Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

26-Jul-2012

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

VALIDATION REPORT OF A RING TEST FOR THE OECD 305 DIETARY EXPOSURE BIOACCUMULATION FISH TEST (PART 1) WITH ADDITIONAL REPORT INCLUDING COMPARATIVE ANALYSIS OF TROUT AND CARP RESULTS (PART II)

Series on Testing and Assessment

No. 175

This document is only available in PDF format.

JT03324997

ENV/JM/MONO(2012)20
Unclassified

English - Or. English

OECD Environment, Health and Safety Publications

Series on Testing and Assessment

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PART I

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

PART II

ADDITIONAL REPORT INCLUDING COMPARATIVE ANALYSIS OF TROUT AND CARP RESULTS

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

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ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris 2012

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The Environment, Health and Safety Division publishes free-of-charge documents in ten different series: Testing and Assessment; Good Laboratory Practice and Compliance Monitoring; Pesticides and Biocides; Risk Management; Harmonisation of Regulatory Oversight in Biotechnology; Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; and Safety of Manufactured Nanomaterials. More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (www.oecd.org/ehs/).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organisations .

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. UNDP is an observer. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

This document contains two parts:

- Part I is a *Validation Report of a Ring Test for the OECD Test Guideline 305 Dietary Exposure Bioaccumulation Fish Test*

- Part II is an *Additional Report including Comparative Analysis of Trout and Carp Results*.

The work on the revision of TG 305 was included in the work plan of the Test Guidelines Programme in 2008. The revision was led jointly by chemical regulators from Germany, the Netherlands and the United Kingdom. An OECD expert group was established and two expert meetings took place in London, on 19-20 October 2009, and in Dessau, on 7-8 November 2011.

The validation (ring test) of TG 305 dietary exposure was conducted in 2010 with ten participating laboratories. Ten studies have been conducted using rainbow trout and one laboratory has conducted a further study using carp. Five test substances were used in each study. An additional report containing a comparative analysis of the trout and carp results has been prepared. This analysis includes an intra-laboratory comparison (key testing results for carp and trout from the same laboratory) and an inter-laboratory comparison (key testing results based on average values for trout from the other laboratories); it draws upon previous studies that examine species-specific differences in bioaccumulation in fish, including plausible explanations for any observed differences.

The draft validation report (part I and II) was approved at the 24th meeting of the Working Group of National Coordinators of the Test Guidelines Programme, in April 2012. The Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology agreed to its declassification on 5 July, 2012.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

PART I

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

Contents

INTRODUCTION

1. The OECD TG 305 (1) currently outlines a method for determining a chemical's bioconcentration factor (BCF) in fish. In the test fish are exposed to the chemical dissolved in water and the BCF is calculated as the ratio of the concentration in the fish to the dissolved concentration in water at "steady-state", or by the ratio of the uptake and depuration rate constants. However problems can arise with this test method for poorly soluble substances owing to the inability to prepare stable and measurable concentrations in the exposure medium.

2. In May 2001 a new method for the bioaccumulation testing of poorly water soluble chemicals was presented at the SETAC Europe conference held in Madrid (2). This work built on various reported bioaccumulation studies in the literature using a dosing method involving spiked feed (see for example (3)). A draft protocol for the new method (4) along with a supporting background document (5) was submitted to the EU PBT working group in 2004. Further justification given for the new method was that potential environmental exposure to such poorly soluble substances (typically with log Kow >5) may be largely via the diet (see (6) (7) (8) (9) (10)).

3. OECD Test Guidelines for Testing of Chemicals are periodically reviewed and revised in the light of scientific progress. This is of particular interest when scientific progress enables the refinement of an existing test guideline, e.g., the collection of additional hazard information. The OECD 305 TG is being revised[1] in order that fewer animals are required in the test as a result of experience gained over the years, and so that a new method for poorly water soluble substances can be added.

4. The new method involves exposing test animals to food spiked with the test substance and results in a dietary biomagnification factor[2] or BMF (the ratio of the substance in the animal's body to the concentration in food) rather than a bioconcentration factor. Validation testing needs to be conducted to demonstrate reproducibility of results and provide information on inter-laboratory variation for this new method. Without this validation testing, the method cannot be accepted by the OECD or member countries of the OECD.

5. The Environment Agency of England and Wales (on behalf of Defra) in association with colleagues in the Netherlands (RIVM) and Germany (UBA) has coordinated a ring test of the new method. A total of ten laboratories volunteered to conduct studies for this ring test. Ten studies have been conducted using rainbow trout (*Oncorhynchus mykiss*) and one laboratory has conducted a further study using carp (*Cyprinus carpio*). Five test substances were used in each study. This report considers the results from eight of the ten laboratories. The full results from the remaining two laboratories are not yet available and will be reported separately, either as an addendum to this report or as a standalone report.

ORGANISATION OF THE VALIDATION

Participants in the validation exercise

6. The validation exercise (ring test) was conducted with ten laboratories (Table 1). Full results are currently available for eight of the laboratories.

[1] The revision is being led by a partnership involving the Netherlands, Germany and the United Kingdom.

[2] Biomagnification has traditionally been used to describe the increase in a chemical's concentration in the body of a predator in the wild as a result of consuming prey contaminated with the chemical and other exposure routes. In this laboratory method the fish are consuming commercial fish food and are not exposed to the substance from other routes, so the process has been described as food accumulation.

Table 1 Participating laboratories in the ring test

Laboratory	Country
Astra Zeneca	UK
BASF	Germany
CERI	Japan
Dow Corning	USA
DuPont	USA
EMBSI	USA
Environment Canada	Canada
Fraunhofer-Institute	Germany
L'Oreal/Harlan Laboratories	France/Switzerland
NIVA	Norway

Pre-study phase

7. The pre-study was designed to investigate the analytical recovery of the substances used in the test by each laboratory. The pre-study considered both the recovery from spiked food and recovery from spiked fish. The chemicals included in the pre-study analytical work are given in Table 2.

Table 2 Substances included in the pre-study analytical work

Substance	CAS No.
Hexachlorobenzene	118-74-1
Musk xylene (1,4,6-trinitro-5-tert-butyl-1,3-xylene)	81-15-2
o-Terphenyl	84-15-1
Methoxychlor	72-43-5
Benzo(*a*)pyrene	50-32-8

Feed

8. For the pre-study analytical recovery work samples of food were spiked by EMBSI with around 100 µg/g each of the five test substances and subsamples of the spiked food were distributed to each participating laboratory for extraction and analysis.

Fish

9. EMBSI provided samples of fish that had been exposed to a diet containing the five test substances (100 ppm of each test compound for two weeks) to each participating laboratory for extraction and analysis. The fish were provided as individuals from an exposed population.

Study phase

10. For the main study each laboratory was provided with the test protocol by EMBSI (11).

TEST PERFORMANCE AND CONDITIONS

Test substances

11. The test was carried out using five test chemicals: hexachlorobenzene (CAS 118-74-1), musk xylene (2,4,6-trinitro-5-tert-butyl-1,3-xylene; CAS 81-15-2), *o*-terphenyl (CAS 84-15-1), methoxychlor (CAS 72-43-5) and benzo(*a*)pyrene (CAS 50-32-8).

12. The test substances were obtained by each laboratory from their own suppliers. The purity of the test materials were reported by each laboratory (Table 3).

Table 3 Purities of the test substances used

Laboratory	Substance purity (%)				
	Hexachloro benzene	Musk xylene	*o*-Terphenyl	Methoxychlor	Benzo(*a*) pyrene
Lab 1	99.9	99	99	99.9	≥96
Lab 2	99.7	>99.0	>99	>95.0	99.7
Lab 3	99	99.5	99	"analytical standard"	≥96
Lab 4	99.5	99.5	99.5	99.0	98.4
Lab 5	no data	no data	no data	no data	no data
Lab 6	no data	no data	no data	no data	no data
Lab 7	99.5	99.9	99.0	99.5	95.0
Lab 8	99	98	99	>95	96.0
Lab 9	Data not yet available				
Lab 10	Data not yet available				

Food

13. The test was carried out using proprietary fish food. Each laboratory sourced the food from their normal suppliers.

14. The ideal lipid content of the food should be around 15-20% w/w, according to the test protocol.

Test system

Fish species

15. The fish used in the test were juvenile rainbow trout (*Oncorhynchus mykiss*). Each laboratory sourced the fish from their normal suppliers. In addition to rainbow trout, one laboratory carried out tests using carp (*Cyprinus carpio*).

16. A total of 118 individuals was recommended for the test (10 for initial weight assessment on arrival, 90 for study, 8 for spike recovery analysis and 10 for analytical method development). The stock population was acclimated in dilution water for at least one week prior to the test and were fed throughout on a sufficient diet of the same type to be used in the test.

17. According to the protocol, organisms for the test were randomly selected from the stock population and should be within a weight range of 1 to 8 g at the start of the test. Fish of similar weight were to be selected such that the smallest was no smaller than two-thirds of the weight of the largest. The initial weights (day 0) of the fish used in the test by each laboratory are summarised in Table 4.

Table 4 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 1	Rainbow trout	10	1.25	0.18	0.90 to 1.47	0.61
Lab 2a	Rainbow trout	5	8.41	0.59	7.71 to 9.20	0.84
Lab 2b	Carp	5	5.42	0.20	5.13 to 5.67	0.90
Lab 3	Rainbow trout	10	1.95	0.22	1.68 to 2.25	0.75
Lab 4	Rainbow trout	5	1.17	0.10	1.07 to 1.26	0.85
Lab 5	Rainbow trout	5	6.77	2.12	3.24 to 8.94	0.36
Lab 6	Rainbow trout	5	0.72	0.12	0.56 to 0.86	0.65
Lab 7	Rainbow trout	5	1.20	0.20	0.92 to 1.46	0.63
Lab 8	Rainbow trout	10	1.24	0.16	1.07 to 1.61	0.66
Lab 9	Rainbow trout	Data not yet available				
Lab 10	Rainbow trout	Data not yet available				

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).

18. As can be seen from the table, the mean weight of the fish used in the study were outside of the range recommended in the test protocol for two laboratories (Lab 2a – > 8 g for rainbow trout; Lab

6, <1 g). The range of fish weights was outside that recommended in the test protocol for Lab 5 (smallest fish was just over a third of the weight of the largest fish) and was close to, but just outside the recommended range for a further three laboratories (Lab 1, Lab 6 and Lab 7).

Spiking and analysis of food

19. The food was spiked with the appropriate amounts of all five test substances to provide the nominal concentrations outlined in Table 5. The total dietary concentration was designed to minimize or mitigate possible cumulative toxic effects on the fish whilst at the same time providing sufficient analytical sensitivity. One laboratory carried out experiments using carp at three different concentrations (one exposure at the levels outlined in Table 5, one exposure at five times these concentrations (omitting benzo(*a*)pyrene) and one exposure at ten times these concentrations (omitting benzo(*a*)pyrene)).

Table 5 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

20. Two different methods of spiking the food were used. One involved adding the test substances as a solution/suspension in corn or fish oil (approximately 0.5% corn oil in the feed). The second method involved adding the test substances to the feed as a solution in a solvent followed by evaporation of the solvent. The method used by each laboratory is summarised in Table 6.

Table 6 Methods used for spiking of feed

Laboratory	Method	
	Solvent	Corn oil or fish oil
Lab 1		x
Lab 2		x
Lab 3	x	
Lab 4	x	
Lab 5	x	
Lab 6	x	
Lab 7	x	
Lab 8	x	
Lab 9	Data not yet available	
Lab 10	x	

21. A control diet was prepared in exactly the same way as the spiked diet but without the addition of the test substances to the solvent or corn/fish oil.

22. Samples of the test and control diets were extracted and analysed for the test substances prior to initiation of the study and at the end of the uptake period (three samples on each occasion).

Exposure duration

23. The test was carried out using a 13 day uptake phase[3] followed by a 28 day depuration period.

24. During the uptake phase, the exposed fish were fed the spiked diet at a rate of 3% of the body weight (eight of the laboratories). Two laboratories carried out their experiments using a feeding rate of 1.5% of the body weight (not discussed here; full results of these studies are not yet available). Control fish were fed in the same manner but using feed containing no test substance.

25. During the depuration phase the fish were fed a clean diet at 3% of the body weight (or 1.5% of the body weight) as appropriate.

Fish sampling and analysis

26. Fish were sampled on day 3 of the uptake phase (optional sample) and days 1, 3, 7, 14, 21 and 28 of depuration. At each sampling period five fish from each of the control and treatment groups were sacrificed and analysed for the concentration of test substance. Optional analysis of the gut contents was also carried out in some cases.

27. In order to ensure that the gastro-intestinal tract was cleared of test or control diet, the fish sampled on uptake day 3 were treated as follows. Shortly after being fed their respective diets, five test and control fish were transferred to separate smaller tanks containing clean water. Approximately 5 hours after being fed their day 3,ration they were fed clean feed and were sacrificed for analysis the following morning.

28. The fish for depuration day one were similarly fed clean food approximately 5 hours after being fed their day 13 diet and were sacrificed the following morning (these were designated the day 1 depuration samples). During the rest of the depuration phase fish were sampled just prior to the daily feeding of clean diet.

29. Observations for mortality and any adverse effects on feeding behaviour were performed and recorded daily.

Fish lipid analysis

30. The lipid content of the fish was measured individually on five fish from the stock population at the start of the uptake, five fish from each of the treatment tanks at the end of the uptake phase and five fish from each of the treatment tanks at the end of the depuration phase.

31. Three different methods were used amongst the participating laboratories to determine the lipid contents. These were solvent extract using chloroform/methanol (12), the ASE method (accelerated solvent extraction (13) using hexane) or extraction with cyclohexane/isopropanol (Smedes method (14)). The method used by each laboratory is summarised in Table 7.

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

Table 7 Methods used for lipid measurements

Laboratory	Method		
	Chloroform/ methanol	**ASE**	**Cyclohexane/isopropanol (Smedes method)**
Lab 1		x	
Lab 2	x		
Lab 3		x	
Lab 4			x
Lab 5		x	
Lab 6		x	
Lab 7	x		
Lab 8		x	
Lab 9		Data not yet available	
Lab 10		Data not yet available	

Conditions for validity

32. The test protocol used indicated that the test is acceptable if:

a. The temperature variation is less than ±2°C in treatment or control groups.

b. The concentrations of dissolved oxygen does not fall below 60% of the air saturation value.

c. The concentration of the test substance in fish food is kept constant over the feeding period with a range of ±20%.

d. Concentrations of the test chemicals are not detected, or are present only at trace levels, in un-spiked food or control fish tissues relative to treated samples.

e. A high degree of homogeneity of substance in food is demonstrated in preliminary analytical work on the spiked diet; concentration for the same substance between the three samples must not vary by more than ±15%.

f. Mortality or other adverse effects/disease in both the control and test group should be ≤10% at the end of the test. Average growth in both test and control groups should be similar.

Summary of the various approaches used

33. As discussed above, although the same basic protocol was followed in all cases, the ring test was designed to investigate the effects of differences in the method of spiking of the test substance onto food, method of determination of the lipid content, differences in the feeding rate and, to a lesser extent, differences between test species on the overall results of the test. A summary of these possible variables by laboratory is given in Table 8.

Table 8 **Summary of similarities and differences between the methods used by the various laboratories**

Laboratory	Species	Mean fish weight (g) at day 0	Spiking method for food	Feeding rate (% body weight)	Method for lipid	Gut contents analysed?
Lab 1	Rainbow trout	1.25	Corn/fish oil	3	ASE	No
Lab 2a	Rainbow trout	8.41	Corn/fish oil	3	Chloroform/ methanol	No
Lab 2b	Carp	5.42	Corn/fish oil	3	Chloroform/ methanol	No
Lab 3	Rainbow trout	1.95	Solvent	3	ASE	No
Lab 4	Rainbow trout	1.17	Solvent	3	Cyclohexane/ isopropanol (Smedes method)	Yes
Lab 5	Rainbow trout	6.77	Solvent	3	ASE	No
Lab 6	Rainbow trout	0.72	Solvent	3	ASE	No
Lab 7	Rainbow trout	1.20	Solvent	3	Chloroform/ methanol	No
Lab 8	Rainbow trout	1.24	Solvent	3	ASE	Yes
Lab 9	Rainbow trout	[1]	[1]	1.5	[1]	[1]
Lab 10	Rainbow trout	[1]	Solvent	1.5	[1]	[1]

[1] Data not yet available.

RESULTS OF THE PRE-STUDY PHASE

34. The raw data were submitted mainly via Excel spreadsheets or short reports. In some cases a more detailed study report was also provided. The key raw data from the studies have been summarised by laboratory in the Appendix to this report.

Pre-study phase - feed analysis

35. Each participating laboratory was provided with subsamples of food that had been spiked with each test substance at a nominal concentration of 100 µg/g. The spiked food samples were prepared by EMBSI. The samples were extracted and analysed by each laboratory. The results of this analysis are summarised in Table 9.

36. The repeat analyses carried out by each laboratory on the spiked food samples provided by EMBSI all fell within the 15% variation required by the draft guideline. The range of variability for each substance, expressed as the relative standard deviation, was as follows:

Hexachlorobenzene	1.01 – 4.34%
Musk xylene	0.51 – 4.06%
o-Terphenyl	0.58 – 4.40%
Methoxychlor	2.33 – 8.70%
Benzo(*a*)pyrene	0.81 – 5.88%

37. For each substance an analysis of variance was conducted on the mean concentrations from the laboratories. These analyses found that the mean concentrations were not the same, in all cases at $p<<0.001$. The Neuman-Keuls multiple range test was used to identify which means could be grouped together, using a significance level of 0.05. In some cases the tests did not provide unequivocal results so the results presented here should be considered as indicative. The means are grouped by laboratory as below, running from low to high concentrations.

Hexachlorobenzene	6; 1,3,4,8,7,5; 9,2
Musk xylene	7; 1; 6; 4,2,3,8,9; 5
o-Terphenyl	7,6,1; 8; 4,9; 3,5,2
Methoxychlor	1; 4,7,2,6,5,3,8; 9
Benzo(*a*)pyrene	7;6,8,1; 2,3,4,5; 9

38. The spiked concentration of each substance was nominally 100 mg/kg. For each substance, the t-test has been used to compare the mean concentrations determined by each laboratory with this nominal value. The results significantly different (at 0.05) from this value were as follows:

Hexachlorobenzene	three results (labs 2, 6 and 9)
Musk xylene	six results (labs 1, 5, 6, 7, 8, 9)
o-Terphenyl	five results (labs 1, 5, 6, 7, 8)
Methoxychlor	three results (labs 1, 8, 9)
Benzo(*a*)pyrene	seven results (labs 3, 4, 5, 6, 7, 8, 9)

39. The five substances were not detectable by any laboratory in two/three samples of control food.

40. In addition to the spiked food supplied by EMBSI, several laboratories carried out recovery experiments on food samples that had been spiked within their laboratory. The results of this analysis are summarised in Table 10. These samples show a high degree of homogeneity with the relative standard deviation of <10%.

Table 9 **Summary of pre-study phase – analysis of food samples spiked by EMBSI**

Laboratory	Parameter	Hexachloro benzene	Musk xylene	*o*-Terphenyl	Methoxychlor	Benzo(*a*)pyrene
Lab 1	Mean concentration[a] (µg/g)	96.7	91.5	88.8	84.6	98.6
	Standard deviation	3.2	3.4	3.0	3.3	5.8
	Relative standard deviation (%)	3.3	3.7	3.4	3.9	5.9
	No of samples analysed	5	5	5	5	5
Lab 2	Mean concentration[a] (µg/g)	105.0	100.4	104.6	101.2	102.4
	Standard deviation	3.2	1.4	3.4	6.9	2.6
	Relative standard deviation (%)	3.0	1.4	3.2	6.8	2.5
	No of samples analysed	5	5	5	5	5
Lab 3	Mean concentration[a] (µg/g)	98.8	101.0	101.3	103.8	102.8
	Standard deviation	4.2	3.1	1.8	3.5	1.9
	Relative standard deviation (%)	4.3	3.1	1.7	3.4	1.8
	No of samples analysed	5	5	5	5	5
Lab 4	Mean concentration[a] (µg/g)	99.3	99.5	98.8	98.3	103.5
	Standard deviation	2.2	2.6	2.2	6.2	2.1
	Relative standard deviation (%)	2.2	2.7	2.2	6.3	2.0
	No of samples analysed	4	4	4	4	4
Lab 5	Mean concentration[a] (µg/g)	100.3	107.7	104.3	103.4	103.7
	Standard deviation	4.4	4.4	0.6	3.8	2.0
	Relative standard deviation (%)	4.3	4.1	0.6	3.7	1.9
	No of samples analysed	5	5	5	5	5

Laboratory	Parameter	Hexachloro benzene	Musk xylene	*o*-Terphenyl	Methoxychlor	Benzo(*a*)pyrene
Lab 6	Mean concentration[a] (µg/g)	89.5	95.1	87.6	101.3	94.3
	Standard deviation	2.1	2.1	1.2	3.0	2.2
	Relative standard deviation (%)	2.3	2.2	1.4	2.9	2.3
	No of samples analysed	5	5	5	5	5
Lab 7	Mean concentration[a] (µg/g)	100.3	81.4	86.3	99.3	86.3
	Standard deviation	2.2	2.6	3.8	8.6	1.7
	Relative standard deviation (%)	2.2	3.2	4.4	8.7	2.0
	No of samples analysed	5	5	5	5	5
Lab 8	Mean concentration[a] (µg/g)	99.5	104.0	94.6	105.0	97.3
	Standard deviation	1.0	2.2	1.9	2.4	1.1
	Relative standard deviation (%)	1.0	2.2	2.0	2.3	1.1
	No of samples analysed	5	5	5	5	5
Lab 9	Mean concentration[a] (µg/g)	103.4	104.6	99.7	115.6	116.7
	Standard deviation	1.4	0.5	0.6	3.4	1.0
	Relative standard deviation (%)	1.4	0.5	0.6	2.9	0.8
	No of samples analysed	7	7	7	7	7
Lab 10	Mean concentration[a] (µg/g)			Data not yet available		
	Standard deviation					
	Relative standard deviation (%)					
	No of samples analysed					

Note: a) As the spiked concentration was nominally 100 µg/g for all test substances the results also represent the nominal percentage recovery.

Table 10 **Summary of pre-study phase – analysis of food samples spiked by individual laboratories**

Laboratory	Parameter	Hexachloro benzene	Musk xylene	*o*-Terphenyl	Methoxychlor	Benzo(*a*)pyrene
Lab 2 (spiked using solvent)	Mean recovery[a] (%)	94.2	94.2	90.3	116.0	97.3
	Standard deviation	0.7	2.1	1.2	5.7	0.7
	Relative standard deviation (%)	0.8	2.3	1.3	4.9	0.7
	No of samples analysed	2	2	2	2	2
Lab 2 (spiked using corn oil)	Mean recovery[a] (%)	73.7	83.6	83.6	102.7	85.6
	Standard deviation	2.8	6.8	6.8	4.7	6.4
	Relative standard deviation (%)	3.8	8.1	8.1	4.6	7.4
	No of samples analysed	5	5	5	5	5
Lab 2 (spiked using the combined method)	Mean recovery[a] (%)	103.2	98.6	101.1	125.6	100.1
	Standard deviation	3.6	3.9	3.6	3.4	5.4
	Relative standard deviation (%)	3.5	3.9	3.6	2.7	5.4
	No of samples analysed	5	5	5	5	5
Lab 3 (spiked using solvent – batch I)	Mean recovery[a] (%)	91.3	85.7	82.6	88.8	83.0
	Standard deviation	2.8	1.2	1.7	1.0	1.9
	Relative standard deviation (%)	3.1	1.4	2.1	1.1	2.2
	No of samples analysed	3	3	3	3	3
Lab 3 (spiked using solvent – batch II)	Mean recovery[a] (%)	103.8	98.7	95.0	102.7	97.6
	Standard deviation	1.6	1.3	0.5	1.1	1.6
	Relative standard deviation (%)	1.6	1.3	0.5	1.0	1.6
	No of samples analysed	3	3	3	3	3

Laboratory	Parameter	Hexachloro benzene	Musk xylene	*o*-Terphenyl	Methoxychlor	Benzo(*a*)pyrene
Lab 3 (spiked using corn oil – batch I)	Mean recovery[a] (%)	113.0	127.3	123.4	141.4	126.6
	Standard deviation	2.3	5.0	4.0	1.6	2.4
	Relative standard deviation (%)	2.1	3.9	3.2	1.1	1.9
	No of samples analysed	3	3	3	3	3
Lab 3 (spiked using corn oil – batch II)	Mean recovery[a] (%)	118.9	131.4	124.8	145.6	127.2
	Standard deviation	5.2	7.2	5.5	7.3	4.2
	Relative standard deviation (%)	4.4	5.5	4.4	5.0	3.3
	No of samples analysed	3	3	3	3	3
Lab 4	Mean recovery[a] (%)	98.2	102.4	99.3	86.2	86.8
	Standard deviation	4.0	4.1	3.9	2.7	3.7
	Relative standard deviation (%)	4.1	4.0	3.9	3.2	4.2
	No of samples analysed	3	3	3	3	3
Lab 8	Mean recovery[a] (%)	96.5	109.1	97.5	113.6	97.1
	Standard deviation	2.4	1.7	2.5	2.8	2.1
	Relative standard deviation (%)	2.5	1.6	2.6	2.5	2.2
	No of samples analysed	3	3	3	3	3

Note: a) As the spiked concentration was nominally 100 µg/g for all test substances the results also represent the nominal percentage recovery

Pre-study phase - fish analysis

41. Each participating laboratory was provided with individual fish samples from a population that had been fed a diet of food spiked with each test substance. The fish samples were provided by EMBSI. The samples were extracted and analysed by each laboratory. The results of this analysis are summarised in Table 11.

42. For each substance an analysis of variance was conducted on the mean concentrations from the laboratories. Where the analysis found that the mean concentrations were not the same, the Neuman-Keuls multiple range test was used to identify which laboratory means could be grouped together, using a significance level of 0.05. In some cases the tests did not provide unequivocal results so the results presented here should be considered as indicative. The results are presented below. Where groups of means are presented, they run from low to high concentrations.

Hexachlorobenzene	means not all the same ($p<<0.001$)
	Groups: 5; 7,8,1,4,3,2
Musk xylene	means not all the same ($p<<0.001$)
	Groups: 5,1,8,7,2,4; 3
o-Terphenyl	means not all the same ($p=0.17$)
	Groups: no unequivocal groups could be established
Methoxychlor	means not significantly different ($p=0.5$)
Benzopyrene	means not significantly different ($p=0.23$)

43. The five substances were not detectable in samples of control fish that had been fed on clean diet except for the following: Lab 7 detected musk xylene at 0.033 µg/g in one out of three samples; Lab 8 detected methoxychlor at 0.38 µg/g in one out of two samples; Lab 3 detected hexachlorobenzene at 0.18 µg/g in one out of two samples (traces of o-terphenyl and benzo[a]pyrene were detectable in some samples but the concentration was below the limit of quantification).

Pre-study phase – fish lipid analysis

44. Each participating laboratory was provided with individual fish samples form a population that had been fed a diet of food spiked with each test substance. The fish samples were provided by EMBSI. The lipid content of the fish were determined by each laboratory. The results of this analysis are summarised in Table 12.

Pre-study phase – spiked fish recovery analysis

45. As part of the pre-study phase, some of the laboratories carried out recovery tests from spiked fish. The available results are summarised in Table 13.

Table 11 **Summary of pre-study phase – analysis of fish provided by EMBSI[a]**

Laboratory	Parameter	Hexachloro benzene	Musk xylene	*o*-Terphenyl	Methoxychlor	Benzo(*a*)pyrene
Lab 1	Mean concentration (µg/g)	10.53	5.06	4.48	1.77	0.101
	Standard deviation	4.98	3.33	2.12	2.28	0.084
	Relative standard deviation (%)	4.74	65.8	47.4	129.2	83.0
	No of samples analysed	8	8	8	8	8
Lab 2	Mean concentration (µg/g)	16.76	7.71	7.28	1.79	0.233
	Standard deviation	3.40	2.81	2.26	1.14	0.305
	Relative standard deviation (%)	20.3	36.5	31.1	63.7	131.3
	No of samples analysed	8	8	8	8	8
Lab 3	Mean concentration (µg/g)	14.55	11.85	6.65	2.84	0.299
	Standard deviation	3.33	3.50	2.20	2.16	0.439
	Relative standard deviation (%)	22.9	29.5	33.1	76.1	147.0
	No of samples analysed	8	8	8	8	8
Lab 4	Mean concentration (µg/g)	14.10	7.94	6.81	2.30	0.276
	Standard deviation	3.73	2.61	3.30	1.86	0.235
	Relative standard deviation (%)	26.5	32.8	48.4	80.8	85.2
	No of samples analysed	8	8	8	8	8
Lab 5	Mean concentration (µg/g)	5.64	4.05	3.87	1.85	0.091
	Standard deviation	1.26	1.09	0.46	0.85	0.044
	Relative standard deviation (%)	22.3	26.9	11.8	46.1	48.6
	No of samples analysed	8	8	8	8	8

Laboratory	Parameter	Hexachloro benzene	Musk xylene	*o*-Terphenyl	Methoxychlor	Benzo(*a*)pyrene
Lab 6	Mean concentration (µg/g)	9.40	6.11	5.72	2.05	0.089
	Standard deviation	2.66	2.55	2.32	1.36	0.106
	Relative standard deviation (%)	28.3	41.8	40.6	66.5	119
	No of samples analysed	8	8	8	8	8
Lab 7	Mean concentration (µg/g)	9.58	7.49	4.99	2.95	0.266
	Standard deviation	1.67	1.68	1.05	1.59	0.189
	Relative standard deviation (%)	17.4	22.4	21.1	53.9	70.9
	No of samples analysed	8	8	8	8	8[b]
Lab 8	Mean concentration (µg/g)	9.81	5.11	5.07	1.50	0.063
	Standard deviation	3.37	2.81	2.54	1.11	0.027
	Relative standard deviation (%)	34.4	54.9	50.0	73.9	43.0
	No of samples analysed	7	7	7	7	7[b]
Lab 9	Mean concentration (µg/g)			Data not yet available		
	Standard deviation					
	Relative standard deviation (%)					
	No of samples analysed					
Lab 10	Mean concentration (µg/g)			Data not yet available		
	Standard deviation					
	Relative standard deviation (%)					
	No of samples analysed					

Note: a) fish were fed with a spiked diet consisting of all test substances at nominal concentrations of 100 ppm for two weeks.

b) Not detectable in one sample. Not detectable results were not included in the calculation of the mean concentration.

Table 12 Summary of pre-study phase – analysis of fish lipid content

Laboratory	Parameter	Result
Lab 1	Mean lipid content (%)	3.00
	Standard deviation	0.83
	Relative standard deviation (%)	27.7
	No of samples analysed	5 control fish
Lab 2	Mean lipid content (%)	3.79
	Standard deviation	0.66
	Relative standard deviation (%)	17.3
	No of samples analysed	5 control fish
Lab 3 – ASE/hexane method	Mean lipid content (%)	3.04
	Standard deviation	0.44
	Relative standard deviation (%)	14.4
	No of samples analysed	5 control fish
Lab 3 – Smedes method	Mean lipid content (%)	3.63
	Standard deviation	0.21
	Relative standard deviation (%)	5.7
	No of samples analysed	4 control fish
Lab 4	Mean lipid content (%)	Not conducted
	Standard deviation	
	Relative standard deviation (%)	
	No of samples analysed	
Lab 5	Mean lipid content (%)	2.94
	Standard deviation	0.69
	Relative standard deviation (%)	23.3
	No of samples analysed	8 control fish
Lab 6	Mean lipid content (%)	4.10
	Standard deviation	0.53
	Relative standard deviation (%)	13.0
	No of samples analysed	5 fish
Lab 7	Mean lipid content (%)	10.60 (10.67 control only)
	Standard deviation	1.99 (3.59 control only)
	Relative standard deviation (%)	18.8 (33.7 control only)
	No of samples analysed	11 (three control fish and 8 exposed fish)

Laboratory	Parameter	Result
Lab 8	Mean lipid content (%)	4.41
	Standard deviation	0.67
	Relative standard deviation (%)	15.2
	No of samples analysed	5 control fish
Lab 9	Mean lipid content (%)	Data not yet available
	Standard deviation	
	Relative standard deviation (%)	
	No of samples analysed	
Lab 10	Mean lipid content (%)	Data not yet available
	Standard deviation	
	Relative standard deviation (%)	
	No of samples analysed	

Table 13 **Summary of pre-study phase – spiked fish recovery samples**

Laboratory	Parameter	Hexachloro benzene	Musk xylene	*o*-Terphenyl	Methoxychlor	Benzo(*a*)pyrene
Lab 1	Mean recovery (%)	79.4	102.9	89.3	103.8	89.1
	Standard deviation	2.6	10.5	2.4	1.2	8.1
	Relative standard deviation (%)	3.3	10.2	2.6	1.2	9.1
	No of samples analysed	3	3	3	3	3
Lab 2	Mean recovery (%)	70.1	83.5	83.0	78.9	74.7
	Standard deviation	2.1	2.9	2.6	1.1	0.3
	Relative standard deviation (%)	2.9	3.5	3.2	1.4	0.4
	No of samples analysed	2	2	2	2	2
Lab 3	Mean recovery (%)	88.9	106.5	101.3	105.7	96.6
	Standard deviation	1.0	3.4	1.0	9.2	1.5
	Relative standard deviation (%)	1.1	3.2	1.0	8.7	1.6
	No of samples analysed	5	5	5	5	5
Lab 4	Mean recovery (%)	89.7	84.9	90.2	99.7	103.6
	Standard deviation	3.1	14.0	3.0	13.4	4.9
	Relative standard deviation (%)	3.5	16.5	3.4	13.5	4.7
	No of samples analysed	4	4	4	4	4
Lab 5	Mean recovery (%)			Not conducted		
	Standard deviation					
	Relative standard deviation (%)					
	No of samples analysed					

Laboratory	Parameter	Hexachloro benzene	Musk xylene	*o*-Terphenyl	Methoxychlor	Benzo(*a*)pyrene
Lab 6	Mean recovery (%)			Not conducted		
	Standard deviation					
	Relative standard deviation (%)					
	No of samples analysed					
Lab 7	Mean recovery (%)			Not conducted		
	Standard deviation					
	Relative standard deviation (%)					
	No of samples analysed					
Lab 8	Mean recovery (%)	67.1	81.1	78.2	88.1	64.2
	Standard deviation	6.6	1.2	2.5	0.6	2.1
	Relative standard deviation (%)	9.8	1.5	3.2	0.7	3.3
	No of samples analysed	3	3	3	3	3
Lab 9	Mean recovery (%)			Data not yet available		
	Standard deviation					
	Relative standard deviation (%)					
	No of samples analysed					
Lab 10	Mean recovery (%)			Data not yet available		
	Standard deviation					
	Relative standard deviation (%)					
	No of samples analysed					

RESULTS OF THE OECD 305 RING TEST

46. The raw data were submitted mainly via Excel spreadsheets or short reports. In some cases a more detailed study report was also provided. The key raw data from the studies have been summarised by laboratory in the Appendix to this report. Data are currently available for Lab 1 to Lab 8. Data for Lab 9 and 10 will be reported separately.

Food recovery prior to start and at end of the study

47. 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 14. In some cases the data were presented as a percentage recovery. In these cases the concentration has been "back calculated" from the target (spiked) concentrations reported using these percentages. Similar samples were required at the end of the uptake period.

48. 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.

49. These samples from the start of the study show that most laboratories achieved homogeneous mixtures of the substances within the food. However, some of the samples from Lab 5 from the end of the uptake phase show that this may not have been the case for this laboratory as high relative standard deviations (>15%) were obtained for hexachlorobenzene and musk xylene. In addition, the concentration of musk xylene measured at the end of the uptake phase was only around 4% of the nominal concentration. Unfortunately only results from the end of the uptake phase were available for this laboratory and so the variability in the concentrations present in food at the start of the test is not known. It is understood that the laboratory only had a limited amount of musk xylene available for the study and so had to test at a lower concentration than the target concentration.

50. Overall the results show that both methods of administration of the test substance (solvent or corn oil/fish oil) to the food can result in homogeneous samples of spiked food.

51. The results from these analyses at the start of the study have been compared between the laboratories. The data used here are those included in the "Feed and observations" tab of the spreadsheets submitted by the laboratories, identified as before uptake, supplemented with data from the pre-test recovery analyses where no before uptake data were included. In the absence of definitive information, this assumes that the pre-test recovery analyses in these cases were carried out on food as used in the test. This may need to be checked. Data for seven laboratories are included.

52. For each substance an analysis of variance was conducted on the mean concentrations from the laboratories. These analyses found that the mean concentrations were not the same, in all cases at $p<<0.001$. The Neuman-Keuls multiple range test was used to identify which means could be grouped together, using a significance level of 0.05. In some cases the tests did not provide unequivocal results so the results presented here should be considered as indicative. The means are grouped by laboratory as below, running from low to high concentrations.

Hexachlorobenzene	7; 6; 8,4,2,1; 3
Musk xylene	7; 2,8; 1; 6,3,4
o-Terphenyl	7; 2; 6; 4,8; 3; 1
Methoxychlor	2; 8,6,4; 1; 3,7

Benzopyrene	4; 7,1,8,2; 6,3

53. For each substance, the t-test has been used to compare the mean concentrations determined by each laboratory with the nominal target value. The results significantly different (at 0.05) from this value were as follows:

Hexachlorobenzene	25 mg/kg; five results (labs 3, 4, 6, 7 and 8)
Musk xylene	50 mg/kg; five results (labs 2, 4, 6, 7 and 8)
o-Terphenyl	50 mg/kg; five results (labs 1, 2, 4, 6 and 7)
Methoxychlor	100 mg/kg; five results (labs 1, 2, 3, 4 and 6)
Benzopyrene	150 mg/kg; three results (labs 4, 7 and 8)

54. The results from analyses of food at the end of the uptake period have been compared between the laboratories. The data used here are those included in the "Feed and observations" tab of the spreadsheets submitted by the laboratories, identified as end of uptake. Data for seven laboratories are included.

55. For each substance an analysis of variance was conducted on the mean concentrations from the laboratories. These analyses found that the mean concentrations were not the same, in all cases at $p<<0.001$. The Neuman-Keuls multiple range test was used to identify which means could be grouped together, using a significance level of 0.05. In some cases the tests did not provide unequivocal results so the results presented here should be considered as indicative. The means are grouped by laboratory as below, running from low to high concentrations.

Hexachlorobenzene	6,8,2,4,7,1; 5
Musk xylene	5; 2,8,7; 6,1,4
o-Terphenyl	2,6,8,7,4; 1; 5
Methoxychlor	5; 2,6,8,1,4; 7
Benzopyrene	4,7,8,2,6; 5,1

56. For each substance, the t-test has been used to compare the mean concentrations determined by each laboratory with the nominal target value. The results significantly different (at a significance level of 0.05) from this value were as follows:

Hexachlorobenzene	25 mg/kg; three results (labs 2, 4 and 6)
Musk xylene	50 mg/kg; six results (labs 1, 2, 4, 5, 6 and 7)
o-Terphenyl	50 mg/kg; three results (labs 2, 6 and 8)
Methoxychlor	100 mg/kg; three results (labs 2, 5 and 7)
Benzopyrene	150 mg/kg; five results (labs 1, 2, 4, 7 and 8)

57. The available results show that the concentrations in food were generally stable over the course of the uptake phase. Exceptions to this were the results for Lab 7 for musk xylene, o-terphenyl and methoxychlor, which appeared to increase by more than 20% between the start and the end of the uptake phase[4]. The mean measured concentrations (based on the samples at the start and end of the uptake phase where available) are summarised in Table 15.

The lipid content of the food used by each laboratory covered a range of 6.4% to 21.1%. The data are summarised in Table 16. 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. In the current ring test, the lipid content of the food used by Lab 1 and Lab 5 was below this recommended range and that used by Lab 7 was slightly above this recommended range.

[4] This is based on the assumption that the chemical recoveries pre-study relate to the same food as the end of uptake measurements, in the absence of definitive information; this may need to be checked.

Table 14 **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 1	Target (spiked) concentration (µg/g)	25.6		50.2		51.5		105.0		149.9	
	Mean measured concentration (µg/g)	26.5[c]	26.0	50.0[c]	55.1	57.7[c]	52.1	113.4[c]	97.3	136.8[c]	167.4
	Standard deviation	0.6	0.7	1.8	1.1	0.4	1.3	3.1	2.6	6.2	3.6
	Relative standard deviation (%)	2.1	2.8	3.5	1.9	0.7	2.6	2.7	2.7	4.5	2.2
	No of samples analysed	3	3	3	3	3	3	3	3	3	3
	Mean recovery (%)	103.7	101.6	99.6	109.7	112.0	101.1	108.0	92.7	91.2	111.7
Lab 2a – rainbow trout	Target (spiked) concentration (µg/g)	25		50		50		100		150	
	Mean measured concentration (µg/g)	24.1	23.3	43.0	43.7	42.9	42.0	83.6	87.1	139.7	136.7
	Standard deviation	0.4	0.5	1.8	1.1	1.4	1.1	2.5	1.2	4.2	2.1
	Relative standard deviation (%)	1.6	2.1	4.1	2.6	3.3	2.7	3.0	1.4	3.0	1.5
	No of samples analysed	3	3	3	3	3	3	3	3	3	3
	Mean recovery (%)	96.5	93.3	86.0	87.4	85.7	84.0	83.6	87.1	93.1	91.1
Lab 2b – carp (level 1)	Target (spiked) concentration (µg/g)	250		500		500		1,000		0	
	Mean measured concentration (µg/g)	211.7	219.0	410.0	388.0	411.0	421.0	777.3	779.7		
	Standard deviation	3.8	12.1	4.4	17.4	6.2	10.5	8.5	22.1		
	Relative standard deviation (%)	1.8	5.5	1.1	4.5	1.5	2.5	1.1	2.8		
	No of samples analysed	3	3	3	3	3	3	3	3		
	Mean recovery (%)	84.7	87.6	82.0	77.6	82.2	84.2	77.7	78.0		

Laboratory	Parameter	Hexachloro benzene		Musk xylene		*o*-Terphenyl		Methoxychlor		Benzo(*a*)pyrene	
		Start	**End**	**Start**	**End**	**Start**	**End**	**Start**	**End**	**Start**	**End**
Lab 2b – carp (level 2)	Target (spiked) concentration (µg/g)	125		250		250		500		0	
	Mean measured concentration (µg/g)	112.7	116.3	233.7	212.0	263.3	226.0	458.7	427.3		
	Standard deviation	6.4	3.1	13.3	8.9	12.7	2.7	25.6	11.0		
	Relative standard deviation (%)	5.6	2.6	5.7	4.2	5.4	1.2	5.6	2.6		
	No of samples analysed	3	3	3	3	3	3	3	3		
	Mean recovery (%)	90.1	93.1	93.5	84.8	94.5	90.4	91.7	85.5		
Lab 2b – carp (level 3)	Target (spiked) concentration (µg/g)	25		50		50		100		150	
	Mean measured concentration (µg/g)	24.7	24.1	47.7	48.6	48.7	47.7	89.2	92.1	151.0	148.7
	Standard deviation	0.6	0.9	1.7	1.4	0.8	1.7	0.8	2.0	2.7	4.7
	Relative standard deviation (%)	2.5	3.8	3.6	3.0	1.6	3.6	0.8	2.2	1.8	3.2
	No of samples analysed	3	3	3	3	3	3	3	3	3	3
	Mean recovery (%)	98.8	96.7	95.4	97.3	96.7	95.4	89.2	92.1	100.7	99.1
Lab 3	Target (spiked) concentration (µg/g)	25		50		50		100		150	
	Mean measured concentration (µg/g)	30.5[a]	nd[a]	57.0[a]	nd[a]	53.9[a]	nd[a]	129.8[a]	nd[a]	161.2[a]	nd[a]
	Standard deviation	0.6		1.8		1.6		0.6		5.1	
	Relative standard deviation (%)	2.1		3.1		2.9		0.4		3.2	
	No of samples analysed	2		2		2		2		2	
	Mean recovery (%)	120.2		113.9		107.8		129.8		107.5	

Laboratory	Parameter	Hexachloro benzene		Musk xylene		*o*-Terphenyl		Methoxychlor		Benzo(*a*)pyrene	
		Start	**End**	**Start**	**End**	**Start**	**End**	**Start**	**End**	**Start**	**End**
Lab 4	Target (spiked) concentration (µg/g)	25.7		50.9		50.9		101		150	
	Mean measured concentration (µg/g)	23.3	23.4	58.7	57.4	47.7	47.7	98.2	99.3	129.0	129.8
	Standard deviation	0.07	0.1	1.3	0.7	0.2	0.04	0.5	1.4	1.0	1.1
	Relative standard deviation (%)	0.3	0.5	2.2	3.1	0.4	0.1	0.5	1.5	0.8	0.9
	No of samples analysed	3	3	3	3	3	3	3	3	3	3
	Mean recovery (%)	90.6	90.9	115.3	112.8	93.7	93.6	97.3	98.3	85.5	86.1
Lab 5	Target (spiked) concentration (µg/g)	25		50		50		100		150	
	Mean measured concentration (µg/g)	nd	37.8	nd	2.0	nd	59.1	nd	70.4	nd	163.6
	Standard deviation		6.7		1.5		6.2		5.3		17.3
	Relative standard deviation (%)		**17.6**		**72.0**		10.5		7.5		10.6
	No of samples analysed		3		3		3		3		
	Mean recovery (%)		151.3		4.1		118.2		70.4		109.1
Lab 6	Target (spiked) concentration (µg/g)	25		50		50		100		150	
	Mean measured concentration (µg/g)	22.3	21.8	55.3	54.0	45.7	45.4	96.9	96.7	154.7	148.0
	Standard deviation	0.7	0.4	1.5	0.3	0.5	0.8	0.2	1.8	4.0	2.0
	Relative standard deviation (%)	2.9	1.9	2.7	0.6	1.1	1.8	0.2	1.9	2.6	1.4
	No of samples analysed	3	3	3	3	3	3	3	3	3	3
	Mean recovery (%)	89.7	87.3	110.7	108.1	91.4	90.7	96.9	96.7	103.1	98.7

Laboratory	Parameter	Hexachloro benzene		Musk xylene		*o*-Terphenyl		Methoxychlor		Benzo(*a*)pyrene	
		Start	**End**	**Start**	**End**	**Start**	**End**	**Start**	**End**	**Start**	**End**
Lab 7	Target (spiked) concentration (µg/g)	25		50		50		100		150	
	Mean measured concentration (µg/g)	20.5	23.6	38.0	47.4	37.6	46.5	130.3	193.7	132.7	132.1
	Standard deviation	0.3	1.2	0.7	0.7	0.5	1.6	12.2	11.6	0.5	5.9
	Relative standard deviation (%)	1.2	4.9	1.7	1.5	1.4	3.5	9.4	6.0	0.4	4.5
	No of samples analysed	3	3	3	3	3	3	3	3	3	3
	Mean recovery (%)	82.0	94.5	76.0	94.9	75.2	93.1	130.3	193.7	88.4	88.1
Lab 8	Target (spiked) concentration (µg/g)	25		50		50		100		150	
	Mean measured concentration (µg/g)	23.2	23.2	45.8	44.2	48.0	46.2	96.8	92.2	139.5	134.0
	Standard deviation	0.4	1.8	0.9	2.7	1.2	1.1	3.1	9.5	3.3	4.1
	Relative standard deviation (%)	1.6	7.7	1.9	6.1	2.5	2.3	3.3	10.3	2.4	3.1
	No of samples analysed	3	3	3	3	3	3	3	3	3	3
	Mean recovery (%)	92.7	92.7	91.5	88.4	95.9	92.3	96.8	92.2	93.0	89.3

Notes: nd = Not determined.

a) These samples were analysed at the start of the uptake phase as the food was prepared some time before the study started. An earlier analysis of the same spiked food (two samples) gave a mean recovery of 123.8% (relative standard deviation 3.9%) for hexachlorobenzene, 111.8% (relative standard deviation 2.7%) for hexachlorobenzene, 108.2% (relative standard deviation 0.5%) for o-terphenyl, 133.0 % (relative standard deviation 3.4%) for methoxychlor and 114.8% (relative standard deviation 8.9%) for benzo[a]pyrene. No analysis of the food was undertaken at the end of the uptake phase but the laboratory considered that the concentrations of the substances were stable over the time period of the study based on these earlier results.

b) The laboratory carried out extra sampling at the start of the uptake phase. Three samples were analysed in duplicate. The mean measured concentrations in these samples were 23.3 µg/g (relative standard deviation 1.8%) for hexachlorobenzene, 58.7 µg/g (relative standard deviation 3.7%) for musk xylene, 47.7 µg/g (relative standard deviation 1.9%) for o-terphenyl, 98.3 µg/g (relative standard deviation 0.8%) for methoxychlor and 129.0 µg/g (relative standard deviation 2.3%) for benzo[a]pyrene.

c) These results are based on the analysis of three samples prior to the start of the test. The laboratory reported the following mean concentrations at the start of the uptake phase: 24.5 µg/g for hexachlorobenzene, 52.2 µg/g for musk xylene, 48.4 µg/g for o-terphenyl, 89.5 µg/g for methoxychlor and 151.7 µg/g for benzo[a]pyrene (only the mean concentrations were reported).

Table 15 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 1	25.2	53.6	50.2	93.4	159.6
Lab 2a – trout	23.7	43.4	42.4	85.3	138.2
Lab 2b – carp (level 1)	215.3	399.0	416.0	778.5	0
Lab 2b – carp (level 2)	114.5	222.8	231.2	443.0	0
Lab 2b – carp (level 3)	24.4	48.2	48.0	90.7	149.8
Lab 3	30.5	56.4	54.0	131.4	166.7
Lab 4	23.3	58.2	47.7	98.6	129.3
Lab 5	37.8	2.0[b]	59.1	70.4	163.6
Lab 6	22.1	54.7	45.5	96.8	151.3
Lab 7	22.1	42.7	42.1	162.0	132.4
Lab 8	23.2	45.0	47.1	94.5	136.8

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 the laboratory carried out extra analysis of the food immediately at the start of the uptake phase, the mean concentration in these samples was used as the mean at the start of the study. For the remaining laboratories the mean concentration in the homogeneity samples, which may or may not have been from the food used in the study, was used as the mean concentration at the start of the study.

b) It is understood that the laboratory had only a limited amount of musk xylene available and so had to test at a lower concentration than the target concentration.

Table 16 Summary of lipid contents of feed used

Laboratory	Lipid content (%)
Lab 1	6.38
Lab 2a – trout	16.4
Lab 2b – carp	16.8
Lab 3	15.3
Lab 4	16.4
Lab 5	11
Lab 6	15
Lab 7	21.1
Lab 8	16.9

Feeding rate

58. The feeding rates used by each laboratory are summarised in Table 17. Where samples were taken on day three of uptake the amount of feed given was generally adjusted to account for the growth of the fish. However not all laboratories sampled the fish on day 3 of uptake.

Table 17 Summary of feeding rates used

Laboratory	Nominal feeding rate (by weight)	Comment
Lab 1	3%	Not adjusted until end of uptake (day 13)
Lab 2a – trout	3%	Not adjusted until end of uptake (day 13)
Lab 2b – carp (level 1)	3%	Not adjusted until end of uptake (day 13)
Lab 2b – carp (level 2)	3%	Not adjusted until end of uptake (day 13)
Lab 2b – carp (level 3)	3%	Not adjusted until end of uptake (day 13)
Lab 3	3%	Adjusted on day 3 and 13 of uptake
Lab 4	3%	Mistakenly fed at 4.5% from day 0 to day 3. Corrected from day 3 onwards and adjusted on day 13
Lab 5	3%	Not adjusted until end of uptake (day 13)?
Lab 6	3%	Adjusted on day 3 and 13 of uptake
Lab 7	3%	Adjusted on day 3 and 13 of uptake
Lab 8	3%	Adjusted on day 3 and 13 of uptake

Experimental conditions

59. The experimental conditions used in the test are summarised in **Table 18**. The temperatures generally fulfilled the criterion that the variation should be less than ±2°C in the treatment or control groups. However, Lab 5 carried out their experiments at 9°C which is outside of the temperature

range recommended for rainbow trout (given as 13-17°C in the standard operating procedure) and the temperature used by Lab 7 (12°C) was slightly below this recommended range.

60. The concentrations of dissolved oxygen remained >60% of the air saturation value in all tests.

Table 18 Summary of experimental conditions

Laboratory	Temperature (°C)		Dissolved oxygen (mg/l)		pH	
	Mean	Range	Mean	Range	Mean	Range
Lab 1	14.0	13.5-14.7	8.5	7.4-9.6	7.3	7.0-7.5
Lab 2a – trout	15.2	15.0-16.1	100%[a]		7.0	6.7-7.4
Lab 2b – carp (level 1)	24.8	24.6-25.0	91.9%[a]	86.3-94.9%[a]	7.6	7.5-7.8
Lab 2b – carp (level 2)	24.7	24.5-25.0	91.5%[a]	87.5-93.7%[a]	7.6	7.4-7.8
Lab 2b – carp (level 3)	24.7	24.5-25.0	92.5%[a]	86.3-94.9%[a]	7.5	7.4-7.6
Lab 3	15.0	14.3-15.4	8.8	8.6-9.0	7.9	7.7-8.0
Lab 4	14.5	14.0-15.0	9.3	8.6-9.5	7.9	7.8-8.0
Lab 5	9.0	8.1-11.1	10.8	10.2-11.8	7.5	7.4-7.5
Lab 6	14.8	14.2-15.1	9.0	8.5-9.2	7.7	7.5-8.0
Lab 7	12.0	11.8-12.3	70%[a]	60-82%[a]	7.5	7.3-7.6
Lab 8	15.0	14.9-15.4	8.2	8.0-8.4	7.0	6.8-7.2

Note: a) Percentage of saturation.

Biological effects

61. The exposed and control fish were observed daily throughout the study for incidences of mortality, effects on feeding behaviour and other adverse effects. Most laboratories noted no incidences of adverse effects in either the control or exposed groups throughout the study. However the following were noted by two laboratories.

62. Lab 5 noted reduced feeding behaviour compared to the control group in the exposed fish between uptake day 9 and depuration day 8.

63. Lab 8 noted occasional instances of dark discoloured fish in both the control (two fish on uptake day 8 to 10) and exposed group (1 fish at various times during depuration) during the study. In addition, three fish died in both the control and exposed groups but these instances of mortality were thought to be the result of damage from siphoning or bullying.

64. Overall, most of the studies appear to have been unaffected by toxicity from exposure to the test substances. The incidences of discoloured fish in one study occurred in both the control group and so were likely not treatment related. However, the reduced feeding behaviour seen by Lab 5 towards the end of the uptake phase may have affected (reduced) the exposure of the fish to the test substances.

Lipid contents of fish

65. The lipid contents of the fish were 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. In most cases the lipid contents of the fish was found to increase as the test progressed. The data are summarised in **Table 19**.

