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VALIDATION REPORT OF A RING TEST FOR THE OECD 305 DIETARY EXPOSURE BIOACCUMULATION FISH TEST

ADDITIONAL REPORT INCLUDING RESULTS USING A LOWER FEEDING RATE

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No. 191

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SUMMARY OF MAIN FINDINGS USING THE 1.5% FEEDING RATE

1. Overall, the results from the two laboratories using the 1.5% feeding rate show that this feeding rate is viable for use in the test (with the tested species). Hence, this suggests that the recommended feeding rate of the fish in TG 305 of 1-2 % is appropriate. The results obtained are generally comparable with the 3% feeding rate used in the earlier validation studies (Part I of the *Validation Report of a ring test for the OECD 305 dietary exposure bioaccumulation fish test*) but the lower feeding rate has the advantage of reducing the growth rate constant for the fish.

2. The following points were also evident and may need to be considered when interpreting the results of the test, or when comparing the results of tests at different feeding rate.

- There were some important differences in how the two laboratories carried out the feeding study using the 1.5% feeding rate. For example, a difference in the designated day of first feeding between the two laboratories and whole fish (Laboratory 9) versus fish minus gut contents (Laboratory 10) being analysed. Despite this there is broad agreement between the results of the two laboratories. The fact that the fish in the Laboratory 10 study were analysed minus the gut contents means that the results from this Laboratory are not confounded by the possible presence of undigested spiked food in the gut.

- There was some evidence for a higher assimilation efficiency at the lower feeding rate but this difference was not statistically significant.

- There was some evidence for a higher BMF_L at the higher feeding rate. Although this is consistent with the equations used to derive the BMF_L if the potential for differences in parameters arising from biological differences connected to changes in feeding rate are not considered, this difference was not statistically significant in the current study.

- The growth rate constant was statistically significantly lower at the lower feeding rate compared to the higher feeding rate. There was also some evidence for lower fish lipid contents at the lower feeding rate than the higher feeding rate, but this was not statistically significant.

- The feeding rate had a statistically significant effect on the growth-corrected depuration rate constant, with the rate constant being higher at the lower feeding rate than the higher feeding rate. It was thought that this results from differences in the lipid content of the fish between the two feeding rates and an inverse relationship (statistically significant for o-terphenyl and methoxychlor, not statistically significant for hexachlorobenzene and musk xylene) was found between the growth corrected depuration rate constant and the fish lipid content. This suggests that lipid normalisation of the data is important when comparing results from different feeding studies.

- The dependence of the growth-corrected depuration rate constant on fish lipid content is also a relevant factor to consider if the rate constant is to be used for purposes other than deriving a BMF value, for example where it is used to derive a BCF. Lipid normalisation of the data may be appropriate.

INTRODUCTION

3. This Annex reports the results from the OECD TG 305 ring test for two laboratories (Lab 9 and Lab 10) utilising a lower feeding rate (1.5% w/w). The results are reported and analysed in a similar way to those in the main report and comparisons are made between the results using the two feeding rates.

4. The available results from the pre-study phase for these two laboratories are given in the main body of the report. The pre-study results are not considered further here.

5. The test substances were obtained by each laboratory from their own suppliers. The purity of the test materials are shown below (Table 1).

Table 1 Purities of the test substances used

Laboratory	Substance purity (%)				
	Hexachloro benzene	Musk xylene	*o*-Terphenyl	Methoxychlor	Benzo(*a*) pyrene
Lab 9	99.9	98.2	99.9	99.9	99.9
Lab 10	no data	no data	no data	no data	no data

6. The fish used in the test were juvenile rainbow trout (*Oncorhynchus mykiss*).

7. The initial weights (day 0) of the fish used in the test by each laboratory are summarised in Table 2.

Table 2 Details of fish used in the tests

Laboratory	Species	No of fish sampled[a]	Mean weight (g)	Standard deviation (g)	Range (g)	Ratio (smallest: largest)
Lab 9	Rainbow trout	5	1.49	0.20	1.21 to 1.70	0.70
Lab 10[b]	Rainbow trout	10	1.97	0.83	0.96 to 3.19	0.30

Note: a) The fish used for this analysis were the day 0 fish from the control group (5 fish) or the day 0 fish from both the control and exposed group (10 fish; where available).

b) The fish weights from Lab 10 are the weights of whole fish minus the guts.

8. According to the test protocol used, the weights of the fish at the start of the test should be within a range of 1 to 8 g. Both laboratories met this requirement. However, the protocol also recommended that fish of similar weight be selected such that the smallest fish was no smaller than two-thirds of the weight of the largest. As can be seen from the table the range of fish weights was outside that recommended in the test protocol for Lab 10 (smallest fish was just under a third of the weight of the largest fish) but was within this range for Lab 9.

Spiking and analysis of food

9. The food was spiked with the appropriate amounts of all five test substances to provide the nominal concentrations outlined in Table 3. The method used for spiking the food is summarised in

10. Table 4.

Table 3 Nominal concentrations in food

Substance	Nominal concentration (µg/g)
Hexachlorobenzene	25
Musk xylene (1,4,6-trinitro-5-tert-butyl-1,3-xylene)	50
o-Terphenyl	50
Methoxychlor	100
Benzo(*a*)pyrene	150

Table 4 Methods used for spiking of feed

Laboratory	Method	
	Solvent	Corn oil or fish oil
Lab 9	x	
Lab 10	x	

Exposure duration

11. The test was carried out using a 13 day uptake phase[1] followed by a 28 day depuration period. The test protocol used designated day 0 as the first day of feeding. However the raw data from Lab 10 indicate that for their experiment day 0 was designated as the day before first feeding. Therefore there was potentially one less day exposure in the study by Lab 10 than in that by Lab 9 (and the other laboratories reported in the main report).

12. During the uptake phase, the exposed fish were fed the spiked diet at a rate of 1.5% of the body weight (i.e. half the rate used in the studies reported in the main report). Control fish were fed in the same manner but using feed containing no test substance.

13. During the depuration phase the fish were fed a clean diet at 1.5% of the body weight.

Fish lipid analysis

14. No information was reported for Lab 9 on the method used for lipid analysis. For Lab 10 the lipid were analysed using the ASE method.

[1] As the start of the uptake phase was designated day 0, day 13 of the uptake actually corresponds to the fourteenth day of exposure.

RESULTS OF THE OECD 305 RING TEST

15. The raw data were submitted via Excel spreadsheets. The key raw data from the Lab 9 and Lab 10 studies have been summarised by laboratory in the Appendix to this report.

Food recovery prior to start and at end of the study

16. Prior to the start of the test, each laboratory was required to spike the food with the five test chemicals and analyse three subsamples of the spiked food to check for spiking homogeneity and recovery. The results of these analyses are summarised in Table 5.

17. The standard operating procedure indicated that the variation in the concentration should not be more than 15% between the three samples at the start of the study and between the three samples taken at the end of the uptake period, and the mean measured concentration of each of the five test substances should not vary by more than 20% between the start of the test and the end of the uptake period. The samples from the start of the study show that both laboratories achieved homogeneous mixtures of the substances within the food.

18. The available results from Lab 9 show that the concentrations in food were generally stable over the course of the uptake phase. No data were available from Lab 10 to investigate the stability.

19. The overall mean concentrations measured in the food over the exposure period are summarised in Table 6.

20. The lipid content of the food used by each laboratory is summarised in Table 7. The standard operating procedure for the test recommended that the lipid content of the food should ideally be in the range 15 to 20% but that slightly lower lipid contents are not ruled out. The lipid content of the food used by Lab 10 was below this recommended range.

Table 5 Summary of food concentrations prior to the start and end of the study

Laboratory	Parameter	Hexachloro benzene		Musk xylene		*o*-Terphenyl		Methoxychlor		Benzo(*a*)pyrene	
		Start	**End**	**Start**	**End**	**Start**	**End**	**Start**	**End**	**Start**	**End**
Lab 9	Target (spiked) concentration (µg/g)	25		50		50		100		150	
	Mean measured concentration (µg/g)	26.5	23.2	52.2	58.0	50.8	47.5	95.8	110.3	142.3	131.3
	Standard deviation	0.46	0.42	1.17	2.21	1.18	0.62	4.44	0.58	2.89	1.15
	Relative standard deviation (%)	1.73	1.80	2.24	3.81	2.33	1.31	4.63	0.52	2.03	0.88
	No of samples analysed	3	3	3	3	3	3	3	3	3	3
	Mean recovery (%)	106.0	92.7	104.5	116.0	101.5	95.0	95.8	110.3	94.9	87.6
Lab 10	Target (spiked) concentration (µg/g)	25		50		50		100		150	
	Mean measured concentration (µg/g)	18.8	nd	49.8	nd	45.0	nd	121.2	nd	184.8	nd
	Standard deviation	0.85	nd	2.52	nd	1.84	nd	5.13	nd	14.5	nd
	Relative standard deviation (%)	4.52	nd	5.07	nd	4.10	nd	4.23	nd	7.83	nd
	No of samples analysed	5	nd	5	nd	5	nd	5	nd	5	nd
	Mean recovery (%)	75.1	nd	99.6	nd	89.9	nd	121.2	nd	123.2	nd

Notes: nd = Not determined.

Table 6 Summary of mean measured food concentrations[a]

Laboratory	Actual concentration in food (µg/g)				
	Hexachloro benzene	Musk xylene	o-Terphenyl	Methoxy chlor	Benzo[a] pyrene
Lab 9	24.8	55.1	49.1	103.1	136.8
Lab 10	18.8	49.8	45.0	121.2	184.8

Note: a) The mean concentration was estimated as the average of the mean concentration at the start of the study and the mean concentration at the end of the study. Where no analysis at the end of the uptake phase was carried out the mean concentration measured at the start of the test is reported.

Table 7 Summary of lipid contents of feed used

Laboratory	Lipid content (%)
Lab 9	16[a]
Lab 10	12.4

Note: a) No lipid analysis was carried out. The reported figure is that of the supplier of the food.

Feeding rate

21. The feeding rates used by each laboratory are summarised in Table 8 along with details of any adjustments made to take into account the growth of the fish.

Table 8 Summary of feeding rates used

Laboratory	Nominal feeding rate (by weight)	Comment
Lab 9	1.5%	Adjusted on day 3, 8 and 13 of uptake
Lab 10	1.5%	No information provided on whether adjustments were made to the feeding rate during the uptake phase

Experimental conditions

22. The experimental conditions used in the test are summarised in **Table 9**. For Lab 9 the temperatures generally fulfilled the criterion that the variation should be less than ±2°C in the treatment or control groups and the concentrations of dissolved oxygen remained >60% of the air saturation value in all tests. No data were reported for Lab 10.

23.

Table 9 Summary of experimental conditions

Laboratory	Temperature (°C)		Dissolved oxygen (percentage of saturation)		pH	
	Mean	Range	Mean	Range	Mean	Range
Lab 9	12.4	11.0-12.9	83%[a]	81-90%[a]	8.0	7.8-8.0
Lab 10	nd	nd	nd	nd	nd	nd

Note: nd = No data.

Biological effects

24. The exposed and control fish were observed daily throughout the study for incidences of mortality, effects on feeding behaviour and other adverse effects by Lab 9. There were no noted incidences of adverse effects in either the control or exposed groups throughout the study for this laboratory. No information was provided for Lab 10.

Lipid contents of fish

25. According to the standard operating procedure used, the lipid contents of the fish should be measured in five fish at the start of the test and in five fish from the control and exposed group on day 13 of uptake and day 28 of depuration. The data from the two studies are summarised in **Table 10**. For Lab 10 the lipid contents of the fish was found to increase as the test progressed. The data for Lab 9 were more variable.

26. The mean concentrations reported in **Table 10** represent the mean value of the three sampling points (start of test, uptake day 13 and depuration day 28) for the lipid content rather than the time weighted average lipid content. The lipid contents of the fish over the depuration phase only (mean value of the uptake day 13 and depuration day 28 data) are shown in parentheses in **Table 10**.

