8.1×10^{-3}
Lab 2a – trout	0.0399	0.0165	0.0234	0.025	0.50	0.005	19.1	1.4×10^{-5}
Lab 2b – carp (level 1)[a]	0.0603	0.0334	0.0269	0.030	0.79	0.009	30.0	0.045
Lab 2b – carp (level 2)[a]	0.0561	0.0334	0.0227	0.024	0.78	0.007	30.7	0.047
Lab 2b – carp (level 3)[a]	0.0486	0.0334	0.0152	0.015	0.17	0.020	129.4	0.5
Lab 3	0.0537	0.044	0.0097	0.010	0.09	0.006	60.1	0.11
Lab 4	0.0517	0.0386	0.0131	0.022	0.31	0.006	28.4	1.5×10^{-3}
Lab 5	0.0407	0.0158	0.0249	0.032	0.30	0.009	28.9	1.7×10^{-3}
Lab 6	0.0625	0.0367	0.0258	0.031	0.43	0.007	22.4	1.4×10^{-4}
Lab 7	0.0491	0.0307	0.0184	0.019	0.18	0.008	40.5	0.02
Lab 8	0.0579	0.0483	0.0096	0.012	0.18	0.005	40.6	0.02
Mean (all data at 3% feeding rate)			0.018*	0.022*				
Standard deviation (all data at 3% feeding rate)			0.007	0.008				
Mean (trout data at 3% feeding rate)			0.017*	0.021*				
Standard deviation (trout data at 3% feeding rate)			0.007	0.008				

Laboratory	OECD 305 method			Alternative method				
	k_2 (day^{-1})	k_{growth} (day^{-1})	k_{2g} (day^{-1})	k_{2g} (day^{-1}) from slope	R^2 value of regression	Standard error in slope (k_{2g})	Relative standard error (%)	Statistical significance of slope (p)
Mean (trout data at 3% feeding rate minus Lab 5)			0.016*	0.020*				
Standard deviation (trout data at 3% feeding rate minus Lab 5)			0.006	0.007				
Lab 9	0.0589	0.020	0.0389	0.0318	0.65	0.0044	13.8	8.52×10^{-8}
Lab 10	0.0485	0.017	0.0315	0.0326	0.30	0.0093	28.5	0.00157
Mean (trout data at 1.5% feeding rate)			0.0352	0.0322				
Standard deviation (trout data at 1.5% feeding rate)			0.0052	0.0006				

Notes: * Indicates that the mean values of k_{2g} obtained by the two methods are statistically significantly different using the paired t-test (two-tail) with alpha = 0.05.
[a] Levels 1, 2 and 3 refer to different concentrations of the test substances in the food, with level 1 having the highest concentrations.

Table 2.8 Summary of growth-corrected depuration rate constants (k_{2g}) for musk xylene

Laboratory	OECD 305 method			Alternative method				
	k_2 (day^{-1})	k_{growth} (day^{-1})	k_{2g}(day^{-1})	k_{2g} (day^{-1}) from slope	R^2 value of regression	Standard error in slope (k_{2g})	Relative standard error (%)	Statistical significance of slope (p)
Lab 1	0.0904	0.0366	0.0538	0.059	0.69	0.007	12.5	1.1×10^{-8}
Lab 2a – trout	0.0734	0.0165	0.0569	0.058	0.79	0.006	9.6	4.2×10^{-11}
Lab 2b – carp (level 1)[a]	0.14	0.0334	0.1066	0.109	0.98	0.010	9.0	1.6×10^{-3}
Lab 2b – carp (level 2)[a]	0.131	0.0334	0.0976	0.099	0.98	0.007	7.2	8.0×10^{-4}
Lab 2b – carp (level 3)[a]	0.111	0.0334	0.0776	0.078	0.92	0.013	17.3	0.010
Lab 3	0.083	0.044	0.039	0.039	0.55	0.007	17.0	2.4×10^{-6}
Lab 4	0.067	0.0386	0.0284	0.038	0.45	0.008	20.9	4.9×10^{-5}
Lab 5	0.647	0.0158	0.6312	0.667	0.87	0.072	10.7	4.1×10^{-7}
Lab 6	0.105	0.0367	0.0683	0.074	0.80	0.007	10.0	1.9×10^{-10}
Lab 7	0.105	0.0307	0.0743	0.074	0.63	0.011	14.5	1.7×10^{-7}
Lab 8	0.0948	0.0483	0.0465	0.049	0.77	0.005	10.5	3.7×10^{-10}
Mean (all data at 3% feeding rate)			0.116	0.122				
Standard deviation (all data at 3% feeding rate)			0.172	0.182				
Mean (trout data at 3% feeding rate)			0.125	0.132				
Standard deviation (trout data at 3% feeding rate)			0.205	0.216				

Laboratory	OECD 305 method			Alternative method				
	k_2 (day^{-1})	k_{growth} (day^{-1})	k_{2g}(day^{-1})	k_{2g} (day^{-1}) from slope	R^2 value of regression	Standard error in slope (k_{2g})	Relative standard error (%)	Statistical significance of slope (p)
Mean (trout data at 3% feeding rate minus Lab 5)			0.052*	0.056*				
Standard deviation (trout data at 3% feeding rate minus Lab 5)			0.016	0.015				
Lab 9	0.110	0.020	0.090	0.0832	0.87	0.0060	7.2	4.69×10^{-14}
Lab 10	0.105	0.017	0.088	0.0896	0.59	0.0142	15.8	7.75×10^{-7}
Mean (trout data at 1.5% feeding rate)			0.089	0.0864				
Standard deviation (trout data at 1.5% feeding rate)			0.0014	0.0045				

Notes: * Indicates that the mean values of k_{2g} obtained by the two methods are statistically significantly different using the paired t-test (two-tail) with alpha = 0.05.
[a] Levels 1, 2 and 3 refer to different concentrations of the test substances in the food, with level 1 having the highest concentrations.

Table 2.9 Summary of growth-corrected depuration rate constants (k_{2g}) for o-terphenyl

Laboratory	OECD 305 method			Alternative method				
	k_2 (day^{-1})	k_{growth} (day^{-1})	k_{2g}(day^{-1})	k_{2g} (day^{-1}) from slope	R^2 value of regression	Standard error in slope (k_{2g})	Relative standard error (%)	Statistical significance of slope (p)
Lab 1	0.0872	0.0366	0.0506	0.055	0.59	0.009	15.6	6.1×10^{-7}
Lab 2a – trout	0.0691	0.0165	0.0526	0.054	0.52	0.010	18.3	7.7×10^{-6}
Lab 2b – carp (level 1)[a]	0.29	0.0334	0.2566	0.260	1.00	0.008	3.0	6.2×10^{-5}
Lab 2b – carp (level 2)[a]	0.351	0.0334	0.3176	0.305	0.97	0.040	13.2	0.017
Lab 2b – carp (level 3)[a]	0.297	0.0334	0.2636	0.248	0.99	0.016	6.6	4.3×10^{-3}
Lab 3	0.104	0.044	0.060	0.060	0.46	0.013	21.1	6.5×10^{-5}
Lab 4	0.077	0.0386	0.0384	0.048	0.20	0.018	37.3	0.012
Lab 5	0.0445	0.0158	0.0287	0.035	0.32	0.010	27.7	1.2×10^{-3}
Lab 6	0.133	0.0367	0.0963	0.102	0.56	0.018	17.6	5.4×10^{-6}
Lab 7	0.0775	0.0307	0.0468	0.047	0.49	0.009	19.4	1.9×10^{-5}
Lab 8	0.113	0.0483	0.0647	0.067	0.48	0.013	19.8	2.7×10^{-5}
Mean (all data at 3% feeding rate)			0.116	0.117				
Standard deviation (all data at 3% feeding rate)			0.107	0.102				
Mean (trout data at 3% feeding rate)			0.055*	0.059*				
Standard deviation (trout data at 3% feeding rate)			0.020	0.020				

Laboratory	OECD 305 method			Alternative method				
	k_2 (day^{-1})	k_{growth} (day^{-1})	k_{2g}(day^{-1})	k_{2g} (day^{-1}) from slope	R^2 value of regression	Standard error in slope (k_{2g})	Relative standard error (%)	Statistical significance of slope (p)
Mean (trout data at 3% feeding rate minus Lab 5)			0.058*	0.062*				
Standard deviation (trout data at 3% feeding rate minus Lab 5)			0.019	0.019				
Lab 9	0.109	0.020	0.089	0.0804	0.77	0.0091	11.3	5.13×10^{-9}
Lab 10	0.126	0.017	0.109	0.111	0.65	0.0152	13.7	6.47×10^{-8}
Mean (trout data at 1.5% feeding rate)			0.099	0.0957				
Standard deviation (trout data at 1.5% feeding rate)			0.014	0.0216				

Note: * Indicates that the mean values of k_{2g} obtained by the two methods are statistically significantly different using the paired t-test (two-tail) with alpha = 0.05.
[a] Levels 1, 2 and 3 refer to different concentrations of the test substances in the food, with level 1 having the highest concentrations.

Table 2.10 Summary of growth-corrected depuration rate constants (k_{2g}) for methoxychlor

Laboratory	OECD 305 method			Alternative method				
	k_2 (day^{-1})	k_{growth} (day^{-1})	k_{2g}(day^{-1})	k_{2g} (day^{-1}) from slope	R^2 value of regression	Standard error in slope (k_{2g})	Relative standard error (%)	Statistical significance of slope (p)
Lab 1	0.15	0.0366	0.1134	0.114	0.56	0.023	20.4	1.0×10^{-4}
Lab 2a – trout	0.116	0.0165	0.0995	0.101	0.68	0.013	12.9	2.0×10^{-8}
Lab 2b – carp (level 1)[a]	0.31	0.0334	0.2766	0.289	0.97	0.037	12.9	0.016
Lab 2b – carp (level 2)[a]	0.294	0.0334	0.2606	0.249	0.94	0.044	17.7	0.030
Lab 2b – carp (level 3)[a]	0.264	0.0334	0.2306	0.212	0.84	0.092	43.6	0.26
Lab 3	0.102	0.044	0.058	0.056	0.26	0.019	33.7	6.6×10^{-3}
Lab 4	0.0922	0.0386	0.0536	0.063	0.21	0.025	39.4	0.018
Lab 5	0.0046	0.0158	-0.0112	-0.005	0.003	0.017	362.3	0.78
Lab 6	0.225	0.0367	0.1883	0.182	0.78	0.022	12.1	9.8×10^{-8}
Lab 7	0.148	0.0307	0.1173	0.118	0.80	0.011	9.5	3.4×10^{-11}
Lab 8	0.11	0.0483	0.0617	0.065	0.26	0.021	32.8	5.1×10^{-3}
Mean (all data at 3% feeding rate)			0.132	0.131				
Standard deviation (all data at 3% feeding rate)			0.094	0.091				
Mean (trout data at 3% feeding rate)			0.085	0.087				
Standard deviation (trout data at 3% feeding rate)			0.048	0.044				

Laboratory	OECD 305 method			Alternative method				
	k_2 (day^{-1})	k_{growth} (day^{-1})	k_{2g}(day^{-1})	k_{2g} (day^{-1}) from slope	R^2 value of regression	Standard error in slope (k_{2g})	Relative standard error (%)	Statistical significance of slope (p)
Mean (trout data at 3% feeding rate minus Lab 5)			0.099	0.100				
Standard deviation (trout data at 3% feeding rate minus Lab 5)			0.048	0.044				
Lab 9	[b]	0.020	[b]	[b]	[b]	[b]	[b]	[b]
Lab 10	0.199	0.017	0.182	0.184	0.62	0.0270	14.7	2.17×10^{-7}
Mean			0.182	0.184				
Standard deviation								

Notes: * Indicates that the mean values of k_{2g} obtained by the two methods are statistically significantly different using the paired t-test (two-tail) with alpha = 0.05.
[a] Levels 1, 2 and 3 refer to different concentrations of the test substances in the food, with level 1 having the highest concentrations.
[b] No data owing to analytical difficulties.

Table 2.11 Summary of growth-corrected depuration rate constants (k_{2g}) for benzo[a]pyrene

Laboratory	OECD 305 method			Alternative method				
	k_2 (day^{-1})	k_{growth} (day^{-1})	k_{2g}(day^{-1})	k_{2g} (day^{-1}) from slope	R^2 value of regression	Standard error in slope (k_{2g})	Relative standard error (%)	Statistical significance of slope (p)
Lab 1	0.986	0.0366	0.9494	0.964	0.80	0.240	24.9	0.016
Lab 2a – trout	2.094	0.0165	2.0775	2.112	0.91	0.235	11.1	1.9×10^{-5}
Lab 2b – carp (level 1)[a]								
Lab 2b – carp (level 2)[a]								
Lab 2b – carp (level 3)[a]								
Lab 3	b	b	b	b	b	b	b	b
Lab 4	b	b	b	b	b	b	b	b
Lab 5	2.066	0.0158	2.0502	2.029	0.72	0.443	21.8	1.8×10^{-3}
Lab 6	1.684	0.0367	1.6473	1.568	0.91	0.221	14.1	8.6×10^{-4}
Lab 7	b	b	b	b	b	b	b	b
Lab 8	1.179	0.0483	1.1307	1.179	0.87	0.160	13.6	7.9×10^{-5}
Mean (all data at 3% feeding rate)			1.571	1.570				
Standard deviation (all data at 3% feeding rate)			0.518	0.506				
Mean (trout data at 3% feeding rate)			1.571	1.570				
Standard deviation (trout data at 3% feeding rate)			0.518	0.506				

Laboratory	OECD 305 method			Alternative method				
	k_2 (day^{-1})	k_{growth} (day^{-1})	k_{2g}(day^{-1})	k_{2g} (day^{-1}) from slope	R^2 value of regression	Standard error in slope (k_{2g})	Relative standard error (%)	Statistical significance of slope (p)
Mean (trout data at 3% feeding rate minus Lab 5)			1.451	1.456				
Standard deviation (trout data at 3% feeding rate minus Lab 5)			0.512	0.504				
Lab 9	[b]	0.020	[b]	[b]	[b]	[b]	[b]	[b]
Lab 10	0.077[c]	0.017	0.060[c]	0.062[c]	0.12[c]	0.031[c]	50[c]	0.059[c]
	0.298[d]		0.281[d]	0.151[d]	0.02[d]	0.359[d]	238[d]	0.685[d]
Mean (trout data at 1.5% feeding rate)			0.060[c]	0.062[d]				
			0.281[d]	0.151[d]				
Standard deviation (trout data at 3% feeding rate)								

Note: * Indicates that the mean values of k_{2g} obtained by the two methods are statistically significantly different using the paired t-test (two-tail) with alpha = 0.05.
[a] Levels 1, 2 and 3 refer to different concentrations of the test substances in the food, with level 1 having the highest concentrations.
[b] Owing to rapid depuration, there were insufficient data points available to derive the depuration curve.
[c] Derived using all reported data during the depuration period.
[d] Derived using only the data reported for depuration days 1 and 3.

2.1.2 Fish lipids

The fish lipid data taken from OECD (2012b, 2013) are summarised in Table 2.12.

Investigation of the effects of the lipid content on the kinetic parameters generated in the OECD 305 ring test is problematic as (a) the lipid contents of the fish, in most cases, increased with increasing time in the test and (b) only a limited number of lipid measurements were taken during the study (usually at the start of the test, the end of the uptake period and the end of the depuration period). For the analysis in OECD (2012b, 2013), the lipid content was taken to be the mean of all the measurements (usually at three time points) during the study. Although these values represent the arithmetic mean of the available measurements they do not necessarily represent the true, time-weighted average, lipid content over the experiment as the sampling times were not evenly distributed throughout the entire test period.

In order to obtain a more meaningful estimate of the time-weighted average lipid content of the fish, plots have been constructed of the measured lipid concentrations in fish against time using each individual data point for both the control and exposed fish. Such plots for each laboratory are shown in Figures 2.1 to 2.13, along with the relevant linear equation[1] obtained by regression analysis. The relevant statistics from the linear regression analysis are shown in Table 2.13. It is interesting to note that all studies except for Lab 9 showed an increase in the lipid content with time. For Lab 9 the analysis suggests that the lipid content decreased slightly with time; however, the slope of the regression line is not statistically significantly different from zero.

Using these regression equations, it is then possible to calculate the approximate lipid concentration in fish on each day during the uptake and depuration phases and then to obtain an estimate of the actual (time-weighted) mean concentration during the study. This has been done for the depuration phase[2] and the resulting time-weighted mean concentrations are summarised in Table 2.14.

As discussed in OECD (2012b) there are a number of uncertainties in the data obtained in some of the studies carried out by Lab 5, and so the data from this laboratory are not considered further in the following analysis.

[1] There is no fundamental reason why the lipid concentration should be linearly related to time. A linear function has been assumed here for simplicity. As can be seen from the plots this appears to be a reasonable approximation over the timescale of the study. However, it should be noted that the number of time points is small (usually three) and so it is difficult to reliably distinguish between a linear function and alternative functions in such plots.

[2] As the k_2 and k_{2g} values have been determined using data from the depuration phase, it is most appropriate to calculate the mean lipid content over the depuration phase also.

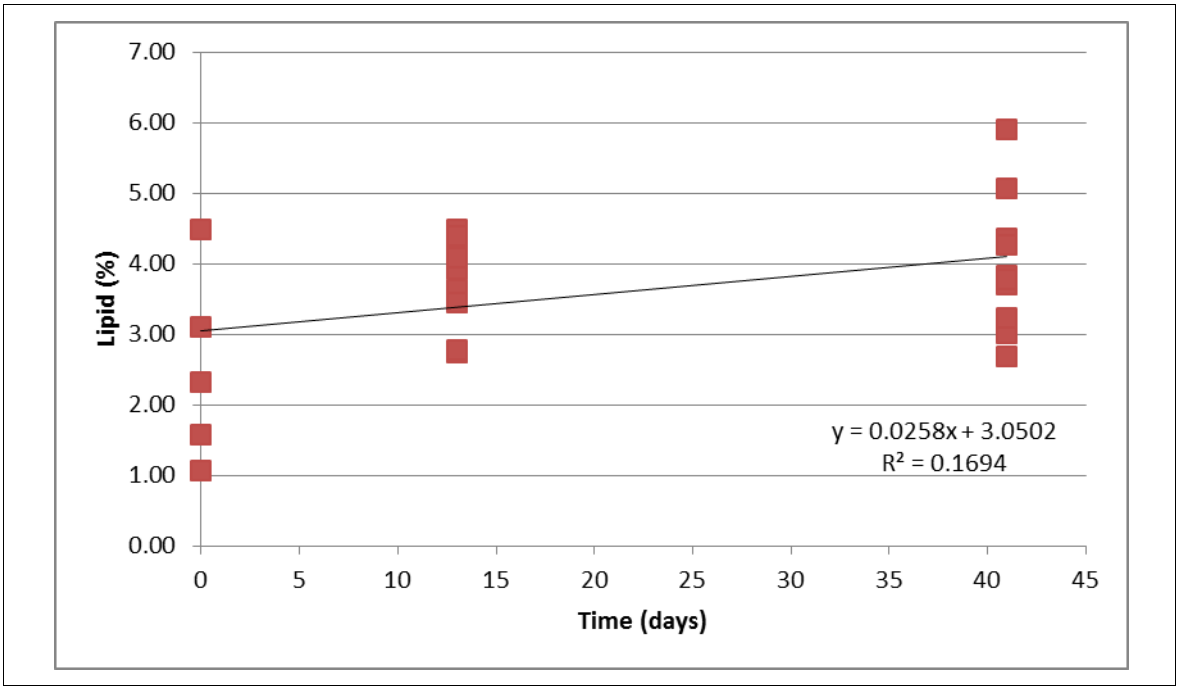

Figure 2.1 Plot of percentage lipid against time for Lab 1

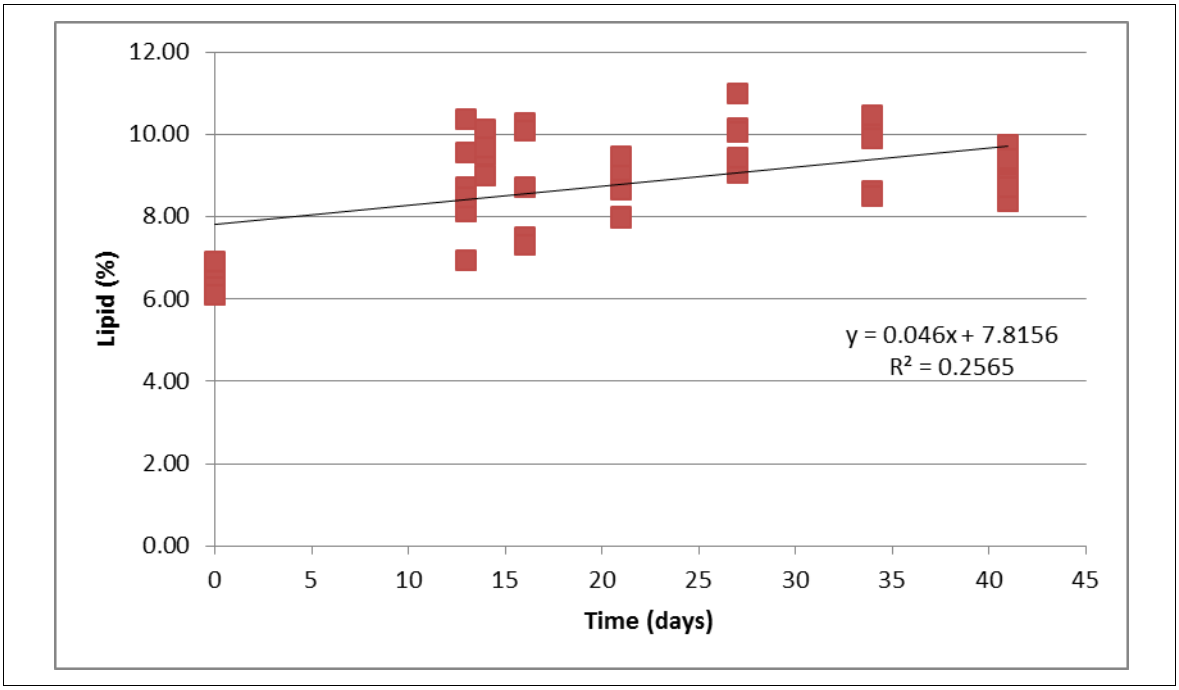

Figure 2.2 Plot of percentage lipid against time for Lab 2a – trout

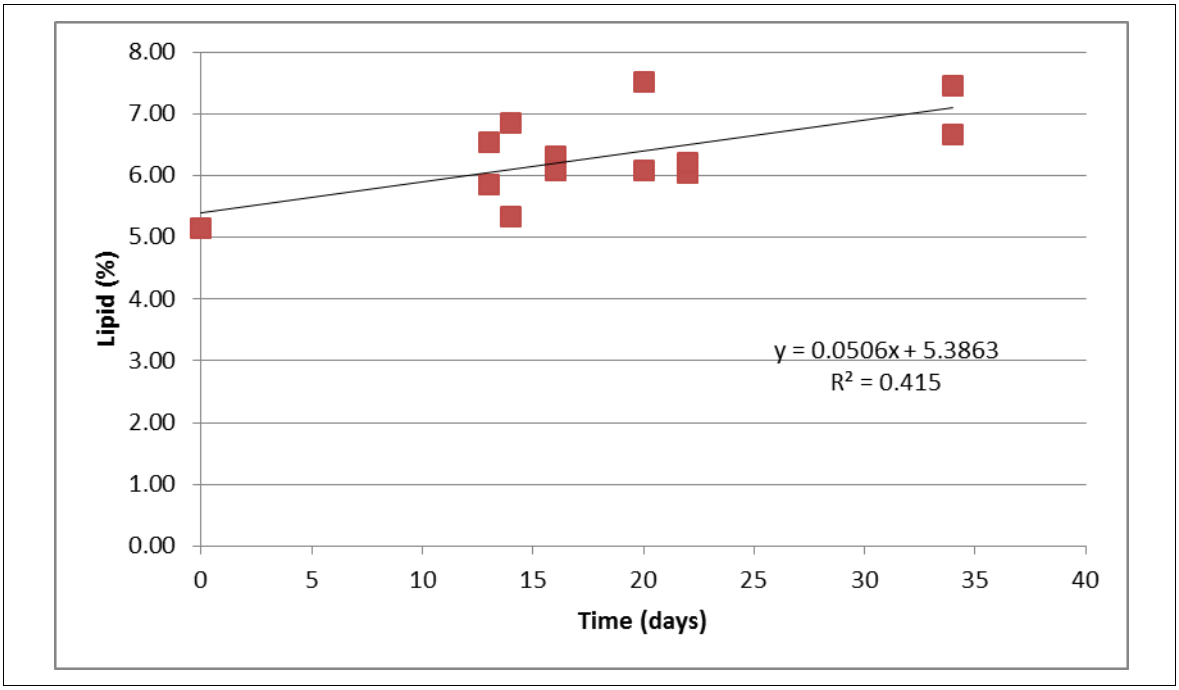

Figure 2.3 Plot of percentage lipid against time for Lab 2b – carp (level 1)

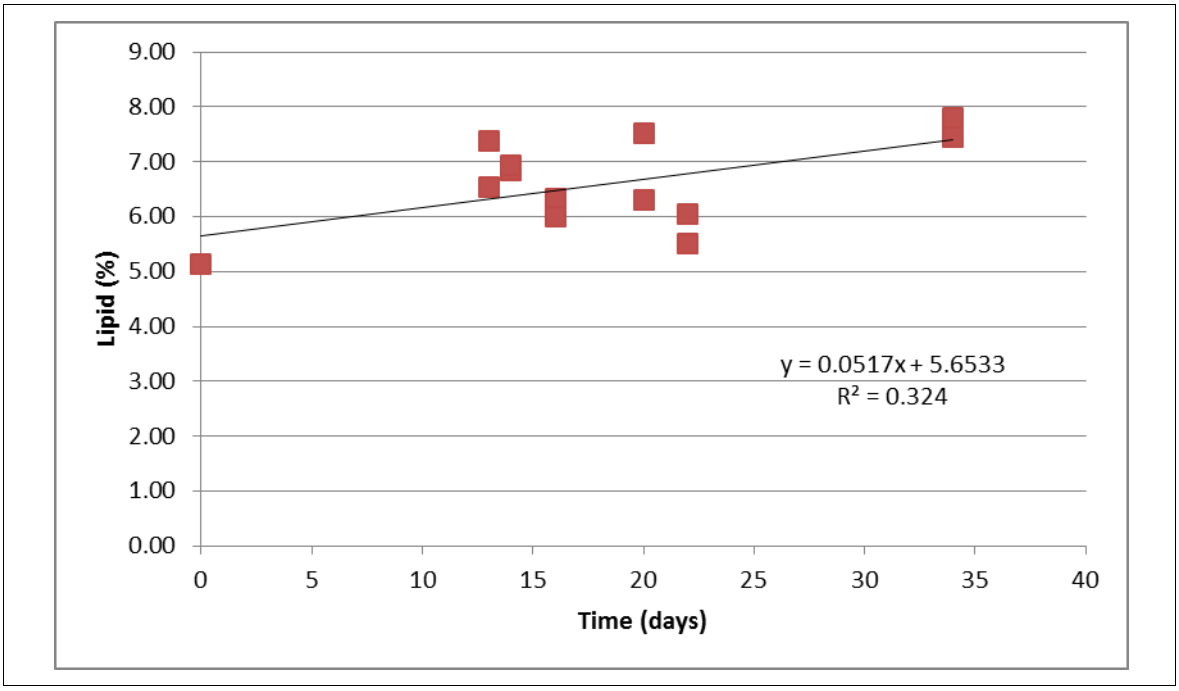

Figure 2.4 Plot of percentage lipid against time for Lab 2b – carp (level 2)