66. The mean concentrations reported in **Table 19** 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 largest increases in lipid content were seen in the results of Lab 6 (an increase of around 3.7 times between the start of the test and the end of depuration) and Lab 8 (an increase of around 2.9 times between the start of the test and the end of depuration). The increase in lipid content in the fish for the remaining laboratories was generally in the range 1.3 to 1.8 times over the course of the test. 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 [ ] in **Table 19**.

Table 19 Summary of fish lipid contents determined during the test

Laboratory	Group	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[a] [9.07[a]]
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	As above		5.85	single value reported	6.66 (day 21 value)	single value reported	5.91[c] [6.04[c]]
	Exposed – level 2	As above		7.37	single value reported	7.81 (day 21 value)	single value reported	6.44[d] [6.66[e]]
	Exposed – level 3	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]
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]

Laboratory	Group	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 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]

Note: a) 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.

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.

Measured concentrations in fish

67. The concentrations measured in fish at the various timepoints are summarised in **Table 20**.

Table 20 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 1	Up	3	1.55	0.37	2.47	0.82	2.34	0.63	1.90	0.47	0.31	0.25
	Dep	1	3.31	1.11	4.26	1.59	3.61	1.43	2.85	1.17	0.21	0.08
		3	3.10	0.86	3.33	1.27	2.56	0.80	2.20	0.85	0.03	b
		7	2.73	0.65	2.54	0.72	2.19	1.14	0.42	0.10	nd	
		14	1.80	0.24	1.06	0.20	0.94	0.40	0.11	0.10	nd	
		21	1.48	0.40	0.76	0.29	0.59	0.19	0.28	b	nd	
		28	0.82	0.28	0.36	0.16	0.33	0.11	0.12	b	nd	
Lab 2a – trout	Dep	1	5.48	0.72	9.50	1.06	5.51	0.87	7.68	1.58	0.61	0.17
		3	5.04	0.64	8.25	1.64	5.67	2.58	8.69	2.65	0.012	0.010
		8	3.74	0.47	5.32	0.93	2.32	0.15	4.84	1.48	nd	
		14	3.92	0.31	4.90	0.55	3.67	1.31	3.34	1.34	nd	
		21	2.38	0.33	2.03	0.40	1.53	0.47	1.11	0.65	nd	
		28	1.81	0.22	1.30	0.16	0.93	0.57	0.67	0.55	nd	
Lab 2b – carp (level 1)	Dep	1	20.47	a	29.84	a	19.52	a	6.85	a	-	
		3	17.25	a	22.26	a	13.64	a	3.45	a	-	
		7	18.44	a	17.56	a	4.46	a	1.46	a	-	
		9	16.39	a	13.15	a	2.67	a	0.48	a	-	
		21	6.05	a	1.84	a	0.06	a	<0.05	a	-	

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 2b – carp (level 2)	Dep	1	12.30	a	19.97	a	9.63	a	3.86	a	-	
		3	10.22	a	12.63	a	3.26	a	1.42	a	-	
		7	7.99	a	7.50	a	0.93	a	0.50	a	-	
		9	8.04	a	5.91	a	0.54	a	0.34	a	-	
		21	3.85	a	1.34	a	<0.05	a	<0.05	a	-	
Lab 2b – carp (level 3)	Dep	1	2.45	a	2.76	a	0.62	a	0.60	a	<0.004	
		3	1.46	a	2.29	a	0.31	a	0.20	a	<0.004	
		7	1.58	a	1.10	a	0.11	a	0.11	a	<0.004	
		9	2.10	a	1.43	a	0.05	a	<0.05	a	<0.004	
		21	0.76	a	0.29	a	<0.05	a	<0.05	a	<0.004	
Lab 3	Up	3	1.52	0.37	3.50	0.60	2.28	0.55	2.17	0.95	1.27	0.47
	Dep	1	4.87	0.94	9.43	1.74	6.30	2.03	6.36	2.95	1.83	0.56
		3	4.23	0.69	7.59	1.48	3.92	0.74	3.68	3.16	nd	
		7	2.72	0.47	4.02	0.87	2.08	0.86	1.41	1.75	nd	
		14	2.23	0.61	2.76	0.80	0.90	0.38	0.50	0.55	nd	
		21	1.51	0.22	1.50	0.26	0.78	0.38	0.56	0.24	nd	
		28	1.10	0.29	1.01	0.41	0.36	0.24	0.27	0.09	nd	

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 4	Up	3	1.73	0.15	3.14	0.33	2.37	0.42	2.65	1.65	0.57	0.27
	Dep	1	4.64	1.21	6.38	2.00	4.50	2.46	4.16	2.35	0.15	0.13
		3	4.16	0.20	4.99	0.26	3.73	0.92	4.39	2.29	nd	
		7	3.28	0.27	3.29	0.26	3.16	0.53	1.29	1.52	nd	
		14	2.22	0.39	1.89	0.20	1.32	1.07	0.15	0.06	nd	
		21	1.30	0.24	0.77	0.19	0.66	0.51	0.50	0.02	nd	
		28	1.31	0.33	1.39	0.29	0.93	0.36	0.42	0.36	nd	
Lab 5	Dep	1	8.37	4.01	0.18	0.09	13.84	7.06	4.84	2.81	3.34	4.03
		3	9.23	5.77	0.02	0.00	8.51	5.27	5.22	4.72	0.02	0.004
		7	9.67	2.82	0.002	0.001	8.43	3.70	7.60	6.15	nd	
		14	6.94	3.09	nd		7.28	2.56	13.46	19.17	nd	
		21	6.67	3.11	nd		7.11	3.53	4.72	2.52	nd	
		28	2.16	0.57	nd		2.38	0.53	4.00	2.34	nd	
Lab 6	Up	3	1.21	0.06	2.79	0.32	1.81	0.34	1.55	0.64	0.92	0.37
	Dep	1	5.26	0.60	5.74	0.41	3.09	0.20	2.27	0.44	1.18	0.18
		3	3.65	1.12	3.43	1.04	1.95	0.83	1.28	0.65	0.05	0.03
		7	2.67	0.37	2.44	0.30	1.05	0.39	0.22	0.09	nd	
		14	2.02	0.57	1.10	0.26	0.58	0.17	0.09	0.06	nd	
		21	1.18	0.19	0.52	0.16	0.29	0.25	0.01	0.00	nd	
		28	0.85	0.25	0.32	0.15	0.13	0.13	0.01	b	nd	

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 7	Up	3	0.47	0.05	0.48	0.05	0.77	0.06	1.46	0.20	0.63	b
	Dep	1	2.13	0.23	2.35	0.55	2.65	0.32	3.22	1.00	0.04	b
		3	2.00	0.57	2.10	0.76	2.29	0.70	2.86	0.88	nd	
		7	1.28	0.54	1.01	0.66	1.25	0.57	1.67	1.17	nd	
		14	0.94	0.22	0.36	0.10	0.62	0.25	0.61	0.23	nd	
		21	0.76	0.13	0.27	0.09	0.58	0.15	0.22	0.13	nd	
		28	0.56	0.15	0.14	0.05	0.32	0.14	0.06	0.04	nd	
Lab 8	Up	3	1.41	0.19	2.56	0.22	2.06	0.28	1.66	0.66	0.46	0.05
	Dep	1	2.91	0.39	5.04	0.91	3.58	1.19	3.39	1.95	0.59	0.32
		3	3.66	0.33	4.49	0.52	3.59	1.07	2.90	1.35	0.05	0.00
		7	3.13	0.26	3.33	0.26	2.44	0.58	2.28	1.01	0.05	0.00
		14	1.56	0.41	1.48	0.53	0.91	0.80	0.36	0.38	0.03	0.00
		21	1.18	0.13	0.90	0.15	0.58	0.37	0.19	0.14	0.05	0.00
		28	0.73	0.07	0.39	0.08	0.28	0.25	0.32	0.15	0.05	0.00

Notes: a) Only single (mean) values were given in the data spreadsheet.

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

SD = Standard deviation.

Up = Uptake phase.

Dep = Depuration phase.

Nd = Below the limit of detection/quantification.

Overall depuration rate constants

68. 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 to **Table 25** but all other values were included except where it was indicated in the data set that the value was an outlier.

69. The plots were constructed using the individual data points at each time point (normally five concentration measurements were taken at each sampling point). However, the Lab 2b carp data were presented as single, mean, concentration values at each time point and so the relevant parameters were derived from these mean concentrations rather than the individual concentrations.

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

71. It should be noted that for benzo[a]pyrene data for only a limited number of timepoints were available (at best two time points, with the concentrations measured at the second time point being close to or below the limit of detection). This means that the slope 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 many cases. 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.

Table 21 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_o]	[C_0] (µg/g)					
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)	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)	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)	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

Table 22 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 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)	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)	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)	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

Table 23 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 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)	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)	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)	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

Table 24 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 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)	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)	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)	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

Table 25 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 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 (level 1)	a	a	a	a	a	a	a	a
Lab 2b – carp (level 2)	a	a	a	a	a	a	a	a
Lab 2b – carp (level 3)	a	a	a	a	a	a	a	a
Lab 3	a	a	a	a	a	a	a	a
Lab 4	a	a	a	a	a	a	a	a
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	a	a	a	a	a	a	a	a
Lab 8	1.179	0.541	1.72	0.92	0.120	0.268	0.902 to 1.456	-0.077 to 1.160

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

Growth rate constants

A summary of the fish growth data is given in **Table 26** (fish weights) and **Table 27** (fish lengths).

Table 26 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 1	Up	0	1.31	0.13	1.19	0.22	1.25	0.18
		3	1.70	0.29	1.57	0.29	1.64	0.28
		13	2.00	0.38	2.20	0.56	2.10	0.46
	Dep	1	1.95	0.21	2.45	0.34	2.20	0.37
		3	2.23	0.23	2.15	0.45	2.19	0.34
		7	2.84	0.63	3.26	0.63	3.05	0.63
		14	4.19	1.92	3.92	0.56	4.05	1.34
		21	5.54	1.70	5.00	1.18	5.27	1.41
		28	5.54	1.48	6.05	1.92	5.79	1.69
Lab 2a – trout	Up	0	8.41	0.59	As control		As control	
		13	9.70	0.71	9.86	1.15	9.78	0.91
	Dep	1	9.24	1.07	10.74	1.41	9.99	1.42
		3	8.71	0.88	10.37	1.49	9.54	1.45
		8	11.72	1.36	11.41	1.43	11.57	1.33
		14	12.52	1.81	15.14	2.11	13.83	2.31
		21	14.22	1.78	14.32	1.80	14.27	1.69
		28	14.94	1.23	15.14	0.68	15.04	0.94
Lab 2b – carp (level 1)	Up	0	5.42	0.20	As control		As control	
		13	7.29	0.78	7.21	0.72	7.11	0.88
	Dep	1	7.09	1.24	7.08	0.66	6.90	0.86
		3	7.79	0.73	7.37	1.04	7.41	0.89
		7	8.16	0.76	7.76	0.60	8.31	0.98
		9	9.73	1.55	8.52	0.78	9.48	1.23
		21	14.98	0.77	12.79	1.29	13.41	1.53

Laboratory	Day		Control group		Test group		Combined group	
			Mean weight (g)	SD	Mean weight (g)	SD	Mean weight (g)	SD
Lab 2b – carp (level 2)	Up	0	As above		As above		As above	
		13	As above		7.14	1.22	As above	
	Dep	1	As above		6.83	0.94	As above	
		3	As above		7.03	0.84	As above	
		7	As above		8.26	1.34	As above	
		9	As above		9.89	1.25	As above	
		21	As above		12.65	1.39	As above	
Lab 2b – carp (level 3)	Up	0	As above		As above		As above	
		13	As above		6.81	0.96	As above	
	Dep	1	As above		6.60	0.70	As above	
		3	As above		7.45	1.05	As above	
		7	As above		9.06	0.84	As above	
		9	As above		9.78	1.00	As above	
		21	As above		13.20	1.60	As above	
Lab 3	Up	0	1.83	0.20	2.07	0.19	1.95	0.22
		3	2.77	0.18	2.47	0.41	2.62	0.34
		13	3.56	0.32	3.72	0.48	3.64	0.39
	Dep	1	3.86	0.52	3.99	0.43	3.92	0.45
		3	4.60	0.42	4.31	0.44	4.45	0.43
		7	5.01	0.75	4.78	0.61	4.89	0.66
		14	6.97	1.40	6.91	1.32	6.94	1.28
		21	9.62	1.30	9.28	2.61	9.45	1.95
		28	12.82	2.51	13.14	2.81	12.98	2.60
Lab 4	Up	0	1.17	0.10	As control		As control	
		3	1.59	0.18	1.57	0.15	1.58	0.16
		14	2.40	0.44	2.25	0.63	2.32	0.52
	Dep	1	2.61	0.38	2.77	0.68	2.69	0.52
		3	2.42	0.32	3.05	0.39	2.73	0.47
		7	3.04	0.65	3.38	0.48	3.21	0.56
		14	4.94	1.46	4.60	1.07	4.77	1.22
		21	6.17	0.74	5.81	0.76	5.99	0.73
		28	6.87	1.46	5.88	1.65	6.38	1.60

Laboratory	Day		Control group		Test group		Combined group	
			Mean weight (g)	SD	Mean weight (g)	SD	Mean weight (g)	SD
Lab 5	Up	0	6.77	2.12	As control		As control	
		13	7.30	2.67	8.37	2.95	7.84	2.71
	Dep	1	12.22	3.72	9.85	1.97	11.04	3.07
		3	9.97	1.15	10.68	2.56	10.32	1.91
		7	11.26	3.08	9.19	3.30	10.22	3.20
		14	9.68	3.63	13.11	4.23	11.39	4.13
		21	11.39	2.87	8.70	3.02	10.05	3.12
		28	17.99	7.06	15.64	7.44	16.48	7.12
Lab 6	Up	0	0.72	0.12	As control		As control	
		3	0.95	0.12	0.86	0.05	0.90	0.10
		13	1.59	0.34	1.64	0.36	1.61	0.33
	Dep	1	1.48	0.31	1.37	0.36	1.42	0.32
		3	1.66	0.15	1.79	0.13	1.73	0.15
		7	1.90	0.30	1.84	0.34	1.87	0.31
		14	2.16	0.14	2.76	0.40	2.46	0.42
		21	3.39	0.61	3.21	0.92	3.30	0.74
		28	4.27	0.78	3.76	0.66	4.01	0.75
Lab 7	Up	0	1.20	0.20	As control		As control	
		3	1.34	0.27	1.12	0.29	1.23	0.29
		14	1.76	0.16	1.79	0.25	1.78	0.20
	Dep	1	1.42	0.34	1.77	0.48	1.59	0.43
		3	1.60	0.34	1.66	0.21	1.63	0.27
		7	1.94	0.29	2.31	0.70	2.13	0.54
		14	2.70	0.43	2.64	0.30	2.67	0.35
		21	2.97	0.44	3.24	0.49	3.11	0.46
		28	3.96	0.50	3.78	0.87	3.89	0.64

Laboratory	Day		Control group		Test group		Combined group	
			Mean weight (g)	SD	Mean weight (g)	SD	Mean weight (g)	SD
Lab 8	Up	0	1.21	0.13	1.27	0.21	1.24	0.16
		3	1.72	0.15	1.38	0.29	1.55	0.28
		13	1.85	0.46	2.31	0.35	2.08	0.46
	Dep	1	2.53	0.33	2.65	0.50	2.59	0.41
		3	2.70	0.50	2.61	0.08	2.66	0.34
		7	3.41	1.02	3.52	0.53	3.46	0.77
		14	5.17	0.80	4.56	0.68	4.87	0.77
		21	6.72	0.70	6.13	1.24	6.43	1.00
		28	9.96	3.25	8.57	0.94	9.23	2.38

Notes: Up = Uptake phase. Dep = Depuration phase.

Table 27 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 1	Up	0	50.2	1.64	49.4	2.97	49.8	2.30
		3	54.2	3.96	52.0	3.67	53.1	3.78
		13	55.2	3.19	58.2	4.15	56.7	3.83
	Dep	1	57.4	2.51	60.6	3.29	59.0	3.23
		3	57.0	2.35	57.0	5.05	57.0	3.71
		7	63.0	4.36	66.4	3.36	64.7	4.08
		14	71.8	8.38	70.0	3.39	70.9	6.10
		21	77.0	8.19	76.2	4.55	76.6	6.26
		28	80.0	7.48	81.9	8.25	81.0	7.73
Lab 2a – trout[a]	Up	0	93.0	2.74	As control		As control	
		13	94.0	2.63	95.4	4.54	94.7	3.58
	Dep	1	94.3	3.61	98.2	3.07	96.3	3.79
		3	95.8	4.32	99.0	2.67	97.4	3.78
		8	103.8	2.28	101.0	4.30	102.4	3.57
		14	105.4	2.07	111.8	4.09	108.6	4.55
		21	111.2	4.55	110.2	2.49	110.7	3.50
		28	112.4	3.58	112.4	3.91	112.4	3.53
Lab 2a – carp (level 1)[a]	Up	0	74.8	1.10	As control		As control	
		13	84.8	3.35	84.0	1.87		
	Dep	1	79.2	7.46	81.4	0.89		
		3	86.4	2.61	82.4	3.51		
		7	87.2	0.84	86.2	3.03		
		9	89.2	5.89	87.2	2.28		
		21	102.0	2.00	100.6	3.21		
Lab 2a – carp (level 2)[a]	Up	0	As above[c]		As above[c]		As above[c]	
		13	As above[c]		81.4	4.51	As above[c]	
	Dep	1	As above[c]		80.0	3.39	As above[c]	
		3	As above[c]		83.4	2.51	As above[c]	
		7	As above[c]		86.6	4.67	As above[c]	
		9	As above[c]		90.8	2.78	As above[c]	
		21	As above[c]		98.6	5.46	As above[c]	

Laboratory	Day		Control group		Test group		Combined group	
			Mean length (mm)	SD	Mean length (mm)	SD	Mean length (mm)	SD
Lab 2a – carp (level 3)[a]	Up	0	As above[c]		As above[c]		As above[c]	
		13	As above[c]		82.2	2.17	As above[c]	
	Dep	1	As above[c]		80.6	3.72	As above[c]	
		3	As above[c]		85.0	3.08	As above[c]	
		7	As above[c]		88.8	4.32	As above[c]	
		9	As above[c]		90.0	2.92	As above[c]	
		21	As above[c]		101.5	4.24	As above[c]	
Lab 3	Up	0	54.0	1.58	54.8	1.10	54.4	1.35
		3	60.2	1.92	58.8	2.39	59.5	2.17
		13	68.8	1.64	68.6	3.21	68.7	2.41
	Dep	1	69.2	3.49	68.0	2.83	68.6	3.06
		3	73.8	3.42	72.8	3.11	73.3	3.13
		7	76.0	3.39	74.2	3.35	75.1	3.31
		14	83.2	5.40	84.2	5.40	83.7	5.12
		21	94.0	3.94	91.6	9.04	92.8	6.70
		28	101.6	6.79	101.8	7.13	101.7	6.78
Lab 4[b]	Up	0	49.6	1.1	As control		As control	
		3	52.4	1.9	51.8	1.6	52.1	1.7
		14	60.0	2.6	58.8	3.8	59.4	3.2
	Dep	1	61.8	2.9	63.8	4.3	62.8	3.6
		3	61.4	2.1	64.4	2.3	62.9	2.6
		7	65.2	4.2	66.8	3.6	66.0	3.8
		14	76.6	8.0	74.0	6.3	75.3	6.9
		21	82.2	2.7	80.4	4.6	81.3	3.7
		28	84.2	4.9	80.1	5.9	82.2	5.7
Lab 5	Up	0	No data		As control		As control	
		13	No data					
	Dep	1	96.3	8.40	88.0	6.47	92.1	8.29
		3	90.6	3.04	95.0	8.37	92.8	6.37
		7	94.3	9.15	91.3	11.21	92.8	9.77
		14	90.2	14.22	101.3	10.32	95.7	13.10
		21	97.0	8.61	90.3	10.83	93.7	9.89
		28	112.0	17.48	103.4	16.72	106.5	16.86

Laboratory	Day		Control group		Test group		Combined group	
			Mean length (mm)	SD	Mean length (mm)	SD	Mean length (mm)	SD
Lab 6	Up	0	43.4	1.95	As control		As control	
		3	46.3	2.65	46.2	1.10	46.3	1.91
		13	54.8	2.95	58.0	2.92	56.4	3.24
	Dep	1	53.6	4.16	52.2	4.60	52.9	4.20
		3	49.2	14.27	56.0	1.58	52.6	10.22
		7	59.6	3.85	58.0	3.46	58.8	3.55
		14	61.2	1.10	66.6	3.21	63.9	3.63
		21	71.0	2.55	69.2	5.26	70.1	4.01
		28	77.2	4.05	73.8	4.96	75.5	4.74
Lab 7	Up	0	51.0	2.74	As control		As control	
		3	52.2	4.32	51.6	4.93	51.9	4.38
		14	56.4	2.07	56.6	1.82	56.5	1.84
	Dep	1	53.6	4.56	56.0	5.52	54.8	4.94
		3	54.0	4.64	56.0	3.94	55.0	4.19
		7	58.6	2.88	63.2	6.22	60.9	5.17
		14	64.2	3.83	65.6	3.51	64.9	3.54
		21	65.2	3.11	70.0	3.16	67.6	3.89
		28	74.2	2.90	72.8	5.42	73.7	3.91
Lab 8	Up	0	41.6	0.89	41.0	1.87	41.3	1.42
		3	45.6	2.19	44.0	2.92	44.8	2.57
		13	48.0	3.74	50.8	2.39	49.4	3.31
	Dep	1	51.6	1.95	52.8	2.28	52.2	2.10
		3	53.8	3.77	53.4	1.82	53.6	2.80
		7	57.8	5.89	59.0	3.54	58.4	4.62
		14	66.0	4.30	65.0	3.24	65.5	3.63
		21	72.8	2.59	71.4	4.34	72.1	3.45
		28	82.4	10.39	78.9	3.70	80.6	7.63

Notes: Up = Uptake phase. Dep = Depuration phase.

a) The values reported for Lab 2 in the raw data were all a factor of 10 higher than these values. It is assumed that this was an error in the raw data (as the length data reported were not consistent with the fish weights and the data from other laboratories) and so have been reduced by a factor of 10 for this analysis. The data are then consistent with those from other laboratories

b) The values reported for Lab 4 were given in the raw data as mm but appear to be cm. It is assumed that this was an error in the raw data and for this analysis the data have been recalculated assuming the original unit was cm. The data are then consistent with those from other laboratories.

c) There was only one control group for this these tests. The combined group includes the control fish and the fish from the three treatment levels.

72. 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 28**.

73. 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).

74. This test revealed no statistically significant differences between the growth rate constant over the uptake phase and depuration phase for Lab 1, Lab 2a – trout, Lab 3, Lab 4, Lab 5 and Lab 7. For these laboratories, 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 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} for these laboratories in subsequent calculations.

75. For Lab 8, a statistically significant difference in the growth rate constant determined for the uptake phase and depuration phase was apparent for the control group, but not the test group or the combined group. Further no statistically significant differences were evident between the growth rate constant derived over the entire test period for the control group compared with the test group. For this laboratory, the growth rate constant derived from the combined control and test group over the entire test period is used as the k_{growth} for this laboratory in subsequent calculations.

76. For Lab 6, statistically significant differences were evident between the rate constant derived for the uptake phase and that derived for the depuration phase for the control group, the test group and the combined group. This indicates that for this data set it is not appropriate to combine the uptake and depuration data. No statistically significant differences were evident between the growth rate constant determined for the depuration phase alone for the control and test group and so the most appropriate value for k_{growth} for this study is taken to be that derived from the depuration phase of the study using the combined data for the control and test group[5].

77. For Lab 2a – carp, statistically significant differences were evident between the growth rate constant derived during the uptake phase and depuration phase for the control group, the test level 3 group and the combined (control plus test levels 1, 2 and 3) group. Similar to the case with Lab 6, this indicates that it may not be appropriate to combine data for the uptake phase with that for the depuration phase. No statistically significant differences were evident between the growth rate constant derived for the control group and that derived for each of the test level 1, test level 2 and test level 3 groups over the depuration phase and so the most appropriate value for k_{growth} for this test is taken to be the value for the combined group over the depuration phase.

78. The preferred values for the k_{growth} are highlighted in **bold** in **Table 28**.

[5] The effect of using growth rate constant of 0.0598 day^{-1} for the combined group for the uptake phase alone on the derived assimilation efficiencies and BMF values is shown in *[italics]* in **Table 29** and **Table 30**.

Table 28 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	0.0357	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)	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)	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)	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

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 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
	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

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 α=0.05).

Values in **bold** are the preferred values.

a) There was only one control group for this these tests. The combined group includes the control fish and the fish from the three treatment levels.

Assimilation efficiency and biomagnification factor

79. The assimilation efficiency and biomagnification factor were calculated for each study using the following equations. The resulting values are summarised in **Table 29** 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.

80. The value of I used in the calculations was the nominal value (0.03 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 on day 3 of the uptake.

81. 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 29** 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 [ ] in **Table 29**.

82. 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). Therefore it could be argued that fish were actually exposed for a total of 14 days. The derived assimilation efficiencies and BMF_L values assuming 14 days in the calculations are shown in **Table 30**. 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 substances (e.g. hexachlorobenzene and to a lesser extent musk xylene and o-terphenyl).

83. Some of the laboratories measured the concentration of each substance on day 3 of the uptake. These data allow the assimilation efficiency on day 3 to be estimated (using the day 3 concentration in place of C_0 in the above equation and setting the duration of the feeding period, t, to be 3 days or 4 days (i.e. assuming four feedings occurred between day 0 and day 3 for the latter case). The assimilation efficiencies calculated this way are shown in **Table 31** assuming 3 days in the calculation and **Table 32** assuming 4 days in the calculation.

84. As can be seen from **Table 31** and **Table 32**, the effect of assuming either 3 or 4 days in the calculation has a marked effect on the derived assimilation efficiency, with the values estimated using 3 days being higher than those estimated using 4 days. This suggests that it is important to clarify the exact number of days that should be used in the calculation when estimating the assimilation efficiency (and hence the BMF_L) value for short time periods.

85. The mean assimilation efficiencies calculated for trout are generally similar to those calculated for carp particularly for musk xylene and o-terphenyl. The mean assimilation efficiency calculated for hexachlorobenzene is slightly lower for carp (e.g. 0.38 or 0.36) than for trout (e.g. 0.60 or 0.57). However a larger difference between carp and trout is evident in the BMF_L values (where the mean BMF_L value is generally a factor of 2 or more larger for trout than carp). This may indicate increased metabolism of the substances in carp compared with trout. A more detailed investigation of the similarities and differences between the trout and carp data is being carried out elsewhere.

86. The assimilation efficiencies calculated for benzo[a]pyrene are, in some cases, above 1. This is theoretically impossible and almost certainly results from difficulties in extrapolating a reliable C_0 value for this substance owing to its very rapid depuration (see paragraph 71). Therefore the results obtained for benzo[a]pyrene are considered to be unreliable.

87. 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 trout data (minus Lab 5). 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).

	Assimilation efficiency		**BMF_L (A)**		**BMF_L (B)**	
	Mean	Rel. SD	Mean	Rel. SD	Mean	Rel. SD
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 man fish lipid content over the depuration phase.

88. As the carp data were generated within one laboratory, the variability in the derived assimilation efficiency and BMF_L provides a measure of intralaboratory variability. These are summarised below for the data derived using t=13 days. It should be noted that the three tests available were carried out using three different test substance concentrations in this case.

	Assimilation efficiency		**BMF_L (A)**		**BMF_L (B)**	
	Mean	Rel. SD	Mean	Rel. SD	Mean	Rel. SD
Hexachlorobenzene	0.38	11%	1.45	14%	1.41	14%
Musk xylene	0.44	27%	0.38	16%	0.37	16%
o-Terphenyl	0.50	60%	0.15	67%	0.14	71%
Methoxychlor	0.10	30%	0.03	33%	0.03	33%

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

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

Table 29 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 over the entire test period; values in [ ] are normalised to the mean fish lipid content during depuration)				
		HCB	MX	oTP	MC	BaP	HCB	MX	oTP	MC	BaP	HCB	MX	oTP	MC	BaP
Lab 1	0.0366	0.50	0.35	0.29	0.13	0.11	1.09	0.20	0.17	0.04	0.003	2.00 [1.75]	0.36 [0.31]	0.31 [0.28]	0.06 [0.06]	0.006 [0.005]
Lab 2a – trout	0.0165	0.79	0.95	0.55	0.65	2.42	1.01	0.50	0.31	0.20	0.04	1.89 [1.83]	0.94 [0.91]	0.59 [0.56]	0.37 [0.35]	0.07 [0.06]
Lab 2b – carp (level 1)	0.0334	0.41	0.54	0.74	0.13		0.46	0.15	0.09	0.01		1.30 [1.27]	0.43 [0.42]	0.25 [0.24]	0.04 [0.04]	
Lab 2b – carp (level 2)	0.0334	0.40	0.48	0.59	0.10		0.52	0.15	0.06	0.01		1.36 [1.32]	0.38 [0.37]	0.14 [0.14]	0.03 [0.03]	
Lab 2b – carp (level 3)	0.0334	0.33	0.31	0.17	0.06		0.65	0.12	0.02	0.01		1.69 [1.64]	0.31 [0.30]	0.05 [0.05]	0.02 [0.02]	
Lab 3	0.044	0.54	0.66	0.43	0.11		1.67	0.50	0.22	0.06		3.71 [3.26]	1.12 [0.98]	0.48 [0.42]	0.13 [0.12]	
Lab 4	0.0386	0.70	0.36	0.33	0.10		1.61	0.38	0.26	0.06		3.82 [3.38]	0.91 [0.81]	0.62 [0.55]	0.13 [0.12]	
Lab 5	0.0158	0.86	1.96	0.63	0.17	4.65	1.03	0.09	0.66	-0.46	0.07	1.14 [1.07]	0.10 [0.10]	0.73 [0.68]	-0.51 [-0.48]	0.08 [0.07]
Lab 6	0.0367	0.77	0.43	0.35	0.14	2.33	0.89	0.19	0.11	0.02	0.04	3.63 [2.77]	0.77 [0.59]	0.45 [0.34]	0.09 [0.07]	0.17 [0.13]
[Lab 6][a]	*0.0598*	*0.77*	*0.43*	*0.35*	*0.14*	*2.33*	*8.51*	*0.29*	*0.14*	*0.03*	*0.04*	*34.7*	*1.16*	*0.59*	*0.11*	*0.18*
Lab 7	0.0307	0.32	0.24	0.23	0.14		0.52	0.10	0.15	0.04		1.87 [1.78]	0.35 [0.33]	0.54 [0.51]	0.13 [0.13]	

Laboratory	k_{growth} used (day^{-1})	Assimilation efficiency					BMF_g					BMF_L (using the mean fish lipid content over the entire test period; values in [ ] are normalised to the mean fish lipid content during depuration)				
		HCB	MX	oTP	MC	BaP	HCB	MX	oTP	MC	BaP	HCB	MX	oTP	MC	BaP
Lab 8	0.0483	0.60	0.58	0.45	0.14	0.49	1.86	0.37	0.21	0.07	0.01	4.77 [3.84]	0.95 [0.77]	0.54 [0.43]	0.17 [0.14]	0.03 [0.03]
Overall	Mean	0.56	0.62	0.43	0.17	2.00	1.03	0.25	0.20	0.00	0.03	2.47 [2.17]	0.60 [0.53]	0.43 [0.38]	0.06 [0.05]	0.07 [0.06]
	SD	0.19	0.49	0.18	0.16	1.82	0.50	0.16	0.17	0.16	0.03	1.26 [0.96]	0.34 [0.29]	0.21 [0.19]	0.21 [0.20]	0.06 [0.05]
Overall minus Lab 5 data	Mean	0.53	0.49	0.41	0.17	1.34	1.03	0.27	0.16	0.05	0.02	2.60 [2.28]	0.65 [0.58]	0.40 [0.35]	0.12 [0.11]	0.07 [0.06]
	SD	0.18	0.21	0.18	0.17	1.21	0.52	0.16	0.09	0.06	0.02	1.24 [0.94]	0.31 [0.27]	0.20 [0.17]	0.10 [0.10]	0.07 [0.06]
Trout data minus Lab 5	Mean	0.60	0.51	0.38	0.20	1.34	1.24	0.32	0.20	0.07	0.02	3.10 [2.66]	0.77 [0.67]	0.50 [0.44]	0.16 [0.14]	0.07 [0.06]
	SD	0.17	0.24	0.11	0.20	1.21	0.49	0.16	0.07	0.06	0.02	1.16 [0.87]	0.30 [0.27]	0.10 [0.11]	0.10 [0.10]	0.07 [0.06]
Carp data	Mean	0.38	0.44	0.50	0.10		0.54	0.14	0.05	0.01		1.45 [1.41	0.38 [0.37]	0.15 [0.14]	0.03 [0.03]	
	SD	0.04	0.12	0.30	0.03		0.10	0.02	0.03	0.00		0.21 [0.20]	0.06 [0.06]	0.10 [0.10]	0.01 [0.01]	

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

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

a) Values in *italics* are those obtained using the growth rate constant for the uptake phase alone for Lab 6 (see paragraph 76). These values are not included in the calculation of the means.

Table 30 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 over the entire test period; values in [ ] are normalised to the mean fish lipid content during depuration)				
		HCB	MX	oTP	MC	BaP	HCB	MX	oTP	MC	BaP	HCB	MX	oTP	MC	BaP
Lab 1	0.0366	0.47	0.34	0.28	0.13	0.11	1.04	0.19	0.17	0.03	0.003	1.90 [1.66]	0.34 [0.30]	0.30 [0.26]	0.06 [0.06]	0.006 [0.005]
Lab 2a – trout	0.0165	0.74	0.91	0.52	0.63	2.42	0.95	0.48	0.30	0.19	0.04	1.79 [1.73]	0.90 [0.87]	0.56 [0.54]	0.36 [0.34]	0.07 [0.06]
Lab 2b – carp (level 1)	0.0334	0.39	0.53	0.74	0.13		0.43	0.15	0.09	0.01		1.24 [1.21]	0.42 [0.41]	0.25 [0.24]	0.04 [0.04]	
Lab 2b – carp (level 2)	0.0334	0.38	0.47	0.59	0.10		0.50	0.14	0.06	0.01		1.30 [1.25]	0.37 [0.36]	0.14 [0.14]	0.03 [0.03]	
Lab 2b – carp (level 3)	0.0334	0.31	0.30	0.17	0.06		0.61	0.12	0.02	0.01		1.60 [1.55]	0.30 [0.29]	0.05 [0.05]	0.02 [0.02]	
Lab 3	0.044	0.51	0.63	0.42	0.11		1.59	0.48	0.21	0.06		3.53 [3.10]	1.08 [0.95]	0.47 [0.41]	0.13 [0.11]	
Lab 4	0.0386	0.67	0.35	0.32	0.10		1.53	0.37	0.25	0.05		3.63 [3.21]	0.87 [0.77]	0.59 [0.53]	0.13 [0.11]	
Lab 5	0.0158	0.81	1.96	0.60	0.16	4.65	0.98	0.09	0.62	-0.43	0.07	1.08 [1.01]	0.10 [0.10]	0.69 [0.65]	-0.48 [-0.45]	0.08 [0.07]
Lab 6	0.0367	0.73	0.42	0.34	0.14	2.33	0.85	0.18	0.11	0.02	0.04	3.46 [2.64]	0.74 [0.57]	0.43 [0.33]	0.09 [0.07]	0.17 [0.13]
[Lab 6][a]	0.0598	*0.73*	*0.42*	*0.34*	*0.14*	*2.33*	*8.12*	*0.28*	*0.14*	*0.03*	*0.04*	*33.1*	*1.12*	*0.57*	*0.11*	*0.18*
Lab 7	0.0307	0.30	0.23	0.22	0.14		0.49	0.09	0.14	0.04		1.78 [1.69]	0.33 [0.32]	0.52 [0.49]	0.13 [0.12]	

Laboratory	k_{growth} used (day^{-1})	Assimilation efficiency					BMF_g					BMF_L(using the mean fish lipid content over the entire test period; values in [ ] are normalised to the mean fish lipid content during depuration)				
		HCB	MX	oTP	MC	BaP	HCB	MX	oTP	MC	BaP	HCB	MX	oTP	MC	BaP
Lab 8	0.0483	0.57	0.56	0.44	0.13	0.49	1.77	0.36	0.20	0.06	0.01	4.54 [3.66]	0.92 [0.74]	0.52 [0.42]	0.17 [0.13]	0.03 [0.03]
Overall	Mean	0.53	0.61	0.42	0.17	2.00	0.98	0.24	0.20	0.01	0.03	2.35 [2.06]	0.58 [0.52]	0.41 [0.37]	0.06 [0.05]	0.07 [0.06]
	SD	0.18	0.49	0.18	0.16	1.82	0.47	0.15	0.16	0.15	0.03	1.20 [0.92]	0.33 [0.28]	0.20 [0.18]	0.20 [0.19]	0.06 [0.05]
Overall minus Lab 5 data	Mean	0.51	0.47	0.40	0.17	1.35	0.98	0.26	0.15	0.05	0.02	2.48 [2.17]	0.63 [0.56]	0.38 [0.34]	0.12 [0.10]	0.07 [0.06]
	SD	0.17	0.20	0.18	0.16	1.23	0.50	0.15	0.09	0.05	0.02	1.19 [0.89]	0.30 [0.26]	0.19 [0.17]	0.10 [0.09]	0.07 [0.06]
Trout data minus Lab 5	Mean	0.57	0.49	0.36	0.20	1.34	1.17	0.31	0.20	0.07	0.02	2.95 [2.53]	0.74 [0.64]	0.48 [0.43]	0.15 [0.14]	0.07 [0.06]
	SD	0.16	0.23	0.10	0.19	1.21	0.46	0.15	0.07	0.06	0.02	1.11 [0.83]	0.29 [0.26]	0.10 [0.10]	0.10 [0.10]	0.07 [0.06]
Carp data	Mean	0.36	0.43	0.50	0.10		0.52	0.14	0.05	0.01		1.38 [1.34]	0.37 [0.36]	0.15 [0.14]	0.03 [0.03]	
	SD	0.04	0.12	0.30	0.03		0.09	0.02	0.03	0.003		0.20 [0.19]	0.06 [0.06]	0.10 [0.10]	0.01 [0.01]	

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

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

a) Values in *italics* are those obtained using the growth rate constant for the uptake phase alone for Lab 6 (see paragraph 76). These values are not included in the calculation of the means.

Table 31 Summary of derived assimilation efficiencies at day 3 of the uptake (using 3 days for the uptake period)

Laboratory	Assimilation efficiency				
	HCB	**MX**	**oTP**	**MC**	**BaP**
Lab 1	0.74	0.58	0.59	0.28	0.07
Lab 2a – trout	nd	nd	nd	nd	nd
Lab 2b – carp (level 1)	nd	nd	nd	nd	nd
Lab 2b – carp (level 2)	nd	nd	nd	nd	nd
Lab 2b – carp (level 3)	nd	nd	nd	nd	nd
Lab 3	0.60	0.78	0.55	0.21	na
Lab 4	0.89	0.66	0.62	0.34	na
Lab 5	nd	nd	nd	nd	nd
Lab 6	0.67	0.66	0.54	0.24	0.34
Lab 7	0.25	0.15	0.23	0.12	na
Lab 8	0.74	0.73	0.57	0.23	0.14
Mean	0.65	0.59	0.51	0.24	0.18
Standard deviation	0.22	0.23	0.14	0.07	0.14

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 32 Summary of derived assimilation efficiencies at day 3 of the uptake (using 4 days for the uptake period (t+1))

Laboratory	Assimilation efficiency				
	HCB	**MX**	**oTP**	**MC**	**BaP**
Lab 1	0.57	0.46	0.46	0.23	0.07
Lab 2a – trout	nd	nd	nd	nd	nd
Lab 2b – carp (level 1)	nd	nd	nd	nd	nd
Lab 2b – carp (level 2)	nd	nd	nd	nd	nd
Lab 2b – carp (level 3)	nd	nd	nd	nd	nd
Lab 3	0.46	0.61	0.43	0.17	na
Lab 4	0.68	0.51	0.48	0.27	na
Lab 5	nd	nd	nd	nd	nd
Lab 6	0.52	0.52	0.43	0.20	0.34
Lab 7	0.20	0.11	0.18	0.10	na
Lab 8	0.57	0.57	0.45	0.18	0.13
Mean	0.50	0.46	0.40	0.19	0.18
Standard deviation	0.17	0.18	0.11	0.06	0.14

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)

Analysis of gut contents

89. Two laboratories (Lab 4 and Lab 8) carried out a separate analysis of the amount of substance in the gut and the remaining fish carcass on day 3 of the uptake phase and day 1 of the depuration phase. The samples were collected in the same manner as in the main test to allow that the gastro-intestinal tract was cleared of test diet prior to sampling.

90. The results of these samples are summarised in **Table 33** (Lab 4) and

Table 34 (Lab 8). The tables also show the contribution that the amount of substance present in the gut samples makes to the total concentration (weighted to take into account the gut weights and weight of the carcass).

91. Lab 5 carried out analysis of the gut contents (the contents of the guts were removed from three fish) on day 1 of depuration. The mean concentrations (± standard deviation) found in three samples were 27.0 (±6.2) µg/g for hexachlorobenzene, <0.1 µg/g for musk xylene, 38.9 (±8.8) µg/g for o-terphenyl, 39.6 (±11.2) µg/g for methoxychlor and 53.3 (±11.4) µg/g for benzo[a]pyrene.

92. Lab 5 also analysed the amount of substance present in the guts and at all timepoints during the depuration phase. The concentrations found in the gut samples (and the concentration in the fish minus the guts) are summarised in **Table 35** (means and standard deviations of five samples (four samples in one case)). The weight of the gut was not given and so it is not possible to determine the fraction of the total concentration that results from the amount of substance in the gut samples.

93. The results show that in most cases substance in the gut represented a substantial proportion of the total substance present in the fish. .

Table 33 Summary of gut and carcass concentrations from Lab 4

Sample		**Weight (g)**	**Concentration (µg/g)**				
			HCB	**MX**	**oTP**	**MC**	**BaP**
Uptake day 3	Sample 1 - guts	0.27	5.41	10.80	7.42	13.70	3.93
	Sample 1 - carcass	1.43	1.09	1.70	1.28	1.89	0
	Sample 1 - total	1.70	1.78	3.15	2.26	3.77	0.62
Uptake day 3	Sample 2 – guts	0.23	4.34	9.39	7.41	14.30	5.77
	Sample 2 – carcass	1.30	1.42	2.38	2.12	3.31	0
	Sample 2 - total	1.53	1.86	3.43	2.92	4.96	0.87
Uptake day 3	Sample 3 - guts	0.17	4.15	8.74	6.26	5.38	1.56
	Sample 3 - carcass	1.10	1.16	1.98	1.59	0.82	0
	Sample 3 - total	1.27	1.56	2.88	2.22	1.43	0.21
Uptake day 3	Sample 4 – guts	0.26	3.48	6.97	4.47	4.46	2.47
	Sample 4 – carcass	1.37	1.22	1.96	1.31	0.52	0
	Sample 4 - total	1.63	1.58	2.76	1.81	1.15	0.39
Uptake day 3	Sample 5 – guts	0.28	4.24	9.02	6.50	6.34	4.08
	Sample 5 – carcass	1.26	1.34	2.27	1.77	0.95	0
	Sample 5 - total	1.54	1.87	3.50	2.63	1.93	0.74
Depuration day 1	Sample 6 - guts	0.33	11.30	17.50	6.90	7.92	0.27
	Sample 6 – carcass	2.16	3.43	4.82	2.00	2.40	0
	Sample 6 – total	2.49	4.47	6.50	2.65	3.13	0.04
Depuration day 1	Sample 7 – guts	0.38	15.20	22.80	19.90	16.80	0.24
	Sample 7 – carcass	2.28	4.10	5.51	4.80	4.58	0
	Sample 7 - total	2.66	5.69	7.98	6.96	6.33	0.03

Sample		Weight (g)	Concentration (µg/g)				
			HCB	MX	oTP	MC	BaP
Depuration day 1	Sample 8 - guts	0.24	4.72	6.21	2.88	1.60	1.39
	Sample 8 – carcass	1.57	2.29	2.44	1.31	0.45	0
	Sample 8 – total	1.81	2.61	2.94	1.52	0.60	0.18
Depuration day 1	Sample 9 - guts	0.49	11.10	16.70	15.70	12.90	1.06
	Sample 9 – carcass	2.83	4.29	5.70	5.41	4.80	0
	Sample 9 – total	3.32	5.30	7.32	6.93	6.00	0.16
Depuration day 1	Sample 10 - guts	0.60	13.50	20.60	12.50	12.50	1.92
	Sample 10 – carcass	2.82	3.35	4.32	2.74	3.11	0
	Sample 10 – total	3.42	5.13	7.18	4.45	4.76	0.34
			Gut contribution expressed as a percentage of the total concentration (%)				
			HCB	**MX**	**oTP**	**MC**	**BaP**
Uptake day 3	Sample 1		48.38	54.54	52.26	57.78	100.00
	Sample 2		35.10	41.11	38.21	43.32	100.00
	Sample 3		35.60	40.55	37.83	50.44	100.00
	Sample 4		35.12	40.29	39.30	61.99	100.00
	Sample 5		41.29	46.89	44.94	59.75	100.00
	Mean		39.10	44.68	42.51	54.66	100.00
	Standard deviation		5.81	6.15	6.16	7.68	0.00
Depuration day 1	Sample 6		33.48	35.68	34.52	33.52	100.00
	Sample 7		38.19	40.82	40.86	37.94	100.00
	Sample 8		23.96	28.01	25.15	35.21	100.00
	Sample 9		30.94	33.66	33.44	31.76	100.00
	Sample 10		46.16	50.36	49.26	46.10	100.00
	Mean		34.55	37.70	36.65	36.90	100.00
	Standard deviation		8.28	8.43	9.00	5.62	0.00
Overall	Mean		36.82	41.19	39.58	45.78	100.00
	Standard deviation		8.28	8.43	9.00	5.62	0.00

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

Table 34 Summary of gut and carcass concentrations from Lab 8

Sample		Weight (g)	Concentration (µg/g)				
			HCB	MX	oTP	MC	BaP
Uptake day 3	Sample 1 - guts	0.25	0.00	9.19	6.19	5.34	16.64
	Sample 1 - carcass	1.65	1.21	2.18	1.66	1.72	0.29
	Sample 1 - total	1.90	1.05	4.19	2.99	2.83	4.41
Uptake day 3	Sample 2 – guts	0.29	0.00	8.21	5.82	3.22	1.66
	Sample 2 – carcass	1.36	1.23	2.43	1.92	1.01	0.35
	Sample 2 - total	1.65	1.01	4.36	3.25	1.76	0.76
Depuration day 1	Sample 3 - guts	0.51	7.26	14.68	10.13	7.99	4.26
	Sample 3 – carcass	2.16	2.05	3.76	2.56	1.27	<0.050
	Sample 3 – total	2.67	5.38	10.58	7.27	5.13	0.82
Depuration day 1	Sample 4 – guts	0.34	6.55	14.53	9.80	17.75	<0.050
	Sample 4 – carcass	1.60	2.09	4.17	2.95	4.64	<0.050
	Sample 4 - total	1.94	3.96	8.40	5.78	9.90	<0.050
			Gut contribution expressed as a percentage of the total concentration (%)				
			HCB	**MX**	**oTP**	**MC**	**BaP**
Uptake day 3	Sample 1		0.00	28.88	27.22	24.89	49.65
	Sample 2		0.00	32.80	31.16	31.84	37.77
	Mean		0.00	30.84	29.19	28.36	43.71
	Standard deviation		0.00	2.78	2.79	4.92	8.40
Depuration day 1	Sample 3		25.92	26.66	26.77	29.93	100.00
	Sample 4		29.12	30.45	29.83	31.59	na
	Mean		27.52	28.55	28.30	30.76	na
	Standard deviation		2.26	2.68	2.16	1.17	na
Overall	Mean		13.76	29.70	28.75	29.56	na
	Standard deviation		15.94	2.59	2.10	3.23	na

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

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

na = not applicable (not possible to estimate)

Table 35 Summary of gut and carcass concentrations from Lab 5

Sample		Concentration (µg/g)				
		HCB	**MX**	**oTP**	**MC**	**BaP**
Depuration day 1	Gut – mean	8.5	0.2	14.7	5.3	5.2
	Gut – standard deviation	2.1	0.03	3.9	2.2	4.3
	Carcass - mean	1.6	0.02	2.1	0.6	0.2
	Carcass –standard deviation	0.5	0.01	0.7	0.4	0.3
Depuration day 3	Gut – mean	7.6	<0.01	6.6	5.2	<0.03
	Gut – standard deviation	5.1		4.9	4.9	
	Carcass - mean	1.6	0.02	1.9	1.0	0.02
	Carcass –standard deviation	0.7	0.00	0.7	0.9	0.004
Depuration day 7	Gut – mean	8.3	<0.01	7.5	7.2	<0.03
	Gut – standard deviation	2.7		3.5	6.0	
	Carcass - mean	1.4	0.002	0.9	0.4	<0.015
	Carcass –standard deviation	0.3	0.001	0.2	0.2	
Depuration day 14	Gut – mean	6.0	<0.01	6.6	12.9	<0.03
	Gut – standard deviation	2.9		2.5	18.7	
	Carcass - mean	1.0	<0.002	0.6	0.6	<0.015
	Carcass –standard deviation	0.2		0.2	0.5	
Depuration day 21	Gut – mean	6.0	<0.01	6.3	3.1	<0.03
	Gut – standard deviation	2.9		3.2	2.0	
	Carcass - mean	0.7	<0.002	0.8	1.6	<0.015
	Carcass –standard deviation	0.2		0.3	0.7	
Depuration day 28	Gut – mean	1.6	<0.01	2.0	3.9	<0.03
	Gut – standard deviation	0.5		0.4	2.3	
	Carcass - mean	0.5	<0.002	0.4	0.1	<0.015
	Carcass –standard deviation	0.1		0.1	0.0	

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

94. It is important to note that the presence of substance in the gut samples does not necessarily mean that the substance was present in undigested food alone (representing non-absorbed substance). For example, fecal elimination could be occurring following absorption, resulting in the presence in the samples. This is evident from the data for hexachlorobenzene, o-terphenyl and methoxychlor in **Table 35** where concentrations of these substances were measurable in the gut contents up until day 28 of the depuration. Thus, in these cases the measured concentrations in the gut are unlikely to represent undigested food containing the test substances, certainly after day 3.

95. In terms of confounding the results of the studies, the most likely impact from the presence of undigested food would be on the depuration day 1 values. Here, although measures were taken in the study to ensure the guts were cleared of food containing the test substances, it cannot totally be ruled out that some undigested food containing the test substances was still present in the analysed fish. In order to determine the significance of this, the depuration rate constants and ln [C_0] values have been determined omitting the depuration day 1 values (using a plot of ln [fish concentration] against time from day 3 onwards). The results of this analysis are summarised in **Table 36** to **Table 39**, along with the equivalent values obtained over the entire depuration period (taken from **Table 21** to **Table 24**). Unfortunately it was not possible to carry out this analysis using the benzo[a]pyrene data owing to insufficient data points from depuration day 3 onwards.

Table 36 Comparison of overall depuration rate constants and C_0 values for hexachlorobenzene with and without depuration day 1 data

Laboratory	Omitting depuration day 1 values			Including depuration day 1 values		
	k_2 (day^{-1})	ln [C_0]	R^2 value of regression	k_2 (day^{-1})	ln [C_0]	R^2 value of regression
Lab 1	0.0524	1.321	0.78	0.0502	1.275	0.78
Lab 2a – trout	0.0400	1.739	0.86	0.0399	1.736	0.88
Lab 2b – carp (level 1)	0.0649	3.238	0.90	0.0603	3.170	0.90
Lab 2b – carp (level 2)	0.0542	2.501	0.99	0.0561	2.529	0.99
Lab 2b – carp (level 3)	0.0434	0.762	0.61	0.0486	0.839	0.71
Lab 3	0.0508	1.473	0.82	0.0537	1.533	0.85
Lab 4	0.0511	1.525	0.86	0.0517	1.535	0.85
Lab 5	0.0494	2.461	0.47	0.0407	2.282	0.36
Lab 6	0.0584	1.424	0.84	0.0625	1.509	0.87
Lab 7	0.0457	0.631	0.64	0.0491	0.701	0.72
Lab 8	0.0654	1.488	0.93	0.0579	1.332	0.90
Lab 9	Data not yet available					
Lab 10	Data not yet available					

Table 37 Summary of overall depuration rate constants and C_0 values for musk xylene

Laboratory	Omitting depuration day 1 values			Including depuration day 1 values		
	k_2 (day^{-1})	ln [C_o]	R^2 value of regression	k_2 (day^{-1})	ln [C_o]	R^2 value of regression
Lab 1	0.0893	1.438	0.86	0.0904	1.460	0.88
Lab 2a – trout	0.0741	2.354	0.91	0.0734	2.340	0.93
Lab 2b – carp (level 1)	0.145	3.743	0.98	0.140	3.654	0.98
Lab 2b – carp (level 2)	0.124	2.896	0.99	0.131	2.994	0.99
Lab 2b – carp (level 3)	0.111	1.123	0.95	0.111	1.124	0.96
Lab 3	0.0786	2.087	0.87	0.083	2.178	0.90
Lab 4	0.062	1.597	0.70	0.067	1.706	0.75
Lab 5	0.459	-2.832	0.95[a]	0.647	-1.705	0.86
Lab 6	0.100	1.511	0.91	0.105	1.609	0.92
Lab 7	0.099	0.648	0.75	0.105	0.766	0.82
Lab 8	0.097	1.807	0.95	0.0948	1.761	0.95

Note: a) Data for two time points only available.

Table 38 Summary of overall depuration rate constants and C_o values for o-terphenyl

Laboratory	Omitting depuration day 1 values			Including depuration day 1 values		
	k_2 (day^{-1})	ln [C_o]	R^2 value of regression	k_2 (day^{-1})	ln [C_o]	R^2 value of regression
Lab 1	0.0841	1.160	0.78	0.0872	1.224	0.82
Lab 2a – trout	0.0698	1.801	0.61	0.0691	1.786	0.68
Lab 2b – carp (level 1)	0.299	3.581	0.99	0.290	3.444	0.99
Lab 2b – carp (level 2)	0.301	2.074	0.99	0.351	2.443	0.98
Lab 2b – carp (level 3)	0.288	-0.287	0.99	0.297	-0.217	0.99
Lab 3	0.0932	1.414	0.73	0.104	1.614	0.79
Lab 4	0.0749	1.322	0.36	0.0770	1.365	0.43
Lab 5	0.0416	2.342	0.38	0.0445	2.402	0.40
Lab 6	0.130	1.014	0.67	0.133	1.086	0.73
Lab 7	0.0718	0.758	0.70	0.0775	0.875	0.78
Lab 8	0.118	1.572	0.72	0.113	1.473	0.75

Table 39 Summary of overall depuration rate constants and C_o values for methoxychlor

Laboratory	Omitting depuration day 1 values			Including depuration day 1 values		
	k_2 (day^{-1})	ln [C_o]	R^2 value of regression	k_2 (day^{-1})	ln [C_o]	R^2 value of regression
Lab 1	0.130	0.455	0.56	0.150	0.762	0.66
Lab 2a – trout	0.125	2.607	0.72	0.116	2.412	0.74
Lab 2b – carp (level 1)	0.314	2.281	0.93	0.310	2.249	0.97
Lab 2b – carp (level 2)	0.240	1.051	0.99	0.294	1.456	0.97
Lab 2b – carp (level 3)	0.154	-1.125	1[a]	0.264	-0.466	0.89
Lab 3	0.0785	0.70	0.42	0.102	1.176	0.56
Lab 4	0.0775	0.509	0.27	0.0922	0.813	0.38
Lab 5	0.0094	1.631	0.009	0.0046	1.531	0.003
Lab 6	0.211	0.344	0.83	0.225	0.572	0.87
Lab 7	0.152	1.460	0.88	0.148	1.389	0.90
Lab 8	0.103	0.837	0.41	0.110	0.988	0.51

Note: a) Two data points only.