Table 10 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) (± standard deviation) (values in [ ] are the means during depuration)
		Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
Lab 9	Control	5.25	2.54	nd	nd	3.17	2.91	4.21±2.80 [3.17±2.91]
	Exposed	nd	nd	4.17	0.50	4.60	1.03	4.38±0.79 [4.38±0.79]
Lab 10[b]	Control	0.81	0.54	1.63	0.34	1.56	1.00	1.61±1.40 [2.02±1.54]
	Exposed	1.11	0.82	2.04	0.42	2.48	0.90	1.93±0.89 [2.26±0.70]

Note: nd = No data

a) Five fish were analysed in the control and exposed groups at each time point.

b) 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.

Measured concentrations in fish

27. The concentrations measured in fish at the various timepoints are summarised in **Table 11**. The mean values and standard deviations are based on only those samples where the concentration measured was above the limit of detection.

28. The concentrations reported for Lab 10 are based on the fish body weight without guts rather than the whole fish weight.

29. No data were generated for methoxychlor for Lab 9 owing to analytical difficulties.

Table 11 Summary of measured concentrations in fish

Laboratory	Sampling day		Hexachlorobenzene		Musk xylene		o-Terphenyl		Methoxychlor		Benzo[a]pyrene	
			Mean (µg/g)	SD	Mean (µg/g)	SD	Mean (µg/g)	SD	Mean (µg/g)	SD	Mean (µg/g)	SD
Lab 9	Up	3	1.31	0.14	2.31	0.29	2.20	0.23	a		0.40	b
		8	2.22	0.30	3.30	0.48	2.64	0.39	a		nd	
	Dep	1	3.44	0.68	4.77	0.89	3.78	0.71	a		0.33	0.06
		3	2.83	0.21	3.42	0.39	2.60	0.50	a		nd	
		7	2.27	0.37	2.27	0.28	1.96	0.28	a		nd	
		14	1.35	0.19	0.88	0.24	0.56	0.21	a		nd	
		21	0.98	0.21	0.49	0.19	0.47	0.16	a		nd	
		28	0.70	0.12	0.25	0.06	0.21	0.002	a		nd	
Lab 10	Up	0[c]	0.012	d	0.029	d	0.023	d	0.037	d	0.012	d
		3	0.48	0.25	0.72	0.44	0.75	0.43	0.68	0.52	0.37	0.12
		8	1.46	0.53	2.18	0.50	1.99	0.58	2.01	1.37	1.60	0.92
		13	1.60	0.91	1.98	1.48	1.81	126	1.51	1.33	0.40	0.21
	Dep	1	2.19	0.35	2.93	0.62	2.56	0.72	0.96	0.75	0.05	0.04
		3	1.15	0.38	1.05	0.54	0.88	0.41	0.19	0.17	0.05	0.08
		7	1.65	0.51	1.49	0.53	1.48	0.62	0.62	0.60	0.11	0.18
		14	0.87	0.15	0.53	0.25	0.41	0.16	0.015	0.011	0.002	0.001
		21	0.69	0.14	0.31	0.16	0.22	0.14	0.008	0.010	0.007	0.011
		28	0.54	0.28	0.16	0.12	0.087	0.072	0.009	0.011	0.044	0.085

Notes: Up = Uptake phase. Dep = Depuration phase. Nd = Below the limit of detection/quantification.

a) No data generated owing to analytical difficulties.

b) Only one value above the limit of detection/quantification.

c) The day 0 value for this laboratory was reported to be the day before first feeding.

d) Single sample analysed.

SD = Standard deviation.

Overall depuration rate constants

30. The overall depuration rate constants were derived for each study from a plot of ln [fish concentration] against time for the depuration phase. The value for C_0 (the concentration in fish at the start of the depuration phase) was derived from the intercept of the plots (intercept = ln [C_0]). The slopes and intercepts were determined by linear regression. Not detected values or "less than" values were not included in the regression analysis.

31. The plots were constructed using the individual data points at each time point (normally five concentration measurements were taken at each sampling point).

32. In all cases the plots appeared to be linear with no obvious deviations from the expected first order kinetics.

33. It should be noted that for benzo[a]pyrene data only a limited number of timepoints were available. For Lab 9 the data showed rapid depuration and there were insufficient data points above the limit of detection with which to estimate the depuration rate constant.

34. For Lab 10, the concentrations of benzo[a]pyrene were reported to be measureable at all time points during the depuration and the depuration rate constant has been determined based on all of these data points. This gave an overall depuration rate constant of 0.073 day^{-1}. However it is evident from the data that the concentrations in the fish rapidly declined to background levels by day 3 of depuration. Therefore in addition the depuration rate constant has been estimated for this substance using only the depuration day 1 and day 3 values. This gives an overall depuration rate constant of 0.298 day^{-1}, which is more in line the expected trend in the overall depuration rate constant increasing with the increasing potential for metabolism of the substances (benzo[a]pyrene is expected to be the most readily metabolised of the substances tested and so would be expected to have the highest overall depuration rate constant (as was found in the ring test using the 3% feeding rate; see the main report)) . In both cases it is important to note that the slopes of the plots (k_2) and the intercept of the plots (ln [C_0]) are uncertain. As these two parameters are used in the subsequent calculation of the assimilation efficiencies and BMF values these uncertainties need to be taken into account when considering the data. In particular, it is doubtful that the extrapolation back to the ln [C_0] value is reliable in either case. This results from the very rapid depuration for this substance meaning that it is difficult to define the depuration curve accurately with the sampling schedule used, as was found in the studies run at the 3% feeding rate.

Table 12 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] (µg/g)					
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 13 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] (µg/g)					
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 14 Summary of overall depuration rate constants and C_o 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] (µg/g)					
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 15 Summary of overall depuration rate constants and C_o 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] (µg/g)					
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 16 Summary of overall depuration rate constants and C_o 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] (µg/g)					
Lab 9	a	a	a	a	a	a	a	a
Lab 10	0.077[b]	-3.740	0.024	0.17	0.032	0.502	0.011 to 0.143	-4.769 to -2.711
	0.298[c]	-2.945	0.053	0.07	0.38	0.85	-1.175 to 0.578	-4.904 to -0.985

Note: a) Owing to rapid depuration, there were insufficient data points available to derive the depuration curve.

b) Derived using all reported data during the depuration period.

c) Derived using only the data reported for depuration days 1 and 3.

Growth rate constants

A summary of the fish growth data is given in **Table 17** (fish weights) and **Table 18** (fish lengths).

The fish weights for Laboratory 10 are minus the guts (the guts were excised prior to analysis).

Table 17 Summary of fish weights

Laboratory	Day		Control group		Test group		Combined group	
			Mean weight (g)	SD	Mean weight (g)	SD	Mean weight (g)	SD
Lab 9	Up	0	1.49	0.20	As control		As control	
		3	1.78	0.12	1.80	0.33	1.79	0.24
		8	1.72	0.27	1.82	0.23	1.77	0.24
		13	2.16	0.19	2.43	0.31	2.29	0.28
	Dep	1	1.98	0.31	1.77	0.25	1.87	0.29
		3	2.36	0.46	2.11	0.15	2.23	0.35
		7	2.18	0.25	2.44	0.24	2.31	0.27
		14	2.68	0.40	2.73	0.62	2.71	0.50
		21	3.12	0.38	3.23	0.59	3.17	0.47
		28	3.54	0.45	3.64	0.50	3.59	0.46
Lab 10	Up	0	0.96	a	As control		0.96	a
		3	2.80	1.21	2.09	0.94	2.36	1.02
		8	2.09	0.10	2.11	0.96	2.11	0.78
		13	3.80	1.09	2.52	0.88	2.88	1.05
	Dep	1	2.31	0.16	1.96	0.51	2.06	0.46
		3	2.57	1.49	2.63	0.72	2.61	0.85
		7	2.24	0.21	2.81	0.70	2.65	0.64
		14	4.39	0.76	3.56	1.11	3.80	1.04
		21	3.81	1.61	3.26	0.96	3.42	1.06
		28	3.51	0.76	3.40	1.26	3.44	1.07

Notes: a) Single fish weighed at day 0.

Up = Uptake phase. Dep = Depuration phase.

Table 18 Summary of fish lengths

Laboratory	Day		Control group		Test group		Combined group	
			Mean length (mm)	SD	Mean length (mm)	SD	Mean length (mm)	SD
Lab 9	Up	0	48.2	2.59	As control		As control	
		3	50.2	1.10	49.8	2.86	50.0	2.05
		8	47.2	1.30	48.4	2.30	47.8	1.87
		14	51.6	1.14	52.6	3.29	52.1	2.38
	Dep	1	50.4	3.21	48.4	3.21	49.4	3.20
		3	53.8	2.59	52.8	2.17	53.3	2.31
		7	54.6	1.14	55.4	3.21	55.0	2.31
		14	57.2	3.42	57.0	3.74	57.1	3.38
		21	59.0	2.00	60.2	4.09	59.6	3.10
		28	62.7	2.21	63.3	2.91	63.0	2.53
Lab 10	Up	0	As test		49.0	a	As test	
		3	65.6	12.0	60.2	10.3	62.3	10.5
		8	59.5	0.71	59.4	9.61	59.4	7.85
		13	74.5	7.78	64.2	10.4	67.1	10.4
	Dep	1	62.0	2.83	56.6	7.02	58.1	6.41
		3	63.0	15.6	63.2	5.26	63.1	7.67
		7	63.5	2.12	69.6	6.11	67.9	5.87
		14	77.5	6.36	70.4	8.65	724	8.28
		21	76.0	11.3	71.0	7.31	72.4	7.93
		28	69.0	7.07	68.8	9.28	68.9	8.11

Notes: a) Single fish weighed at day 0.

Up = Uptake phase. Dep = Depuration phase.

35. Growth rate constants were derived for each study from a plot of ln [1/fish weight (g)] against time. The growth rate constant is obtained directly from the slope of such plots. The plots were constructed using the individual data points at each time point (normally weight measurements were taken at each sampling point, with ten at day 28 of depuration). The growth rate constant was determined separately for the uptake phase, depuration phase, and total experimental phase, for both the control group, the test group and the combined control and test group where possible. The derived rate constants are summarised in **Table 19**.

36. A test for statistically significant differences between the growth rate constant determined during the uptake phase alone and the depuration phase alone was carried out for the data for the control group, the test group and the combined (control plus test) group using the t-test (α=0.05).

37. This test revealed no statistically significant differences between the growth rate constant over the uptake phase and depuration phase for either laboratory. A further test for statistically

significant differences was made between the growth rate constant derived for the control group over the entire test period (uptake and depuration) and the growth rate constant derived for the test group over the same period. This test revealed no statistically significant differences between the control and test group for either laboratory and so the growth rate constant derived from the combined control and test group over the entire test period is used as the k_{growth} in subsequent calculations.

38. The preferred values for the k_{growth} are highlighted in **bold** in **Table 19**.The k_{growth} values obtained are 0.020 day^{-1} for Laboratory 9 and 0.017 day^{-1} for Laboratory 10. The mean (± standard deviation) k_{growth} for rainbow trout using the lower feeding rate of 1.5% body weight is therefore 0.019 (±0.002) day^{-1}.

Table 19 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 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: Values in **bold** are the preferred values.

Assimilation efficiency and biomagnification factor

39. The assimilation efficiency and biomagnification factor were calculated for each study using the following equations. The resulting values are summarised in **Table 20** for the data at day 13 of the uptake phase.

$$\alpha = \left(\frac{C_0 \times k_2}{I \times C_{food}}\right) \times \left(\frac{1}{1 - e^{-k_2 \times t}}\right)$$

$$BMF_g = \frac{I \times \alpha}{k_{2g}}$$

$$BMF_L = BMF_g \times \frac{F_{l,food}}{F_{l,fish}}$$

where: α = assimilation efficiency.

C_0 = concentration in fish at time zero of the depuration phase (µg/g).

k_2 = overall (not growth corrected) depuration rate constant (day^{-1}).

k_{2g} = growth corrected depuration rate constant (day^{-1}).

I = food ingestion rate (g food/g fish/day).

C_{food} = concentration in food (µg/g).

t = duration of the feeding period (day). In this case t = 13 days (see below).

BMF_g = growth corrected biomagnification factor.

BMF_L = lipid normalised growth corrected biomagnification factor.

$F_{l,\ food}$ = fraction of lipid in food.

$F_{l,\ fish}$ = fraction of lipid in fish.