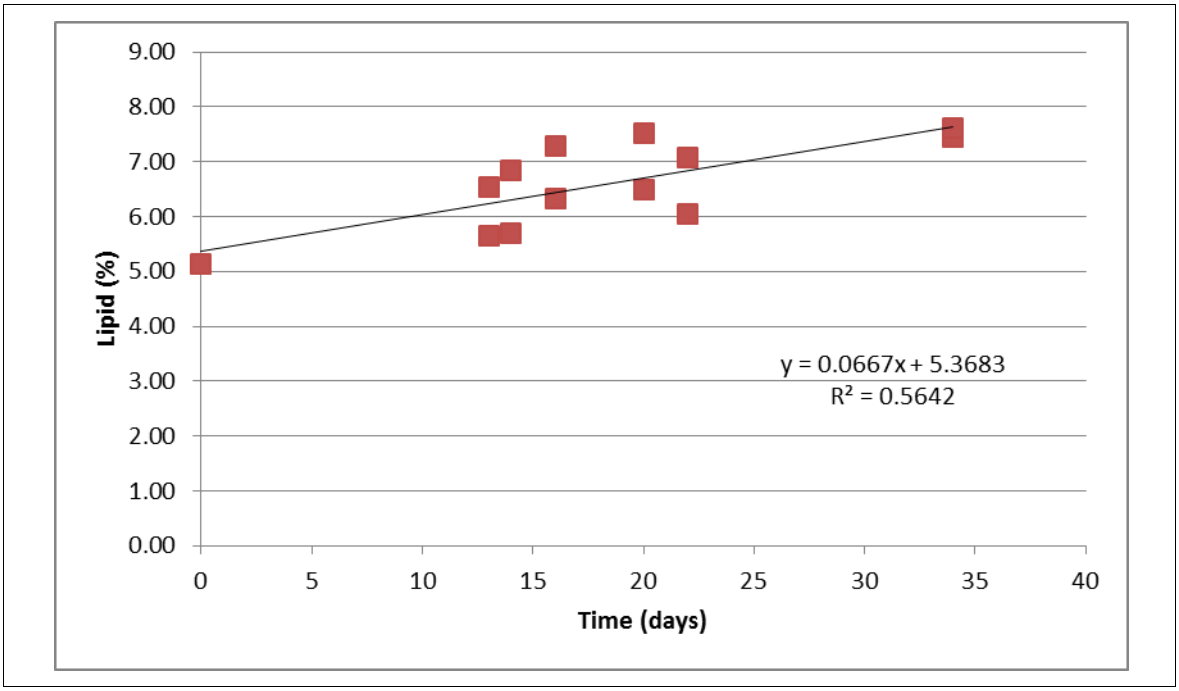

Figure 2.5 Plot of percentage lipid against time for Lab 2b – carp (level 3)

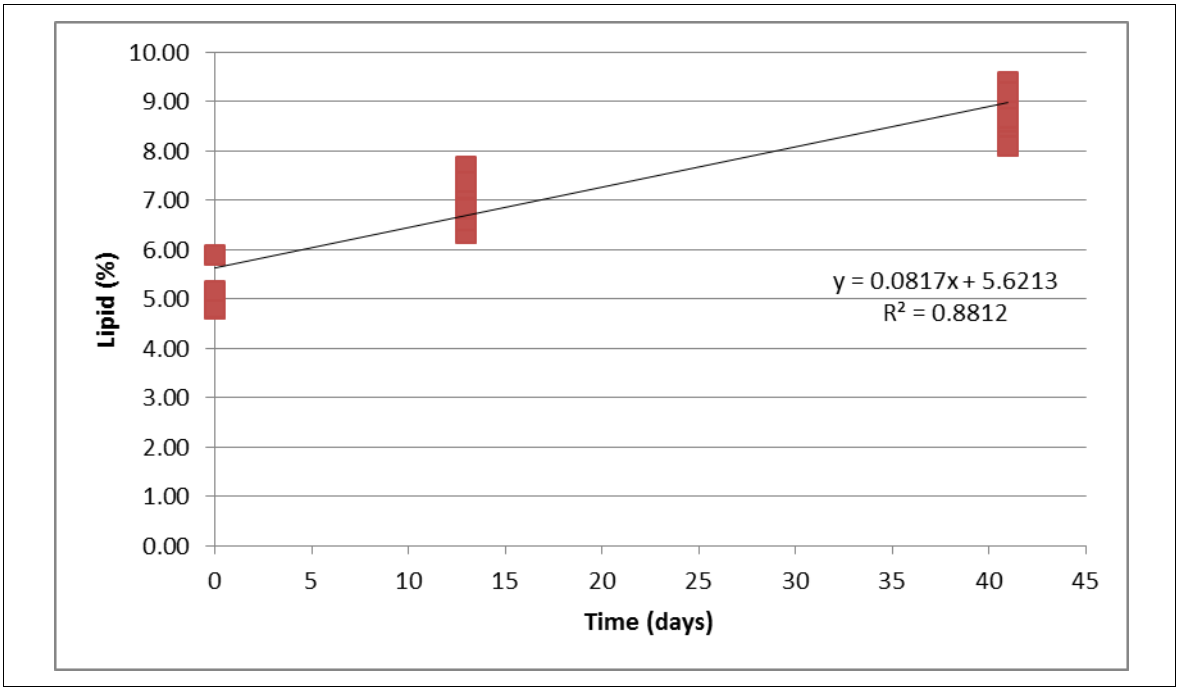

Figure 2.6 Plot of percentage lipid against time for Lab 3

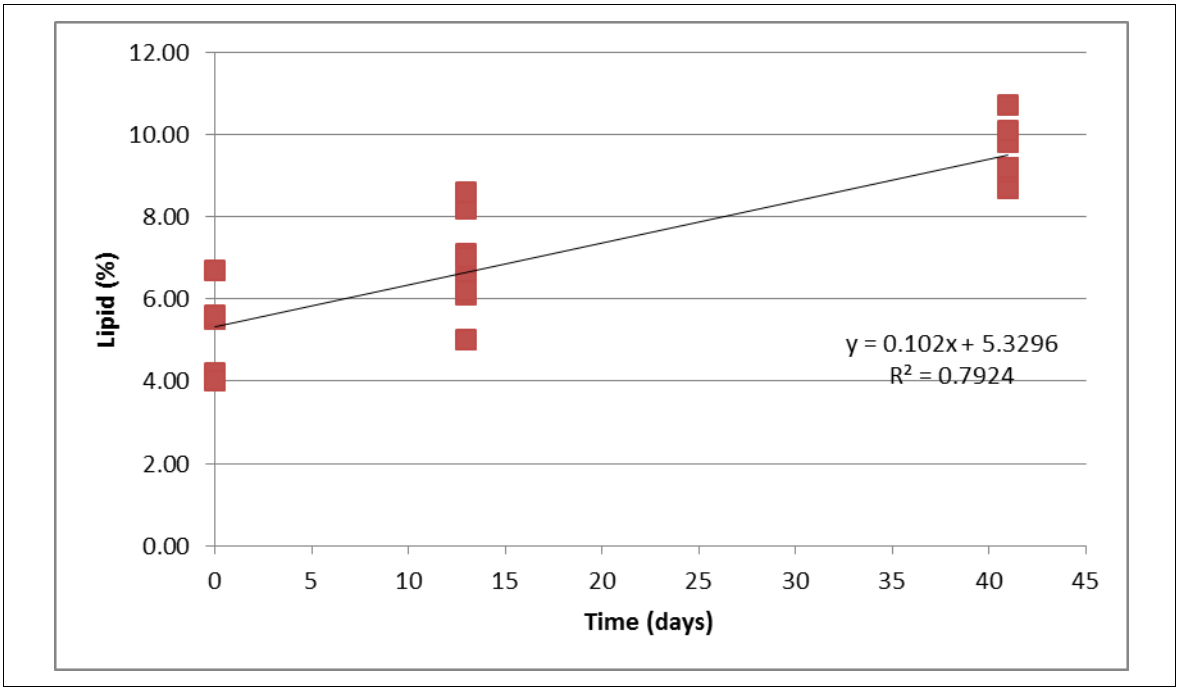

Figure 2.7 Plot of percentage lipid against time for Lab 4

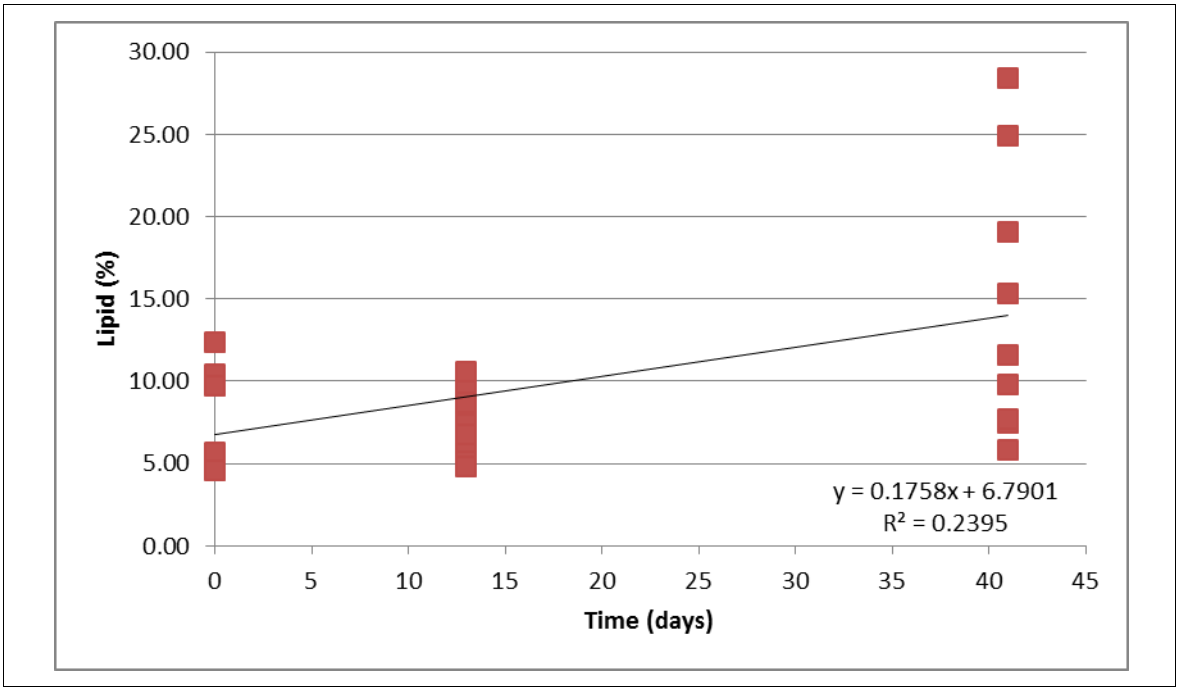

Figure 2.8 Plot of percentage lipid against time for Lab 5

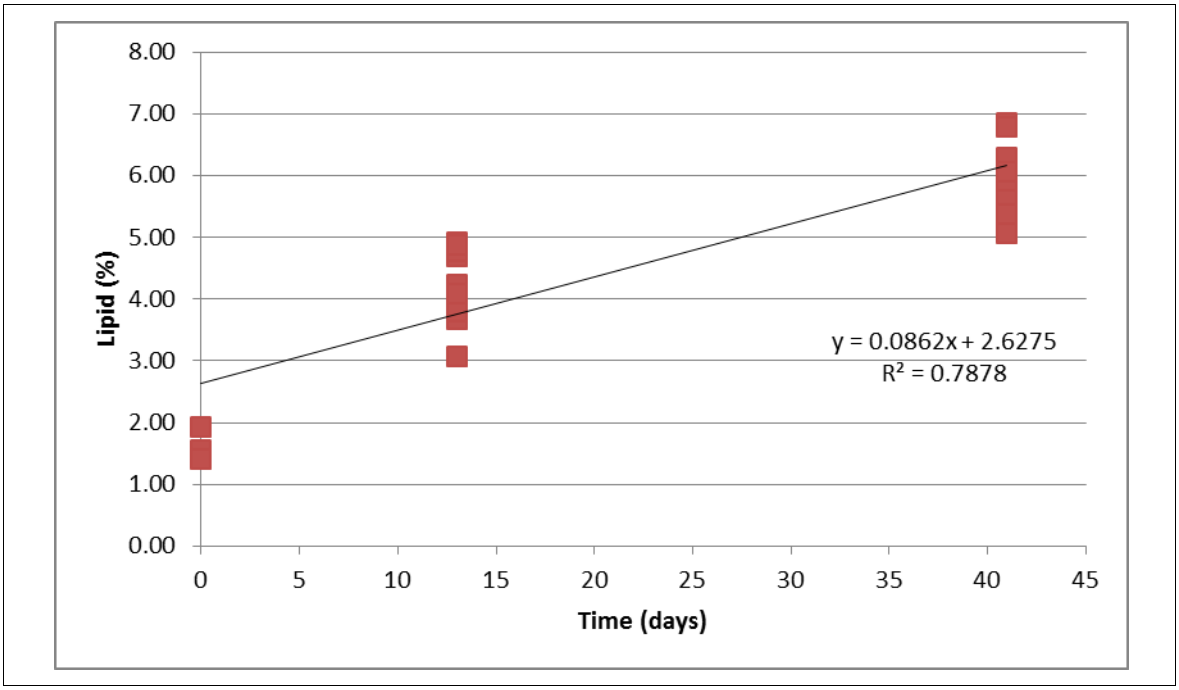

Figure 2.9 Plot of percentage lipid against time for Lab 6

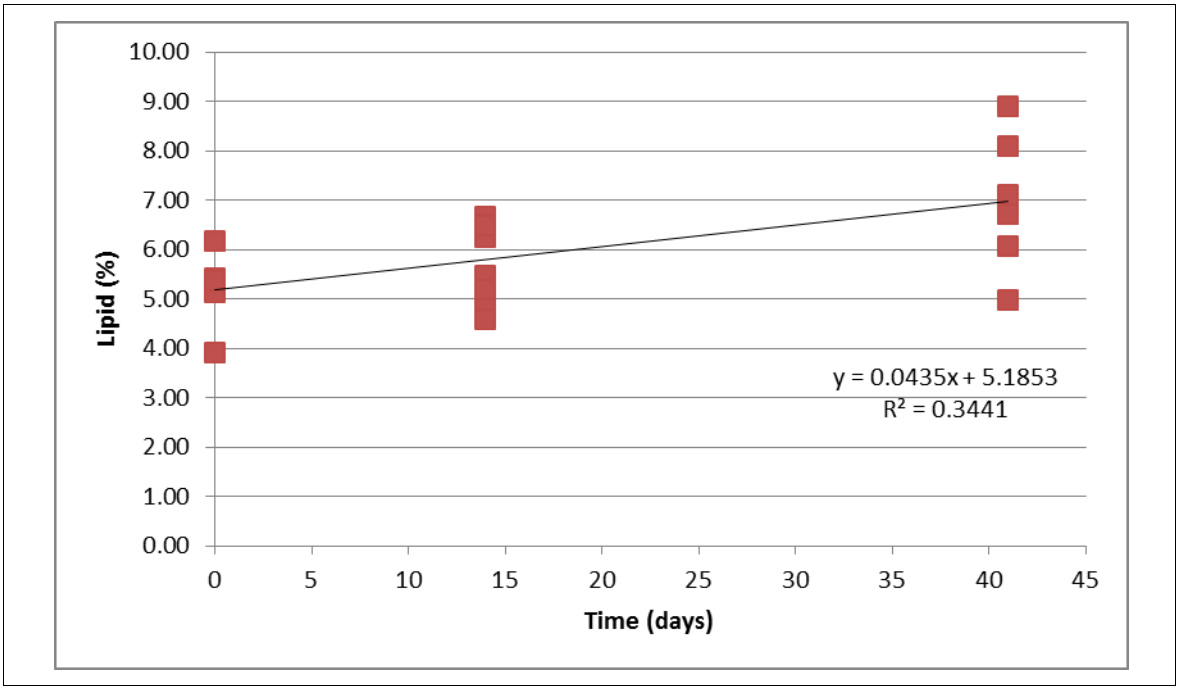

Figure 2.10 Plot of percentage lipid against time for Lab 7

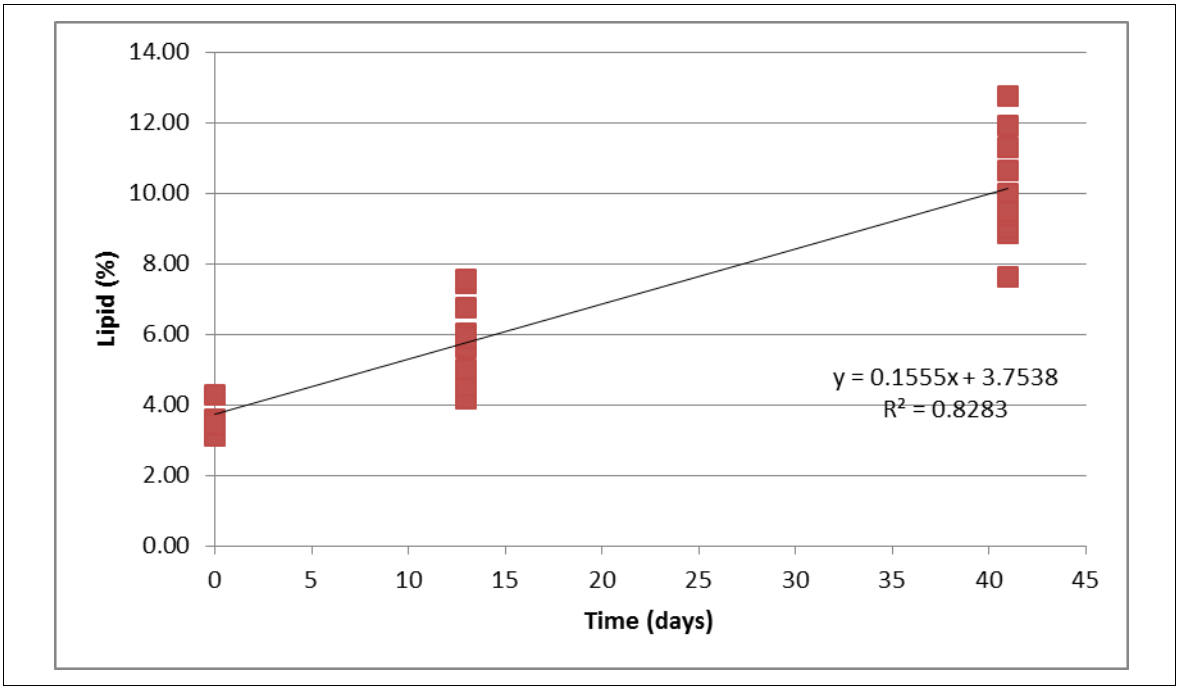

Figure 2.11 Plot of percentage lipid against time for Lab 8

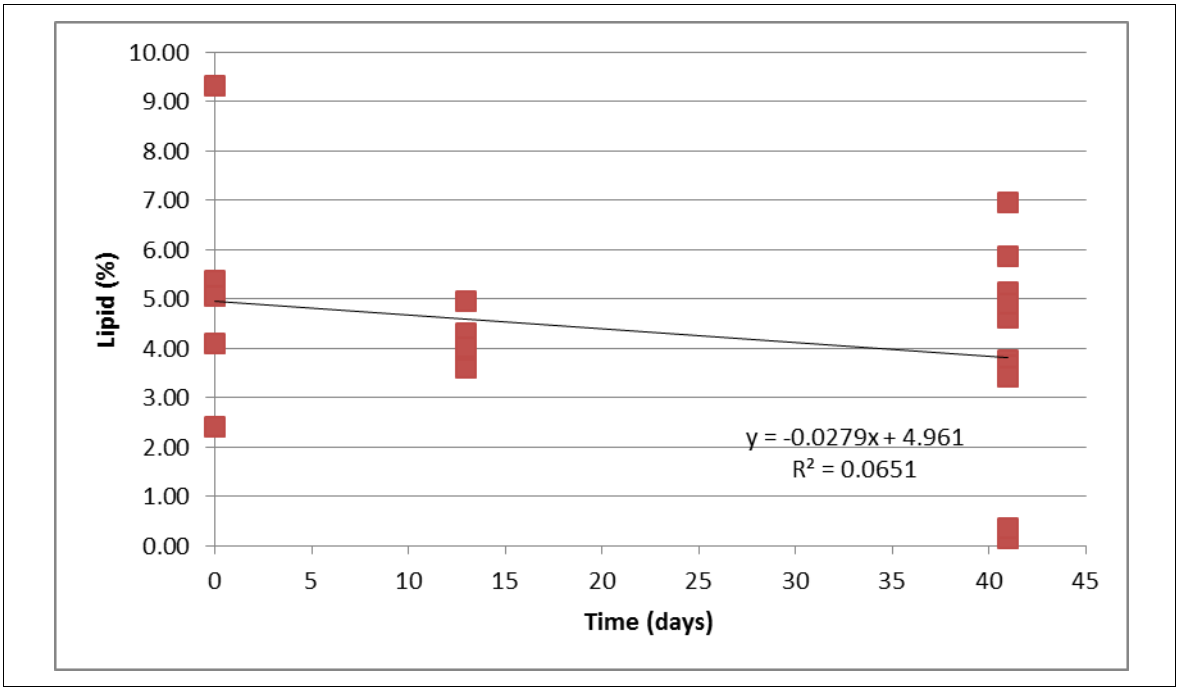

Figure 2.12 Plot of percentage lipid against time for Lab 9

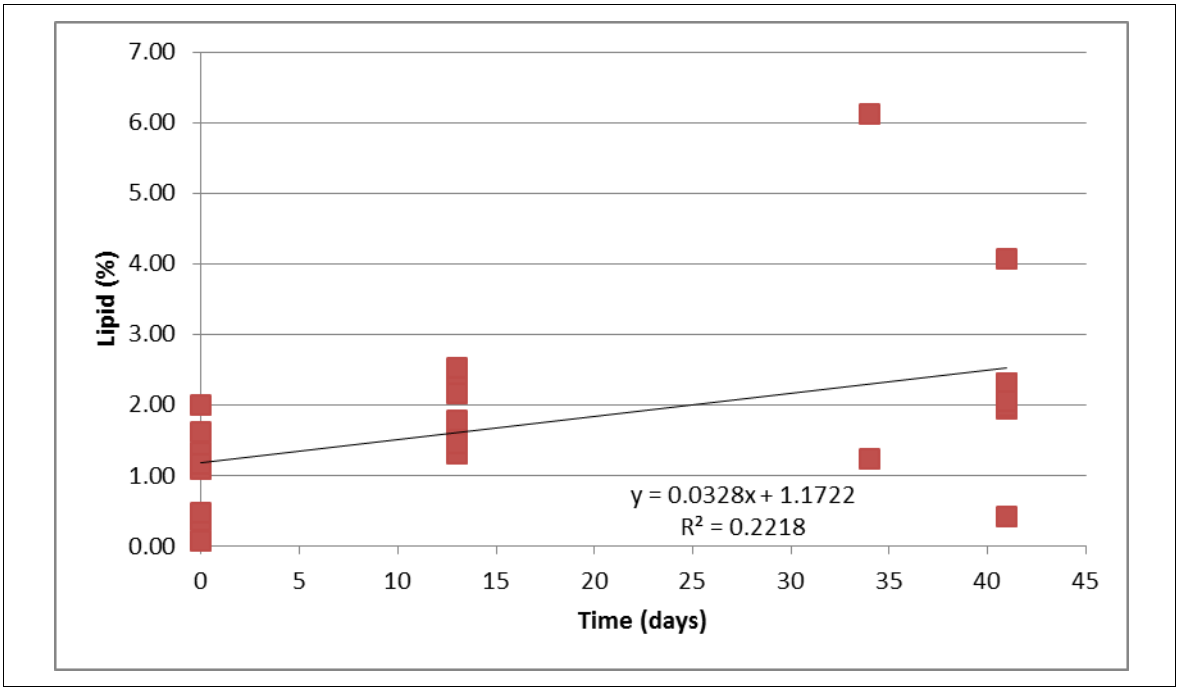

Figure 2.13 Plot of percentage lipid against time for Lab 10

Table 2.12 Summary of fish lipid contents determined during the test

Laboratory	Group[a]	Lipid content – day 0 (% w/w)		Lipid content – uptake day 13 (% w/w)		Lipid content – depuration day 28 (% w/w)		Overall mean over the entire study (% w/w) (values in [ ] are the means during depuration)
		Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
Lab 1	Control	2.51	1.34	3.89	0.69	3.67	0.61	3.36 [3.78]
	Exposed	As above		3.67	0.62	4.30	1.22	3.49 [3.99]
Lab 2a – trout	Control	6.41	0.31	9.05	0.90	9.32	0.32	8.26 [9.19]
	Exposed	As above		8.15	0.72	8.83	0.37	8.74[f] [9.07[f]]
Lab 2b – carp	Control	5.14	single value reported	6.54	single value reported	7.46 (day 21 value)	single value reported	6.55[b] [6.79[b]]
	Exposed – level 1 (high)	As above		5.85	single value reported	6.66 (day 21 value)	single value reported	5.91[c] [6.04[c]]
	Exposed – level 2 (medium)	As above		7.37	single value reported	7.81 (day 21 value)	single value reported	6.44[d] [6.66[e]]
	Exposed – level 3 (low)	As above		5.64	single value reported	7.61 (day 21 value)	single value reported	6.42[e] [6.64[e]]
Lab 3	Control	5.2	0.44	7.2	0.30	8.9	0.36	7.1 [8.05]
	Exposed	As above		6.9	0.43	8.8	0.51	6.9 [7.85]
Lab 4	Control	5.2	1.11	7.3 (day 14 value)	1.07	9.6	0.76	7.37 [8.45]
	Exposed	As above		6.2	0.73	9.4	0.56	6.9 [7.8]

Laboratory	Group[a]	Lipid content – day 0 (% w/w)		Lipid content – uptake day 13 (% w/w)		Lipid content – depuration day 28 (% w/w)		Overall mean over the entire study (% w/w) (values in [ ] are the means during depuration)
		Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
Lab 5	Control	8.56	3.29	7.74	1.89	15.3	8.5	10.5 [11.5]
	Exposed	As above		7.82	2.00	13.4	8.7	9.94 [10.6]
Lab 6	Control	1.63	0.27	4.70	0.27	6.02	0.57	4.11 [5.36]
	Exposed	As above		3.68	0.38	6.03	0.63	3.78 [4.96]
Lab 7	Control	5.20	0.82	6.01	0.63	7.03	1.56	6.08 [6.52]
	Exposed	As above		5.55	0.89	6.73	(single value)	5.83 [6.14]
Lab 8	Control	3.6	0.4	5.8	1.2	9.8	1.6	6.4 [7.8]
	Exposed	As above		6.0	1.2	10.4	1.6	6.6 [8.2]
Lab 9	Control	5.25	2.54	nd	nd	3.17	2.91	4.21 [3.17]
	Exposed	nd	nd	4.17	0.50	4.60	1.03	4.38 [4.38]
Lab 10[g]	Control	0.81	0.54	1.63	0.34	1.56	1.00	1.61 [2.02]
	Exposed	1.11	0.82	2.04	0.42	2.48	0.90	1.93 [2.26]

Notes: nd = No data

[a] In most cases, five fish were analysed in the control and exposed groups at each time point.

[b] The laboratory sampled the lipid content also on day 1, 3, 7 and 9 of the depuration phase for the control group. The mean lipid contents measured were respectively 6.85%, 6.32%, 7.51% and 6.04% at these sampling times. The overall mean value reported includes these intermediate sampling point values.

[c] The laboratory sampled the lipid content also on day 1, 3, 7 and 9 of the depuration phase for the exposed group. The mean lipid contents measured were respectively 5.33%, 6.08%, 6.09% and 6.20% at these sampling times. The overall mean value reported includes these intermediate sampling point values.

[d] The laboratory sampled the lipid content also on day 1, 3, 7 and 9 of the depuration phase for the exposed group. The mean lipid contents measured were respectively 6.94%, 6.00%, 6.31% and 5.51% at these sampling times. The overall mean value reported includes these intermediate sampling point values.

[e] The laboratory sampled the lipid content also on day 1, 3, 7 and 9 of the depuration phase for the exposed group. The mean lipid contents measured were respectively 5.70%, 7.29%, 6.50% and 7.07% at these sampling times. The overall mean value reported includes these intermediate sampling point values.

[f] The laboratory sampled the lipid content also on day 1, 3, 8, 14 and 21 of the depuration phase for the exposed group. The mean lipid contents measured were respectively 9.59%, 8.78%, 8.64%, 9.94% and 9.56% at these sampling times. The overall mean value reported includes these intermediate sampling point values.

[g] Five fish were sampled except for the exposed group at day 0 (four fish) and the control group at depuration day 28 (three fish). An additional lipid measurement was made in two fish from the control group at day 21 of depuration. The mean lipid concentration measured at this sampling point was 3.68% (standard deviation ± 3.45%). These values were included in the calculation of the overall mean.