96. The results of this analysis show that in some cases omitting the depuration day 1 value leads to a higher k_2 value (and consequently a higher ln [C_0] value) than when it is included, whereas in other cases the opposite is true[6]. However in most cases, omitting the depuration day 1 value results in a lower correlation coefficient (R^2) for the regression than when it is included (the few exceptions to this occur mainly when the number of time points for which data are available are very low when the day 1 value is omitted). This implies that inclusion of the depuration day 1 values strengthen the regression and suggests that these concentration values are consistent with the concentration values obtained at later depuration times. Overall this analysis does not provide any strong evidence that the presence of undigested food in the gut is skewing the depuration day 1 concentrations and the resulting kinetic calculations.

Estimation of growth corrected depuration rate constants using an alternative method

97. 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 40** to **Table 44** for the ring test data.

98. An alternative method for estimating the growth corrected depuration rate constant has recently been proposed (16), 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

[6] It is important to bear in mind here that within each experiment the food contained all five test substances, therefore the presence of undigested food in the gut, if significant, would be expected to impact all substances in the same way.

method has been used here to obtain a further estimate of the growth corrected depuration rate constant. The values determined are shown in **Table 40** to **Table 44** for the ring test data[7].

99. As can be seen from the data reported, the agreement in the growth rate constant between the two methods is good. As noted in (16), the alternative method generally tends to provide a slightly higher estimate of the k_{2g} value than the normal method for the substances that are more slowly depurated (e.g. hexachlorobenzene and musk xylene). Growth makes a larger relative contribution to the overall depuration for these substances than the more rapidly depurated substances and so this difference between the two methods may result from uncertainties (or assumptions) inherent in the growth correction (for example if growth dilution does not follow strict first order kinetics).

100. Similar to the case with the assimilation efficiency and BMF_L, a measure of the interlaboratory variability in the derived k_{2g} value can be obtained from the mean and relative standard deviation of these values for the trout data (minus Lab 5). These are summarised below for both the rate constant subtraction method and the alternative method.

	Rate constant subtraction		**Alternative method**	
	Mean (day^{-1})	Rel. SD	Mean (day^{-1})	Rel. SD
Hexachlorobenzene	0.016	38%	0.020	35%
Musk xylene	0.052	31%	0.056	27%
o-Terphenyl	0.058	33%	0.062	31%
Methoxychlor	0.099	48%	0.100	44%

101. The carp data were all generated within the same laboratory and so the variability in the mean k_{2g} provides a measure of the intralaboratory variability (taking into account that different concentrations were used in each of the three studies). The mean and relative standard deviation of the k_{2g} values are summarised below.

	Rate constant subtraction		**Alternative method**	
	Mean (day^{-1})	Rel. SD	Mean (day^{-1})	Rel. SD
Hexachlorobenzene	0.022	27%	0.023	32%
Musk xylene	0.094	16%	0.095	17%
o-Terphenyl	0.28	12%	0.27	11%
Methoxychlor	0.26	9%	0.25	15%

102. It is interesting to note that the k_{2g} values obtained with carp are substantially larger than those obtained with trout for methoxychlor and o-terphenyl in particular, but also musk xylene. This indicates faster depuration of these substance in carp compared to trout. The carp data of hexachlorobenzene are generally similar to those for trout.

[7] Some of the ring test data have been considered previously in (16). Some small differences may exist between the k_{growth} values (and hence k_{2g} values) reported in (16) and reported here owing to differences in how the growth rate constant was determined.

Table 40 Summary of growth corrected depuration rate constants (k_{2g}) for hexachlorobenzene

Laboratory	Draft 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)	0.0603	0.0334	0.0269	0.030	0.79	0.009	30.0	0.045
Lab 2b – carp (level 2)	0.0561	0.0334	0.0227	0.024	0.78	0.007	30.7	0.047
Lab 2b – carp (level 3)	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)			0.018*	0.022*				
Standard deviation (all data)			0.007	0.008				
Mean (trout data)			0.017*	0.021*				
Standard deviation (trout data)			0.007	0.008				

Laboratory	Draft 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 minus Lab 5)			0.016*	0.020*				
Standard deviation (trout data minus Lab 5)			0.006	0.007				

Note: * Indicates that the mean values are statistically significantly different using the paired t-test (two-tail) with alpha = 0.05.

Table 41 Summary of growth corrected depuration rate constants (k_{2g}) for musk xylene

Laboratory	Draft 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)	0.14	0.0334	0.1066	0.109	0.98	0.010	9.0	1.6×10^{-3}
Lab 2b – carp (level 2)	0.131	0.0334	0.0976	0.099	0.98	0.007	7.2	8.0×10^{-4}
Lab 2b – carp (level 3)	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)			0.116	0.122				
Standard deviation (all data)			0.172	0.182				
Mean (trout data)			0.125	0.132				
Standard deviation (trout data)			0.205	0.216				

Laboratory	Draft 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 minus Lab 5)			0.052*	0.056*				
Standard deviation (trout data minus Lab 5)			0.016	0.015				

Note: * Indicates that the mean values are statistically significantly different using the paired t-test (two-tail) with alpha = 0.05.

Table 42 Summary of growth corrected depuration rate constants (k_{2g}) for o-terphenyl

Laboratory	Draft 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)	0.29	0.0334	0.2566	0.260	1.00	0.008	3.0	6.2×10^{-5}
Lab 2b – carp (level 2)	0.351	0.0334	0.3176	0.305	0.97	0.040	13.2	0.017
Lab 2b – carp (level 3)	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)			0.116	0.117				
Standard deviation (all data)			0.107	0.102				
Mean (trout data)			0.055*	0.059*				
Standard deviation (trout data)			0.020	0.020				

Laboratory	Draft 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 minus Lab 5)			0.058	0.062*				
Standard deviation (trout data minus Lab 5)			0.019	0.019				

Note: * Indicates that the mean values are statistically significantly different using the paired t-test (two-tail) with alpha = 0.05.

Table 43 Summary of growth corrected depuration rate constants (k_{2g}) for methoxychlor

Laboratory	Draft 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)	0.31	0.0334	0.2766	0.289	0.97	0.037	12.9	0.016
Lab 2b – carp (level 2)	0.294	0.0334	0.2606	0.249	0.94	0.044	17.7	0.030
Lab 2b – carp (level 3)	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)			0.132	0.131				
Standard deviation (all data)			0.094	0.091				
Mean (trout data)			0.085	0.087				
Standard deviation (trout data)			0.048	0.044				

Laboratory	Draft 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 minus Lab 5)			0.099	0.100				
Standard deviation (trout data minus Lab 5)			0.048	0.044				

Table 44 Summary of growth corrected depuration rate constants (k_{2g}) for benzo[a]pyrene

Laboratory	Draft 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)								
Lab 2b – carp (level 2)								
Lab 2b – carp (level 3)								
Lab 3	a	a	a	a	a	a	a	a
Lab 4	a	a	a	a	a	a	a	a
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	a	a	a	a	a	a	a	a
Lab 8	1.179	0.0483	1.1307	1.179	0.87	0.160	13.6	7.9×10^{-5}
Mean (all data)			1.571	1.570				
Standard deviation (all data)			0.518	0.506				
Mean (trout data)			1.571	1.570				
Standard deviation (trout data)			0.518	0.506				

Laboratory	Draft 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 minus Lab 5)			1.451	1.456				
Standard deviation (trout data minus Lab 5)			0.512	0.504				

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

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

103. 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 (in some cases) 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 (and taking into account any actual adjustments made to the amount of feed given) 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. The results of this analysis are summarised in Table 45, along with the assimilation efficiencies obtained using the derived mean feeding rate.

104. The corresponding values for the BMF_L estimated using the mean feeding rate are summarised in **Table 46**.

105. As can be seen by a comparison with the data in **Table 29** and **Table 30**, the derived assimilation efficiencies using the estimated mean feeding rate over the uptake phase are higher than those obtained using the nominal feeding rate. However, the BMF_L obtained is the same regardless of whether the mean feeding rate or the nominal feeding rate is used in the calculation.

106. The reason for this results from the fact that the assimilation efficiency is, in effect, an intermediary value which is obtained by using the feeding rate (I) in the denominator (see paragraph 79). The BMF_L is subsequently obtained by multiplying the assimilation efficiency by the feeding rate, thus the value of I used cancels out in the overall calculation of the BMF_L.

107. It should be noted, however, that this is not the same as saying that the BMF_L is independent of the feeding rate, but rather that within one experimental set of data, the feeding rate cancels out in the calculation of the BMF_L. According to the equations in paragraph 79, if the assimilation efficiency is a constant for a given chemical, the C_o value should be directly proportional to the feeding rate. Thus it is important that the feeding rate remains constant throughout the test. Further, again assuming that the assimilation efficiency is a constant for a given chemical, the BMF_L value is also indicated to be directly proportional to the feeding rate which implies that different BMF_L values should be obtained using different feeding rates. The two remaining studies that are not yet available, run at a lower feeding rate of 1.5%, should give information on this implied relationship.

Table 45 **Estimates of mean feeding rates used and the calculated assimilation efficiency**

Laboratory	Estimated mean feeding rate over the uptake phase	Calculated assimilation efficiency (t = 13 days)					Calculated assimilation efficiency (t = 14 days)				
		HCB	MX	oTP	MC	BaP	HCB	MX	oTP	MC	BaP
Lab 1	0.024	0.62	0.44	0.36	0.17	0.13	0.59	0.42	0.35	0.16	0.13
Lab 2a – trout	0.027	0.88	1.06	0.61	0.72	2.69	0.83	1.01	0.58	0.70	2.69
Lab 2b – carp (level 1)	0.026	0.46	0.61	0.85	0.15		0.44	0.60	0.84	0.14	
Lab 2b – carp (level 2)	0.026	0.45	0.54	0.67	0.11		0.43	0.53	0.67	0.11	
Lab 2b – carp (level 3)	0.026	0.37	0.35	0.19	0.07		0.35	0.34	0.19	0.07	
Lab 3	0.025	0.64	0.78	0.52	0.14		0.61	0.75	0.50	0.13	
Lab 4	0.028	0.75	0.39	0.35	0.11		0.71	0.37	0.34	0.10	
Lab 5	0.027	0.95	2.17	0.70	0.19	5.15	0.90	2.17	0.66	0.18	5.15
Lab 6	0.024	0.96	0.54	0.44	0.18	2.93	0.92	0.52	0.43	0.18	2.93
Lab 7	0.026	0.36	0.27	0.27	0.16		0.34	0.26	0.25	0.16	
Lab 8	0.025	0.72	0.70	0.55	0.17	0.60	0.68	0.67	0.53	0.16	0.60
Mean (all data)		0.65	0.71	0.50	0.20	2.30	0.62	0.70	0.49	0.19	2.30
Standard deviation (all data)		0.22	0.53	0.20	0.18	2.02	0.21	0.53	0.20	0.17	2.02

Laboratory	Estimated mean feeding rate over the uptake phase	Calculated assimilation efficiency (t = 13 days)					Calculated assimilation efficiency (t = 14 days)				
		HCB	MX	oTP	MC	BaP	HCB	MX	oTP	MC	BaP
Mean (all data minus Lab 5)		0.62	0.57	0.48	0.20	1.59	0.59	0.55	0.47	0.19	1.59
Standard deviation (all data minus Lab 5))		0.21	0.23	0.20	0.19	1.43	0.20	0.22	0.20	0.18	1.43
Mean (trout data minus Lab 5)		0.70	0.60	0.44	0.23	1.59	0.67	0.57	0.43	0.23	1.59
Standard deviation (trout data minus Lab 5)		0.19	0.27	0.12	0.22	1.43	0.18	0.26	0.12	0.21	1.43
Mean (carp data)		0.43	0.50	0.57	0.11		0.41	0.49	0.57	0.11	
Standard deviation (carp data)		0.05	0.14	0.34	0.04		0.05	0.13	0.34	0.04	

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

Table 46 **Estimates of BMF_L using the mean feeding rate (normalised using the mean fish lipid over the entire test duration)**

Laboratory	Estimated mean feeding rate over the uptake phase	BMF_L (t = 13 days)					BMF_L (t = 14 days)				
		HCB	MX	oTP	MC	BaP	HCB	MX	oTP	MC	BaP
Lab 1	0.024	2.00	0.36	0.31	0.06	0.006	1.90	0.34	0.30	0.06	0.006
Lab 2a – trout	0.027	1.89	0.94	0.59	0.37	0.07	1.79	0.90	0.56	0.36	0.07
Lab 2b – carp (level 1)	0.026	1.30	0.43	0.25	0.04		1.24	0.42	0.25	0.04	
Lab 2b – carp (level 2)	0.026	1.36	0.38	0.14	0.03		1.30	0.37	0.14	0.03	
Lab 2b – carp (level 3)	0.026	1.69	0.31	0.05	0.02		1.60	0.30	0.05	0.02	
Lab 3	0.025	3.71	1.12	0.48	0.13		3.53	1.08	0.47	0.13	
Lab 4	0.028	3.82	0.91	0.62	0.13		3.63	0.87	0.59	0.13	
Lab 5	0.027	1.14	0.10	0.73	-0.51	0.08	1.08	0.10	0.69	-0.48	0.08
Lab 6	0.024	3.63	0.77	0.45	0.09	0.17	3.46	0.74	0.43	0.09	0.17
Lab 7	0.026	1.87	0.35	0.54	0.13		1.78	0.33	0.52	0.13	
Lab 8	0.025	4.77	0.95	0.54	0.17	0.03	4.54	0.92	0.52	0.17	0.03
Mean (all data)		2.47	0.60	0.43	0.06	0.07	2.35	0.58	0.41	0.06	0.07
Standard deviation (all data)		1.26	0.34	0.21	0.21	0.06	1.20	0.33	0.20	0.20	0.06

Laboratory	Estimated mean feeding rate over the uptake phase	BMF_L (t = 13 days)					BMF_L (t = 14 days)				
		HCB	MX	oTP	MC	BaP	HCB	MX	oTP	MC	BaP
Mean (all data minus Lab 5)		2.60	0.65	0.40	0.12	0.07	2.48	0.63	0.38	0.12	0.07
Standard deviation (all data minus Lab 5)		1.24	0.31	0.20	0.10	0.07	1.19	0.30	0.19	0.10	0.07
Mean (trout data minus Lab 5)		3.10	0.77	0.50	0.16	0.07	2.95	0.74	0.48	0.15	0.07
Standard deviation (trout data minus Lab 5)		1.16	0.30	0.10	0.10	0.07	1.11	0.29	0.10	0.10	0.07
Mean (carp data)		1.45	0.38	0.15	0.03		1.38	0.37	0.15	0.03	
Standard deviation (carp data)		0.21	0.06	0.10	0.01		0.20	0.06	0.10	0.01	

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

Differences in sampling schedule at start of depuration between the ring test and that proposed in the draft OECD 305 Test method

108. There are a number of methodological differences between the protocol followed in the ring test and that proposed in the draft OECD 305 Test Methodology. The most important of these, in terms of interpretation of the results, relates to the sampling at the early stages of depuration and the calculation of C_0.

109. For the ring test, the Standard Operating Procedure used sampled fish on depuration day 1. For these samples, the fish were fed spiked diet on day 13 of the uptake phase, followed by a feeding of clean food approximately five hours after their last treatment (i.e. also on day 13). The fish were then sacrificed the following morning (a similar approach was also used for the optional fish sampled on uptake day 3). The aim of this was to ensure sufficient time had passed to enable any undigested food containing the test substances to have cleared the guts before analysis. For the remaining time intervals during the depuration period the fish were sampled just prior to the daily feeding. For the analysis here, day 13 of uptake corresponds to day 0 of depuration and the C_0 values have been estimated for this time period.

110. The latest draft of the OECD 305 Test Methodology suggests a different sampling strategy for the fish at the start of the depuration. Here an experimental day is defined as starting at the time of feeding and ending shortly before the time of next feeding (approximately 24 hours later). Thus on the last day of uptake, the fish are fed a diet of spiked food and 24 hours later (designated day 0 of depuration) the fish are fed a diet of clean food. The sampling of fish should occur at the end of an experimental day. Thus the samples taken on day 1 will occur shortly before the second feeding with clean diet. Accrding to the TG, sample points from the depuration phase (i.e. beginning with the sample taken just before the second feeding with clean food) are used to derive C_0 and k_2; the measured C_0 (the sample taken shortly before the first feeding with clean food) is used as a comparison and to check if uptake has occurred during the uptake phase (with removal of the GI tract).

111. This difference in approach was introduced to the ring test in an attempt to remove an additional source of variability. The deviation was not included in the TG because it added extra complexity to the test, and possibly could introduce more variability than its omission in practice (although it appeared to have little influence on results). These differences mean that the C_0 in the draft OECD 305 Test Methodology may not correspond exactly with the C_0 in the ring test. Further, the difference in the times of feeding of the first clean diet between the ring test and that proposed in the draft OECD 305 Test Methodology means that the ring test data cannot be used to test the appropriateness of the proposed sampling close to the start of depuration. In hindsight this situation is less than ideal; nonetheless, the analysis carried out above (see paragraphs 95 and 96, and Table 36 - Table 39) suggests that the effect of this difference is likely only to be small (omission of the first depuration sampling point from the ring test data analysis did not provide any strong evidence that the studies' derived parameters were skewed, in this case by the possible presence of undigested food in the gut). It should be noted that the protocol followed in the OECD 305 Test Methodology was more commonly used in the literature, and also in the submission of the method to the EU PBT working group in 2004.

HISTORICAL BMF DATA – INTRALABORATORY VARIABILITY

112. In order to investigate the possible intralaboratory variability in the proposed OECD 305 method historic data from studies using the proposed method (or a variation of the proposed method) with hexachlorobenzene have been kindly provided by Lab 6. The results from analyses of these data are summarised in Table 47 (using the stated uptake period in the dataset (t)) and Table 48 (using t+1 for the uptake period).

113. As can be seen from the data, the relative standard deviation in the growth corrected depuration rate constant (k_{2g}) and the assimilation efficiency are both around 26-27% for the trout data (omitting the first study which is considered to be an outlier). However, the relative standard deviation around the BMF_L is higher at around 42%. In all cases, the BMF_L for hexachlorobenzene is >1.

114. A summary of studies carried out by Lab 2 with hexachlorobenzene have also kindly been provided. It was not possible to re-analyse these data as part of this current evaluation and so the results as provided by Lab 2 are presented in Table 49. The tests were carried out using carp and exposure was to either food containing hexachlorobenzene alone or food containing a mixture of hexachlorobenzene and other substances. From these data, the relative standard deviations are around 32% in the k_{2g}, 12% in the assimilation efficiency and 22% in the BMF_L. Again the BMF_L values are all >1 for hexachlorobenzene.

Table 47 **Summary of data for hexachlorobenzene from Lab 6 analysed using the stated uptake period (t)**

Species	Initial fish weight (g) at beginning of depuration period	Conc. in food (µg/g)	Mean fish lipid content (%)	Food lipid content (%)	Nominal feeding rate (% w/w)	Growth rate constant (day^{-1})	Uptake period (days)	Depur-ation period (days)	k_{2g} (day^{-1})	Assimil-ation efficiency	BMF_L
Rainbow trout[b]	*1.0*	*100*	*2.7*	*15.6*	*3*	*0.033*	*10*	*14*	*0.050*	*1.32*	*4.38*
Rainbow trout	2.1	26.5	4.0	15.6	3	0.030	13	21	0.026	0.65	2.97
Rainbow trout	0.9	8.0	2.4	15.6	3	0.052	10	10	0.038	0.55	2.83
Rainbow trout	1.1	24.3	2.6	15.6	3	0.035	13	21	0.056	0.64	2.04
Rainbow trout	1.4	28.6	2.9	15.6	3	0.028	12	21	0.031	0.56	2.90
Rainbow trout	1.8	22.3	3.1	15.6	3	0.036	14	21	0.035	0.30	1.30
Rainbow trout	1.9	26.9	5.1	15.6	3	0.022	11	21	0.037	0.47	1.16
Rainbow trou	1.3	24.6	3.0	15.6	3	0.039	10	21	0.036	0.47	1.28
Rainbow trout[a]	1.6	22.1	3.8	15.6	3	0.037	13	28	0.026	0.77	3.63
Carp	*3.4*	*28.2*	*3.2*	*15.6*	*3*	*0.016*	*11*	*21*	*0.032*	*0.19*	*0.88*
Mean (omitting first data point[b] and carp data)									0.036	0.55	2.26
Standard deviation									0.010	0.14	0.95
Relative standard deviation (%)									26.9	25.9	41.8

Note: a) Data from the ring test.
b) The data from the first study reported are considered to be outliers as the assimilation efficiency is above 1.

Table 48 **Summary of data for hexachlorobenzene from Lab 6 analysed using the stated uptake period+1 (t+1)**

Species	Initial fish weight (g) at beginning of depuration period	Conc. in food (µg/g)	Mean fish lipid content (%)	Food lipid content (%)	Nominal feeding rate (% w/w)	Growth rate constant (day^{-1})	Uptake period (days)	Depur-ation period (days)	k_{2g} (day^{-1})	Assimil-ation efficiency	BMF_L
Rainbow trout[b]	*1.0*	*100*	*2.7*	*15.6*	*3*	*0.033*	*10 (+1)*	*14*	*0.050*	*1.24*	*4.13*
Rainbow trout	2.1	26.5	4.0	15.6	3	0.030	13 (+1)	21	0.026	0.62	2.83
Rainbow trout	0.9	8.0	2.4	15.6	3	0.052	10 (+1)	10	0.038	0.52	2.67
Rainbow trout	1.1	24.3	2.6	15.6	3	0.035	13 (+1)	21	0.056	0.62	1.96
Rainbow trout	1.4	28.6	2.9	15.6	3	0.028	12 (+1)	21	0.031	0.53	2.75
Rainbow trout	1.8	22.3	3.1	15.6	3	0.036	14 (+1)	21	0.035	0.29	1.25
Rainbow trout	1.9	26.9	5.1	15.6	3	0.022	11 (+1)	21	0.037	0.44	1.09
Rainbow trou	1.3	24.6	3.0	15.6	3	0.039	10 (+1)	21	0.036	0.44	1.22
Rainbow trout[a]	1.6	22.1	3.8	15.6	3	0.037	13 (+1)	28	0.026	0.73	3.46
Carp	*3.4*	*28.2*	*3.2*	*15.6*	*3*	*0.016*	*11 (+1)*	*21*	*0.032*	*0.18*	*0.83*
Mean (omitting first data point[b] and carp data)									0.036	0.52	2.15
Standard deviation									0.010	0.14	0.90
Relative standard deviation (%)									26.9	26.1	41.7

Note: a) Data from the ring test.
b) The data from the first study reported are considered to be outliers as the assimilation efficiency is above 1.

Table 49 **Summary of data for hexachlorobenzene from Lab 2 for carp**

Exposure method	Initial fish weight (g) at beginning of depuration period	Conc. in food (µg/g)	Mean fish lipid content (%)	Food lipid content (%)	Nominal feeding rate (% w/w)	Growth rate constant (day^{-1})	Uptake period (days)	Depur-ation period (days)	k_{2g} (day^{-1})	Assimil-ation efficiency	BMF_L
Single diet	Not given	96.8	4.51	15.4	0.03	0.0231	10	Not given	0.069	0.86	1.28
Single diet	Not given	106	4.48	15.4	0.03	0.0171	10	Not given	0.070	0.76	1.12
Single diet	Not given	94.1	5.34	15.4	0.03	0.0291	10	Not given	0.056	0.78	1.21
Single diet	Not given	101	4.84	15.4	0.03	0.0298	10	Not given	0.039	0.73	1.80
Single diet	Not given	103	4.83	15.4	0.03	0.0288	10	Not given	0.039	0.75	1.83
Combined diet	Not given	24.1	6.75	16.6	0.03	0.0238	10	Not given	0.033	0.59	1.31
Mean									0.051	0.74	1.42
Standard deviation									0.016	0.09	0.31
Relative standard deviation (%)									31.6	11.8	21.7

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

Estimation of BCF from the ring test data

115. 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 (17). 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 50** to **Table 62** for the estimates using the overall depuration rate constant (resulting in a BCF that is not growth corrected). The equivalent calculations using the growth corrected depuration rate constant are shown in Appendix 3.

116. The methods used for the estimates of k_1 were as follows (see (17) for further details).

Method 1	Sijm *et al* 1995 (30).
Method 2	Omega/Hendriks, 2001 (31)
Method 6	QEAFDCHN/Thomann 1989 (32)
Method 7	BASS/Barber, 2001 (33)
Method 8	FGETS/Barber *et al*., 2001 (33)
Method 9	Erickson and McKim, 1990a (34)
Method 10	Erickson and McKim, 1990b (35)
Method 13	Hayton and Barron 1990 (36)
Method 15	Streit and Sire 1993 (37)
Method 17	Barber, 2003 observed (38)
Method 18	Barber 2003 calibrated (38)
Method 21	Spacie and Hamelink 1982 (39)
Method 22	Tolls and Sijm 1995 (40)

Table 50 **Summary of estimated BCF from the ring test using Method 1** (Sijm et al 1995 (30))

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 1	1.25	2.1	484	410	9,645	8,169	5,356	4,537	5,552	4,703	3,228	2,734	491	416
Lab 2a – trout	8.41	9.78	263	251	6,593	6,282	3,584	3,415	3,807	3,628	2,268	2,161	126	120
Lab 2b – carp (level 1)	5.42	7.11	303	278	5,021	4,603	2,163	1,983	1,044	957	977	895		
Lab 2b – carp (level 2)	5.42	7.11	303	278	5,397	4,948	2,311	2,119	863	791	1,030	944		
Lab 2b – carp (level 3)	5.42	7.11	303	278	6,230	5,712	2,728	2,501	1,019	935	1,147	1,051		
Lab 3	1.95	3.64	420	344	7,820	6,404	5,060	4,144	4,038	3,307	4,117	3,372		
Lab 4	1.17	2.32	495	397	9,565	7,683	7,381	5,929	6,422	5,159	5,364	4,308		
Lab 5	6.77	7.84	282	269	6,928	6,610	436	416	6,337	6,046	61,300	58,488	136	130
Lab 6	0.72	1.61	578	446	9,242	7,144	5,501	4,252	4,343	3,357	2,567	1,984	343	265
Lab 7	1.2	1.78	491	432	9,990	8,806	4,672	4,118	6,329	5,579	3,314	2,922		
Lab 8	1.24	2.08	485	411	8,384	7,105	5,120	4,339	4,296	3,640	4,413	3,740	412	349
Mean BCF all data					7,711	6,679	4,028	3,432	4,005	3,464	8,157	7,509	302	256
Standard deviation					1,784	1,290	1,976	1,540	2,165	1,883	17,685	16,947	164	131
Mean BCF for trout (minus Lab 5 data)					8,749	7,371	5,239	4,391	4,970	4,196	3,610	3,032	343	287

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
Standard deviation					1,218	916	1,139	763	1,108	933	1,088	837	157	128
Mean BCF for carp					5,549	5,088	2,401	2,201	975	894	1,051	964		
Standard deviation					619	567	293	269	98	90	87	80		

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/day 14).

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

Table 51 **Summary of estimated BCF from the ring test using Method 2** (Omega/Hendriks, 2001 (31))

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 1	1.25	2.1	c	c	7,516	6,602	3,822	3,357	4,284	3,763	2,383	2,093	386	339
Lab 2a – trout	8.41	9.78	c	c	5,871	5,654	2,923	2,814	3,357	3,233	1,914	1,843	113	109
Lab 2b – carp (level 1)	5.42	7.11	c	c	4,336	4,052	1,710	1,598	893	834	799	747		
Lab 2b – carp (level 2)	5.42	7.11	c	c	4,661	4,355	1,828	1,708	738	689	843	787		
Lab 2b – carp (level 3)	5.42	7.11	c	c	5,380	5,027	2,157	2,016	872	815	938	877		
Lab 3	1.95	3.64	c	c	6,287	5,379	3,725	3,187	3,214	2,750	3,136	2,683		
Lab 4	1.17	2.32	c	c	7,420	6,252	5,243	4,418	4,933	4,157	3,942	3,322		
Lab 5	6.77	7.84	c	c	6,077	5,858	350	337	5,503	5,305	50,945	49,110	121	117
Lab 6	0.72	1.61	c	c	6,929	5,667	3,777	3,089	3,224	2,637	1,824	1,491	260	212
Lab 7	1.2	1.78	c	c	7,763	7,034	3,324	3,012	4,870	4,413	2,440	2,211		
Lab 8	1.24	2.08	c	c	6,529	5,737	3,652	3,209	3,313	2,911	3,257	2,862	324	284
Mean BCF all data					6,252	5,601	2,955	2,613	3,200	2,864	6,584	6,184	241	212
Standard deviation					1,137	889	1,346	1,109	1,706	1,552	14,749	14,263	122	102
Mean BCF for trout (minus Lab 5 data)					6,902	6,046	3,781	3,298	3,885	3,409	2,699	2,358	271	236

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
Standard deviation					703	601	719	522	787	707	774	632	117	100
Mean BCF for carp					4,792	4,478	1,898	1,774	834	779	860	804		
Standard deviation					534	499	232	216	84	79	71	67		

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/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, 214 to 410 l kg^{-1} for Methoxychlor and 228 to 437 l kg^{-1} day^{-1} for benzo[a]pyrene.

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

Table 52 **Summary of estimated BCF from the ring test using Method 6** (QEAFDCHN/Thomann 1989 (32))

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 1	1.25	2.1	757	665	15,072	13,238	8,369	7,351	8,677	7,621	5,044	4,430	767	674
Lab 2a – trout	8.41	9.78	470	452	11,774	11,338	6,400	6,163	6,798	6,547	4,050	3,900	224	216
Lab 2b – carp (level 1)	5.42	7.11	524	490	8,695	8,125	3,745	3,499	1,808	1,689	1,691	1,580		
Lab 2b – carp (level 2)	5.42	7.11	524	490	9,346	8,733	4,002	3,740	1,494	1,396	1,783	1,666		
Lab 2b – carp (level 3)	5.42	7.11	524	490	10,788	10,081	4,724	4,414	1,765	1,650	1,986	1,856		
Lab 3	1.95	3.64	677	579	12,607	10,786	8,156	6,978	6,510	5,569	6,637	5,678		
Lab 4	1.17	2.32	769	648	14,878	12,538	11,481	9,675	9,990	8,418	8,343	7,031		
Lab 5	6.77	7.84	496	478	12,186	11,747	767	739	11,145	10,744	107,816	103,933	240	231
Lab 6	0.72	1.61	868	710	13,896	11,363	8,271	6,764	6,530	5,340	3,860	3,156	516	422
Lab 7	1.2	1.78	764	693	15,567	14,106	7,280	6,596	9,863	8,937	5,165	4,680		
Lab 8	1.24	2.08	758	666	13,094	11,505	7,997	7,027	6,709	5,895	6,892	6,056	643	565
Mean BCF all data					12,537	11,233	6,472	5,722	6,481	5,800	13,933	13,088	478	422
Standard deviation					2,279	1,783	2,948	2,429	3,456	3,143	31,214	30,185	242	202

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
Mean BCF for trout (minus Lab 5 data)					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
Mean BCF for carp					9,610	8,979	4,157	3,884	1,689	1,578	1,820	1,701		
Standard deviation					1,071	1,001	507	474	170	159	151	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/day 14).

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

Table 53 **Summary of estimated BCF from the ring test using Method 7** (BASS/Barber, 2001 (33))

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 1	1.25	2.1	669	615	13,317	12,250	7,395	6,803	7,667	7,052	4,457	4,100	678	624
Lab 2a – trout	8.41	9.78	492	480	12,327	12,031	6,701	6,540	7,118	6,947	4,240	4,138	235	229
Lab 2b – carp (level 1)	5.42	7.11	528	505	8,754	8,380	3,771	3,609	1,820	1,742	1,703	1,630		
Lab 2b – carp (level 2)	5.42	7.11	528	505	9,410	9,008	4,030	3,857	1,504	1,440	1,796	1,719		
Lab 2b – carp (level 3)	5.42	7.11	528	505	10,862	10,398	4,756	4,552	1,777	1,701	2,000	1,914		
Lab 3	1.95	3.64	622	563	11,589	10,481	7,498	6,781	5,984	5,412	6,101	5,518		
Lab 4	1.17	2.32	676	605	13,069	11,705	10,085	9,032	8,775	7,859	7,328	6,564		
Lab 5	6.77	7.84	509	497	12,514	12,222	787	769	11,446	11,178	110,723	108,138	247	241
Lab 6	0.72	1.61	731	642	11,690	10,269	6,958	6,113	5,493	4,826	3,247	2,853	434	381
Lab 7	1.2	1.78	673	632	13,705	12,862	6,409	6,015	8,683	8,149	4,547	4,267		
Lab 8	1.24	2.08	669	616	11,561	10,637	7,061	6,497	5,924	5,450	6,085	5,599	568	522
Mean BCF all data					11,709	10,931	5,950	5,506	6,017	5,614	13,839	13,313	432	399
Standard deviation					1,556	1,420	2,472	2,193	3,230	3,077	32,187	31,494	195	173

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
Mean BCF for trout (minus Lab 5 data)					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
Mean BCF for carp					9,675	9,262	4,185	4,006	1,701	1,628	1,833	1,754		
Standard deviation					1,079	1,032	511	489	172	164	152	145		

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/day 14).

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

Table 54 **Summary of estimated BCF from the ring test using Method 8** (FGETS/Barber *et al*., 2001 (33))

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 1	1.25	2.1	654	595	13,019	11,846	7,229	6,578	7,495	6,819	4,357	3,964	663	603
Lab 2a – trout	8.41	9.78	462	449	11,578	11,264	6,294	6,123	6,685	6,504	3,982	3,874	221	215
Lab 2b – carp (level 1)	5.42	7.11	500	476	8,298	7,899	3,574	3,402	1,726	1,642	1,614	1,536		
Lab 2b – carp (level 2)	5.42	7.11	500	476	8,920	8,490	3,820	3,636	1,426	1,357	1,702	1,620		
Lab 2b – carp (level 3)	5.42	7.11	500	476	10,296	9,800	4,508	4,291	1,685	1,604	1,895	1,804		
Lab 3	1.95	3.64	603	538	11,224	10,019	7,262	6,482	5,795	5,173	5,909	5,275		
Lab 4	1.17	2.32	661	584	12,794	11,295	9,872	8,716	8,590	7,584	7,174	6,334		
Lab 5	6.77	7.84	481	468	11,807	11,496	743	723	10,799	10,514	104,467	101,714	233	226
Lab 6	0.72	1.61	723	624	11,561	9,986	6,881	5,944	5,433	4,693	3,211	2,774	429	371
Lab 7	1.2	1.78	658	613	13,409	12,481	6,271	5,836	8,496	7,907	4,449	4,141		
Lab 8	1.24	2.08	654	596	11,304	10,288	6,904	6,284	5,792	5,272	5,950	5,415	555	505
Mean BCF all data					11,292	10,442	5,760	5,274	5,811	5,370	13,155	12,586	420	384
Standard deviation					1,604	1,405	2,444	2,130	3,106	2,931	30,340	29,604	195	171

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
Mean BCF for trout (minus Lab 5 data)					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
Mean BCF for carp					9,171	8,729	3,967	3,776	1,612	1,534	1,737	1,653		
Standard deviation					1,022	973	484	461	163	155	144	137		

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/day 14).

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

Table 55 **Summary of estimated BCF from the ring test using Method 9** (Erickson and McKim, 1990a (34))

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 1	1.25	2.1	649	599	12,935	11,923	7,183	6,621	7,447	6,864	4,329	3,990	659	607
Lab 2a – trout	8.41	9.78	481	470	12,065	11,782	6,558	6,405	6,967	6,803	4,150	4,053	230	225
Lab 2b – carp (level 1)	5.42	7.11	516	494	8,553	8,197	3,684	3,530	1,779	1,704	1,664	1,594		
Lab 2b – carp (level 2)	5.42	7.11	516	494	9,194	8,810	3,937	3,773	1,469	1,408	1,754	1,681		
Lab 2b – carp (level 3)	5.42	7.11	516	494	10,612	10,170	4,647	4,453	1,737	1,664	1,954	1,872		
Lab 3	1.95	3.64	606	549	11,277	10,224	7,296	6,615	5,823	5,279	5,937	5,383		
Lab 4	1.17	2.32	656	589	12,691	11,398	9,793	8,795	8,521	7,653	7,116	6,391		
Lab 5	6.77	7.84	498	487	12,238	11,959	770	752	11,193	10,938	108,276	105,810	241	236
Lab 6	0.72	1.61	708	624	11,329	9,985	6,744	5,943	5,324	4,692	3,147	2,774	420	371
Lab 7	1.2	1.78	654	614	13,310	12,511	6,224	5,850	8,433	7,926	4,416	4,151		
Lab 8	1.24	2.08	650	599	11,229	10,353	6,858	6,323	5,754	5,305	5,911	5,450	551	508
Mean BCF all data					11,403	10,665	5,790	5,369	5,859	5,476	13,514	13,013	420	389
Standard deviation					1,499	1,378	2,397	2,133	3,148	3,004	31,481	30,820	189	168

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
Mean BCF for trout (minus Lab 5 data)					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
Mean BCF for carp					9,453	9,059	4,089	3,919	1,662	1,592	1,791	1,716		
Standard deviation					1,054	1,010	499	478	168	161	148	142		

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/day 14).

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

Table 56 **Summary of estimated BCF from the ring test using Method 10** (Erickson and McKim, 1990b (35))

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 1	1.25	2.1	541	481	10,784	9,581	5,989	5,321	6,208	5,516	3,609	3,207	549	488
Lab 2a – trout	8.41	9.78	351	339	8,786	8,488	4,776	4,614	5,073	4,901	3,022	2,920	167	162
Lab 2b – carp (level 1)	5.42	7.11	387	364	6,426	6,040	2,768	2,602	1,336	1,256	1,250	1,175		
Lab 2b – carp (level 2)	5.42	7.11	387	364	6,907	6,492	2,958	2,780	1,104	1,038	1,318	1,239		
Lab 2b – carp (level 3)	5.42	7.11	387	364	7,973	7,494	3,491	3,281	1,305	1,226	1,468	1,380		
Lab 3	1.95	3.64	489	424	9,110	7,901	5,894	5,112	4,704	4,080	4,796	4,160		
Lab 4	1.17	2.32	550	470	10,631	9,094	8,203	7,018	7,138	6,106	5,961	5,100		
Lab 5	6.77	7.84	368	356	9,050	8,752	569	551	8,277	8,005	80,069	77,435	178	172
Lab 6	0.72	1.61	614	511	9,823	8,176	5,847	4,867	4,616	3,842	2,729	2,271	365	303
Lab 7	1.2	1.78	546	499	11,129	10,172	5,204	4,757	7,051	6,445	3,692	3,375		
Lab 8	1.24	2.08	542	482	9,367	8,325	5,721	5,085	4,800	4,266	4,931	4,382	460	409
Mean BCF all data					9,090	8,229	4,675	4,181	4,692	4,244	10,259	9,695	344	307
Standard deviation					1,524	1,231	2,080	1,745	2,499	2,302	23,206	22,506	169	143

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
Mean BCF for trout (minus Lab 5 data)					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
Mean BCF for carp					7,102	6,676	3,072	2,888	1,248	1,173	1,345	1,264		
Standard deviation					792	744	375	352	126	118	111	105		

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/day 14).

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

Table 57 **Summary of estimated BCF from the ring test using Method 13** (Hayton and Barron 1990 (36))

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 1	1.25	2.1	482	436	9,605	8,676	5,334	4,818	5,529	4,995	3,214	2,904	489	442
Lab 2a – trout	8.41	9.78	332	322	8,317	8,074	4,521	4,389	4,802	4,662	2,861	2,777	158	154
Lab 2b – carp (level 1)	5.42	7.11	362	343	5,998	5,687	2,583	2,450	1,247	1,183	1,167	1,106		
Lab 2b – carp (level 2)	5.42	7.11	362	343	6,447	6,113	2,761	2,618	1,030	977	1,230	1,166		
Lab 2b – carp (level 3)	5.42	7.11	362	343	7,442	7,056	3,258	3,090	1,218	1,155	1,370	1,299		
Lab 3	1.95	3.64	442	391	8,229	7,282	5,324	4,711	4,249	3,760	4,332	3,834		
Lab 4	1.17	2.32	488	427	9,448	8,261	7,290	6,375	6,343	5,547	5,298	4,632		
Lab 5	6.77	7.84	346	336	8,507	8,266	535	520	7,781	7,560	75,271	73,137	168	163
Lab 6	0.72	1.61	537	459	8,595	7,341	5,116	4,370	4,039	3,450	2,388	2,039	319	272
Lab 7	1.2	1.78	486	450	9,899	9,163	4,629	4,285	6,271	5,805	3,284	3,040		
Lab 8	1.24	2.08	483	436	8,340	7,536	5,094	4,603	4,274	3,862	4,390	3,967	410	370
Mean BCF all data					8,257	7,587	4,222	3,839	4,253	3,905	9,528	9,082	309	280
Standard deviation					1,231	1,051	1,817	1,566	2,269	2,126	21,847	21,278	146	126

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
Mean BCF for trout (minus Lab 5 data)					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
Mean BCF for carp					6,629	6,285	2,868	2,719	1,165	1,105	1,256	1,191		
Standard deviation					739	701	350	332	118	111	104	99		

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/day 14).

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

Table 58 **Summary of estimated BCF from the ring test using Method 15** (Streit and Sire 1993 (37))

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 1	1.25	2.1	394	363	7,844	7,227	4,356	4,013	4,516	4,160	2,625	2,418	399	368
Lab 2a – trout	8.41	9.78	291	284	7,302	7,130	3,969	3,876	4,216	4,117	2,512	2,453	139	136
Lab 2b – carp (level 1)	5.42	7.11	312	299	5,179	4,962	2,231	2,137	1,077	1,032	1,007	965		
Lab 2b – carp (level 2)	5.42	7.11	312	299	5,567	5,333	2,384	2,284	890	852	1,062	1,018		
Lab 2b – carp (level 3)	5.42	7.11	312	299	6,426	6,156	2,814	2,695	1,052	1,007	1,183	1,133		
Lab 3	1.95	3.64	367	333	6,835	6,193	4,422	4,007	3,529	3,198	3,598	3,261		
Lab 4	1.17	2.32	398	357	7,696	6,907	5,939	5,330	5,167	4,638	4,316	3,873		
Lab 5	6.77	7.84	302	295	7,408	7,238	466	455	6,776	6,620	65,547	64,045	146	143
Lab 6	0.72	1.61	430	378	6,874	6,053	4,092	3,603	3,230	2,845	1,909	1,681	255	225
Lab 7	1.2	1.78	396	372	8,071	7,584	3,774	3,546	5,114	4,805	2,678	2,516		
Lab 8	1.24	2.08	394	363	6,809	6,275	4,159	3,832	3,489	3,215	3,584	3,303	334	308
Mean BCF all data					6,910	6,460	3,510	3,253	3,551	3,317	8,184	7,879	255	236
Standard deviation					911	836	1,454	1,293	1,907	1,819	19,057	18,654	115	102

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
Mean BCF for trout (minus Lab 5 data)					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
Mean BCF for carp					5,724	5,484	2,476	2,372	1,006	964	1,084	1,039		
Standard deviation					638	611	302	289	102	97	90	86		

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/day 14).

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

Table 59 **Summary of estimated BCF from the ring test using Method 17** (Barber, 2003 observed (38))

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 1	1.25	2.1	426	384	8,483	7,659	4,711	4,253	4,883	4,409	2,839	2,563	432	390
Lab 2a – trout	8.41	9.78	293	284	7,331	7,116	3,985	3,868	4,233	4,109	2,522	2,448	140	136
Lab 2b – carp (level 1)	5.42	7.11	319	302	5,289	5,014	2,278	2,160	1,100	1,043	1,029	975		
Lab 2b – carp (level 2)	5.42	7.11	319	302	5,685	5,389	2,435	2,308	909	861	1,085	1,028		
Lab 2b – carp (level 3)	5.42	7.11	319	302	6,563	6,221	2,873	2,724	1,074	1,018	1,208	1,145		
Lab 3	1.95	3.64	390	345	7,265	6,424	4,700	4,156	3,751	3,317	3,825	3,382		
Lab 4	1.17	2.32	431	377	8,345	7,292	6,439	5,627	5,603	4,896	4,679	4,089		
Lab 5	6.77	7.84	305	297	7,501	7,287	472	458	6,860	6,665	66,366	64,475	148	144
Lab 6	0.72	1.61	475	405	7,595	6,482	4,521	3,858	3,569	3,046	2,110	1,801	282	241
Lab 7	1.2	1.78	429	397	8,743	8,089	4,088	3,783	5,539	5,125	2,900	2,684		
Lab 8	1.24	2.08	427	385	7,366	6,653	4,499	4,063	3,774	3,409	3,877	3,502	362	327
Mean BCF all data					7,288	6,693	3,727	3,387	3,754	3,445	8,404	8,008	273	247
Standard deviation					1,091	929	1,606	1,382	2,003	1,876	19,262	18,757	129	112

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
Mean BCF for trout (minus Lab 5 data)					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
Mean BCF for carp					5,846	5,542	2,529	2,397	1,027	974	1,107	1,050		
Standard deviation					652	618	309	292	104	98	92	87		

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/day 14).

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

Table 60 **Summary of estimated BCF from the ring test using Method 18** (Barber 2003 calibrated (38))

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 1	1.25	2.1	614	496	12,229	9,886	6,784	5,484	7,039	5,691	4,090	3,306	623	503
Lab 2a – trout	8.41	9.78	281	264	7,042	6,620	3,824	3,594	4,066	3,822	2,421	2,275	134	126
Lab 2b – carp (level 1)	5.42	7.11	336	301	5,579	4,992	2,400	2,148	1,160	1,038	1,085	970		
Lab 2b – carp (level 2)	5.42	7.11	336	301	5,997	5,366	2,565	2,295	958	857	1,144	1,023		
Lab 2b – carp (level 3)	5.42	7.11	336	301	6,923	6,194	3,028	2,709	1,133	1,013	1,274	1,139		
Lab 3	1.95	3.64	512	396	9,527	7,376	6,157	4,767	4,919	3,808	5,012	3,881		
Lab 4	1.17	2.32	631	476	12,201	9,215	9,404	7,103	8,191	6,187	6,837	5,164		
Lab 5	6.77	7.84	307	289	7,546	7,105	474	446	6,901	6,498	66,720	62,824	149	140
Lab 6	0.72	1.61	770	553	12,316	8,855	7,323	5,265	5,787	4,160	3,419	2,458	457	329
Lab 7	1.2	1.78	624	531	12,714	10,817	5,939	5,052	8,054	6,852	4,215	3,586		
Lab 8	1.24	2.08	616	498	10,638	8,605	6,490	5,250	5,450	4,409	5,596	4,526	522	423
Mean BCF all data					9,337	7,730	4,944	4,010	4,878	4,030	9,256	8,287	377	304
Standard deviation					2,796	1,887	2,661	1,936	2,736	2,232	19,154	18,144	223	168

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
Mean BCF for trout (minus Lab 5 data)					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
Mean BCF for carp					6,166	5,517	2,665	2,384	1,084	970	1,167	1,044		
Standard deviation					687	615	325	291	109	98	97	86		

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/day 14).

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

Table 61 **Summary of estimated BCF from the ring test using Method 21** (Spacie and Hamelink 1982 (39))

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 1	664	502	619	533	761	13,231	5,548	7,095	3,554	771
Lab 2a – trout	664	502	619	533	761	16,647	6,833	8,953	4,595	363
Lab 2b – carp (level 1)	664	502	619	533	761	11,015	3,582	2,133	1,719	
Lab 2b – carp (level 2)	664	502	619	533	761	11,840	3,829	1,763	1,813	
Lab 2b – carp (level 3)	664	502	619	533	761	13,667	4,518	2,083	2,019	
Lab 3	664	502	619	533	761	12,369	6,043	5,948	5,226	
Lab 4	664	502	619	533	761	12,848	7,486	8,034	5,781	
Lab 5	664	502	619	533	761	16,320	775	13,902	115,878	368
Lab 6	664	502	619	533	761	10,627	4,777	4,651	2,369	452
Lab 7	664	502	619	533	761	13,528	4,777	7,982	3,602	
Lab 8	664	502	619	533	761	11,472	5,290	5,475	4,846	645
Mean BCF all data						13,051	4,860	6,184	13,764	520
Standard deviation						1,968	1,799	3,611	33,898	181
Mean BCF for trout (minus Lab 5 data)						12,960	5,822	6,877	4,282	558
Standard deviation						1,915	1,031	1,565	1,170	185
Mean BCF for carp						12,174	3,976	1,993	1,851	
Standard deviation						1,357	485	201	153	

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.

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

Table 62 **Summary of estimated BCF from the ring test using Method 22** (Tolls and Sijm 1995 (40))

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 1	778	616	734	648	871	15,501	6,818	8,412	4,322	883
Lab 2a – trout	778	616	734	648	871	19,502	8,397	10,616	5,589	416
Lab 2b – carp (level 1)	778	616	734	648	871	12,905	4,402	2,530	2,091	
Lab 2b – carp (level 2)	778	616	734	648	871	13,871	4,705	2,090	2,205	
Lab 2b – carp (level 3)	778	616	734	648	871	16,011	5,552	2,470	2,456	
Lab 3	778	616	734	648	871	14,491	7,425	7,054	6,356	
Lab 4	778	616	734	648	871	15,051	9,199	9,527	7,031	
Lab 5	778	616	734	648	871	19,119	953	16,485	140,930	421
Lab 6	778	616	734	648	871	12,450	5,870	5,516	2,881	517
Lab 7	778	616	734	648	871	15,848	5,870	9,465	4,380	
Lab 8	778	616	734	648	871	13,439	6,501	6,492	5,893	738
Mean BCF all data						15,290	5,972	7,332	16,739	595
Standard deviation						2,306	2,210	4,282	41,226	207
Mean BCF for trout (minus Lab 5 data)						15,183	7,154	8,155	5,207	639
Standard deviation						2,244	1,267	1,856	1,422	211
Mean BCF for carp						14,262	4,886	2,363	2,251	
Standard deviation						1,590	596	238	186	

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.

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

Available published BCF data

117. A brief search was undertaken for published data on the bioconcentration factors for the substances used in the ring test. This included a search of the EURAS Gold Standard Database[8] of bioconcentration factors, standard textbooks (e.g. (15), although it is not always clear if the values reported are experimental values or estimates) and a set of BCF data that had been made available for other projects (see (17) for a description of these data). The available data are summarised in Table 63

Table 63 Summary of available BCF data for the substances used in the ring test

Substance	Species	Lipid content (%)	Fish size (g)	BCF l/kg wet wt.	Comment	Reference
Hexachloro benzene	*Cyrpinus carpio*			27,000	Reported in Gold Standard Database as a steady state value.	MITI
	Cyprinus carpio			30,000	Reported in Gold Standard Database as a steady state value.	MITI
	Cyrpinus carpio		30	18,100	Taken from BCF data set used in (17). Average BCF (probably same study as in the Gold Standard Database above).	CERI
	Cyprinus carpio		30	19,000	Taken from BCF data set used in (17). Average BCF (probably same study as in the Gold Standard Database above).	CERI
	Gambius affinis	3.1	0.19	3,730	Reported in Gold Standard Database as steady state value. The kinetic BCF is given as 3,776 l/kg wet wt.	(18)
	Lepomis cyanellus			21,900	Taken from (15).	(25)
	Oncorhynchus mykiss	8.20	349	12,000	Taken from BCF data set used in (17).	(19)
	Oncorhynchus mykiss	8.70	400	20,000	Taken from BCF data set used in (17).	(19)

[8] http://ambit.sourceforge.net/euras/

Substance	Species	Lipid content (%)	Fish size (g)	BCF l/kg wet wt.	Comment	Reference
	Oncorhynchus mykiss			7,760	Taken from (15). Reported to be a kinetic BCF.	(20)
	Oncorhynchus mykiss			5,370	Taken from (15).	(25)
	Pimephales promelas			18,600	Taken from (15).	(25)
	Pimephales promelas		0.15	17,700	Taken from BCF data set used in (17).	(21)
	Pimephales promelas		0.17	21,400	Taken from BCF data set used in (17).	(21)
	Pimephales promelas		0.16	22,500	Taken from BCF data set used in (17).	(21)
	Pimephales promelas		0.16	26,700	Taken from BCF data set used in (17).	(21)
	Pimephales promelas	9.28	0.13	25,000	Taken from BCF data set used in (17).	(22)
	Pimephales promelas	9.52	0.13	32,000	Taken from BCF data set used in (17).	(22)
	Pimephales promelas	9.27	0.13	37,000	Taken from BCF data set used in (17).	(22)
	Pimephales promelas	8.52	0.13	39,000	Taken from BCF data set used in (17).	(22)
	Pimephales promelas	9.13	0.13	50,000	Taken from BCF data set used in (17).	(22)
	Poecillia reticulata	5.00	0.15	26,900	Taken from BCF data set used in (17).	(23)
	Unknown fish			1,230	Taken from (15).	(26)
	Unknown fish (may be killifish)			18,600	Taken from (15).	(27)
	Unknown fish			8,500	Taken from (15). Flow-through test.	(28), (29)
	Unknown fish			288	Taken from (15). Static test.	(28), (29)
Musk xylene	*Cyrpinus carpio*	3.4		5,750	Reported in Gold Standard Database as a steady state value.	MITI
	Cyprinus carpio	3.4		6,610	Reported in Gold Standard Database as a steady state value.	MITI

Substance	Species	Lipid content (%)	Fish size (g)	BCF l/kg wet wt.	Comment	Reference
	Cyrpinus carpio	3.4	30	3,230	Taken from BCF data set used in (17). Average BCF (probably same study as in the Gold Standard Database above).	CERI
	Cyprinus carpio	3.4	30	4,090	Taken from BCF data set used in (17). Average BCF (probably same study as in the Gold Standard Database above).	CERI
o-Terphenyl	*Cyrpinus carpio*			1,000	Reported in Gold Standard Database as a steady state value.	MITI
	Cyprinus carpio			5,500	Reported in Gold Standard Database as a steady state value.	MITI
Methoxy chlor						
Benzo[*a*] pyrene	*Lepomis macrochirus*		0.5	490	Taken from BCF data set used in (17). Kinetic BCF corrected for metabolites.	