40. The value of I used in the calculations was the nominal value (0.015 g food/g fish/day). As the fish grew during the test, the actual feeding rate would decrease during the course of the test, particularly in the studies where no adjustment was made to the feeding rate during the uptake, although this would not affect the calculation of BMF.

41. The fraction of lipid in fish is problematic as in most cases the lipid content of the fish increased over the duration of the test and so it is not clear which is the appropriate value to use. The values of BMF_L in **Table 20** have been estimated using the average values of the determinations of lipid carried out over the entire test period (it should be noted that these values do not necessarily represent the time weighted average lipid content over the test). As the lipid content of the fish was generally increasing over the entire experimental period, the average lipid content over the depuration phase would be higher than these values, which would result in a lower BMF_L. The effect of using the average lipid content over the depuration phase on the BMF_L is shown in parentheses in **Table 20**.

42. It should be noted that although the uptake period was 13 days, there were actually 14 daily feedings during the uptake phase (i.e. feeding with the spiked diet was started on day 0 of the study) for Laboratory 9. For Laboratory 10 day 0 was designated the day before first feeding (i.e. feeding

with the spiked diet was started on day 1 of the test) and so there were 13 daily feedings in this experiment. It could therefore be argued that fish in the Laboratory 9 experiment were actually exposed for a total of 14 days (a similar argument was used in the main report for the studies using the higher feeding rate). The derived assimilation efficiencies and BMF_L values assuming 14 days in the calculations for Laboratory 9 are shown in **Table 21** (this assumption is not appropriate for the Laboratory 10 study). As can be seen the assumption of 14 days rather than 13 days makes only a minor difference to the derived parameters with this difference only being noticeable for the more slowly depurating substance hexachlorobenzene.

43. Both of the laboratories measured the concentration of each substance in fish on day 3 and day 8 of the uptake, and Laboratory 10 also measured the concentration in fish on day 13 of uptake . These data allow the assimilation efficiency on days 3, 8 and 13 to be estimated (using the day 3, 8 or 13 concentration in place of C_0 in the above equation and setting the duration of the feeding period, t, to be 3, 8 or 13 days days (or for Laboratory 9, 4 or 9 days i.e. assuming four feedings occurred between day 0 and day 3 or 9 feedings occurred between day 0 and day 8). The assimilation efficiencies calculated this way are shown in **Table 22** assuming 3, 8 and 13 days in the calculation and **Table 23** assuming 4 and 9 days in the calculation (Laboratory 9 only).

44. A measure of the interlaboratory variability in the derived assimilation efficiency and BMF_L value can be obtained from the mean and relative standard deviation of these values for the two laboratories. These are summarised below for the data derived using t=13 days. The BMF_L values have been calculated using both the mean fish lipid content over the entire experimental period (A) and the mean fish lipid content over the depuration phase (B). The results from the Laboratories using the higher feeding rate (taken from the main report) are also shown for comparison. The data show a similar or lower relative standard deviation (interlaboratory variability) at the lower feeding rate than obtained at the higher feeding rate.

	Assimilation efficiency		**BMF_L (A)**		**BMF_L (B)**	
	Mean	Rel. SD	Mean	Rel. SD	Mean	Rel. SD
Results from the 1.5% feeding rate studies (Laboratory 9 and 10)						
Hexachlorobenzene	0.83	30%	1.73	27%	1.47	12%
Musk xylene	0.62	48%	0.48	8%	0.43	16%
o-Terphenyl	0.60	28%	0.44	1%	0.38	19%
Methoxychlor	0.05	a	0.028	a	0.022	a

[Note: a) single value only]

Results from the 3% feeding rate studies (taken from main report – excluding the results from Laboratory 5)

Hexachlorobenzene	0.60	28%	3.10	37%	2.66	33%
Musk xylene	0.51	47%	0.77	39%	0.67	40%
o-Terphenyl	0.38	29%	0.50	20%	0.44	25%
Methoxychlor	0.20	100%	0.16	63%	0.14	71%

(A) = Normalised using the mean fish lipid content over the entire experimental period.

(B) = Normalised using the mean fish lipid content over the depuration phase.

45. It should be noted that there are some important differences in how the two laboratories carried out the feeding study using the 1.5% feeding rate. In particular, as noted above, Laboratory 9 designated day 0 of the study as the day of first feeding (as was done with the studies using the 3% feeding rate in the main report) whereas Laboratory 10 designated day 0 as the day before first

feeding. Therefore the fish in the Laboratory 9 study received one more feed of contaminated food than the fish in the Laboratory 10 study. Further in the Laboratory 9 study whole fish were analysed whereas in the Laboratory 10 study the gut contents were excised prior to analysis. This makes direct comparison between the results of the two studies (and the results of the Laboratory 10 study with those carried out at the higher feeding rate in the main report) problematic. Despite this there is broad agreement between the results of Laboratory 9 and Laboratory 10. The fact that the fish in the Laboratory 10 study were analysed minus the gut contents means that the results from this Laboratory are not confounded by the possible presence of undigested spiked food in the gut.

Table 20 Summary of derived assimilation efficiencies and BMF values (estimated using 13 days for the uptake period)

Laboratory	k_{growth} used (day^{-1})	Assimilation efficiency					BMF_g					BMF_L (using the mean fish lipid content of exposed fish over the entire test period; values in [ ] are normalised to the mean fish lipid content of exposed fish during depuration)				
		HCB	MX	oTP	MC	BaP	HCB	MX	oTP	MC	BaP	HCB	MX	oTP	MC	BaP
Lab 9	0.020	1.00	0.83	0.72	-	-	0.39	0.14	0.12	-	-	1.41 [1.41]	0.51 [0.51]	0.44 [0.44]	-	-
Lab 10	0.017	0.67	0.42	0.51	0.06	0.001[a] 0.006[b]	0.32	0.07	0.07	0.005	0.0003[a] 0.0003[b]	2.05 [1.75]	0.46 [0.40]	0.45 [0.38]	0.03 [0.03]	0.002[a] [0.001][a] 0.002[b] [0.002][b]
Overall	Mean	0.83	0.63	0.61	0.05	0.001[a]	0.35	0.11	0.10	0.005	0.0003[a] 0.0003[b]	1.73 [1.58]	0.48 [0.45]	0.44 [0.41]	0.03 [0.03]	0.002[a] [0.001][a] 0.002[b] [0.002][b]
	SD	0.23	0.29	0.15			0.05	0.05	0.04			0.45 [0.24]	0.03 [0.08]	0.007 [0.04]		

Notes: HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl. MC = Methoxychlor.

BaP = Benzo[a]pyrene. SD = Standard deviation.

a) Estimated using the overall depuration rate constant and Co value derived from all data points during depuration.

b) Estimated using the overall depuration rate constant and Co value derived from the depuration day 1 and 3 data points only.

Table 21 Summary of derived assimilation efficiencies and BMF values (estimated using 14 days for the uptake period (t+1))

Laboratory	k_{growth} used (day^{-1})	Assimilation efficiency					BMF_g					BMF_L (using the mean fish lipid content of exposed fish over the entire test period; values in [ ] are normalised to the mean fish lipid content of exposed fish during depuration)				
		HCB	MX	oTP	MC	BaP	HCB	MX	oTP	MC	BaP	HCB	MX	oTP	MC	BaP
Lab 9	0.020	0.95	0.81	0.70	-	-	0.37	0.13	0.12	-	-	1.34 [1.34]	0.49 [0.49]	0.43 [0.43]	-	-
Lab 10	0.017	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a

Notes: a) Calculations assuming a 14 day uptake period are not appropriate for this Laboratory (see text).

HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl. MC = Methoxychlor.

BaP = Benzo[a]pyrene. SD = Standard deviation.

Table 22 Summary of derived assimilation efficiencies at day 3, 8 and 13 of the uptake (using 3, or 13 days for the uptake period)

Laboratory	Day	Assimilation efficiency				
		HCB	**MX**	**oTP**	**MC**	**BaP**
Lab 9	3	1.28	1.09	1.17	nd	na
	8	0.94	0.75	0.67	nd	na
Lab 10	3	0.61	0.37	0.44	0.17	0.05
	8	0.78	0.54	0.58	0.28	0.10
	13	0.59	0.37	0.42	0.18	0.02
Mean		0.84	0.63	0.66	0.21	0.05
Standard deviation		0.28	0.30	0.30	0.06	0.04

Notes: HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl.

MC = Methoxychlor. BaP = Benzo[a]pyrene.

SD = Standard deviation. nd = no data (analysis not conducted)

na = not applicable (not possible to estimate)

Table 23 Summary of derived assimilation efficiencies at day 3 and day 8 of the uptake (using 4 or 9 days for the uptake period (t+1))

Laboratory	Day	Assimilation efficiency				
		HCB	**MX**	**oTP**	**MC**	**BaP**
Lab 9	4	0.99	0.86	0.92	nd	na
	9	0.85	0.70	0.63	nd	na
Mean		0.82	0.78	0.77	0.20	0.05
Standard deviation		0.09	0.12	0.21	0.06	0.04

Notes: HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl.

MC = Methoxychlor. BaP = Benzo[a]pyrene.

SD = Standard deviation. nd = no data (analysis not conducted)

na = not applicable (not possible to estimate)

Estimation of growth corrected depuration rate constants using an alternative method

46. The growth corrected depuration rate constant (k_{2g}) is usually determined in the draft OECD 305 Test Guideline as the difference between the overall depuration rate constant (k_2) and the growth rate constant (k_{growth}). The values of k_{2g} estimated using this method are shown in **Table 24** to **Table 28** for the two laboratories.

47. An alternative method for estimating the growth corrected depuration rate constant has recently been proposed (1), and has subsequently been added to the draft OECD 305 Test Guideline. The alternative method obtains the growth corrected depuration rate constant directly from the slope

of a plot of ln [amount of chemical/fish] against time from the depuration phase. This alternative method has been used here to obtain a further estimate of the growth corrected depuration rate constant. The values determined are shown in **Table 24** to **Table 28**.

48. As can be seen from the data reported, the agreement in the growth rate constant between the two methods is generally good.

Table 24 Summary of growth corrected depuration rate constants (k_{2g}) for hexachlorobenzene

Laboratory	Rate constant subtraction 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 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	0.0537		0.0352	0.0322				
Standard deviation	0.0074		0.0052	0.0006				

Table 25 Summary of growth corrected depuration rate constants (k_{2g}) for musk xylene

Laboratory	Rate constant subtraction 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 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	0.108		0.089	0.0864				
Standard deviation	0.004		0.0014	0.0045				

Table 26 Summary of growth corrected depuration rate constants (k_{2g}) for o-terphenyl

Laboratory	Rate constant subtraction 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 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	0.118		0.099	0.0957				
Standard deviation	0.012		0.014	0.0216				

Table 27 Summary of growth corrected depuration rate constants (k_{2g}) for methoxychlor

Laboratory	Rate constant subtraction 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 9	a	0.020	a	a	a	a	a	a
Lab 10	0.199	0.017	0.182	0.184	0.62	0.0270	14.7	2.17×10^{-7}
Mean	0.199		0.182	0.184				
Standard deviation								

Note: a) No data owing to analytical difficulties.

Table 28 Summary of growth corrected depuration rate constants (k_{2g}) for benzo[a]pyrene

Laboratory	Rate constant subtraction 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 9	a	0.020	a	a	a	a	a	a
Lab 10	0.077[b]	0.017	0.060[b]	0.062[b]	0.12[b]	0.031[b]	50[b]	0.059[b]
	0.298[c]		0.281[c]	0.151[c]	0.02[c]	0.359[c]	238[c]	0.685[c]
Mean (all data)	0.077[b]		0.060[b]	0.062[b]				
	0.298[c]		0.281[c]	0.151[c]				
Standard deviation (all data)								

Note: a) Owing to rapid depuration, there were insufficient data points available to derive the depuration curve.

b) Derived using all reported data during the depuration period.

c) Derived using only the data reported for depuration days 1 and 3.

COMPARISON BETWEEN THE RESULTS OBTAINED USING THE 1.5% BY WEIGHT FEEDING RATE AND THE RESULTS OBTAINED USING THE 3% BY WEIGHT FEEDING RATE

49. This section compares the various bioaccumulation parameters obtained in the test using the 1.5% feeding rate with those reported in the main report obtained using the 3% feeding rate.