Table 2.13 Summary of regression analysis on the plots of percentage fish lipid against time

Laboratory	Slope (% day^{-1})	Intercept (%)	R^2 value of regression	p-value of slope[a]	Standard error in slope	Standard error in intercept	95% Confidence interval – slope	95% Confidence interval – intercept
Lab 1	0.026	3.05	0.17	0.041	0.012	0.32	0.001 to 0.050	2.38 to 3.72
Lab 2a – trout	0.046	7.82	0.26	1.8×10^{-4}	0.011	0.29	0.023 to 0.069	7.24 to 8.39
Lab 2b – carp (level 1)[b]	0.051	5.39	0.42	0.017	0.018	0.37	0.011 to 0.091	4.58 to 6.19
Lab 2b – carp (level 2)[b]	0.052	5.65	0.32	0.042	0.023	0.46	0.002 to 0.101	4.65 to 6.66
Lab 2b – carp (level 3)[b]	0.067	5.37	0.56	0.0031	0.018	0.36	0.028 to 0.106	4.58 to 6.16
Lab 2b – carp (all levels)	0.055	5.43	0.34	0.0023	0.016	0.33	0.022 to 0.087	4.76 to 6.11
Lab 3	0.082	5.62	0.88	4.0×10^{-12}	0.006	0.17	0.069 to 0.095	5.27 to 5.97
Lab 4	0.102	5.33	0.79	2.6×10^{-9}	0.011	0.30	0.079 to 0.124	4.72 to 5.94
Lab 5	0.176	6.79	0.24	0.015	0.067	1.77	0.037 to 0.314	3.12 to 10.46
Lab 6	0.086	2.63	0.79	1.6×10^{-8}	0.010	0.28	0.066 to 0.107	2.05 to 3.20
Lab 7	0.043	5.19	0.34	0.0052	0.014	0.33	0.014 to 0.072	4.49 to 5.88
Lab 8	0.155	3.75	0.83	2.9×10^{-10}	0.014	0.40	0.125 to 0.186	2.92 to 4.58
Lab 9	-0.028	4.96	0.07	0.278*	0.025	0.74	-0.080 to 0.024	3.40 to 6.52
Lab 10	0.033	1.17	0.22	0.010	0.012	0.29	0.009 to 0.057	0.58 to 1.77

Notes: [a] A p-value <0.05 means that the slope is statistically significantly different from zero with a 95% certainty.
* Slope not statistically significantly different from zero.
[b] Levels 1, 2 and 3 refer to different concentrations of the test substances in the food, with level 1 having the highest concentrations.

Table 2.14 Estimates of the time-weighted average fish lipid concentration obtained from the regression analysis

Laboratory	Time-weighted average mean lipid (%)	Standard deviation[a]
Lab 1	3.76	0.21
Lab 2a – trout	9.08	0.38
Lab 2b – carp (level 1)[b]	6.60	0.31
Lab 2b – carp (level 2)[b]	6.89	0.32
Lab 2b – carp (level 3)[b]	6.97	0.30
Lab 2b – carp (all levels)	6.74	0.34
Lab 3	7.87	0.67
Lab 4	8.13	0.84
Lab 5	11.63	1.45
Lab 6	5.00	0.71
Lab 7	6.38	0.36
Lab 8	8.03	1.28
Lab 9	4.19	0.23
Lab 10	2.07	0.27

Notes: [a] The standard deviation reflects that standard deviation around the mean estimate of the lipid concentration using an estimate of the lipid content for each day during the depuration phase. This provides an indication of the variation of the lipid content during the depuration phase in each series of experiments.
[b] Levels 1, 2 and 3 refer to different concentrations of the test substances in the food, with level 1 having the highest concentrations.

2.2 Background to lipid normalisation of depuration rate constants

It is common practice to normalise a fish BCF value to a 'standard' lipid content using the following equation:

$$BCF_L = \frac{BCF_{Exp} \times F_{L,std}}{F_{L,exp}}$$ Equation 1

Where BCF_L = lipid-normalised BCF

BCF_{Exp} = BCF determined in the experiment

$F_{L, std}$ = the standard fractional lipid content

$F_{L, exp}$ = the fractional lipid content of the fish in the experiment.

The experimental BCF can also be expressed kinetically as the ratio of the experimental uptake rate constant (k_1) and the overall depuration rate constant (k_2) as follows:

$$BCF_L = \frac{k_{1,exp}}{k_{2,exp}} \times \frac{F_{L,std}}{F_{L,exp}}$$ Equation 2

Where $k_{1,\,exp}$ = The experimentally determined (or estimated; see later) uptake rate constant

$k_{2,\,exp}$ = The experimentally determined overall depuration rate constant.

When considering the effect of lipid normalisation on the depuration rate constant a number of assumptions have to be made. These are outlined below. If any one of these assumptions is not met for a given substance then lipid normalisation of the depuration rate constant (or of the BCF itself) may not be appropriate or needs to be carried out carefully.

1. The uptake into the fish is a first-order process in the concentration in water and the overall depuration process in the fish is a first-order process in the concentration in fish.
2. The uptake rate constant, k_1, is independent of the lipid content. [This is assumed in many, but not all, of the methods for predicting k_1 considered in Crookes and Brooke (2011) and Brooke *et al*. (2012)].
3. The overall depuration rate constant is made up of the following processes: respiratory elimination, faecal elimination, metabolism and growth dilution; all of these processes are first-order processes in the concentration in fish. In this case $k_2 = k_r + k_e + k_m + k_g$, where k_r is the rate constant for respiratory elimination, k_e that for faecal elimination, k_m that for metabolism and k_g the rate constant for growth dilution.
4. The individual rate constants that make up k_2 all show the same lipid dependence.

Taking these into account, a lipid-normalised overall depuration rate constant ($k_{2,\,L}$) can be defined as follows:

$$BCF_L = \frac{k_{1,exp}}{k_{2,L}} = \frac{k_{1,exp}}{k_{2,exp}} \times \frac{F_{L,std}}{F_{L,exp}} \qquad \text{Equation 3}$$

Where $k_{2.\,L}$ = lipid-normalised overall depuration rate constant.

Rearranging gives the following:

$$k_{2,L} = k_{2,exp} \times \frac{F_{L,exp}}{F_{L,std}} \qquad \text{Equation 4}$$

Of the above four assumptions, the fourth needs particular consideration. For respiratory elimination in particular, and also faecal elimination, a dependence on the lipid content of the fish can be envisaged as they can be considered to involve passive transfer of the substance from lipid stores within the fish to water (transfer across the gills) or faeces (transfer across the gut). However, for the other processes a dependence on the fish lipid is not so obvious. For example, for rapidly growing fish, the rate constant for growth dilution (k_g) will contribute significantly to the overall k_2 value and there would appear to be no apparent reason why k_g should be dependent on the lipid content of the fish. Similarly for rapidly metabolised substances, k_m would contribute significantly to the overall k_2 value and it is not obvious why metabolism would be dependent on the fish lipid content.[3] This means that lipid normalisation of BCF data (whether steady-state or kinetic values) obtained with rapidly growing fish or with substances that are rapidly metabolised needs careful consideration.

[3] Although internal concentrations of a substance 'available' for metabolism may be affected by differing partitioning kinetics or rates of mobilisation from lipid stores; these rates could be affected by the amount of lipid.

As the OECD 305 test guideline now estimates the rate constant for growth dilution separately from the overall depuration rate constant, it is relatively straight forward to factor out the effects of growth dilution, that is, to estimate a growth-corrected depuration rate constant ($k_{2g} = k_2 - k_g$, if first-order kinetics are followed) and a growth-corrected BCF value, and thus any uncertainties resulting from the lipid dependence of growth dilution can be eliminated. However, uncertainties for rapidly metabolised substances still remain as it is difficult in the standard test system used in the OECD 305 test to distinguish between elimination via the three remaining processes.

For growth-corrected BCF data, Equations 3 and 4 can be rewritten in terms of the growth-corrected depuration rate constant, leading to the following equation for the lipid-normalised growth-corrected depuration rate constant ($k_{2g,\,L}$):

$$k_{2g,L} = k_{2g,exp} \times \frac{F_{L,exp}}{F_{L,std}} \qquad \text{Equation 5}$$

Where $k_{2g,\,exp}$ = The experimentally determined growth-corrected depuration rate constant

$k_{2g,\,L}$ = The lipid-normalised growth-corrected depuration rate constant.

In the following section, Equation 5 has been used to lipid normalise the growth-corrected depuration rate constants obtained in the OECD 305 ring test to investigate (a) whether differences in lipid explains some of the variability seen between the tests and (b) the most appropriate lipid measurements from the OECD 305 guideline for carrying out such normalisation.

2.3 Analysis of lipid-normalised growth-corrected depuration rate constants from the OECD 305 ring test

A previous report of the OECD 305 ring test (OECD 2013) found some evidence for a decrease in the growth-corrected depuration rate constant with increasing lipid content using the arithmetic mean concentration at the three sampling points (start of test, end of uptake phase and end of depuration phase) as a measure of the lipid content in the fish. However, a number of other measures of lipid content can be derived from the study. These are the following:

- The mean lipid content in the control/exposed fish at the start of the test (day 0 values). These were usually a subsample of the control fish population, but in one case both control and exposed fish populations were sampled.
- The mean lipid content of the exposed fish at the end of the uptake phase (day 13 values).
- The mean lipid content of the exposed fish at the end of the depuration phase, that is day 41 for trout (28 day depuration period) or day 34 for carp (21 day depuration period).
- The arithmetic mean lipid contents of the exposed population based on the above three sampling points.
- The arithmetic mean lipid contents of the exposed population based on the sampling points at the end of uptake and end of depuration only.
- The time-weighted average lipid from the total (control and exposed) population based on the regression analysis outlined in section 2.1.2.

The previous analysis suggested an inverse relationship between the growth-corrected depuration rate constant and lipid (i.e. the depuration rate constant tended to decrease with an increase in lipid; such an inverse relationship can also be inferred from Equation 2 above, which suggests $1/k_{2g}$ should be linear related to the experimental lipid content[4]). Plots showing the dependence of 1/growth-corrected depuration rate constant obtained using trout in the OECD 305 ring test (minus the data for Lab 5) are shown in Figures 2.14 to 2.19 for each lipid derivation. For these plots the growth-corrected depuration rate constant was obtained using the rate constant subtraction method (similar plots can be constructed for the growth-corrected depuration rate constant obtained using the alternative method). The relevant statistics for the linear regression equations are summarised in Table 2.15.

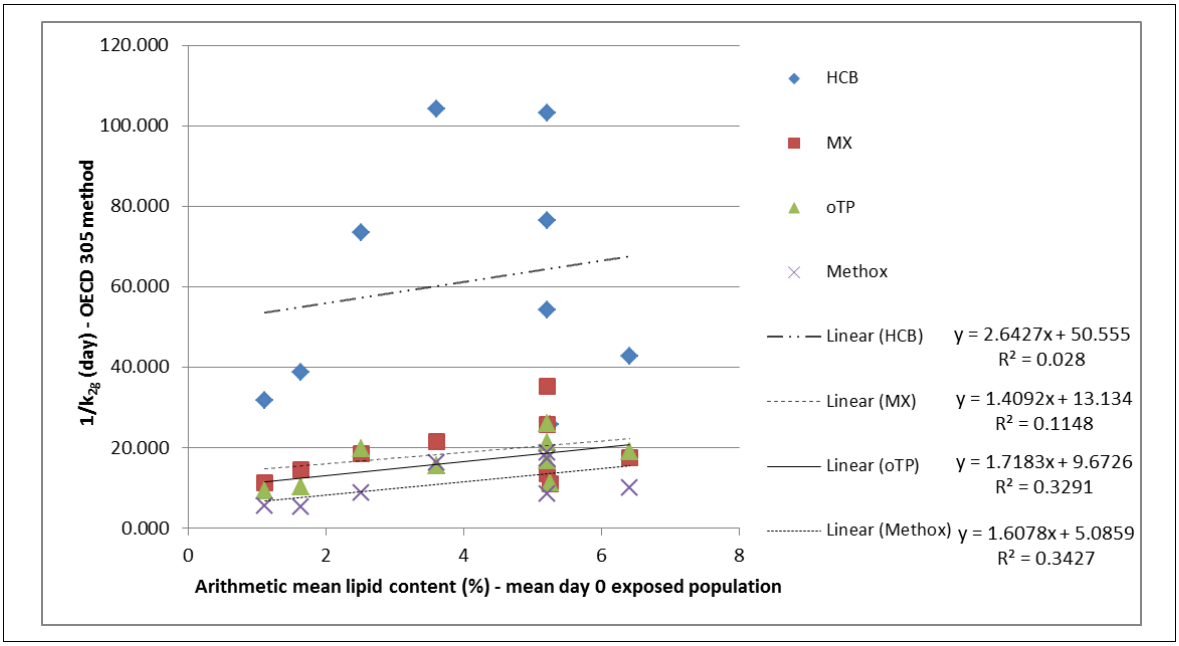

Figure 2.14 Plot of 1/growth-corrected depuration rate constant against fish lipid for trout – lipid content is the mean value of the exposed fish at the start of the test

[4] In the previous analysis in OECD (2012b) and unpublished (2012) trends in k_{2g} with lipid were investigated using plots of k_{2g} versus lipid content. These plots showed a general trend to a decrease in k_{2g} with increasing lipid (i.e. a similar inverse relationship to that observed in this report). However, such plots would be expected to be non-linear at low lipid contents (i.e. k_{2g} directly proportional to 1/lipid). Plotting $1/k_{2g}$ against lipid (as is done in the current analysis) should therefore result in a linear function across the whole range of lipid contents.

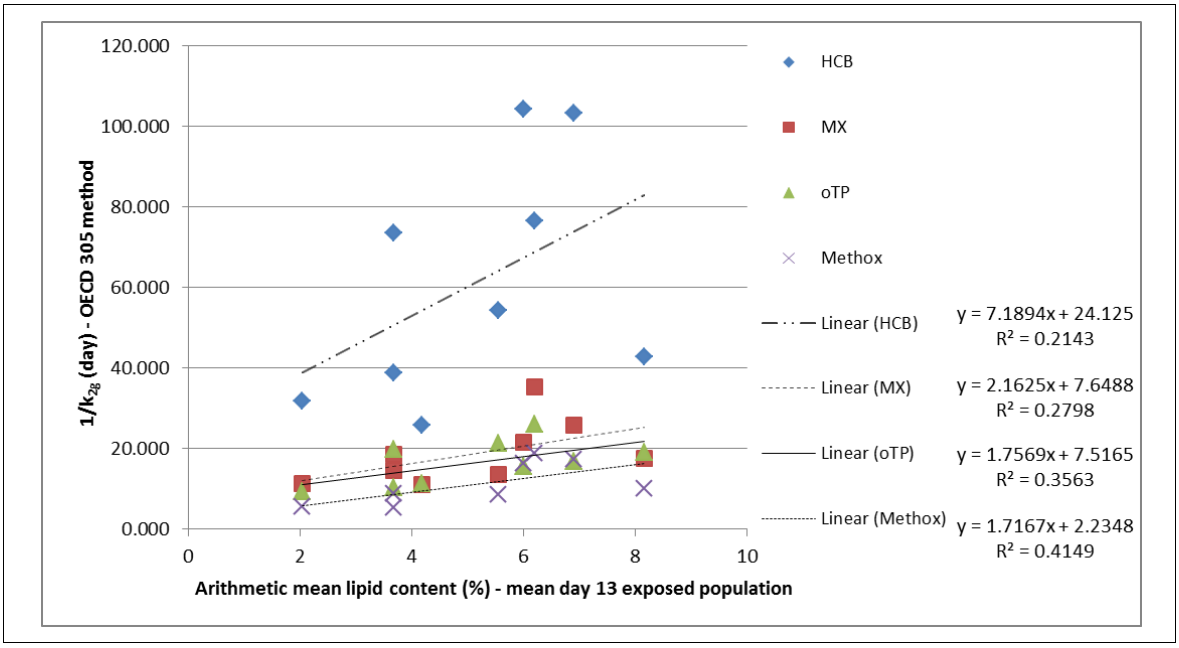

Figure 2.15 Plot of 1/growth-corrected depuration rate constant against fish lipid for trout – lipid content is the mean value of the exposed fish at the end of the uptake phase

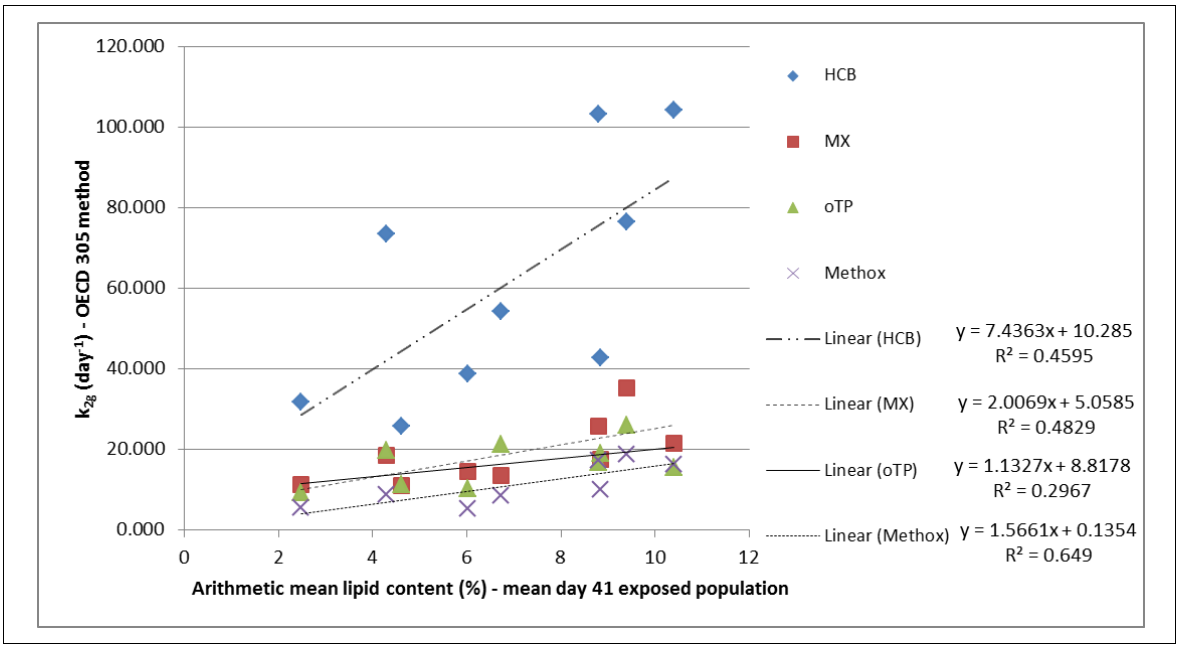

Figure 2.16 Plot of 1/growth-corrected depuration rate constant against fish lipid for trout – lipid content is the mean value of the exposed fish at the end of the depuration phase

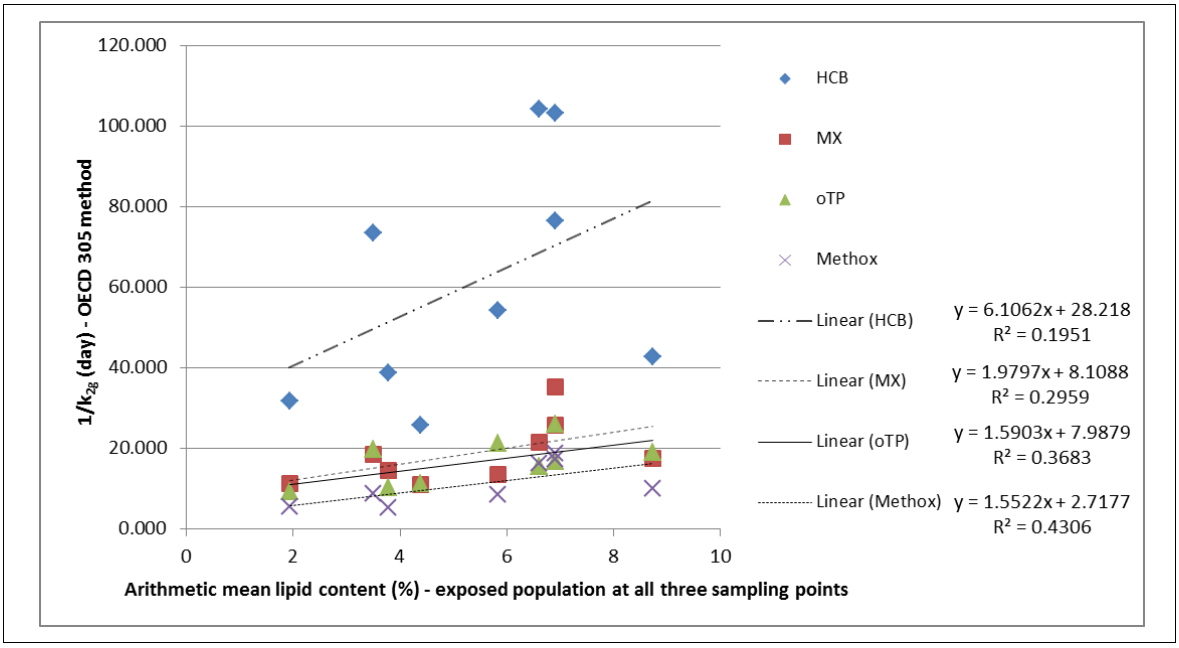

Figure 2.17 Plot of 1/growth-corrected depuration rate constant against fish lipid for trout – lipid content is the arithmetic mean value of the exposed fish for sampling points at the start of the test, end of uptake and depuration phases

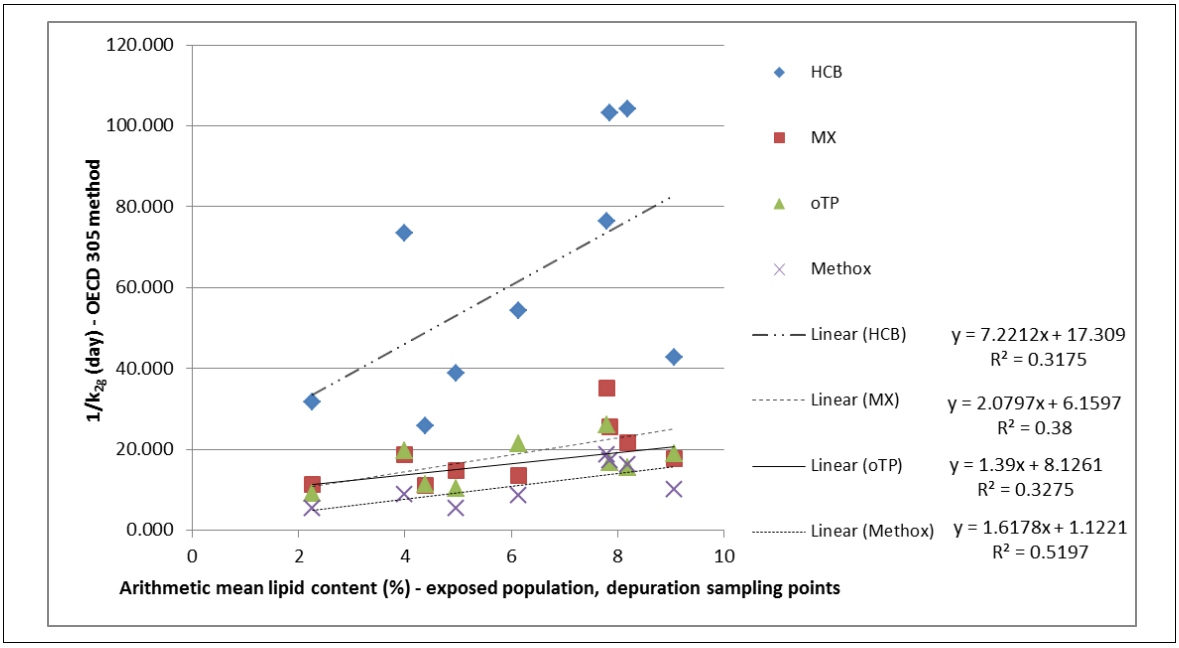

Figure 2.18 Plot of 1/growth-corrected depuration rate constant against fish lipid for trout – lipid content is the arithmetic mean value of the exposed fish for sampling points at the end of uptake and depuration phases

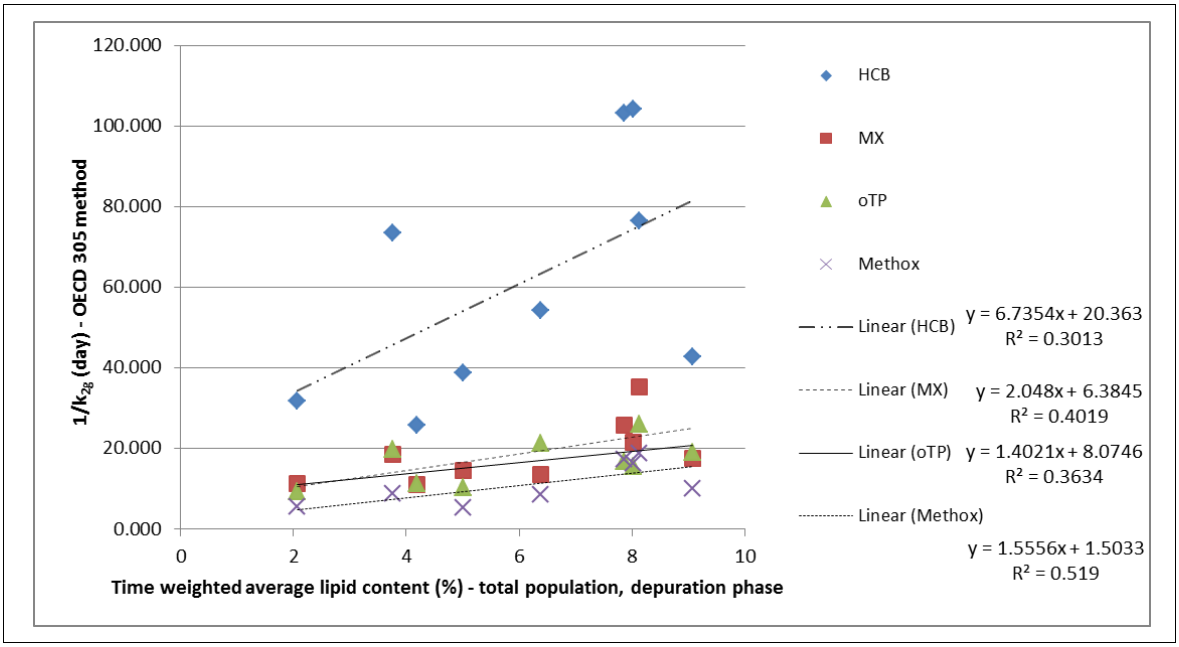

Figure 2.19 Plot of 1/growth-corrected depuration rate constant against fish lipid for trout – lipid content is the estimated time-weighted average of the exposed and control fish during the depuration phase

Table 2.15 Summary of regression analysis of the plots of 1/growth-corrected depuration rate constant (trout; rate constant subtraction method) against fish lipid

Substance[a]	Slope (day/%)	Intercept (day)	R^2 value of regression	Standard error in slope	p-value of slope[b]	95% Confidence interval – slope
Lipid content – mean day 0 value of the exposed population (start of uptake)						
HCB	2.64	50.55	0.028	5.89	0.67	-11.3 to 16.6
MX	1.41	13.13	0.11	1.48	0.37	-2.09 to 4.91
oTP	1.72	9.67	0.33	0.93	0.11	-0.47 to 3.91
Methox	1.61	5.09	0.34	0.91	0.13	0.62 to 3.83
Lipid content – mean day 13 value of the exposed population (end of uptake)						
HCB	7.19	24.12	0.21	5.20	0.21	-5.11 to 19.5
MX	2.16	7.65	0.28	1.31	0.14	-0.94 to 5.26
oTP	1.76	7.52	0.36	0.89	0.090	-0.35 to 3.87
Methox	1.72	2.33	0.41	0.83	0.085	-0.32 to 3.75
Lipid content – mean day 41 value of the exposed population (end of depuration)						
HCB	7.44	10.29	0.46	3.05	0.045[b]	0.23 to 14.6
MX	2.00	5.06	0.48	0.78	0.038[b]	0.15 to 3.86
oTP	1.13	8.82	0.30	0.66	0.13	-0.43 to 2.69
Methox	1.57	0.14	0.65	0.47	0.015[b]	0.42 to 2.72

Substance[a]	Slope (day/%)	Intercept (day)	R^2 value of regression	Standard error in slope	p-value of slope[b]	95% Confidence interval – slope
Lipid content – arithmetic mean value of the exposed population based on the sampling points at the start of the test, end of uptake and end of depuration						
HCB	6.11	28.22	0.20	4.69	0.23	-4.98 to 17.2
MX	1.98	8.11	0.30	1.15	0.13	-0.75 to 4.71
oTP	1.59	7.99	0.37	0.79	0.083	-0.27 to 3.45
Methox	1.55	2.72	0.43	0.73	0.077	-0.23 to 3.34
Lipid content – arithmetic mean value of the exposed population based on the sampling points at the end of uptake and end of depuration						
HCB	7.22	17.31	0.32	4.00	0.11	-2.24 to 16.7
MX	2.08	6.16	0.38	1.00	0.077	-0.29 to 4.45
oTP	1.39	8.13	0.33	0.75	0.11	-0.39 to 3.17
Methox	1.62	1.12	0.52	0.63	0.044[b]	0.064 to 3.17
Lipid content – estimated time-weighted average lipid content of the control and exposed population over the depuration phase						
HCB	6.74	20.36	0.30	3.88	0.13	-2.43 to 15.9
MX	2.05	6.38	0.40	0.94	0.067	-0.19 to 4.28
oTP	1.40	8.07	0.36	0.70	0.086	-0.26 to 3.06
Methox	1.56	1.50	0.52	0.61	0.044[b]	0.060 to 3.05

Notes: [a] HCB = hexachlorobenzene, MX = musk xylene, oTP = o-terphenyl, Methox = methoxychlor.
[b] A p-value <0.05 means that the slope is statistically significantly different from zero with a 95% certainty.