Note: MITI/CERI – Value taken from the Japanese MITI database or the Chemicals Evaluation and Research Institute (CERI) (Biodegradation and Bioconcentration of Existing Substances under the Chemical Substances Control Law) database. These are now available from the National Institute of Technology and Evaluation at http://www.safe.nite.go.jp/english/kizon/KIZON_start_hazkizon.html.

Comparison of predicted BCF data with experimental BCF data

118. It is not always clear whether or not the experimental BCF data have been corrected for growth dilution. However, as growth correction was not routinely carried out (at least up until recently) for BCF studies, for the comparison it is assumed that the experimental BCF data relate to values that have not been growth corrected. Therefore the comparison is made between the experiment BCF data and the predicted BCF data using the overall depuration rate constant (k_2 rather than k_{2g}). The possible consequence of this is considered further below.

119. 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. The mean predicted BCFs using

the ring test data are in the range 6,046 to 15,183 depending on the method used. Thus the predicted BCFs cover a similar range as the experimental BCFs for this species. For carp (*Cyprinus carpio*) the experimental BCF values are in the range 19,000 to 30,000 l/kg. The predicted BCFs using the ring test data are generally lower than this range i.e. mean values between 4,478 and 14,262 l/kg.

120. For musk xylene, the experimental BCF values are in the range 3,230 to 6,610 l/kg for carp. The mean BCFs predicted using the ring test data cover the range 1,774 to 4,886 l/kg. No experimental data are available for rainbow trout.

121. For o-terphenyl, the experimental BCF values are in the range 1,000 to 5,000 l/kg, again with carp. The predicted BCF values are in the range 779 to 2,363 l/kg.

122. No experimental data with rainbow trout or carp were located for methoxychlor or benzo[a]pyrene and so it was not possible to carry out a comparison of the experimental data with the predicted data for these substances.

123. Overall the predictions obtained from the ring test 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 it is worth noting that in all cases, experimental BCF values are available that are higher than predicted from the ring test data. One possible explanation for this may be that the growth of the fish in the feeding study may have been higher than in the corresponding BCF studies. This possibility has not been investigated further in the current work.

DISCUSSION OF DATA

Investigation of correlations between experimental variables and derived bioaccumulation parameters

124. In order to investigate possible correlations between experimental variables and the derived bioaccumulation parameters from the study a series of plots have been constructed. The data used for these plots are taken from the ring test studies that used a nominal 3% by weight feeding rate. Where relevant the data have been analysed both including and excluding the data for Lab 2b as they refer to carp rather than trout. Similarly where relevant the data have been analysed both including and excluding the data for Lab 5 as some of the data for this laboratory (for example the data for musk xylene and methoxychlor) appear to be outliers compared with the results from other laboratories. Where the mean lipid content is used, this is the mean lipid content of the fish over the entire experimental duration.

125. It is important to note for this analysis that the number of data points available is relatively limited. Therefore it is difficult to conclude definitively if any of the trends apparent are real or not. However, this analysis is considered useful as it allows some of the underlying factors that may have influence the data to be tentatively identified, and to see how these factors may co-vary or may have propagated into the various derived kinetic and bioaccumulation parameters.

126. The dependence of the derived growth rate constant on the food lipid, the mean fish lipid and the initial fish weight is shown in Figure 1, Figure 2 and Figure 3 respectively. From visual inspection of these plots, no clear trend is apparent between the growth rate constant and the food lipid content[9]. However a trend towards a decrease in the growth rate constant with an increase in the fish lipid content is apparent in Figure 2 and a trend towards a decrease in the growth rate constant with an increase in initial fish weight is apparent in Figure 3. Linear regression analysis indicates that the slope of Figure 2 is not significantly different from 0 ($p>0.05$) for both the complete data set and the trout (minus Lab 5)[10] data set but that the slope of Figure 3 is significantly different from 0 ($p>0.05$) for both data sets[11].

[9] Linear regression of the data set yields a slope that is not significantly different from 0 ($p>0.05$) for both the full data set and the trout (minus Lab 5) data set. The regression equations derived were: full data set k_{growth} = 0.0003×% food lipid + 0.029 (R^2 = 0.012); trout data (minus Lab 5) k_{growth} = -0.0003×% food lipid + 0.040 (R^2 = 0.017).

[10] The regression equations derived were: full data set k_{growth} = -0.003×% fish lipid + 0.054 (R^2 = 0.34); trout data (minus Lab 5) k_{growth} = -0.002×% fish lipid + 0.047 (R^2 = 0.11).

[11] The regression equations derived were: full data set k_{growth} = -0.003×fish weight+ 0.043 (R^2 = 0.60); trout data (minus Lab 5) k_{growth} = -0.003×fish weight + 0.043 (R^2 = 0.64).

Figure 1 Plot of growth rate constant against food lipid content

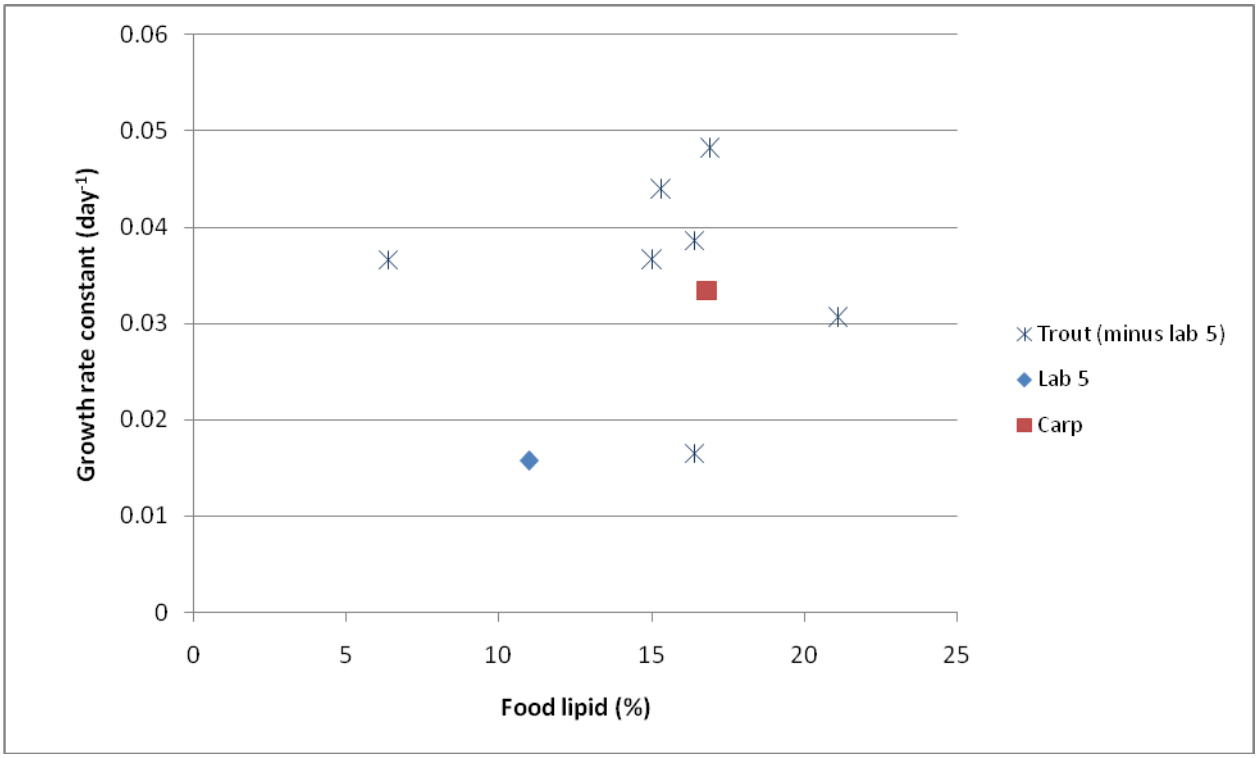


Figure 2 Plot of growth rate constant against fish lipid

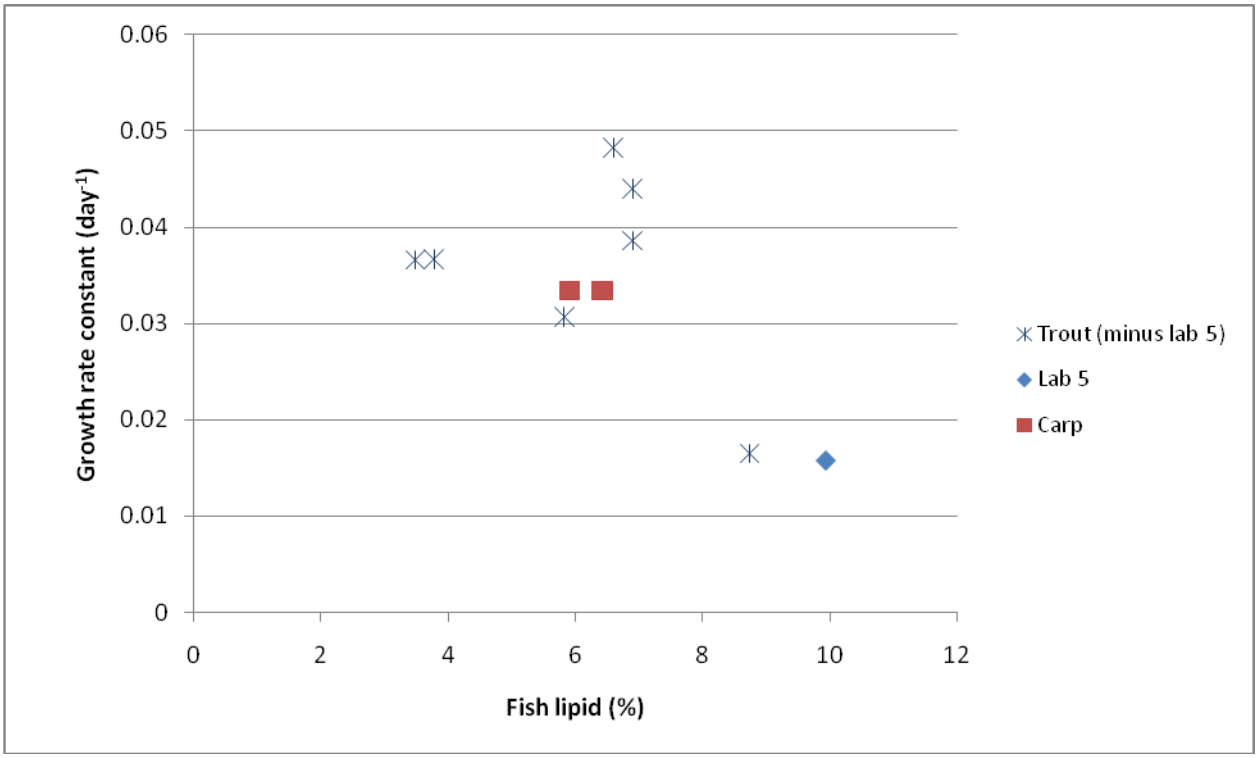
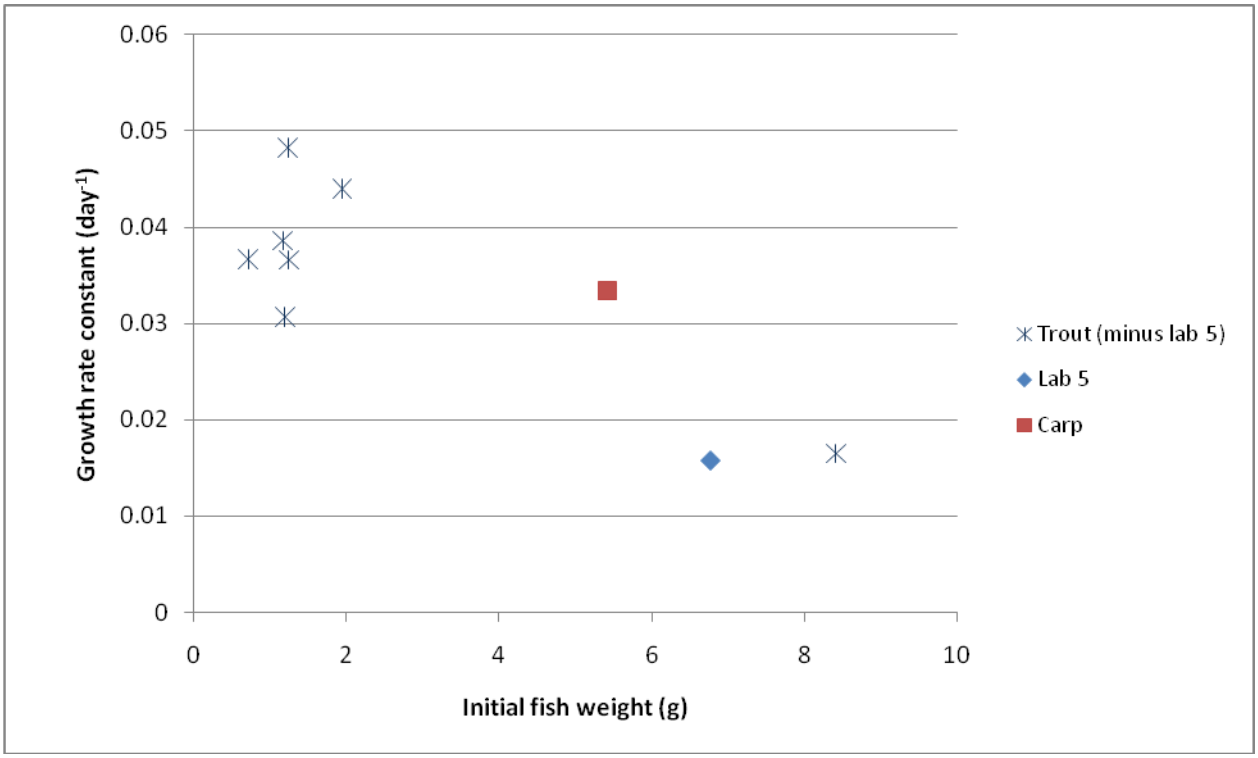


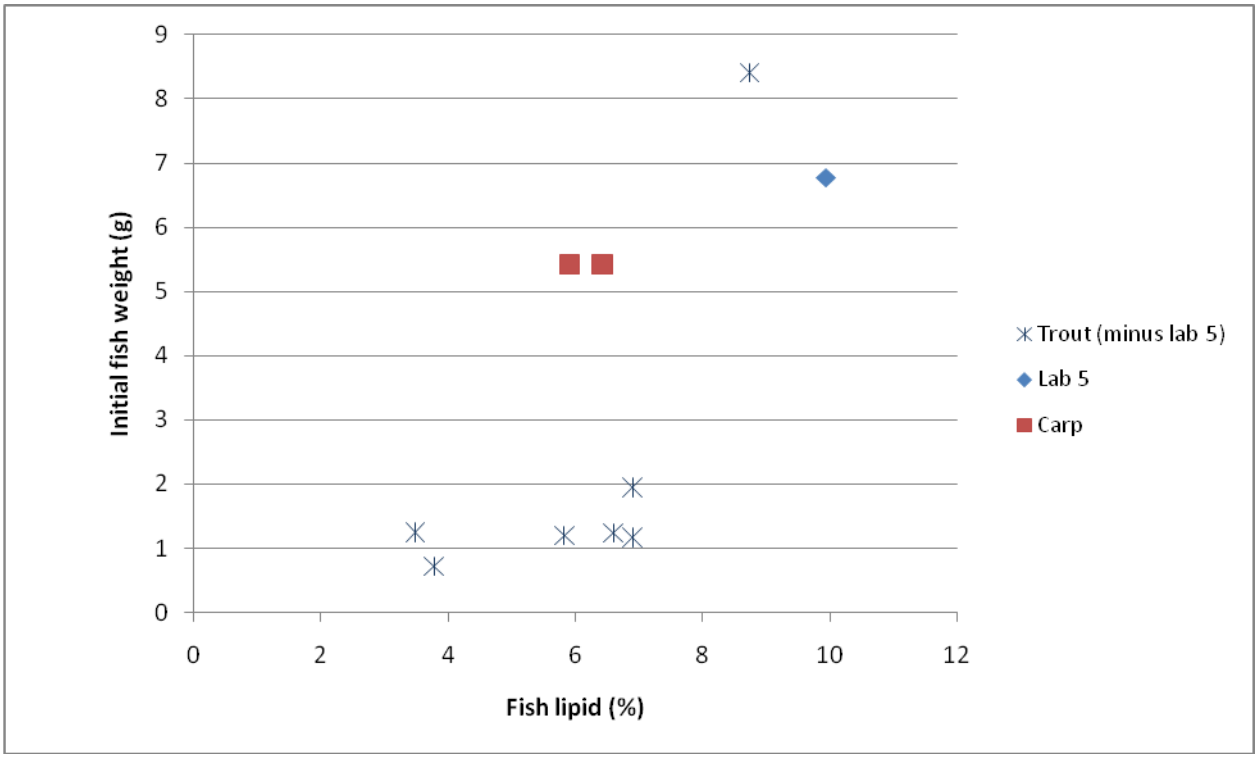
Figure 3 Plot of growth rate constant against initial fish weight



127. In order to see if there is a correlation between the initial fish weight and the mean fish lipid content, a plot of fish weight against lipid content was constructed. This is shown in Figure 4. This shows a trend in increasing mean lipid content with increasing initial fish weight (linear regression analysis indicates a slope significantly different from 0 ($p<0.05$) for the combined data set only[12]) and so the trends noted in Figure 2 and Figure 3 may not be independent.

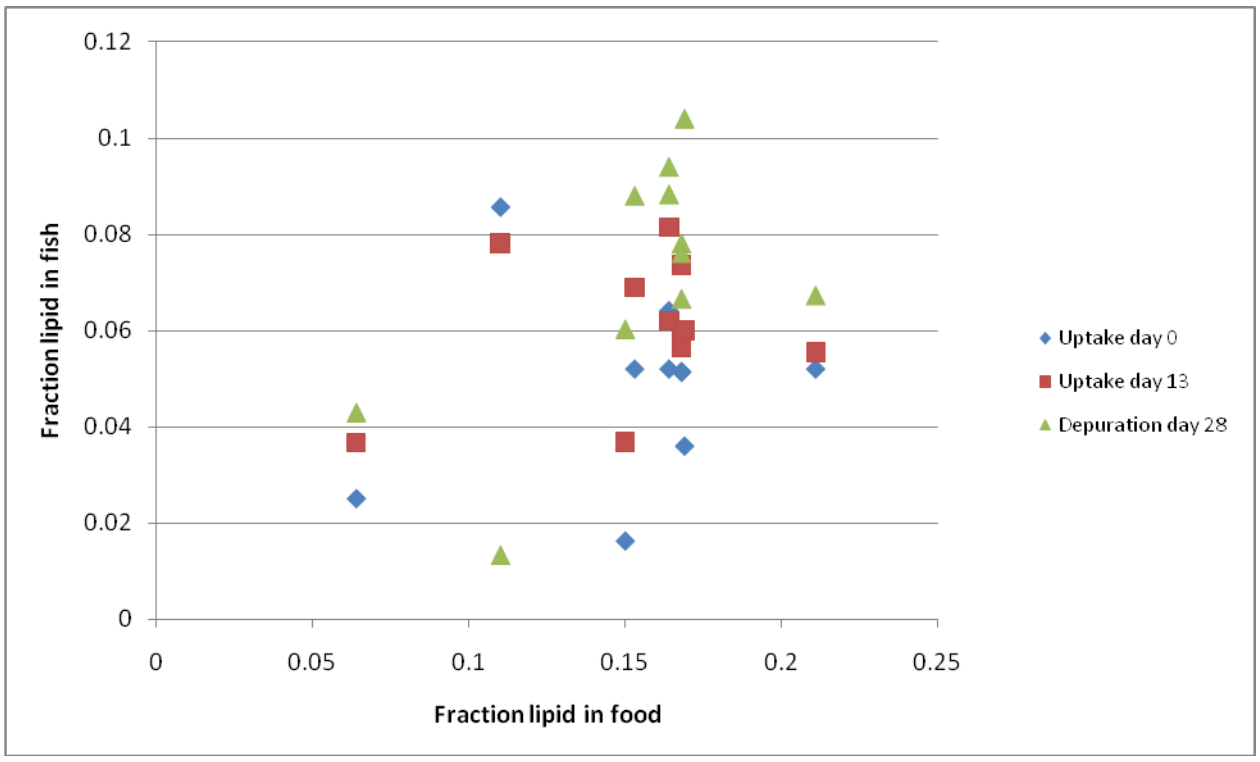
[12] The regression equations derived were: full data set fish weight = 1.02×% fish lipid - 3.0 ($R^2 = 0.46$); trout data (minus Lab 5) fish weight = 1.02×% fish lipid – 3.9 ($R^2 = 0.48$).

Figure 4 **Plot of initial fish weight against fish lipid**



128. Plots showing the correlation between the food lipid content and the fish lipid content are shown in Figure 5 for the start of the uptake, end of the uptake and end of the depuration periods. The plots show a general trend towards increasing fish lipid with increasing food lipid.

Figure 5 **Plot of fish lipid content against food lipid content**



129. Linear regression analysis of the data for Figure 5 show that the slopes[13] of the best fit line are all positive, but are not statistically significantly different from zero for the data for uptake day 0 and uptake day 13. However for depuration day 28 the slope is statistically significant ($p>0.05$). This suggests that the food lipid may have a larger influence on the fish lipid as the feeding time is increased.

130. Plots of the overall depuration rate constant against fish lipid content for each substances are shown in Figure 6 to Figure 10. These plots tend to suggest a slight decreasing trend in the overall depuration rate constant with increasing fish lipid content for hexachlorobenzene[14], musk xylene (if the Lab 5 data point is ignored[15]), o-terphenyl[16] and methoxchlor[16]. For benzo[a]pyrene the opposite trend is suggested[16] however, as discussed earlier, the depuration seen for this substance was very rapid and it is doubtful that the overall depuration rate constant can be reliably measured using this experimental design (owing to a very limited number of sampling points before the concentration fell to non-detectable levels). Given that the lipid content of the fish correlates somewhat with the initial fish weight (Figure 4), similar plots can be constructed (not shown) of overall depuration rate constant against initial fish weight.

131. The plots also suggest that the carp data are comparable to the trout data for hexachlorobenzene and musk xylene but the overall depuration rate constants with carp for o-terphenyl and methoxychlor are generally higher than those obtained with trout. This could possibly relate to increased metabolism of o-terphenyl and methoxychlor in carp compared with trout.

[13] The regression equations derived were: uptake day 0 fraction fish lipid = 0.065×fraction food lipid + 0.039 (R^2 = 0.02), uptake day 13 fraction fish lipid = 0.10×fraction food lipid + 0.045 (R^2 = 0.07), depuration day 28 fraction fish lipid = 0.41×fraction food lipid + 0.008 (R^2 = 0.37).

[14] Linear regression analysis indicates a slope significantly different from 0 ($p<0.05$) for the combined data set but not the trout (minus Lab 5) data set alone. The regression equations derived for hexachlorobenzene were: full data set k_2=-0.003×% fish lipid + 0.069 (R^2 = 0.45); trout data (minus Lab 5) k_2 = -0.002×% fish lipid + 0.066 (R^2 = 0.34).

[15] Linear regression analysis indicates that the slope is not significantly different from 0 ($p>0.05$) for the trout (minus Lab 5) data set. The regression equations derived for musk xylene were: full data set k_2=0.053×% fish lipid - 0.19 (R^2 = 0.35); trout data (minus Lab 5) k_2 = -0.005×% fish lipid + 0.12 (R^2 = 0.41).

[16] Linear regression analysis indicates that the slope is not significantly different from 0 ($p>0.05$) for both the combined data set and the trout (minus Lab 5) data set. The regression equations derived for o-terphenyl were: full data set k_2=-0.012×% fish lipid + 0.23 (R^2 = 0.043); trout data (minus Lab 5) k_2 = -0.006×% fish lipid + 0.13 (R^2 = 0.25). The regression equations derived for methoxychlor were: full data set k_2=-0.027×% fish lipid + 0.34 (R^2 = 0.27); trout data (minus Lab 5) k_2 = -0.018×% fish lipid + 0.24 (R^2 = 0.55). The regression equations derived for benzo[a]pyrene were: full data set k_2=0.12×% fish lipid + 0.76 (R^2 = 0.54); trout data (minus Lab 5) k_2 = 0.12×% fish lipid + 0.79 (R^2 = 0.38).

Figure 6 **Plot of overall depuration rate constant against fish lipid content for hexachlorobenzene**

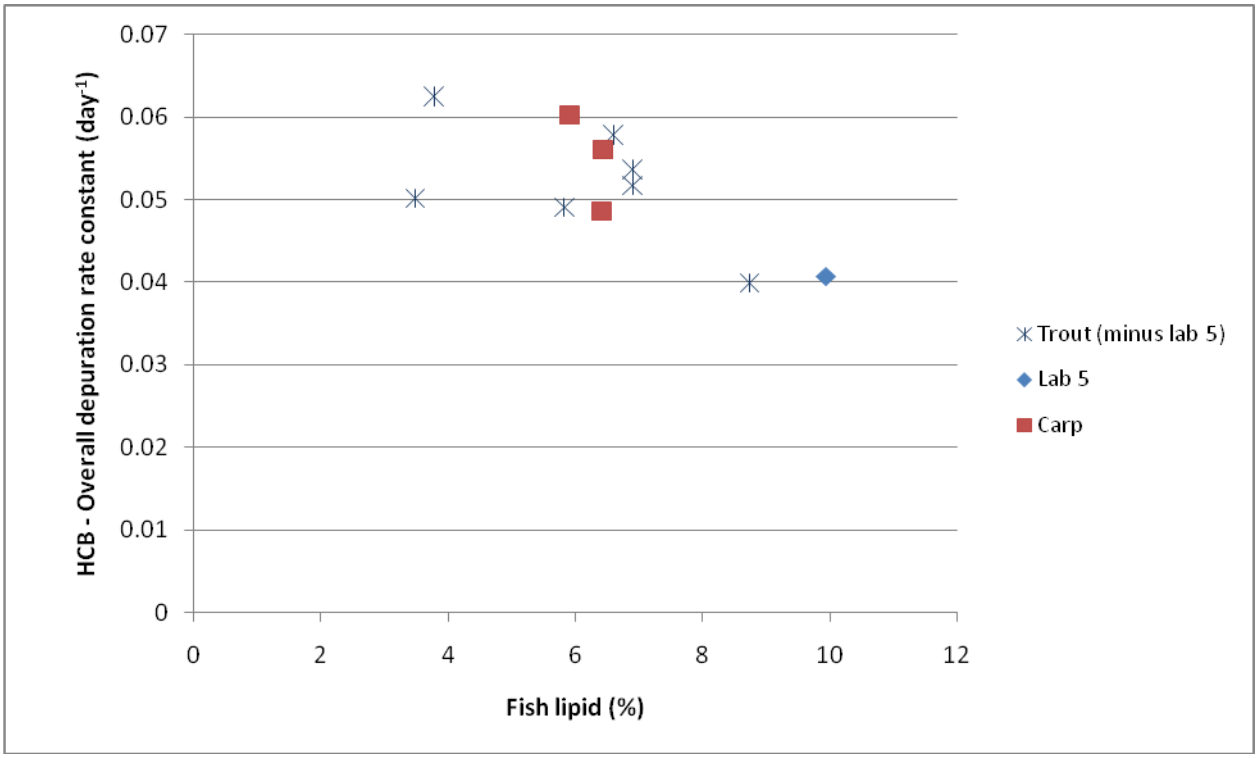


Figure 7 **Plot of overall depuration rate constant against fish lipid content for musk xylene**

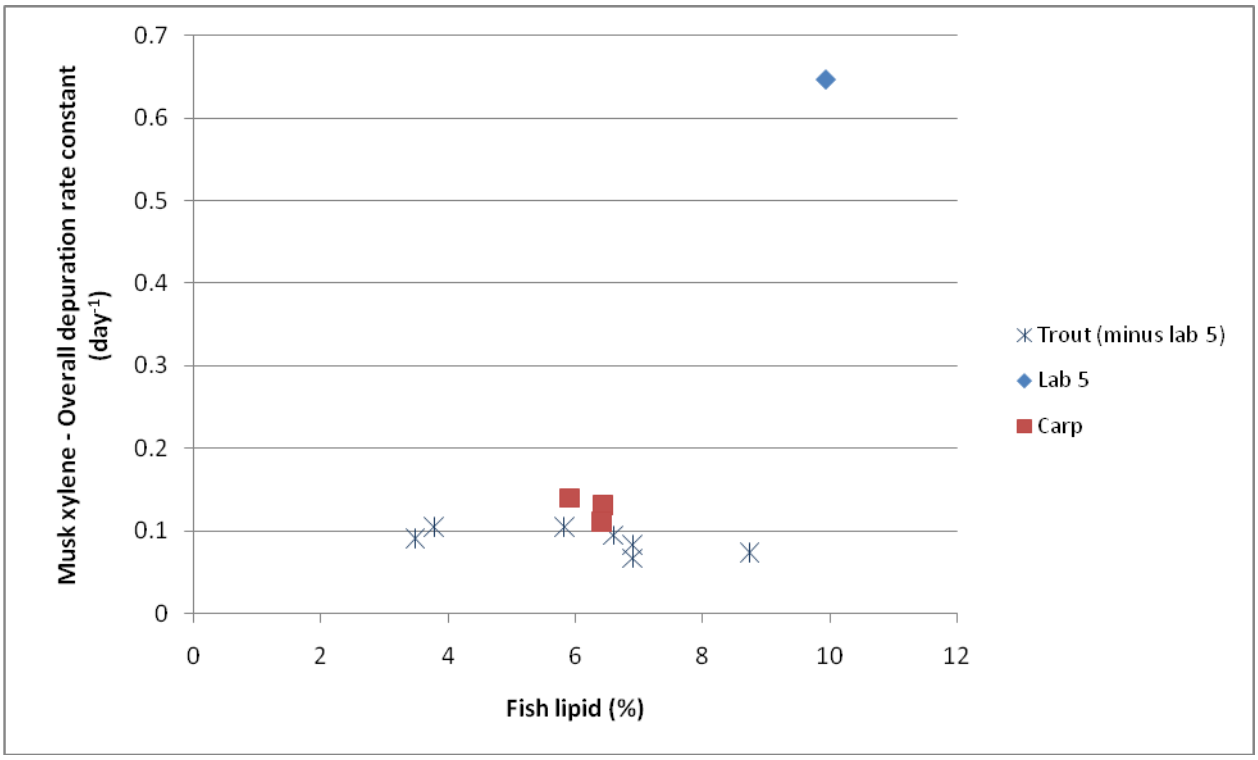


Figure 8 **Plot of overall depuration rate constant against fish lipid for o-terphenyl**

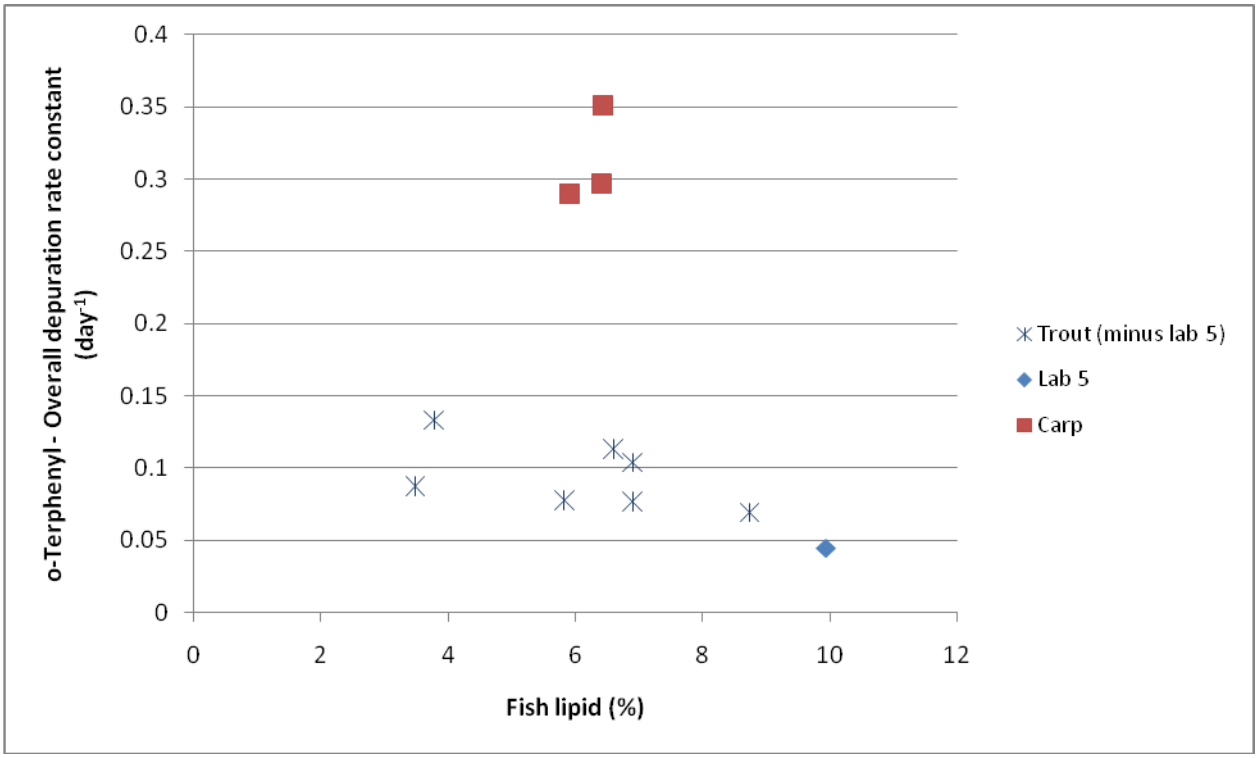


Figure 9 **Plot of overall depuration rate constant against fish lipid for methoxychlor**

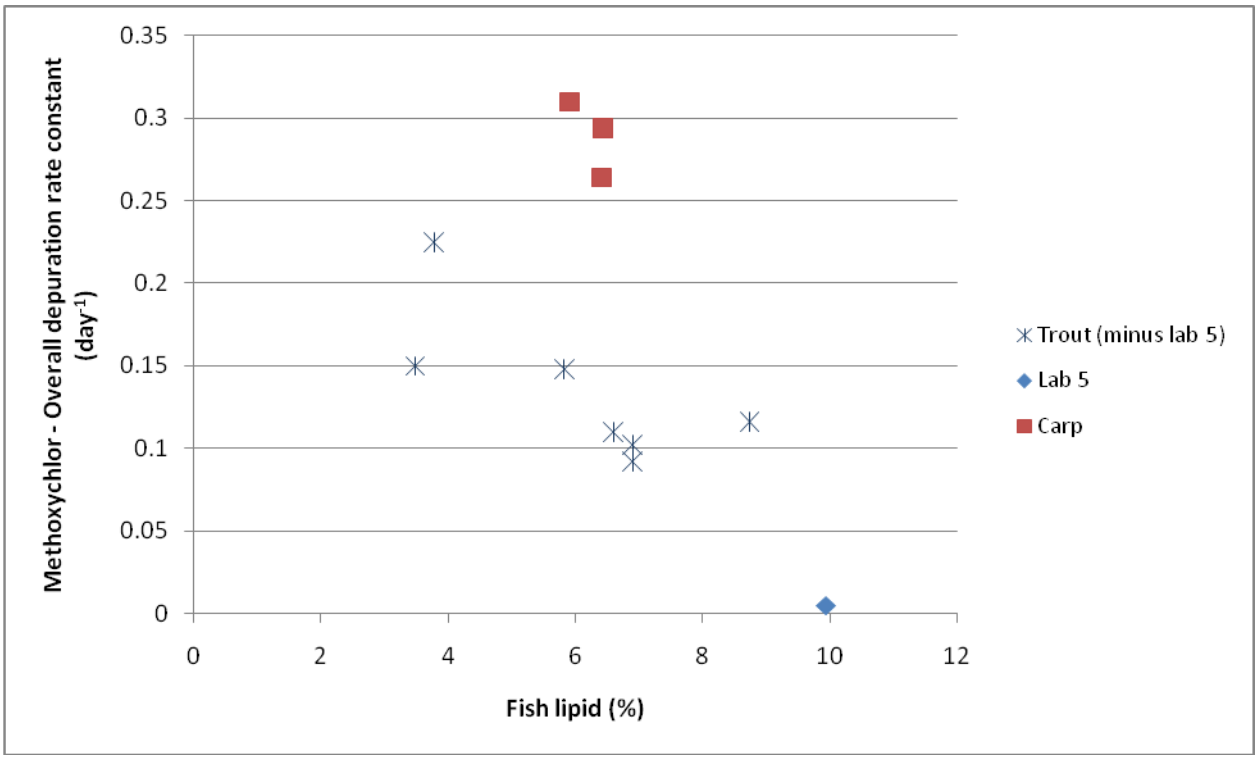
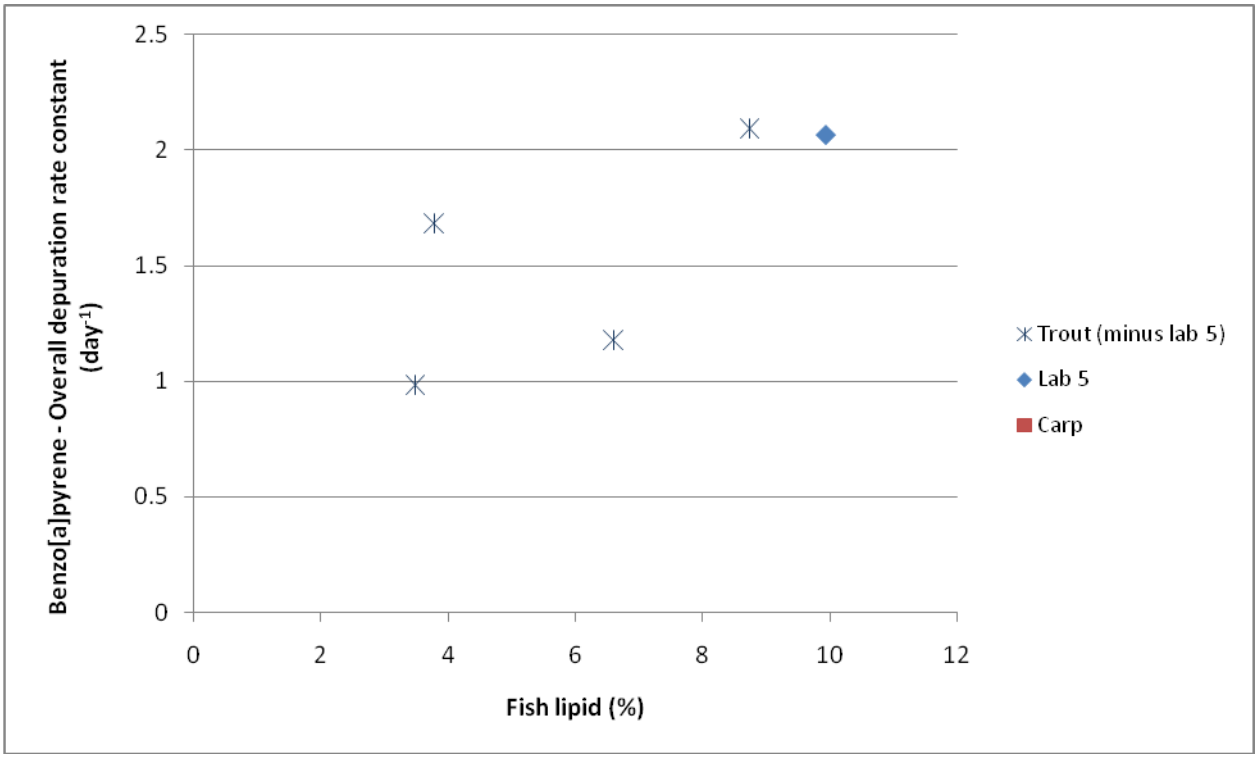


Figure 10 **Plot of overall depuration rate constant against fish lipid for benzo[a]pyrene**



132. Similar plots for the growth corrected depuration rate constant against fish lipid content are shown in Figure 11 to Figure 15. In these cases any trend in the growth corrected depuration rate constant with lipid content is even less evident than with the overall depuration rate constant. In this case linear regression analysis revealed that the slopes of all plots (based on either the combined data set or the trout (minus Lab 5) data set were not significantly different from 0 ($p>0.05$)[17]. This suggests that any apparent trend in the overall depuration rate constant with lipid content may relate to the fact that the growth rate constant component of the overall depuration rate constant appears to decrease with increasing lipid content (as evidenced by Figure 2).

133. Bioaccumulation theory would suggest that, at least for substances that are not rapidly metabolised, the growth corrected depuration rate constant would be expected to decrease (and the corresponding elimination half-life increase) with increasing lipid content of the fish (see (17) for a discussion). This was not clearly evident in the data analysed in Figure 11 to Figure 15.

[17] The regression equations derived were as follows.

Hexachlorobenzene: full data set k_{2g}= 0.0005×% fish lipid + 0.015 (R^2 = 0.018); trout data set (minus Lab 5) k_{2g} = -0.0004×% fish lipid + 0.019 (R^2 = 0.012).

Musk xylene: full data set k_{2g}= 0.056×% fish lipid – 0.24 (R^2 = 0.36); trout data set (minus Lab 5) k_{2g} = -0.0033×% fish lipid + 0.072 (R^2 = 0.14).

o-Terphenyl: full data set k_{2g}= -0.009×% fish lipid + 0.17 (R^2 = 0.024); trout data set (minus Lab 5) k_{2g} = -0.0044×% fish lipid + 0.085 (R^2 = 0.19).

Methoxychlor: full data set k_{2g}= -0.024×% fish lipid + 0.29 (R^2 = 0.22); trout data set (minus Lab 5) k_{2g} = -0.016×% fish lipid + 0.20 (R^2 = 0.40).

Benzo[a]pyrene: full data set k_{2g}= 0.13×% fish lipid – 0.71 (R^2 = 0.54); trout data set (minus Lab 5) k_{2g} = 0.13×% fish lipid + 0.74 (R^2 = 0.14).

Figure 11 Plot of growth corrected depuration rate constant against lipid content for hexachlorobenzene

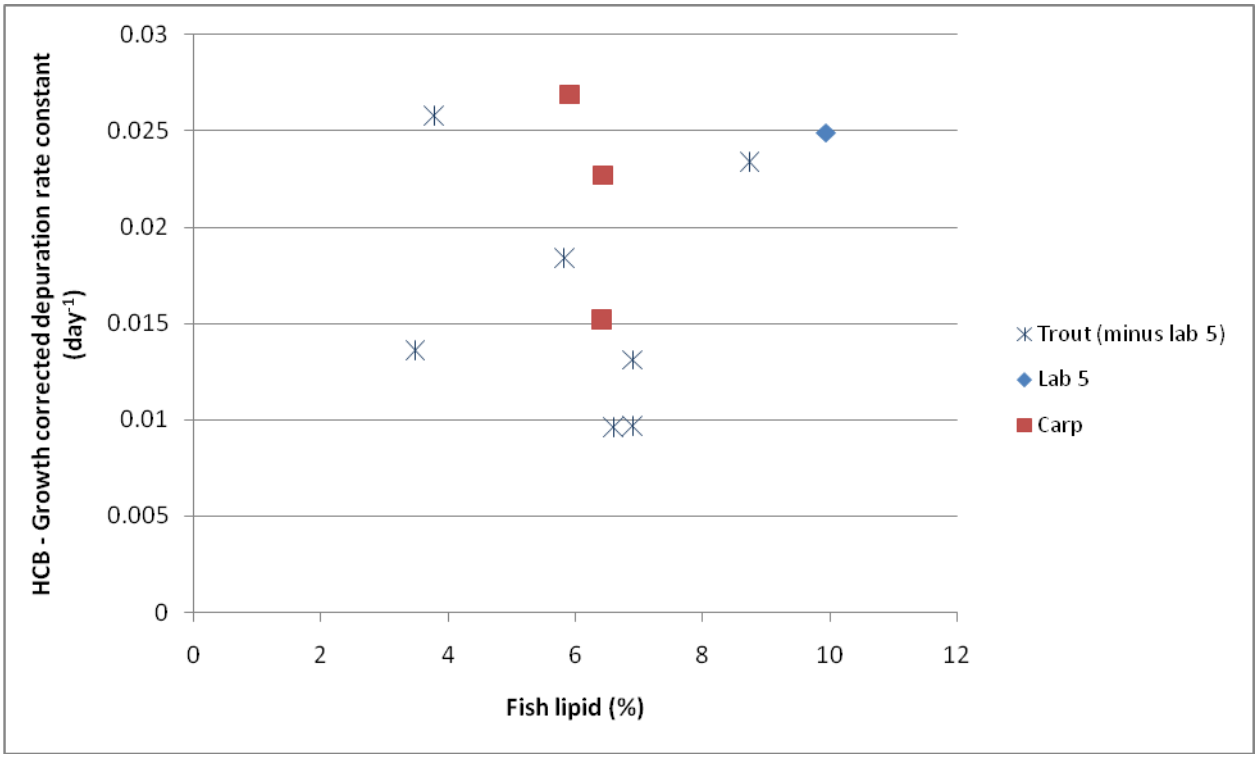


Figure 12 Plot of growth corrected depuration rate constant against lipid content for musk xylene

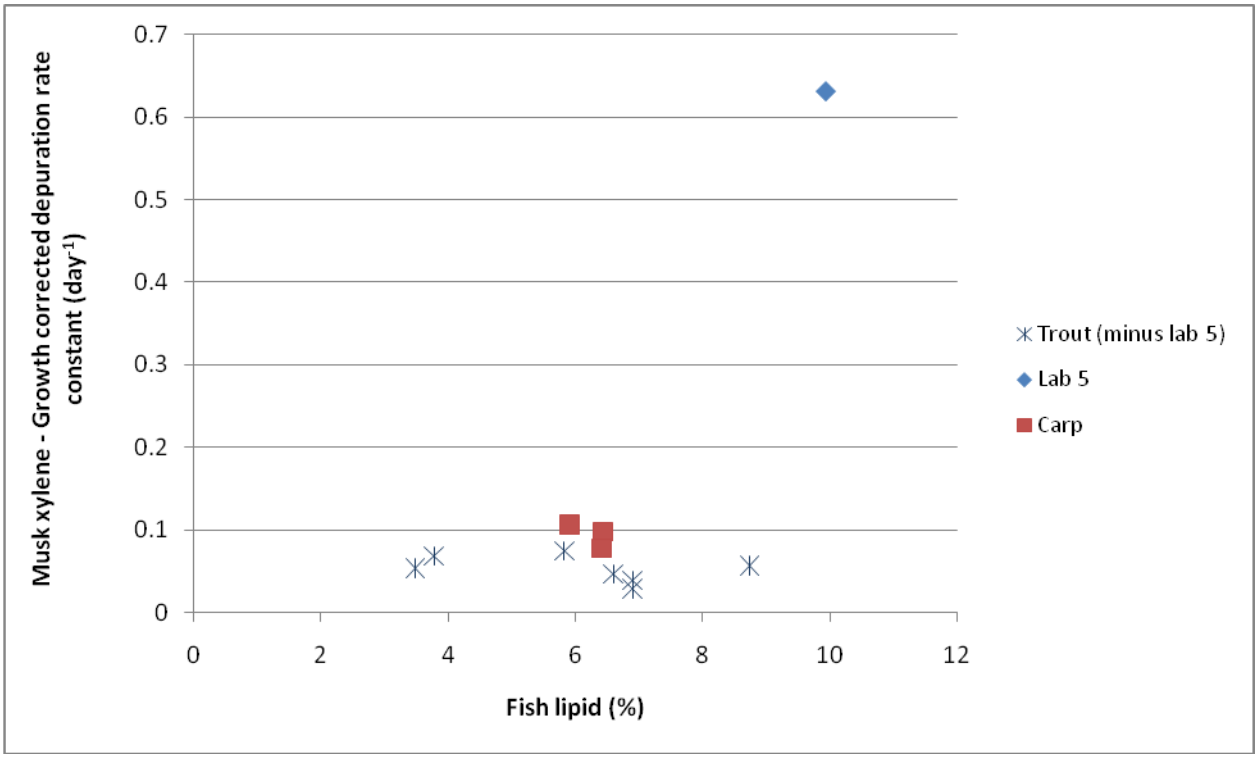


Figure 13 Plot of growth corrected depuration rate constant against fish lipid content for o-terphenyl

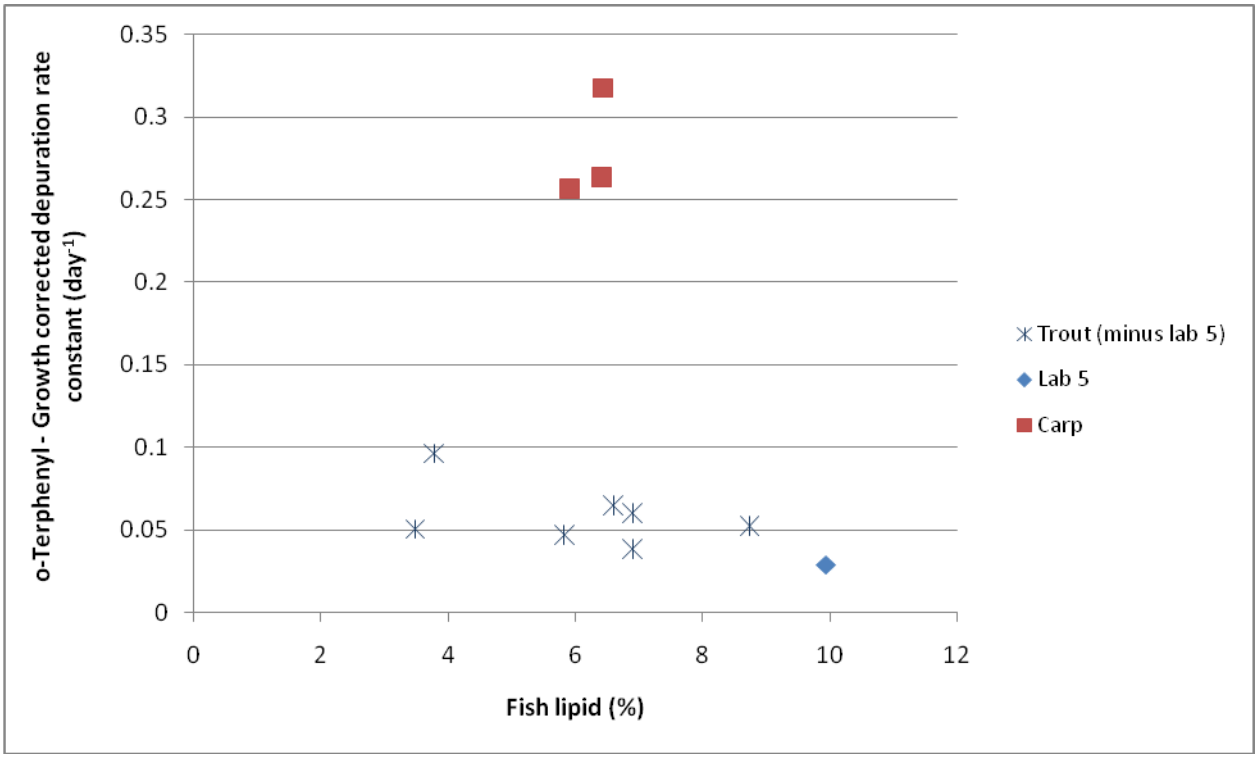


Figure 14 Plot of growth corrected depuration rate constant against fish lipid content for methoxychlor

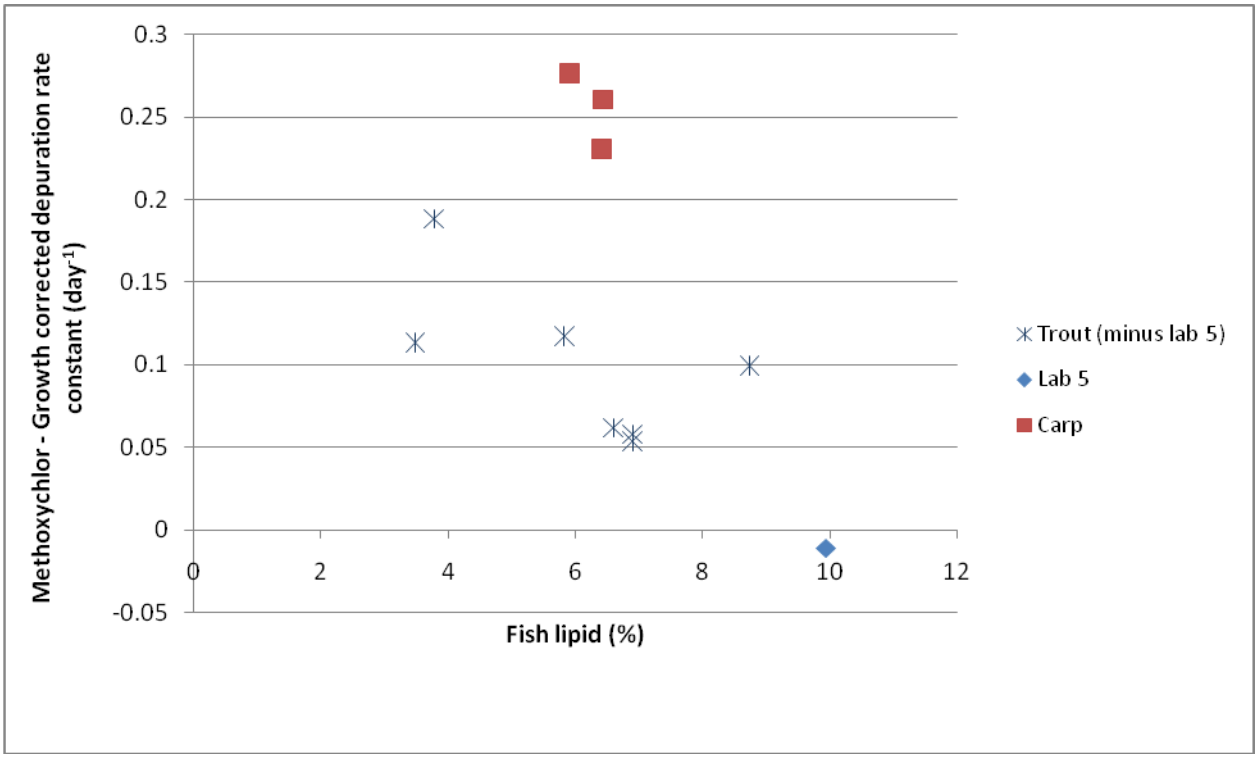
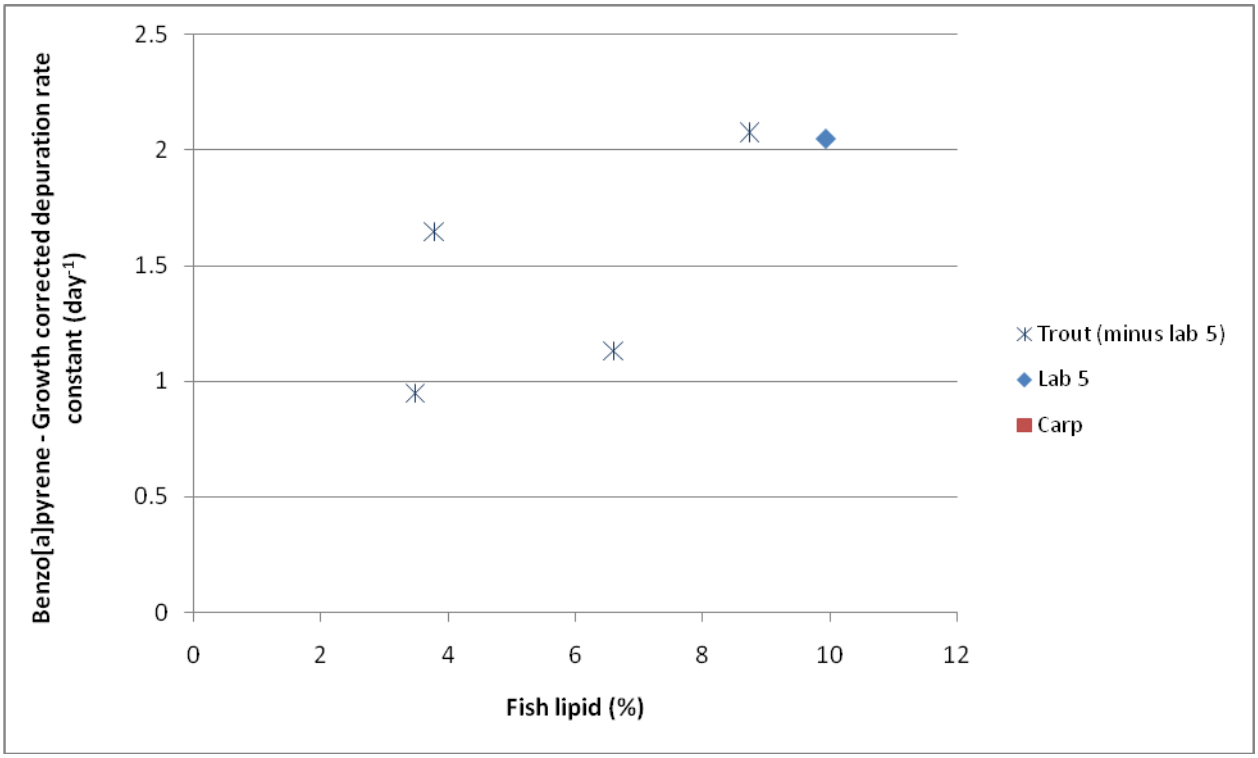


Figure 15 Plot of growth corrected depuration rate constant against fish lipid content for benzo[a]pyrene



134. Correlations between the derived assimilation efficiency and the fish lipid content, fish initial weight and food lipid content are shown in Figure 16 to Figure 18. For these plots the assimilation efficiency derived using t=13 days has been used.