50. The 3% feeding rate data used for the comparisons were the data obtained using rainbow trout not including Lab 5.

51. Unless otherwise noted, statistically significant differences were tested using the t-test with alpha = 0.05.

Growth rate constant (k_{growth})

52. The mean (± standard deviation) for the growth rate constant k_{growth} was 0.019±0.002 day^{-1} at the 1.5% feeding rate compared with 0.036±0.010 day^{-1} at the 3% feeding rate. The k_{growth} was statistically significantly lower at the 1.5% feeding rate compared with the 3% feeding rate.

Assimilation efficiencies

53. For hexachlorobenzene, the mean (± standard deviation) assimilation efficiency (estimated using 13 days in the calculation) at the 1.5% feeding rate was determined to be 0.84±0.23 compared with 0.60±0.17 at the 3% feeding rate. Although the mean value was higher at the lower feeding rate, this difference was not statistically significant.

54. For musk xylene, the mean (± standard deviation) assimilation efficiency (estimated using 13 days in the calculation) at the 1.5% feeding rate was determined to be 0.63±0.29 compared with 0.51±0.24 at the 3% feeding rate. Although the mean value was slightly higher at the lower feeding rate, this difference was not statistically significant.

55. For o-terphenyl, the mean (± standard deviation) assimilation efficiency (estimated using 13 days in the calculation) at the 1.5% feeding rate was determined to be 0.57±0.21 compared with 0.38±0.11 at the 3% feeding rate. Although the mean value was higher at the lower feeding rate, this difference was not statistically significant.

56. No meaningful comparison could be made between the assimilation efficiency obtained at the two feeding rates for methoxychlor and benzo[a]pyrene.

57. Overall, the available data suggest that the feeding rate has limited effect on the assimilation efficiency. Although the 1.5% feeding rate resulted in higher mean assimilation efficiencies than the 3% feeding rate, this difference was not statistically significant.

BMF_L

58. The comparisons here are made between the growth corrected and lipid normalised BMF (BMF_L) estimated using 13 days for the uptake period and using the mean fish lipid content over the entire test period.

59. For hexachlorobenzene, the mean (± standard deviation) BMF_L at the 1.5% feeding rate was determined to be 1.73±0.45 compared with 3.10±1.16 at the 3% feeding rate. Although the mean value was higher at the higher feeding rate, this difference was not statistically significant.

60. For musk xylene, the mean (± standard deviation) BMF_L at the 1.5% feeding rate was determined to be 0.48±0.03 compared with 0.77±0.30 at the 3% feeding rate. Although the mean value was higher at the higher feeding rate, this difference was not statistically significant.

61. For o-terphenyl, the mean (± standard deviation) BMF_L at the 1.5% feeding rate was determined to be 0.44±0.003 compared with 0.50±0.10 at the 3% feeding rate. Although the mean value was higher at the higher feeding rate, this difference was not statistically significant.

62. It should also be noted that a direct comparison of the mean BMF_L values across the two feeding rates is complicated by the fact that, as explained earlier, the fish in the Lab 10 experiment appear to have had one day less feeding of contaminated food than in the other studies. In order to investigate the possible effects of this, a comparison has also been made between the mean BMF_L values for the 1.5% feeding rate obtained using 14 days as the uptake period for Lab 9 and 13 days as the uptake period for Lab 10 with the mean BMF_L values for the 3% feeding rate obtained using 14 days as the uptake period.

63. Using this approach, the mean (± standard deviation) BMF_L for hexachlorobenzene at the 1.5% feeding rate is 1.70±0.50 compared with 2.95±1.11 at the 3% feeding rate. Again, although the mean value was higher at the higher feeding rate, this difference was not statistically significant.

64. For musk xylene, the mean (± standard deviation) BMF_L at the 1.5% feeding rate was determined to be 0.48±0.02 compared with 0.74±0.29 at the 3% feeding rate. Although the mean value was higher at the higher feeding rate, this difference was not statistically significant.

65. For o-terphenyl, the mean (± standard deviation) BMF_L at the 1.5% feeding rate was determined to be 0.44±0.01 compared with 0.48±0.10 at the 3% feeding rate. Although the mean value was higher at the higher feeding rate, this difference was not statistically significant.

66. Overall, the available data suggest that the feeding rate has limited effect on the BMF_L. Although the 3% feeding rate resulted in higher mean BMF_L values than the 1.5% feeding rate, this difference was not statistically significant.

67. From a purely theoretical point of view, a dependence of the BMF_L value on the feeding rate would be expected from the equations given in paragraph 39 if the feeding rate does not affect the assimilation efficiency or the growth corrected depuration rate constant. The generally higher BMF_L values obtained at the higher feeding rate are consistent with this theory.

Overall depuration rate constant (k_2)

68. For hexachlorobenzene, the mean (± standard deviation) for the overall depuration rate constant was 0.054±0.007 day^{-1} at the 1.5% feeding rate compared with 0.052±0.007 at the 3% feeding rate. The two mean values were not statistically significantly different.

69. For musk xylene, the mean (± standard deviation) for the overall depuration rate constant was 0.108±0.004 day^{-1} at the 1.5% feeding rate compared with 0.088±0.015 at the 3% feeding rate. The two mean values were not statistically significantly different.

70. For o-terphenyl, the mean (± standard deviation) for the overall depuration rate constant was 0.118±0.012 day^{-1} at the 1.5% feeding rate compared with 0.094±0.023 at the 3% feeding rate. The two mean values were not statistically significantly different.

71. Overall, the available data suggest that the feeding rate has limited effect on the overall depuration rate constant. At first sight, this finding is somewhat surprising given that the overall

depuration rate constant has a component from growth dilution and, as indicated in paragraph 52, there is a significant difference in the growth rate constants obtained at the two feeding rates. However, there are also differences in lipid content of the fish between the two feeding rates that should also be considered in this context (see below).

Growth-corrected depuration rate constant (k_{2g})

72. The comparisons here are made between the growth-corrected depuration rate constants obtained using the rate constant subtraction method.

73. For hexachlorobenzene, the mean (± standard deviation) for the growth-corrected depuration rate constant was 0.035±0.005 day^{-1} at the 1.5% feeding rate compared with 0.016±0.006 at the 3% feeding rate. The mean value at the 1.5% feeding rate was statistically significantly higher than the mean value at the 3% feeding rate.

74. For musk xylene, the mean (± standard deviation) for the overall depuration rate constant was 0.089±0.001 day^{-1} at the 1.5% feeding rate compared with 0.052±0.016 at the 3% feeding rate. The mean value at the 1.5% feeding rate was statistically significantly higher than the mean value at the 3% feeding rate.

75. For o-terphenyl, the mean (± standard deviation) for the overall depuration rate constant was 0.099±0.014 day^{-1} at the 1.5% feeding rate compared with 0.058±0.019 at the 3% feeding rate. The mean value at the 1.5% feeding rate was statistically significantly higher than the mean value at the 3% feeding rate.

76. Overall, the results of this analysis show that the feeding rate has a statistically significant effect on the growth corrected depuration rate constant, with the rate constant being significantly higher at the lower feeding rate.

77. The reason for a dependence of the growth corrected depuration rate constant on the feeding rate is not entirely clear. One possible explanation may be that at the higher feeding rate, the fish have higher lipid contents and so depuration processes such as loss via respiration may be slower (loss via respiration can be envisaged as involving partitioning from the lipid phases into the aqueous phase followed by loss across the gills). In order to test whether this is a possible explanation, a comparison has been made between the lipid contents of the fish at the two feeding rates.

78. For the 1.5% feeding rate, the mean (±standard deviation) lipid content of the fish (based on the overall values determined over the course of the study in the exposed population for each laboratory) was 3.12%±1.73%. For the 3% feeding rate the mean lipid content was 6.03%±1.86%. The mean lipid content was statistically significantly higher at the higher feeding rate. When considering this comparison it is important to bear in mind that the actual (time weighted average) mean lipid content in each laboratory cannot be estimated from the available data and so the values used are only approximate. Nevertheless it is evident that the fish at the higher feeding rate generally had higher lipid contents and this is likely to explain the apparent dependence of the growth corrected depuration rate constant on the feeding rate.

79. In order to explore further the possible dependence of the growth-corrected depuration rate constant on the lipid content, plots were constructed of the k_{2g} value versus lipid content (mean over total test duration for exposed fish) for hexachlorobenzene, musk xylene, o-terphenyl and methoxychlor by combining the data with trout from both feeding rates (omitting the data from Lab 5). These are shown below.

Figure 1 Plot of growth-corrected depuration rate constant (k_{2g}) against fish lipid content for hexachlorobenzene

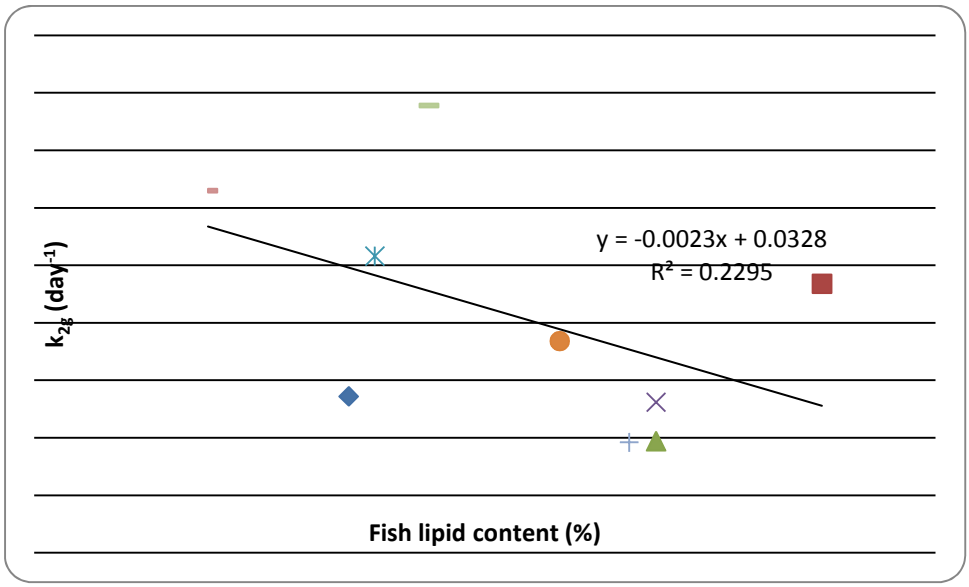


Figure 2 Plot of growth-corrected depuration rate constant (k_{2g}) against fish lipid content for musk xylene

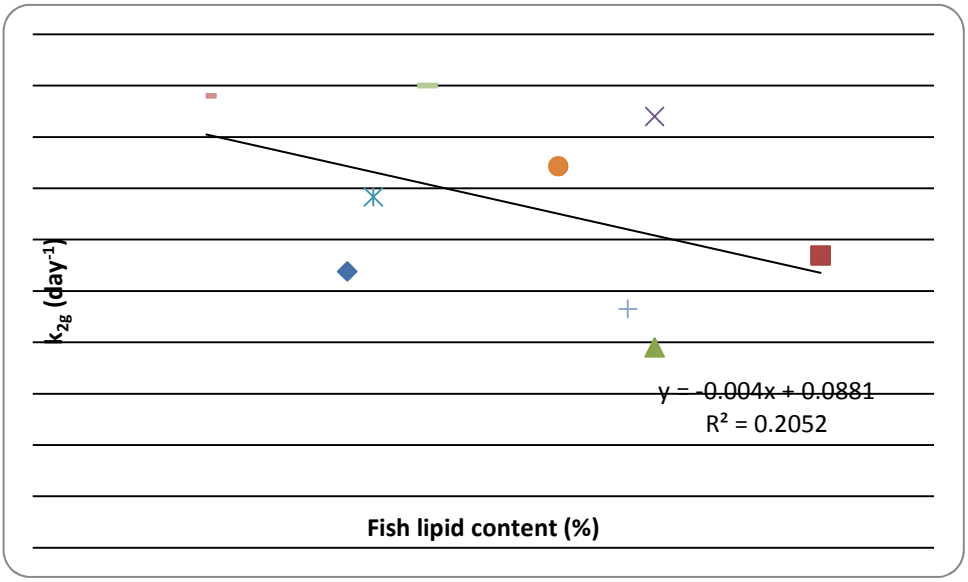


Figure 3 Plot of growth-corrected depuration rate constant (k_{2g}) against fish lipid content for o-terphenyl