The analysis illustrates that in all cases the $1/k_{2g}$ shows an increasing trend with increasing lipid content. However, linear regression analysis indicates that the slope of the linear fit to the data is not always statistically significantly different from 0, and in many cases the correlation coefficient is relatively small. This is particularly the case when the fish lipid content is represented by the mean values of the exposed population at day 0 (start of uptake) alone, day 13 (end of uptake) alone and the arithmetic mean of the sampling points at the start of uptake, end of uptake and end of depuration. For the other three methods for expressing the lipid content (mean value of exposed population at day 41 (end of depuration), arithmetic mean value of exposed population based on sampling points at the end of uptake and end of depuration, and the estimated time-weighted average lipid content of the control and exposed population over the depuration phase), in each case the slope of the regression equation is statistically significantly different from 0 for at least one of the four substances, with the mean lipid content of the exposed population at day 41 giving statistically significant positive slopes for three of the four substances. Given that the growth-corrected depuration rate constant is determined over the depuration period, it is perhaps not surprising that correlations with lipid content are more evident when measures of the lipid content during the depuration phase are used (as opposed to measures of the lipid content during the uptake phase).

To investigate further whether lipid differences in the fish can explain some of the differences in the growth-corrected depuration rate constants obtained by each laboratory, lipid-normalised growth-corrected depuration rate constants have been calculated for each laboratory from the ring test (except for Lab 5) using Equation 5 based on the above different measures of lipid content. The purpose of this was to determine whether the standard deviation around the mean value could be reduced by lipid normalisation compared with non-normalised data. The relevant lipid-normalised growth-corrected depuration rate constants are shown in Tables 2.16 to 2.19 for hexachlorobenzene, musk xylene, o-terphenyl and methoxychlor using the growth-corrected depuration rate constants obtained by the rate constant subtraction method ($k_{2g}=k_2-k_g$). The equivalent analysis using the growth-corrected depuration rate constants obtained by the alternative method outlined in Brooke and Crookes (2012) are shown in Tables 2.20 to 2.23. The results for benzo[a]pyrene were not considered in this analysis as the uncertainty over the depuration rate constants derived in the studies was high (only a limited number of data points were available). In all cases the depuration rate constants have been normalised to a standard lipid content of 5%.

Table 2.16 Summary of lipid-normalised and growth-corrected depuration rate constants obtained by the rate constant subtraction method for hexachlorobenzene

Laboratory	k_{2g} – not lipid normalised (day^{-1})	$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:					
		Mean day 0 lipid of exposed population	Mean day 13 lipid of exposed population	Mean lipid of exposed population at end of depuration	Arithmetic mean lipid of exposed population at the three sampling points	Arithmetic mean lipid of exposed population at the start and end of depuration	Time-weighted average lipid of total population over depuration period
Lab 1	0.014	0.007	0.010	0.012	0.009	0.011	0.010
Lab 2a – trout	0.023	0.030	0.038	0.041	0.041	0.042	0.042
Lab 2b – carp (level 1)	0.027	0.028	0.031	0.036	0.032	0.032	0.036
Lab 2b – carp (level 2)	0.023	0.023	0.035	0.035	0.029	0.030	0.031
Lab 2b – carp (level 3)	0.015	0.016	0.017	0.023	0.020	0.020	0.021
Lab 3	0.010	0.010	0.013	0.017	0.013	0.015	0.015
Lab 4	0.013	0.014	0.016	0.025	0.018	0.020	0.021
Lab 5	*0.025*	*0.043*	*0.039*	*0.067*	*0.050*	*0.053*	*0.058*
Lab 6	0.026	0.008	0.019	0.031	0.020	0.026	0.026
Lab 7	0.018	0.019	0.020	0.025	0.021	0.023	0.023
Lab 8	0.010	0.007	0.012	0.020	0.013	0.016	0.015
Lab 9	0.039	0.041	0.032	0.036	0.034	0.034	0.033
Lab 10	0.032	0.007	0.013	0.016	0.012	0.014	0.013

Laboratory	k_{2g} – not lipid normalised (day^{-1})	$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:					
		Mean day 0 lipid of exposed population	Mean day 13 lipid of exposed population	Mean lipid of exposed population at end of depuration	Arithmetic mean lipid of exposed population at the three sampling points	Arithmetic mean lipid of exposed population at the start and end of depuration	Time-weighted average lipid of total population over depuration period
Mean trout – both feeding rates	0.020	0.016	0.019	0.025	0.020	0.022	0.022
Relative standard deviation	50.1%	76.2%	50.5%	**39.8%**	52.8%	**46.1%**	**46.7%**
Mean trout – 3% feeding rate	0.016	0.014	0.018	0.024	0.019	0.022	0.022
Relative standard deviation	39.9%	62.3%	51.7%	39.9%	53.7%	47.4%	47.8%
Mean trout 1.5% feeding rate	0.035	0.024	0.023	0.026	0.023	0.024	0.023
Relative standard deviation	14.9%	100.1%	61.2%	55.5%	67.0%	58.1%	60.6%
Mean carp 3% feeding rate	0.022	0.022	0.028	0.031	0.027	0.028	0.029
Relative standard deviation	27.4%	27.4%	34.0%	**23.0%**	**24.1%**	**23.7%**	**25.1%**

Notes: The data for Lab 5 were not included in the calculations of the means and standard deviations – see text.
Values in **bold** show an improvement in the relative standard deviation compared with the non-normalised data.
Levels 1, 2 and 3 in the Lab 2b carp studies refer to test substance concentrations in the food (level 1 being the highest).

Table 2.17 Summary of lipid-normalised and growth-corrected depuration rate constants obtained by the rate constant subtraction method for musk xylene

Laboratory	k_{2g} – not lipid normalised (day^{-1})	$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:					
		Mean day 0 lipid of exposed population	Mean day 13 lipid of exposed population	Mean lipid of exposed population at end of depuration	Arithmetic mean lipid of exposed population at the three sampling points	Arithmetic mean lipid of exposed population at the start and end of depuration	Time-weighted average lipid of total population over depuration period
Lab 1	0.054	0.027	0.039	0.046	0.038	0.043	0.040
Lab 2a – trout	0.057	0.073	0.093	0.100	0.099	0.103	0.103
Lab 2b – carp (level 1)	0.107	0.110	0.125	0.142	0.126	0.129	0.141
Lab 2b – carp (level 2)	0.098	0.100	0.151	0.152	0.126	0.130	0.134
Lab 2b – carp (level 3)	0.078	0.080	0.088	0.118	0.100	0.103	0.108
Lab 3	0.039	0.041	0.054	0.069	0.054	0.061	0.061
Lab 4	0.028	0.030	0.035	0.053	0.039	0.044	0.046
Lab 5	*0.631*	*1.081*	*0.987*	*1.692*	*1.255*	*1.338*	*1.468*
Lab 6	0.068	0.022	0.050	0.082	0.052	0.068	0.068
Lab 7	0.074	0.077	0.082	0.100	0.087	0.091	0.095
Lab 8	0.047	0.033	0.056	0.097	0.061	0.076	0.075
Lab 9	0.090	0.095	0.075	0.083	0.079	0.079	0.075
Lab 10	0.088	0.020	0.036	0.044	0.034	0.040	0.036

Laboratory	k_{2g} – not lipid normalised (day^{-1})	$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:					
		Mean day 0 lipid of exposed population	Mean day 13 lipid of exposed population	Mean lipid of exposed population at end of depuration	Arithmetic mean lipid of exposed population at the three sampling points	Arithmetic mean lipid of exposed population at the start and end of depuration	Time-weighted average lipid of total population over depuration period
Mean trout – both feeding rates	0.061	0.046	0.058	0.075	0.060	0.067	0.067
Relative standard deviation	35.1%	59.8%	36.3%	**30.5%**	38.7%	**33.2%**	**34.8%**
Mean trout – 3% feeding rate	0.052	0.043	0.059	0.078	0.061	0.070	0.070
Relative standard deviation	30.6%	51.9%	36.6%	**28.8%**	38.2%	32.5%	33.4%
Mean trout 1.5% feeding rate	0.089	0.057	0.055	0.063	0.056	0.059	0.056
Relative standard deviation	1.59%	93.0%	49.9%	43.8%	56.3%	46.6%	49.3%
Mean carp 3% feeding rate	0.094	0.097	0.121	0.138	0.117	0.121	0.128
Relative standard deviation	15.8%	15.8%	26.3%	**12.8%**	**12.9%**	**12.6%**	**13.5%**

Notes: The data for Lab 5 were not included in the calculations of the means and standard deviations – see text.
Values in **bold** show an improvement in the relative standard deviation compared with the non-normalised data.
Levels 1, 2 and 3 in the Lab 2b carp studies refer to test substance concentrations in the food (level 1 being the highest).

Table 2.18 Summary of lipid-normalised and growth-corrected depuration rate constants obtained by the rate constant subtraction method for o-terphenyl

Laboratory	k_{2g} – not lipid normalised (day^{-1})	$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:					
		Mean day 0 lipid of exposed population	Mean day 13 lipid of exposed population	Mean lipid of exposed population at end of depuration	Arithmetic mean lipid of exposed population at the three sampling points	Arithmetic mean lipid of exposed population at the start and end of depuration	Time-weighted average lipid of total population over depuration period
Lab 1	0.051	0.025	0.037	0.044	0.035	0.040	0.038
Lab 2a – trout	0.053	0.067	0.086	0.093	0.092	0.095	0.096
Lab 2b – carp (level 1)	0.257	0.264	0.300	0.342	0.303	0.310	0.339
Lab 2b – carp (level 2)	0.318	0.326	0.491	0.496	0.409	0.423	0.438
Lab 2b – carp (level 3)	0.264	0.271	0.297	0.401	0.338	0.350	0.367
Lab 3	0.060	0.062	0.083	0.106	0.083	0.094	0.094
Lab 4	0.038	0.040	0.048	0.072	0.053	0.060	0.062
Lab 5	*0.029*	*0.049*	*0.045*	*0.077*	*0.057*	*0.061*	*0.067*
Lab 6	0.096	0.031	0.071	0.116	0.073	0.096	0.096
Lab 7	0.047	0.049	0.052	0.063	0.055	0.057	0.060
Lab 8	0.065	0.047	0.078	0.135	0.085	0.106	0.104
Lab 9	0.089	0.093	0.074	0.082	0.078	0.078	0.075
Lab 10	0.109	0.024	0.044	0.054	0.042	0.049	0.045

Laboratory	k_{2g} – not lipid normalised (day^{-1})	$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:					
		Mean day 0 lipid of exposed population	Mean day 13 lipid of exposed population	Mean lipid of exposed population at end of depuration	Arithmetic mean lipid of exposed population at the three sampling points	Arithmetic mean lipid of exposed population at the start and end of depuration	Time-weighted average lipid of total population over depuration period
Mean trout – both feeding rates	0.067	0.049	0.064	0.085	0.066	0.075	0.074
Relative standard deviation	36.5%	46.2%	**28.8%**	**35.5%**	**30.8%**	**31.8%**	**32.7%**
Mean trout – 3% feeding rate	0.058	0.046	0.065	0.090	0.068	0.078	0.079
Relative standard deviation	32.0%	33.3%	**29.5%**	35.6%	**30.6%**	32.2%	**31.8%**
Mean trout 1.5% feeding rate	0.099	0.059	0.059	0.068	0.060	0.064	0.060
Relative standard deviation	14.3%	83.2%	35.5%	28.9%	42.3%	31.9%	34.8%
Mean carp 3% feeding rate	0.279	0.287	0.363	0.413	0.350	0.361	0.381
Relative standard deviation	12.0%	12.0%	30.6%	18.8%	15.4%	15.9%	13.3%

Notes: The data for Lab 5 were not included in the calculations of the means and standard deviations – see text.
Values in **bold** show an improvement in the relative standard deviation compared with the non-normalised data.
Levels 1, 2 and 3 in the Lab 2b carp studies refer to test substance concentrations in the food (level 1 being the highest).

Table 2.19 Summary of lipid-normalised and growth-corrected depuration rate constants obtained by the rate constant subtraction method for methoxychlor

Laboratory	k_{2g} – not lipid normalised (day^{-1})	$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:					
		Mean day 0 lipid of exposed population	Mean day 13 lipid of exposed population	Mean lipid of exposed population at end of depuration	Arithmetic mean lipid of exposed population at the three sampling points	Arithmetic mean lipid of exposed population at the start and end of depuration	Time-weighted average lipid of total population over depuration period
Lab 1	0.113	0.057	0.083	0.098	0.079	0.090	0.085
Lab 2a – trout	0.100	0.128	0.162	0.176	0.174	0.180	0.181
Lab 2b – carp (level 1)	0.277	0.284	0.324	0.368	0.327	0.334	0.365
Lab 2b – carp (level 2)	0.261	0.268	0.403	0.407	0.336	0.347	0.359
Lab 2b – carp (level 3)	0.231	0.237	0.260	0.351	0.296	0.306	0.321
Lab 3	0.058	0.060	0.080	0.102	0.080	0.091	0.091
Lab 4	0.054	0.056	0.066	0.101	0.074	0.084	0.087
Lab 5	*-0.011*	*-0.019*	*-0.018*	*-0.030*	*-0.022*	*-0.024*	*-0.026*
Lab 6	0.188	0.061	0.139	0.227	0.142	0.187	0.188
Lab 7	0.117	0.122	0.130	0.158	0.137	0.144	0.150
Lab 8	0.062	0.044	0.074	0.128	0.081	0.101	0.099
Lab 9	nd	nd	nd	nd	nd	nd	nd
Lab 10	0.182	0.040	0.074	0.090	0.070	0.082	0.075

Laboratory	k_{2g} – not lipid normalised (day^{-1})	$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:					
		Mean day 0 lipid of exposed population	Mean day 13 lipid of exposed population	Mean lipid of exposed population at end of depuration	Arithmetic mean lipid of exposed population at the three sampling points	Arithmetic mean lipid of exposed population at the start and end of depuration	Time-weighted average lipid of total population over depuration period
Mean trout – both feeding rates	0.109	0.071	0.101	0.135	0.105	0.120	0.120
Relative standard deviation	48.6%	**47.8%**	**36.2%**	**35.8%**	**38.1%**	**36.6%**	**38.4%**
Mean trout – 3% feeding rate	0.099	0.075	0.105	0.141	0.110	0.125	0.126
Relative standard deviation	48.3%	**45.3%**	**36.0%**	**34.3%**	**36.9%**	**35.5%**	**36.3%**
Mean trout 1.5% feeding rate	0.182	0.040	0.074	0.090	0.070	0.082	0.075
Relative standard deviation	nd	nd	nd	nd	nd	nd	nd
Mean carp 3% feeding rate	0.256	0.263	0.329	0.375	0.320	0.329	0.349
Relative standard deviation	9.1%	9.1%	21.7%	**7.6%**	**6.5%**	**6.3%**	**6.8%**

Notes: The data for Lab 5 were not included in the calculations of the means and standard deviations – see text.
Levels 1, 2 and 3 in the Lab 2b carp studies refer to test substance concentrations in the food (level 1 being the highest).
nd – No data.
Values in **bold** show an improvement in the relative standard deviation compared with the non-normalised data.

Table 2.20 Summary of lipid-normalised and growth-corrected depuration rate constants obtained by the alternative method for hexachlorobenzene

Laboratory	k_{2g} – not lipid normalised (day^{-1})	$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:					
		Mean day 0 lipid of exposed population	Mean day 13 lipid of exposed population	Mean lipid of exposed population at end of depuration	Arithmetic mean lipid of exposed population at the three sampling points	Arithmetic mean lipid of exposed population at the start and end of depuration	Time-weighted average lipid of total population over depuration period
Lab 1	0.018	0.009	0.013	0.015	0.013	0.014	0.014
Lab 2a – trout	0.025	0.032	0.041	0.044	0.044	0.045	0.045
Lab 2b – carp (level 1)	0.030	0.031	0.035	0.040	0.035	0.036	0.040
Lab 2b – carp (level 2)	0.024	0.025	0.037	0.037	0.031	0.032	0.033
Lab 2b – carp (level 3)	0.015	0.015	0.017	0.023	0.019	0.020	0.021
Lab 3	0.010	0.010	0.014	0.018	0.014	0.016	0.016
Lab 4	0.022	0.023	0.027	0.041	0.030	0.034	0.036
Lab 5	*0.032*	*0.055*	*0.050*	*0.086*	*0.064*	*0.068*	*0.074*
Lab 6	0.031	0.010	0.023	0.037	0.023	0.031	0.031
Lab 7	0.019	0.020	0.021	0.026	0.022	0.023	0.024
Lab 8	0.012	0.009	0.014	0.025	0.016	0.020	0.019
Lab 9	0.032	0.033	0.027	0.029	0.028	0.028	0.027
Lab 10	0.033	0.007	0.013	0.016	0.013	0.015	0.013

Laboratory	k_{2g} – not lipid normalised (day^{-1})	$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:					
		Mean day 0 lipid of exposed population	Mean day 13 lipid of exposed population	Mean lipid of exposed population at end of depuration	Arithmetic mean lipid of exposed population at the three sampling points	Arithmetic mean lipid of exposed population at the start and end of depuration	Time-weighted average lipid of total population over depuration period
Mean trout – both feeding rates	0.022	0.017	0.021	0.028	0.022	0.025	0.025
Relative standard deviation	37.6%	60.7%	42.9%	38.9%	46.1%	41.7%	43.7%
Mean trout – 3% feeding rate	0.020	0.016	0.022	0.030	0.023	0.026	0.026
Relative standard deviation	37.2%	55.9%	45.0%	38.9%	47.6%	42.8%	43.8%
Mean trout 1.5% feeding rate	0.032	0.020	0.020	0.023	0.020	0.021	0.020
Relative standard deviation	1.76%	91.0%	47.0%	40.7%	53.4%	43.6%	46.3%
Mean carp 3% feeding rate	0.023	0.024	0.030	0.033	0.029	0.029	0.031
Relative standard deviation	32.8%	32.8%	37.4%	**27.7%**	**29.3%**	**28.8%**	**30.4%**

Notes: The data for Lab 5 were not included in the calculations of the means and standard deviations – see text.
Values in **bold** show an improvement in the relative standard deviation compared with the non-normalised data.
Levels 1, 2 and 3 in the Lab 2b carp studies refer to test substance concentrations in the food (level 1 being the highest).

Table 2.21 Summary of lipid-normalised and growth-corrected depuration rate constants obtained by the alternative method for musk xylene

Laboratory	k_{2g} – not lipid normalised (day^{-1})	$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:					
		Mean day 0 lipid of exposed population	Mean day 13 lipid of exposed population	Mean lipid of exposed population at end of depuration	Arithmetic mean lipid of exposed population at the three sampling points	Arithmetic mean lipid of exposed population at the start and end of depuration	Time-weighted average lipid of total population over depuration period
Lab 1	0.059	0.030	0.043	0.051	0.041	0.047	0.044
Lab 2a – trout	0.058	0.074	0.095	0.102	0.101	0.105	0.105
Lab 2b – carp (level 1)	0.109	0.112	0.128	0.145	0.129	0.132	0.144
Lab 2b – carp (level 2)	0.099	0.102	0.153	0.155	0.128	0.132	0.136
Lab 2b – carp (level 3)	0.078	0.080	0.088	0.119	0.100	0.104	0.109
Lab 3	0.039	0.041	0.054	0.069	0.054	0.061	0.061
Lab 4	0.038	0.040	0.047	0.071	0.052	0.059	0.062
Lab 5	*0.667*	*1.142*	*1.043*	*1.788*	*1.326*	*1.414*	*1.551*
Lab 6	0.074	0.024	0.054	0.089	0.056	0.073	0.074
Lab 7	0.074	0.077	0.082	0.100	0.086	0.091	0.094
Lab 8	0.049	0.035	0.059	0.102	0.065	0.080	0.079
Lab 9	0.083	0.087	0.069	0.077	0.073	0.073	0.070
Lab 10	0.090	0.020	0.037	0.044	0.035	0.040	0.037

Laboratory	k_{2g} – not lipid normalised (day^{-1})	$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:					
		Mean day 0 lipid of exposed population	Mean day 13 lipid of exposed population	Mean lipid of exposed population at end of depuration	Arithmetic mean lipid of exposed population at the three sampling points	Arithmetic mean lipid of exposed population at the start and end of depuration	Time-weighted average lipid of total population over depuration period
Mean trout – both feeding rates	0.063	0.048	0.060	0.078	0.063	0.070	0.070
Relative standard deviation	29.8%	53.0%	31.4%	**27.7%**	34.1%	**29.4%**	31.4%
Mean trout – 3% feeding rate	0.056	0.046	0.062	0.083	0.065	0.074	0.074
Relative standard deviation (%)	26.6%	46.3%	30.7%	**24.1%**	32.6%	27.1%	28.1%
Mean trout 1.5% feeding rate	0.086	0.054	0.053	0.060	0.054	0.057	0.053
Relative standard deviation (%)	5.2%	89.0%	43.8%	37.5%	50.4%	40.4%	43.2%
Mean carp 3% feeding rate	0.095	0.098	0.123	0.140	0.119	0.122	0.130
Relative standard deviation (%)	16.6%	16.6%	26.7%	**13.3%**	**13.6%**	**13.3%**	**14.3%**

Notes: The data for Lab 5 were not included in the calculations of the means and standard deviations – see text.
Values in **bold** show an improvement in the relative standard deviation compared with the non-normalised data.
Levels 1, 2 and 3 in the Lab 2b carp studies refer to test substance concentrations in the food (level 1 being the highest).

Table 2.22 Summary of lipid-normalised and growth-corrected depuration rate constants obtained by the alternative method for o-terphenyl

Laboratory	k_{2g} – not lipid normalised (day^{-1})	$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:					
		Mean day 0 lipid of exposed population	Mean day 13 lipid of exposed population	Mean lipid of exposed population at end of depuration	Arithmetic mean lipid of exposed population at the three sampling points	Arithmetic mean lipid of exposed population at the start and end of depuration	Time-weighted average lipid of total population over depuration period
Lab 1	0.055	0.028	0.040	0.047	0.038	0.044	0.041
Lab 2a – trout	0.054	0.069	0.088	0.095	0.094	0.098	0.098
Lab 2b – carp (level 1)	0.260	0.267	0.304	0.346	0.307	0.314	0.343
Lab 2b – carp (level 2)	0.305	0.314	0.472	0.476	0.393	0.406	0.420
Lab 2b – carp (level 3)	0.248	0.255	0.280	0.377	0.318	0.329	0.346
Lab 3	0.060	0.062	0.083	0.106	0.083	0.094	0.094
Lab 4	0.048	0.050	0.060	0.090	0.066	0.075	0.078
Lab 5[a]	*0.035*	*0.060*	*0.055*	*0.094*	*0.070*	*0.074*	*0.081*
Lab 6	0.102	0.033	0.075	0.123	0.077	0.101	0.102
Lab 7	0.047	0.049	0.052	0.063	0.055	0.058	0.060
Lab 8	0.067	0.048	0.080	0.139	0.088	0.110	0.108
Lab 9	0.080	0.084	0.067	0.074	0.070	0.070	0.067
Lab 10	0.111	0.025	0.045	0.055	0.043	0.050	0.046

Laboratory	k_{2g} – not lipid normalised (day^{-1})	$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:					
		Mean day 0 lipid of exposed population	Mean day 13 lipid of exposed population	Mean lipid of exposed population at end of depuration	Arithmetic mean lipid of exposed population at the three sampling points	Arithmetic mean lipid of exposed population at the start and end of depuration	Time-weighted average lipid of total population over depuration period
Mean trout – both feeding rates	0.069	0.050	0.066	0.088	0.068	0.078	0.077
Relative standard deviation	33.9%	39.7%	**26.3%**	35.4%	**28.8%**	**30.9%**	**32.1%**
Mean trout – 3% feeding rate	0.062	0.049	0.068	0.095	0.072	0.083	0.083
Relative standard deviation	30.7%	**30.3%**	**26.1%**	33.8%	**27.7%**	**29.7%**	**29.5%**
Mean trout 1.5% feeding rate	0.096	0.055	0.056	0.065	0.057	0.060	0.057
Relative standard deviation	22.6%	77.5%	27.4%	**20.7%**	34.4%	23.8%	26.7%
Mean carp 3% feeding rate	0.271	0.279	0.352	0.400	0.340	0.350	0.370
Relative standard deviation	11.1%	11.1%	29.7%	17.0%	13.7%	14.1%	11.8%

Notes: The data for Lab 5 were not included in the calculations of the means and standard deviations – see text.
Values in **bold** show an improvement in the relative standard deviation compared with the non-normalised data.
Levels 1, 2 and 3 in the Lab 2b carp studies refer to test substance concentrations in the food (level 1 being the highest).

Table 2.23 Summary of lipid-normalised and growth-corrected depuration rate constants obtained by the alternative method for methoxychlor

Laboratory	k_{2g} – not lipid normalised (day^{-1})	$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:					
		Mean day 0 lipid of exposed population	Mean day 13 lipid of exposed population	Mean lipid of exposed population at end of depuration	Arithmetic mean lipid of exposed population at the three sampling points	Arithmetic mean lipid of exposed population at the start and end of depuration	Time-weighted average lipid of total population over depuration period
Lab 1	0.114	0.057	0.084	0.098	0.080	0.091	0.086
Lab 2a – trout	0.101	0.129	0.165	0.178	0.177	0.183	0.183
Lab 2b – carp (level 1)	0.289	0.297	0.338	0.385	0.342	0.349	0.381
Lab 2b – carp (level 2)	0.249	0.256	0.385	0.389	0.321	0.332	0.343
Lab 2b – carp (level 3)	0.212	0.218	0.239	0.323	0.272	0.282	0.296
Lab 3	0.056	0.058	0.077	0.099	0.077	0.088	0.088
Lab 4	0.063	0.066	0.078	0.118	0.087	0.098	0.102
Lab 5	*-0.005*	*-0.009*	*-0.008*	*-0.013*	*-0.010*	*-0.011*	*-0.012*
Lab 6	0.182	0.059	0.134	0.219	0.138	0.181	0.182
Lab 7	0.118	0.123	0.131	0.159	0.138	0.145	0.151
Lab 8	0.065	0.047	0.078	0.135	0.086	0.107	0.104
Lab 9	nd	nd	nd	nd	nd	nd	nd
Lab 10	0.184	0.041	0.075	0.091	0.071	0.083	0.076

Laboratory	k_{2g} – not lipid normalised (day^{-1})	$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:					
		Mean day 0 lipid of exposed population	Mean day 13 lipid of exposed population	Mean lipid of exposed population at end of depuration	Arithmetic mean lipid of exposed population at the three sampling points	Arithmetic mean lipid of exposed population at the start and end of depuration	Time-weighted average lipid of total population over depuration period
Mean trout – both feeding rates	0.110	0.073	0.103	0.137	0.107	0.122	0.122
Relative standard deviation	45.9%	46.9%	**34.1%**	**33.1%**	**36.3%**	**34.1%**	**36.0%**
Mean trout – 3% feeding rate	0.100	0.077	0.107	0.144	0.112	0.127	0.128
Relative standard deviation	44.4%	**44.2%**	**33.6%**	**31.1%**	**34.8%**	**32.7%**	**33.6%**
Mean trout 1.5% feeding rate	0.184	0.041	0.075	0.091	0.071	0.083	0.076
Relative standard deviation	nd	nd	nd	nd	nd	nd	nd
Mean carp 3% feeding rate	0.250	0.257	0.321	0.366	0.312	0.321	0.340
Relative standard deviation	nd	nd	nd	nd	nd	nd	nd

Notes: The data for Lab 5 were not included in the calculations of the means and standard deviations – see text.
nd – No data.
Values in **bold** show an improvement in the relative standard deviation compared with the non-normalised data.
Levels 1, 2 and 3 in the Lab 2b carp studies refer to test substance concentrations in the food (level 1 being the highest).