Figure 16 Plot of assimilation efficiency against fish lipid content

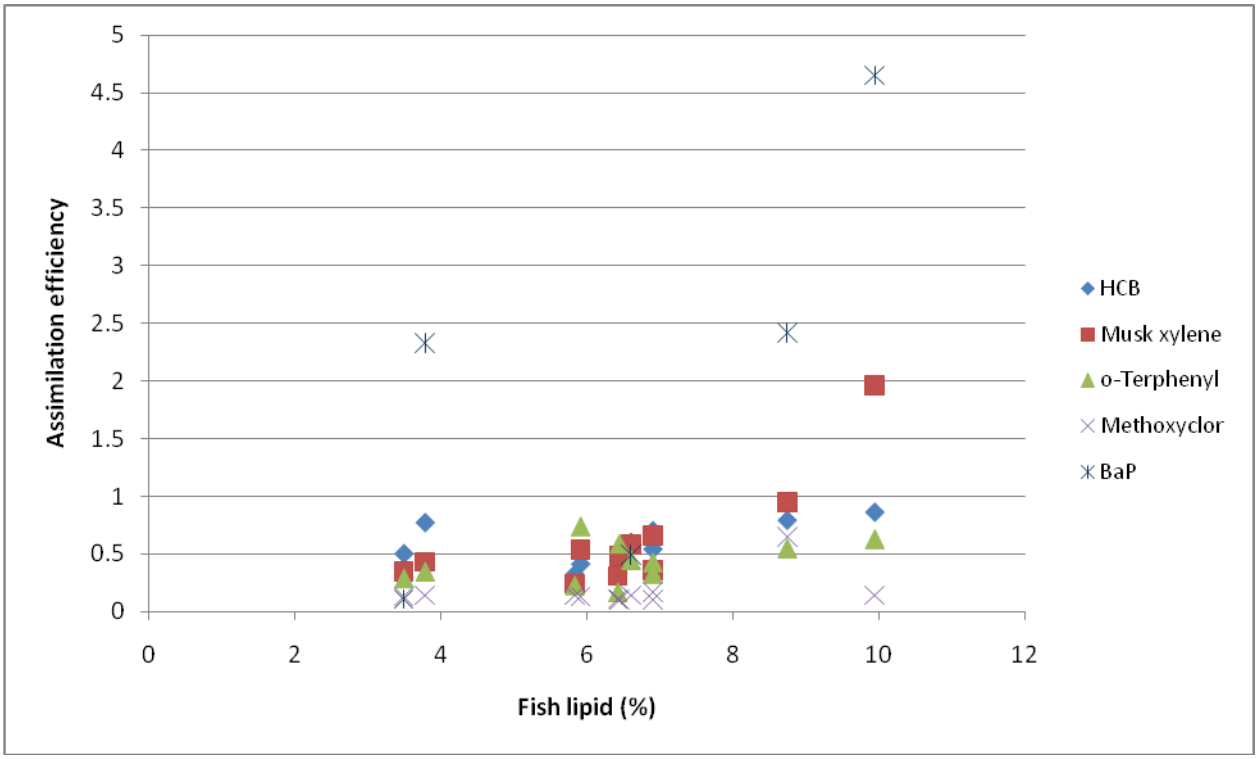


Figure 17 **Plot of assimilation efficiency against initial fish weight**

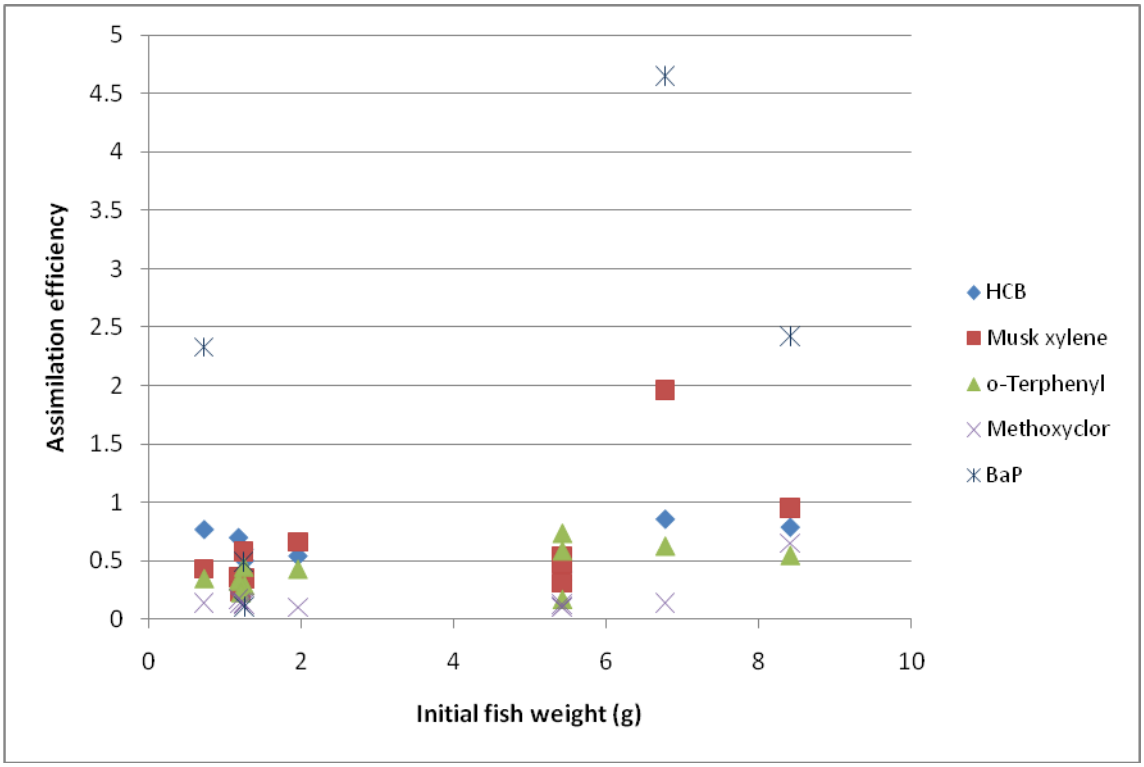
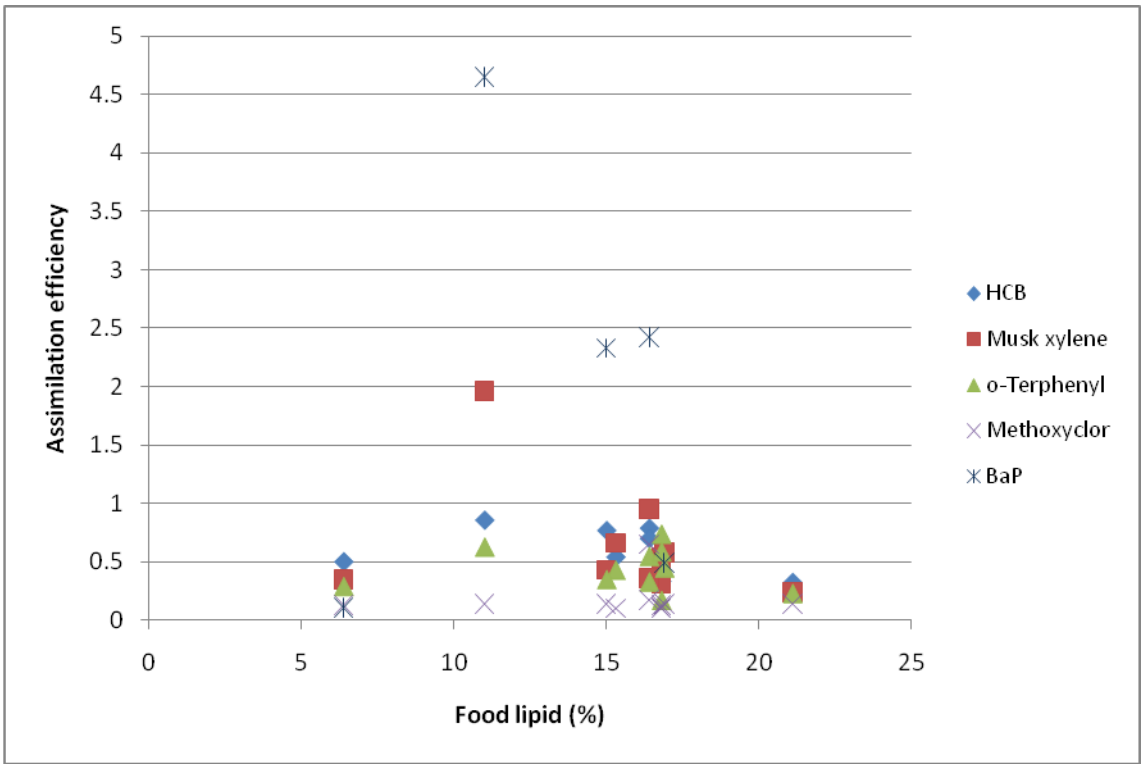


Figure 18 **Plot of assimilation efficiency against food lipid content**



135. When considering this analysis, it is important to take account of the fact that some of the assimilation efficiencies calculated are above 1 (which is theoretically impossible). This is particularly the case for some of the benzo[a]pyrene data, but also one data point with musk xylene (Lab 5 data point).

136. The assimilation efficiency shows a general increasing trend with increasing fish lipid. Linear regression analysis[18] indicates that the plots for all chemicals have a positive slope but this slope is not significantly different from 0 ($p>0.05$), except for musk xylene when the Lab 5 data (assimilation efficiency is close to 2 for this data point) is included in the regression.

137. The plots of assimilation efficiency against initial fish weight similarly show an increasing trend in the assimilation efficiency with increasing initial fish weight. Linear regression analysis[19] indicates that the slopes of these plots for all chemicals are positive but this slope is significantly different from 0 ($p<0.05$) only for the trout (minus Lab 5) data sets for musk xylene and methoxychlor.

138. There is no discernable trend in the assimilation efficiency with food lipid. Linear regression analysis[20] indicates that the slopes of most plots are very close to 0, and the slopes are not significantly different from 0 ($p>0.05$).

139. Plots of the derived growth corrected BMF (BMF_g) and the lipid normalised growth corrected BMF (BMF_L) against the fish lipid content, the fish initial weight and the food lipid content are shown in Figure 19 to Figure 24. Again for these plots the BMF derived using the assimilation efficiency calculated assuming t=13 days has been used.

[18] The regression equations derived were as follows.

Hexachlorobenzene: full data set Assimilation efficiency = 0.043×% fish lipid + 0.28 (R^2 = 0.17); trout data set (minus Lab 5) Assimilation efficiency = 0.023×% fish lipid + 0.46 (R^2 = 0.066).

Musk xylene: full data set Assimilation efficiency = 0.20×% fish lipid – 0.67 (R^2 = 0.59); trout data set (minus Lab 5) Assimilation efficiency = 0.090×% fish lipid - 0.035 (R^2 = 0.49).

o-Terphenyl: full data set Assimilation efficiency = 0.044×% fish lipid + 0.15 (R^2 = 0.21); trout data set (minus Lab 5) Assimilation efficiency = 0.040×% fish lipid + 0.14 (R^2 = 0.47).

Methoxychlor: full data set Assimilation efficiency = 0.035×% fish lipid - 0.048 (R^2 = 0.17); trout data set (minus Lab 5) Assimilation efficiency = 0.067×% fish lipid - 0.20 (R^2 = 0.41).

Benzo[a]pyrene: full data set Assimilation efficiency = 0.44×% fish lipid – 0.87 (R^2 = 0.49); trout data set (minus Lab 5) Assimilation efficiency = 0.18×% fish lipid + 0.32 (R^2 = 0.14).

[19] The regression equations derived were as follows.

Hexachlorobenzene: full data set Assimilation efficiency = 0.010×fish weight + 0.53 (R^2 = 0.022); trout data set (minus Lab 5) Assimilation efficiency = 0.027×% fish weight + 0.54 (R^2 = 0.20).

Musk xylene: full data set Assimilation efficiency = 0.099×fish weight + 0.27 (R^2 = 0.32); trout data set (minus Lab 5) Assimilation efficiency = 0.074×fish weight + 0.34 (R^2 = 0.71).

o-Terphenyl: full data set Assimilation efficiency = 0.036×fish weight + 0.30 (R^2 = 0.33); trout data set (minus Lab 5) Assimilation efficiency = 0.029×fish weight + 0.30 (R^2 = 0.55).

Methoxychlor: full data set Assimilation efficiency = 0.031×fish weight + 0.069 (R^2 = 0.29); trout data set (minus Lab 5) Assimilation efficiency = 0.070×fish weight + 0.051 (R^2 = 0.95).

Benzo[a]pyrene: full data set Assimilation efficiency = 0.33×fish weight + 0.78 (R^2 = 0.44); trout data set (minus Lab 5) Assimilation efficiency = 0.18×fish weight + 0.82 (R^2 = 0.29).

[20] The regression equations derived were as follows.

Hexachlorobenzene: full data set Assimilation efficiency = -0.018×% food lipid + 0.84 (R^2 = 0.12); trout data set (minus Lab 5) Assimilation efficiency = -0.0039×% food lipid + 0.66 (R^2 = 0.011).

Musk xylene: full data set Assimilation efficiency = -0.044×% food lipid + 1.30 (R^2 = 0.12); trout data set (minus Lab 5) Assimilation efficiency = 0.0027×% food lipid + 0.47 (R^2 = 0.0024).

o-Terphenyl: full data set Assimilation efficiency = -0.0026×% food lipid + 0.47 (R^2 = 0.003); trout data set (minus Lab 5) Assimilation efficiency = 0.0016×% food lipid + 0.35 (R^2 = 0.0042).

Methoxychlor: full data set Assimilation efficiency = 0.0038×% food lipid + 0.12 (R^2 = 0.008); trout data set (minus Lab 5) Assimilation efficiency = 0.0056×% food lipid + 0.12 (R^2 = 0.016).

Benzo[a]pyrene: full data set Assimilation efficiency = 0.043×% food lipid + 1.43 (R^2 = 0.011); trout data set (minus Lab 5) Assimilation efficiency = 0.14×% food lipid - 0.63 (R^2 = 0.011).

Figure 19 **Plot of growth corrected BMF (BMF_g) against fish lipid content**

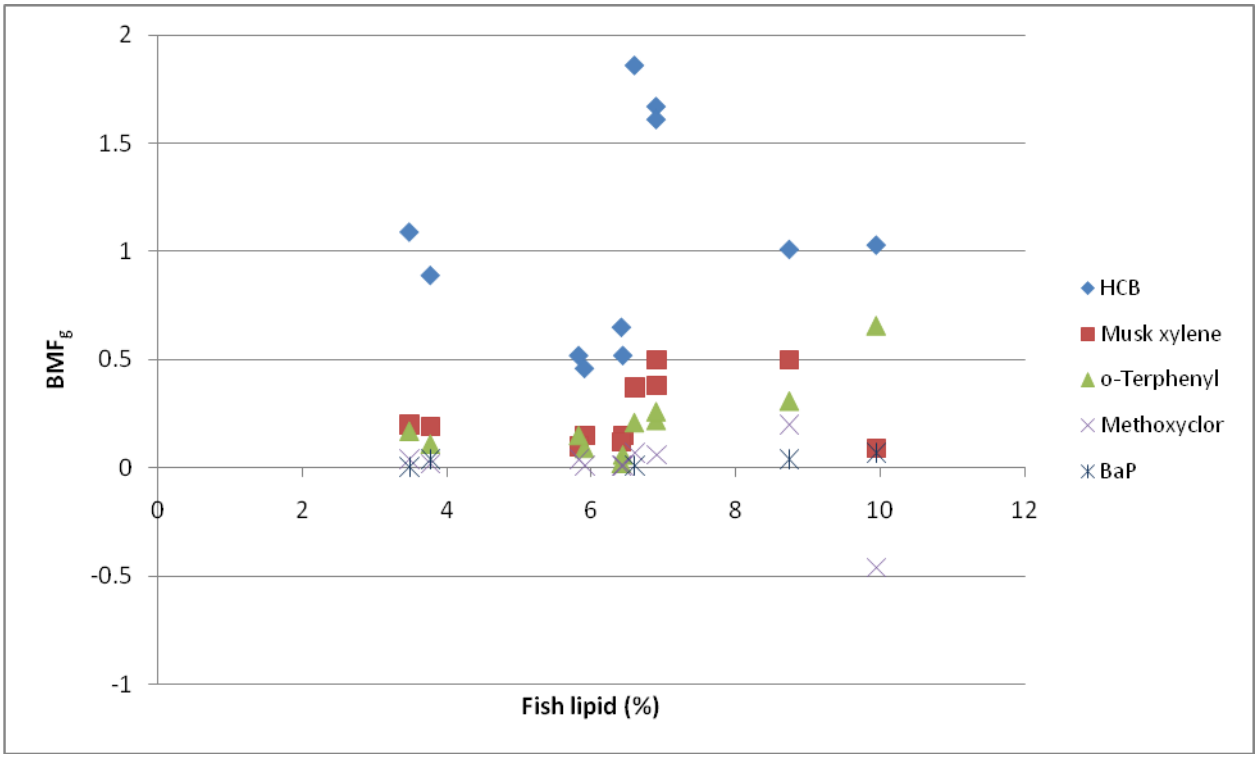


Figure 20 **Plot of growth corrected BMF (BMF_g) against initial fish weight**

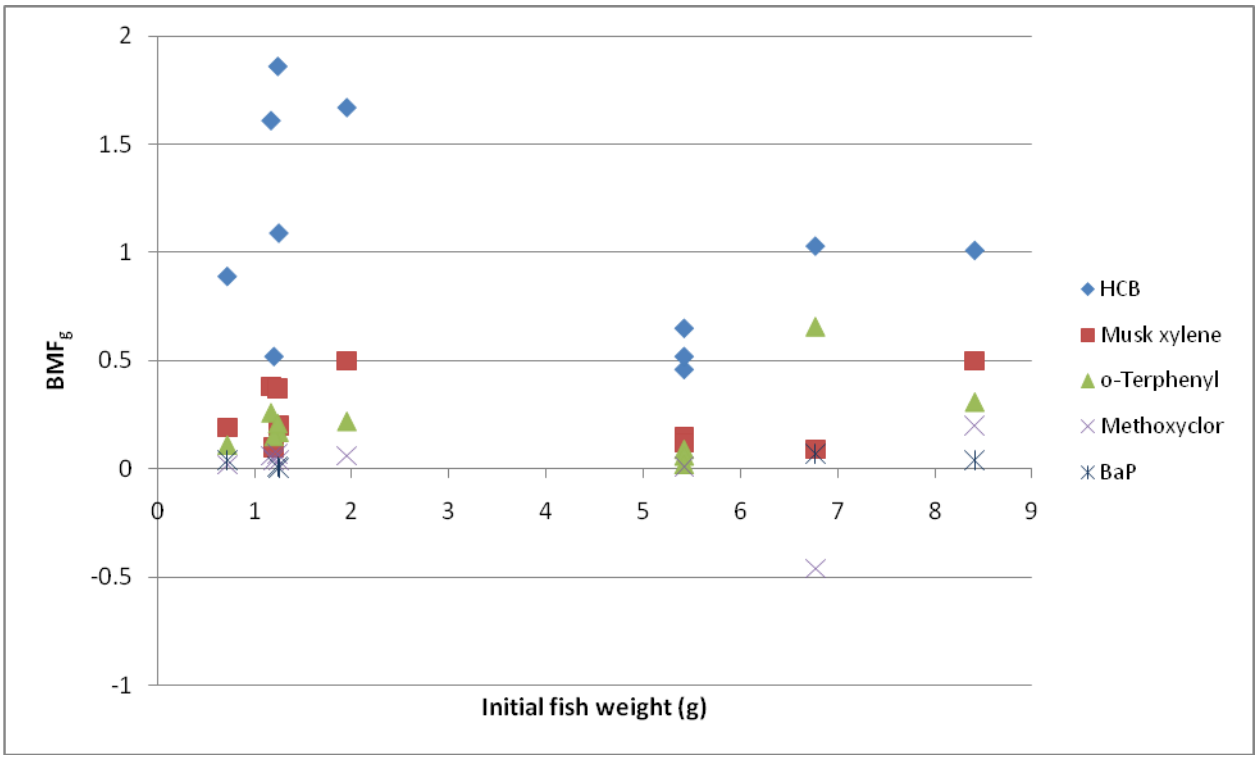


Figure 21 **Plot of growth corrected BMF (BMF_g) against food lipid content**

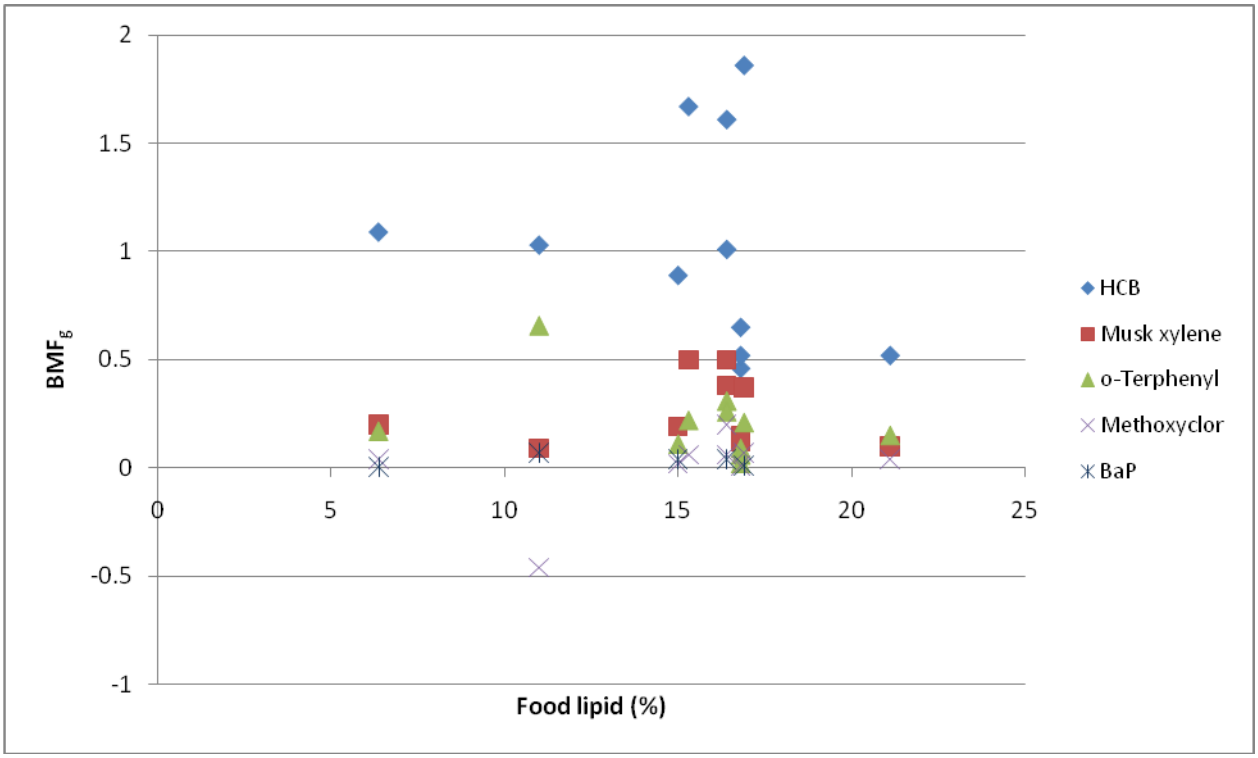


Figure 22 **Plot of lipid normalised growth corrected BMF (BMF_L) against fish lipid content**

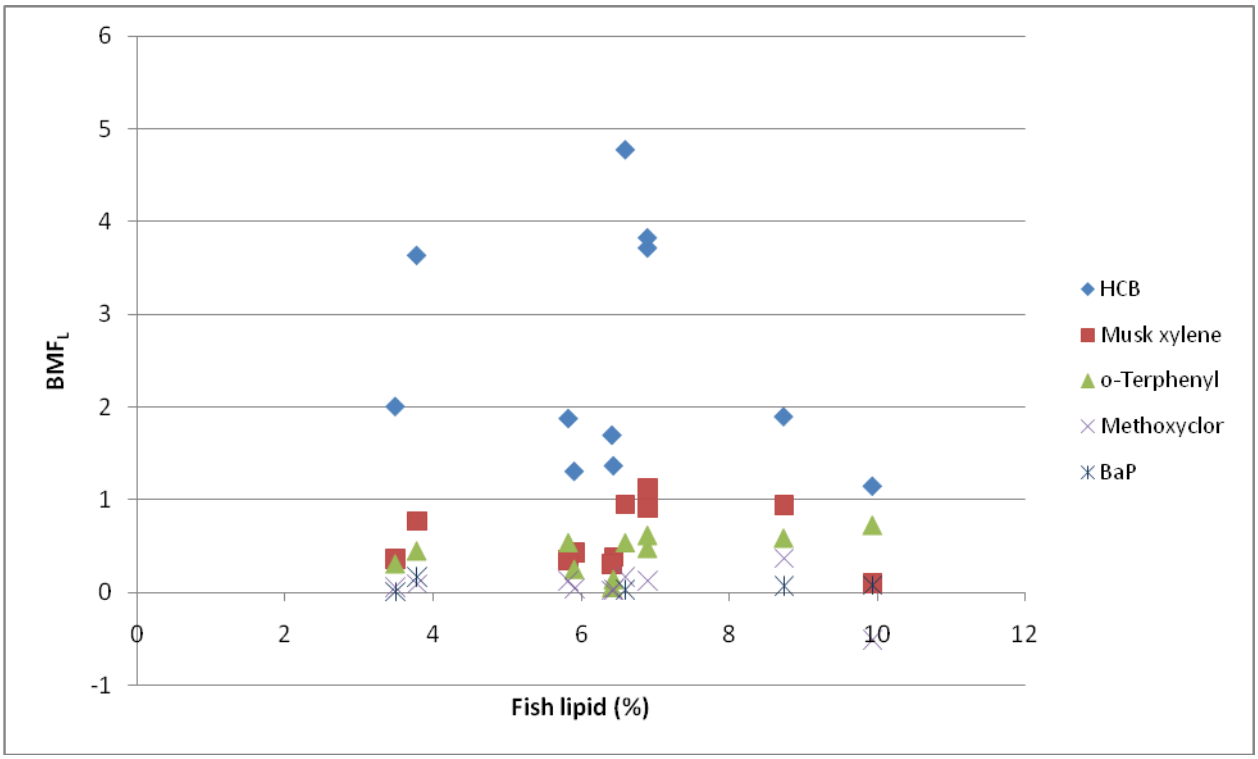


Figure 23 **Plot of growth corrected and lipid normalised BMF (BMF_L) against initial fish weight**

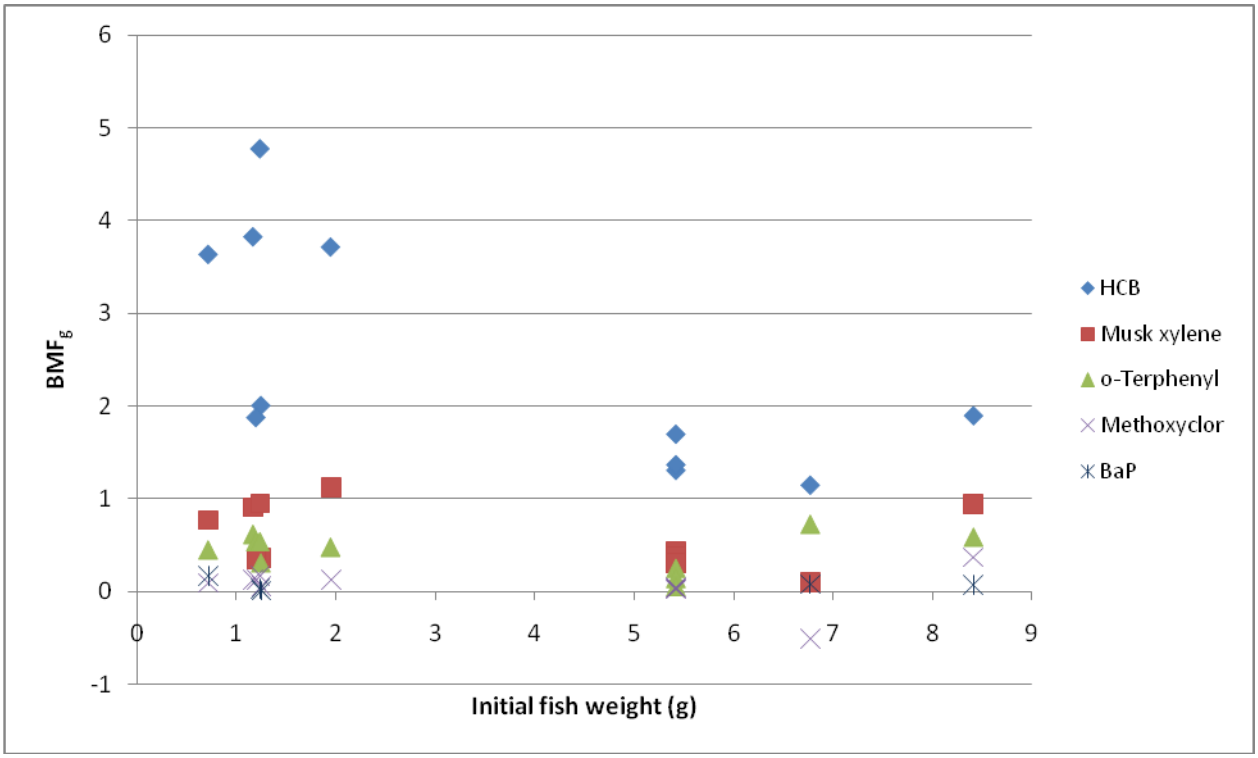
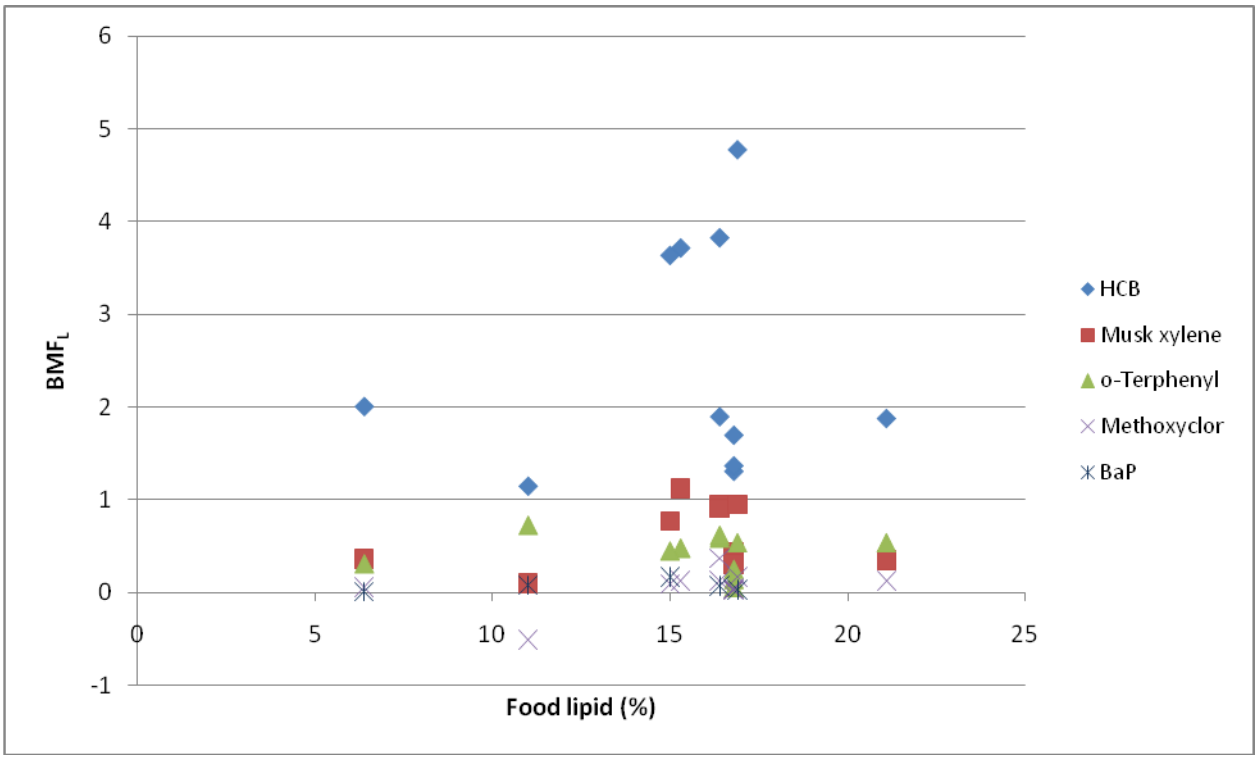


Figure 24 **Plot of growth corrected and lipid normalised BMF (BMF_L) against food lipid content**



140. The plots of BMF_g against fish lipid (Figure 19) showed a generally increasing trend in the BMF_g with increasing fish lipid content. Linear regression analysis revealed that the slopes of the plots were all positive (with the exception of the full data set for methoxychlor)[21] and that the slopes were significantly different from 0 ($p<0.05$) for the trout data set (minus Lab 5) for musk xylene, o-terphenyl and methoxychlor. In addition the slope of the plot for o-terphenyl using the full data set was also significantly different from 0 ($p<0.05$).

141. The plots of BMF_g against initial fish weight (Figure 20) showed no clear overall trend, although the trout data set (minus Lab 5) suggested an increasing trend in the BMF_g with initial fish weight for musk xylene, o-terphenyl, methoxychlor and benzo[a]pyrene but a decreasing trend in the BMF_g with increasing initial fish weight for hexachlorobenzene. Linear regression analysis[22] showed that only in the case of methoxychlor with the trout data set (minus Lab 5) was the slope significantly different from 0 ($p<0.05$).

142. The plots of BMF_g against food lipid content (Figure 21) were again inconclusive with regards to any trends. Linear regression analysis showed a general increasing trend in the BMF_g with increasing food lipid content for the trout data set (minus Lab 5) for musk xylene, o-terphenyl, methoxychlor and benzo[a]pyrene, whereas the opposite trend was found for hexachlorobenzene[23]. However, in all cases the slopes of the plots were not significantly different from 0 ($p>0.05$) for both the full data set and the trout data set (minus Lab 5).

143. Given that the proposed OECD 305 Test Guideline normalises the BMF to both the fish and food lipid content a dependence of the BMF_g on both the fish lipid content (where a increasing trend in the BMF_g with increasing fish lipid would be expected) and the food lipid content (where a decreasing trend in the BMF_g with increasing food lipid would be expected) would be predicted. This dependence was only evident on the fish lipid data in the analysis carried out here.

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[21] The regression equations derived were as follows.

Hexachlorobenzene: full data set $BMF_g = 0.043\times\%$ fish lipid + 0.75 ($R^2 = 0.025$); trout data set (minus Lab 5) $BMF_g = 0.084\times\%$ fish lipid + 0.73 ($R^2 = 0.10$).

Musk xylene: full data set $BMF_g = 0.019\times\%$ fish lipid + 0.13 ($R^2 = 0.051$); trout data set (minus Lab 5) $BMF_g = 0.068\times\%$ fish lipid - 0.078 ($R^2 = 0.60$).

o-Terphenyl: full data set $BMF_g = 0.067\times\%$ fish lipid - 0.23 ($R^2 = 0.52$); trout data set (minus Lab 5) $BMF_g = 0.032\times\%$ fish lipid + 0.012 ($R^2 = 0.77$).

Methoxychlor: full data set $BMF_g = -0.037\times\%$ fish lipid + 0.24 ($R^2 = 0.18$); trout data set (minus Lab 5) $BMF_g = 0.026\times\%$ fish lipid - 0.084 ($R^2 = 0.63$).

Benzo[a]pyrene: full data set $BMF_g = 0.0064\times\%$ fish lipid – 0.009 ($R^2 = 0.49$); trout data set (minus Lab 5) $BMF_g = 0.0028\times\%$ fish lipid + 0.0076 ($R^2 = 0.12$).

[22] The regression equations derived were as follows.

Hexachlorobenzene: full data set $BMF_g = -0.074\times$fish weight + 1.29 ($R^2 = 0.17$); trout data set (minus Lab 5) $BMF_g = -0.025\times$fish weight + 1.29 ($R^2 = 0.020$).

Musk xylene: full data set $BMF_g = -0.0034\times$fish weight + 0.26 ($R^2 = 0.004$); trout data set (minus Lab 5) $BMF_g = 0.033\times$fish weight + 0.24 ($R^2 = 0.33$).

o-Terphenyl: full data set $BMF_g = 0.019\times$fish weight + 0.13 ($R^2 = 0.09$); trout data set (minus Lab 5) $BMF_g = 0.018\times$fish weight + 0.16 ($R^2 = 0.54$).

Methoxychlor: full data set $BMF_g = -0.014\times$fish weight + 0.055 ($R^2 = 0.058$); trout data set (minus Lab 5) $BMF_g = 0.021\times$fish weight - 0.021 ($R^2 = 0.95$).

Benzo[a]pyrene: full data set $BMF_g = 0.005\times$fish weight + 0.014 ($R^2 = 0.43$); trout data set (minus Lab 5) $BMF_g = 0.003\times\%$ fish lipid + 0.015 ($R^2 = 0.27$).

[23] The regression equations derived were as follows.

Hexachlorobenzene: full data set $BMF_g = -0.026\times\%$ food lipid + 1.42 ($R^2 = 0.039$); trout data set (minus Lab 5) $BMF_g = -0.013\times\%$ food lipid + 1.44 ($R^2 = 0.015$).

Musk xylene: full data set $BMF_g = 0.014\times\%$ food lipid + 0.21 ($R^2 = 0.004$); trout data set (minus Lab 5) $BMF_g = 0.0015\times\%$ food lipid + 0.30 ($R^2 = 0.002$).

o-Terphenyl: full data set $BMF_g = -0.017\times\%$ food lipid + 0.46 ($R^2 = 0.13$); trout data set (minus Lab 5) $BMF_g = 0.002\times\%$ food lipid + 0.18 ($R^2 = 0.013$).

Methoxychlor: full data set $BMF_g = 0.016\times\%$ food lipid - 0.24 ($R^2 = 0.14$); trout data set (minus Lab 5) $BMF_g = 0.002\times\%$ food lipid + 0.039 ($R^2 = 0.023$).

Benzo[a]pyrene: full data set $BMF_g = 0.0009\times\%$ food lipid + 0.020 ($R^2 = 0.023$); trout data set (minus Lab 5) $BMF_g = 0.0024\times\%$ food lipid - 0.0092 ($R^2 = 0.36$).

144. The plots of the growth corrected and lipid normalised BMF (BMF_L) against fish lipid (Figure 22) show an apparent trend of increasing BMF_L with increasing fish lipid content for the trout data set (minus Lab 5) for all substances except benzo[a]pyrene. Linear regression analysis showed that the slopes of these plots (except for benzo[a]pyrene) were positive[24] but that the slope was only significantly different from 0 for o-terphenyl and methoxychlor (both using the trout data set minus Lab 5). As these BMF_L data have been normalised to the fish lipid content no significant trend with fish lipid would be expected.

145. The plots of the BMF_L against initial fish weight are shown in Figure 23. Linear regression analysis[25] revealed that slopes of the plots were significantly different from 0 ($p<0.05$) only for hexachlorobenzene (using the full data set but not the trout data set (minus Lab 5)) and methoxychlor (using the trout data set (minus Lab 5)). The plots using the full data sets generally showed a negative slope (decreasing BMF_L with increasing initial fish weight) but the plots using the trout data (minus Lab 5) showed a more mixed picture, with positive slopes for musk xylene, o-terphenyl and methoxychlor but negative slopes for hexachlorobenzene and benzo[a]pyrene.

146. The plots of the BMF_L against food lipid are shown in Figure 24. These plots show an apparent trend of increasing BMF_L with increasing food lipid for the trout data set (minus Lab 5) for all substances. Linear regression analysis[26] showed that the slopes of these were positive but that the slope was only significantly different from 0 for o-terphenyl (using the trout data set minus Lab 5). As these BMF_L values have been normalised to the food lipid content no significant trend with food lipid would be expected.

147. Another experimental variable that could potentially influence the results of the test is temperature. However it is not possible to carry out an analysis of the effect of this variable on the results as most of the tests with trout were carried out over a relatively narrow temperature range (12-15°C; with the exception of Lab 5 which were carried out at 9°C) and the tests with carp were all

[24] The regression equations derived were as follows.

Hexachlorobenzene: full data set $BMF_L = -0.15\times\%$ fish lipid + 3.43 ($R^2 = 0.048$); trout data set (minus Lab 5) $BMF_L = 0.033\times\%$ fish lipid + 2.90 ($R^2 = 0.003$).

Musk xylene: full data set $BMF_L = -0.0008\times\%$ fish lipid + 0.61 ($R^2 = 1.7\times10^{-5}$); trout data set (minus Lab 5) $BMF_L = 0.10\times\%$ fish lipid + 0.16 ($R^2 = 0.39$).

o-Terphenyl: full data set $BMF_L = 0.055\times\%$ fish lipid + 0.074 ($R^2 = 0.23$); trout data set (minus Lab 5) $BMF_L = 0.045\times\%$ fish lipid + 0.223 ($R^2 = 0.66$).

Methoxychlor: full data set $BMF_L = -0.038\times\%$ fish lipid + 0.31 ($R^2 = 0.11$); trout data set (minus Lab 5) $BMF_L = 0.045\times\%$ fish lipid - 0.12 ($R^2 = 0.69$).

Benzo[a]pyrene: full data set $BMF_L = -0.0015\times\%$ fish lipid + 0.081 ($R^2 = 0.005$); trout data set (minus Lab 5) $BMF_L = -0.006\times\%$ fish lipid + 0.095 ($R^2 = 0.026$).

[25] The regression equations derived were as follows.

Hexachlorobenzene: full data set $BMF_L = -0.31\times$fish weight + 3.58 ($R^2 = 0.48$); trout data set (minus Lab 5) $BMF_L = -0.19\times$fish weight + 3.53 ($R^2 = 0.20$).

Musk xylene: full data set $BMF_L = -0.038\times$fish weight + 0.74 ($R^2 = 0.097$); trout data set (minus Lab 5) $BMF_L = 0.033\times$fish weight + 0.70 ($R^2 = 0.087$).

o-Terphenyl: full data set $BMF_L = -0.009\times$fish weight + 0.46 ($R^2 = 0.013$); trout data set (minus Lab 5) $BMF_L = 0.014\times$fish weight + 0.47 ($R^2 = 0.13$).

Methoxychlor: full data set $BMF_L = -0.017\times$fish weight + 0.12 ($R^2 = 0.047$); trout data set (minus Lab 5) $BMF_L = 0.035\times$fish weight + 0.075 ($R^2 = 0.89$).

Benzo[a]pyrene: full data set $BMF_L = -0.0002\times$fish weight + 0.070 ($R^2 = 0.002$); trout data set (minus Lab 5) $BMF_L = -0.0011\times$fish weight + 0.072 ($R^2 = 0.003$).

[26] The regression equations derived were as follows.

Hexachlorobenzene: full data set $BMF_L = 0.042\times\%$ food lipid + 1.83 ($R^2 = 0.016$); trout data set (minus Lab 5) $BMF_L = 0.039\times\%$ food lipid + 2.49 ($R^2 = 0.022$).

Musk xylene: full data set $BMF_L = 0.021\times\%$ food lipid + 0.28 ($R^2 = 0.06$); trout data set (minus Lab 5) $BMF_L = 0.016\times\%$ food lipid + 0.53 ($R^2 = 0.053$).

o-Terphenyl: full data set $BMF_L = -0.002\times\%$ food lipid + 0.45 ($R^2 = 0.001$); trout data set (minus Lab 5) $BMF_L = 0.019\times\%$ food lipid + 0.21 ($R^2 = 0.65$).

Methoxychlor: full data set $BMF_L = 0.023\times\%$ food lipid – 0.29 ($R^2 = 0.17$); trout data set (minus Lab 5) $BMF_L = 0.008\times\%$ food lipid + 0.030 ($R^2 = 0.13$).

Benzo[a]pyrene: full data set $BMF_L = 0.006\times\%$ food lipid – 0.004 ($R^2 = 0.16$); trout data set (minus Lab 5) $BMF_L = 0.006\times\%$ food lipid - 0.019 ($R^2 = 0.19$).

carried out at 24.7-24.8°C. Similarly another potential variable, pH, was similar in all tests (range of mean pH was 7.0 to 7.9).

148. Overall the analysis here is relatively inconclusive. The fact that the lipid content and the initial fish weight appear to co-vary means that it is not possible to distinguish between trends in the data related purely to the lipid content and trends in the data related purely to the fish weight. There are indications from the data for the following trends, however it is difficult to conclude definitively which of these trends are real and which result from uncertainties in the data.

a. No clear trend is apparent in the growth rate constant in relation to the fish lipid content.

b. There is a trend towards a decrease in the growth rate constant with an increase in the fish lipid content.

c. There is a trend towards a decrease in the growth rate constant with an increase in the initial fish weight.

d. There is a correlation between the food lipid content and the fish lipid content, particularly at the end of the study period.

e. There is a trend towards a decrease in the overall depuration rate constant with increasing lipid content of the fish (or initial fish weight) for hexachlorobenzene, musk xylene, o-terphenyl and methoxychlor. This may relate, at least in part, to the apparent decrease in the growth rate constant component with increasing lipid content of the fish.

f. No trend was apparent in the growth corrected depuration rate constant with fish lipid content.

g. The assimilation efficiency shows a general increasing trend with increasing fish lipid content and initial fish weight.

h. There is no discernable trend in the variation of the assimilation efficiency with food lipid content.

i. The BMF_g generally showed an increasing trend with increasing lipid content.

j. No overall trend was evident between the BMF_g and initial fish weight or food lipid content.

k. An apparent trend towards increasing BMF_L with increasing fish lipid content was observed for hexachlorobenzene, musk xylene, o-terphenyl and methoxychlor.

l. No overall trend was evident between the BMF_L and initial fish weight.

m. An apparent trend towards increasing BMF_L with increasing food lipid was observed for all substances.

SUMMARY AND RECOMMENDATIONS

149. The results of the ring test using trout are summarised below for hexachlorobenzene, musk xylene, o-terphenyl and methoxychlor. These values are derived for the data for rainbow trout (minus the data for Lab 5; using t=13 days and the nominal feeding rate) and provide a measure of interlaboratory variability. The results for benzo[a]pyrene are uncertain owing to a rapid depuration of this substance.

	Assimilation efficiency		**BMF_L (A)**		**BMF_L (B)**	
	Mean	Rel. SD	Mean	Rel. SD	Mean	Rel. SD
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.

150. The relative standard deviation in the growth corrected depuration rate constant (k_{2g}) for the trout data (minus Lab 5) was in the range 31% to 48% depending on the substance when using the rate constant subtraction method or 27% to 44% when using the alternative method.

151. Measures of intralaboratory variability can be obtained from the carp data generated as part of the ring test, and from two other data sets generated within two of the laboratories (one with carp and one with trout) using hexachlorobenzene. For the trout data from Lab 6, the relative standard deviation in the growth corrected depuration rate constant (k_{2g}) and the assimilation efficiency are both around 26-27% for hexachlorobenzene. However, the relative standard deviation around the BMF_L is higher at around 42%.

152. The carp data for Lab 2 from the ring test show a relative standard deviation of between 9 and 27% in the k_{2g} (using the rate constant subtraction method; range is 11% to 32% using the alternative method), between 11% and 60% in the assimilation efficiency and between 14% and around 70% in the BMF_L, depending on the substance. For the second set of data for Lab 2 with hexachlorobenzene, the relative standard deviations are around 32% in the k_{2g}, 12% in the assimilation efficiency and 22% in the BMF_L.

153. The BMF_L values derived decrease in the order hexachlorobenzene > musk xylene > o-terphenyl > methoxychlor. The BMF_L derived for hexachlorobenzene is generally >1 whereas the values for o-terphenyl and methoxychlor (and benzo[a]pyrene) are generally <1. Most values for the BMF_L derived for musk xylene are <1 but a few values >1 were also derived. The trend in the BMF_L values derived is as would be expected based on the known bioaccumulation potential of these substances.

154. The BCF values estimated from the depuration data from the ring test are generally consistent with the available experimental BCF data for these substances.

155. It is recommended that the following points are considered in the new OECD 305 Test Guideline.

- Fish growth was extensive with both rainbow trout and carp over the course of the studies at a 3% feeding rate. Although the results can be corrected for growth dilution, other aspects, such as increases in lipid content are not so easy to address. Although growth of fish cannot be avoided when using juveniles, it should be considered to reduce the growth as much as practical. Although no data are available as yet from the ring test to demonstrate it, it is

likely that the growth of the fish will be related to the feeding rate used. Therefore a reduction of the feeding rate used may reduce the growth of the fish.

- There is some evidence from the ring test that the increase in lipid content seen in the fish may be related to the lipid content of the feed used. Therefore it could be considered to limit the recommended lipid content of the feed. Clearly, such recommendations would also need to take into account the lipid contents of available commercial feeds.

- There is some evidence for increased depuration (possibly by metabolism) for the more rapidly depurated substances in carp compared to trout. It is understood that possible differences between carp and trout are being considered in a separate study.

- The sampling schedule used in the depuration phase of the ring test may not be appropriate for rapidly depurating substances.

- The relevant times to use in the various equations to calculate the kinetic parameters are not always clear. This particularly relates to the following two aspects.

    - The number of days for the length of the uptake phase to be used in the calculation of the assimilation efficiency.

    - The timing of the start of the depuration phase for extrapolation of the C_0 values.

  It is recommended that clear guidance on how these parameters are calculated is given in the OECD 305 Test Guideline.

- If a reliable estimate of the assimilation efficiency is required it is important to take into account the actual feeding rate throughout the uptake phase for fish that are growing, i.e. it is necessary to correct the nominal feeding rate for growth of the fish. This is less important for the BMF_L.

- The importance of using fish of a similar weight at the start of the test should be stressed. A high standard deviation in the mean starting weight is, based on the limited evidence in the current ring test, likely to be maintained throughout the experimental period and this will propagate through and contribute to the uncertainty in the growth rate constant. Further, although there is no direct evidence of this from the ring test, a wider range of fish weights at the start of the test may increase the chances of unequal feeding (on a weight per weight basis) of the smaller fish versus the larger fish during the test, resulting in variation of exposure of individual fish to the test substance.

156. It should be noted that the fish sampling protocol used in the ring test differed from that currently proposed in the draft OECD 305 Test Guideline. In the ring test no equivalent end of uptake sample was included, and the first depuration sample differed in pre-treatment and timing. This means it was not possible to investigate fully the appropriateness of the proposed sampling method for these two sample points. Nonetheless, analysis carried out in this report suggests that the effect of this difference is likely only to be small (omission of the first depuration sampling point from the ring test data analysis did not provide any strong evidence that the studies' derived parameters were skewed, for example by presence of undigested food in the gut).

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ANNEX 1 – SUMMARY OF RAW DATA FROM THE PRE-STUDY PHASE

LABORATORY 1

FEED ANALYSIS RESULTS (PRE-STUDY ANALYTICAL VALIDATION)

Analytical method description

The method provided was followed, with the following small differences:

1) The internal standard mixture was not available, and therefore not used.
2) Samples were diluted 1:100 with acetone/dichloromethane prior to measurement to reduce matrix effects.
3) Matrix calibration solutions were used.

Results of spiked diet analysis – EMBSI

The analytical results of the fish food spiked by EMBSI are given in the table below.

	HCB (ppm)	Musk xylene (ppm)	o-Terphenyl (ppm)	Methoxy chlor (ppm)	B[a]P (ppm)
Sample 1	93.8	88.4	86.1	81.4	93.1
Sample 2	97.9	92.8	89.3	84.5	98.6
Sample 3	94.2	87.7	86.4	82.5	93.8
Sample 4	101.6	95.8	93.5	90.0	107.5
Sample 5	96.2	92.7	88.5	84.5	99.9
Control 1	0	0	0	0	0
Control 2	0	0	0	0	0
Average	96.7	91.5	88.7	84.6	98.6
STDEV	3.1	3.4	3.0	3.3	5.8
RSD (%)	3.3	3.7	3.3	3.9	5.8

FISH ANALYSIS RESULTS

Analytical method description

The method for the analysis of hexachlorobenzene (HCB), musk xylene, o-terphenyl, methoxyclor and benzo[a]pyrene (B[a]P) was successfully implemented. No other details were given. By inference this was the method as outlined by the lead laboratory, EMBSI.