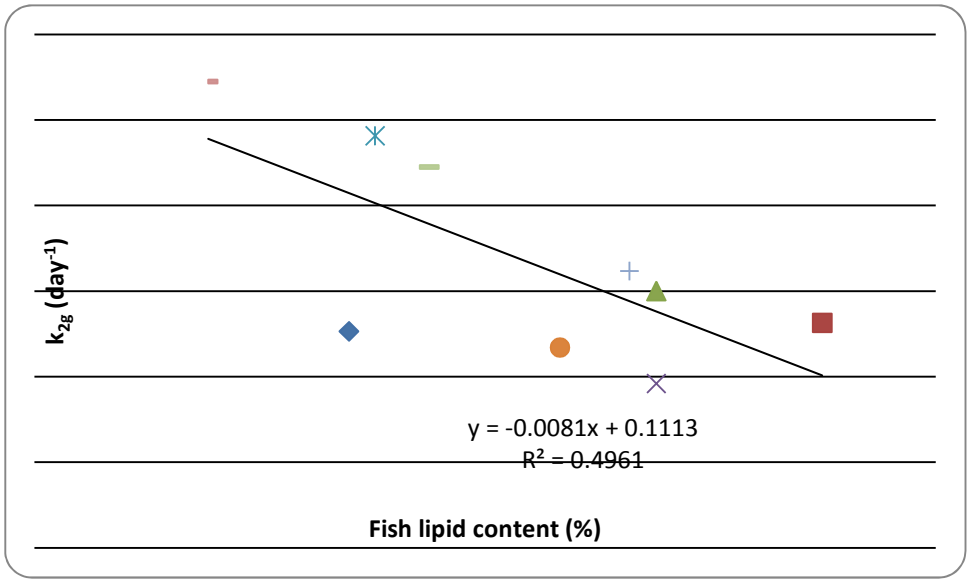
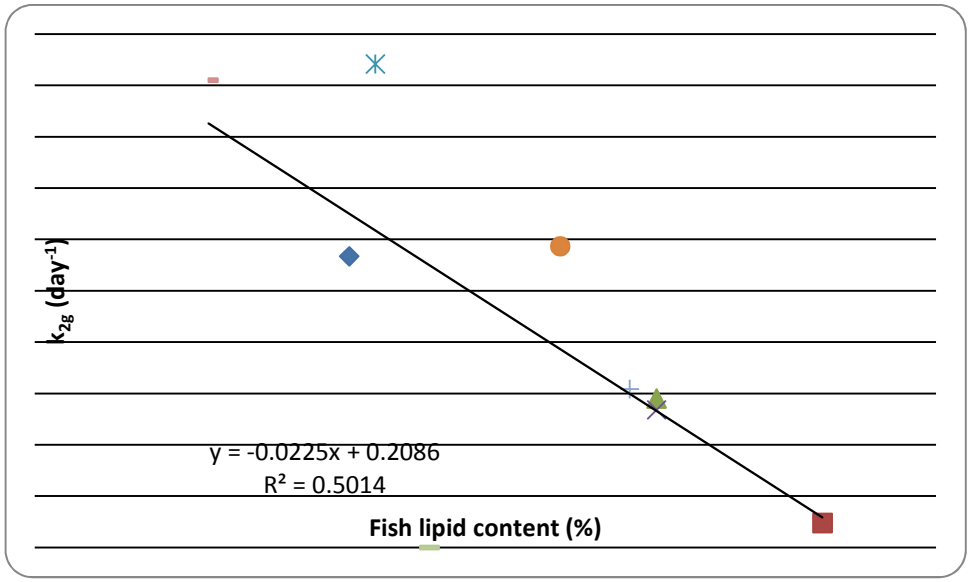


Figure 4 Plot of growth-corrected depuration rate constant (k_{2g}) against fish lipid content for methoxychlor



80. In all cases the plots show a trend for decreasing k_{2g} with increasing fish lipid content. Linear regression analysis (95% confidence level) indicated that the slopes of the plots were not statistically significantly different from zero for hexachlorobenzene and musk xylene but were statistically significantly different from zero for both o-terphenyl and methoxychlor.

81. In terms of the overall results of the study (e.g. BMF_L) any dependence of the k_{2g} value on the lipid is effectively accounted for in the lipid normalisation processes (indeed lipid normalisation requires there to be a dependence of the depuration rate constant on lipid). However, if the k_{2g} values are to be used for purposes other than deriving a BMF_L (for example for estimation of a BCF) then the possible impact of the lipid content on their values may need to be considered.

Investigation of the effect of not correcting the feeding rate, *I*, for growth of the fish during the uptake phase

82. The draft OECD 305 Test Guideline recommends that a constant feeding rate is used in the study. However, in the ring test the weight of fish (and hence adjustments to the amount of feed given) was determined only on day 0, 3, 8 and 13 and at sampling times during the depuration phase. In order to investigate the possible effect of growth on the actual feeding rate in the study, calculations have been carried out using the known growth rate constant from each study to estimate the likely actual feeding rate on each day of the uptake phase of the study, and hence to estimate the likely mean feeding rate over the duration of the uptake phase.

83. Assuming that adjustments to the amount of feed given were made on both uptake days 3 and 8 by both laboratories, the actual feeding rate can be estimated to be around 1.4% compared with the nominal feeding rate of 1.5% by weight for both laboratories. If it is assumed that no corrections were made to the amount of food given during the uptake phase the actual feeding rate can be estimated to be around 1.3%. These estimated actual feeding rates are close to the nominal feeding rates and indicates that the need for correction of the amount of food given to take account of growth of the fish is less important at the 1.5% feeding rate compared with the 3% feeding rate used in the main report. This results from the significantly lower growth of the fish at a 1.5% feeding rate compared with that seen at the 3% feeding rate.

ESTIMATION OF BCF VALUES FROM THE RING TEST DATA AND COMPARISON WITH PUBLISHED BCF DATA

Estimation of BCF from the ring test data at 1.5% feeding rate

84. The equivalent uptake rate constant from water (k_1) for each of the substances has been estimated using the "best" methods identified in the Environment Agency review of such methods (2). Where needed the fish weight was set to the initial fish weight at the start of the study. In addition, calculations were also performed using the fish weight at the end of the uptake phase. The corresponding BCF was then estimated as the ratio of the k_1 value to the overall depuration rate constant or the growth corrected depuration rate constant determined in the OECD 305 ring test studies. The results are summarised in **Table 29** to **Table 41** for the estimates using the overall depuration rate constant (resulting in a BCF that is not growth corrected).

85. The methods used for the estimates of k_1 were as follows (see (2) for further details).

Method 1 Sijm *et al.*, 1995 (3)

Method 2 Omega/Hendriks, 2001 (4)

Method 6 QEAFDCHN/Thomann, 1989 (5)

Method 7 BASS/Barber, 2001 (6)

Method 8 FGETS/Barber *et al*., 1991 (7)

Method 9 Erickson and McKim, 1990a (8)

Method 10 Erickson and McKim, 1990b (9)

Method 13 Hayton and Barron, 1990 (10)

Method 15 Streit and Sire, 1993 (11)

Method 17 Barber, 2003 observed (12)

Method 18 Barber, 2003 calibrated (12)

Method 21 Spacie and Hamelink, 1982 (13)

Method 22 Tolls and Sijm, 1995 (14)

86. Also shown in the Tables are the mean non-growth corrected BCFs estimated from the ring test data at the 3% feeding rate (trout data minus Lab 5). As can be seen the agreement between the estimated non-growth corrected BCFs at the two feeding rates is generally good.

Table 29 Summary of estimated BCF from the ring test using Method 1 – Sijm *et al.*, 1995 (3)

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (not growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Lab 9	1.49	2.88	458	371	7,771	6,293	4,161	3,370	4,199	3,401	-	-	-	-
Lab 10	0.96	1.05	527	512	10,863	10,556	5,017	4,876	4,181	4,063	2,647	2,573	-	-
Mean BCF (1.5% feeding rate)					9,317	8,424	4,589	4,123	4,190	3,732	2,647	2,573	-	-
Standard deviation					2,186	3,014	606	1,065	12	468	-	-	-	-
Mean BCF for trout (3% feeding rate – from main report)					8,749	7,371	5,239	4,391	4,970	4,196	3,610	3,032	343	287
Standard deviation					1,218	916	1,139	763	1,108	933	1,088	837	157	128

Note: 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).

- No estimate possible.

HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl. MC = Methoxychlor. BaP = Benzo[a]pyrene.

Table 30 Summary of estimated BCF from the ring test using Method 2 – Omega/Hendriks, 2001 (4)

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (not growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Lab 9	1.49	2.88	c	c	6,131	5,199	3,006	2,549	3,280	2,782	-	-	-	-
Lab 10	0.96	1.05	c	c	8,310	8,126	3,515	3,437	3,167	3,097	1,919	1,877	-	-
Mean BCF (1.5% feeding rate)					7,220	6,663	3,260	2,993	3,224	2,939	1,919	1,877	-	-
Standard deviation					1,541	2,069	360	628	80	223	-	-	-	-
Mean BCF for trout (3% feeding rate – from main report)					6,902	6,046	3,781	3,298	3,885	3,409	2,699	2,358	271	236
Standard deviation					703	601	719	522	787	707	774	632	117	100

Note: 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).

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 306-403 l kg^{-1} day^{-1} for hexachlorobenzene, 280-369 l kg^{-1} day^{-1} for musk xylene, 303-399 l kg^{-1} day^{-1} for o-terphenyl, 290-382 l kg^{-1} day^{-1} for methoxychlor and 309-407 l kg^{-1} day^{-1} for benzo[a]pyrene.

- No estimate possible.

HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl. MC = Methoxychlor. BaP = Benzo[a]pyrene.

Table 31 Summary of estimated BCF from the ring test using Method 6 – QEAFDCHN/Thomann, 1989 (5)

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (not growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Lab 9	1.49	2.88	724	614	12,294	10,426	6,583	5,583	6,643	5,634	-	-	-	-
Lab 10	0.96	1.05	808	790	16,664	16,295	7,697	7,527	6,414	6,272	4,061	3,971	-	-
Mean BCF (1.5% feeding rate)					14,479	13,361	7,140	6,555	6,529	5,953	4,061	3,971	-	-
Standard deviation					3,090	4,150	788	1,375	162	451	-	-	-	-
Mean BCF for trout (3% feeding rate – from main report)					13,841	12,125	8,279	7,222	7,868	6,904	5,713	4,990	538	469
Standard deviation					1,410	1,205	1,575	1,144	1,595	1,433	1,639	1,337	233	198

Note: 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).

- No estimate possible.

HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl. MC = Methoxychlor. BaP = Benzo[a]pyrene.

Table 32 Summary of estimated BCF from the ring test using Method 7 – BASS/Barber, 2001 (6)

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (not growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Lab 9	1.49	2.88	650	584	11,034	9,923	5,908	5,313	5,962	5,362	-	-	-	-
Lab 10	0.96	1.05	698	688	14,382	14,176	6,643	6,548	5,536	5,457	3,505	3,455	-	-
Mean BCF (1.5% feeding rate)					12,708	12,050	6,276	5,931	5,749	5,409	3,505	3,455	-	-
Standard deviation					2,368	3,008	520	873	301	67	-	-	-	-
Mean BCF for trout (3% feeding rate – from main report)					12,466	11,462	7,444	6,826	7,092	6,528	5,144	4,720	479	439
Standard deviation					898	1,002	1,224	1,019	1,344	1,301	1,403	1,240	191	172

Note: 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).

- No estimate possible.

HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl. MC = Methoxychlor. BaP = Benzo[a]pyrene.