Taking the relative standard deviation of the non-normalised k_{2g} as the starting point, the following can be seen from this analysis (for trout for both feeding rates combined and carp, this information is summarised in Table 2.24):

- Using the growth-corrected depuration rate constant obtained by the rate constant subtraction method:
    - For hexachlorobenzene, lipid normalising the growth-corrected depuration rate constant reduces the relative standard deviation compared with the non-lipid-normalised data for the combined (i.e. both feeding rates) trout dataset when the mean lipid of the exposed group at the end of the depuration phase, arithmetic mean of the exposed population at the start and end of depuration and the time-weighted average lipid content of the control and exposed population during depuration are used. The lowest relative standard deviation is obtained using the mean lipid content of the exposed population measured at the end of depuration (giving a relative standard deviation of 39.8% compared with 50.1% for the non-normalised data). Similar findings are evident for the carp data. When the data for trout for the two feeding rates are considered separately there is no apparent improvement in the relative standard deviation by lipid normalisation.
    - For musk xylene, lipid normalisation of the growth-corrected depuration rate constant results in a slight improvement of the relative standard deviation for the combined trout dataset using the same measures of lipid content as for hexachlorobenzene. The biggest improvement is again with the mean lipid content of the exposed population at the end of the depuration period (relative standard deviation of 30.5% compared with 35.1% for the non-normalised data). This measure of lipid also results in a slight improvement in the relative standard deviation of the trout data at the 3% feeding rate (from 30.6% to 28.8%) but not at the 1.5% feeding rate. The relative standard deviation of the carp data is improved by lipid normalisation using four of the measures of lipid content but in this case the best improvement (from 15.8% to 12.6%) is obtained using the arithmetic mean of the measurements at the end of uptake/start of depuration and the end of depuration, although in this case the differences between the various methods are relatively small.
    - For *o*-terphenyl, lipid normalisation of the growth-corrected depuration rate constant results in a slight improvement of the relative standard deviation for the combined trout dataset using the following measures of the lipid content: the mean of exposed population on day 13 of uptake (end of uptake), the mean of exposed population at the end of depuration, the arithmetic mean of the exposed population at the three sampling points during the whole study, the arithmetic mean of the exposed population at the start and end of depuration, and the time-weighted average lipid of the total population over the depuration period. The largest improvement (from 36.5% to 28.8%) in the relative standard deviation is obtained using the mean lipid content on day 13 of uptake (end of uptake). The lipid content on day 13 of uptake also resulted in the greatest improvement of the relative standard deviation for the trout at the 3% feeding rate (from 32.0% to 29.5%). However, no improvement of the relative standard deviation by lipid normalisation was evident for the trout data at the 1.5% feeding rate or for the carp data.
    - For methoxychlor, lipid normalisation of the growth-corrected depuration rate constant resulted in an improvement of the relative standard

deviation for all measures of the lipid content. The largest improvement (from 48.6% to 35.8%) in the relative standard deviation was found using the mean lipid of the exposed population at the end of depuration. The same findings were also apparent for the trout at the 3% feeding rate (no comparison could be made at the 1.5% feeding rate). For the carp data, the relative standard deviation was improved using the mean lipid of the exposed population at the end of depuration, the arithmetic mean of the exposed population at the three sampling points during the whole study, the arithmetic mean lipid content at the start and end of depuration, and the time-weighted average lipid of the whole population over the depuration period. The largest improvement in the relative standard deviation (from 9.12% to 6.3%) was evident with the arithmetic mean lipid of the exposed population at the start and end of depuration; however, the improvement using this measure of the lipid content over the other measures was small.

- Using the growth-corrected depuration rate constant obtained by the alternative (Brooke and Crookes 2012) method:
    - For hexachlorobenzene, lipid normalising the growth-corrected depuration rate constant did not reduce the relative standard deviation for the trout data compared with the non-normalised data for any of the methods investigated. A slight improvement in the relative standard deviation was seen for the carp data when the mean lipid of the exposed population at the end of depuration was used for normalisation (relative standard deviation of 27.7% compared with 32.8% for the non-normalised data), and similar slight improvements were also evident using the arithmetic mean lipid of the exposed population at the three sampling points over the whole experimental period, the arithmetic mean lipid of the exposed population at the start and end of depuration, and the time-weighted average lipid content of the total population over the depuration period.
    - For musk xylene, lipid normalisation of the growth-corrected depuration rate constant results in a slight improvement of the relative standard deviation for the combined trout dataset using the mean lipid content of the exposed population at the end of depuration (relative standard deviation of 27.7% compared with 29.8% for the non-normalised data) and the arithmetic mean of the exposed population at the start and end of depuration (relative standard deviation of 29.4%). The mean lipid content of the exposed population at the end of depuration also results in a slight improvement in the relative standard deviation of the trout data at the 3% feeding rate (from 26.6% to 24.1%) but not at the 1.5% feeding rate. The relative standard deviation of the carp data is improved by lipid normalisation using four of the measures of lipid content but in this case the best improvement (from 16.6% to 13.3%) is obtained using both the mean lipid content of the exposed population at the end of the depuration and the arithmetic mean of the measurements at the end of uptake/start of depuration and the end of depuration, although in this case the differences between the various methods are relatively small.
    - For *o*-terphenyl, lipid normalisation of the growth-corrected depuration rate constant results in an improvement of the relative standard deviation for the combined trout dataset using the following measures of the lipid content: the mean of exposed population on day 13 of uptake (end of uptake), the arithmetic mean of the exposed population at the

three sampling points during the whole study, the arithmetic mean of the exposed population at the start and end of depuration, and the time-weighted average lipid of the total population over the depuration period. The largest improvement (from 33.9% to 26.3%) in the relative standard deviation is obtained using the mean lipid content on day 13 of the uptake (end of uptake). The lipid content on day 13 of the uptake also resulted in the greatest improvement of the relative standard deviation for the trout at the 3% feeding rate (from 30.7% to 26.1%). The relative standard deviation for the trout data at the 1.5% feeding rate was improved from 22.6% to 20.7% only by normalising using the mean lipid of the exposed population at the end of the depuration phase. No improvement of the relative standard deviation by lipid normalisation was evident for the carp data.

- For methoxychlor, lipid normalisation of the growth-corrected depuration rate constant resulted in an improvement of the relative standard deviation for the combined trout dataset using the mean lipid content of the exposed population on day 13 of uptake (end of uptake), the mean of the exposed population at the end of depuration, the arithmetic mean of the exposed population at the three sampling points over the whole study, the arithmetic mean of the exposed population at the start and end of depuration, and the time-weighted average over the depuration period. The largest improvement (from 45.9% to 33.1%) in the relative standard deviation was found using the mean lipid of the exposed population at the end of the depuration. For the 3% feeding rate, all six measures of lipid content resulted in an improvement of the relative standard deviation compared with non-normalised data but again the largest improvement was using the mean lipid of the exposed population at the end of depuration (improvement in the relative standard deviation was from 44.4% to 31.1%). No comparison could be made at the 1.5% feeding rate or for the carp data.

Table 2.24 Summary of the improvement in relative standard deviation of growth-corrected depuration rate constant according to different lipid normalisation methods

TROUT – combined feeding rate groups						
k_{2g} from rate constant subtraction method						
Test substance	**$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:**					
	Mean day 0 lipid of exposed population	**Mean day 13 lipid of exposed population**	**Mean lipid of exposed population at end of depuration**	**Arithmetic mean lipid of exposed population at the three sampling points**	**Arithmetic mean lipid of exposed population at the start and end of depuration**	**Time-weighted average lipid of total population over depuration period**
Hexachlorobenzene	-	-	**Rel SD** ↓	-	Rel SD ↓	Rel SD ↓
Musk xylene	-	-	**Rel SD** ↓	-	Rel SD ↓	Rel SD ↓
o-terphenyl	-	**Rel SD** ↓	Rel SD ↓	Rel SD ↓	Rel SD ↓	Rel SD ↓
Methoxychlor	Rel SD ↓	Rel SD ↓	**Rel SD** ↓	Rel SD ↓	Rel SD ↓	Rel SD ↓
k_{2g} from alternative method						
Test substance	**$k_{2g, L}$, (day^{-1}) normalised to a 5% lipid content based on:**					
	Mean day 0 lipid of exposed population	**Mean day 13 lipid of exposed population**	**Mean lipid of exposed population at end of depuration**	**Arithmetic mean lipid of exposed population at the three sampling points**	**Arithmetic mean lipid of exposed population at the start and end of depuration**	**Time-weighted average lipid of total population over depuration period**
Hexachlorobenzene	-	-	-	-	-	-
Musk xylene	-	-	Rel SD ↓	-	Rel SD ↓	-
o-terphenyl	-	**Rel SD** ↓	-	Rel SD ↓	Rel SD ↓	Rel SD ↓
Methoxychlor	-	Rel SD ↓	**Rel SD** ↓	Rel SD ↓	Rel SD ↓	Rel SD ↓

CARP						
k_{2g} from rate constant subtraction method						
Test substance	**$k_{2g,L}$, (day^{-1}) normalised to a 5% lipid content based on:**					
	Mean day 0 lipid of exposed population	**Mean day 13 lipid of exposed population**	**Mean lipid of exposed population at end of depuration**	**Arithmetic mean lipid of exposed population at the three sampling points**	**Arithmetic mean lipid of exposed population at the start and end of depuration**	**Time-weighted average lipid of total population over depuration period**
Hexachlorobenzene	-	-	**Rel SD** ↓	Rel SD ↓	Rel SD ↓	Rel SD ↓
Musk xylene	-	-	Rel SD ↓	Rel SD ↓	**Rel SD** ↓	Rel SD ↓
o-terphenyl	-	**-**	-	-	-	-
Methoxychlor			Rel SD ↓	Rel SD ↓	**Rel SD** ↓	Rel SD ↓
k_{2g} from alternative method						
Test substance	**$k_{2g,L}$, (day^{-1}) normalised to a 5% lipid content based on:**					
	Mean day 0 lipid of exposed population	**Mean day 13 lipid of exposed population**	**Mean lipid of exposed population at end of depuration**	**Arithmetic mean lipid of exposed population at the three sampling points**	**Arithmetic mean lipid of exposed population at the start and end of depuration**	**Time-weighted average lipid of total population over depuration period**
Hexachlorobenzene	-	-	**Rel SD** ↓	Rel SD ↓	Rel SD ↓	Rel SD ↓
Musk xylene	-	-	**Rel SD** ↓	Rel SD ↓	**Rel SD** ↓	Rel SD ↓
o-terphenyl	-	**-**	-	-	-	-
Methoxychlor				No comparison possible		

Notes: Rel SD ↓ signifies an improvement in the standard deviation relative to the non-lipid-normalised k_{2g} standard deviation. Values in **bold** showed the greatest improvement.

- No improvement in the standard deviation relative to the non-lipid-normalised k_{2g} standard deviation.

As discussed in section 2.2, a dependence of the depuration rate constant on the lipid content can be envisaged. The analysis carried out here is complicated by the fact that the lipid content varied (tended to increase) during the course of the depuration. Thus if the depuration rate constant is dependent on the lipid content this would also be expected to vary during the course of the experiment (from theoretical considerations the depuration rate constant would be expected to decrease as the lipid content increases). For the analysis carried out on the datasets various measures of the lipid content have been considered. However, in reality the lipid content of the fish may vary continually during the experiment and so, in principle, at any given point in time during the experiment the depuration rate constant may also vary. As the analysis has been carried out using discrete values of the depuration rate constant and lipid contents this may introduce a further source of error into the analysis. However, owing to the relatively high variability in the experimental data, it is considered unlikely that any continual variation in the depuration rate constant caused by continual variation in the fish lipid content would be detectable in practice; further, it is most likely that any uncertainty introduced into the analysis from this source would be relatively small compared with the uncertainty resulting from the variability in the experimental measurements.

Overall, no one method for lipid normalisation consistently results in an improvement in the relative standard deviation of the lipid-normalised growth-corrected depuration rate constant compared with the non-normalised growth-corrected depuration rate constant. However, it is evident that lipid normalisation using measures of the lipid content during depuration (particularly the mean lipid at the end of depuration, the arithmetic mean of the start and end of depuration along with the time-weighted average lipid over the depuration phase) tend to produce a (modest) improvement in the relative standard deviation for most substances. This is in general agreement with the trends found in $1/k_{2g}$ with lipid content considered earlier, where generally stronger correlations were evident with data over the depuration phase than the uptake phase.

It is also evident from this analysis that normalisation to the lipid content at day 0 of the experiment tends to result in an increase in the relative standard deviation of the mean growth-corrected depuration rate constant for most of the substances tested. This confirms the expectation that the lipid content at day 0 is not a good measure of the lipid content in the fish during depuration. Therefore it could be considered in a future revision of the OECD 305 test guideline that this sampling point could be omitted and replaced by further lipid measurements during the depuration phase of the study.

It should also be noted that the relative standard deviations around the mean lipid-normalised k_{2g} values are frequently still relatively high (typically 20–30% or more) and so lipid alone does not appear to account for much of the variability seen in the experimental data.

Overall, based on the results of this analysis, when lipid normalisation is required, it is recommended that a measure of the lipid during the depuration phase is used for the normalisation. The analysis carried out suggests that either the mean lipid content at the end of the depuration phase, the arithmetic mean concentration of the two sampling points over the depuration phase, or the time-weighted average lipid over the depuration phase are appropriate measures for carrying out this normalisation.

2.4 Other datasets

2.4.1 Inoue *et al*. (2012)

The paper by Inoue *et al*. (2012) contains a series of growth-corrected depuration rate constants for hexachlorobenzene obtained using a draft of the OECD 305 dietary study

methodology. Other substances were also included in the study, but only single values for the depuration rate constant were reported for these substances. However, hexachlorobenzene was used as a reference substance in a number of tests and so multiple values are given for this substance.[5]

The species tested was carp and the feeding rate used was 3% body weight. The concentration of hexachlorobenzene in the diet was nominally 25 μg g^{-1} or 100 μg g^{-1}. The uptake phase of the study was either 10 or 13 days and the depuration phase of the study was between 14 and 38 days. The growth-corrected depuration rate constant was obtained from the data by the rate constant subtraction method. The growth-corrected depuration rate constants determined for hexachlorobenzene are summarised in Table 2.25. The fish lipid contents are also given in this table. These lipid contents were given as the mean values based on measurements at the beginning/end of uptake and the end of depuration and so represent the arithmetic mean of the sampling points across the whole study. The raw lipid data were not given and so it is not possible to estimate other measures of the lipid content for this study.

A plot of $1/k_{2g}$ versus lipid content is given in Figure 2.20. This indicates a positive correlation between the $1/k_{2g}$ and fish lipid, although the p-value of the slope of the plot is 0.12 indicating that the slope is not statistically significantly different from zero at the 95% confidence level.

Lipid-normalised growth-corrected depuration rate constants ($k_{2g,\ L}$) have been calculated from the data reported in Inoue *et al.* (2012) and these are shown in Table 2.25 (all values normalised to a 5% lipid content). Lipid normalisation results in an improvement in the relative standard deviation around the mean value (23.5% compared with 31.5% for the non-normalised data). This is consistent with the results reported in the previous section for the OECD 305 ring test data.

[5] It is possible that some, but not all, of these results are part of the OECD 305 ring test dataset.

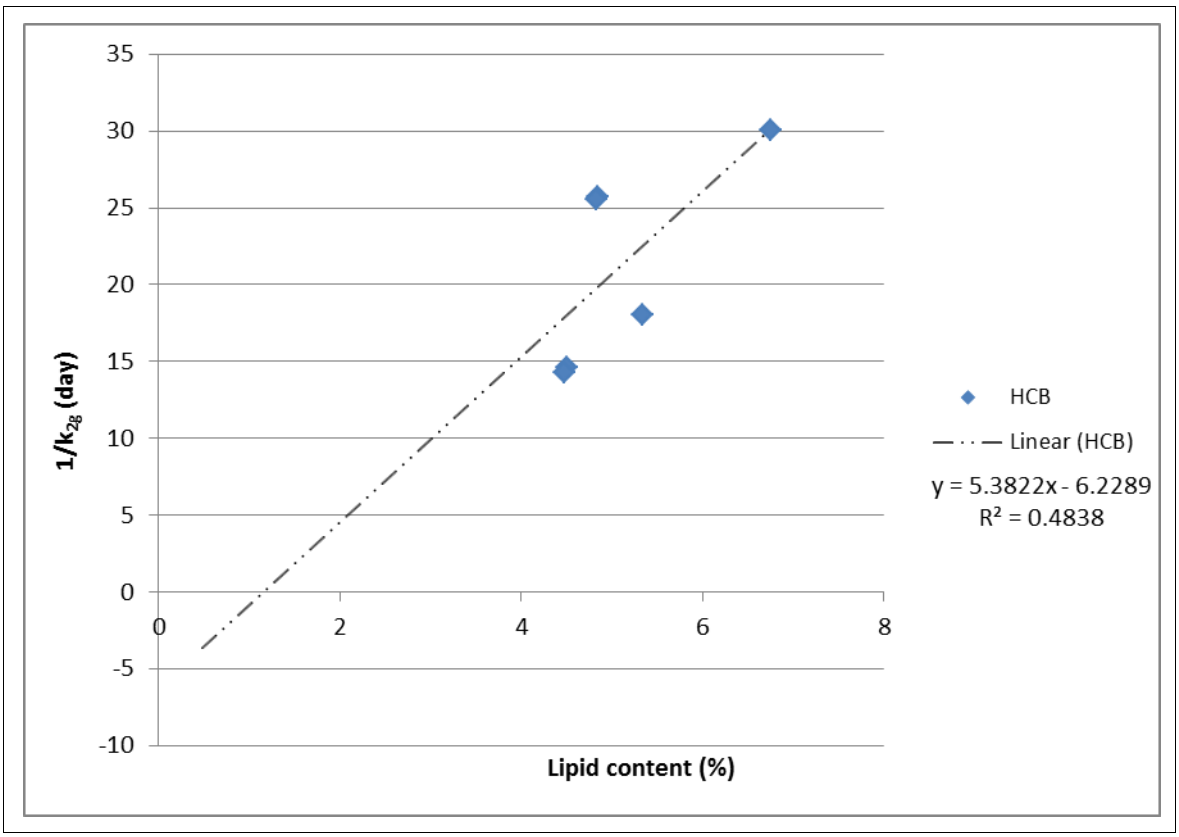

Figure 2.20 Plot of 1/growth-corrected depuration rate constant against fish lipid for the hexachlorobenzene data obtained by Inoue *et al*. (2012)

Table 2.25 Inoue *et al.* (2012) dataset for hexachlorobenzene

Concentration in diet (μg g^{-1})	Mean fish lipid content (%)	k_2 (day^{-1})	k_g (day^{-1})	k_{2g} (day^{-1})	$k_{2g,L}$ (day^{-1})
96.8	4.51	0.0918	0.0231	0.0686	0.0619
106	4.48	0.0871	0.0171	0.0700	0.0627
94.1	5.34	0.0846	0.0291	0.0555	0.0593
101	4.84	0.0686	0.0298	0.0389	0.0377
103	4.83	0.0680	0.0288	0.0392	0.0379
24.1	6.75	0.0571	0.0238	0.0333	0.0450
Mean		0.0762		0.0509	0.0507
Standard deviation		0.0136		0.0161	0.0119
Relative standard deviation		17.8%		31.5%	23.5%

2.5 Overall conclusion

The analysis carried out indicates that there is a correlation between $1/k_{2g}$ and fish lipid. A correlation between $1/k_{2g}$ and fish lipid would be expected from theoretical considerations. However, in most cases the experimental correlation is relatively weak (and often not statistically significant). A possible reason for a weak correlation is that the lipid content of the fish is itself variable within most experimental studies and so this complicates the analysis of the data (and itself likely introduces further variability into the data). Thus these correlations need to be seen within the context of the overall variability of the datasets.

A dependence of the k_{2g} value on the fish lipid suggests that lipid normalisation of the k_{2g} value is appropriate when they are used outside of the OECD 305 dietary study, for example when used to estimate a BCF value (lipid normalisation is effectively already included in the calculation of a BMF_L value using the OECD 305 methodology). When lipid normalisation is carried out, the standard deviation around the mean value of the growth-corrected depuration rate constant is generally reduced for lipid-normalised data compared with non-normalised data when an appropriate measure of the fish lipid during depuration is used. The analysis carried out suggests that either the mean lipid content at the end of the depuration phase, the arithmetic mean concentration of the two sampling points over the depuration phase, or the time-weighted average lipid over the depuration phase are appropriate measures for carrying out this normalisation.

3 Selection of the most appropriate depuration rate constant for BCF estimation

Based on the analysis carried out in section 2, it can be seen that three 'measures' of depuration can be generated from the OECD 305 dietary test. These are:

- The overall depuration rate constant (k_2; day^{-1}).
- The growth-corrected depuration rate constant (k_{2g}; day^{-1}).
- The growth-corrected and lipid-normalised depuration rate constant ($k_{2g,L}$; day^{-1}).

As well as their use in calculating the biomagnification factor, depuration rate constants obtained using the OECD 305 dietary test can be used to calculate an 'equivalent' BCF value by using an estimate of the rate constant for uptake from water (k_1). A detailed evaluation of the methods for estimating the k_1 is given in Crookes and Brooke (2011) and Brooke *et al*. (2012) and so these are not discussed further here. Which of the three depuration rate constants is most appropriate for use in the BCF estimation must be examined. The options are shown below:

$$BCF = \frac{k_1}{k_2}$$ where the BCF is not growth corrected or lipid normalised

$$BCF_g = \frac{k_1}{k_{2g}}$$ where BCF_g is growth corrected but not lipid normalised

$$BCF_{g,L} = \frac{k_1}{k_{2g,L}}$$ where $BCF_{g,L}$ is growth corrected and normalised to a specific lipid content[6]

Based on the preceding discussion, lipid normalisation of the depuration rate constant appears to be appropriate. However, comparisons of the indicative BCFs predicted using the above equations with experimental BCF data may be confounded by the fact that most of the available experimental data is neither lipid normalised nor growth corrected.

A previous comparison of the predicted BCF with experimental BCF using the ring test data (OECD 2012b, 2013) found that the predicted growth-corrected BCF was generally much higher than the experimental BCF but this would be expected if the experimental BCF data were not themselves growth corrected. The previous comparison, however, did not consider the effect of lipid normalisation on the growth-corrected depuration rate constant. Therefore, BCFs have been estimated using the growth-corrected and lipid-normalised depuration rate constant and compared with (a) the predicted BCFs presented in OECD (2012b, 2013) and (b) the available experimental BCF data. Details of the predicted, growth-corrected and lipid-normalised

[6] This assumes that the k_1 value does not have a lipid dependence, which is thought to be true at higher log K_{OW} values (see Crookes and Brooke (2011) and Brooke *et al*. (2012)).

bioconcentration factors ($BCF_{g,L}$) are given in Appendix A for the OECD 305 dataset. The lipid content used for the normalisation was the time-weighted average concentration over the depuration period (as estimated in the preceding section) and the data were normalised to a standard lipid content of 5%. No predictions were carried out for benzo[a]pyrene owing to the limited data available from the ring test.

As in the previous studies, the following methods were used to estimate the k_1 values (see Crookes and Brooke 2011 for further details).

- Method 1 Sijm *et al*. (1993, 1994, 1995)
- Method 2 Omega/Hendriks *et al*. (2001)
- Method 6 QEAFDCHN/Thomann (1989)
- Method 7 BASS/Barber (2001)
- Method 8 FGETS/Barber *et al*. (1991)
- Method 9 Erickson and McKim (1990a)
- Method 10 Erickson and McKim (1990b)
- Method 13 Hayton and Barron (1990)
- Method 15 Streit and Siré (1993)
- Method 17 Barber (2003) observed
- Method 18 Barber (2003) calibrated
- Method 21 Spacie and Hamelink (1982)
- Method 22 Tolls and Sijm (1995)

A comparison of the predicted BCF values with experimental BCF data is not straightforward as it is not always clear whether or not the experimental BCF data have been corrected for growth dilution or have been lipid normalised. However, as growth correction was not routinely carried out (at least up until recently) for BCF studies, it is likely that many of the available experimental data are not growth corrected (and hence the growth-corrected BCF would be expected to be larger in many cases than the reported BCF values).

The available experimental BCF data for the ring test chemicals was considered in OECD (2012b) and these data are also considered here. The largest set of experimental BCF data is for hexachlorobenzene. However, many of the data are for species other than rainbow trout or carp. The available experimental data with rainbow trout (*Oncorhynchus mykiss*) are in the range 5,370 to 20,000 L kg^{-1}. The mean predicted lipid-normalised and growth-corrected $BCF_{g,L}$ using the ring test data are in the range 18,200 to 42,700 L kg^{-1} depending on the k_1 estimation method used. Thus the predicted $BCF_{g,L}$ values are of a similar order to, but higher than, the experimental data. This may reflect the lack of growth correction of the experimental data, which may be significant for a slowly depurating substance such as hexachlorobenzene. For carp (*Cyprinus carpio*) the experimental BCF values in OECD (2012b) are in the range 19,000 to 30,000 L kg^{-1}. The predicted $BCF_{g,L}$ using the ring test data are generally consistent with this range (i.e. mean values between 8,800 and 28,000 L kg^{-1}).

For musk xylene, the experimental BCF values are in the range 3,230 to 6,610 L kg^{-1} for carp. The mean $BCF_{g,L}$ predicted using the ring test data cover the range 1,770 to 4,860 L kg-1. The agreement between the predicted values and the experimental data is generally good in this case, particularly as it is not clear if the experimental data have been lipid normalised or growth corrected. No experimental data are available for rainbow trout.

For *o*-terphenyl, the experimental BCF values given in OECD (2012b) are in the range 1,000 to 5,000 L kg-1, again with carp. The predicted $BCF_{g,L}$ values are in the range

640 to 1,400 L kg-1, which again is consistent with the experimental data (given the uncertainties in the experimental data). No data were available for rainbow trout.

No experimental data with rainbow trout or carp were given in OECD (2012b) for methoxychlor and so it is not possible to carry out a comparison of the experimental data with the predicted data for these substances.

A recent study by Inuoe *et al.* (2012) provides a dataset of substances that have been tested by both water exposure and dietary exposure under a reasonably standard set of conditions (using carp). This dataset allows further comparisons to be made between the BCFs estimated using an estimated k_1 value along with the depuration rate constant obtained from dietary exposure. The relevant data are shown in Table 3.1.

The BCF values given in the Inoue *et al.* (2012) paper are reported to be steady-state values based on whole body wet weights. In addition, Inoue *et al.* (2012) report the BCF values normalised to a 5% lipid content (BCF_L in Table 3.1). It is not clear whether these data have been growth corrected but, as the data are reported to be steady-state values (rather than kinetics) the likelihood is that the data have not been growth corrected.

For hexachlorobenzene the experimental BCF and BCF_L values are 17,000 and 27,000 L kg-1 respectively. The mean BCF values predicted using the estimates of k_1 along with the depuration data generated by Inoue *et al.* (2012) from the feeding studies are about 6,600 L kg-1 (range 3,260 to 14,140 L kg-1) for the BCF (not growth corrected or lipid normalised), 10,500 L kg-1 (range 4,270 to 24,240 L kg-1) for the BCF_g (growth corrected but not lipid normalised) and 10,200 L kg-1 (range 4,760 to 21,500 L kg-1) for the $BCF_{g, L}$. The predicted values are therefore lower than the experimental data; however, the growth-corrected (and lipid-normalised values) are of a similar order to the experimental data. For comparison, normalising the predicted BCF to a 5% lipid content (by using the estimate of k_1 and the k_{2g} value, normalised to a 5% lipid content) results in a mean predicted BCF_L (not growth-corrected but lipid-normalised BCF) of 6,480 L kg-1 (range 3,310 to 12,290 L kg-1) for this dataset.

For Binox M (4,4'-methylenebis(2,6-di-tert-butylphenol)) the experimental BCF and BCF_L values are 9,200 and 8,100 L kg-1 respectively. The mean BCF values predicted using the estimates of k_1 along with the depuration data from the feeding studies are about 14,390 L kg-1 (range 6,410 to 42,420 L kg-1) for the BCF (not growth corrected or lipid normalised), 28,190 L kg-1 (range 12,550 to 83,080 L kg-1) for the BCF_g (growth corrected but not lipid normalised) and 31,250 L kg-1 (range 13,910 to 92,110 L kg-1) for the $BCF_{g, L}$. The predicted values are therefore higher than the experimental data, particularly when growth correction is taken into account (this may reflect the fact that the experimental BCF data are unlikely to have been growth corrected). For comparison, normalising the predicted BCF to a 5% lipid content (by using the estimate of k_1 and the k_{2g} value, normalised to a 5% lipid content) results in a mean predicted BCF_L (not growth-corrected but lipid-normalised BCF) of 15,960 L kg-1 (range 7,100 to 47,030 L kg-1) for this dataset.