Results

Results of the fish samples provided by EMBSI

The results of the fish samples provided by EMBSI are summarised in the table below.

Sample description	Fish weight (g)	HCB (ppm)*	Musk xylene (ppm)*	o-Ter-phenyl (ppm)*	Methoxy-chlor (ppm)*	B[a]P (ppm)*
Treated Fish 1	1.395	5.6	1.8	2.4	0.33	0.14
Treated Fish 2	1.822	8.2	3.2	3.5	0.72	0.02
Treated Fish 3	1.681	9.0	3.6	3.8	0.58	0.07
Treated Fish 4	2.135	22.1	12.7	9.4	7.3	0.16
Treated Fish 5	2.138	11.5	6.0	4.9	1.7	0.27
Treated Fish 6	2.091	10.4	5.2	4.4	1.5	0.07
Treated Fish 7	1.615	8.3	4.0	3.5	1.0	0.04
Treated Fish 8	1.552	9.1	4.0	3.9	1.0	0.04
Control Fish 1	2.331	<LOD	ND	<LOD	ND	ND
Control Fish 2	1.916	<LOD	ND	<LOD	ND	ND
Average (ppm)		10.5	5.0	4.5	1.8	0.10
STDEV (ppm)		5.0	3.3	2.1	2.3	0.08
RSD (%)		47	66	47	129	83

Note: * Not corrected for recovery.

Results of fortified samples

The results of the fortification samples are demonstrated in the table below.

	Control fish (Laboratory 1)	Fish A (Laboratory 1)		Fish B (Laboratory 1)		Fish C (Exxon #155)	
Fortification Level (ppm)	0	0.40		0.52		0.24	
Fish weight (g)	1.34	1.26		0.96		2.103	
Recovery	**(ppm)**	**(ppm)**	**(%)**	**(ppm)**	**(%)**	**(ppm)**	**(%)**
HCB	ND	0.33	81	0.32	80	0.18	76
Musk Xylene	ND	0.38	96	0.39	97	0.27	115
o-Terphenyl	ND	0.37	91	0.36	90	0.21	87
Methoxychlor	ND	0.42	104	0.42	105	0.24	103
B[a]P	ND	0.37	93	0.38	94	0.19	80

[Note given in test report: As a deviation to the provided method, ^{13}C hexachlorobenzene was used as internal standard as the recommended internal standard mixture is not yet available.]

Lipid analysis

The results of the lipid determination by accelerated solvent extraction (ASE) with hexane for the fish samples obtained from EMBSI are given below.

	Fish weight (g)	Sample weight (minced fish) (g)	Lipid amount (mg)	mg lipid/g fish (mg/g)
Sample 3	2.285	1.858	69.3	37.30
Sample 4	1.947	1.700	31.5	18.53
Sample 5	1.783	1.590	40.6	25.53
Sample 6	1.666	1.474	44.3	30.05
Sample 7	2.435	2.238	86.0	38.43
Blank	0.000	0.000	0.6	-
Average				29.97
STDEV				8.30
RSD (%)				27.7

Note: Lipid contents were calculated based on the sample weights. The blank value was negligible and was therefore not subtracted from the sample results.

LABORATORY 2

FEED ANALYSIS RESULTS (PRE-STUDY ANALYTICAL VALIDATION)

Analytical method description

Diet extraction procedure

1) Weigh out 0.500 g of spiked diet.
2) Add 15.0 mL extraction solvent (acetone).
3) Homogenise extraction, using homogeniser (Kinematica) for one minute.
4) Centrifuge (7,000 ×g) for 5 minutes.
5) Filter the supernatant with absorbent cotton.
6) Bring to 25 mL using volumetric flasks with acetone. Resulting concentration of each compound is 2.00 µg/mL.
7) Transfer 1.0 mL using whole pipette to 10 mL volumetric flasks.
8) Bring to 10 mL with hexane.
9) Transfer 1.0 mL of the extract to an amber GC autosampler vial.
10) Add 10 µL of a 200 µg/mL SV internal standard solution. Analyse by GC-MS.

Recovery test of diet sample

Recovery test was conducted as follows:

1) Weigh out 0.500 g of control diet.
2) Add 250 µL of standard solution (200 mg/L of each compound in acetone).

Following procedure is same as *Diet extraction procedure*. The recovery test was conducted in duplicate.

Analysis was carried out by GC-MS (QP-2010 shimadzu).

Results

Results of spiked diet analysis – EMBSI

The analytical results of spiked diet provided by EMBSI are shown in the table below. No peak was found in any of the peak positions of test substances in control diet.

	HCB (µg/g)	Musk xylene (µg/g)	o-Terphenyl (µg/g)	Methoxy chlor (µg/g)	B[a]P (µg/g)
Sample 1	103	101	104	92.8	103
Sample 2	105	102	103	95.4	105
Sample 3	109	101	110	103	103
Sample 4	107	99.3	105	109	103

	HCB (µg/g)	Musk xylene (µg/g)	o-Terphenyl (µg/g)	Methoxy chlor (µg/g)	B[a]P (µg/g)
Sample 5	101	98.5	101	106	98.1
Control 1					
Control 2					
Average	104	100	105	101	104
STDEV	2.8	1.2	3.1	6.2	2.2
RSD (%)	2.7	1.2	3.0	6.1	2.1

Recovery test of spiked diet samples

The recovery rate from fish feed is shown in the table below.

	HCB (%)	Musk xylene (%)	o-Terphenyl (%)	Methoxychlor (%)	B[a]P (%)
Sample 1	94.7	92.7	89.4	112	96.8
Sample 2	93.7	95.7	91.1	120	97.8
Sample 3					
Sample 4					
Sample 5					
Control 1					
Control 2					
Average	94.2	94.2	90.2	116	97.3
STDEV					
RSD					

<u>Preparation of spiked diet</u>

Preparation of spiked diet using corn oil suspension

1) Combine 0. 10 g of each test compound in a 5.0 mL volumetric flask.
2) Bring to volume with corn oil. Resulting concentration of each compound is 20 mg/mL (2% w/v).
3) Add micro-stir bar and stopper.
4) Mix rapidly on stir plate overnight to suspend/emulsify solid compounds in corn oil. There may be partial dissolution of the compounds in corn oil.
5) Place 50 g of unspiked fish feed in amber glass bottle.
6) Add 0.5 mL of corn oil suspension using a wide bore syringe or pipette.
7) Add an additional 50 g of fish diet and cap bottle. The concentration of each compound in the diet will be 100 µg/g.
8) Shake the bottle by hand to homogenize the corn oil suspension throughout the fish feed.

9) Place the bottle containing the spiked diet on a mechanical tumbler and tumble slowly overnight.

Concentrations of the test substances in spiked diet prepared using corn oil suspension are shown in the table below.

	HCB (µg/g)	Musk xylene (µg/g)	o-Terphenyl (µg/g)	Methoxy chlor (µg/g)	B[a]P (µg/g)
Sample 1	76.4	95.4	93.4	108	95.5
Sample 2	76.0	82.3	85.2	106	87.9
Sample 3	72.6	81.8	85.1	104	79.3
Sample 4	69.6	78.3	80.8	97.4	82.3
Sample 5	74.1	80.3	83.1	98.3	82.8
Control 1					
Control 2					
Average	73.7	83.6	85.5	103	85.6
STDEV	2.5	6.1	4.3	4.1	5.7
RSD (%)	3.4	7.3	5.0	4.0	6.7

Note: Concentrations of four substances were lower than the setting concentrations.

Preparation of spiked diet using solvent and corn oil (combination method)

1) Weigh out 0.10 g of each test substances in a 100 mL volumetric flask.
2) Bring to volume with acetone. Resulting concentration of each compound is 1000 µg/mL.
3) Place 0.5 mL of corn oil in boiling flask.
4) Add 10 mL of each test substance solution using whole pipette.
5) Evaporate the solvent using rotary evaporator at 40 °C.
6) Add 50 g of unspiked fish feed in boiling flask.
7) Shake the bottle by hand.
8) Add an additional 50 g of unspiked fish feed in boiling flask. The concentration of each compound in the diet will be 100 µg/g.
9) Place the bottle containing the spiked diet on a mechanical tumbler and tumble slowly overnight.

Concentrations of test substances in spiked diet prepared using solvent and corn oil (combination method) are shown in the table below.

	HCB (µg/g)	Musk xylene (µg/g)	o-Terphenyl (µg/g)	Methoxy chlor (µg/g)	B[a]P (µg/g)
Sample 1	102	94.8	100	121	92.7
Sample 2	106	94.1	96.4	127	96.6
Sample 3	100	102	100	124	101
Sample 4	108	102	106	130	105
Sample 5	100	100	103	126	105
Control 1					

	HCB (µg/g)	Musk xylene (µg/g)	o-Terphenyl (µg/g)	Methoxy chlor (µg/g)	B[a]P (µg/g)
Control 2					
Average	103	98.6	101	125	100
STDEV	3.3	3.4	3.2	3.0	4.9
RSD (%)	3.2	3.5	3.1	2.4	4.9

Note: High homogeneity was seen for four substances. However, the concentration of methoxychlor was higher than the setting concentration.

FISH ANALYSIS RESULTS

Analytical method description

Fish extraction procedure

Fish extraction:

1) Weigh the fish.
2) Chop the fish using scissors.
3) Refine the fish sample using homogeniser (Kinematica) for two minutes.
4) Weigh out 5.00 g of sample into 40 mL extraction vial.
5) Add 25 mL extraction solvent (acetone).
6) Solvent extraction, using homogeniser (Kinematica) for one minute.
7) Centrifuge (7,000 ×*g*) for 5 minutes.
8) Dehydrate with sodium sulfate.
9) Filter the supernatant with filter paper.
10) Bring to 50 mL using volumetric flasks with acetone.

Fish extract clean-up:

1) Transfer 10 mL of fish extraction to boiling flask using whole pipette.
2) Evaporate the solvent using a rotary evaporator at 40 °C.
3) Add 5 mL of hexane/ethyl acetate (1:1, v/v) solution.
4) Condition silica SPE (Sep-Pak® Plus Silica cartridge) cartridges with 10 mL of hexane/ethyl acetate (1:1, v/v).
5) Add fish extracts (from 3) to the SPE cartridges and collect cartridge eluent.
6) Elute cartridges with additional 10 mL of hexane/ethyl acetate (1:1, v/v) and collect the eluent.
7) Evaporate the solvent using a rotary evaporator at 40 °C.
8) Bring to 2 mL using volumetric flasks with hexane.
9) Transfer 1.0 mL of the extract to an amber GC auto sampler vial.
10) Add 10 µL of a 2.00 µg/mL SV internal standard solution. Analyse by GC-MS (QP-2010 shimadzu).

Recovery test of fish sample

Recovery test was conducted as follows.

1) Chop the fish using scissors.
2) Weigh out 10.0 g of fish sample.
3) Add 100 µL of standard solution (1000 mg/L of each compound in acetone).
4) Refine the fish sample using homogeniser (Kinematica) for two minutes.
5) Weigh out 5.00 g of sample into 40 mL extraction vial.

Following procedure is same as *Fish extraction procedure*. The recovery test was conducted in duplicate.

Fish lipid determination

1) Weigh the fish.
2) Chop the fish using scissors.
3) Refine the fish sample using homogenizer for two minutes.
4) Weigh out 1.00 g of sample.
5) Add 45 mL of chloroform/methanol (1:2, v/v) solution.
6) Homogenize extraction, using homogenizer for two minutes.
7) Add 15 mL of chloroform solution.
8) Solvent extraction, using homogenizer for two minutes.
9) Filter using filter paper.
10) Add 60 mL of chloroform/methanol (1:1, v/v) solution to the residue.
11) Filter using filter paper.
12) Collect the filtrate to a separation funnel.
13) Add 44 mL of refined water.
14) Shake the separation funnel by hand.
15) Permit contents to settle for more than 15 hours.
16) Collect the bottom layer and dehydrate with sodium sulfate.
17) Evaporate the solvent using rotary evaporator at 40 °C.
18) Add 40 mL of chloroform.
19) Transfer to another boiling flask.
20) Evaporate the solvent using rotary evaporator at 40 °C.
21) Place the contents in the vacuum desiccators for more than 4 hours.
22) Calculate lipid content.

Results

Results of the fish samples provided by EMBSI

The results from the analysis of the incurred fish supplied by EMBSI are shown in the table below. No peak was found in any of the peak positions of test substances in control fish.

Sample	HCB (µg/g)	Musk xylene (µg/g)	o-Terphenyl (µg/g)	Methoxychlor (µg/g)	B[a]P (µg/g)	Fish weight (g)
Incurred Fish 1	23.8	13.5	11.8	3.7	0.41	2.51
Incurred Fish 2	15.7	6.9	6.6	1.1	0.10	1.44
Incurred Fish 3	16.0	8.4	7.2	3.4	0.93	2.39

Sample	HCB (µg/g)	Musk xylene (µg/g)	o-Terphenyl (µg/g)	Methoxychlor (µg/g)	B[a]P (µg/g)	Fish weight (g)
Incurred Fish 4	16.4	6.5	7.1	1.4	0.05	1.82
Incurred Fish 5	12.7	3.8	3.6	0.6	0.04	1.50
Incurred Fish 6	13.6	6.4	6.4	1.4	0.09	1.62
Incurred Fish 7	17.5	9.1	8.0	1.0	0.14	2.04
Incurred Fish 8	18.4	7.1	7.5	1.7	0.10	1.69
Control Fish 1						
Control Fish 2						
Average	16.8	7.7	7.3	1.8	0.23	1.88
STDEV	3.2	2.6	2.1	1.0	0.29	0.4
RSD (%)	18.9	34.1	29.3	57.7	122.6	-

Recovery test of spiked fish samples

Recovery rate is shown in the table below. [Units not given in test report – assumed to be %]

Sample	HCB (%)	Musk xylene (%)	o-Terphenyl (%)	Methoxychlor (%)	B[a]P (%)
Spiked Fish 1	71.5	81.4	81.1	78.1	74.9
Spiked Fish 2	68.6	85.5	84.8	79.7	74.5
Average	70.1	83.5	83.0	78.9	74.7

Lipid analysis

The individual lipid content for each of the five control fish samples is shown in the table below.

Sample	Lipid content (%)
Control Fish 1	4.41
Control Fish 2	4.03
Control Fish 3	4.32
Control Fish 4	3.07
Control Fish 5	3.10
Average	3.79
STDEV	0.59
RSD (%)	15.5

LABORATORY 3

FEED ANALYSIS RESULTS (PRE-STUDY ANALYTICAL VALIDATION)

Analytical method description

Laboratory 3 was the only partner laboratory taking part in the ring test to compare the two techniques; solvent spiked and corn oil spiked fish feed samples. The standard protocol for diet preparation was used.

Calibration solutions and the prepared samples, each of 25 mL were diluted 1:100 with toluene prior to injection, calibration units: µg/mL.

Preparation of spiked diet

Preparation of spiked diet using corn oil suspension

See *Feed analysis results* for Laboratory 2 (the same protocol was used for the preparation of spiked diet using corn oil suspension). Two batches of corn oil spiked feed samples were produced as part of this study.

Preparation of spiked diet using corn oil suspension

40ml of acetone containing 10mg of each test item were sprayed portion wise on 100g of fish food with a pump spray bottle to achieve the required nominal dose level of 100µg/g. The food/test substance was constantly mixed during the spiking procedure in a stainless steel mixing bowl. The freshly-dosed fish food was left in the bowl in a laboratory hood for two days (stirred occasionally) to allow the excess acetone to evaporate. The spiked diet was transferred into a brown glass bottle and kept refrigerated until use. Two batches of solvent spiked feed samples were produced as part of this study.

Results

Results of spiked diet analysis – EMBSI

The analytical results of the fish food spiked by EMBSI are given in the table below. The analytical results for the corresponding two blanks (control fish feed) were <LOQ for all analytes. The LOQ was set to 20 mg/kg corresponding to the concentration of lowest calibration solution and a sample weight of 1.0 g.

	HCB (mg/kg)	Musk xylene (mg/kg)	o-Terphenyl (mg/kg)	Methoxychlor (mg/kg)	B[a]P (mg/kg)
Sample 1	104.41	101.10	102.96	100.67	104.58
Sample 2	100.58	102.35	100.75	109.80	104.78

	HCB (mg/kg)	Musk xylene (mg/kg)	o-Terphenyl (mg/kg)	Methoxychlor (mg/kg)	B[a]P (mg/kg)
Sample 3	99.58	105.40	102.64	103.23	100.35
Sample 4	95.60	98.77	101.43	102.19	102.14
Sample 5	93.77	97.47	98.56	103.22	101.93
Control 1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Control 2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Average					
STDEV					
RSD (%)					

Note: Nominal concentration 100 mg/kg.

Recovery test of spiked diet samples

	HCB (%)	Musk xylene (%)	o-Terphenyl (%)	Methoxychlor (%)	B[a]P (%)
Sample 1	104.4	101.1	103.0	100.7	104.6
Sample 2	100.6	102.4	100.8	109.8	104.8
Sample 3	99.6	105.4	102.6	103.2	100.3
Sample 4	95.6	98.8	101.4	102.2	102.1
Sample 5	93.8	97.5	98.6	103.2	101.9
Control 1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Control 2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Average	98.8	101.0	101.3	103.8	102.8
STDEV	4.20	3.11	1.76	3.50	1.89
RSD	4.25	3.08	1.74	3.37	1.84

Note: Nominal concentration 100 mg/kg.

Recoveries – average values per sample and overall

	Average (%) (Sample)	STDEV (%) (Sample)	RSD (%) (Sample)
Sample 1	102.7	1.82	1.77
Sample 2	103.7	3.83	3.69
Sample 3	102.2	2.33	2.28
Sample 4	100.0	2.84	2.84
Sample 5	99.0	3.75	3.79
Control 1	<LOQ	<LOQ	<LOQ
Control 2	<LOQ	<LOQ	<LOQ
Average (%) (overall)		101.5	
STDEV (%) (overall)		3.28	
RSD (%) (overall)		3.23	

Note: Nominal concentration 100 mg/kg.

Solvent spiked fish feed

Batch 1 – analytical results

Calibration solutions and the prepared samples, each of 25 mL were diluted 1:100 with toluene prior to injection, calibration units: µg/mL.

	HCB (mg/kg)	Musk xylene (mg/kg)	o-Terphenyl (mg/kg)	Methoxychlor (mg/kg)	B[a]P (mg/kg)
Sample 1	94.46	85.78	84.46	87.70	82.53
Sample 2	89.49	86.86	81.86	89.29	85.09
Sample 3	89.76	84.52	81.29	89.54	81.49
Sample 4					
Sample 5					
Control 1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Control 2					
Average					
STDEV					
RSD (%)					

Note: Nominal concentration 100 mg/kg.

Recovery test of spiked diet samples

	HCB (%)	Musk xylene (%)	o-Terphenyl (%)	Methoxychlor (%)	B[a]P (%)
Sample 1	94.5	85.8	84.5	87.7	82.5
Sample 2	89.5	86.9	81.9	89.3	85.1
Sample 3	89.8	84.5	81.3	89.5	81.5
Sample 4					
Sample 5					
Control 1					
Control 2					
Average	91.2	85.7	82.5	88.8	83.0
STDEV	2.8	1.2	1.7	1.0	1.8
RSD (%)	3.1	1.4	2.0	1.1	2.2

Note: Nominal concentration 100 mg/kg.

Recoveries – average values per sample and overall

	Average (%) (Sample)	STDEV (%) (Sample)	RSD (%) (Sample)
Sample 1	87.0	4.6	5.3
Sample 2	86.5	3.2	3.7
Sample 3	85.3	4.2	4.9
Sample 4			
Sample 5			
Control 1			
Control 2			
Average (%) (overall)		86.3	
STDEV (%) (overall)		3.8	
RSD (%) (overall)		4.4	

Note: Nominal concentration 100 mg/kg.

Batch 2 – analytical results

	HCB (mg/kg)	Musk xylene (mg/kg)	o-Terphenyl (mg/kg)	Methoxychlor (mg/kg)	B[a]P (mg/kg)
Sample 1	102.94	100.15	94.45	101.68	99.05
Sample 2	105.73	98.22	95.32	102.70	95.87
Sample 3	102.92	97.73	95.25	103.85	97.83
Sample 4					
Sample 5					
Control 1					
Control 2					
Average					
STDEV					
RSD (%)					

Note: Nominal concentration 100 mg/kg.

Recovery test of spiked diet samples

	HCB (%)	Musk xylene (%)	o-Terphenyl (%)	Methoxychlor (%)	B[a]P (%)
Sample 1	102.9	100.1	94.5	101.7	99.1
Sample 2	105.7	98.2	95.3	102.7	95.9
Sample 3	102.9	97.7	95.3	103.8	97.8
Sample 4					
Sample 5					
Control 1					
Control 2					
Average	103.9	98.7	95.0	102.7	97.6
STDEV	1.6	1.3	0.5	1.1	1.6
RSD (%)	1.6	1.3	0.5	1.1	1.6

Note: Nominal concentration 100 mg/kg.

Recoveries – average values per sample and overall

	Average (%) (Sample)	STDEV (%) (Sample)	RSD (%) (Sample)
Sample 1	99.7	3.3	3.3
Sample 2	99.6	4.5	4.5
Sample 3	99.5	3.7	3.7
Sample 4			
Sample 5			
Control 1			
Control 2			
Average (%) (overall)		99.6	
STDEV (%) (overall)		3.6	
RSD (%) (overall)		3.6	

Note: Nominal concentration 100 mg/kg.

Corn oil fish feed

Batch 1 – analytical results

Calibration solutions and the prepared samples, each of 25 mL were diluted 1:100 with toluene prior to injection, calibration units: µg/mL.

	HCB (mg/kg)	Musk xylene (mg/kg)	o-Terphenyl (mg/kg)	Methoxychlor (mg/kg)	B[a]P (mg/kg)
Sample 1	112.05	128.17	122.53	142.17	127.62
Sample 2	115.55	131.75	127.84	142.55	128.29
Sample 3	111.22	122.01	120.01	139.63	123.85
Sample 4					
Sample 5					
Control 1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Control 2					
Average					
STDEV					
RSD (%)					

Note: Nominal concentration 100 mg/kg.

Recovery test of spiked diet samples

	HCB (%)	Musk xylene (%)	o-Terphenyl (%)	Methoxychlor (%)	B[a]P (%)
Sample 1	112.1	128.2	122.5	142.2	127.6
Sample 2	115.6	131.8	127.8	142.5	128.3
Sample 3	111.2	122.0	120.0	139.6	123.9
Sample 4					
Sample 5					
Control 1					
Control 2					
Average	112.9	127.3	123.5	141.4	126.6
STDEV	2.3	4.9	4.0	1.6	2.4
RSD (%)	2.0	3.9	3.2	1.1	1.9

Note: Nominal concentration 100 mg/kg.

Recoveries – average values per sample and overall

	Average (%) (Sample)	STDEV (%) (Sample)	RSD (%) (Sample)
Sample 1	126.5	10.9	8.6
Sample 2	129.2	9.7	7.5
Sample 3	123.3	10.3	8.4
Sample 4			
Sample 5			
Control 1			
Control 2			
Average (%) (overall)		126.3	
STDEV (%) (overall)		9.9	
RSD (%) (overall)		7.8	

Note: Nominal concentration 100 mg/kg.

Batch 2 – analytical results

	HCB (mg/kg)	Musk xylene (mg/kg)	o-Terphenyl (mg/kg)	Methoxychlor (mg/kg)	B[a]P (mg/kg)
Sample 1	124.40	138.50	130.20	153.05	131.23
Sample 2	118.34	131.48	125.00	145.26	127.64
Sample 3	114.06	124.08	119.27	138.53	122.91
Sample 4					
Sample 5					
Control 1					
Control 2					
Average					
STDEV					
RSD (%)					

Note: Nominal concentration 100 mg/kg.

Recovery test of spiked diet samples

	HCB (%)	Musk xylene (%)	o-Terphenyl (%)	Methoxychlor (%)	B[a]P (%)
Sample 1	124.4	138.5	130.2	153.1	131.2
Sample 2	118.3	131.5	125.0	145.3	127.6
Sample 3	114.1	124.1	119.3	138.5	122.9
Sample 4					
Sample 5					
Control 1					
Control 2					
Average (compound)	118.9	131.4	124.8	145.6	127.3
STDEV	5.2	7.2	5.5	7.3	4.2
RSD (%)	4.4	5.5	4.4	5.0	3.3

Note: Nominal concentration 100 mg/kg.

Recoveries – average values per sample and overall

	Average (%) (Sample)	STDEV (%) (Sample)	RSD (%) (Sample)
Sample 1	135.5	11.0	8.1
Sample 2	129.5	10.0	7.7
Sample 3	123.8	9.1	7.4
Sample 4			
Sample 5			
Control 1			
Control 2			
Average (%) (overall)		129.6	
STDEV (%) (overall)		10.6	
RSD (%) (overall)		8.2	

Note: Nominal concentration 100 mg/kg.

FISH ANALYSIS RESULTS

Analytical method description

Fish extraction and analysis by GC/EI-MS

Sample extraction/preparation was carried out as follows:

1) Whole fish (weights approx. 2 to 16 g) were mixed with 4.5 to 8 g hydromatrix for homogenization/drying.
2) ASE extraction at 120°C in 22 or 33 mL cells; solvent: acetone/methylene chloride (1:1, v/v).
3) Drying of the extracts with Na_2SO_4 and volume setting to 50, 100 or 200 mL.
4) Cleanup of the extracts analogous to the Analytical validation protocol using Sep-Pak® Vac 12cc (2g) Silica cartridges.
5) Addition of 20 µL of a IS solution containing the internal standards (IS) in a conc. of 4 µg/mL.
6) Concentrating the SPE eluates to almost dryness by a gentle stream of N_2 and reconstitution of the residues in 500 µL toluene.

Spiked diet extraction and analysis

1g spiked diet was extracted with 25 mL acetone/methylene chloride (1:1, v/v) by USE (ultrasonic solvent extraction with cooling by ice), after centrifugation for 5 min at 1500 rpm 10 µL of the clear extract were pipetted together in autosampler vials with 1 mL toluene and 10 µL IS solution (containing the internal standards (IS) in a conc. of 4 µg/mL). Analysis was carried out by GC/EI-MS.

Results

Results of the fish samples provided by EMBSI

The results from the analysis of the incurred fish supplied by EMBSI are shown in the table below.

Sample	HCB (µg/g)	Musk xylene (µg/g)	o-Terphenyl (µg/g)	Methoxychlor (µg/g)	B[a]P (µg/g)	Fish (g)
Incurred Fish 1	10.9	7.7	5.2	1.2	0.17	1.824
Incurred Fish 2	19.3	16.7	8.7	4.7	0.76	2.724
Incurred Fish 3	14.8	11.7	5.6	1.4	0.07	1.535
Incurred Fish 4	14.9	12.2	6.0	1.72	0.05	1.796
Incurred Fish 5	11.4	8.3	4.0	1.19	0.02	1.361
Incurred Fish 6	16.4	14.0	9.3	5.6	0.03	1.989
Incurred Fish 7	18.1	15.8	9.6	5.9	1.2	2.372
Incurred Fish 8	10.6	8.4	4.8	0.98	0.09	1.604
Control Fish 1						
Control Fish 2						
Average	14.5	11.8	6.6	2.8	0.29	1.90
STDEV	3.33	3.50	2.21	2.16	0.43	0.45
RSD (%)	22.9	29.5	33.3	76.0	146	24

Recovery test of spiked fish samples

Validation of the method included spiking five control fish (Rainbow trout; ca. 2 g/4 fish) with 250 µL of a solution of 40 bzw, 4 µg/ml in toluene, respectively. The recoveries from each spiked fish are given in the table below.

Sample	HCB (%)	Musk xylene (%)	o-Terphenyl (%)	Methoxy chlor (%)	B[a]P (%)
Fortification level (mg/kg fresh weight)	5.0	5.0	5.0	0.5	0.5
Spiked Control Fish 1	87.3	101.6	102.3	111.9	98.3
Spiked Control Fish 2	89.6	111.1	101.7	118.8	98.1
Spiked Control Fish 3	88.9	105.5	101.9	97.6	95.9
Spiked Control Fish 4	89.6	107.6	99.7	100.1	94.7
Spiked Control Fish 5	89.3	106.7	100.9	100.1	96.0
Average (mg/kg fresh weight)	88.9	106.5	101.3	105.7	96.6
STDEV (mg/kg fresh weight)	0.98	3.43	1.04	9.20	1.57
RSD (%)	1.1	3.2	1.0	8.7	1.6

Lipid analysis

Results from the lipid analysis (using ASE extraction with 100% n-hexane) of five control fish are shown in the table below.

Sample	Fish fresh weight (g)	Total lipid content (mg)	Total lipid content (%)
Control Fish 1	2.198	60.5	2.75
Control Fish 2	1.904	59.4	3.12
Control Fish 3	2.110	76.0	3.60
Control Fish 4	1.395	45.3	3.25
Control Fish 5	1.702	42.2	2.48
Blank	-	3.0	
Average			
STDEV			
RSD (%)			

An alternative lipid extraction process (Smedes, 1999) was used to analyse four control fish. In this protocol chloroform/methanol were replaced by propan-2-ol and cyclohexane. The total lipid contents were found to be a bit higher than the results obtained with ASE using n-hexane as the solvent and are summarised in the table below.

Sample	Fish fresh weight (g)	Total lipid content (mg)	Total lipid content (%)

Control Fish 1	2.1	81.70	3.8
Control Fish 2	1.9	66.40	3.5
Control Fish 3	1.8	61.80	3.4
Control Fish 4	1.9	72.50	3.8
Average			
STDEV			
RSD (%)			

LABORATORY 4

FEED ANALYSIS RESULTS (PRE-STUDY ANALYTICAL VALIDATION)

Analytical method description

The sample was prepared by weighing 1 g of the spiked diet and extracting with 25 ml acetone/dichloromethane (1:1, v/v), ad internal standard solution to 0.5ml extract. Analysis was carried out by GC/MS (HP6890 GC/MS method).

Results

Results of spiked diet analysis – EMBSI

The analytical results of the fish food spiked by EMBSI are given in the table below.

	HCB (µg/g)	Musk xylene (µg/g)	o-Terphenyl (µg/g)	Methoxychlor (µg/g)	B[a]P (µg/g)
Sample 1	100	101	100	92	103
Sample 2	97	96	96	94	101
Sample 3	102	102	101	105	106
Sample 4	98	99	98	102	104
Sample 5					
Control 1					
Control 2					
Average	99	100	99	98	103
STDEV					
RSD (%)	2.0	2.5	2.0	6.5	1.9

Note: The control diet was free of interferences.

[Results were reported for four samples only. Standard deviation was not given in the test report.]

Results of spiked diet analysis – Laboratory 4

The analytical results of the spiked diet provided by Laboratory 4 are given in the table below.

	HCB	Musk xylene	o-Terphenyl	Methoxychlor	B[a]P
Spiked conc. (µg/g)	102.5	96.6	101.6	98.2	105.2
Sample 1 (%)	93.6	97.7	94.8	83.8	82.6
Sample 2 (%)	100.6	105.3	101.9	89.2	89.3

	HCB	Musk xylene	o-Terphenyl	Methoxychlor	B[a]P
Sample 3 (%)	100.5	104.2	101.1	85.6	88.5
Sample 4 (%)					
Sample 5 (%)					
Control 1 (%)					
Control 2 (%)					
Average (%)	98.2	102.4	99.3	86.2	86.8
STDEV					
RSD (%)	4.1	4.0	3.9	3.2	4.2

Note: The control diet was free of interferences.

[Results were reported for three samples only. Standard deviation was not given in the test report.]

Recovery test of spiked diet samples

Results of spiked diet samples prepared in the analytical laboratory by adding a definite volume of stock solution to the diet are given in the table below.

Chemical	m/z	Spiked conc. (µg/g)	Recovery (%)
HCB	252	116.8	95.6
Musk xylene	230	116.7	99.6
o-Terphenyl	227	128.6	95.9
Methoxychlor	284	98.7	111.7
B[a]P	282	91.7	103.4

FISH ANALYSIS RESULTS

Analytical method description

The complete fish was extracted 5 min by an Ultra Turrax with 20 ml extraction-solution (acetone/dichloromethane, 1:1). After centrifugation an aliquot of 5 ml was cleaned up by a silica gel-SPE-column according to the method provided. The eluate was evaporated to dryness (N-EVAP) and the residue re-dissolved in extraction-solution. After adding the internal standard mixture the solution was injected into the GC/MS-system (HP6890 GC/MS method).

Results

Results of the fish samples provided by EMBSI

Sample	Sample weight (g)	HCB (mg/kg)	Musk Xylene (mg/kg)	o-Ter-phenyl (mg/kg)	Methoxy-chlor (mg/kg)	B[a]P (mg/kg)
Day1 Dep Treatment Fish 1	1.76	10.52	5.73	3.33	0.92	0.23
Day1 Dep Treatment Fish 2	1.94	12.25	6.53	4.84	0.88	0.14
Day1 Dep Treatment Fish 3	1.36	10.99	5.34	4.61	1.12	0.18
Day1 Dep Treatment Fish 4	1.98	12.20	7.36	5.81	1.48	0.29
Day1 Dep Treatment Fish 5	2.20	17.87	10.69	9.88	3.91	0.19
Day1 Dep Treatment Fish 6	1.63	13.30	7.06	6.26	1.87	0.10
Day1 Dep Treatment Fish 7	1.56	14.30	7.83	6.29	1.99	0.24
Day1 Dep Treatment Fish 8	2.83	21.39	12.96	13.46	6.21	0.84
Day1 Dep Control Fish 1	1.81	<LOD	<LOD	<LOD	<LOD	<LOD
Day1 Dep Control Fish 2	1.98	<LOD	<LOD	<LOD	<LOD	<LOD
Day1 Dep Control Fish 3	1.58	<LOD	<LOD	<LOD	<LOD	<LOD
Day1 Dep Control Fish 4	2.14	<LOD	<LOD	<LOD	<LOD	<LOD
Day1 Dep Control Fish 5	2.13	<LOD	<LOD	<LOD	<LOD	<LOD
Day1 Dep Control Fish 6	2.00	<LOD	<LOD	<LOD	<LOD	<LOD
Day1 Dep Control Fish 7	1.64	<LOD	<LOD	<LOD	<LOD	<LOD
Day1 Dep Control Fish 8	1.91	<LOD	<LOD	<LOD	<LOD	<LOD
Day1 Dep Control Fish 9	2.39	<LOD	<LOD	<LOD	<LOD	<LOD
Day1 Dep Control Fish 10	1.74	<LOD	<LOD	<LOD	<LOD	<LOD
Day1 Dep Control Fish 11	2.32	<LOD	<LOD	<LOD	<LOD	<LOD
Average		14.10	7.94	6.81	2.30	0.28
STDEV						
RSD (%)						

Note: LOD is defined as the lowest calibration solution 20 ng/ml.

[Standard deviation and RSD were not given in the test report]

Recovery test of spiked fish samples

The fish were provided by the laboratory. The fortification of the fish was carried out in the analytical laboratory by adding a definite volume of a stock solution into the fish.

Chemical	m/z	Spiked conc. (mg/kg)	Recovery (%)	Mean Recovery (%)
HCB	252	1.05	89.7	89.7
			85.6	
		209.1	93.1	
			90.3	
Musk xylene	230	0.99	105.3	84.9
			73.5	
		197.4	81.5	
			79.2	
o-Terphenyl	227	1.23	91.3	90.2
			85.9	
		245.2	93	
			90.7	
Methoxychlor	284	0.99	111.2	99.7
			108.1	
		197.2	81.4	
			98.1	
B[a]P	282	0.97	108	103.6
			106.5	
		194.0	97.1	
			102.6	

Lipid analysis

[No data have been provided by Laboratory 4]

LABORATORY 5

FEED ANALYSIS RESULTS (PRE-STUDY ANALYTICAL VALIDATION)

Analytical method description

No details about sample preparation or the analytical method were given in the study report.

Results

Results of spiked diet analysis – EMBSI

The analytical results of the fish food spiked by EMBSI are given in the table below.

	HCB (ppm)	Musk xylene (ppm)	o-Terphenyl (ppm)	Methoxychlor (ppm)	B[a]P (ppm)
Sample 1	99.0	115.1	103.9	102.4	103.1
Sample 2	94.9	104.7	103.5	108.0	105.5
Sample 3	107.0	107.1	104.6	106.7	103.1
Sample 4	100.1	107.6	104.6	100.8	105.9
Sample 5	100.5	104.2	105.0	99.2	101.0
Control 1	-	-	-	-	-
Control 2					
Average	100.3±4.4	107.8±4.4	104.3±0.6	103.4±3.8	103.7±2.0
STDEV					
RSD (%)					

[Standard deviation and RSD were not given in the test report.]

FISH ANALYSIS RESULTS (SPIKED FOOD ANALYSIS)

Analytical method description

Fish extraction

Whole fish were homogenised with approx. 3 g hydromatix and dried overnight. Homogenised samples were transferred to 22 ml ASE cells and sample beakers were ‘rinsed’ with hydromatrix. The ASE cells were filled with additional hydromatix. The samples were extracted using acetone/dichloromethane (50:50). Extracts were reduced under nitrogen to 10 ml and stored at 4°C.

Fish extract clean up

Silica SPE (2g/12ml) cartridges were conditioned with 8 ml of acetone/dichloromethane (50:50) followed by 8 ml hexane and the cartridge dried under vacuum. The cartridges were filled with 8 ml hexane and 1 ml sample extract was added. The cartridges were then eluted with 2 × 2 ml of dichloromethane/hexane (50/59) and 80 ng internal standard was added to each cleaned-up extract. The extracts were evaporated under nitrogen to approx. 1 ml and transferred to GC vials in preparation for analysis. Vials were stored at -20°C until analysis.

Extract analysis

GC-ToF-MS (Waters GCT) analysis was used for the analysis. Accurate masses were quantified for the test chemicals and internal standards.

Lipid determination

Fish were weighed into a beaker and homogenised with approx. 3 g hydromatrix and the samples left to dry overnight. The samples were transferred to 22 ml ASE cells and the beakers 'rinsed' with additional hydromatrix and the cells filled with additional hydromatrix. The samples were extracted using ASE conditions. The extracts were evaporated to dryness under nitrogen and dried at 105 °C to a constant weight.

Results

Results of the fish samples provided by EMBSI

The results from the analysis of the incurred fish supplied by EMBSI are shown in the table below.

Sample	HCB (µg/g)	Musk xylene (µg/g)	o-Terphenyl (µg/g)	Methoxychlor (µg/g)	B[a]P (µg/g)
Treated Fish 1	6.583	3.338	4.183	0.153	0.043
Treated Fish 2	5.238	4.674	3.667	1.850	0.139
Treated Fish 3	4.131	5.834	3.568	2.186	0.057
Treated Fish 4	6.335	3.238	4.144	1.605	0.098
Treated Fish 5	5.165	3.214	3.712	1.365	0.087
Treated Fish 6	7.314	2.978	4.449	2.804	0.164
Treated Fish 7	3.782	5.328	3.035	2.772	0.095
Treated Fish 8	6.532	3.770	4.189	2.057	0.042
Control Fish 1	0.000	0.000	0.000	0.000	0.000
Control Fish 2	0.000	0.000	0.000	0.000	0.000
Average	5.635	4.047	3.868	1.849	0.091
STDEV	1.258	1.089	0.458	0.853	0.044
RSD (%)	4.495	3.889	1.634	3.045	0.158

Lipid analysis

Results from the lipid analysis of eight control fish are shown in the table below.

Sample	Lipid content (%)
Control Fish 1	2.79
Control Fish 2	2.99
Control Fish 3	2.65
Control Fish 4	2.02
Control Fish 5	2.99
Control Fish 6	2.27
Control Fish 7	3.83
Control Fish 8	3.99
Average	2.94
STDEV	0.69
RSD	23.35

LABORATORY 6

FEED ANALYSIS RESULTS (PRE-STUDY ANALYTICAL VALIDATION)

Analytical method description

No details about sample preparation or the analytical method were given in the study report.

Results

Results of spiked diet analysis – EMBSI

The analytical results of the fish food spiked by EMBSI are given in the table below.

	HCB (µg/g)	Musk xylene (µg/g)	o-Terphenyl (µg/g)	Methoxychlor (µg/g)	B[a]P (µg/g)
Sample 1	90.7	93.8	88.6	99.1	94.3
Sample 2	88.5	92.1	86.6	99.0	91.5
Sample 3	92.3	97.0	89.1	105	97.4
Sample 4	89.0	96.6	87.2	104	95.0
Sample 5	86.9	96.0	86.4	99.3	93.1
Control 1	ND	ND	ND	ND	ND
Control 2	ND	ND	ND	ND	ND
Average	89.5	95.1	87.6	101	94.3
STDEV	2.1	2.1	1.2	3.0	2.2
RSD (%)	2.3	2.2	1.4	3.0	2.3

Note: All test compounds were not detected (ND) in each of the two control diet samples analysed.

FISH ANALYSIS RESULTS

Results

Results of the fish samples provided by EMBSI

The results from the analysis of the incurred fish supplied by EMBSI are shown in the table below.

Sample	HCB (µg/g)	Musk xylene (µg/g)	o-Terphenyl (µg/g)	Methoxychlor (µg/g)	B[a]P (µg/g)	Fish (g)
Incurred Fish 1	7.05	4.25	3.86	0.643	0.0735	1.989
Incurred Fish 2	7.73	3.77	3.93	1.40	0.0304	1.323
Incurred Fish 3	8.82	5.72	5.34	1.17	0.0428	1.607
Incurred Fish 4	9.89	7.07	6.97	4.12	0.0299	1.686
Incurred Fish 5	8.65	4.61	3.41	1.35	0.0331	1.503
Incurred Fish 6	10.1	7.17	7.41	2.64	0.0426	2.933
Incurred Fish 7	15.4	11.6	10.2	3.98	0.340	2.364
Incurred Fish 8	7.52	4.66	4.67	1.07	0.120	1.796
Average	9.40	6.11	5.72	2.05	0.0890	1.90
STDEV	2.7	2.6	2.3	1.4	0.11	0.52
RSD (%)	29	43	40	68	124	27

Lipid analysis

Results from the lipid analysis are shown in the table below.

Sample	Weight of fish (g)	Lipid (% w/w wet)
Sample 1	2.184	4.02
Sample 2	1.92	3.75
Sample 3	1.951	4.63
Sample 4	2.338	3.46
Sample 5	2.494	4.66
Average	2.091	4.10
STDEV	0.247	0.53
RSD (%)	12	13

LABORATORY 7

FEED ANALYSIS RESULTS (PRE-STUDY ANALYTICAL VALIDATION)

Analytical method description

Spike diet. 1 g of sample extracted, final sample volume 25 mL.

Results

Results of spiked diet analysis – EMBSI

The analytical results of the fish food spiked by EMBSI are given in the table below.

	HCB (µg/g ww)	Musk xylene (µg/g ww)	o-Terphenyl (µg/g ww)	Methoxychlor (µg/g ww)	B[a]P (µg/g ww)
Sample 1	99.3	81.3	92.3	84.8	84.5
Sample 2	99.0	82.7	86.5	99.7	88.2
Sample 3	98.0	77.0	81.7	107.2	85.7
Sample 4	103.5	82.5	85.2	103.7	88.0
Sample 5	101.5	83.7	85.7	101.2	85.0
Control 1	ND	ND	ND	ND	ND
Control 2	ND	ND	ND	ND	ND
Average	100.2	81.4	86.3	99.3	86.3
STDEV	2.2	2.6	3.8	8.6	1.7
RSD (%)	2.2	3.2	4.4	8.7	2.0

FISH ANALYSIS RESULTS (SPIKED FOOD ANALYSIS)

Analytical method description

No details about sample preparation or the analytical method were given in the study report.

Results

Results of the fish samples provided by EMBSI

The results from the analysis of the incurred fish supplied by EMBSI are shown in the table below.

Sample	HCB (µg/kg ww)	Musk Xylene (µg/kg ww)	o-Terphenyl (µg/kg ww)	Methoxy chlor (µg/kg ww)	B[a]P (µg/kg ww)
Dosed Fish 1	7800	5600	4240	2250	64
Dosed Fish 2	8380	6440	4290	2930	137
Dosed Fish 3	12000	10100	6790	5720	542
Dosed Fish 4	12100	9660	6420	3790	448
Dosed Fish 5	9530	7920	5120	4310	127
Dosed Fish 6	8700	7180	4470	2300	390
Dosed Fish 7	8250	5710	4050	1130	<32
Dosed Fish 8	9870	7330	4530	1160	155
Control Fish 1	<30	<30	<30	<118	<30
Control Fish 2	<25	33	<25	<97	<25
Control Fish 3	<31	<31	<31	<124	<31
Average	9579	7493	4989	2949	266
STDEV	1667	1676	1050	1588	189
RSD (%)	17.40	22.37	21.05	53.86	70.97

[The following information was also given in the spreadsheet, but not actually presented in a summary table.]

Sample	Mass extracted (g)	Lipid content (no units, assumed to be %)
Dosed Fish 1	1.941	12.9
Dosed Fish 2	1.727	9.0
Dosed Fish 3	2.325	10.0
Dosed Fish 4	2.432	11.8
Dosed Fish 5	1.967	11.7
Dosed Fish 6	1.576	9.5
Dosed Fish 7	1.469	9.3
Dosed Fish 8	1.668	10.4
Control Fish 1	1.603	8.3
Control Fish 2	1.944	8.9
Control Fish 3	1.523	14.8

LABORATORY 8

FEED ANALYSIS RESULTS (PRE-STUDY ANALYTICAL VALIDATION)

Analytical method description

Diet extraction procedure

1) Weigh out 0.500 g of spiked diet.
2) Transfer to PTFE centrifuge tube using 25.0 mL extraction solvent (dichloromethane).
3) Shake for 2 minutes.
4) Sonicate for 30 minutes.
5) Shake for 2 minutes.
6) Centrifuge for 30 minutes at 40,000 g at 5°C.
7) Filter 5.0 mL through PTFE filter vial.
8) Transfer 1.0 mL of the extract to an amber GC autosampler vial.
9) Add 10 µL of a 200 mg/L SV internal standard solution. Analyse by GC-MS.

Recovery test of diet sample

The recovery test was conducted as follows:

1) Weigh out 0.500 g of control diet.
2) Add 50 µL of standard solution (1000 mg/L of each compound in DCM).
3) Leave for 30-60 min in fume hood to evaporate to dryness.

Continue from step 2 in the *diet extraction procedure*. The recovery test was conducted in triplicate.

Analysis was carried out by GC-MS (HP 6890 Series Plus).

Results

Results of spiked diet analysis – EMBSI

The analytical results of the fish food spiked by EMBSI are given in the table below. No peaks were present for any compounds in the control diet.

	HCB (µg/g)	Musk xylene (µg/g)	o-Terphenyl (µg/g)	Methoxychlor (µg/g)	B[a]P (µg/g)
Sample 1	100.9	103	96.5	106	98.8
Sample 2	99.2	105	95.2	106	95.9
Sample 3	99.6	104	92.4	102	97.6
Sample 4	98.1	101	92.8	103	97.0
Sample 5	99.6	107	95.9	108	97.0

	HCB (µg/g)	Musk xylene (µg/g)	o-Terphenyl (µg/g)	Methoxychlor (µg/g)	B[a]P (µg/g)
Control 1					
Control 2					
Average	99.5	104	94.6	105	97
STDEV	1.0	2.2	1.9	2.7	1.0
RSD (%)	1.0	2.1	2.0	2.6	1.1

Recovery test of spiked diet samples

The recovery rate from fish feed is shown in the table below.

	HCB (%)	Musk xylene (%)	o-Terphenyl (%)	Methoxychlor (%)	B[a]P (%)
Sample 1	99.3	107	100	111	100
Sample 2	95.3	110	97.3	117	96.4
Sample 3	94.9	110	95.1	113	95.4
Sample 4					
Sample 5					
Control 1					
Control 2					
Average	97	109	98	114	97
STDEV	2.4	1.8	2.5	2.8	2.2
RSD	2.5	1.6	2.6	2.5	2.2

FISH ANALYSIS RESULTS (SPIKED FISH RESULTS)

Analytical method description

Spiking

Inject a pre-weighed fish with 28 µl of a 357 mg/L mixed standard in acetone.

Fish extraction

1) Cut fish into small pieces.
2) Homogenise fish in a beaker with approximately 3 g of hydromatrix using a metal spatula.
3) Leave to dry overnight in a fume hood with occasional stirring.
4) Transfer mixture to an 11 ml ASE cell loaded with cellulose filter.
5) Rinse beaker with small amount of excess hydromatrix followed by approximately 2 ml of 1:1 acetone/dichloromethane and add to ASE cell.
6) Add additional hydromatrix to reduce dead volume if required.

7) Extract cell using acetone/dichloromethane (1:1).
8) Collect extract and reduce to 10 ml under N_2 at 37°C. Store refrigerated.
9) Condition 2 g silica SPE cartridge with 8 ml of 1:1 acetone/dichloromethane followed by 8 ml of hexane.
10) Dry cartridge.
11) Fill SPE cartridge with 8 ml of hexane and add 1 ml of fish extract and collect eluent.
12) Wash cartridge with 2 × 2 ml of 1:1 dichloromethane/hexane and combine eluents.
13) Apply vacuum to remove last bit of solvent.
14) Reduce cleaned fish extract to1 ml and add 10 µl of 100 mg/L SV internal standard.

Analysis was carried out by GC-MS.

Lipid extraction

1) Cut fish into small pieces.
2) Homogenise fish in a beaker with approximately 3 g of hydromatrix using a metal spatula.
3) Leave to dry overnight in a fume hood with occasional stirring.
4) Transfer mixture to an 11 ml ASE cell loaded with cellulose filter.
5) Rinse beaker with small amount of excess hydromatrix, followed by approximately 2 ml of hexane and add to ASE cell.
6) Add additional hydromatrix to reduce dead volume if required.
7) Extract cell using hexane.
8) Extract one blank cell full of hydromatrix per lipid batch.
9) Evaporate collected extracts to dryness at approximately 60°C under N_2.
10) Dry extracts to constant weight at approximately 150°C.

Results

Results of the fish samples provided by EMBSI

The results from the analysis of the incurred fish supplied by EMBSI are shown in the table below. These results have not been corrected for loss during recovery.

Sample	HCB (µg/g)	Musk xylene (µg/g)	o-Terphenyl (µg/g)	Methoxychlor (µg/g)	B[a]P (µg/g)	Fish (g)
Incurred Fish 1	7.610	3.237	3.996	1.034	0.000	1.497
Incurred Fish 2	15.801	9.886	10.125	3.805	0.101	2.524
Incurred Fish 3	13.211	8.357	6.658	1.094	0.087	2.174
Incurred Fish 4	7.373	3.233	3.421	1.767	0.053	1.422
Incurred Fish 5	9.460	4.257	4.723	1.078	0.066	1.999
Incurred Fish 6	7.729	3.763	3.621	1.424	0.046	1.681
Incurred Fish 7	7.474	3.034	2.972	0.302	0.027	1.733

Control Fish 1	0.000	0.000	0.000	0.000	0.000	2.268
Control Fish 2	0.000	0.000	0.000	0.376	0.000	2.345
Average	9.808	5.110	5.074	1.501	0.054	1.861
STDEV	3.370	2.806	2.535	1.110	0.034	0.394
RSD (%)	34	55	50	74	63	21

Recovery test of spiked fish samples

Validation of the method included spiking three control fish at a level of 10 µg. The recoveries from each spiked fish are given in the table below.

Sample	HCB (%)	Musk xylene (%)	o-Terphenyl (%)	Methoxy chlor (%)	B[a]P (%)	Fish (g)
Spiked Control Fish 1	69	81	81	88	63	2.531
Spiked Control Fish 2	72	83	78	89	67	2.082
Spiked Control Fish 3	60	80	76	87	63	2.007
Average	67	81	78	88	64	2.207
STDEV	6.5	1.2	2.6	0.6	2.1	0.283
RSD (%)	9.7	1.5	3.3	0.7	3.2	13

Lipid analysis

Results from the lipid analysis (using ASE) of five control fish are shown in the table below.

Sample	Weight of fish taken from vial (g)	Lipid content (%)
Control Fish 1	1.903	4.88
Control Fish 2	1.778	5.33
Control Fish 3	1.606	4.08
Control Fish 4	1.592	3.69
Control Fish 5	1.8498	4.08
Average	1.746	4.414
STDEV	0.141	0.669
RSD (%)	8.1	15.2

LABORATORY 9

FEED ANALYSIS RESULTS (PRE-STUDY ANALYTICAL VALIDATION)

Results

Results of spiked diet analysis – EMBSI

The analytical results of the fish food spiked by EMBSI are given in the table below.

	HCB (µg/g)	Musk xylene (µg/g)	o-Terphenyl (µg/g)	Methoxychlor (µg/g)	B[a]P (µg/g)
Sample 1	104	105	101	112	117
Sample 2	102	104	100	121	117
Sample 3	102	104	99.5	113	118
Sample 4	102	104	99.2	112	117
Sample 5	105	105	99.7	117	115
Sample 6	104	105	99.2	116	116
Sample 7	105	105	99.5	118	117
Control 1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Control 2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Control 3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Average	103	105	100	116	117
STDEV	1.40	0.53	0.63	3.41	0.95
RSD (%)	1.35	0.51	0.63	2.95	0.81

Note: LOQ = 5.00 ppm based on concentration of lowest calibration standard (0.2 ppm) adjusted for a dilution factor of 25.0.

ANNEX 2 – SUMMARY OF THE RAW DATA FROM THE OECD 305 RING TEST

LABORATORY 1

RING TEST STUDY RESULTS

Pre-study measurements

Dilution water characteristics

Source:	Local tap water (non-chlorinated well water of drinking water quality), reduced for total hardness by ion exchange.
TOC:	0.31 mg/L
Total suspended solids:	Not reported in spreadsheet.

Fish species

Rainbow trout (*Oncorhynchus mykiss*)

Fish food

Source/manufacturer:	Hokovit-502
Crude protein:	49.9%
Crude fat:	10.3%
Crude fibre:	1.6%
Moisture:	Not provided
Ash:	12.9%
Manufacturer reported impurities:	Vitamins and additives reported.

Test substances

Chemical	CAS Number	Purity (%)
Hexachlorobenzene (HCB)	118-74-1	99.9
Musk xylene	81-15-2	99
o-Terphenyl	84-15-1	99
Methoxychlor	72-43-5	99.9
Benzo(a)pyrene (B[a]P)	50-32-8	>96

Spiked feed preparation

Preparation method: i) Corn oil

Extra details on spiking method: the spiking method was carried out according to the SOP provided.