Table 33 Summary of estimated BCF from the ring test using Method 8 – FGETS/Barber *et al.*, 1991 (7)

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (not growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Lab 9	1.49	2.88	633	561	10,747	9,532	5,754	5,104	5,807	5,151	-	-	-	-
Lab 10	0.96	1.05	686	675	14,138	13,909	6,530	6,425	5,442	5,354	3,446	3,390	-	-
Mean BCF (1.5% feeding rate)					12,442	11,721	6,142	5,764	5,625	5,252	3,446	3,390	-	-
Standard deviation					2,398	3,095	549	934	258	143	-	-	-	-
Mean BCF for trout (3% feeding rate – from main report)					12,127	11,025	7,245	6,566	6,898	6,279	5,005	4,540	467	423
Standard deviation					913	963	1,225	985	1,317	1,255	1,379	1,196	190	169

Note: 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).

- No estimate possible.

HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl. MC = Methoxychlor. BaP = Benzo[a]pyrene.

Table 34 Summary of estimated BCF from the ring test using Method 9 – Erickson and McKim, 1990a (8)

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (not growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Lab 9	1.49	2.88	632	570	10,725	9,671	5,743	5,178	5,795	5,226	-	-	-	-
Lab 10	0.96	1.05	677	667	13,955	13,760	6,446	6,356	5,372	5,297	3,401	3,354	-	-
Mean BCF (1.5% feeding rate)					12,340	11,715	6,094	5,767	5,583	5,261	3,401	3,354	-	-
Standard deviation					2,284	2,892	497	833	300	50	-	-	-	-
Mean BCF for trout (3% feeding rate – from main report)					12,119	11,168	7,237	6,650	6,895	6,360	5,001	4,599	465	428
Standard deviation					870	980	1,184	992	1,306	1,267	1,362	1,208	185	167

Note: 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).

- No estimate possible.

HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl. MC = Methoxychlor. BaP = Benzo[a]pyrene.

Table 35 Summary of estimated BCF from the ring test using Method 10 – Erickson and McKim, 1990b (9)

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (not growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Lab 9	1.49	2.88	520	448	8,831	7,599	4,728	4,069	4,772	4,106	-	-	-	-
Lab 10	0.96	1.05	575	563	11,855	11,615	5,476	5,365	4,563	4,471	2,889	2,831	-	-
Mean BCF (1.5% feeding rate)					10,343	9,607	5,102	4,717	4,668	4,289	2,889	2,831	-	-
Standard deviation					2,138	2,840	528	917	148	258	-	-	-	-
Mean BCF for trout (3% feeding rate – from main report)					9,947	8,820	5,948	5,253	5,656	5,022	4,106	3,631	385	340
Standard deviation					910	826	1,086	813	1,119	1,025	1,161	966	164	141

Note: 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).

- No estimate possible.

HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl. MC = Methoxychlor. BaP = Benzo[a]pyrene.

Table 36 Summary of estimated BCF from the ring test using Method 13 – Hayton and Barron, 1990 (10)

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (not growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Lab 9	1.49	2.88	466	409	7,909	6,951	4,235	3,722	4,274	3,756	-	-	-	-
Lab 10	0.96	1.05	508	499	10,469	10,287	4,836	4,752	4,030	3,960	2,552	2,507	-	-
Mean BCF (1.5% feeding rate)					9,189	8,619	4,535	4,237	4,152	3,858	2,552	2,507	-	-
Standard deviation					1,810	2,359	425	728	172	144	-	-	-	-
Mean BCF for trout (3% feeding rate – from main report)					8,919	8,048	5,330	4,793	5,073	4,583	3,681	3,313	344	310
Standard deviation					706	711	921	724	976	920	1,022	875	142	125

Note: 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).

- No estimate possible.

HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl. MC = Methoxychlor. BaP = Benzo[a]pyrene.

Table 37 Summary of estimated BCF from the ring test using Method 15 – Streit and Sire, 1993 (11)

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (not growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Lab 9	1.49	2.88	383	345	6,502	5,859	3,482	3,137	3,514	3,166	-	-	-	-
Lab 10	0.96	1.05	411	405	8,465	8,346	3,910	3,855	3,258	3,212	2,063	2,034	-	-
Mean BCF (1.5% feeding rate)					7,483	7,102	3,696	3,496	3,386	3,189	2,063	2,034	-	-
Standard deviation					1,388	1,758	303	507	181	33	-	-	-	-
Mean BCF for trout (3% feeding rate – from main report)					7,347	6,767	4,387	4,030	4,180	3,854	3,032	2,786	282	259
Standard deviation					528	593	719	601	792	768	826	732	112	101

Note: 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).

- No estimate possible.

HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl. MC = Methoxychlor. BaP = Benzo[a]pyrene.

Table 38 Summary of estimated BCF from the ring test using Method 17 – Barber, 2003 observed (12)

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (not growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Lab 9	1.49	2.88	411	361	6,984	6,134	3,740	3,284	3,774	3,314	-	-	-	-
Lab 10	0.96	1.05	449	441	9,249	9,087	4,272	4,197	3,560	3,498	2,254	2,215	-	-
Mean BCF (1.5% feeding rate)					8,116	7,610	4,006	3,741	3,667	3,406	2,254	2,215	-	-
Standard deviation					1,601	2,088	377	646	151	130	-	-	-	-
Mean BCF for trout (3% feeding rate)					7,875	7,102	4,706	4,230	4,479	4,044	3,250	2,924	304	273
Standard deviation					626	628	815	639	863	812	903	772	125	110

Note: 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).

- No estimate possible.

HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl. MC = Methoxychlor. BaP = Benzo[a]pyrene.

Table 39 Summary of estimated BCF from the ring test using Method 18 – Barber, 2003 calibrated (12)

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (not growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Lab 9	1.49	2.88	571	436	9,699	7,402	5,188	3,959	5,240	4,000	-	-	-	-
Lab 10	0.96	1.05	684	659	14,105	13,596	6,508	6,273	5,429	5,233	3,435	3,311	-	-
Mean BCF (1.5% feeding rate)					11,902	10,499	5,848	5,116	5,334	4,616	3,435	3,311	-	-
Standard deviation					3,116	4,380	934	1,636	133	872	-	-	-	-
Mean BCF for trout (3% feeding rate – from main report)					10,953	8,768	6,560	5,216	6,215	4,990	4,513	3,600	434	345
Standard deviation					2,065	1,428	1,671	1,040	1,583	1,237	1,453	1,043	211	163

Note: 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).

- No estimate possible.

HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl. MC = Methoxychlor. BaP = Benzo[a]pyrene.

Table 40 Summary of estimated BCF from the ring test using Method 21 – Spacie and Hamelink, 1982 (13)

Lab	Estimated k_1 (l kg day^{-1})[a]					Estimated BCF (not growth corrected)[a]				
	HCB	**MX**	**oTP**	**MC**	**BaP**	**HCB**	**MX**	**oTP**	**MC**	**BaP**
Lab 9	664	502	619	533	761	11,277	4,559	5,676	-	-
Lab 10	664	502	619	533	761	13,695	4,777	4,910	2,679	-
Mean BCF (1.5% feeding rate)						12,486	4,668	5,293	2,679	-
Standard deviation						1,710	154	545	-	-
Mean BCF for trout (3% feeding rate – from main report)						12,960	5,822	6,877	4,282	558
Standard deviation						1,915	1,031	1,565	1,170	185

Note: a) For this method the predicted k1 value is dependent on the log Kow of the substance only. Therefore the same k_1 value and BCF would be obtained at the start and end of the uptake phase.

- No estimate possible.

HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl. MC = Methoxychlor. BaP = Benzo[a]pyrene.

Table 41 Summary of estimated BCF from the ring test using Method 22 – Tolls and Sijm, 1995 (14)

Lab	Estimated k_1 (l kg day^{-1})[a]					Estimated BCF (not growth corrected)[a]				
	HCB	**MX**	**oTP**	**MC**	**BaP**	**HCB**	**MX**	**oTP**	**MC**	**BaP**
Lab 9	778	616	734	648	871	13,211	5,603	6,730	-	-
Lab 10	778	616	734	648	871	16,044	5,870	5,822	3,258	-
Mean BCF (1.5% feeding rate)						14,628	5,736	6,276	3,258	-
Standard deviation						2,003	189	642	-	-
Mean BCF for trout (3% feeding rate – from main report)						15,183	7,154	8,155	5,207	639
Standard deviation						2,244	1,267	1,856	1,422	211

Note: a) For this method the predicted k1 value is dependent on the log Kow of the substance only. Therefore the same k_1 value and BCF would be obtained at the start and end of the uptake phase.

- No estimate possible.

HCB = Hexachlorobenzene. MX = Musk xylene. oTP = o-Terphenyl. MC = Methoxychlor. BaP = Benzo[a]pyrene.

LITERATURE

(1) Brooke D. and Crookes M. (2011). Depuration rate constant as an indicator of bioaccumulation potential. Draft Report for the Environment Agency for England and Wales.

(2) Crookes M. and Brooke D. (2011). Estimation of fish bioconcentration factor (BCF) from depuration data. Science Report SCHO0811BUCE-E-E, Environment Agency for England and Wales (available from http://publications.environment-agency.gov.uk/PDF/SCHO0811BUCE-E-E.pdf).

(3) Sijm D.T.H.M, Verberne 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. Toxicol. Appl. Pharmacol., **131**, 130-135.

(4) Hendriks A.J., Van Der Linde A., 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. Environ. Toxicol. Chem., **20**, 1399-1420.

(5) Thomann R.V. (1989). Bioaccumulation model of organic chemical distribution in aquatic food chains. Environ. Sci. Technol., **23**, 699-707.

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

(7) 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. Can. J. Fish. Aquat. Sci., **48**, 318-337.

(8) Erickson R.J. and McKim J.M. (1990a). A simple flow-limited model for exchange of organic chemicals at fish gills. Environ. Toxicol. Chem., **9**, 159-165.

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

(10) Hayton W.L. and Baron M.G. (1990). Rate-limiting barriers to xenobiotic uptake by the gill. Environ. Toxicol. Chem., **9**, 151-157.

(11) 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.

(12) Barber M.C. (2003). A review and comparison of models for predicting dynamic chemical bioconcentration in fish. Environ. Toxicol. Chem., **22**, 1963-1992.

(13) Spacie A. and Hamelink J.L. (1982). Alternative models for describing the bioconcentration of organics in fish. Environ. Toxicol. Chem., **1**, 309-323.

(14) Tolls J. and Sijm D.T.H.M. (1995). A preliminary evaluation of the relationship between bioconcentration and hydrophobicity for surfactants. Environ. Toxicol. Chem., **14**, 1675-1685.

ANNEX 1 – SUMMARY OF THE RAW DATA FROM THE OECD 305 RING TEST FOR THE 1.5% FEEDING RATE

LABORATORY 9

RING TEST STUDY RESULTS

Pre-study measurements

Dilution water characteristics

Source: Haskell Well Water
TOC: none detected at 0.50 mg/L method detection limit
Total suspended solids: Not reported in spreadsheet.

Fish species

Rainbow trout (*Oncorhynchus mykiss*)

Fish food

Source/manufacturer: Aqua Max Starter Fingerling 300 5D03

Crude protein: 50%
Crude fat: 16%
Crude fibre: 3%
Moisture: 4.3%
Ash: No data
Manufacturer reported impurities: None

Test substances

Chemical	CAS Number	Purity (%)
Hexachlorobenzene (HCB)	118-74-1	99.9
Musk xylene	81-15-2	98.2
o-Terphenyl	84-15-1	99.9
Methoxychlor	72-43-5	99.9

Benzo(a)pyrene (B[a]P)	50-32-8	99.9

Spiked feed preparation

Preparation method: ii) Solvent (acetone)

Chemical recoveries pre-study

Chemical	Target concentration (µg/g)	Sample 1 (µg/g) pre-study	Sample 2 (µg/g) pre-study	Sample 3 (µg/g) pre-study
HCB	25	26.6	26.9	26
Musk xylene	50	52.7	53.1	50.9
o-Terphenyl	50	51.4	51.5	49.4
Methoxychlor	100	98.8	97.9	90.7
B[a]P	150	144	144	139

Analytical method description

None given.

Phys-chem measurements in the study

Temperature

The temperature ranged from 11.0-12.9°C (average = 12.4°C) in the water bath throughout the uptake and depuration phases.