Table 3.1 Summary of estimated BCFs from the Inoue *et al.* (2012) dataset

Parameter		Substance								
		HCB[b]	Binox M	PeCB	NIP	Solvent Blue 36	DNPD	MX	oTP	MC
Initial fish weight (g)[a]		3.23	3.23	3.23	3.23	3.23	3.23	3.23	3.23	3.23
Log Kow[b]		5.86	8.99	5.22	4.32	6.07	6.39	4.45	5.52	5.67
Method 1	Predicted k_1 (L kg^{-1} day^{-1})	357	357	357	357	357	357	357	357	357
	Predicted BCF (L kg^{-1})	4,823	7,570	1,678	1,328	1,787	1,401	2,749	961	950
	Predicted BCF_g (L kg^{-1})	7,633	14,827	1,824	1,489	2,099	1,580	3,365	1,026	1,015
	Predicted $BCF_{g,L}$ (L kg^{-1})	7,398	16,437	2,036	1,395	2,169	1,635	2,492	760	752
Method 2	Predicted k_1 (L kg^{-1} day^{-1})	299	302	287	213	300	301	231	295	297
	Predicted BCF (L kg^{-1})	4,034	6,407	1,349	793	1,501	1,182	1,777	792	790
	Predicted BCF_g (L kg^{-1})	6,383	12,548	1,467	889	1,764	1,332	2,175	846	843
	Predicted $BCF_{g,L}$ (L kg^{-1})	6,187	13,912	1,637	833	1,822	1,379	1.611	627	624
Method 6	Predicted k_1 (L kg^{-1} day^{-1})	597	597	597	597	597	597	597	597	597
	Predicted BCF (L kg^{-1})	8,055	12,643	2,802	2,218	2,984	2,340	4,590	1,604	1,587
	Predicted BCF_g (L kg^{-1})	12,748	24,761	3,046	2,487	3,506	2,638	5,619	1,714	1,694
	Predicted $BCF_{g,L}$ (L kg^{-1})	12,356	27,451	3,400	2,329	3,622	2,731	4,162	1,269	1,255
Method 7	Predicted k_1 (L kg^{-1} day^{-1})	574	574	574	574	574	574	574	574	574
	Predicted BCF (L kg^{-1})	7,745	12,156	2,694	2,133	2,869	2,250	4,414	1,542	1,526
	Predicted BCF_g (L kg^{-1})	12,257	23,808	2,929	2,392	3,371	2,537	5,403	1,648	1,629
	Predicted $BCF_{g,L}$ (L kg^{-1})	11,880	26,395	3,269	2,239	3,483	2,626	4,002	1,221	1,207

Parameter		Substance								
		HCB[b]	Binox M	PeCB	NIP	Solvent Blue 36	DNPD	MX	oTP	MC
Method 8	Predicted k_1 (L kg^{-1} day^{-1})	550	550	550	550	550	550	550	550	550
	Predicted BCF (L kg^{-1})	7,422	11,649	2,581	2,044	2,749	2,156	4,229	1,478	1,462
	Predicted BCF_g (L kg^{-1})	11,746	22,815	2,807	2,292	3,230	2,431	5,177	1,579	1,561
	Predicted $BCF_{g,L}$ (L kg^{-1})	11,384	25,293	3,132	2,146	3,337	2,516	3,835	1,170	1,156
Method 9	Predicted k_1 (L kg^{-1} day^{-1})	559	559	559	559	559	559	559	559	559
	Predicted BCF (L kg^{-1})	7,551	11,852	2,626	2,080	2,797	2,194	4,303	1,504	1,488
	Predicted BCF_g (L kg^{-1})	11,951	23,213	2,856	2,332	3,287	2,473	5,268	1,607	1,588
	Predicted $BCF_{g,L}$ (L kg^{-1})	11,583	25,735	3,187	2,183	3,396	2,560	3,902	1,190	1,177
Method 10	Predicted k_1 (L kg^{-1} day^{-1})	436	436	436	436	436	436	436	436	436
	Predicted BCF (L kg^{-1})	5,886	9,238	2,047	1,621	2,180	1,710	3,354	1,172	1,160
	Predicted BCF_g (L kg^{-1})	9,314	18,092	2,226	1,817	2,562	1,928	4,106	1,252	1,238
	Predicted $BCF_{g,L}$ (L kg^{-1})	9,028	20,057	2,484	1,702	2,646	1,995	3,041	928	917
Method 13	Predicted k_1 (L kg^{-1} day^{-1})	400	400	400	400	400	400	400	400	400
	Predicted BCF (L kg^{-1})	5,403	8,481	1,879	1,488	2,001	1,570	3,079	1,076	1,065
	Predicted BCF_g (L kg^{-1})	8,551	16,610	2,043	1,669	2,352	1,770	3,769	1,150	1,137
	Predicted $BCF_{g,L}$ (L kg^{-1})	8,288	18,414	2,281	1,562	2,430	1,832	2,792	852	842

Parameter		Substance								
		HCB[b]	Binox M	PeCB	NIP	Solvent Blue 36	DNPD	MX	oTP	MC
Method 15	Predicted k_1 (L kg^{-1} day^{-1})	339	339	339	339	339	339	339	339	339
	Predicted BCF (L kg^{-1})	4,575	7,180	1,591	1,260	1,695	1,329	2,607	911	901
	Predicted BCF_g (L kg^{-1})	7,240	14,063	1,730	1,413	1,991	1,498	3,191	973	962
	Predicted $BCF_{g,L}$ (L kg^{-1})	7,017	15,591	1,931	1,323	2,057	1,551	2,364	721	713
Method 17	Predicted k_1 (L kg^{-1} day^{-1})	353	353	353	353	353	353	353	353	353
	Predicted BCF (L kg^{-1})	4,768	7,483	1,658	1,313	1,766	1,385	2,717	949	939
	Predicted BCF_g (L kg^{-1})	7,545	14,655	1,803	1,472	2,075	1,561	3,326	1,014	1,003
	Predicted $BCF_{g,L}$ (L kg^{-1})	7,313	16,248	2,012	1,379	2,144	1,616	2,464	751	743
Method 18	Predicted k_1 (L kg^{-1} day^{-1})	416	416	416	414	416	416	415	416	416
	Predicted BCF (L kg^{-1})	5,615	8,815	1,952	1,539	2,080	1,632	3,189	1,118	1,106
	Predicted BCF_g (L kg^{-1})	8,887	17,264	2,123	1,726	2,444	1,839	3,903	1,195	1,181
	Predicted $BCF_{g,L}$ (L kg^{-1})	8,613	19,139	2,369	1,616	2,525	1,904	2,891	885	875
Method 21	Predicted k_1 (L kg^{-1} day^{-1})	694	2,002	559	412	745	830	431	619	651
	Predicted BCF (L kg^{-1})	9,369	42,422	2,624	1,532	3,726	3,257	3,313	1,663	1,731
	Predicted BCF_g (L kg^{-1})	14,827	83,083	2,853	1,718	4,379	3,671	4,055	1,777	1,848
	Predicted $BCF_{g,L}$ (L kg^{-1})	14,371	92,110	3,184	1,609	4,523	3,801	3,004	1,316	1,369

Parameter		Substance								
		HCB[b]	**Binox M**	**PeCB**	**NIP**	**Solvent Blue 36**	**DNPD**	**MX**	**oTP**	**MC**
Method 22	Predicted k_1 (L kg^{-1} day^{-1})	807	1,944	674	524	856	937	543	734	765
	Predicted BCF (L kg^{-1})	10,894	41,194	3,166	1,947	4,281	3,673	4,178	1,972	2,035
	Predicted BCF_g (L kg^{-1})	17,241	80,679	3,442	2,183	5,030	4,141	5,114	2,107	2,172
	Predicted $BCF_{g,L}$ (L kg^{-1})	16,711	89,445	3,841	2,040	5,196	4,287	3,788	1,561	1,609
Summary of predictions of BCF (L kg^{-1})	Overall mean	**6,626**	**14,392**	**2,204**	**1,638**	**2,493**	**2,006**	**3,423**	**1,288**	**1,288**
	Standard deviation	2,330	12,345	570	422	837	757	859	360	377
	Lowest value	3,255	6,407	1,349	793	1,501	1,182	1,777	792	790
	Highest value	14,135	42,422	3,166	2,218	4,281	3,673	4,590	1,972	2,035
Summary of predicted BCF_g (L kg^{-1})	Overall mean	**10,486**	**28,186**	**2,396**	**1,837**	**2,930**	**2,261**	**4,190**	**1,376**	**1,375**
	Standard deviation	4,441	24,177	619	473	984	854	1,052	384	402
	Lowest value	4,269	12,548	1,467	889	1,764	1,332	2,175	846	843
	Highest value	24,237	83,083	3,442	2,487	5,030	4,141	5,619	2,107	2,172
Summary of predicted $BCF_{g,L}$ (L kg^{-1})	Overall mean	**10,164**	**31,248**	**2,674**	**1,720**	**3,027**	**2,341**	**3,104**	**1,019**	**1,018**
	Standard deviation	3,883	26,804	691	443	1,017	884	779	285	298
	Lowest value	4,764	13,912	1,637	833	1,822	1,379	1,611	627	624
	Highest value	21,489	92,110	3,841	2,329	5,196	4,287	4,162	1,561	1,609
Experimental BCF (reported by Inoue *et al.* 2012)	BCF (L kg^{-1})	**17,000**	**9,200**	**5,100**	**3,400**	**5,300**	**1,100**	**4,300**	**1,400**	**620**
	BCF (L kg^{-1}), normalised to 5% lipid content	**27,000**	**8,100**	**7,400**	**4,900**	**5,300**	**1,500**	**6,900**	**1,200**	**810**

Notes: [a] The fish weight at day 0 only is given. This was stated to be 3.23±0.66 g (mean ± standard deviation).
[b] Log K_{ow} values are given by Inoue *et al.* (2012).
[c] The predicted BCF values for hexachlorobenzene represent the mean value from six determinations of the depuration rate constant.
HCB = hexachlorobenzene; Binox M = 4,4'-methylenebis(2,6-di-tert-butylphenol); PeCB = pentachlorobenzene; NIP = 2,4-dichloro-1-(4-nitrophenoxy) benzene; Solvent Blue 36 = 1,4-bis(isopropylamino)anthraquinone; DNPD = N,N'-di-2-naphthyl-p-phenylenediamine; MX = musk xylene; oTP = *o*-terphenyl; MC = methoxychlor.

For pentachlorobenzene the experimental BCF and BCF_L values are 5,100 and 7,400 L kg-1 respectively. The mean BCF values predicted using the estimates of k_1 along with the depuration data from the feeding studies are about 2,200 L kg-1 (range 1,350 to 3,170 L kg-1) for the BCF (not growth corrected or lipid normalised), 2,400 L kg-1 (range 1,470 to 3,440 L kg-1) for the BCF_g (growth corrected but not lipid normalised) and 2,670 L kg-1 (range 1,640 to 3,840 L kg-1) for the $BCF_{g, L}$. The predicted values are therefore lower than the experimental data but of a similar order. In this case growth correction of the data results in only a modest increase in the predicted BCF. For comparison, normalising the predicted BCF to a 5% lipid content (by using the estimate of k_1 and the k_{2g} value, normalised to a 5% lipid content) results in a mean predicted BCF_L (not growth-corrected but lipid-normalised BCF) of 2,460 L kg-1 (range 1,510 to 3,530 L kg-1) for this dataset.

For 2,4-dichloro-1-(4-nitrophenoxy) benzene the experimental BCF and BCF_L values are 3,400 and 4,900 L kg-1 respectively. The mean BCF values predicted using the estimates of k_1 along with the depuration data from the feeding studies are about 1,640 L kg-1 (range 790 to 2,220 L kg-1) for the BCF (not growth corrected or lipid normalised), 1,840 L kg-1 (range 890 to 2,490 L kg-1) for the BCF_g (growth corrected but not lipid normalised) and 1,720 L kg-1 (range 830 to 2,330 L kg-1) for the $BCF_{g, L}$. The predicted values are therefore lower than the experimental data but of a similar order. In this case growth correction of the data results in only a modest increase in the predicted BCF. For comparison, normalising the predicted BCF to a 5% lipid content (by using the estimate of k_1 and the k_{2g} value, normalised to a 5% lipid content) results in a mean predicted BCF_L (not growth-corrected but lipid-normalised BCF) of 1,530 L kg-1 (range 740 to 2,080 L kg-1) for this dataset.

For Solvent Blue 36 the experimental BCF and BCF_L values are both 5,300 L kg-1. The mean BCF values predicted using the estimates of k_1 along with the depuration data from the feeding studies are about 2,490 L kg-1 (range 1,500 to 4,280 L kg-1) for the BCF (not growth corrected or lipid normalised), 2,930 L kg-1 (range 1,760 to 5,030 L kg-1) for the BCF_g (growth corrected but not lipid normalised) and 3,030 L kg-1 (range 1,820 to 5,200 L kg-1) for the $BCF_{g, L}$. The predicted values are therefore lower than the experimental data but of a similar order. In this case growth correction of the data results in only a modest increase in the predicted BCF. For comparison, normalising the predicted BCF to a 5% lipid content (by using the estimate of k_1 and the k_{2g} value, normalised to a 5% lipid content) results in a mean predicted BCF_L (not growth-corrected but lipid-normalised BCF) of 2,580 L kg-1 (range 1,550 to 4,420 L kg-1) for this dataset.

For N,N'-di-2-naphthyl-p-phenylenediamine the experimental BCF and BCF_L values are 1,100 and 1,500 L kg-1 respectively. The mean BCF values predicted using the estimates of k_1 along with the depuration data from the feeding studies are about 2,010 L kg-1 (range 1,180 to 3,670 L kg-1) for the BCF (not growth corrected or lipid normalised), 2,260 L kg-1 (range 1,330 to 4,140 L kg-1) for the BCF_g (growth corrected but not lipid normalised) and 2,340 L kg-1 (range 1,380 to 4,290 L kg-1) for the $BCF_{g, L}$. The predicted values are therefore slightly higher than the experimental data but of a similar order. In this case growth correction of the data results in only a modest increase in the predicted BCF. For comparison, normalising the predicted BCF to a 5% lipid content (by using the estimate of k_1 and the k_{2g} value, normalised to a 5% lipid content) results in a mean predicted BCF_L (not growth-corrected but lipid-normalised BCF) of 2,080 L kg-1 (range 1,220 to 3,802 L kg-1) for this dataset.

For musk xylene the experimental BCF and BCF_L values are 4,300 and 6,900 L kg-1 respectively. The mean BCF values predicted using the estimates of k_1 along with the depuration data from the feeding studies are about 3,420 L kg-1 (range 1,780 to 4,590 L kg-1) for the BCF (not growth corrected or lipid normalised), 4,190 L kg-1 (range 2,180 to 5,620 L kg-1) for the BCF_g (growth corrected but not lipid normalised) and

3,100 L kg-1 (range 1,610 to 4,160 L kg-1) for the $BCF_{g, L}$. The predicted values are therefore slightly lower than the experimental data but of a similar order. In this case growth correction of the data results in only a modest increase in the predicted BCF. For comparison, normalising the predicted BCF to a 5% lipid content (by using the estimate of k_1 and the k_{2g} value, normalised to a 5% lipid content) results in a mean predicted BCF_L (not growth-corrected but lipid-normalised BCF) of 2,540 L kg-1 (range 1,320 to 3,400 L kg-1) for this dataset.

For *o*-terphenyl the experimental BCF and BCF_L values are 1,400 and 1,200 L kg-1 respectively. The mean BCF values predicted using the estimates of k_1 along with the depuration data from the feeding studies are 1,290 L kg-1 (range 790 to 1,970 L kg-1) for the BCF (not growth corrected or lipid normalised), 1,380 L kg-1 (range 850 to 2,110 L kg-1) for the BCF_g (growth corrected but not lipid normalised) and 1,020 L kg-1 (range 630 to 1,560 L kg-1) for the $BCF_{g, L}$. The predicted values are therefore in good agreement with the experimental data. In this case growth correction of the data results in only a modest increase in the predicted BCF. For comparison, normalising the predicted BCF to a 5% lipid content (by using the estimate of k_1 and the k_{2g} value, normalised to a 5% lipid content) results in a mean predicted BCF_L (not growth-corrected but lipid-normalised BCF) of 950 L kg-1 (range 590 to 1,460 L kg-1) for this dataset.

For methoxychlor the experimental BCF and BCF_L values are 620 and 810 L kg-1 respectively. The mean BCF values predicted using the estimates of k_1 along with the depuration data from the feeding studies are 1,290 L kg-1 (range 790 to 2,040 L kg-1) for the BCF (not growth corrected or lipid normalised), 1,380 L kg-1 (range 840 to 2,170 L kg-1) for the BCF_g (growth corrected but not lipid normalised) and 1,020 L kg-1 (range 300 to 620 L kg-1) for the $BCF_{g, L}$. The predicted values are therefore slightly higher than the experimental data but of a similar order. In this case growth correction of the data results in only a modest increase in the predicted BCF. For comparison, normalising the predicted BCF to a 5% lipid content (by using the estimate of k_1 and the k_{2g} value, normalised to a 5% lipid content) results in a mean predicted BCF_L (not growth-corrected but lipid-normalised BCF) of 950 L kg-1 (range 590 to 1,510 L kg-1) for this dataset.

Overall, the predictions obtained from the ring test data and the Inoue *et al*. (2012) data are reasonably consistent with the available experimental data. The inherent variability in the available experimental data means that it is difficult to draw conclusions on the most appropriate method for estimating the BCF. However, given the preceding discussions and the general agreement with the experimental data, it is recommended that the lipid-normalised and growth-corrected depuration rate constant ($k_{2g, L}$) is used in these calculations (resulting in a $BCF_{g, L}$ value) as this is consistent with the current recommendations in the European Chemicals Agency's REACH guidance document (which recommends that BCF values should, where possible, be lipid normalised and growth corrected) and also the OECD test guideline (which also includes lipid normalisation and growth correction as standard). Growth correction can be considered as a worst-case approach as it tends to increase the resulting BCF in all cases. However, lipid normalisation can either increase or decrease the resulting BCF depending on (a) the lipid content of the fish used in the test and (b) the 'standard' lipid content to which the data are normalised.

It is also relevant to note that growth correction only has a significant effect on the predicted BCF value for substances where depuration is relatively slow (i.e. where the k_g contributes significantly to the k_2). Lipid normalisation, however, will affect all substances equally.

It is also important to note that, as discussed in section 2.2, lipid normalisation may not be appropriate in all cases as it requires that all of the depuration processes (other than growth dilution which is factored out) show a similar dependence on the lipid content of

the fish. This may not necessarily be the case for substances that are rapidly metabolised, for instance.

4 Estimation of errors in derived bioaccumulation parameters

4.1 Propagation of errors

One way to estimate the errors in bioaccumulation parameters is to consider how the errors propagate through the various calculations that are sequentially carried out. Such an approach was used recently by Inoue *et al.* (2012). The approach taken in Inoue *et al.* (2012) was based on a paper by Ku (1966) but no further details were given. The approach outlined below is also based on Ku (1966).

Ku (1966) outlines the theory of propagation of errors and provides a series of formulae for approximating the variance[7] in a derived value from the standard deviations of the mean values of variables used to derive that value. It is important to note that these formulae are only approximations to the true variance in the derived value. In particular the following should be noted:

- The distribution of values around the mean approximates to a normal distribution. This may not be the case for exponential terms in particular (the equations used to estimate bioaccumulation parameters contains one such term).
- The standard deviation of a given value is small in comparison to its mean. This may not always be the case in a bioaccumulation study.
- The approach does not take into account the potential co-variance of parameters, for example the dependence of the depuration rate constant on a fish's lipid content. Such co-variance is not explored here.[8]

To use the formulae in Ku (1966) the equations used to derive the BMF (and other parameters) in the feeding study must be broken down into simpler 'units' so that the variance (and standard deviation) in these 'units' can be calculated and then combined to give the overall variance and standard deviation in the derived parameter. This is outlined in Table 4.1.

As part of this project, these equations have been implemented in a spreadsheet to allow the errors in the parameters derived in the OECD 305 test to be estimated based on the known errors in the parameters measured during the study (spreadsheet available separately).

Other methods, for example based on Monte Carlo analysis, could potentially be used to explore the propagation of errors in the derived bioaccumulation parameters. These often require specialist software and so may not be generally accessible. However, these should be considered, where available, alongside the approach outlined here as they are not necessarily subject to the same assumptions and approximations.

[7] Variance is the square of the standard deviation.

[8] This is because (i) co-variance for all but a few parameters has not been proved with test data and (ii) the approach is an approximation; an attempt to incorporate additional terms to capture all possible combinations of co-variance would likely make the approach unworkable.

Table 4.1 Estimation of propagation of errors

Equation/term	Propagation of error formula		
	Variance	Standard deviation	Key and notes
$k_{2g} = k_2 - k_g$	$v_{k2g} = s_{k2}^2 + s_{kg}^2$	$s_{k2g} = \sqrt{s_{k2}^2 + s_{kg}^2}$	k_2 = overall depuration rate constant k_g = rate constant for growth dilution k_{2g} = growth-corrected depuration rate constant v_{k2g} = estimated variance in k_{2g} s_{k2g} = estimated standard deviation in k_{2g} s_{k2} = known standard deviation in the k_2. This can be approximated to the standard error in the slope of the ln [concentration] versus time plot for the depuration phase s_{kg} = known standard deviation in the k_g. This can be approximated to the standard error in the slope of the ln [1/fish weight] versus time plot Note: if the k_{2g} is obtained by the alternative method the standard deviation can be approximated directly from the standard error in the slope of the ln [amount per fish] versus time plot for the depuration phase

Equation/term	Propagation of error formula		
	Variance	Standard deviation	Key and notes
$x = -k_2 \times t$	$v_x = s_{k2}^2 \times t^2$	$s_x = s_{k2} \times t$	x = the experimentally determined value of $-k_2 \times t$ v_x = estimated variance in the term $-k_2 \times t$ s_x = estimated standard deviation in the term $-k_2 \times t$ s_{k2} = known standard deviation in the k_2. t = duration of uptake phase – the error in this term is ignored (t is taken to be a constant within any one experiment)
$e^{-k2t} = e^x$	$v_{e-k2t} = e^{2x} \times s_x^2$ (see Note a)	$s_{e-k2t} = \sqrt{e^{2x} \times s_x^2}$ (see Note a)	v_{e-k2t} = estimated variance in the term e^{-k2t} s_{e-k2t} = estimated standard deviation in the term e^{-k2t} x = the experimentally determined value of $-k_2 \times t$ s_x = estimated standard deviation in the term $-k_2 \times t$ (estimated above)

Equation/term	Propagation of error formula		
	Variance	Standard deviation	Key and notes
$j = 1 - e^{-k2t}$	$v_j = 1 + e^{2x} \times s_x^2$	$s_j = \sqrt{1 + e^{2x} \times s_x^2}$	j = the experimentally determined value of $1–e^{–k2t}$ v_j = estimated variance in the term $1–e^{–k2t}$ s_j = estimated standard deviation in the term $1–e^{–k2t}$ x = the experimentally determined value of $–k_2×t$ s_x = estimated standard deviation in the term $–k_2×t$ (estimated above)
$f = \frac{1}{1 - e^{-k2t}} = \frac{1}{j}$	$v_f = \frac{1 + e^{2x} \times s_x^2}{\left(1 - e^{-k2t}\right)^4}$	$s_f = \sqrt{\frac{1 + e^{2x} \times s_x^2}{\left(1 - e^{-k2t}\right)^4}}$	f = the experimentally determined value of $1/(1–e^{–k2t})$ v_f = estimated variance in the term $1/(1–e^{–k2t})$ s_j = estimated standard deviation in the term $1/(1–e^{–k2t})$ x = the experimentally determined value of $–k_2×t$ s_x = estimated standard deviation in the term $–k_2×t$ (estimated above)

Equation/term	Propagation of error formula		
	Variance	Standard deviation	Key and notes
$y = C_0 \times k_2$	$v_y = (C_0 \times k_2)^2 \times \left(\frac{s_{C0}^2}{C_0^2} + \frac{s_{k2}^2}{k_2^2} \right)$	$s_y = \sqrt{(C_0 \times k_2)^2 \times \left(\frac{s_{C0}^2}{C_0^2} + \frac{s_{k2}^2}{k_2^2} \right)}$	y = the experimentally determined value of $C_0 \times k_2$ v_y= estimated variance in the term $C_0 \times k_2$ s_y = estimated standard deviation in the term $C_0 \times k_2$ C_0 = the concentration in fish at the start of the depuration phase. This is estimated from the intercept of the ln [concentration] versus time plot or from the measurements in fish at the end of uptake/start of depuration k_2 = the overall depuration rate constant determined experimentally

Equation/term	Propagation of error formula		
	Variance	Standard deviation	Key and notes
			s_{C0} = standard deviation in the C_0 value. This can be estimated from the ln [concentration] time plot (although as the data are log transformed this may not be normally distributed) or estimated from the measured concentration in the fish at the start of depuration directly s_{k2} = known standard deviation in the k_2. This can be approximated to the standard error in the slope of the ln [concentration] versus time plot for the depuration phase
$z = I \times C_{food}$	$v_z = (I \times C_{food})^2 \times \left(\frac{s_I^2}{I^2} + \frac{s_{Cfood}^2}{C_{food}^2} \right)$	$s_z = \sqrt{(I \times C_{food})^2 \times \left(\frac{s_I^2}{I^2} + \frac{s_{Cfood}^2}{C_{food}^2} \right)}$	z = the experimentally determined value of $I \times C_{food}$ v_z= estimated variance in the term $I \times C_{food}$ s_z = estimated standard deviation in the term $I \times C_{food}$ C_{food} = the concentration in food. This is determined experimentally in the test I = mean feeding rate. This can be estimated from the known amounts of food added, and the known (or estimated, using the growth rate constant) fish weights

Equation/term	Propagation of error formula		
	Variance	Standard deviation	Key and notes
			on each day of uptake s_{Cfood} = standard deviation in the C_{food} value. This can be directly obtained from the analytical data s_I = the standard deviation in the feeding rate. This can be estimated from the known amounts of food added, and the known (or estimated, using the growth rate constant) fish weights on each day of uptake
$w = \frac{C_0 \times k_2}{I \times C_{food}} = \frac{y}{z}$	$v_w = \left(\frac{y}{z}\right)^2 \times \left(\frac{s_y^2}{y^2} + \frac{s_z^2}{z^2}\right)$	$s_w = \sqrt{\left(\frac{y}{z}\right)^2 \times \left(\frac{s_y^2}{y^2} + \frac{s_z^2}{z^2}\right)}$	w = the experimentally determined value of $C_0 \times k_2/(I \times C_{food})$ v_w= estimated variance in the term $C_0 \times k_2/(I \times C_{food})$ s_w = estimated standard deviation in the term $C_0 \times k_2/(I \times C_{food})$ y = the experimentally determined value of $C_0 \times k_2$ z = the experimentally determined value of $I \times C_{food}$ s_y = estimated standard deviation in the term $C_0 \times k_2$ (see above) s_y = estimated standard deviation in the term $I \times C_{food}$ (see above)

Equation/term	Propagation of error formula		
	Variance	Standard deviation	Key and notes
$\alpha = \frac{C_0 \times k_2}{I \times C_{food}} \times \frac{1}{1-e^{-k2t}}$ =	$v_\alpha = (w \times f)^2 \times \left(\frac{s_w^2}{w^2} + \frac{s_f^2}{f^2} \right)$	$s_\alpha = \sqrt{(w \times f)^2 \times \left(\frac{s_w^2}{w^2} + \frac{s_f^2}{f^2} \right)}$	α = calculated assimilation efficiency v_α= estimated variance in the assimilation efficiency s_α = estimated standard deviation in the assimilation efficiency f = the experimentally determined value of $1/(1-e^{-k2t})$ s_j = estimated standard deviation in the term $1/(1-e^{-k2t})$ (see above) w = the experimentally determined value of $C_0 \times k_2/(I \times C_{food})$ s_w = estimated standard deviation in the term $C_0 \times k_2/(I \times C_{food})$ (see above)
$h = I \times \alpha$	$v_h = (I \times \alpha)^2 \times \left(\frac{s_I^2}{I^2} + \frac{s_\alpha^2}{\alpha^2} \right)$	$s_h = \sqrt{(I \times \alpha)^2 \times \left(\frac{s_I^2}{I^2} + \frac{s_\alpha^2}{\alpha^2} \right)}$	h = calculated value of I×α v_h= estimated variance in the calculated value of I×α s_h= estimated standard deviation in the calculated value of I×α α = calculated assimilation efficiency I = feeding rate s_α = estimated standard deviation in the assimilation efficiency (see

Equation/term	Propagation of error formula		
	Variance	Standard deviation	Key and notes
			above) s_I = the standard deviation in the feeding rate. This can be estimated from the known amounts of food added, and the known (or estimated using the growth rate constant) fish weights on each day of uptake
$BMF = \frac{I \times \alpha}{k_{2g}} = \frac{h}{k_{2g}}$	$v_{BMF} = \left(\frac{h}{k_{2g}}\right)^2 \times \left(\frac{s_h^2}{h^2} + \frac{s_{k2g}^2}{k_{2g}^2}\right)$	$s_{BMF} = \sqrt{\left(\frac{h}{k_{2g}}\right)^2 \times \left(\frac{s_h^2}{h^2} + \frac{s_{k2g}^2}{k_{2g}^2}\right)}$	BMF = calculated BMF (growth corrected but not lipid normalised) v_{BMF} = estimated variance in the BMF s_{BMF} = estimated standard deviation in the BMF h = calculated value of I×α s_h = estimated standard deviation in the calculated value of I×α (see above) k_{2g} = growth-corrected depuration rate constant s_{k2g} = estimated standard deviation in the growth-corrected depuration rate constant (see above)
$r = BMF \times L_{food}$	$v_r = (BMF \times L_{food})^2 \times \left(\frac{s_{BMF}^2}{BMF^2} + \frac{s_{Lfood}^2}{L_{food}^2}\right)$	$s_r = \sqrt{(BMF \times L_{food})^2 \times \left(\frac{s_{BMF}^2}{BMF^2} + \frac{s_{Lfood}^2}{L_{food}^2}\right)}$	r = the value of BMF×L_{food}, where L_{food} is the lipid fraction of food used