Chemical recoveries pre-study

Chemical	Target concentration (µg/g)	Sample 1 (% theoretical) pre-study	Sample 2 (% theoretical) pre-study	Sample 3 (% theoretical) pre-study
HCB	25.6	101	105	105
Musk xylene	50.2	104	98	97
o-Terphenyl	51.5	112	111	113
Methoxychlor	105.0	110	105	109
B[a]P	149.9	93	87	95

Analytical method description

See description given in the *Feed analysis result*s section in Annex 1.

Phys-chem measurements in the study

Temperature

The temperature ranged from 13.5-14.7°C (average = 14.0°C) in the test group and 13.6-14.9°C (average = 14.1°C) in the control throughout the uptake and depuration phases.

Dissolved oxygen

	Day	Test group (mg/L)	Control (mg/L)
Uptake	0	8.2	8.3
	7	9.2	9.8
	13	8.3	7.9
Depuration	7	7.4	8.1
	14	8.5	8.3
	21	8.6	8.3
	28	9.6	9.5

pH

	Day	Test group	Control
Uptake	0	7.0	7.0
	7	7.4	7.5
	13	7.4	7.2

	Day	Test group	Control
Depuration	7	7.5	7.3
	14	7.3	7.1
	21	7.1	7.1
	28	7.3	7.2

Other experimental conditions

Test flow rate:	320 L/24 h
Size of test vessels (approx.):	50 L
Number volumes replacement per day (approx.):	6.4
Loading rate, control tank:	0.22 g/L
Loading rate, test tank:	0.26 g/L

Fish data

Control

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (mg/g)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	1.40	50	44.8					
	0	1.20	49	23.3					
	0	1.18	49	10.7					
	0	1.29	50	15.8					
	0	1.47	53	31					
	3	1.68	55		0	0	0	0	0
	3	1.60	54		0	0	0	0	0
	3	1.72	53						
	3	2.14	60						
	3	1.35	49						
	13	1.78	53	44.9					
	13	1.48	51	43.6					
	13	2.45	59	27.4					
	13	2.21	56	39.4					
	13	2.07	57	39.3					
Depuration	1	1.84	55		0	0	0	0	0

Test stage				Concentration (µg/g)				
Day	Weight (g)	Total length (mm)	Lipid (mg/g)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
1	2.10	58		0	0	0	0	0
1	2.19	61		0	0	0	0	0
1	1.66	55		0	0	0	0	0
1	1.96	58		0	0	0	0	0
3	2.02	56		0	0	0	0	0
3	2.49	61		0	0	0	0	0
3	2.38	57		0	0	0	0	0
3	2.32	56		0	0	0	0	0
3	1.96	55		0	0	0	0	0
7	3.12	62		0	0	0	0	0
7	3.16	64		0	0	0	0	0
7	3.51	70		0	0	0	0	0
7	1.94	59		0	0	0	0	0
7	2.48	60		0	0	0	0	0
14	3.28	68		0	0	0	0	0
14	7.62	86		0	0	0	0	0
14	3.44	71		0	0	0	0	0
14	3.15	64		0	0	0	0	0
14	3.46	70		0	0	0	0	0
21	6.51	78		0	0	0	0	0
21	7.66	88		0	0	0	0	0

	Test stage				Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (mg/g)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	21	3.27	66		0	0	0	0	0
	21	5.70	80		0	0	0	0	0
	21	4.57	73		0	0	0	0	0
	28	5.92	85		0	0	0	0	0
	28	5.00	78		0	0	0	0	0
	28	5.48	81		0	0	0	0	0
	28	3.37	70		0	0	0	0	0
	28	6.49	85		0	0	0	0	0
*	28	7.32	88	26.8					
*	28	6.67	86	38.3					
*	28	3.96	70	37.1					
*	28	3.78	70	43.5					
*	28	7.45	87	37.7					

Test

	Test stage				Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (mg/g)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
Uptake	0	0.90	45						
	0	1.29	50						
	0	1.03	48						

Test stage					Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (mg/g)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
	0	1.42	52						
	0	1.32	52						
	3	1.96	57		1.76	2.96	2.64	2.39	0.05
	3	1.21	48		0.89	1.03	1.28	1.15	0.38
	3	1.55	52		1.67	2.68	2.58	1.85	0.43
	3	1.42	49		1.75	3.04	2.90	2.13	0.63
	3	1.72	54		1.68	2.64	2.32	1.99	0.07
	13	2.36	59	44.0					
	13	1.72	55	40.8					
	13	2.92	63	27.8					
	13	1.56	53	36.3					
	13	2.45	61	34.5					
Depuration	1	2.90	65		5.19	7.00	6.05	3.55	0.17
	1	2.73	63		2.77	3.57	3.64	1.53	0.22
	1	2.15	57		3.33	4.15	3.07	2.77	0.33
	1	2.23	59		2.34	2.94	2.46	1.99	0.21
	1	2.24	59		2.91	3.61	2.82	4.42	0.10
	3	1.75	55		4.06	4.47	3.33	3.53	0.00
	3	2.04	55		2.45	2.00	2.01	2.06	0.00
	3	1.72	51		2.39	2.41	1.78	1.69	0.00
	3	2.54	60		2.58	2.90	2.17	1.31	0.03

Test stage				Concentration (µg/g)				
Day	Weight (g)	Total length (mm)	Lipid (mg/g)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
3	2.71	64		4.00	4.85	3.51	2.43	0.00
7	2.75	62		3.87	3.72	3.87	0.33	0.00
7	3.14	67		2.49	2.74	2.81	0.38	0.00
7	3.60	69		2.61	2.20	1.81	0.58	0.00
7	2.64	64		2.44	2.06	1.26	0.47	0.00
7	4.15	70		2.24	1.96	1.22	0.37	0.00
14	3.43	66		1.48	0.80	0.28	0.00	0.00
14	3.83	69		1.80	1.04	0.91	0.04	0.00
14	4.20	73		1.69	0.98	0.98	0.11	0.00
14	3.40	68		2.09	1.22	1.22	0.14	0.00
14	4.73	74		1.94	1.29	1.29	0.27	0.00
21	5.17	77		1.83	1.22	0.35	0.28	0.00
21	6.13	80		1.50	0.63	0.63	0.00	0.00
21	3.76	72		1.37	0.64	0.64	0.00	0.00
21	3.80	71		1.84	0.85	0.85	0.00	0.00
21	6.12	81		0.87	0.45	0.45	0.00	0.00
28	4.70	78		1.22	0.60	0.44	0.12	0.00
28	6.95	88		0.70	0.27	0.27	0.00	0.00
28	5.02	78		0.94	0.44	0.44	0.00	0.00
28	3.24	67		0.77	0.32	0.32	0.00	0.00
28	6.17	83		0.46	0.18	0.18	0.00	0.00

Test stage					Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (mg/g)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
*	28	5.00	81	30.2					
*	28	10.05	97	42.7					
*	28	5.55	77	59.1					
*	28	5.70	80	50.6					
*	28	8.07	90	32.4					

Control – Average results lipid and concentrations

Test stage			Concentration (µg/g)				
	Day	Lipid (mg/g)	HCB	Musk xylene	o-Terphenyl	Methoxychlor	B[a]P
Uptake	0	25.12					
	3		0	0	0	0	0
	13	38.92					
Depuration	1		0	0	0	0	0
	3		0	0	0	0	0
	7		0	0	0	0	0
	14		0	0	0	0	0
	21		0	0	0	0	0
	28		0	0	0	0	0
	28	36.68					

Test – Average results lipid and concentrations

Test stage			Concentration (µg/g)				
	Day	**Lipid (mg/g)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxychlor**	**B[a]P**
Uptake	0						
	3		1.55	2.47	2.34	1.90	0.31
	13	36.7					
Depuration	1		3.31	4.26	3.61	2.85	0.21
	3		3.10	3.33	2.56	2.20	0.01
	7		2.73	2.54	2.19	0.42	0.00
	14		1.80	1.06	0.94	0.11	0.00
	21		1.48	0.76	0.59	0.06	0.00
	28		0.82	0.36	0.33	0.02	0.00
	28	43.0					

Feed and test observations

Feed analysis

Lipid content	Grab #1 (mg/g)	Grab #2 (mg/g)	Grab #3 (mg/g)	Grab #4 (mg/g)	Grab #5 (mg/g)	Grab #6 (mg/g)	Average (mg/g)
Study start	26.7*	63.30	62.70	61.30	66.00	68.70	64.40
End of uptake	61.30	26*	58.00	67.30	66.00	--	63.15

Note: *Outlier value, not used for the calculation of the mean.

Method used for lipid determination

Accelerated solvent extraction (ASE) method was used.

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	25.41	26.82	25.82
Musk xylene	50	54.94	56.17	54.09
o-Terphenyl	50	50.96	53.55	51.68
Methoxychlor	100	95.05	100.2	96.69
B[a]P	150	163.5	170.6	168.1

Other data included in the spreadsheet

Chemical	Mean at end uptake (µg/g)	Mean at start uptake phase (µg/g)	Total mean (µg/g)
HCB	26.02	24.46	25.24
Musk xylene	55.07	52.22	53.64
o-Terphenyl	52.06	48.42	50.24
Methoxychlor	97.31	89.53	93.42
B[a]P	167.4	151.7	159.55

Feed quantities (for 3% 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	1.29	1.32	1.30	3% of day 0 weight
	3	1.70	1.57	1.64	up to day 13
	13	2.00	2.20	2.10	
Depuration	1	2.45	1.95	2.20	3% adjusted each time interval
	3	2.15	2.23	2.19	3% adjusted each time interval
	7	3.26	2.84	3.05	3% adjusted each time interval
	14	3.92	4.19	4.05	3% adjusted each time interval
	21	5.00	5.54	5.27	3% adjusted each time interval
	28	5.54	6.05	5.79	3% adjusted each time interval

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 2A

RING TEST STUDY RESULTS

Pre-study measurements

Dilution water characteristics

Source:	Dechlorination water.
TOC:	<0.3 mg/L
Total suspended solids:	Not reported in spreadsheet.

Fish species

Rainbow trout (*Oncorhynchus mykiss*)

Fish food

Source/manufacturer:	Finfish starter 55-15/zeigler
Crude protein:	>55.0%
Crude fat:	>15.0%
Crude fibre:	<2.0%
Moisture:	Not reported in spreadsheet.
Ash:	Not reported in spreadsheet.
Manufacturer reported impurities:	Not reported in spreadsheet.

Test substances

Chemical	CAS Number	Purity (%)
Hexachlorobenzene (HCB)	118-74-1	99.7
Musk xylene	81-15-2	>99.0
o-Terphenyl	84-15-1	>99
Methoxychlor	72-43-5	>95.0
Benzo(a)pyrene (B[a]P)	50-32-8	99.7

Spiked feed preparation

Preparation method: i) Corn/fish oil

Extra details on spiking method:

1) Combine 0.10 g of each test compound in a 5.0 mL volumetric flask.

2) Bring to volume with corn oil. Resulting concentration of each compound is 20 mg/mL (2% w/v).
3) Add micro-stir bar and stopper.
4) Mix rapidly on stir plate overnight to suspend/emulsify solid compounds in corn oil. There may be partial dissolution of the compounds in corn oil.
5) Place 50 g of unspiked fish feed in amber glass bottle.
6) Add 0.5 mL of corn oil suspension using a wide bore syringe or pipette.
7) Add an additional 50 g of fish diet and cap bottle. The concentration of each compound in the diet will be 100 µg/g.
8) Shake the bottle by hand to homogenize the corn oil suspension throughout the fish feed.
9) Place the bottle containing the spiked diet on a mechanical tumbler and tumble slowly overnight.

Chemical recoveries pre-study

Chemical	Target concentration (µg/g)	Sample 1 (%) pre-study	Sample 2 (%) pre-study	Average (%)
HCB	25	92.5	88.5	90.5
Musk xylene	50	95.5	94.1	94.8
o-Terphenyl	50	94.4	91.9	93.2
Methoxychlor	100	101	101	101.0
B[a]P	150	92.3	94.2	93

Analytical method description

The *Feed method* was followed (see *Feed analysis result*s section in Annex 1 for full description), with the following small differences:

1) 1.00 g of spiked diet was weighed out (rather than 0.50 g).
2) Resulting concentration of each compound is 4.00 µg/mL (rather than 2.00 µg/mL).
3) Transfer 1.0 mL using whole pipette to 20 mL volumetric flasks and bring to 20 mL with hexane.

The *Fish extraction procedure* was followed (see *Fish analysis results* section in Annex 1 for full description).

Phys-chem measurements in the study

Temperature

The temperature ranged from 15.0-16.1°C in both the test group and the control throughout the uptake and depuration phases (average = 15.2°C in the test group and 15.3°C in the control).

Dissolved oxygen

	Day	Test group (% of air saturation)	Control (% of air saturation)
Uptake	1	100	100
	8	100	100
	13	100	100
Depuration	8	100	100
	14	100	100
	21	100	100
	28	100	100

pH

	Day	Test group	Control
Uptake	1	7.4	7.5
	8	6.9	6.9
	13	6.7	6.7
Depuration	8	7.0	7.0
	14	7.0	7.1
	21	7.2	7.2
	28	6.7	6.8

Other experimental conditions

Test flow rate:	1600 mL/min
Size of test vessels (approx.):	100 L
Number volumes replacement per day (approx.):	23

Fish data

Control

Test stage					Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	8.50	950	6.43					
	0	7.97	900	6.29					
	0	9.20	950	6.30					
	0	8.68	950	6.10					
	0	7.71	900	6.91					
	13	8.81	905	10.38					
	13	9.28	922	9.55					
	13	10.31	965	8.26					
	13	10.52	964	8.37					
	13	9.59	945	8.68					
Depuration	1	9.26	960		0.00	0.00	0.00	0.00	0.00
	1	8.34	909		0.00	0.00	0.00	0.00	0.00
	1	8.17	899						
	1	9.63	969						
	1	10.80	977						
	3	9.27	980		0.00	0.00	0.00	0.00	0.00

	Test stage				Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	3	9.96	1020		0.00	0.00	0.00	0.00	0.00
	3	8.17	930						
	3	8.31	950						
	3	7.82	910						
	8	13.85	1070		0.00	0.00	0.00	0.00	0.00
	8	10.77	1030		0.00	0.00	0.00	0.00	0.00
	8	11.13	1030						
	8	12.28	1050						
	8	10.59	1010						
	14	14.80	1070		0.00	0.00	0.00	0.00	0.00
	14	13.60	1080		0.00	0.00	0.00	0.00	0.00
	14	10.00	1030						
	14	12.00	1040						
	14	12.20	1050						
	21	12.80	1070		0.00	0.00	0.00	0.00	0.00
	21	14.60	1120		0.00	0.00	0.00	0.00	0.00
	21	14.80	1120						
	21	12.20	1070						
	21	16.70	1180						
*	28	16.60	1180	9.23	0.00	0.00	0.00	0.00	0.00
*	28	14.60	1140	9.36	0.00	0.00	0.00	0.00	0.00

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
*	28	13.20	1100	8.86					
*	28	15.30	1100	9.38					
*	28	15.00	1100	9.75					

Test

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	8.50	950	6.43					
	0	7.97	900	6.29					
	0	9.20	950	6.30					
	0	8.68	950	6.10					
	0	7.71	900	6.91					
	13	9.65	936	8.11					
	13	8.00	882	6.93					
	13	11.01	989	8.53					
	13	10.17	984	8.73					
	13	10.48	979	8.47					
Depuration	1	10.09	956	9.69	6.48	9.76	4.61	9.73	0.84
	1	12.52	1024	8.99	5.04	8.76	6.23	7.40	0.70
	1	8.82	949	9.51	4.56	8.06	4.55	8.72	0.58

Test stage				Concentration (µg/g)				
Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
1	11.57	997	10.11	5.63	10.42	5.82	5.72	0.51
1	10.72	986	9.67	5.66	10.48	6.33	6.84	0.41
3	10.20	956	8.72	5.21	8.38	4.91	8.46	0.0042
3	12.22	1020	7.51	4.85	7.16	4.35	6.87	0.0222
3	9.44	992	10.26	5.13	8.66	4.81	8.04	0.0043
3	11.45	1010	10.09	5.88	10.67	10.25	13.25	0.0248
3	8.54	970	7.33	4.12	6.37	4.03	6.84	0.0059
8	13.72	1060	8.67	4.33	5.94	2.27	5.49	-
8	10.44	1010	7.99	3.33	4.92	2.35	4.20	-
8	10.78	990	9.13	4.17	6.30	2.38	7.13	-
8	11.85	1040	9.45	3.50	5.49	2.50	3.58	-
8	10.24	950	7.97	3.37	3.93	2.10	3.82	-
14	17.30	1150	10.15	3.94	5.18	1.65	1.74	-
14	12.00	1050	9.05	3.57	4.15	4.14	4.23	-
14	16.50	1140	10.07	4.06	5.53	3.72	3.34	-
14	15.80	1140	9.44	3.69	4.54	3.56	2.36	-
14	14.10	1110	11.00	4.35	5.12	5.27	5.04	-
21	14.30	1100	8.62	1.87	1.55	1.76	0.78	-
21	14.50	1100	8.49	2.25	1.65	1.75	1.29	-
21	17.20	1140	10.35	2.60	2.21	1.35	1.00	-
21	12.90	1100	10.45	2.69	2.42	0.80	2.11	-

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
	21	12.70	1070	9.90	2.51	2.31	2.01	0.37	-
*	28	15.40	1100	8.39	1.67	1.30	0.64	0.11	-
*	28	14.60	1090	8.81	1.70	1.21	1.30	1.03	-
*	28	15.40	1120	8.82	2.05	1.33	1.68	0.96	-
*	28	14.30	1120	9.40	1.58	1.12	0.82	1.20	-
*	28	16.00	1190	8.71	2.04	1.55	0.21	0.06	-

Additional data given in the spreadsheet – Lipid correction

	Test stage				Concentration (µg/g) lipid correction				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	8.50	950	6.43					
	0	7.97	900	6.29					
	0	9.20	950	6.30					
	0	8.68	950	6.10					
	0	7.71	900	6.91					
	13	9.65	936	8.11					
	13	8.00	882	6.93					
	13	11.01	989	8.53					
	13	10.17	984	8.73					
	13	10.48	979	8.47					
Depuration	1	10.09	956	9.69	0.669220738	1.006850433	0.476133614	1.004360566	0.086894702
	1	12.52	1024	8.99	0.560844745	0.974402006	0.693541245	0.823428348	0.078076979
	1	8.82	949	9.51	0.479957747	0.848025063	0.478137608	0.916983997	0.060727736
	1	11.57	997	10.11	0.557165672	1.031046115	0.575973827	0.565338846	0.050680292
	1	10.72	986	9.67	0.58547145	1.083681286	0.65452311	0.707690513	0.042768589
	3	10.20	956	8.72	0.597845977	0.960718348	0.563313693	0.970397635	0.000482091

	Test stage				Concentration (µg/g) lipid correction				
	Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	3	12.22	1020	7.51	0.645865568	0.953613856	0.579846442	0.914360518	0.00295174
	3	9.44	992	10.26	0.499573513	0.84449082	0.469244849	0.783684499	0.000420784
	3	11.45	1010	10.09	0.582358561	1.057411136	1.015421463	1.31285084	0.002461223
	3	8.54	970	7.33	0.562462642	0.869176373	0.550369801	0.932744721	0.000799162
	8	13.72	1060	8.67	0.498881048	0.684967351	0.262000299	0.633182972	ND
	8	10.44	1010	7.99	0.416515484	0.616177982	0.293955667	0.525574266	ND
	8	10.78	990	9.13	0.456579485	0.690322809	0.261187534	0.781432417	ND
	8	11.85	1040	9.45	0.370343387	0.581333632	0.264295867	0.378483846	ND
	8	10.24	950	7.97	0.423399171	0.493383873	0.263062592	0.479198174	ND
	14	17.30	1150	10.15	0.388378175	0.51039346	0.162620377	0.171747226	ND
	14	12.00	1050	9.05	0.393997522	0.458215646	0.457002311	0.467831151	ND
	14	16.50	1140	10.07	0.403016544	0.548843067	0.369005434	0.331945897	ND
	14	15.80	1140	9.44	0.391311853	0.480426311	0.376842014	0.250006856	ND
	14	14.10	1110	11.00	0.395467728	0.465104558	0.479224717	0.45847601	ND
	21	14.30	1100	8.62	0.216524814	0.179577573	0.203753598	0.090145607	ND
	21	14.50	1100	8.49	0.264466918	0.19458086	0.206691916	0.15248187	ND
	21	17.20	1140	10.35	0.250842627	0.213219605	0.130500379	0.096609394	ND
	21	12.90	1100	10.45	0.257769106	0.231933435	0.076630358	0.201605	ND
	21	12.70	1070	9.90	0.253599233	0.233756254	0.202820454	0.037073643	ND
*	28	15.40	1100	8.39	0.198835729	0.154847529	0.075948291	0.012557505	ND
*	28	14.60	1090	8.81	0.193400338	0.137222711	0.147060338	0.117187286	ND

	Test stage				Concentration (µg/g) lipid correction				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
*	28	15.40	1120	8.82	0.232714548	0.150497859	0.191021002	0.108991277	ND
*	28	14.30	1120	9.40	0.168138137	0.119523318	0.087694784	0.127400381	ND
*	28	16.00	1190	8.71	0.233849826	0.178437134	0.023869826	0.006427149	ND

ND = not detected

Control – Average results lipid and concentrations

Test stage			Concentration (µg/g)				
	Day	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxychlor	B[a]P
Uptake	0	6.41					
	13	9.05					
Depuration	1		0	0	0	0	0
	3		0	0	0	0	0
	8		0	0	0	0	0
	14		0	0	0	0	0
	21		0	0	0	0	0
*	28	9.32	0	0	0	0	0

Test – Average results lipid and concentrations

Test stage			Concentration (µg/g)				
	Day	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxychlor	B[a]P
Uptake	0	6.41					
	13	8.15					
Depuration	1	9.59	5.48	9.50	5.51	7.68	0.61
	3	8.78	5.04	8.25	5.67	8.69	0.01
	8	8.64	3.74	5.32	2.32	4.84	-
	14	9.94	3.92	4.90	3.67	3.34	-
	21	9.56	2.38	2.03	1.53	1.11	-
*	28	8.83	1.81	1.30	0.93	0.67	-

Test – Average results lipid correction

Test stage			Concentration (µg/g /lipid)				
	Day	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxychlor**	**B[a]P**
Uptake	0	6.41					
	13	8.15					
Depuration	1	9.59	0.57	0.99	0.57	0.80	0.06
	3	8.78	0.57	0.94	0.65	0.99	0.00
	8	8.64	0.43	0.62	0.27	0.56	-
	14	9.94	0.39	0.49	0.37	0.34	-
	21	9.56	0.25	0.21	0.16	0.12	-
*	28	8.83	0.20	0.15	0.11	0.08	-

Feed and test observations

Feed analysis [N.B. Units not given in spreadsheet but assumed to be %]

Lipid content	Grab (%)	Grab (%)	Grab (%)	Average %
Study start (test)	16.21	16.22	16.71	16.38
Study start (control)	15.42	16.31	16.16	15.96333333
End of uptake (test)	15.82	15.68	16.39	15.96333333
End of uptake (control)	18.07	17.69	16.56	17.44

Method used for lipid determination

The *Fish lipid determination procedure* was followed (see *Fish analysis results* section in Annex 1 for full description). The lipids were extracted using methanol/chloroform.

Chemical concentrations – Before uptake

Chemical	Target concentration (µg/g)	Concentration 1 before uptake (µg/g)	Concentration 2 before uptake (µg/g)	Concentration 3 before uptake (µg/g)	Average (µg/g)
HCB	25	23.7	24.3	24.4	24.1
Musk xylene	50	43.3	41.1	44.6	43.0
o-Terphenyl	50	43.3	41.3	44.0	42.9
Methoxychlor	100	83.0	81.4	86.3	83.6
B[a]P	150	141	143	135	140

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)	Average (µg/g)
HCB	25	23.1	23.0	23.9	23.3
Musk xylene	50	43.9	44.7	42.5	43.7
o-Terphenyl	50	42.5	42.8	40.7	42.0
Methoxychlor	100	86.9	88.4	86.0	87.1
B[a]P	150	136	139	135	137

Feed quantities (for 3% 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	8.412	8.412	8.412	13.8798
	13	9.862	9.702	9.782	14.673
Depuration	1	10.744	9.24	9.992	13.4892
	3	10.37	8.706	9.538	11.4456
	8	11.406	11.724	11.565	12.14325
	14	15.14	12.52	13.83	12.447
	21	14.32	14.22	14.27	10.7025
	28	15.14	14.94	15.04	6.768

Note: Feeding regime: ration split to two feedings.

Daily observations

No mortality was observed in the test group or the control group. [A dash (-) is given in the corresponding columns in the spreadsheet – we have assumed that this means that no adverse effects were noted.]

LABORATORY 2B

RING TEST STUDY RESULTS

Pre-study measurements

Dilution water characteristics

Source:	Ground water from the premises of Laboratory 2B.
TOC:	Not reported in spreadsheet.
Total suspended solids:	Not reported in spreadsheet.

Fish species

Carp (presumed to be *Cyrinus carpio*)

Fish food

See *Ring test study results* for Laboratory 2A for details.

Test substances

See *Ring test study results* for Laboratory 2A for details.

Spiked feed preparation

See *Ring test study results* for Laboratory 2A for details (the same protocol was used).

Chemical recoveries pre-study

See *Ring test study results* for Laboratory 2A for details.

Analytical method description

See *Ring test study results* for Laboratory 2A for details (the same protocol was used).

Phys-chem measurements in the study

Temperature

The temperature ranged from 24.3-25.0°C (average = 24.6°C) in the control group, 24.6-25.0°C (average = 24.8°C) for Level 1 and 24.5-25.0°C (average = 24.7°C) for both Level 2 and Level 3.

Dissolved oxygen

	Day	Level 1 (% of air saturation)	Level 2 (% of air saturation)	Level 3 (% of air saturation)	Control (% of air saturation)
Uptake	1	86.3	88.8	86.3	88.8
	7	92.5	87.5	92.5	92.5
	13	93.7	92.5	93.7	94.9
Depuration	7	93.7	93.7	93.7	92.5
	14	94.9	93.7	94.9	93.7
	21	90.0	92.5	93.7	91.2

pH

	Day	Level 1	Level 2	Level 3	Control
Uptake	1	7.5	7.5	7.4	7.4
	7	7.6	7.4	7.4	7.4
	13	7.6	7.5	7.5	7.5
Depuration	7	7.8	7.8	7.6	7.6
	14	7.6	7.6	7.6	7.6
	21	7.5	7.5	7.5	7.5

Other experimental conditions

See *Ring test study results* for Laboratory 2A for details.

Fish data

Control

	Test stage				Concentration (µg/g) Level 1				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	5.67	760	5.14					-
	0	5.13	730						-
	0	5.51	750						-
	0	5.36	750						-
	0	5.43	750						-
	13	6.80	820	6.54					-
	13	8.09	890						-
	13	7.91	870						-
	13	6.23	810						-
	13	7.43	850						-
Depuration	1	6.86	670	6.85	0.00	0.00	0.00	0.00	-
	1	6.63	810						-
	1	8.61	840						-
	1	5.39	780						-
	1	7.94	860						-
	3	6.84	860	6.32	0.00	0.00	0.00	0.00	-

	Test stage				Concentration (µg/g) Level 1				
	Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	3	8.74	900						-
	3	7.50	840						-
	3	7.60	840						-
	3	8.27	880						-
	7	9.30	880	7.51	0.00	0.00	0.00	0.00	-
	7	8.00	870						-
	7	7.84	870						-
	7	8.39	880						-
	7	7.26	860						-
	9	8.85	820	6.04	0.00	0.00	0.00	0.00	-
	9	8.78	890						-
	9	12.10	960						-
	9	8.41	850						-
	9	10.50	940						-
*	21	14.60	1010	7.46	0.00	0.00	0.00	0.00	-
*	21	15.86	1010						-
*	21	14.02	1000						-
*	21	14.76	1050						-
*	21	15.67	1030						-

Note: Benzo(a)pyrene (B[a]P) was excluded from Level 1.

Test (Level 1) [There were four day 7 and six day 9 in the depuration phase. We have assumed that this is a typographical error in the spreadsheet and it should it be five of each as the code numbers of the fish ran from xxxa to xxxe].

Test stage					Concentration (µg/g) Level 1				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	5.67	760	5.14					-
	0	5.13	730						-
	0	5.51	750						-
	0	5.36	750						-
	0	5.43	750						-
	13	7.13	860	5.85					-
	13	6.92	810						-
	13	6.96	850						-
	13	6.58	840						-
	13	8.44	840						-
Depuration	1	6.70	810	5.33	20.47	29.84	19.52	6.85	-
	1	8.25	810						-
	1	6.81	810						-
	1	6.86	830						-
	1	6.79	810						-
	3	8.98	880	6.08	17.25	22.26	13.64	3.45	-

	3	7.07	800						-
	3	6.20	790						-
	3	7.65	820						-
	3	6.96	830						-
	7	7.71	890	6.09	18.44	17.56	4.46	1.46	-
	7	8.48	890						-
	7	8.21	870						-
	7	7.04	830						-
	7 [9]	7.34	830						-
	9	8.37	850	6.20	16.39	13.15	2.67	0.48	-
	9	9.68	870						-
	9	8.03	900						-
	9	8.82	890						-
	9	7.68	850						-
*	21	13.81	1050	6.66	6.05	1.84	0.06	<0.05	-
*	21	13.68	1010						-
*	21	13.62	1020						-
*	21	11.84	980						-
*	21	10.99	970						-

Note: Benzo(a)pyrene (B[a]P) was excluded from Level 1.

Test (Level 2)

	Test stage				Concentration (µg/g) Level 2				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	5.67	760	5.14					-
	0	5.13	730						-
	0	5.51	750						-
	0	5.36	750						-
	0	5.43	750						-
	13	6.96	810	7.37					-
	13	9.20	890						-
	13	6.45	780						-
	13	7.01	810						-
	13	6.07	780						-
Depuration	1	8.15	820	6.94	12.30	19.97	9.63	3.86	-
	1	5.54	740						-
	1	6.55	820						-
	1	7.07	810						-
	1	6.86	810						-
	3	8.30	870	6.00	10.22	12.63	3.26	1.42	-
	3	7.28	850						-
	3	6.06	820						-

	Test stage				Concentration (µg/g) Level 2				
	Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	3	6.65	810						-
	3	6.86	820						-
	7	9.15	880	6.31	7.99	7.50	0.93	0.50	-
	7	7.02	840						-
	7	8.06	850						-
	7	7.01	820						-
	7	10.07	940						-
	9	8.99	880	5.51	8.04	5.91	0.54	0.34	-
	9	9.51	930						-
	9	8.96	880						-
	9	12.00	940						-
	9	10.00	910						-
*	21	12.69	970	7.81	3.85	1.34	<0.05	<0.05	-
*	21	11.96	960						-
*	21	14.53	1060						-
*	21	13.23	1020						-
*	21	10.82	920						-

Note: Benzo(a)pyrene (B[a]P) was excluded from Level 2.

Test (Level 3)

	Test stage				Concentration (µg/g) Level 3				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	5.67	760	5.14					
	0	5.13	730						
	0	5.51	750						
	0	5.36	750						
	0	5.43	750						
	13	6.22	830	5.64					
	13	8.28	840						
	13	7.30	840						
	13	6.02	810						
	13	6.22	790						
Depuration	1	5.47	740	5.70	2.45	2.76	0.62	0.60	<0.004
	1	7.22	820						
	1	6.44	820						
	1	7.08	830						
	1	6.81	820						
	3	8.24	880	7.29	1.46	2.29	0.31	0.20	<0.004
	3	8.71	880						
	3	7.49	850						

	Test stage				Concentration (µg/g) Level 3				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
	3	6.43	830						
	3	6.38	810						
	7	8.99	920	6.50	1.58	1.10	0.11	0.11	<0.004
	7	10.05	930						
	7	9.58	890						
	7	8.88	880						
	7	7.82	820						
	9	10.90	920	7.07	2.10	1.43	0.05	<0.05	<0.004
	9	8.77	870						
	9	10.70	940						
	9	9.67	890						
	9	8.84	880						
*	21	11.35	970	7.61	0.76	0.29	<0.05	<0.05	<0.004
*	21	14.33	1030						
*	21	11.73	974						
*	21	15.00	1070						
*	21	13.61	1030						

Control – Average results lipid and concentrations

Test stage			Concentration (µg/g)				
	Day	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxychlor	B[a]P
Uptake	0	5.14					-
	13	6.54					-
Depuration	1	6.85	0.00	0.00	0.00	0.00	-
	3	6.32	0.00	0.00	0.00	0.00	-
	7	7.51	0.00	0.00	0.00	0.00	-
	9	6.04	0.00	0.00	0.00	0.00	-
*	21	7.46	0.00	0.00	0.00	0.00	-

Note: Benzo(a)pyrene (B[a]P) was excluded from Level 1.

Test – Average results lipid and concentrations (Level 1)

Test stage			Concentration (µg/g)				
	Day	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxychlor	B[a]P
Uptake	0	5.14					-
	13	5.85					-
Depuration	1	5.33	20.47	29.84	19.52	6.85	-
	3	6.08	17.25	22.26	13.64	3.45	-
	7	6.09	18.44	17.56	4.46	1.46	-
	9	6.20	16.39	13.15	2.67	0.48	-

*	21	6.66	6.05	1.84	0.06	<0.05	-

Note: Benzo(a)pyrene (B[a]P) was excluded from Level 1.

Test – Average results lipid and concentrations (Level 1)

Test stage			Concentration (µg/g /lipid)				
	Day	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxychlor**	**B[a]P**
Uptake	0	5.14					-
	13	5.85					-
Depuration	1	5.33	3.84	5.60	3.66	1.29	-
	3	6.08	2.84	3.66	2.24	0.57	-
	7	6.09	3.03	2.88	0.73	0.24	-
	9	6.20	2.64	2.12	0.43	0.08	-
*	21	6.66	0.91	0.28	0.01	#VALUE!	-

Note: Benzo(a)pyrene (B[a]P) was excluded from Level 1.

Test – Average results lipid and concentrations (Level 2)

Test stage			Concentration (µg/g)				
	Day	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxychlor**	**B[a]P**
Uptake	0	5.14					-
	13	7.37					-
Depuration	1	6.94	12.30	19.97	9.63	3.86	-
	3	6.00	10.22	12.63	3.26	1.42	-
	7	6.31	7.99	7.50	0.93	0.50	-

	9	5.51	8.04	5.91	0.54	0.34	-
*	21	7.81	3.85	1.34	<0.05	<0.05	-

Note: Benzo(a)pyrene (B[a]P) was excluded from Level 2.

Test – Average results lipid and concentrations (Level 2)

Test stage			**Concentration (µg/g /lipid)**				
	Day	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxychlor**	**B[a]P**
Uptake	0	5.14					-
	13	7.37					-
Depuration	1	6.94	1.77	2.88	1.39	0.56	-
	3	6.00	1.70	2.11	0.54	0.24	-
	7	6.31	1.27	1.19	0.15	0.08	-
	9	5.51	1.46	1.07	0.10	0.06	-
*	21	7.81	0.49	0.17	ND	ND	-

Note: Benzo(a)pyrene (B[a]P) was excluded from Level 2.

Test – Average results lipid and concentrations (Level 3)

Test stage			Concentration (µg/g)				
	Day	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxychlor	B[a]P
Uptake	0	5.14					
	13	5.64					
Depuration	1	5.70	2.45	2.76	0.62	0.60	<0.004
	3	7.29	1.46	2.29	0.31	0.20	<0.004
	7	6.50	1.58	1.10	0.11	0.11	<0.004
	9	7.07	2.10	1.43	0.05	<0.05	<0.004
*	21	7.61	0.76	0.29	<0.05	<0.05	<0.004

Test – Average results lipid and concentrations (Level 3)

Test stage			Concentration (µg/g /lipid)				
	Day	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxychlor	B[a]P
Uptake	0	5.14					
	13	5.64					
Depuration	1	5.70	0.43	0.48	0.11	0.11	0.00
	3	7.29	0.20	0.31	0.04	0.03	0.00
	7	6.50	0.24	0.17	0.02	0.02	0.00
	9	7.07	0.30	0.20	0.01	ND	0.00

*	21	7.61	0.10	0.04	ND	ND	0.00

Feed and test observations

Feed analysis (Level 1) [Units not given in spreadsheet. Assumed to be %.]

Lipid content	Grab #1 (%)	Grab #2 (%)	Grab #3 (%)	Average (%)
Study start (test)	16.99	17.01	17.06	17.02
Study start (control)	16.49	16.78	16.75	16.67
End of uptake (test)	16.74	16.65	16.59	16.66
End of uptake (control)	16.90	16.95	16.67	16.84

Feed analysis (Level 2)

Lipid content	Grab #1 (%)	Grab #2 (%)	Grab #3 (%)	Average (%)
Study start (test)	16.72	16.45	16.52	16.56
Study start (control)	16.49	16.78	16.75	16.67
End of uptake (test)	17.33	17.24	16.74	17.10
End of uptake (control)	16.90	16.95	16.67	16.84

Feed analysis (Level 3)

Lipid content	Grab #1 (%)	Grab #2 (%)	Grab #3 (%)	Average (%)
Study start (test)	16.61	16.55	16.81	16.66
Study start (control)	16.49	16.78	16.75	16.67
End of uptake (test)	17.37	16.82	16.63	16.94
End of uptake (control)	16.90	16.95	16.67	16.84

Method used for lipid determination

See *Ring test study results* for Laboratory 2A for details (same protocol was used).

Chemical concentrations – Before uptake (Level 1)

Chemical	Target concentration (µg/g)	Concentration 1 before uptake (µg/g)	Concentration 2 before uptake (µg/g)	Concentration 3 before uptake (µg/g)	Average (µg/g)
HCB	250	216	209	210	212
Musk xylene	500	413	405	412	410
o-Terphenyl	500	409	406	418	411
Methoxychlor	1000	774	771	787	777
B[a]P	-	-	-	-	-

Note: Benzo(a)pyrene (B[a]P) was excluded from Level 1.

Chemical concentrations – End of uptake (Level 1)

Chemical	Target concentration (µg/g)	Concentration 1 end uptake (µg/g)	Concentration 2 end uptake (µg/g)	Concentration 3 end uptake (µg/g)	Average (µg/g)
HCB	250	212	233	212	219
Musk xylene	500	408	376	380	388
o-Terphenyl	500	431	422	410	421
Methoxychlor	1000	803	777	759	779
B[a]P	-	-	-	-	-

Note: Benzo(a)pyrene (B[a]P) was excluded from Level 1.

Chemical concentrations – Before uptake (Level 2)

Chemical	Target concentration (µg/g)	Concentration 1 before uptake (µg/g)	Concentration 2 before uptake (µg/g)	Concentration 3 before uptake (µg/g)	Average (µg/g)
HCB	125	120	109	109	113
Musk xylene	250	249	225	227	234
o-Terphenyl	250	251	228	230	236
Methoxychlor	500	488	441	447	459
B[a]P	-	-	-	-	-

Note: Benzo(a)pyrene (B[a]P) was excluded from Level 2.

Chemical concentrations – End of uptake (Level 2)

Chemical	Target concentration (µg/g)	Concentration 1 end uptake (µg/g)	Concentration 2 end uptake (µg/g)	Concentration 3 end uptake (µg/g)	Average (µg/g)
HCB	125	113	117	119	116
Musk xylene	250	219	215	202	212
o-Terphenyl	250	225	229	224	226
Methoxychlor	500	431	436	415	427
B[a]P	-	-	-	-	-

Note: Benzo(a)pyrene (B[a]P) was excluded from Level 2.

Chemical concentrations – Before uptake (Level 3)

Chemical	Target concentration (µg/g)	Concentration 1 before uptake (µg/g)	Concentration 2 before uptake (µg/g)	Concentration 3 before uptake (µg/g)	Average (µg/g)
HCB	25	25.1	25.0	24.0	24.7
Musk xylene	50	45.7	48.7	48.7	47.7
o-Terphenyl	50	47.5	48.7	48.9	48.4
Methoxychlor	100	90.1	88.8	88.8	89.2
B[a]P	150	148	152	153	151

Chemical concentrations – End of uptake (Level 3)

Chemical	Target concentration (µg/g)	Concentration 1 end uptake (µg/g)	Concentration 2 end uptake (µg/g)	Concentration 3 end uptake (µg/g)	Average (µg/g)
HCB	25	23.2	25.0	24.3	24.2
Musk xylene	50	47.0	49.2	49.7	48.6
o-Terphenyl	50	45.8	48.2	49.1	47.7
Methoxychlor	100	89.9	93.9	92.4	92.1
B[a]P	150	145	147	154	149

Feed quantities (for 3% 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	5.42	5.42	5.42	8.94
	13	7.05	7.29	7.17	10.76
Depuration	1	6.84	7.09	6.96	9.40
	3	7.28	7.79	7.54	9.04
	7	8.36	8.16	8.26	8.67
	9	9.39	9.73	9.56	8.61
	21	12.88	14.98	13.93	8.3584

Daily observations

No mortality was observed in the test groups (Levels 1, 2 and 3) or the control group. [A dash (-) is given in the corresponding columns in the spreadsheet – we have assumed that this means that no adverse effects were noted.]

LABORATORY 3

RING TEST STUDY RESULTS

Pre-study measurements

Dilution water characteristics

Source:	Pre-conditioned municipal water.
TOC:	0.7-1.4 mg DOC/L
Total suspended solids:	Not reported in spreadsheet.

Fish species

Not reported in spreadsheet. [But known to be rainbow trout (*Oncorhynchus mykiss*).]

Fish food

Source/manufacturer:	INICIO Plus 0.8mm, Bio Mar
Crude protein:	56.00%
Crude fat:	15.3% (measured by Laboratory 3)
Crude fibre:	0.50%
Moisture:	Not given
Ash:	11.50%
Manufacturer reported impurities:	Not reported in spreadsheet.

Test substances

Chemical	CAS Number	Purity (%)
Hexachlorobenzene (HCB)	118-74-1	99
Musk xylene	81-15-2	99.50
o-Terphenyl	84-15-1	99
Methoxychlor	72-43-5	analytical standard
Benzo(a)pyrene (B[a]P)	50-32-8	≥96 (HPLC)

Spiked feed preparation

Preparation method: ii) Solvent

Extra details on spiking method: none given in the spreadsheet.

Chemical recoveries pre-study [Average values not given in the spreadsheet.]

Chemical	Target concentration (µg/g)	1st analysis* (%) pre-study	2nd analysis** (%) pre-study	Average (%)
HCB	25	127.2	120.30	
Musk xylene	50	109.7	113.90	
o-Terphenyl	50	108.6	107.80	
Methoxychlor	100	136.1	129.80	
B[a]P	150	122.0	107.50	

Notes: * Concentrations (n=5) after feed preparation. Spiked feed was kept frozen (-18°C) for four months until the onset of the experiment.

** Concentrations were reanalysed (n=2) at the onset of the experiment. Feed was kept refrigerated during the experiment.

Analytical method description

Analytical Method description I: Fish Extraction and Analysis by GC/EI-MS

Sample extraction/preparation:

7) Whole fish (weights approx. 2 to 16 g) were mixed with 4.5 to 8 g Hydromatrix for homogenisation/drying.
8) ASE extraction at 120°C in 22 or 33 mL cells; solvent: acetone/methylene chloride (1:1, v/v).
9) Drying of the extracts with Na_2SO_4 and volume setting to 50, 100 or 200 mL.
10) Clean-up of the extracts analogous to the *Analytical validation protoco*l using Sep-Pak® Vac 12cc (2g) Silica cartridges.
11) Addition of 20 µL of an IS solution containing the internal standards (IS) in a conc. of 4 µg/mL.
12) Concentrating the SPE eluates to almost dryness by a gentle stream of N_2 and reconstitution of the residues in 500 µL toluene.

Analytical Method description II: Spiked Diet Extraction and Analysis by GC/EI-MS

Sample extraction/preparation:

1g spiked diet was extracted with 25 mL acetone/methylene chloride (1:1, v/v) by USE (ultrasonic solvent extraction with cooling by ice). After centrifugation for 5 min at 1500 rpm, 10 µL of the clear extract was pipetted together in autosampler vials with 1 mL toluene and 10 µL IS solution (containing the internal standards (IS) at a conc. of 4 µg/mL).

Phys-chem measurements in the study

Temperature

The temperature ranged from 14.6-15.4°C in both the test group and the control group throughout the uptake and depuration phases (average = 15.01 °C in the test group and 14.98 °C in the control).

Dissolved oxygen

	Day	Test group (mg/L)	Control group (mg/L)
Uptake	0	9	8.9
	6	8.8	8.9
	13	8.7	8.7
Depuration	7	8.6	8.3
	14	8.8	8.8
	21	8.9	8.8
	28	9	9

pH

	Day	Test group	Control
Uptake	0	7.94	7.93
	6	7.73	7.76
	13	7.83	7.83
Depuration	7	7.78	7.76
	14	7.79	7.76
	21	7.97	7.96
	28	7.91	7.92

Other experimental conditions

Test flow rate:	18 L/hour
Size of test vessels (approx.):	90 L
Number volumes replacement per day (approx.):	5

Fish data

Control

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	1.8	5.4	4.8					
	0	2.2	5.5	5.1					
	0	1.7	5.2	4.8					
	0	1.9	5.6	5.9					
	0	1.7	5.3	5.1					
	0	2.2	5.6		0	0	0	0	0
	0	2.2	5.5		0	0	0	0	0
	0	1.8	5.5						
	0	1.9	5.3						
	0	2.2	5.5						
	3	2.8	5.9		0	0	0	0	0
	3	2.8	6		0	0	0	0	0
	3	3.0	6.3						
	3	2.7	6.1						
	3	2.5	5.8						
	13	3.7	6.8	7.7					

Test stage					Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	13	3.8	7.1	7.1					
	13	3.2	6.8	6.9					
	13	3.9	7	7.2					
	13	3.2	6.7	7.1					
Depuration	1	4.3	7.1		0	0	0	0	0
	1	3.3	6.5		0	0	0	0	0
	1	3.4	6.6						
	1	4.1	7.1						
	1	4.4	7.3						
	3	5.0	7.8		0	0	0	0	0
	3	4.8	7.5		0	0	0	0	0
	3	4.4	7.2						
	3	4.8	7.5						
	3	4.0	6.9						
	7	4.4	7.3		0	0	0	0	0
	7	5.6	7.8		0	0	0	0	0
	7	6.0	8.1						
	7	4.3	7.4						
	7	4.7	7.4						
	14	5.4	7.8		0	0	0	0	0
	14	9.0	9		0	0	0	0	0

	Test stage				Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	14	6.2	8						
	14	6.5	8						
	14	7.8	8.8						
	21	11.5	10		0	0	0	0	0
	21	8.8	9.1		0	0	0	0	0
	21	8.3	9						
	21	9.2	9.5						
	21	10.4	9.4						
	28	10.3	9.2		0	0	0	0	0
	28	13.4	10.4		0	0	0	0	0
	28	13.5	10.5						
	28	10.9	9.6						
	28	15.4	10.6						
*	28	13.6	10.5	8.8					
*	28	10.3	9.6	9.2					
*	28	17.9	11.5	9.4					
*	28	10.4	9.6	8.9					
*	28	12.6	10.1	8.4					

Test

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	2.2	5.6						
	0	2.2	5.5						
	0	1.8	5.5						
	0	1.9	5.3						
	0	2.2	5.5						
	3	2.1	5.7		1.5	3.7	2.5	3.5	1.3
	3	3.0	6.2		2.1	4.4	2.8	2.6	2.0
	3	2.8	6		1.4	3.3	2.3	1.9	1.1
	3	2.1	5.6		1.1	2.7	1.3	1.0	0.7
	3	2.5	5.9		1.4	3.4	2.4	1.8	1.3
	13	3.6	6.9	7.2					
	13	3.0	6.5	6.3					
	13	3.8	7	6.6					
	13	4.4	7.3	6.8					
	13	3.7	6.6	7.4					
Depuration	1	4.5	7		6.1	11.5	8.8	10.0	1.4
	1	3.6	6.6		4.7	9.2	6.0	6.7	2.7
	1	3.8	6.6		5.2	10.3	7.1	8.3	1.8

Test stage				Concentration (µg/g)				
Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
1	4.4	7.2		4.9	9.4	6.4	3.9	1.3
1	3.7	6.6		3.5	6.8	3.2	2.9	2.1
3	3.6	6.8		3.3	5.8	3.4	1.5	< LOQ
3	4.6	7.5		4.4	7.7	4.2	7.1	< LOQ
3	4.1	7.2		4.2	7.7	2.9	1.1	< LOQ
3	4.7	7.6		5.2	9.8	4.5	7.2	< LOQ
3	4.5	7.3		4.0	6.8	4.5	1.6	< LOQ
7	4.9	7.4		2.6	4.2	2.6	0.5	< LOQ
7	4.3	7.2		2.5	4.1	2.2	0.4	< LOQ
7	5.8	8		3.4	5.2	3.0	4.4	< LOQ
7	4.5	7.3		2.2	2.7	0.8	0.3	< LOQ
7	4.4	7.2		3.0	4.0	1.8	1.3	< LOQ
14	8.6	9		2.3	2.8	1.4	0.2	< LOQ
14	4.9	7.6		1.2	1.4	0.8	< LOQ	< LOQ
14	7.2	8.8		2.5	3.1	0.4	0.2	< LOQ
14	6.9	8.4		2.8	3.6	1.1	0.3	< LOQ
14	6.9	8.3		2.3	2.9	0.9	1.3	< LOQ
21	8.1	8.9		1.9	1.9	1.0	0.8	< LOQ
21	12.5	10.1		1.4	1.3	1.0	0.5	< LOQ
21	6.4	8.1		1.4	1.5	0.3	< LOQ	< LOQ
21	11.6	10.1		1.4	1.3	1.1	0.3	< LOQ

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
	21	7.8	8.6		1.4	1.5	0.5	0.7	< LOQ
	28	17.1	11.3		1.2	1.1	0.3	0.3	< LOQ
	28	10.2	9.5		1.5	1.7	0.2	0.3	< LOQ
	28	14.6	10.5		0.8	0.6	< LOQ	0.1	< LOQ
	28	12.7	10.1		0.8	0.7	0.6	0.3	< LOQ
	28	11.2	9.6		1.2	1.0	< LOQ	< LOQ	< LOQ
*	28	10.3	9.5	9.4					
*	28	15.9	11	8.6					
*	28	17.1	11	8.1					
*	28	12.1	9.8	8.7					
*	28	10.3	9.5	9.2					

Note: $LOQ_{defined}$ 0.1 mg/kg FW for HCB, Musk xylene, o-Terphenyl, Methoxychlor and $LOQ_{defined}$ 0.01 mg/kg FW for B[a]P.

Feed and test observations

Feed analysis

Lipid content study start: 15.65%
Lipid content end of uptake: 15.00%

Method used for lipid determination

ASE method.

Chemical concentrations – Start of uptake

Chemical	Target concentration (µg/g)	Concentration 1 start of uptake (µg/g)	Concentration 2 start of uptake (µg/g)	Average concentration (µg/g)
HCB	25	30.5	29.6	30.05
Musk xylene	50	58.2	55.7	56.95
o-Terphenyl	50	55.0	52.8	53.90
Methoxychlor	100	130.2	129.4	129.80
B[a]P	150	164.8	157.6	161.20

[There is a note in the spreadsheet explaining that because of the delay of the feeding experiment, the experimental diet was analysed several times including a final analysis at the onset of the actual test trial. The extended storage period was found to have no significant effect on the quality of the test diet. Unfortunately, there was no sub-sample of the diet taken for analysis at the end of the uptake period. However, with respect to the former analysis, Laboratory 3 is convinced that the quality of the test diet did not change during the experimental uptake period.]

Feed quantities (for 3% 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	1.91	2.02	1.96	2.76
	3	2.77	2.47	2.62	3.30
	13	3.56	3.72	3.64	4.04
Depuration	1	3.86	3.99	3.92	3.76
	3	4.6	4.31	4.46	3.61
	7	5.01	4.78	4.89	3.23
	14	6.97	6.91	6.94	3.54
	21	9.63	9.28	9.45	3.4

	28	12.68	13.16		

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 4

RING TEST STUDY RESULTS

Pre-study measurements

Dilution water characteristics

Source:	Non-chlorinated charcoal filtered tap water mixed with deionised water to achieve a hardness of 1 mmol/L.
TOC:	1.7 mg/L (measured)
Total suspended solids:	0.039 g/L (measured)

Fish species

Rainbow trout (*Oncorhynchus mykiss*)

Fish food

Source/manufacturer:	Ecostart 17
Crude protein:	50%
Crude fat:	16%
Crude fibre:	1%
Moisture:	Not given
Ash:	10%
Manufacturer reported impurities:	BioMar

Test substances

Chemical	CAS Number	Purity (%)
Hexachlorobenzene (HCB)	118-74-1	99.5±0.5
Musk xylene	81-15-2	99.5±0.5
o-Terphenyl	84-15-1	99.5±0.5
Methoxychlor	72-43-5	99.0±0.5
Benzo(a)pyrene (B[a]P)	50-32-8	98.40

Spiked feed preparation

Preparation method: ii) Solvent
Extra details on spiking method:

1) Actual weights of the test substances used for diet preparation: HCB 2.57 mg; Musk xylene 5.09 mg, o-Terphenyl 5.09 mg, Methoxychlor 10.10 mg, Benzo(a)pyrene 15.08 mg.
2) These weighed amounts were transferred into a volumetric flask with the help of dichloromethane, made up to 25 mL with dichloromethane and completely dissolved.
3) 100.06 g fish diet was placed into the flask of a rotation evaporator (internal surface of the flask structured to mix contents during rotation).
4) The solution with the test substances was sucked into the rotating flask together with air through a caburator by the underpressure in the flask, to reach very fine distribution.
5) By this procedure the solution was sprayed homogeneously on the rotating diet and the diet appeared homogeneously wet.
6) The apparatus and tubing were washed twice with approx. 5 mL dichloromethane to ensure that no test substance was lost.
7) The diet was dried in the rotation evaporator for approx. 2 hours and was then completely dry (weight = 99.50 g).
8) No crystalline test substance was observed.
9) Control diet was treated in the same way with dichloromethane only.

Chemical recoveries pre-study

Chemical	Target concentration (µg/g)	Sample 1 (% theoretical) pre-study	Sample 2 (% theoretical) pre-study	Sample 3 (% theoretical) pre-study
HCB	25.7	90.4	90.5	90.9
Musk xylene	50.9	112.4	117.3	116.15
o-Terphenyl	50.9	93.3	94.05	93.6
Methoxychlor	101.0	97.1	96.85	97.85
B[a]P	150.8	85.2	86.3	85.05

Analytical method description

See description given in the *Feed analysis results* section in Annex 1.

Phys-chem measurements in the study

Temperature

The temperature ranged from 14-15°C in both the test group and the control throughout the uptake and depuration phases (average = 14.49 °C for both the test group and the control).