Dissolved oxygen

	Day	Control (% of saturation)	Test group (% of saturation)
Uptake	0	89.4	90.3
	7	82.3	82.3
	13	80.5	80.5
Depuration	7	82.3	80.5
	14	82.3	81.4
	21	84.1	84.1
	28	83.2	82.3

pH

	Day	Control	Test group
Uptake	0	7.9	8.0
	7	8.0	8.0
	13	8.0	7.9
Depuration	7	8.0	8.0
	14	8.0	8.0
	21	7.8	7.8
	28	8.0	8.0

Other experimental conditions

Test flow rate: 475 ml min^{-1}
Size of test vessels (approx.): 40 L
Number volumes replacement per day (approx.): 15

Fish data

Control

	Test stage				Concentration (µg/g) (see note)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (w/w) (see note)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	1.59226	50	5.382					
	0	1.70283	51	2.414					
	0	1.21122	45	5.053					
	0	1.61137	49	4.108					
	0	1.35478	46	9.308					
	3	1.85563	50		242	242	242		242
	3	1.62644	49		277	277	277		277
	3	1.74363	50		258	258	258		258
	3	1.75222	50						
	3	1.93953	52						
	8	2.08807	48						
	8	1.91405	49						
	8	1.46536	47						
	8	1.64078	46						
	8	1.49503	46						
	13	2.08874	52						
	13	2.20635	52						

Test stage					Concentration (µg/g) (see note)				
	Day	Weight (g)	Total length (mm)	Lipid (w/w) (see note)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	13	2.44355	53						
	13	1.92779	51						
	13	2.13060	50						
Depuration	1	2.50414	56						
	1	1.68354	48						
	1	1.87892	50						
	1	1.84299	49						
	1	1.97292	49						
	3	2.91533	57						
	3	2.45561	53						
	3	1.77580	52						
	3	2.03062	51						
	3	2.63452	56						
	7	2.30026	55						
	7	2.22198	55						
	7	2.51101	56						
	7	1.89804	53						
	7	1.97886	54						
	14	2.19621	53						
	14	2.43155	56						
	14	2.98356	59						

Test stage				Concentration (µg/g) (see note)				
Day	Weight (g)	Total length (mm)	Lipid (w/w) (see note)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
14	3.18751	62						
14	2.58827	56						
21	2.64865	58						
21	3.09446	62						
21	2.90373	57						
21	3.29919	58						
21	3.63503	60						
28	4.35645	67		103	103	103		103
28	3.92552	63		115	115	115		115
28	3.21953	63		140	140	140		140
28	3.29162	62						
28	3.49019	64						
28	2.94241	60	0.15					
28	3.27462	60	0.36					
28	3.92365	65	4.611					
28	3.84158	62	6.961					
28	3.12274	61	3.772					

Test

	Test stage				Concentration (µg/g) (see note)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (w/w) (see note)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	1.59226	50						
	0	1.70283	51						
	0	1.21122	45						
	0	1.61137	49						
	0	1.35478	46						
	3	1.92166	50		1370	2360	1890		<234
	3	2.10231	52		1270	2340	1890		402
	3	1.28418	46		1520	2720	1970		<351
	3	1.65868	48		1220	2210	1520		<271
	3	2.03724	53		1160	1930	1480		<221
	8	1.63309	48		1950	3030	2140		<276
	8	1.68398	46		2430	3310	2980		<267
	8	1.66758	47		2370	3820	3080		<270
	8	1.90877	49		2500	3710	2540		<236
	8	2.18403	52		1850	2650	2460		<206
	13	2.49596	56	3.61					
	13	2.53227	52	4.957					
	13	2.33467	50	4.299					
	13	2.80270	56	3.973					
	13	1.96593	49	4.012					

	Test stage				Concentration (µg/g) (see note)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (w/w) (see note)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Depuration	1	2.19673	54		3200	4220	3020		272
	1	1.68247	47		3120	4470	3960		399
	1	1.61203	46		3830	5540	4720		282
	1	1.58069	47		4400	5850	4170		357
	1	1.77203	48		2650	3770	3050		<229
	3	2.04771	50		2500	2850	2120		<220
	3	2.17639	54		2790	3740	3030		<207
	3	2.31615	55		3070	3740	2280		<194
	3	1.90457	51		2890	3200	2330		<236
	3	2.08457	54		2900	3590	3250		<216
	7	2.56427	58		2660	2740	2340		<176
	7	2.31524	55		2580	2630	1960		<194
	7	2.45295	54		2240	2180	1950		<184
	7	2.74309	59		2140	2250	2010		<164
	7	2.12540	51		1730	1550	1550		<212
	14	2.61570	57		1200	806	524		<172
	14	2.73286	59		1580	1050	601		<165
	14	1.83104	51		1140	616	312		<246
	14	2.92622	57		1500	1150	458		<154
	14	3.55725	61		1340	754	887		<127
	21	3.59584	61		1040	566	486		<125

Test stage				Concentration (µg/g) (see note)				
Day	Weight (g)	Total length (mm)	Lipid (w/w) (see note)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
21	3.74386	65		704	291	354		<120
21	3.51775	62		1220	757	686		<128
21	2.99999	59		1110	505	353		<150
21	2.29713	54		840	318	<196		<196
28	3.81491	65		775	268	207		<118
28	4.03550	64		769	232	210		<112
28	4.44971	68		568	189	<101		<101
28	3.65204	65		577	207	<123		<123
28	4.20007	67		808	333	<107		<107
28	3.40297	62	3.664					
28	3.52781	61	5.139					
28	2.91722	60	5.858					
28	3.42145	61	3.428					
28	2.98215	60	4.892					

Notes: The lipid contents are given as w/w in the spreadsheet. It has been assumed that this refers to percentage by weight.

The concentrations are given as µg/g in the spreadsheet. However the values reported appear to be around 1,000 times higher than reported in other studies and so it has been assumed that the concentrations are actually µg/kg.

Feed and test observations

Feed analysis

Lipid content was taken from manufacturers literature.

Chemical concentrations – End of uptake

Chemical	Target concentration (µg/g)	Concentration 1 end uptake (µg/g)	Concentration 2 end uptake (µg/g)	Concentration 3 end uptake (µg/g)
HCB	25	22.7	23.3	23.5
Musk xylene	50	55.5	58.8	59.7
o-Terphenyl	50	46.8	48	47.7
Methoxychlor	100	110	111	110
B[a]P	150	130	132	132

Feed quantities (for 1.5% of body weight)

	Day	Average mass of test group fish (g)	Average mass of control fish (g)	Average mass of test and control fish (g)	Mass of food fed to each group (g)
Uptake	0	NA	1.49449	1.49449	1.23296
	3	1.80081	1.78349	1.79215	1.34411
	8	1.81549	1.72066	1.76807	1.19345
	13				
Depuration	1	2.09755	2.06795	2.08275	1.093
	3	2.10588	2.36238	2.23413	1.00536
	7	2.44019	2.18203	2.31111	0.86667
	14	2.73261	2.67742	2.70502	0.81151
	21	3.23091	3.11621	3.17356	0.71405
	28				

Daily observations

No mortality, adverse effects or changes in feeding behaviour were observed in the test group or the control group throughout the uptake and depuration phases.

LABORATORY 10

RING TEST STUDY RESULTS

Pre-study measurements

Dilution water characteristics

No data.

Fish species

Rainbow trout (*Oncorhynchus mykiss*)

Fish food

No data on source.

Crude protein: No data
Crude fat: See below
Crude fibre: No data
Moisture: No data
Ash: No data
Manufacturer reported impurities: No data

The lipid contents were determined (ASE Method) in two samples of control feed and two samples of test feed as follows:

Fish Feed 1	12.51%
Fish Feed 2	12.28%
Control Feed 1	12.35%
Control Feed 2	12.55%

Test substances

Chemical	CAS Number	Purity (%)
Hexachlorobenzene (HCB)	118-74-1	No data
Musk xylene	81-15-2	No data
o-Terphenyl	84-15-1	No data
Methoxychlor	72-43-5	No data
Benzo(a)pyrene (B[a]P)	50-32-8	No data

Spiked feed preparation

Preparation method: ii) Solvent

Chemical recoveries pre-study

Chemical	Target concentration (µg/g)	Sample 1 (µg/g)	Sample 2 (µg/g)	Sample 3 (µg/g)	Sample 4 (µg/g)	Sample 5 (µg/g)
HCB	25	19	20	19	18	18
Musk xylene	50	46	50	51	51	52
o-Terphenyl	50	46	47	46	43	43
Methoxychlor	100	114	125	120	127	120
B[a]P	150	194	196	195	164	176

Note: It is not clear from the spreadsheet if these samples were pre-study or at the end of uptake.

Analytical method description

None given.

Phys-chem measurements in the study

Temperature

No data.

Dissolved oxygen

No data.

pH

No data.

Other experimental conditions

Test flow rate: No data.
Size of test vessels (approx.): No data.
Number volumes replacement per day (approx.): No data.

Fish data

Note: The data for this Laboratory were not reported on the standard spreadsheet. The data have been reproduced here as they appear in the spreadsheet (weights, concentrations and lipids reported spearparately).

#	Type of treatment	Sample ID	Day of dosing	Whole body fish mass after gut has been removed (g)	Length (mm) snout to end of tail fin.	Comment
1	control	C1 f1 d0	0	2.504	70	day 0 refers to measurements on the day before dose was administered.
2	control	C1 f2 d0	0	1.545	54	
3	control	C2 f1 d0	0	1.359	55	
4	control	C3 f1 d0	0	1.099	48	
5	control	C4 f1 d0	0	3.188	69	
6	dosed	D1 f1 d0	0	0.957	49	
7	dosed	D1 f2 d0	0	1.379	55	
8	dosed	D2 f1 d0	0	1.917	60	
9	dosed	D3 f1 d0	0	2.961	69	
10	dosed	D4 f1 d0	0	2.814	68	
11	control	C f1 d3	3	4.051	78	
12	control	C f2 d3	3	2.706	65	
13	control	C f3 d3	3	3.034	68	
14	control	C f4 d3	3	1.511	52	
15	control	C f5 d3	3	1.644	54	

16	dosed	D f1 d3	3	0.876	44	
17	dosed	D f2 d3	3	1.873	60	
18	dosed	D f3 d3	3	3.268	71	
19	dosed	D f4 d3	3	1.692	59	
20	dosed	D f5 d3	3	2.750	67	
21	control	C f1 d8	8	2.284	63	
22	control	C f2 d8	8	2.022	59	
23	control	C f3 d8	8	1.479	55	
24	control	C f4 d8	8	2.166	60	
25	control	C f5 d8	8	4.070	78	
26	dosed	D f1d8	8	1.801	57	
27	dosed	D f2 d8	8	3.712	75	
28	dosed	D f3 d8	8	1.183	49	
29	dosed	D f4 d8	8	2.121	60	
30	dosed	D f5 d8	8	1.749	56	
31	control	C f1 d13	13	4.569	80	day 13 is last day dose was administered
32	control	C f2 d13	13	3.860	75	
33	control	C f3 d13	13	3.027	69	
34	control	C f4 d13	13	3.432	70	
35	control	C f5 d13	13	3.308	70	
36	control	C f6 d13	13	2.005	60	
37	control	C f7 d13	13	1.751	57	
38	control	C f8 d13	13	2.487	68	

39	control	C f9 d13	13	1.389	54	
40	control	C f10 d13	13	2.267	64	
41	dosed	D f1d13	13	3.260	70	
42	dosed	D f2 d13	13	3.530	69	
43	dosed	D f3 d13	13	2.895	69	
44	dosed	D f4 d13	13	1.846	60	
45	dosed	D f5 d13	13	1.324	48	
46	dosed	D f6 d13	13	2.028	55	
47	dosed	D f7 d13	13	1.959	58	
48	dosed	D f8 d13	13	3.268	74	
49	dosed	D f9 d13	13	2.076	58	
50	dosed	D f10 d13	13	2.566	64	
51	control	C1 f1 d14	14	3.349	72	day 14 is first day of clean feed.
52	control	C1 f2 d14	14	2.429	64	
53	control	C2 f3 d14	14	2.198	60	
54	control	C3 f4 d14	14	1.803	55	
55	control	C4 f5 d14	14	1.914	60	
56	dosed	D1 f1d14	14	1.339	47	
57	dosed	D2 f2 d14	14	2.349	63	
58	dosed	D2 f3 d14	14	2.517	63	
59	dosed	D3 f4 d14	14	2.065	58	
60	dosed	D4 f5 d14	14	1.516	52	
61	control	C1 f1 d16	16	3.627	74	
62	control	C2 f2 d16	16	2.451	63	