Equation/term	Propagation of error formula		
	Variance	Standard deviation	Key and notes
			v_r = estimated variance in BMF×L_{food} s_r = estimated standard deviation in BMF×L_{food} s_{BMF} = estimated standard deviation in the BMF (see above) S_{Lfood} = estimated standard deviation in the lipid content of food. This can be estimated directly from the food lipid measurements in the study. If only a single lipid value is available the standard deviation can be assumed to be zero
$BMF_L = \frac{BMF \times L_{food}}{L_{fish}} = \frac{r}{L_{fish}}$	$v_{BMF_L} = \left(\frac{r}{L_{fish}}\right)^2 \times \left(\frac{s_r^2}{r^2} + \frac{s_L^2}{L_{fish}^2}\right)$	$v_{BMF_L} = \sqrt{\left(\frac{r}{L_{fish}}\right)^2 \times \left(\frac{s_r^2}{r^2} + \frac{s_L^2}{L_{fish}^2}\right)}$	BMF_L = lipid-normalised and growth-corrected biomagnification factor v_{BMFL} = estimated variance in the BMF_L L_{fish} = mean lipid content of the fish s_{Lfish} = standard deviation in the mean lipid concentration of the fish. This can be estimated directly from the fish lipid measurements (preferably during the depuration phase) r = the value of BMF×L_{food}, where

Equation/term	Propagation of error formula		
	Variance	Standard deviation	Key and notes
			L_{food} is the lipid fraction of the food s_r = estimated standard deviation in BMF×L_{food} (see above)
$BCF = \frac{k_1}{k_2}$	$v_{BCF} = \left(\frac{k_{1`}}{k_2}\right)^2 \times \left(\frac{s_{k1}^2}{k_1^2} + \frac{s_{k2}^2}{k_2^2}\right)$	$s_{BCF} = \sqrt{\left(\frac{k_{1`}}{k_2}\right)^2 \times \left(\frac{s_{k1}^2}{k_1^2} + \frac{s_{k2}^2}{k_2^2}\right)}$	BCF = bioconcentration factor (not lipid normalised or growth corrected) v_{BCF} = estimated variance in the BCF s_{BCF} = estimated standard deviation in the BCF k_1 = rate constant for uptake from water, either determined experimentally or estimated k_2 = overall depuration rate constant s_{k1} = standard deviation in the k_1. This may be difficult to estimate if the k_1 is a predicted value. Similarly the standard deviation around the experimental value may be difficult to estimate if it is obtained by curve fitting for example s_{k2} = known standard deviation in the k_2. This can be approximated to the standard error in the slope of the ln [concentration] versus time plot for the depuration phase

Equation/term	Propagation of error formula		
	Variance	Standard deviation	Key and notes
$BCF_g = \frac{k_1}{k_{2g}}$	$v_{BCFg} = \left(\frac{k_{1`}}{k_{2g}}\right)^2 \times \left(\frac{s_{k1}^2}{k_1^2} + \frac{s_{k2g}^2}{k_{2g}^2}\right)$	$s_{BCFg} = \sqrt{\left(\frac{k_{1`}}{k_{2g}}\right)^2 \times \left(\frac{s_{k1}^2}{k_1^2} + \frac{s_{k2g}^2}{k_{2g}^2}\right)}$	BCF_g = growth-corrected bioconcentration factor (not lipid normalised) v_{BCFg} = estimated variance in the BCFg s_{BCFg} = estimated standard deviation in the BCFg k_1 = rate constant for uptake from water, either determined experimentally or estimated k_{2g} = growth-corrected depuration rate constant s_{k1} = standard deviation in the k_1. This may be difficult to estimate if the k_1 is a predicted value. Similarly the standard deviation around the experimental value may be difficult to estimate if it is obtained by curve fitting for example s_{k2g} = deviation in the k_{2g} (see above)
$k_{2g,L} = k_{2g} \times \frac{L}{0.05}$	$v_{k2g,L} = \left(\frac{1}{0.05} \times k_{2g} \times L\right)^2 \times \left(\frac{s_{k2g}^2}{k_{2g}^2} + \frac{s_L^2}{L^2}\right)$	$s_{k2g,L} = \sqrt{\left(\frac{1}{0.05} \times k_{2g} \times L\right)^2 \times \left(\frac{s_{k2g}^2}{k_{2g}^2} + \frac{s_L^2}{L^2}\right)}$	$k_{2g,L}$ = growth-corrected and lipid-normalised depuration rate constant (normalised to a ‘standard’ lipid fraction of 0.05). $v_{k2g,L}$ = estimated variance in the

Equation/term	Propagation of error formula		
	Variance	Standard deviation	Key and notes
			$k_{2g,L}$ $s_{k2g,L}$ = estimated standard deviation in the $k_{2g,L}$ k_{2g} = growth-corrected depuration rate constant s_{k2g} = standard deviation in the k_{2g} (see above) L = lipid content of the fish s_L = standard deviation in the mean fish lipid content (see above)
$BCF_{g,L} = \frac{k_1}{k_{2g,L}}$	$v_{BCFg,L} = \left(\frac{k_{1`}}{k_{2g,L}}\right)^2 \times \left(\frac{s_{k1}^2}{k_1^2} + \frac{s_{k2g,L}^2}{k_{2g,L}^2}\right)$	$s_{BCFg} = \sqrt{\left(\frac{k_{1`}}{k_{2g,L}}\right)^2 \times \left(\frac{s_{k1}^2}{k_1^2} + \frac{s_{k2g,L}^2}{k_{2g,L}^2}\right)}$	$BCF_{g,L}$ = growth-corrected and lipid-normalised bioconcentration factor $v_{BCFg,L}$ = estimated variance in the $BCF_{g,L}$ $s_{BCFg,L}$ = estimated standard deviation in the $BCF_{g,L}$ k_1 = rate constant for uptake from water, either determined experimentally or estimated $k_{2g,L}$ = growth-corrected and lipid-normalised depuration rate constant s_{k1} = standard deviation in the k_1. This may be difficult to estimate if the k_1 is a predicted value.

Equation/term	Propagation of error formula		
	Variance	Standard deviation	Key and notes
			Similarly the standard deviation around the experimental value may be difficult to estimate if it is obtained by curve fitting for example $s_{k2g,L}$ = standard deviation in the $k_{2g,L}$ (see above)

Note: [a] The assumption of a normal distribution of values around the mean may not be appropriate for exponential terms.

4.2 An example

The following example on how the uncertainty in the derived bioaccumulation parameters can be estimated is based on the results for Lab 1 with hexachlorobenzene from the OECD 305 ring test dataset with trout. The statistical calculations on the raw data were performed using the data analysis add-in in Microsoft Excel® 2010. For the example the standard deviation and standard error have effectively been used interchangeably[9] in order to facilitate the calculations. This is a necessary simplification and means that this approach may not be entirely statistically robust; however, it should allow an indication of the uncertainty in each term to be estimated, and the relative importance of these uncertainties at each stage of the calculations to be estimated in a pragmatic way using information that should be readily available from the OECD 305 test.

The overall depuration rate constant (k_2) is obtained from a plot of ln [concentration] versus time. The intercept corresponds to the natural logarithm of the concentration in the fish at the end of the uptake phase/start of depuration (ln [C_0]). For the example, the following values are obtained by linear regression analysis of the raw data (see Table 2.1).

$k_2 = 0.0502$ day^{-1}	Standard error = 0.005 day^{-1}
ln [C_0 (µg g^{-1})] = 1.275	Standard error = 0.079
$C_0 = 3.58$ µg g^{-1}	{Standard deviation estimate = 1.2 µg g^{-1} – see below}

For C_0 the regression analysis results in the standard error in ln [C_0]. However, for the subsequent calculations the standard error (or standard deviation) in C_0 is needed. Although taking the antilogarithm of the standard error in ln [C_0] will result in a measure of the uncertainty in C_0, this will not be normally distributed (as is a requirement for the propagation of errors methodology) and so cannot be used directly in this analysis. Two possible alternatives exist. Firstly, it could be assumed that the uncertainty in C_0 can be ignored (i.e. setting the standard error or standard deviation in C_0 to zero). However, this would result in an underestimate in the uncertainty in the terms derived from C_0. The other alternative would be to obtain another measure of the uncertainty in C_0. For example, in experiments where the concentration in fish is measured directly at the end of uptake (or beginning of depuration) the standard deviation of the direct measurements could be used. In this case, for Lab 1 the concentrations were measured in fish only on day 1 of depuration. Here the mean and standard deviation of the concentrations measured were 3.31 µg g^{-1} and 1.11 µg g^{-1} (i.e. the standard deviation was 33.5% of the mean value). If the same relative standard deviation is applied to the C_0 value of 3.58 µg g^{-1} the standard deviation in the C_0 can be estimated to be around 1.2 µg g^{-1}. This value is used in the subsequent calculations as a pragmatic value rather than a statistically robust value.

The rate constant for growth dilution (k_g) is obtained from the slope of a plot of ln [1/fish weight] versus time. For the example, the following value is obtained by linear regression analysis of the raw data (see Table 2.6).

$k_g = 0.0366$ day^{-1}	Standard error = 0.0017 day^{-1}

[9] Standard deviation is an estimate of how individual values within the sample differ from the sample mean. Standard error is an estimate of how close the sample mean is to the population mean. Standard deviation decreases with larger sample sizes whereas standard error should be unaffected by sample size.

Using the appropriate propagation of errors formula in Table 4.1, the uncertainty in the derived growth-corrected depuration rate constant (k_{2g}) can be estimated as follows.

$k_{2g} = 0.0502–0.0366 = 0.0136$ day^{-1}

Estimated standard error = $\sqrt{(0.005^2+0.0017^2)} = 0.0053$ day^{-1}

As can be seen in this example, the uncertainty in the k_{2g} value is dominated by the uncertainty in the k_2 value. The uncertainty in the k_g value makes only a relatively small contribution to the overall uncertainty.

The estimation of the uncertainty in the assimilation efficiency is more complicated. As indicated in Table 4.1, the calculation needs to be broken down into several discrete steps. Using the same terminology as in Table 4.1, the following values can be estimated. The calculations assume that the duration of the uptake phase (t) was 13 days and that the standard deviation (or standard error) around this value is zero.

$x = –k_2 \times t = –0.0502 \times 13 = –0.653$

Estimated standard deviation/error = $0.005 \times 13 = 0.065$

$e^{–k2t} = e^x = 0.521$

Estimated standard deviation/error = $\sqrt{(e^{2x–0.653} \times 0.065^2)} = 0.034$

$j = 1–e^{–k2t} = 1–0.521 = 0.479$

Estimated standard deviation/error = $\sqrt{(e^{2x–0.653} \times 0.065^2)} = 0.034$

$f = 1/(1–e^{–k2t}) = 1/0.479 = 2.09$

Estimated standard deviation/error = $\sqrt{((e^{2x–0.653} \times 0.065^2)/(0.479^4))} = 0.147$

$y = C_0 \times k_2 = 3.58 \times 0.0502 = 0.18$ µg g^{-1} day^{-1}

Estimated standard deviation/error =
$\sqrt{(0.18^2)} \times ((1.2^2/3.58^2)+(0.005^2/(0.0502^2)) = 0.063$ µg g^{-1} day^{-1}

For the calculation of the z term ($z = I \times C_{food}$), the uncertainty in both of these terms can be estimated directly from the raw data from the OECD 305 test. The standard deviation in C_{food} can be obtained from the repeat measurements of the concentration in the food during the study. The uncertainty in I (the feeding rate) is more problematic as it depends on whether (and how often) adjustments are made for the growth of the fish during the uptake phase. In the previous evaluation of the OECD 305 ring test (OECD 2012b), the average feeding rate over the uptake phase was estimated using the known fish growth rate constant and the amount of food added daily. If this approach is taken, then the mean and standard deviation of the actual feeding rate can readily be calculated. The relevant data derived from the OECD 305 ring test data for Lab 1 are shown below (see OECD 2012b for further details).

$C_{food} = 25.2$ µg g^{-1}

Standard deviation = 1.1 µg g^{-1}

I = 0.024 g g^{-1} day^{-1}

Standard deviation = 0.004 g g^{-1} day^{-1}

Using these experimentally determined values, the uncertainty in the z term can be estimated as follows.

$z = I \times C_{food} = 0.024 \times 25.2 = 0.60$ µg g^{-1} day^{-1}

Estimated standard deviation/error = $\sqrt{(0.60^2)} \times ((0.004^2/0.024^2)+(1.1^2/25.2^2))$

$= 0.10$ µg g^{-1} day^{-1}

In this case the overall uncertainty is dominated by the uncertainty in the feeding rate, with the uncertainty in the concentration in food making only a minor contribution to the total.

Continuing the approach outlined in Table 4.1, the uncertainty in the further derived parameters can be estimated as follows.

$w = C_0 \times k_2/(I \times C_{food}) = y/z = 0.18/0.60 = 0.30$ day^{-1}

Estimated standard deviation/error = $\sqrt{(0.30^2 \times ((0.063^2/0.18^2)+(0.10^2/0.60^2))}$

$= 0.12$ day^{-1}

$\alpha = (C_0 \times k_2/(I \times C_{food})) \times (1/(1-e^{-k2t})) = w \times f = 0.30 \times 2.09 = 0.62$[10]

Estimated standard deviation/error = $\sqrt{(0.62^2 \times ((0.12^2/0.30^2)+(0.147^2/2.09^2))}$ $= 0.25$

In this case the uncertainty in the assimilation efficiency is dominated by the uncertainty in the w term rather than the f term.

$h = I \times \alpha = 0.024 \times 0.62 = 0.015$ g g^{-1} day^{-1}

Estimated standard deviation/error = $\sqrt{(0.015^2 \times ((0.004^2/0.024^2)+(0.25^2/0.62^2))} = 0.0064$ g g^{-1} day^{-1}

$BMF = I \times \alpha/k_{2g} = h/k_{2g}$ $0.015/0.0136 = 1.1$

Estimated standard deviation/error = $\sqrt{(1.1^2 \times ((0.0064^2/0.015^2)+(0.0053^2/0.0136^2))} = 0.63$

In this case the uncertainty in k_{2g} contributes around 44% of the total estimated uncertainty in the BMF. The uncertainty in the lipid-normalised BMF can be estimated as follows. The lipid content of the food was determined in the experiment to be 0.0638 but the uncertainty in the value was not known. For the calculation here the uncertainty is assumed to be zero.

$r = BMF \times L_{food} = 1.1 \times 0.0638 = 0.070$

Estimated standard deviation/error = $\sqrt{(0.070^2 \times ((0.63^2/1.1^2)+(0^2/0.0638^2))}$ $= 0.040$

The uncertainty in the experimental fish lipid content can be obtained from the raw data generated during the OECD 305 ring test. For this example the mean and standard deviation of the fish lipid contents at the end of the depuration phase from the exposed population have been used.[11] The values are shown below (taken from Table 2.12).

Lipid content (L) = 0.043 (fractional lipid content)

Standard deviation = 0.0122 (based on measurements in the exposed population and the end of the depuration phase)

$BMF_L = BMF \times L_{food}/L_{fish} = r/L_{fish} = 0.070/0.043 = 1.6$

Estimated standard deviation/error = $\sqrt{(1.6^2 \times ((0.040^2/0.070^2)+(0.0122^2/0.043^2))} = 1.0$

[10] Note: This value differs slightly from the value given in OECD (2012b) as the mean estimated feeding rate has been used here whereas in most of the calculations in OECD (2012b) the nominal feeding rate was used in most of the calculations.
[11] Other measures of lipid content could equally be used.

The uncertainty in a BCF estimated using the data from the OECD 305 ring test could be estimated in a similar way. However, such estimates would be dependent on knowing (or being able to estimate) the uncertainty in the predicted k_1 value. This is currently very difficult to predict in a meaningful way.

5 Conclusions and recommendations

The analysis of depuration data has shown that the growth-corrected depuration rate constant shows a dependence on the lipid content of the fish, in accordance with bioaccumulation theory. This suggests that the growth-corrected depuration rate constant should be normalised to a ‘standard’ lipid content to allow comparisons to be made between different studies for this parameter. This lipid normalisation is an important consideration when using the growth-corrected depuration rate constant from an OECD 305 feeding study to calculate a BCF value using an estimate of the uptake rate constant (it will not influence the calculation of biomagnification factor (BMF), which is corrected for the lipid content of both fish and food). The analysis suggests that either the mean lipid content at the end of the depuration phase, the arithmetic mean concentration of the two sampling points over the depuration phase, or the time-weighted average lipid over the depuration phase are appropriate measures for carrying out this normalisation.

Selection of the most appropriate depuration rate constant for BCF estimation is difficult as most of the experimental BCF data have not been growth corrected and it is not always clear whether they have been normalised to a standard lipid content. However, based on the analysis here, the most appropriate depuration rate constant to use for such purposes is the growth-corrected and lipid-normalised depuration rate constant. This is in keeping with the basis for bioconcentration and biomagnification factors recommended in the OECD 305 test guideline.

Equations for approximating the propagation of errors in the bioaccumulation parameters derived from an OECD 305 dietary study are given. However, these are subject to a number of assumptions which may not be valid in all cases. It is therefore recommended that these equations are used cautiously and other methods, such as those based on Monte Carlo analysis, are explored (e.g. distributions other than normal distributions can theoretically be considered).

References

Barber, M.C. (2001) *Bioaccumulation and aquatic system simulator (BASS) user's manual*. Beta Test Version 2.1. EPA/600/R-01/035. Athens, GA: United States Environmental Protection Agency, Office of Research and Development.

Barber, M.C. (2003) A review an comparison of models for predicting dynamic chemical bioconcentration in fish. *Environmental Toxicology and Chemistry*, 22, 1963–1992.

Barber, M.C., Suárez, L.A. and Lassiter, R.R. (1991) Modelling bioaccumulation of organic pollutants in fish with an application to PCBs in Lake Ontario salmonids. *Canadian Journal of Fisheries and Aquatic Science*, 48, 318–337.

Brooke, D. and Crookes, M. (2012) *Depuration rate constant: growth correction and use as an indicator of bioaccumulation potential* [online]. Product Code LIT 7371, Environment Agency for England and Wales. Available at: http://a0768b4a8a31e106d8b0-50dc802554eb38a24458b98ff72d550b.r19.cf3.rackcdn.com/LIT_7371_44228a.pdf [Accessed 7 December 2012].

Brooke, D.N., Crookes, M.J. and Merckel, D.A.S. (2012) Methods for predicting the rate constant for uptake of organic chemicals from water by fish. *Environmental Toxicology and Chemistry*, 31(11), 2465–2471.

Crookes, M. and Brooke, D. (2011) *Estimation of fish bioconcentration factor (BCF) from depuration data* [online]. Science Report SCHO0811BUCE-E-E, Environment Agency for England and Wales. Available at: http://a0768b4a8a31e106d8b0-50dc802554eb38a24458b98ff72d550b.r19.cf3.rackcdn.com/scho0811buce-e-e.pdf [Accessed 7 December 2012].

Erickson, R.J. and McKim, J.M. (1990a) A simple flow-limited model for exchange of organic chemicals at fish gills. *Environmental Toxicology and Chemistry*, 9, 159–165.

Erickson, R.J. and McKim, J.M. (1990b) A model for exchange of organic chemicals at fish gills: flow of diffusion limitations. *Aquatic Toxicology*, 18, 175–198.

Hayton, W.L. and Barron, M.G. (1990) Rate-limiting barriers to xenobiotic uptake by the gill. *Environmental Toxicology and Chemistry*, 9, 151–157.

Hendriks, A.J., Van der Linde, E., Cornelissen, G. and Sijm, D.T.H.M. (2001) The power of size. 1. Rate constants and equilibrium ratios for accumulation of organic substances related to octanol–water partition ratio and species weight. *Environmental Toxicology and Chemistry*, 20, 1399–1420.

Inoue, Y., Hashizume, N., Yoshida, T., Murakami, H., Suzuki, Y., Koga, Y., Takeshige, R., Kikushima, E., Yakata, N. and Otsuka, M. (2012) Comparison of bioconcentration and biomagnification factors for poorly water-soluble chemicals using common carp (*Cyprinus carpio* L.). *Archive of Environmental Contamination and Toxicology*, 63(2), 241–248.

Ku, H.H. (1966) Notes on the use of propagation of error formulas. *Journal of Research of the National Bureau of Standards*, 70C, 263–273.

OECD (2012a) *OECD guidelines for the testing of chemicals. OECD 305: Bioaccumulation in fish: aqueous and dietary exposure*. Adopted 2 October 2012 [online]. Organisation for Economic Co-operation and Development. Available at: http://www.oecd-ilibrary.org/docserver/download/9712191e.pdf?expires=1354887265&id=id&accname=

guest&checksum=0333E1302D4366655FCFB4D9D522851C [Accessed 7 December 2012].

OECD (2012b) *Validation report of a ring test for the OECD 305 dietary exposure bioaccumulation fish test (Part I) with additional report including comparative analysis of trout and carp results (Part II)* [online]. OECD Environment, Health and Safety Publications, Series on Testing and Assessment No. 175, ENV/JM/MONO(2012)20. Organisation for Economic Co-operation and Development. Available at: http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2012)20&doclanguage=en [Accessed 9 October 2012].

OECD (2013) *Validation report of a ring test for the OECD 305 dietary exposure bioaccumulation fish test. Additional report including results using a lower feeding rate* [online]. Series on Testing and Assessment No. 191, ENV/JM/MONO(2013)15. Available at: http://www.oecd.org/officialdocuments/displaydocument/?cote=ENV/JM/MONO(2013)15&doclanguage=en [Accessed 3 June 2014].

Sijm, D.T.H.M., Pärt, P. and Opperhuizen, A. (1993) The influence of temperature on the uptake rate constants of hydrophobic compounds determined by the isolated perfused gills of rainbow trout. *Aquatic Toxicology*, 25, 1–14.

Sijm, D.T.H.M., Verberne, M.E., De Jonge, W.J., Pärt P. and Opperhuizen, A. (1994) Experimentally determined blood and water flow limitations for uptake of hydrophobic compounds using perfused gills of rainbow trout: allometric applications. *Aquatic Toxicology*, 30, 325–341.

Sijm, D.T.H., Verbrene, M.E., De Jonge, W.J., Pärt, P. and Opperhuizen, A. (1995) Allometry in the uptake of hydrophobic chemicals determined in vivo and in isolated perfused gills. *Toxicology and Applied Pharmacology*, 131, 130–135.

Spacie, A. and Hamelink, J.L. (1982) Alternative models for describing the bioconcentration of organics in fish. *Environmental Toxicology and Chemistry*, 1, 309–323.

Streit, B. and Siré, E.-O. (1993) On the role of blood proteins for uptake, distribution, and clearance of waterborne lipophilic xenobiotics by fish – a linear system analysis. *Chemosphere*, 26, 1031–1039.

Thomann, R.V. (1989) Bioaccumulation model of organic chemical distribution in aquatic food chains. *Environmental Science and Technology*, 23**,** 699–707.

Tolls, J. and Sijm, D.T.H.M. (1995) A preliminary evaluation of the relationship between bioconcentration and hydrophobicity for surfactants. *Environmental Toxicology and Chemistry*, 14**,** 1675–1685.

Appendix A: Estimates for the growth-corrected and lipid-normalised BCF for the substances used in the OECD 305 ring test

Table A.1 Summary of estimated growth-corrected and lipid-normalised BCF from the ring test using Method 1

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g,L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Lab 1	1.25	2.1	484	410	48,416	41,010	12,104	10,253	12,741	10,792	5,696	4,825
Lab 2a – trout	8.41	9.78	263	251	6,264	5,968	2,554	2,434	2,740	2,611	1,453	1,385
Lab 2b – carp (level 1)	5.42	7.11	303	278	8,410	7,711	2,103	1,928	893	819	830	761
Lab 2b – carp (level 2)	5.42	7.11	303	278	9,767	8,955	2,260	2,072	691	634	843	773
Lab 2b – carp (level 3)	5.42	7.11	303	278	14,418	13,219	2,803	2,570	825	756	943	865
Lab 3	1.95	3.64	420	344	27,996	22,928	13,547	11,094	4,467	3,659	4,615	3,779
Lab 4	1.17	2.32	495	397	23,549	18,916	10,750	8,636	7,976	6,407	5,684	4,566
Lab 5	6.77	7.84	282	269	4,862	4,639	192	183	4,209	4,016	-10,845	-10,348
Lab 6	0.72	1.61	578	446	22,217	17,173	8,495	6,566	6,017	4,651	3,073	2,375
Lab 7	1.2	1.78	491	432	21,327	18,799	5,163	4,551	8,175	7,206	3,270	2,883
Lab 8	1.24	2.08	485	411	32,361	27,424	6,472	5,485	4,667	3,955	4,903	4,155
Lab 9	1.49	2.88	458	371	13,870	11,233	6,103	4,942	6,103	4,942		
Lab 10	0.96	1.05	527	512	40,526	39,380	14,634	14,221	11,707	11,377	7,024	6,826

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g,L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Mean BCF all data					21,076	18,258	6,706	5,764	5,478	4,756	2,291	1,904
Standard deviation					13,464	11,834	4,805	4,203	3,896	3,470	4,658	4,303
Mean BCF for trout (minus Lab 5 data)					26,281	22,537	8,869	7,576	7,177	6,178	4,465	3,849
Standard deviation					12,923	11,764	4,127	3,754	3,334	3,108	1,781	1,674
Mean BCF for carp					10,865	9,961	2,389	2,190	803	736	872	800
Standard deviation					3,151	2,889	368	337	103	94	62	57

Notes: [a] Estimated using the fish weight at the start of the uptake phase (day 0).
[b] Estimated using the fish weight at the end of the uptake phase (day 13/day 14).
HCB = hexachlorobenzene; MX = musk xylene; oTP = *o*-terphenyl; MC = methoxychlor.

Table A.2 Summary of estimated growth-corrected BCF from the ring test using Method 2

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g,L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Lab 1	1.25	2.1	c	c	37,730	33,140	8,638	7,587	9,831	8,635	4,206	3,694
Lab 2a – trout	8.41	9.78	c	c	5,578	5,371	2,083	2,006	2,416	2,327	1,226	1,181
Lab 2b – carp (level 1)	5.42	7.11	c	c	7,263	6,786	1,663	1,554	764	714	679	634
Lab 2b – carp (level 2)	5.42	7.11	c	c	8,434	7,881	1,787	1,670	591	552	690	645
Lab 2b – carp (level 3)	5.42	7.11	c	c	12,451	11,634	2,217	2,072	705	659	772	721
Lab 3	1.95	3.64	c	c	22,507	19,255	9,973	8,532	3,556	3,042	3,515	3,007
Lab 4	1.17	2.32	c	c	18,266	15,393	7,636	6,435	6,126	5,162	4,178	3,521
Lab 5	6.77	7.84	c	c	4,264	4,111	154	149	3,655	3,523	-9,013	-8,689
Lab 6	0.72	1.61	c	c	16,657	13,622	5,832	4,769	4,467	3,653	2,183	1,785
Lab 7	1.2	1.78	c	c	16,573	15,017	3,674	3,329	6,290	5,700	2,408	2,182
Lab 8	1.24	2.08	c	c	25,204	22,146	4,616	4,056	3,599	3,163	3,618	3,179
Lab 9	1.49	2.88	c	c	10,942	9,280	4,409	3,739	4,767	4,043		
Lab 10	0.96	1.05	c	c	31,003	30,316	10,252	10,025	8,868	8,672	5,092	4,979

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g, L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Mean BCF all data					16,682	14,919	4,841	4,302	4,280	3,834	1,629	1,403
Standard deviation					10,175	9,166	3,370	3,021	2,932	2,674	3,687	3,471
Mean BCF for trout (minus Lab 5 data)					20,495	18,171	6,346	5,609	5,547	4,933	3,303	2,941
Standard deviation					9,890	9,150	2,908	2,679	2,490	2,352	1,270	1,203
Mean BCF for carp					9,383	8,767	1,889	1,765	687	642	714	667
Standard deviation					2,721	2,542	291	272	88	82	51	47

Notes: [a] Estimated using the fish weight at the start of the uptake phase (day 0).
[b] Estimated using the fish weight at the end of the uptake phase (day 13/day 14).
[c] The estimation method used depends on both the fish weight and log Kow of the substance. The range of k_1 values estimated was 226 to 433 L kg^{-1} day^{-1} for hexachlorobenzene, 207 to 397 L kg^{-1} day^{-1} for musk xylene, 223 to 429 L kg^{-1} day^{-1} for *o*-terphenyl and 214 to 410 L kg^{-1} day^{-1} for methoxychlor.
HCB = hexachlorobenzene; MX = musk xylene; oTP = *o*-terphenyl; MC = methoxychlor.

Table A.3 Summary of estimated growth-corrected BCF from the ring test using Method 6

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g,L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Lab 1	1.25	2.1	757	665	75,659	66,456	18,915	16,614	19,910	17,488	8,901	7,818
Lab 2a – trout	8.41	9.78	470	452	11,185	10,771	4,561	4,392	4,894	4,712	2,595	2,499
Lab 2b – carp (level 1)	5.42	7.11	524	490	14,564	13,609	3,641	3,402	1,547	1,445	1,436	1,342
Lab 2b – carp (level 2)	5.42	7.11	524	490	16,913	15,804	3,913	3,656	1,197	1,119	1,460	1,365
Lab 2b – carp (level 3)	5.42	7.11	524	490	24,967	23,329	4,855	4,536	1,429	1,335	1,633	1,526
Lab 3	1.95	3.64	677	579	45,133	38,612	21,838	18,683	7,202	6,162	7,439	6,365
Lab 4	1.17	2.32	769	648	36,629	30,867	16,722	14,092	12,407	10,455	8,841	7,451
Lab 5	6.77	7.84	496	478	8,551	8,243	338	326	7,402	7,136	-19,075	-18,388
Lab 6	0.72	1.61	868	710	33,403	27,316	12,772	10,444	9,047	7,398	4,620	3,778
Lab 7	1.2	1.78	764	693	33,233	30,113	8,046	7,291	12,739	11,543	5,096	4,617
Lab 8	1.24	2.08	758	666	50,541	44,410	10,108	8,882	7,290	6,405	7,658	6,729
Lab 9	1.49	2.88	724	614	21,942	18,609	9,655	8,188	9,655	8,188		
Lab 10	0.96	1.05	808	790	62,170	60,792	22,450	21,953	17,960	17,562	10,776	10,537

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g,L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Mean BCF all data					33,453	29,918	10,601	9,420	8,667	7,765	3,448	2,970
Standard deviation					20,403	18,381	7,380	6,615	5,939	5,416	7,802	7,346
Mean BCF for trout (minus Lab 5 data)					41,099	36,439	13,896	12,282	11,234	9,990	6,991	6,224
Standard deviation					19,833	18,349	6,368	5,867	5,043	4,763	2,688	2,546
Mean BCF for carp					18,815	17,581	4,136	3,865	1,391	1,300	1,510	1,411
Standard deviation					5,456	5,098	637	595	178	166	107	100

Notes: [a] Estimated using the fish weight at the start of the uptake phase (day 0).
[b] Estimated using the fish weight at the end of the uptake phase (day 13/day 14).
HCB = hexachlorobenzene; MX = musk xylene; oTP = *o*-terphenyl; MC = methoxychlor.

Table A.4 Summary of estimated growth-corrected BCF from the ring test using Method 7

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g,L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Lab 1	1.25	2.1	669	615	66,853	61,495	16,713	15,374	17,593	16,183	7,865	7,235
Lab 2a – trout	8.41	9.78	492	480	11,711	11,429	4,775	4,661	5,123	5,000	2,717	2,652
Lab 2b – carp (level 1)	5.42	7.11	528	505	14,664	14,037	3,666	3,509	1,557	1,491	1,446	1,384
Lab 2b – carp (level 2)	5.42	7.11	528	505	17,029	16,301	3,940	3,771	1,205	1,154	1,470	1,408
Lab 2b – carp (level 3)	5.42	7.11	528	505	25,138	24,063	4,888	4,679	1,438	1,377	1,645	1,574
Lab 3	1.95	3.64	622	563	41,489	37,522	20,075	18,156	6,621	5,988	6,839	6,185
Lab 4	1.17	2.32	676	605	32,175	28,818	14,689	13,156	10,898	9,761	7,766	6,956
Lab 5	6.77	7.84	509	497	8,781	8,576	347	339	7,602	7,424	-19,589	-19,132
Lab 6	0.72	1.61	731	642	28,101	24,686	10,744	9,439	7,611	6,686	3,886	3,414
Lab 7	1.2	1.78	673	632	29,258	27,458	7,084	6,648	11,216	10,526	4,486	4,210
Lab 8	1.24	2.08	669	616	44,626	41,060	8,925	8,212	6,436	5,922	6,762	6,221
Lab 9	1.49	2.88	650	584	19,694	17,711	8,665	7,793	8,665	7,793		
Lab 10	0.96	1.05	698	688	53,658	52,889	19,376	19,099	15,501	15,279	9,301	9,167

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g,L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Mean BCF all data					30,244	28,157	9,530	8,833	7,805	7,276	2,883	2,606
Standard deviation					17,296	16,123	6,384	5,924	5,088	4,789	7,596	7,318
Mean BCF for trout (minus Lab 5 data)					36,396	33,674	12,339	11,393	9,963	9,237	6,203	5,755
Standard deviation					17,121	16,222	5,552	5,230	4,266	4,105	2,267	2,177
Mean BCF for carp					18,943	18,134	4,164	3,986	1,400	1,340	1,520	1,455
Standard deviation					5,493	5,258	641	614	179	171	108	104

Notes: [a] Estimated using the fish weight at the start of the uptake phase (day 0).
[b] Estimated using the fish weight at the end of the uptake phase (day 13/day 14).
HCB = hexachlorobenzene; MX = musk xylene; oTP = *o*-terphenyl; MC = methoxychlor.

Table A.5 Summary of estimated growth-corrected BCF from the ring test using Method 8

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g,L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Lab 1	1.25	2.1	654	595	65,353	59,465	16,338	14,866	17,198	15,649	7,689	6,996
Lab 2a – trout	8.41	9.78	462	449	10,999	10,701	4,485	4,363	4,812	4,682	2,552	2,483
Lab 2b – carp (level 1)	5.42	7.11	500	476	13,900	13,230	3,475	3,308	1,476	1,405	1,371	1,305
Lab 2b – carp (level 2)	5.42	7.11	500	476	16,142	15,364	3,734	3,554	1,142	1,087	1,394	1,327
Lab 2b – carp (level 3)	5.42	7.11	500	476	23,828	22,680	4,633	4,410	1,363	1,298	1,559	1,484
Lab 3	1.95	3.64	603	538	40,181	35,867	19,443	17,355	6,412	5,723	6,623	5,912
Lab 4	1.17	2.32	661	584	31,497	27,808	14,379	12,695	10,668	9,419	7,603	6,712
Lab 5	6.77	7.84	481	468	8,285	8,067	327	319	7,172	6,983	-18,483	-17,996
Lab 6	0.72	1.61	723	624	27,790	24,004	10,626	9,178	7,527	6,501	3,843	3,320
Lab 7	1.2	1.78	658	613	28,626	26,644	6,931	6,451	10,973	10,214	4,389	4,085
Lab 8	1.24	2.08	654	596	43,632	39,712	8,726	7,942	6,293	5,728	6,611	6,017
Lab 9	1.49	2.88	633	561	19,181	17,013	8,440	7,486	8,440	7,486		
Lab 10	0.96	1.05	686	675	52,746	51,892	19,047	18,739	15,238	14,991	9,143	8,995

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g, L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Mean BCF all data					29,397	27,111	9,276	8,513	7,593	7,013	2,858	2,553
Standard deviation					17,078	15,787	6,272	5,770	5,009	4,679	7,252	6,949
Mean BCF for trout (minus Lab 5 data)					35,556	32,567	12,046	11,008	9,729	8,932	6,057	5,565
Standard deviation					16,830	15,851	5,443	5,097	4,214	4,036	2,241	2,144
Mean BCF for carp					17,957	17,091	3,948	3,757	1,327	1,263	1,441	1,372
Standard deviation					5,207	4,956	608	579	170	162	103	98

Notes: [a] Estimated using the fish weight at the start of the uptake phase (day 0).
[b] Estimated using the fish weight at the end of the uptake phase (day 13/day 14).
HCB = hexachlorobenzene; MX = musk xylene; oTP = *o*-terphenyl; MC = methoxychlor.

Table A.6 Summary of estimated growth-corrected BCF from the ring test using Method 9

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g,L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Lab 1	1.25	2.1	649	599	64,935	59,855	16,234	14,964	17,088	15,751	7,639	7,042
Lab 2a – trout	8.41	9.78	481	470	11,462	11,193	4,674	4,564	5,014	4,897	2,660	2,597
Lab 2b – carp (level 1)	5.42	7.11	516	494	14,327	13,729	3,582	3,432	1,521	1,458	1,413	1,354
Lab 2b – carp (level 2)	5.42	7.11	516	494	16,638	15,944	3,849	3,688	1,178	1,128	1,437	1,377
Lab 2b – carp (level 3)	5.42	7.11	516	494	24,560	23,536	4,776	4,576	1,405	1,347	1,607	1,540
Lab 3	1.95	3.64	606	549	40,371	36,602	19,534	17,711	6,442	5,841	6,654	6,033
Lab 4	1.17	2.32	656	589	31,244	28,060	14,264	12,810	10,583	9,504	7,542	6,773
Lab 5	6.77	7.84	498	487	8,587	8,392	339	332	7,434	7,265	-19,156	-18,720
Lab 6	0.72	1.61	708	624	27,234	24,002	10,413	9,177	7,376	6,501	3,766	3,319
Lab 7	1.2	1.78	654	614	28,414	26,708	6,879	6,466	10,892	10,238	4,357	4,095
Lab 8	1.24	2.08	650	599	43,344	39,964	8,669	7,993	6,252	5,764	6,567	6,055
Lab 9	1.49	2.88	632	570	19,142	17,260	8,422	7,595	8,422	7,595		
Lab 10	0.96	1.05	677	667	52,063	51,336	18,801	18,538	15,041	14,830	9,024	8,898

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g, L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Mean BCF all data					29,409	27,429	9,264	8,604	7,588	7,086	2,793	2,530
Standard deviation					16,768	15,655	6,195	5,759	4,935	4,652	7,412	7,148
Mean BCF for trout (minus Lab 5 data)					35,357	32,776	11,988	11,091	9,679	8,991	6,026	5,602
Standard deviation					16,613	15,759	5,389	5,083	4,135	3,982	2,197	2,112
Mean BCF for carp					18,508	17,736	4,069	3,899	1,368	1,311	1,485	1,424
Standard deviation					5,367	5,143	627	600	175	168	106	101

Notes: [a] Estimated using the fish weight at the start of the uptake phase (day 0).
[b] Estimated using the fish weight at the end of the uptake phase (day 13/day 14).
HCB = hexachlorobenzene; MX = musk xylene; oTP = *o*-terphenyl; MC = methoxychlor.

Table A.7 Summary of estimated growth-corrected BCF from the ring test using Method 10

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g,L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Lab 1	1.25	2.1	541	481	54,138	48,099	13,535	12,025	14,247	12,658	6,369	5,659
Lab 2a – trout	8.41	9.78	351	339	8,346	8,064	3,403	3,288	3,652	3,528	1,937	1,871
Lab 2b – carp (level 1)	5.42	7.11	387	364	10,763	10,117	2,691	2,529	1,143	1,074	1,062	998
Lab 2b – carp (level 2)	5.42	7.11	387	364	12,499	11,749	2,892	2,718	885	832	1,079	1,015
Lab 2b – carp (level 3)	5.42	7.11	387	364	18,451	17,344	3,588	3,372	1,056	992	1,207	1,135
Lab 3	1.95	3.64	489	424	32,612	28,286	15,780	13,687	5,204	4,514	5,376	4,663
Lab 4	1.17	2.32	550	470	26,172	22,390	11,948	10,221	8,865	7,584	6,317	5,404
Lab 5	6.77	7.84	368	356	6,350	6,141	251	243	5,497	5,316	-14,166	-13,700
Lab 6	0.72	1.61	614	511	23,613	19,655	9,029	7,515	6,395	5,323	3,266	2,718
Lab 7	1.2	1.78	546	499	23,758	21,716	5,752	5,257	9,107	8,324	3,643	3,330
Lab 8	1.24	2.08	542	482	36,158	32,136	7,232	6,427	5,215	4,635	5,479	4,869
Lab 9	1.49	2.88	520	448	15,762	13,563	6,935	5,968	6,935	5,968		
Lab 10	0.96	1.05	575	563	44,228	43,334	15,971	15,648	12,777	12,519	7,666	7,511

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g, L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Mean BCF all data					24,066	21,738	7,616	6,838	6,229	5,636	2,436	2,123
Standard deviation					14,454	13,128	5,253	4,747	4,217	3,875	5,713	5,410
Mean BCF for trout (minus Lab 5 data)					29,421	26,360	9,954	8,893	8,044	7,228	5,007	4,503
Standard deviation					14,110	13,127	4,540	4,203	3,571	3,387	1,902	1,807
Mean BCF for carp					13,905	13,070	3,057	2,873	1,028	966	1,116	1,049
Standard deviation					4,032	3,790	471	442	131	124	79	75

Notes: [a] Estimated using the fish weight at the start of the uptake phase (day 0).
[b] Estimated using the fish weight at the end of the uptake phase (day 13/day 14).
HCB = hexachlorobenzene; MX = musk xylene; oTP = *o*-terphenyl; MC = methoxychlor.

Table A.8 Summary of estimated growth-corrected BCF from the ring test using Method 13

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g,L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Lab 1	1.25	2.1	482	436	48,215	43,554	12,054	10,888	12,688	11,462	5,672	5,124
Lab 2a – trout	8.41	9.78	332	322	7,901	7,671	3,222	3,128	3,457	3,356	1,833	1,780
Lab 2b – carp (level 1)	5.42	7.11	362	343	10,046	9,526	2,512	2,382	1,067	1,012	991	940
Lab 2b – carp (level 2)	5.42	7.11	362	343	11,667	11,062	2,699	2,559	826	783	1,007	955
Lab 2b – carp (level 3)	5.42	7.11	362	343	17,223	16,330	3,349	3,175	985	934	1,127	1,068
Lab 3	1.95	3.64	442	391	29,461	26,068	14,255	12,614	4,701	4,160	4,856	4,297
Lab 4	1.17	2.32	488	427	23,259	20,339	10,618	9,285	7,878	6,889	5,614	4,909
Lab 5	6.77	7.84	346	336	5,970	5,801	236	229	5,168	5,021	-13,317	-12,940
Lab 6	0.72	1.61	537	459	20,662	17,647	7,900	6,747	5,596	4,779	2,857	2,441
Lab 7	1.2	1.78	486	450	21,132	19,560	5,116	4,736	8,100	7,498	3,240	2,999
Lab 8	1.24	2.08	483	436	32,194	29,090	6,439	5,818	4,643	4,196	4,878	4,408
Lab 9	1.49	2.88	466	409	14,116	12,406	6,211	5,459	6,211	5,459		
Lab 10	0.96	1.05	508	499	39,058	38,378	14,104	13,859	11,283	11,087	6,770	6,652

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g,L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Mean BCF all data					21,608	19,802	6,824	6,221	5,585	5,126	2,127	1,886
Standard deviation					12,683	11,661	4,643	4,248	3,714	3,451	5,268	5,030
Mean BCF for trout (minus Lab 5 data)					26,222	23,857	8,880	8,059	7,173	6,543	4,465	4,076
Standard deviation					12,462	11,692	4,023	3,754	3,131	2,990	1,666	1,590
Mean BCF for carp					12,979	12,306	2,853	2,705	959	910	1,042	988
Standard deviation					3,764	3,569	439	417	123	116	74	70

Notes: [a] Estimated using the fish weight at the start of the uptake phase (day 0).
[b] Estimated using the fish weight at the end of the uptake phase (day 13/day 14).
HCB = hexachlorobenzene; MX = musk xylene; oTP = *o*-terphenyl; MC = methoxychlor.

Table A.9 Summary of estimated growth-corrected BCF from the ring test using Method 15

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g,L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Lab 1	1.25	2.1	394	363	39,376	36,277	9,844	9,069	10,362	9,547	4,632	4,268
Lab 2a – trout	8.41	9.78	291	284	6,937	6,774	2,829	2,762	3,035	2,963	1,610	1,572
Lab 2b – carp (level 1)	5.42	7.11	312	299	8,675	8,311	2,169	2,078	921	883	856	820
Lab 2b – carp (level 2)	5.42	7.11	312	299	10,074	9,651	2,331	2,233	713	683	870	833
Lab 2b – carp (level 3)	5.42	7.11	312	299	14,871	14,247	2,892	2,770	851	815	973	932
Lab 3	1.95	3.64	367	333	24,470	22,172	11,840	10,728	3,905	3,538	4,033	3,655
Lab 4	1.17	2.32	398	357	18,947	17,005	8,650	7,763	6,418	5,760	4,574	4,105
Lab 5	6.77	7.84	302	295	5,199	5,079	205	201	4,500	4,397	-11,597	-11,331
Lab 6	0.72	1.61	430	378	16,524	14,551	6,318	5,564	4,475	3,941	2,285	2,012
Lab 7	1.2	1.78	396	372	17,231	16,190	4,172	3,920	6,605	6,206	2,642	2,482
Lab 8	1.24	2.08	394	363	26,284	24,221	5,257	4,844	3,791	3,493	3,982	3,670
Lab 9	1.49	2.88	383	345	11,606	10,458	5,106	4,601	5,106	4,601		
Lab 10	0.96	1.05	411	405	31,579	31,135	11,404	11,243	9,123	8,995	5,474	5,397

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g, L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Mean BCF all data					17,829	16,621	5,617	5,214	4,600	4,294	1,695	1,535
Standard deviation					10,173	9,494	3,758	3,491	2,994	2,821	4,490	4,328
Mean BCF for trout (minus Lab 5 data)					21,439	19,865	7,269	6,722	5,869	5,449	3,654	3,395
Standard deviation					10,077	9,556	3,269	3,082	2,509	2,416	1,333	1,281
Mean BCF for carp					11,207	10,736	2,464	2,360	828	794	899	862
Standard deviation					3,250	3,113	379	363	106	101	64	61

Notes: [a] Estimated using the fish weight at the start of the uptake phase (day 0).
[b] Estimated using the fish weight at the end of the uptake phase (day 13/day 14).
HCB = hexachlorobenzene; MX = musk xylene; oTP = *o*-terphenyl; MC = methoxychlor.

Table A.10 Summary of estimated growth-corrected BCF from the ring test using Method 17

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g,L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Lab 1	1.25	2.1	426	384	42,583	38,446	10,646	9,611	11,206	10,117	5,010	4,523
Lab 2a – trout	8.41	9.78	293	284	6,965	6,761	2,840	2,757	3,047	2,958	1,616	1,569
Lab 2b – carp (level 1)	5.42	7.11	319	302	8,860	8,399	2,215	2,100	941	892	874	828
Lab 2b – carp (level 2)	5.42	7.11	319	302	10,289	9,753	2,380	2,256	728	690	888	842
Lab 2b – carp (level 3)	5.42	7.11	319	302	15,188	14,398	2,953	2,800	869	824	994	942
Lab 3	1.95	3.64	390	345	26,008	22,999	12,584	11,128	4,150	3,670	4,287	3,791
Lab 4	1.17	2.32	431	377	20,544	17,952	9,379	8,195	6,958	6,080	4,959	4,333
Lab 5	6.77	7.84	305	297	5,263	5,114	208	202	4,556	4,427	-11,742	-11,407
Lab 6	0.72	1.61	475	405	18,258	15,582	6,981	5,958	4,945	4,220	2,525	2,155
Lab 7	1.2	1.78	429	397	18,664	17,269	4,519	4,181	7,154	6,620	2,862	2,648
Lab 8	1.24	2.08	427	385	28,434	25,679	5,687	5,136	4,101	3,704	4,308	3,891
Lab 9	1.49	2.88	411	361	12,465	10,947	5,485	4,817	5,485	4,817		
Lab 10	0.96	1.05	449	441	34,505	33,901	12,460	12,242	9,968	9,794	5,981	5,876

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g, L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Mean BCF all data					19,079	17,477	6,026	5,491	4,931	4,524	1,880	1,666
Standard deviation					11,207	10,299	4,101	3,751	3,281	3,048	4,647	4,436
Mean BCF for trout (minus Lab 5 data)					23,158	21,059	7,842	7,114	6,335	5,775	3,943	3,598
Standard deviation					11,009	10,326	3,554	3,315	2,766	2,641	1,472	1,405
Mean BCF for carp					11,446	10,850	2,516	2,385	846	802	919	871
Standard deviation					3,319	3,146	387	367	108	103	65	62

Notes: [a] Estimated using the fish weight at the start of the uptake phase (day 0).
[b] Estimated using the fish weight at the end of the uptake phase (day 13/day 14).
HCB = hexachlorobenzene; MX = musk xylene; oTP = *o*-terphenyl; MC = methoxychlor.

Table A.11 Summary of estimated growth-corrected BCF from the ring test using Method 18

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g,L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Lab 1	1.25	2.1	614	496	61,392	49,629	15,331	12,394	16,154	13,059	7,218	5,835
Lab 2a – trout	8.41	9.78	281	264	6,690	6,289	2,725	2,561	2,927	2,751	1,551	1,458
Lab 2b – carp (level 1)	5.42	7.11	336	301	9,345	8,361	2,334	2,088	992	888	921	824
Lab 2b – carp (level 2)	5.42	7.11	336	301	10,853	9,710	2,508	2,244	768	687	937	838
Lab 2b – carp (level 3)	5.42	7.11	336	301	16,021	14,334	3,112	2,784	917	820	1,047	937
Lab 3	1.95	3.64	512	396	34,107	26,406	16,485	12,763	5,442	4,213	5,618	4,350
Lab 4	1.17	2.32	631	476	30,038	22,687	13,698	10,346	10,173	7,683	7,246	5,473
Lab 5	6.77	7.84	307	289	5,295	4,986	209	197	4,583	4,316	-11,804	-11,115
Lab 6	0.72	1.61	770	553	29,605	21,285	11,307	8,129	8,017	5,764	4,092	2,942
Lab 7	1.2	1.78	624	531	27,143	23,091	6,564	5,584	10,403	8,850	4,159	3,538
Lab 8	1.24	2.08	616	498	41,063	33,216	8,204	6,636	5,922	4,790	6,218	5,029
Lab 9	1.49	2.88	571	436	17,311	13,212	7,608	5,807	7,616	5,813		
Lab 10	0.96	1.05	684	659	52,622	50,724	18,982	18,297	15,200	14,652	9,115	8,786

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g, L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Mean BCF all data					26,268	21,841	8,390	6,910	6,855	5,714	3,026	2,408
Standard deviation					17,718	15,124	6,223	5,294	5,097	4,415	5,449	4,908
Mean BCF for trout (minus Lab 5 data)					33,330	27,393	11,212	9,169	9,095	7,508	5,652	4,676
Standard deviation					16,768	14,992	5,327	4,787	4,399	4,038	2,354	2,196
Mean BCF for carp					12,073	10,802	2,651	2,372	892	798	968	866
Standard deviation					3,501	3,132	408	365	114	102	69	62

Notes: [a] Estimated using the fish weight at the start of the uptake phase (day 0).
[b] Estimated using the fish weight at the end of the uptake phase (day 13/day 14).
HCB = hexachlorobenzene; MX = musk xylene; oTP = *o*-terphenyl; MC = methoxychlor.

Table A.12 Summary of estimated growth-corrected BCF from the ring test using Method 21

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g, L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Lab 1	1.25	2.1	664	664	66,422	66,422	12,538	12,538	16,280	16,280	6,271	6,271
Lab 2a – trout	8.41	9.78	664	664	15,815	15,815	4,869	4,869	6,444	6,444	2,945	2,945
Lab 2b – carp (level 1)	5.42	7.11	664	664	18,450	18,450	3,483	3,483	1,825	1,825	1,460	1,460
Lab 2b – carp (level 2)	5.42	7.11	664	664	21,426	21,426	3,743	3,743	1,412	1,412	1,485	1,485
Lab 2b – carp (level 3)	5.42	7.11	664	664	31,629	31,629	4,644	4,644	1,686	1,686	1,661	1,661
Lab 3	1.95	3.64	664	664	44,281	44,281	16,179	16,179	6,581	6,581	5,858	5,858
Lab 4	1.17	2.32	664	664	31,629	31,629	10,903	10,903	9,978	9,978	6,127	6,127
Lab 5	6.77	7.84	664	664	11,452	11,452	342	342	9,233	9,233	-20,502	-20,502
Lab 6	0.72	1.61	664	664	25,547	25,547	7,375	7,375	6,444	6,444	2,835	2,835
Lab 7	1.2	1.78	664	664	28,879	28,879	5,279	5,279	10,311	10,311	3,554	3,554
Lab 8	1.24	2.08	664	664	44,281	44,281	6,687	6,687	5,948	5,948	5,384	5,384
Lab 9	1.49	2.88	664	664	20,128	20,128	6,687	6,687	8,249	8,249		
Lab 10	0.96	1.05	664	664	51,094	51,094	13,931	13,931	13,748	13,748	7,107	7,107

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g, L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Mean BCF all data					31,618	31,618	7,435	7,435	7,549	7,549	2,015	2,015
Standard deviation					15,868	15,868	4,623	4,623	4,493	4,493	7,377	7,377
Mean BCF for trout (minus Lab 5 data)					36,453	36,453	9,383	9,383	9,331	9,331	5,010	5,010
Standard deviation					16,306	16,306	4,107	4,107	3,640	3,640	1,657	1,657
Mean BCF for carp					23,835	23,835	3,956	3,956	1,641	1,641	1,535	1,535
Standard deviation					6,912	6,912	609	609	210	210	109	109

Notes: [a] Estimated using the fish weight at the start of the uptake phase (day 0).
[b] Estimated using the fish weight at the end of the uptake phase (day 13/day 14).
HCB = hexachlorobenzene; MX = musk xylene; oTP = *o*-terphenyl; MC = methoxychlor.

Table A.13 Summary of estimated growth-corrected BCF from the ring test using Method 22

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g,L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Lab 1	1.25	2.1	778	778	77,814	77,814	15,408	15,408	19,304	19,304	7,627	7,627
Lab 2a – trout	8.41	9.78	778	778	18,527	18,527	5,984	5,984	7,641	7,641	3,582	3,582
Lab 2b – carp (level 1)	5.42	7.11	778	778	21,615	21,615	4,280	4,280	2,164	2,164	1,776	1,776
Lab 2b – carp (level 2)	5.42	7.11	778	778	25,101	25,101	4,599	4,599	1,675	1,675	1,806	1,806
Lab 2b – carp (level 3)	5.42	7.11	778	778	37,054	37,054	5,707	5,707	1,999	1,999	2,020	2,020
Lab 3	1.95	3.64	778	778	51,876	51,876	19,881	19,881	7,804	7,804	7,124	7,124
Lab 4	1.17	2.32	778	778	37,054	37,054	13,398	13,398	11,832	11,832	7,451	7,451
Lab 5	6.77	7.84	778	778	13,416	13,416	420	420	10,949	10,949	-24,934	-24,934
Lab 6	0.72	1.61	778	778	29,929	29,929	9,063	9,063	7,641	7,641	3,448	3,448
Lab 7	1.2	1.78	778	778	33,832	33,832	6,487	6,487	12,226	12,226	4,322	4,322
Lab 8	1.24	2.08	778	778	51,876	51,876	8,217	8,217	7,054	7,054	6,548	6,548
Lab 9	1.49	2.88	778	778	23,580	23,580	8,217	8,217	9,781	9,781		
Lab 10	0.96	1.05	778	778	59,857	59,857	17,120	17,120	16,301	16,301	8,644	8,644

Lab	Fish weight (g)		Estimated k_1 (L kg day^{-1})		Estimated $BCF_{g, L}$ (growth corrected and normalised to a 5% lipid content)							
	a	b	a	b	HCB		MX		oTP		MC	
					a	b	a	b	a	b	a	b
Mean BCF all data					37,041	37,041	9,137	9,137	8,952	8,952	2,451	2,451
Standard deviation					18,589	18,589	5,681	5,681	5,328	5,328	8,972	8,972
Mean BCF for trout (minus Lab 5 data)					42,705	42,705	11,531	11,531	11,065	11,065	6,093	6,093
Standard deviation					19,103	19,103	5,046	5,046	4,317	4,317	2,015	2,015
Mean BCF for carp					27,924	27,924	4,862	4,862	1,946	1,946	1,867	1,867
Standard deviation					8,097	8,097	749	749	249	249	133	133

Notes: [a] Estimated using the fish weight at the start of the uptake phase (day 0).
[b] Estimated using the fish weight at the end of the uptake phase (day 13/day 14).
HCB = hexachlorobenzene; MX = musk xylene; oTP = *o*-terphenyl; MC = methoxychlor.

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