63	control	C2 f3 d16	16	2.964	67
64	control	C3 f4 d16	16	5.106	78
65	control	C4 f5 d16	16	3.809	72
66	dosed	D1 f1d16	16	3.556	70
67	dosed	D2 f2 d16	16	2.095	59
68	dosed	D3 f3 d16	16	3.206	66
69	dosed	D4 f4 d16	16	2.387	64
70	dosed	D4 f5 d16	16	1.894	57
71	control	C1 f1 d20	20	2.390	65
72	control	C2 f2 d20	20	2.095	62
73	control	C3 f3 d20	20	2.932	67
74	control	C3 f4 d20	20	3.195	72
75	control	C4 f5 d20	20	4.188	75
76	dosed	D1 f1d20	20	2.119	63
77	dosed	D2 f2 d20	20	2.035	64
78	dosed	D3 f3 d20	20	3.338	70
79	dosed	D4 f4 d20	20	3.545	77
80	dosed	D4 f5 d20	20	3.022	74
81	control	C1 f1 d27	27	3.853	73
82	control	C2 f2 d27	27	4.931	82
83	control	C3 f3 d27	27	1.936	56
84	control	C4 f4 d27	27	4.998	80
85	control	C4 f5 d27	27	2.668	66
86	dosed	D1 f1 d27	27	2.312	60

87	dosed	D1 f2 d27	27	4.719	80
88	dosed	D2 f3 d27	27	2.595	64
89	dosed	D3 f4 d27	27	4.587	78
90	dosed	D4 f5 d27	27	3.603	70
91	control	C1 f1 d34	34	2.666	68
92	control	C1 f2 d34	34	4.948	84
93	control	C2 f3 d34	34	3.903	77
94	control	C3 f4 d34	34	3.627	79
95	control	C4 f5 d34	34	4.457	78
96	dosed	D1 f1 d34	34	2.169	62
97	dosed	D2 f2 d34	34	4.238	78
98	dosed	D2 f3 d34	34	2.645	67
99	dosed	D3 f4 d34	34	2.968	69
100	dosed	D4 f5 d34	34	4.285	79
101	control	C1 f1 d41	41	4.054	74
102	control	C2 f2 d41	41	2.974	64
103	control	C2 f3 d41	41	3.992	75
104	control	C3 f4 d41	41	5.348	80
105	control	C4 f5 d41	41	2.373	59
106	dosed	D1 f1 d41	41	3.131	71
107	dosed	D2 f2 d41	41	4.046	73
108	dosed	D2 f3 d41	41	2.242	60
109	dosed	D2 f4 d41	41	5.246	81
110	dosed	D2 f5 d41	41	2.358	59

111	dosed	D3 f6 d41	41	2.794	63
112	dosed	D3 f7 d41	41	2.262	61
113	dosed	D3 f8 d41	41	5.595	84
114	dosed	D3 f9 d41	41	3.562	72
115	dosed	D3 f10 d41	41	2.942	66
116	dosed	D4 f11 d41	41	1.956	57
117	dosed	D4 f12 d41	41	2.903	66
118	dosed	D4 f13 d41	41	2.726	65
119	dosed	D4 f14 d41	41	4.664	79
120	control	C1	45	5.472	86
121	control	C2	45	4.657	84
122	control	C3	45	3.514	70
123	control	C4	45	3.028	70
124	control	C5	45	2.650	67
125	control	C6	45	3.614	74
126	control	C7	45	3.593	77
127	control	C8	45	4.600	80
128	control	C9	45	3.979	77

Type of treatment	Sample code	Fish mass (g)	Fish length (mm)	Day	Concentration (µg/g)					Comment
					Hexachloro benzene	Musk xylene	o-Terphenyl	Methoxy chlor	B(a)P	
dosed	D1f1d0	0.957	49	0	0.012	0.029	0.023	0.037	0.012	day zero is day before the dose was first introduced.
dosed	df1d3	0.876	44	3	0.895	1.425	1.482	1.038	0.483	
dosed	df2d3	1.873	60	3	0.408	0.463	0.534	0.331	0.325	
dosed	Df3d3	3.268	71	3	0.214	0.415	0.397	0.258	0.238	
dosed	df4d3	1.692	59	3	0.470	0.433	0.543	0.344	0.298	
dosed	df5d3	2.750	67	3	0.434	0.870	0.807	1.419	0.512	
dosed	df1d8	1.801	57	8	1.711	2.376	2.004	1.177	1.398	
dosed	Df2d8	3.712	75	8	0.710	1.338	1.146	0.392	0.157	
dosed	df3d8	1.183	49	8	1.789	2.267	2.716	3.687	2.623	
dosed	df4d8	2.121	60	8	1.115	2.249	1.847	3.125	1.909	
dosed	df5d8	1.749	56	8	1.970	2.665	2.251	1.686	1.912	
dosed	df1d13	3.260	70	13	1.650	2.414	2.262	3.626	0.614	day 13 is last day dose was administered
dosed	df3d13	2.895	69	13	0.771	0.476	0.541	0.192	0.130	
dosed	Df4d13	1.846	60	13	1.584	1.735	1.545	1.864	0.370	
dosed	df5d13	1.324	48	13	3.068	4.278	3.751	1.160	0.281	
dosed	df8d13	3.268	74	13	0.912	0.977	0.956	0.713	0.612	
dosed	d1f1d14	1.339	47	14	2.297	2.608	2.699	1.107	0.065	day 14 is first day of clean feed
dosed	d2f2d14	2.349	63	14	2.242	3.338	2.786	2.003	0.030	
dosed	d2f3d14	2.517	63	14	1.960	2.830	2.058	0.233	0.011	

dosed	d3f4d14	2.065	58	14	2.700	3.738	3.563	1.241	0.125	
dosed	D4f5d14	1.516	52	14	1.773	2.153	1.707	0.213	0.033	
dosed	d1f1d16	3.556	70	16	1.303	1.200	1.056	0.108	0.033	
dosed	d2f2d16	2.095	59	16	0.533	0.237	0.205	0.016	0.191	
dosed	D3f3d16	3.206	66	16	1.367	1.584	1.293	0.158	0.006	
dosed	d4f4d16	2.387	64	16	1.076	0.808	0.852	0.459	0.005	
dosed	d4f5d16	1.894	57	16	1.478	1.432	0.995	0.227	0.021	
dosed	d1f1d20	2.119	63	20	1.531	1.467	1.483	0.779	0.006	
dosed	d2f2d20	2.035	64	20	2.123	1.889	2.296	1.572	0.006	
dosed	D3 f3 d20	3.338	70	20	1.580	1.167	0.912	0.065	0.100	
dosed	d4f4d20	3.545	77	20	0.893	0.827	0.857	0.554	0.430	
dosed	d4f5d20	3.022	74	20	2.111	2.122	1.866	0.153	0.002	
dosed	d1f1d27	2.312	60	27	1.141	0.913	0.658	0.015	0.002	
dosed	d1f2d27	4.719	80	27	0.829	0.526	0.260	0.001	0.001	
dosed	D2f3d27	2.595	64	27	0.814	0.467	0.358	0.010	0.001	
dosed	d3f4d27	4.587	78	27	0.773	0.216	0.302	0.030	0.003	
dosed	D4f5d27	3.603	70	20	0.791	0.514	0.462	0.017	0.002	See note below
dosed	d1f1d34	2.169	62	34	0.524	0.142	0.048	0.004	0.002	
dosed	d2f2d34	4.238	78	34	0.907	0.558	0.434	0.005	0.001	
dosed	d2f3d34	2.645	67	34	0.678	0.322	0.269	0.005	0.002	

dosed	D3f4d34	2.968	69	34	0.701	0.310	0.157	0.001	0.002
dosed	d4f5d34	4.285	79	34	0.650	0.200	0.172	0.027	0.026
dosed	D1f1d41	3.131	71	41	0.154	0.018	0.013	0.001	0.006
dosed	d2f2d41	4.046	73	41	0.469	0.069	0.016	0.002	0.015
dosed	D2 f3 d41	2.242	60	41	0.479	0.153	0.100	0.012	0.196
dosed	d2f4d41	5.246	81	41	0.881	0.319	0.126	0.027	0.001
dosed	d2f5d41	2.358	59	41	0.730	0.251	0.179	0.001	0.002
control	cf1d3	4.051	78	3	0.010	0.018	0.024	0.032	0.204
control	cf2d3	2.706	65	3	0.001	nd	0.002	0.003	0.019
control	cf5d3	1.644	54	3	0.009	nd	0.012	0.026	0.365
control	cf2d8	2.022	59	8	0.001	nd	0.001	0.003	0.001
control	cf4d8	2.166	60	8	0.001	nd	0.002	nd	0.022
control	cf1d13	4.569	80	13	0.000	nd	0.000	nd	0.000
control	cf3d13	3.027	69	13	0.001	nd	0.001	nd	0.001
control	c1f2d14	2.429	64	14	0.001	nd	0.002	nd	0.010
control	c2f3d14	2.198	60	14	0.002	nd	0.003	nd	0.012
control	c1f1d16	1.516	52	16	0.001	nd	0.001	nd	nd
control	c2f2d16	3.627	74	16	nd	nd	0.001	nd	0.002
control	c1f1d20	2.390	65	20	nd	nd	0.002	nd	0.002
control	c2f2d20	2.095	62	20	0.001	nd	0.001	nd	nd
control	c1f1d27	3.853	73	27	nd	nd	0.000	nd	0.003
control	c2f2d27	4.931	82	27	nd	nd	0.000	nd	nd

control	c1f1d34	2.666	68	34	nd	nd	0.001	nd	nd
control	c1f2d34	4.948	84	34	0.002	nd	0.003	0.002	0.011
control	c1f1d41	4.054	74	41	0.004	0.014	0.020	0.060	0.023
control	c2f2d41	2.974	64	41	nd	nd	0.001	nd	0.004

Note: This value is given as a day 20 value in the spreadsheet. However the sample code and position in the spreadsheet suggests that it is a day 27 value and has been treated as such in the analysis of the data.

Type of treatment	Sample code	Day	whole body fish mass (g)	% Lipid
dosed	D1 f2 d0	0	1.379	0.36
dosed	D2 f1 d0	0	1.917	0.47
dosed	D3 f1 d0	0	2.961	1.62
dosed	D4 f1 d0	0	2.814	1.99
dosed	D f2 d13	13	3.530	2.27
dosed	D f6 d13	13	2.028	2.51
dosed	D f7 d13	13	1.959	1.79
dosed	D f9 d13	13	2.076	2.17
dosed	D f10 d13	13	2.566	1.44
dosed	D3 f7 d41	41	2.262	4.07
dosed	D3 f8 d41	41	5.595	2.00
dosed	D3 f9 d41	41	3.562	2.30
dosed	D3 f10 d41	41	2.942	1.97
dosed	D4 f14 d41	41	4.664	2.06
control	C1 f1 d0	0	2.504	1.32
control	C1 f2 d0	0	1.545	0.39

control	C2f1d0	0	1.359	0.07
control	C3f1d0	0	1.099	1.09
control	C4f1d0	0	3.188	1.16
control	Cf2d13	13	3.860	1.58
control	Cf7d13	13	1.751	1.31
control	Cf8d13	13	2.487	2.21
control	Cf9d13	13	1.389	1.58
control	Cf10d13	13	2.267	1.46
control	C2f3d34	34	3.903	6.12
control	C3f4d34	34	3.627	1.24
control	C2f3d41	41	3.992	2.30
control	C3f4d41	41	5.348	1.94
control	C4f5d41	41	2.373	0.42

Feed and test observations

Feed analysis

Lipid content measured in four samples of feed (see above).

Chemical concentrations

No data (see above).

Feed quantities (for 1.5% of body weight)

No data.

Daily observations

No data.