Dissolved oxygen

	Day	Test group (mg/L)	Control (mg/L)
Uptake	0	8.6	8.7
	7	9.2	9.1
	13	9.4	9.3
Depuration	7	9.4	9.4
	14	9.5	9.3
	21	9.3	9.2
	28	9.4	9.2

pH

	Day	Test group	Control
Uptake	0	7.8	7.8
	6	7.9	7.9
	13	8.0	8.0
Depuration	7	7.9	7.9
	14	7.9	7.9
	21	7.9	7.9
	27	7.9	7.9

Other experimental conditions

Test flow rate:	20.82 L/hour
Size of test vessels (approx.):	80×35×55 cm
Number volumes replacement per day (approx.):	5

Fish data

Control

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	1.26	5.0	4.2					
	0	1.30	5.0	5.5					
	0	1.07	4.8	4.0					
	0	1.13	5.1	6.7					
	0	1.11	4.9	5.6					
	3	1.75	5.3		0	0	0	0	0
	3	1.62	5.3		0	0	0	0	0
	3	1.76	5.5						
	3	1.48	5.1						
	3	1.34	5.0						
	14	1.98	5.9	6.5					
	14	2.12	5.7	6.1					
	14	2.19	5.9	7.1					
	14	3.03	6.4	8.2					
	14	2.66	6.1	8.6					
Depuration	1	2.22	5.8		0	0	0	0	0

Test stage				Concentration (µg/g)				
Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
1	2.88	6.4		0	0	0	0	0
1	3.13	6.5						
1	2.44	6.2						
1	2.39	6.0						
3	2.08	6.0		0	0	0	0	0
3	2.23	6.0		0	0	0	0	0
3	2.43	6.1						
3	2.94	6.5						
3	2.41	6.1						
7	2.19	6.0		0	0	0	0	0
7	3.51	7.0		0	0	0	0	0
7	3.05	6.4						
7	3.80	6.9						
7	2.66	6.3						
14	5.69	8.1		0	0	0	0	0
14	4.24	7.5		0	0	0	0	0
14	2.82	6.4						
14	6.61	8.5						
14	5.35	7.8						
21	7.01	8.4		0	0	0	0	0
21	5.54	8.2		0	0	0	0	0

	Test stage				Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	21	6.87	8.5						
	21	5.43	7.8						
	21	6.02	8.2						
*	28	4.47	7.4		0	0	0	0	0
*	28	7.08	8.6		0	0	0	0	0
*	28	7.82	8.6						
*	28	9.60	9.2						
*	28	7.21	8.5						
	28	8.11	8.9	9.2					
	28	6.52	8.2	9.2					
	28	6.69	8.5	10.0					
	28	5.54	8.0	10.7					
	28	5.65	8.3	8.8					

Test

	Test stage				Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
Uptake	0								
	0								
	0								

Test stage					Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	0								
	0								
	3	1.71	5.3		1.776	3.145	2.255	3.766	0.624
	3	1.56	5.2		1.859	3.434	2.915	4.962	0.867
	3	1.33	4.9		1.560	2.885	2.215	1.428	0.209
	3	1.67	5.3		1.580	2.759	1.814	1.148	0.394
	3	1.59	5.2		1.867	3.497	2.630	1.929	0.742
	13	2.73	6.1	7.0					
	13	1.94	5.7	6.4					
	13	1.40	5.4	5.0					
	13	2.97	6.4	6.4					
	13	2.19	5.8	6.2					
Depuration	1	2.52	6.3		4.473	6.500	2.649	3.132	0.035
	1	2.70	6.4		5.686	7.980	6.957	6.326	0.035
	1	1.81	5.7		2.612	2.940	1.518	0.602	0.184
	1	3.37	6.7		5.295	7.323	6.929	5.995	0.156
	1	3.47	6.8		5.131	7.176	4.452	4.757	0.337
	3	2.71	6.2		4.15	5.24	5.22	1.74	< 0.018
	3	3.29	6.5		4.48	5.27	3.49	7.2	< 0.018
	3	2.66	6.2		3.94	4.88	2.98	6.32	< 0.018
	3	3.00	6.6		4.06	4.65	3.92	3.64	< 0.018

	Test stage				Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	3	3.57	6.7		4.19	4.91	3.04	3.06	< 0.018
	7	3.07	6.4		3.32	3.38	3.45	1.52	< 0.018
	7	3.87	7.0		3.73	3.63	3.62	0.334	< 0.018
	7	2.82	6.3		3.08	3	2.29	0.088	< 0.018
	7	3.88	7.1		3.15	3.05	3.37	3.83	< 0.018
	7	3.28	6.6		3.13	3.38	3.08	0.701	< 0.018
	14	4.42	7.2		2.46	1.98	1.75	0.221	< 0.018
	14	4.07	7.3		2.27	1.93	2.1	0.147	< 0.018
	14	6.00	8.1		2.2	1.9	0.152	0.097	< 0.018
	14	5.26	7.9		2.58	2.08	2.39	0.19	< 0.018
	14	3.23	6.5		1.57	1.56	0.188	0.076	< 0.018
	21	6.01	8.3		1.09	0.522	0.123	< 0.018	< 0.018
	21	6.59	8.5		1.19	0.701	0.901	0.515	< 0.018
	21	4.54	7.3		1.27	0.796	0.096	< 0.018	< 0.018
	21	5.87	8.0		1.71	1.05	1.12	< 0.018	< 0.018
	21	6.02	8.1		1.25	0.773	1.05	0.481	< 0.018
*	28	6.08	8.0		1.7	1.8	1.32	0.254	< 0.018
*	28	4.47	7.4		0.92	1.15	0.519	0.187	< 0.018
*	28	9.22	9.0		1.18	1.11	1.29	1.05	< 0.018
*	28	5.27	7.6		1.6	1.55	0.659	0.247	< 0.018
*	28	4.23	7.5		1.15	1.36	0.868	0.347	< 0.018

Test stage				Concentration (µg/g)				
Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
28	3.73	7.2	8.7					
28	7.66	8.7	9.1					
28	6.21	8.4	9.8					
28	6.40	8.3	10.1					
28	5.54	8.0	9.2					

[The following notes were given in the spreadsheet.

Fish that were fed with diet with test substances were removed on day 3, incubated until day 4 in clean water, sacrificed and the guts were removed. The same procedure was followed with fish sampled on day 13 of the uptake phase.

Lipid content in fish is increased over the test period, if the daily food ration is 3% of the fish body weight.]

Calculation of the total concentration in fish for fish with separate analysis for guts and rest of the fish (results for HCB and Musk xylene)

Test stage					Concentration (µg/g)					
	Day	Weight rest (g)	Weight guts (g)	Lipid (w/w)	HCB rest	HCB guts	HCB fish	Musk xylene rest	Musk xylene guts	Musk xylene fish
Uptake	0									
	0									
	0									
	0									
	0									
	3	1.43	0.27		1.090	5.410	1.776	1.700	10.800	3.145
	3	1.30	0.23		1.420	4.340	1.859	2.380	9.390	3.434
	3	1.10	0.17		1.160	4.150	1.560	1.980	8.740	2.885
	3	1.37	0.26		1.220	3.480	1.580	1.960	6.970	2.759
	3	1.26	0.28		1.340	4.240	1.867	2.270	9.020	3.497
Depuration	1	2.16	0.33		3.430	11.300	4.473	4.820	17.500	6.500
	1	2.28	0.38		4.100	15.200	5.686	5.510	22.800	7.980
	1	1.57	0.24		2.290	4.720	2.612	2.440	6.210	2.940
	1	2.83	0.49		4.290	11.100	5.295	5.700	16.700	7.323
	1	2.82	0.60		3.350	13.500	5.131	4.320	20.600	7.176

Calculation of the total concentration in fish for fish with separate analysis for guts and rest of the fish (results for o-Terphenyl, Methoxychlor and B[a]P)

Test stage					Concentration (µg/g)								
	Day	Weight rest (g)	Weight guts (g)	Lipid (w/w)	o-Ter phenyl rest	o-Ter phenyl guts	o-Ter phenyl fish	Methoxy chlor rest	Methoxy chlor guts	Methoxy chlor fish	B[a]P rest	B[a]P guts	B[a]P fish
Uptake	0												
	0												
	0												
	0												
	0												
	3	1.43	0.27		1.280	7.420	2.255	1.890	13.700	3.766	0.000	3.930	0.624
	3	1.30	0.23		2.120	7.410	2.915	3.310	14.300	4.962	0.000	5.770	0.867
	3	1.10	0.17		1.590	6.260	2.215	0.817	5.380	1.428	0.000	1.560	0.209
	3	1.37	0.26		1.310	4.470	1.814	0.519	4.460	1.148	0.000	2.470	0.394
	3	1.26	0.28		1.770	6.500	2.630	0.949	6.340	1.929	0.000	4.080	0.742
Dep.	1	2.16	0.33		2.000	6.900	2.649	2.400	7.920	3.132	0.000	0.267	0.035
	1	2.28	0.38		4.800	19.900	6.957	4.580	16.800	6.326	0.000	0.244	0.035
	1	1.57	0.24		1.310	2.880	1.518	0.450	1.600	0.602	0.000	1.390	0.184
	1	2.83	0.49		5.410	15.700	6.929	4.800	12.900	5.995	0.000	1.060	0.156
	1	2.82	0.60		2.740	12.500	4.452	3.110	12.500	4.757	0.000	1.920	0.337

Feed and test observations

Feed analysis

Lipid content study start: Not reported
Lipid content end of uptake: 16.40%

[The lipid content of the diet was analysed only once at the end of the uptake period. The lipid content was unchanged during the preparation of the diet mix, and was the same for treated and for untreated diet. Furthermore, the value measured at the end of the feeding period (16.4%) was close to the supplier specification (16% lipids). Therefore, the test lab considered that a single measurement was sufficient to provide information on the lipid content.]

Method used for lipid determination

Extraction with cyclohexane/isopropanol.

Chemical concentrations

Chemical	Target concentration (µg/g)	Concentration 1 start uptake (µg/g)	Concentration 2 end uptake (µg/g)	Mean concentration (µg/g)	% Initial
HCB	25.7	23.27	23.45	23.36	100.8
Musk xylene	50.9	58.68	56.17	57.43	95.7
o-Terphenyl	50.9	47.67	47.62	47.64	99.9
Methoxychlor	101	98.25	100.28	99.27	102.1
B[a]P	150.8	128.98	130.55	129.77	101.2

Feed quantities (for 3% 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				
	3	1.57	1.59	1.58	3.23*
	13				1.90
Depuration	1	2.51	2.3	2.405	1.90
	3	3.05	2.42	2.735	2.17
	7	3.38	3.04	3.21	2.06
	14	4.6	4.94	4.77	1.93
	21	5.81	6.17	5.99	2.15
	28				1.80

Note: Number of fish not considered in this table.

* Too high feeding rate (error in calculation).

[The following information regarding the feeding rate was provided. The feeding was too high from day 0 to day 3. The reason was a mistake in the calculation. With 45 fish of a mean bodyweight of 1.58 g the feeding rate during this time was approx. 4.5% instead of 3% bodyweight.]

Other data included in the spreadsheet

Concentration measurements in freshly prepared diet (double measurement in three samples) are summarised in the table below. [Units not given in spreadsheet – assumed to be (µg/g).]

Sample 1 (µg/g)		**Sample 2 (µg/g)**		**Sample 3 (µg/g)**		**Average (µg/g)**	**STDEV**	**RSD (%)**
23.00	23.4	23	23.5	23.9	22.8	23.27	0.41	1.75
56.70	57.8	58.7	60.7	61.8	56.4	58.68	2.18	3.71
47.20	47.8	47.5	48.2	49	46.3	47.67	0.92	1.92
98.50	97.6	97.7	98	99.6	98.1	98.25	0.73	0.75
127.90	129.1	128.8	131.5	132.4	124.2	128.98	2.90	2.25

Daily observations

No mortality, adverse effects or changes in feeding behaviour were observed in either the test group or the control group.

LABORATORY 5

RING TEST STUDY RESULTS

Pre-study measurements

[Correspondence from the lab stated that the pre-test information hasn't been put into the spreadsheet, but Laboratory 5 could provide it if necessary. Chemical analysis was not performed on the fish at day 3 and Laboratory 5 only performed chemical analysis on the control fish once to save on time and resources.]

Dilution water characteristics

Source:	Tap water.
TOC:	Not reported in spreadsheet.
Total suspended solids:	Not reported in spreadsheet.

Fish species

Not given in spreadsheet. [But confirmed as rainbow trout (*Oncorhynchus mykiss*).]

Fish food

Source/manufacturer:	Trout starter feed
Crude protein:	66%
Crude fat:	11%
Crude fibre:	0.3%
Moisture:	-
Ash:	12.2%
Manufacturer reported impurities:	-

Test substances

Chemical	CAS Number	Purity (%)
Hexachlorobenzene (HCB)	118-74-1	
Musk xylene	81-15-2	
o-Terphenyl	84-15-1	
Methoxychlor	72-43-5	
Benzo(a)pyrene (B[a]P)	50-32-8	

[Note: The purity of the test substances is not reported in the spreadsheet.]

Spiked feed preparation

Preparation method: ii) Solvent

Extra details on spiking method: none given in spreadsheet.

Chemical recoveries pre-study [This table has been left blank in the spreadsheet – see note above.]

Chemical	Target concentration (µg/g)	Sample 1 (% theoretical) pre-study	Sample 2 (% theoretical) pre-study	Sample 3 (% theoretical) pre-study
HCB				
Musk xylene				
o-Terphenyl				
Methoxychlor				
B[a]P				

Analytical method description

[Not given in spreadsheet.]

Phys-chem measurements in the study

Temperature

The temperature ranged from 8.1-11.1°C in the test group throughout the uptake and depuration phases (average = 8.96 °C for the test group). The temperature in the control group has not been reported in the spreadsheet.

Dissolved oxygen [Only one set of dissolved oxygen concentrations, reported as a percentage of air saturation, are reported in the spreadsheet, presumably for the test group. It is likely that these are actually concentrations, and so the units have been changed in the table]

	Day	Test group (mg/L)	Control (mg/L)
Uptake	0	10.2	
	7	10.3	
	13	10.5	
Depuration	7	10.5	
	14	11.3	
	21	11.2	
	28	11.8	

pH [Only one set of pH values are reported in the spreadsheet, presumably for the test group.]

	Day	Test group	Control
Uptake	0	7.49	
	7	7.51	
	13	7.42	
Depuration	7	7.45	
	14	7.53	
	21	7.36	
	28	7.50	

Other experimental conditions

Test flow rate:	200 mL/minute
Size of test vessels (approx.):	100 L
Number volumes replacement per day (approx.):	2.88

Fish data

Control [Units for the lipid content not given – the mean lipid content in the calculation spreadsheet is 7.82% which is consistent with the values reported if they are increased by a factor of 10 – therefore the units appear to be %/10.]

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (w/w)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	8.94	-	1.04					
	0	3.24	-	0.97					
	0	6.98	-	1.24					
	0	7.48	-	0.57					
	0	7.2	-	0.46					
	3								
	3								
	3								
	3								
	3								
	13	8.44	-	0.85					
	13	11.23	-	0.74					
	13	7.06	-	0.59					
	13	4.69	-	1.06					
	13	5.07	-	0.63					
Depuration	1	14.15	100		<0.015	<0.002	<0.01	<0.01	<0.015

Test stage				Concentration (µg/g)				
Day	Weight (g)	Total length (mm)	Lipid (w/w)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
1	17.9	109.1		<0.015	<0.002	<0.01	<0.01	<0.015
1	9.58	89.5						
1	9.76	89.2						
1	9.7	93.5						
3	9.78	91.1						
3	10.08	90						
3	10.25	91.5						
3	11.47	94.4						
3	8.25	86						
7	6.44	78.7						
7	13.45	100.4						
7	12.33	97.1						
7	10.1	94.3						
7	13.98	101.1						
14	7.32	8.44						
14	12.72	10.05						
14	4.65	6.9						
14	13.11	10.49						
14	10.6	9.2						
21	11.98	95.8						
21	6.91	84.5						

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (w/w)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
	21	14.41	105.20						
	21	13.07	105.1						
	21	10.56	94.6						
*	28	27.7	134.9	0.75					
*	28	17.41	111.3	1.91					
*	28	14.63	103.6	0.98					
*	28	8.91	88.8	2.84					
*	28	21.28	121.4	1.16					

Test [Units for the lipid content not given – the mean lipid content in the calculation spreadsheet is 7.82% which is consistent with the values reported if they are increased by a factor of 10 – therefore the units appear to be %/10.]

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (w/w)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	8.94	-	1.04					
	0	3.24	-	0.97					
	0	6.98	-	1.24					
	0	7.48	-	0.57					
	0	7.2	-	0.46					
	3								
	3								

Test stage					Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (w/w)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	3								
	3								
	3								
	13	8.72	-	0.94					
	13	12.15	-	0.48					
	13	4.28	-	0.94					
	13	9.69	-	0.68					
	13	7.02	-	0.87					
Depuration	1	12.06	93.7		13.23993126	0.212162753	21.84249737	6.119727712	3.988423777
	1	10.64	89.4		9.282170984	0.175099736	14.86824292	6.691554427	10.04367606
	1	10.17	90.8		9.581209673	0.202491981	17.4717515	7.686576031	1.842381636
	1	6.7	76.9		7.49579221	0.264550783	12.03752663	2.405496233	0.098613969
	1	9.69	89.3		2.230083954	0.025660586	2.981103664	1.30643742	0.738255601
	3	12.66	102.6		5.338854811	0.014172894	5.855965209	1.681448468	0.022955177
	3	9.45	89.2		9.75953045	0.012067981	7.86289237	12.98028694	0.017552638
	3	10.66	96.2		18.17042205	0.018331529	17.623998	2.604380335	0.022598985
	3	7.11	83.9		9.75866624	0.020081562	6.956072827	6.480526942	0.029582152
	3	13.5	103		3.114516932	0.011576411	4.23225923	2.374676601	0.021220925
	7	14.45	109.4		7.137443622	0.001859562	5.321846746	1.550112709	<0.015
	7	6.87	83		10.74230621	0.002698062	6.60658184	3.404672359	<0.015
	7	10.44	95.1		6.517433053	0.003221373	5.961416984	5.237987518	<0.015

	Test stage				Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (w/w)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	7	7.24	83.6		10.63972357	0.002000506	14.17569327	16.34828337	<0.015
	7	6.93	85.6		13.31928981	0.002334267	10.10212137	11.46309373	<0.015
	14	9.37	94.2		6.08947463	<0.002	6.759640453	1.069709057	<0.015
	14	15.5	111.2		6.711146631	<0.002	8.940727233	8.102983258	<0.015
	14	10.88	96.5		12.18320623	<0.002	9.89975015	47.03665964	<0.015
	14	10.4	91		5.593246732	<0.002	7.566718529	1.334091587	<0.015
	14	19.39	113.5		4.105106382	<0.002	3.238774868	9.761444813	<0.015
	21	6.3	80.4		10.66534621	<0.002	11.04226385	4.103292227	<0.015
	21	8.28	91.5		4.124066203	<0.002	4.210920207	3.915320978	<0.015
	21	12.74	105.6		6.7205497	<0.002	8.504257315	4.594241278	<0.015
	21	5.49	79.4		8.67359842	<0.002	9.149840369	8.896713402	<0.015
	21	10.71	94.4		3.181625816	<0.002	2.639758047	2.103392693	<0.015
*	28	18.94	114.9		1.909282713	<0.002	2.344084064	1.989123321	<0.015
*	28	26.35	123.1		1.562105788	<0.002	1.498057414	1.761150036	<0.015
*	28	18.06	114.4		2.100074932	<0.002	2.679926876	5.584818942	<0.015
*	28	8.6	88.7		2.14528821	<0.002	2.889738855	7.173196241	<0.015
*	28	8.11	87.3		3.085995976	<0.002	2.487041513	3.489464216	<0.015
	28	25.81	122.2	2.49					
	28	12.98	101.2	1.53					
	28	15.96	103.4	0.58					
	28	5.93	75.8	0.77					

Control – Average results lipid and concentrations [Units for the lipid content not given – the mean lipid content in the calculation spreadsheet is 7.82% which is consistent with the values reported if they are increased by a factor of 10 – therefore the units appear to be %/10.]

Test stage			Concentration (µg/g)				
	Day	Lipid (w/w)	HCB	Musk xylene	o-Terphenyl	Methoxychlor	B[a]P
Uptake	0	0.856					
	3		<0.015	<0.002	<0.01	<0.01	<0.015
	13	0.774					
Depuration	1		<0.015	<0.002	<0.01	<0.01	<0.015
	3		<0.015	<0.002	<0.01	<0.01	<0.015
	7		<0.015	<0.002	<0.01	<0.01	<0.015
	14		<0.015	<0.002	<0.01	<0.01	<0.015
	21		<0.015	<0.002	<0.01	<0.01	<0.015
*	28	1.528	<0.015	<0.002	<0.01	<0.01	<0.015

Test – Average results lipid and concentrations [Units for the lipid content not given – the mean lipid content in the calculation spreadsheet is 7.82% which is consistent with the values reported if they are increased by a factor of 10 – therefore the units appear to be %/10.]

Test stage			Concentration (µg/g)				
	Day	Lipid (w/w)	HCB	Musk xylene	o-Terphenyl	Methoxychlor	B[a]P
Uptake	0						
	3		<0.015	<0.002	<0.01	<0.01	<0.015
	13	0.782					
Depuration	1		8.365838	0.175993	13.84022	4.841958	3.34227
	3		9.228398	0.015246	8.506238	5.224264	0.022782

Test stage			Concentration (µg/g)				
	Day	Lipid (w/w)	HCB	Musk xylene	o-Terphenyl	Methoxychlor	B[a]P
	7		9.671239	0.002423	8.433532	7.60083	<0.015
	14		6.936436	<0.002	7.281122	13.46098	<0.015
	21		6.673037	<0.002	7.109408	4.722592	<0.015
*	28	1.3425	2.16055	<0.002	2.37977	3.999551	<0.015

Additional information – Day 1 depuration gut content

Gut content (µg/g)				
HCB	**Musk xylene**	**o-Terphenyl**	**Methoxychlor**	**B[a]P**
19.958	<0.1	31.732	52.378	44.790
31.096	<0.1	36.293	34.776	48.790
30.091	<0.1	48.688	31.668	66.244

Fish carcass (–guts)

Fish (µg/g)				
HCB	**Musk xylene**	**o-Terphenyl**	**Methoxychlor**	**B[a]P**
1.788	0.013	1.859	0.416	0.049
1.347	0.020	1.756	0.378	0.067
1.649	0.017	2.508	0.701	0.180
0.824	0.026	1.199	0.300	0.099
2.230	0.026	2.981	1.306	0.738
1.054	0.014	1.270	0.137	0.023
1.820	0.012	2.250	0.997	0.018
2.403	0.018	2.413	2.604	0.023
1.949	0.020	2.434	0.937	0.030
0.694	0.012	0.966	0.537	0.021
0.876	0.002	0.599	0.342	<0.015
1.526	0.003	0.921	0.312	<0.015
1.378	0.003	0.947	0.422	<0.015
1.728	0.002	1.152	0.735	<0.015
1.374	0.002	0.966	0.309	<0.015
1.016	<0.002	0.685	0.151	<0.015
0.873	<0.002	0.575	0.466	<0.015
1.196	<0.002	0.720	1.472	<0.015
1.164	<0.002	0.863	0.275	<0.015
0.627	<0.002	0.404	0.664	<0.015
0.988	<0.002	1.271	1.541	<0.015
0.663	<0.002	0.724	1.414	<0.015
0.659	<0.002	0.909	2.062	<0.015
0.743	<0.002	0.922	2.291	<0.015
0.438	<0.002	0.385	0.570	<0.015

0.531	<0.002	0.349	0.029	<0.015
0.392	<0.002	0.250	0.099	<0.015
0.520	<0.002	0.348	0.113	<0.015
0.603	<0.002	0.613	0.098	<0.015
0.579	<0.002	0.324	0.013	<0.015

Guts

Guts (µg/g)				
HCB	**Musk xylene**	**o-Terphenyl**	**Methoxychlor**	**B[a]P**
11.452	0.199	19.983	5.704	3.940
7.935	0.155	13.112	6.314	9.976
7.932	0.185	14.964	6.986	1.663
6.672	0.239	10.839	2.106	<0.03
Not analysed	Not analysed	Not analysed	Not analysed	Not analysed
4.285	<0.01	4.586	1.545	<0.03
7.940	<0.01	5.613	11.983	<0.03
15.767	<0.01	15.211	<0.05	<0.03
7.809	<0.01	4.522	5.543	<0.03
2.421	<0.01	3.266	1.837	<0.03
6.261	<0.01	4.722	1.208	<0.03
9.216	<0.01	5.686	3.092	<0.03
5.139	<0.01	5.014	4.816	<0.03
8.912	<0.01	13.023	15.613	<0.03
11.945	<0.01	9.136	11.154	<0.03
5.074	<0.01	6.074	0.919	<0.03
5.838	<0.01	8.365	7.637	<0.03
10.987	<0.01	9.180	45.565	<0.03
4.429	<0.01	6.703	1.059	<0.03
3.478	<0.01	2.835	9.098	<0.03
9.677	<0.01	9.771	2.563	<0.03
3.461	<0.01	3.487	2.502	<0.03
6.061	<0.01	7.596	2.532	<0.03
7.931	<0.01	8.228	6.606	<0.03
2.744	<0.01	2.254	1.533	<0.03
1.378	<0.01	1.995	1.960	<0.03
1.170	<0.01	1.248	1.662	<0.03

1.581	<0.01	2.332	5.471	<0.03
1.542	<0.01	2.276	7.075	<0.03
2.507	<0.01	2.163	3.476	<0.03

The following information was given in correspondence from Laboratory 5: Fish and guts were analysed separately. On day 1 depuration the contents of the guts were removed and the contents of three fish were analysed (see tables above). The concentration of the chemicals is very high in all samples (except the musk). Unfortunately, Laboratory 5 had to expose the musk at a lower concentration than was recommended in the SOP due to availability of the substance, but hopefully that hasn't affected the study. The temperature of the study was less than that used by Laboratory 4 (i.e. 10±2 °C) and decreased during the study due to the effect of seasonal changes on the reservoir water supply used for the study. A marked increase in growth was not seen during the study, which may have been due to the decrease in temperature but it was noted that the feeding behaviour of the fish was less active in the exposure group of fish. In addition, the feeding rates were adjusted based on the fish which were sampled for analysis and therefore may not have been truly representative of the population. Additional considerations/questions that were noted during the test were:

- Should the water be analysed during the exposure phase to account for water borne exposure?
- Removal of the guts/vs gut content.
- Bulk weighing of the fish at days 0, 7, 14 etc. for calculation of feeding rate.
- Transfer fish to clean tanks after the exposure phase.

Feed and test observations

Feed analysis

Lipid content study start: 11%
Lipid content at end of uptake: [Not reported in spreadsheet.]

Method used for lipid determination

ASE method.

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		31.19	44.50	37.81
Musk xylene		3.74	1.15	1.24
o-Terphenyl		52.03	61.90	63.41
Methoxychlor		65.39	75.95	69.75
B[a]P		155.25	183.50	152.18

Feed quantities (for 3% of body weight) [This table has been left blank in the spreadsheet.]

	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				
	3				
	13				
Depuration	1				
	3				
	7				
	14				
	21				
	28				

Daily observations

No mortality, adverse effects or changes in feeding behaviour were observed in the control group. In the test group reduced feeding compared to the controls was observed on uptake days 9-13 (inclusive) and depuration days 1-8 (inclusive). However, no mortality or adverse effects were observed in the test group.

LABORATORY 6

RING TEST STUDY RESULTS

Pre-study measurements

Dilution water characteristics

Source:	Not reported in spreadsheet.
TOC:	Not reported in spreadsheet.
Total suspended solids:	Not reported in spreadsheet.

Fish species

Rainbow trout (*Oncorhynchus mykiss*)

Fish food

Source/manufacturer:	Ziegler Finfish Starter #1
Crude protein:	57.8%
Crude fat:	15.42%
Crude fibre:	0.67%
Moisture:	8.36%
Ash:	8.99%
Manufacturer reported impurities:	Not reported in the spreadsheet.

Test substances

Chemical	CAS Number	Purity (%)
Hexachlorobenzene (HCB)	118-74-1	
Musk xylene	81-15-2	
o-Terphenyl	84-15-1	
Methoxychlor	72-43-5	
Benzo(a)pyrene (B[a]P)	50-32-8	

[Note: The purity of the test substances is not reported in the spreadsheet.]

Spiked feed preparation

Preparation method: [Not reported in the spreadsheet – assumed to be using a solvent].

Extra details on spiking method: none reported in spreadsheet.

Chemical recoveries pre-study

Chemical	Target concentration (µg/g)	Sample 1 (µg/g or %) pre-study	Sample 2 (µg/g or %) pre-study	Sample 3 (µg/g or %) pre-study	Average (%)
HCB	25	22.9	22.3	21.6	22.3
Musk xylene	50	56.9	55.2	53.9	55.3
o-Terphenyl	50	46.3	45.4	45.4	45.7
Methoxychlor	100	97.1	96.8	96.9	96.9
B[a]P	150	157	157	150	155

Analytical method description

[Not given in the spreadsheet.]

Phys-chem measurements in the study

Temperature

The temperature ranged from 14.2-15.1°C in the test group (average = 14.76 °C) and from 14.1-15.1°C (average = 14.8°C) in the control group throughout the uptake and depuration phases.

Dissolved oxygen

	Day	Test group (mg/L)	Control group (mg/L)
Uptake	0	9.14	9.12
	7	8.54	8.33
	13	9.06	9.1
Depuration	7	8.89	8.87
	15	9.12	8.99
	21	9.05	9.21
	28	9.23	9.19

pH

	Day	Test group	Control group
Uptake	0	7.95	7.98
	7	7.96	8.01

	Day	Test group	Control group
Depuration	13	7.61	7.58
	7	7.51	7.53
	15	7.55	7.56
	21	7.65	7.47
	28	7.72	7.65

Other experimental conditions

Test flow rate:	Not reported in the spreadsheet.
Size of test vessels (approx.):	~31 L
Number volumes replacement per day (approx.):	~5-7

Fish data

Control

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	0.855	4.5	1.55					
	0	0.638	4.4	1.93					
	0	0.77	4.4	1.40					
	0	0.767	4.4	not sampled					
	0	0.555	4	not sampled					
	3	0.919	47.6		nd	nd	nd	nd	nd
	3	0.822	4.2		nd	nd	nd	nd	nd
	3	0.985	4.7		nd	nd	nd	nd	nd
	3	0.868	4.6		nd	nd	nd	nd	nd
	3	1.14	4.9		nd	nd	nd	nd	nd
	13	2.177	6	4.92					
	13	1.383	5.3	4.70					
	13	1.55	5.3	4.77					
	13	1.475	5.4	4.87					
	13	1.361	5.4	4.24					
Depuration	1	1.95	6		nd	0.00637	nd	nd	nd

Test stage				Concentration (µg/g)				
Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
1	1.334	5.2		0.00495	0.02380	nd	nd	nd
1	1.508	5.5		0.00547	0.01780	nd	0.00976	0.03850
1	1.495	5.2		nd	nd	nd	nd	nd
1	1.109	4.9		nd	nd	nd	nd	nd
3	1.694	5.5		nd	0.01380	0.00817	nd	nd
3	1.412	4.8		nd	nd	nd	nd	nd
3	1.807	6		nd	nd	nd	0.00021	nd
3	1.759	5.8		nd	nd	nd	nd	nd
3	1.633	2.5		nd	nd	nd	nd	nd
7	1.781	5.7		nd	nd	nd	nd	nd
7	1.804	5.9		nd	0.00780	0.00465	nd	nd
7	1.542	5.5		nd	nd	nd	nd	nd
7	2.334	6.4		nd	nd	nd	nd	nd
7	2.06	6.3		nd	nd	nd	nd	nd
14	2.059	6.1		0.00501	0.01820	0.01300	nd	nd
14	2.191	6.1		nd	nd	nd	nd	nd
14	2.287	6.3		0.00394	nd	nd	nd	nd
14	2.288	6.1		nd	nd	nd	nd	nd
14	1.969	6		nd	nd	nd	nd	nd
21	3.762	7.1		0.00405	0.00602	nd	nd	nd
21	4.237	7.5		nd	nd	nd	nd	nd

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
	21	3.245	7.1		nd	nd	nd	nd	nd
	21	3.003	7		nd	nd	nd	nd	nd
	21	2.704	6.8		nd	nd	nd	nd	nd
*	28	3.364	7.2	5.69					
*	28	3.827	7.3	6.26					
*	28	4.489	8	6.86					
*	28	3.985	7.4	5.37					
*	28	4.456	7.9	5.91					
*t	28	6.003	8.5		0.00447	0.00482	nd	nd	nd
*t	28	3.957	7.9		0.00537	0.00537	nd	nd	nd
*t	28	5.069	8		0.00876	0.00876	0.00443	nd	nd
*t	28	3.93	7.5		nd	nd	nd	nd	nd
*t	28	3.571	7.5		nd	nd	nd	nd	nd

Note: *t = Fish analysed for test substance.

Test

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	0.855	4.5						
	0	0.638	4.4						

Test stage					Concentration (μg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
	0	0.77	4.4						
	0	0.767	4.4						
	0	0.555	4						
	3	0.896	4.5		1.29	3.32	2.30	1.95	1.21
	3	0.786	4.6		1.14	2.78	1.88	2.39	nd
	3	0.904	4.8		1.23	2.48	1.39	1.17	nd
	3	0.869	4.6		1.18	2.71	1.62	1.49	0.503
	3	0.822	4.6		1.23	2.66	1.84	0.752	1.05
	13	1.1798	5.9	3.76					
	13	2.042	6.1	3.67					
	13	1.411	5.4	3.06					
	13	1.641	6	3.80					
	13	1.928	5.6	4.09					
Depuration	1	1.236	5.1		~~0.0597~~	~~0.177~~	~~0.0599~~	~~0.0359~~	~~nd~~
	1	1.964	6		5.93	5.94	3.07	2.72	1.06
	1	1.407	5.2		5.05	6.01	3.30	2.25	1.38
	1	1.173	5		~~0.0966~~	~~0.253~~	~~0.0841~~	~~0.0434~~	~~0.0721~~
	1	1.055	4.8		4.79	5.26	2.91	1.85	1.09
	3	1.928	5.7		3.79	3.79	2.06	2.17	0.0151
	3	1.897	5.8		4.53	3.95	2.31	1.56	0.0346
	3	1.614	5.4		2.25	2.16	1.32	0.600	0.0594

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
	3	1.788	5.6		2.80	2.56	0.969	0.691	0.0846
	3	1.729	5.5		4.90	4.67	3.07	1.39	nd
	7	1.724	5.7		2.69	2.71	0.736	0.256	nd
	7	1.61	5.5		2.30	1.95	1.39	0.319	nd
	7	1.791	5.7		3.25	2.63	0.782	0.0978	nd
	7	1.624	5.7		2.41	2.46	0.782	0.165	nd
	7	2.43	6.4		2.70	2.44	1.56	0.2440	nd
	14	2.458	6.5		1.45	0.753	0.634	0.0566	nd
	14	3.272	7		2.00	0.958	0.697	0.0744	nd
	14	3.032	7		2.98	1.42	0.389	0.0971	nd
	14	2.728	6.5		1.89	1.29	0.763	0.0489	nd
	14	2.296	6.3		1.80	1.10	0.415	0.190	nd
	21	4.758	7.8		1.44	0.679	0.539	0.00692	nd
	21	2.667	6.7		1.33	0.718	0.547	nd	nd
	21	2.669	6.5		1.01	0.371	0.0467	nd	nd
	21	2.601	6.6		1.11	0.420	0.0637	0.00825	nd
	21	3.372	7		1.02	0.427	0.248	nd	nd
*	28	3.364	7.2	6.29					
*	28	3.827	7.3	5.92					
*	28	4.489	8	6.79					
*	28	3.985	7.4	6.07					

Test stage					Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
*	28	4.456	7.9	5.07					
*t	28	2.841	6.5		0.611	0.205	0.0104	nd	nd
*t	28	3.235	7		1.04	0.399	0.0201	nd	nd
*t	28	3.23	7.1		0.573	0.180	0.0810	nd	nd
*t	28	4.845	8.1		0.852	0.277	0.233	0.0136	nd
*t	28	3.369	7.3		1.15	0.538	0.313	nd	nd

Note: *t = fish analysed for test substance.

[Some of the figures in this table have lines through them or are underlined. They are reported here as they appear in the spreadsheet. The figures with lines through them are assumed to be outliers.]

Control – Average results lipid and concentrations

Test stage			Concentration (µg/g)				
	Day	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxychlor**	**B[a]P**
Uptake	0	1.626666667					
	3		nd	nd	nd	nd	nd
	13	4.7					
Depuration	1		0.0052095	0.01599	nd	0.00976	0.0385
	3		nd	0.0138	0.00817	0.00021	nd
	7		nd	0.0078	0.00465	nd	nd
	14		0.004475	0.0182	0.013	nd	nd
	21		0.00405	0.00602	nd	nd	nd
*	28	6.018					
	28		0.00620	0.00632	0.00443	nd	nd

Test – Average results lipid and concentrations

Test stage			Concentration (µg/g)				
	Day	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxychlor**	**B[a]P**
Uptake	0						
	3		1.214	2.79	1.806	1.5504	0.921
	13	3.676					

Test stage			Concentration (µg/g)				
	Day	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxychlor	B[a]P
Depuration	1		3.18526	3.528	1.8848	1.37986	0.900525
	3		3.654	3.426	1.9458	1.2822	0.048425
	7		2.67	2.438	1.05	0.21636	nd
	14		2.024	1.1042	0.5796	0.0934	nd
	21		1.182	0.523	0.28888	0.007585	nd
*	28	6.028					
	28		0.845	0.320	0.132	0.014	nd

Feed and test observations

Feed analysis

Lipid content	Grab #1	Grab #2	Grab #3	Average (mg/g)
Study start	N/A	N/A	N/A	N/A
End of uptake	N/A	N/A	N/A	N/A

Method used for lipid determination

ASE (hexane) method was used.

Chemical concentrations – End of uptake [Target concentrations not given in the spreadsheet.]

Chemical	Target concentration (µg/g)	Conc. 1 end uptake (µg/g)	Conc. 2 end uptake (µg/g)	Conc. 3 end uptake (µg/g)	Average
HCB		22.2	21.9	21.4	21.83333333
Musk xylene		53.8	53.9	54.4	54.03333333
o-Terphenyl		46.1	45.5	44.5	45.36666667
Methoxychlor		97.6	94.6	97.9	96.7
B[a]P		148	146	150	148

Feed quantities (for 3% 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	0.712	0.712	0.712	0.961
	3	0.855	0.947	0.901	1.18
Depuration	13 1	1.566	1.528	1.547	1.374
	3	1.791	1.661	1.726	1.265
	7	1.836	1.904	1.870	1.117
	14	1.566	1.528	1.547	1.095
	21	3.219	3.390	3.305	0.999
	28	3.577	4.265	3.921	

[Highlighted values appear in cells which have been merged and centred in the spreadsheet.]

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 7

RING TEST STUDY RESULTS

Pre-study measurements

Dilution water characteristics

Source:	Dechlorinated municipal water.
TOC:	0.9 mg/L
Total suspended solids:	Not reported in spreadsheet.
Hardness:	112 mg/L as $CaCO_3$.
Alkalinity:	69 mg/L as $CaCO_3$.
Conductivity:	286 umhos/cm
Total residual chlorine:	<10 µg/L

Fish species

Rainbow trout (*Oncorhynchus mykiss*)

Fish food

Source/manufacturer:	Ziegler Bros. Inc
Crude protein:	min. 55.0%
Crude fat:	min. 18.0%
Crude fibre:	max. 2.0%
Moisture:	Unknown
Ash:	Unknown
Manufacturer reported impurities:	None
Other comments:	Finfish Starter #1 Crumble.

Test substances

Chemical	CAS Number	Purity (%)
Hexachlorobenzene (HCB)	118-74-1	99.5
Musk xylene	81-15-2	99.9
o-Terphenyl	84-15-1	99.0
Methoxychlor	72-43-5	99.5
Benzo(a)pyrene (B[a]P)	50-32-8	95.0

Spiked feed preparation

Preparation method: ii) Solvent

Extra details on spiking method:

1) Five stock solutions were prepared in acetone.
2) 5 mL of each stock was added to 100 g of feed.
3) Feed was then rotary evaporated for ~4 hours.

Chemical recoveries pre-study

Chemical	Target concentration (µg/g)	Sample 1 (µg/g or %) pre-study	Sample 2 (µg/g or %) pre-study	Sample 3 (µg/g or %) pre-study
HCB	25	20.752	20.5	20.248
Musk xylene	50	37.754	38.75	37.496
o-Terphenyl	50	38.004	37.75	36.996
Methoxychlor	100	128.513	119	143.236
B[a]P	150	133.263	132.25	132.487

Analytical method description

Both fish tissue extracts and dietary feed extracts were analyzed using a Hewlett Packard 5890 Series II gas chromatograph using a 30 meter long ZB5MS Guardian capillary column (Phenomenex), 0.25 µm coating, 0.25 mm diameter. The column was attached to the GC using a splitless inlet with glass wool packed deactivated liner. Detection was achieved using Hewlett Packard Mass Selective Detectors (MSD – 5970 and 5972) utilizing electron ionization. Standard curves were constructed as per the validation protocol using high purity stock standards purchased from various sources. All compounds of interest were associated with deuterated internal standards, and calibration was considered acceptable if all compounds achieved an R^2 value of 0.99 or better. Feed sample curves required three calibration points while tissue sample curves used at least six calibration points. A calibration verification standard was injected at least every tenth experimental sample and the calculated concentration was required to be within 20% of the true value. GC/MS data was converted to concentration data using the HP Chemstation software.

Phys-chem measurements in the study

Temperature

The temperature ranged from 11.8-12.3°C (average = 12°C) in the test group and from 11.9-12.4°C (average = 12.06 °C) in the control group throughout the uptake and depuration phases.

Dissolved oxygen

	Day	Test group (% of air saturation)	Control group (% of air saturation)
Uptake	0	71	72

	Day	Test group (% of air saturation)	Control group (% of air saturation)
	7	73	82
Depuration	1	75	75
	8	82	82
	15	60	60
	22	62	62
	28	65	65

pH

	Day	Test group	Control group
Uptake	0	7.6	7.6
	7	7.6	7.6
Depuration	1	7.3	7.3
	8	7.3	7.3
	15	7.5	7.5
	22	7.5	7.5
	28	7.5	7.5

Other experimental conditions

Test flow rate:	600 mL/min
Size of test vessels (approx.):	57 L vessels with 42 L of test solution.
Number volumes replacement per day (approx.):	21

Fish data [Weight and length were not recorded in the same worksheet of the spreadsheet for the control or test group as test chemical concentrations. They were reported on the "growth rates" worksheet, and so have been included here. **NB as a result the lipid contents and chemical concentrations may not tally with the reported weights and lengths**]

Control

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	1.303	51	5.14					
	0	1.137	50	5.41					
	0	0.916	47	5.37					
	0	1.461	54	6.18					
	0	1.196	53	3.92					
	3	1.239	52		< 44.37	< 44.37	< 44.37	< 44.37	< 44.37
	3	1.266	52		< 44.37	< 44.37	< 44.37	< 44.37	< 44.37
	3	1.236	51						
	3	1.152	47						
	3	1.813	59						
	13	1.787	58						
	13	1.834	57						
	13	1.527	53						
	13	1.963	58						
	13	1.704	56						

Test stage					Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Depuration	1	1.862	58	6.46	< 29.83	< 29.83	< 29.83	< 29.83	< 29.83
	1	1.603	58	5.21	< 33.19	< 33.19	< 33.19	< 33.19	< 33.19
	1	1.448	54	5.48					
	1	1.019	48	6.24					
	1	1.168	50	6.66					
	3	1.982	60		< 29.50	< 29.50	< 29.50	< 29.50	< 29.50
	3	1.376	51		< 41.45	< 41.45	< 41.45	< 41.45	< 41.45
	3	1.718	56						
	3	1.792	55						
	3	1.132	48						
	7	1.763	57		< 31.69	< 31.69	< 31.69	< 31.69	< 31.69
	7	1.988	58		< 28.56	< 28.56	< 28.56	< 28.56	< 28.56
	7	2.353	61						
	7	2.02	62						
	7	1.585	55						
	14	3.177	66		< 17.64	< 17.64	< 17.64	< 17.64	< 17.64
	14	2.652	65		< 20.95	< 20.95	< 20.95	< 20.95	< 20.95
	14	3.121	69						
	14	2.309	62						
	14	2.257	59						
	21	2.904	66		< 19.42	< 19.42	< 19.42	< 19.42	< 19.42

	Test stage				Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	21	2.806	62		< 22.29	< 22.29	< 22.29	< 22.29	< 22.29
	21	2.402	62						
	21	3.118	67						
	21	3.612	69						
*	28	3.487	70	8.10	< 15.27	< 15.27	< 15.27	< 15.27	< 15.27
*	28	4.438	75	4.97	< 16.64	< 16.64	< 16.64	< 16.64	< 16.64
*	28	3.499	71	7.11					
*	28	3.286	71	8.89					
*	28	3.959	75	6.06					

Test

	Test stage				Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
Uptake	0	1.303	51						
	0	1.137	50						
	0	0.916	47						
	0	1.461	54						
	0	1.196	53						
	3	1.546	57		0.400	0.398	0.671	1.291	0.63
	3	0.941	50		0.488	0.526	0.814	1.477	<LOD

Test stage					Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	3	0.797	45		0.533	0.499	0.807	1.226	<LOD
	3	1.077	50		0.445	0.509	0.797	1.571	<LOD
	3	1.248	56		0.487	0.465	0.769	1.716	<LOD
	13	1.741	56						
	13	2.093	59						
	13	1.837	55						
	13	1.895	58						
	13	1.402	55						
Depuration	1	2.001	59	4.98	2.521	3.332	3.044	4.957	0.038
	1	2.395	62	6.50	1.908	2.086	2.413	2.532	<LOD
	1	1.753	58	4.58	2.094	2.051	2.663	2.543	<LOD
	1	1.109	48	6.49	2.082	2.038	2.876	2.987	<LOD
	1	1.59	53	5.20	2.068	2.245	2.272	3.073	<LOD
	3	1.488	50		1.849	1.975	2.442	1.561	<LOD
	3	2.011	59		2.822	3.228	3.118	2.595	<LOD
	3	1.636	56		2.167	2.278	2.735	3.900	<LOD
	3	1.529	60		1.911	1.913	1.651	3.352	<LOD
	3	1.623	55		1.261	1.130	1.503	2.891	<LOD
	7	3.015	67		1.740	1.536	1.546	3.541	<LOD
	7	2.621	68		1.826	1.780	1.950	1.960	<LOD
	7	1.752	58		1.005	0.533	1.048	1.229	<LOD

	Test stage				Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (%)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	7	2.78	68		1.309	0.986	1.273	1.167	<LOD
	7	1.399	55		0.506	0.195	0.424	0.458	<LOD
	14	2.668	68		0.718	0.283	0.353	0.236	<LOD
	14	2.704	65		0.998	0.442	0.924	0.589	<LOD
	14	3.069	69		1.145	0.367	0.410	0.849	<LOD
	14	2.249	60		1.135	0.468	0.837	0.654	<LOD
	14	2.52	66		0.707	0.230	0.560	0.711	<LOD
	21	3.729	72		0.748	0.196	0.618	0.194	<LOD
	21	3.577	74		0.584	0.175	0.399	0.149	<LOD
	21	2.647	66		0.801	0.314	0.486	0.437	<LOD
	21	2.787	68		0.940	0.391	0.786	0.109	<LOD
	21	3.473	70		0.712	0.261	0.590	0.217	<LOD
*	28	3.257	69		0.692	0.173	0.521	0.129	<LOD
*	28	3.094	68		0.665	0.183	0.390	0.032	<LOD
*	28	3.376	71		0.636	0.168	0.177	0.038	<LOD
*	28	3.727	74		0.342	0.109	0.226	0.042	<LOD
*	28	5.479	83	6.73	0.455	0.080	0.277	0.082	<LOD

Control – Average results lipid and concentrations

Test stage			Concentration (µg/g)				
	Day	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxychlor**	**B[a]P**
Uptake	0	5.204					
	3		0.47069	0.479372813	<LOD	<LOD	<LOD
	13						
Depuration	1	6.01	<LOD	<LOD	<LOD	<LOD	<LOD
	3		<LOD	<LOD	<LOD	<LOD	<LOD
	7		<LOD	<LOD	<LOD	<LOD	<LOD
	14		<LOD	<LOD	<LOD	<LOD	<LOD
	21		<LOD	<LOD	<LOD	<LOD	<LOD
*	28	7.026	<LOD	<LOD	<LOD	<LOD	<LOD

Test – Average results lipid and concentrations

Test stage			Concentration (µg/g)				
	Day	**Lipid (%)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxychlor**	**B[a]P**
Uptake	0						
	3		0.47	0.48	0.771867765	1.456414815	0.630274496
	13						
Depuration	1	5.55	2.134579408	2.350564191	2.653511076	3.218482017	0.03834671
	3		2.002018566	2.104906016	2.28987881	2.859728961	<LOD
	7		1.277072494	1.005859418	1.248502475	1.670950628	<LOD
	14		0.940537428	0.358012398	0.61692733	0.607905171	<LOD
	21		0.757399974	0.267482329	0.575728148	0.221310462	<LOD
*	28	6.73	0.557999848	0.142621694	0.318267943	0.064456361	<LOD

Feed and test observations

Feed analysis [Units not given in the spreadsheet – assumed to be %.]

Lipid content	Grab #1 (%)	Grab #2 (%)	Grab #3 (%)	Average (%)
Study start	20.99	20.8	20.69	20.83
End of uptake	21.31	21.23	21.55	21.36

Method used for lipid determination

A chloroform/methanol (87:13) mixture was used for extraction.

Chemical concentrations – End of uptake [Target concentrations not given in the spreadsheet.]

Chemical	Target concentration (µg/g)	Concentration 1 end uptake (µg/g)	Concentration 2 end uptake (µg/g)	Concentration 3 end uptake (µg/g)
HCB		22.953	22.963	24.98
Musk xylene		47.153	46.913	48.23
o-Terphenyl		48.4	45.431	45.756
Methoxychlor		206.574	183.947	190.692
B[a]P		138.714	127.405	130.096

Feed quantities (for 3% 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	0.966	0.966	0.966	1.304
	3	1.122	1.341	1.232	1.478
	13	1.122	1.341	1.232	1.478
Depuration	1	1.782	1.592	1.687	1.518
	3	1.657	1.6	1.629	1.222
	7	2.313	1.942	2.128	1.277 / 1.213*
	14	2.642	2.703	2.673	1.203 / 0.882*
	21	3.243	2.968	3.106	0.932 / 0.559*
	28	3.243	2.968	3.106	0.952 / 0.559*

Note: *Control/treated.

Daily observations

No mortality was observed in the test group or the control group. [A dash (-) is given in the corresponding columns in the spreadsheet – this is assumed to mean that no adverse effects were seen].

LABORATORY 8

RING TEST STUDY RESULTS

Pre-study measurements

Dilution water characteristics

Source:	Dechlorinated tap water filtered to 5um.
TOC:	0.54 mg/L
Total suspended solids:	Not reported in spreadsheet.
Hardness:	50-62 mg/L as $CaCO_3$

Fish species

Rainbow trout (*Oncorhynchus mykiss*)

Fish food

Source/manufacturer:	Biomar Inicio plus
Crude protein:	56%
Crude fat:	18%
Crude fibre:	0.5%
Moisture:	N/A
Ash:	11.5%
Manufacturer reported impurities:	Not reported in spreadsheet

Test substances

Chemical	CAS Number	Purity (%)
Hexachlorobenzene (HCB)	118-74-1	99
Musk xylene	81-15-2	98
o-Terphenyl	84-15-1	99
Methoxychlor	72-43-5	>95
Benzo(a)pyrene (B[a]P)	50-32-8	96

Spiked feed preparation

Preparation method: ii) Solvent (hexane prepared as per protocol)

Extra details on spiking method: none given in the spreadsheet.

Chemical recoveries pre-study

Chemical	Target concentration (µg/g)	Sample 1 (µg/g or %) pre-study	Sample 2 (µg/g or %) pre-study	Sample 3 (µg/g or %) pre-study	Average (µg/g or %)
HCB	25	23.0	22.9	23.6	23.2
Musk xylene	50	45.8	44.9	46.6	45.8
o-Terphenyl	50	48.6	46.6	48.7	47.9
Methoxychlor	100	95.4	94.6	100.4	96.8
B[a]P	150	137.2	138.1	143.3	139.5

Analytical method description

The *Diet extraction procedure* was followed (see *Feed analysis result*s section in Annex 1 for full description), with the following small differences:

1) 1.000 g of spiked diet was weighed out (rather than 0.500 g).
2) Sonicate for 30 minutes and shake for 2 minutes (carried out three times).
3) Centrifuge for 30 minutes at 4,000 g at 10°C (rather than 40,000 g at 5°C).

The *Recovery test of diet sample* was carried out (see *Feed analysis results* section in Annex 1 for full description).

Phys-chem measurements in the study

Temperature

The temperature ranged from 14.9-15.4°C (average = 15.0°C) in both the test group and the control group throughout the uptake and depuration phases.

Dissolved oxygen

	Day	Test group (mg/L)	Control group (mg/L)
Uptake	0	8.0	8.2
	7	8.2	8.2
	13	8.0	7.6
Depuration	7	8.0	7.8
	14	8.4	8.4
	21	8.2	8.2
	28	8.4	8.2

pH

	Day	Test group	Control group
Uptake	0	7.0	7.0
	7	6.9	6.9
	13	6.8	6.8
Depuration	7	6.9	6.9
	14	7.2	7.3
	21	6.9	6.9
	28	7.1	7.1

Flows

	Day	Test group (mL/min)	Control group (mL/min)
Uptake	0	225	225
	7	230	230
	13	240	230
Depuration	7	230	230
	14	240	230
	21	230	230
	28	240	240

Other experimental conditions

Test flow rate:	234 mL/min
Size of test vessels (approx.):	50 L
Number volumes replacement per day (approx.):	6.7
Light levels:	250 lux over control tank and 300 lux over test tank.

Fish data

Control

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (w/w)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	0	1.3258	42	0.04272					
	0	1.3526	42	0.03600					
	0	1.1685	42	0.03113					
	0	1.1202	42	0.03522					
	0	1.0707	40	0.03443					
	3	1.59	48		-	-	-	-	-
	3	1.92	46		-	-	-	-	-
	3	1.84	46		-	-	-	-	-
	3	1.64	42		<0.10	<0.25	<0.25	<0.10	<0.10
	3	1.60	46		<0.10	<0.25	<0.25	<0.10	<0.10
	13	1.3722	43	0.04177					
	13	1.6814	47	0.06011					
	13	1.5887	47	0.05643					
	13	2.0965	50	0.07430					
	13	2.5237	53	0.05892					
Depuration	1	3.12	55		<0.010	<0.010	<0.050	<0.050	<0.050

Test stage				Concentration (µg/g)				
Day	Weight (g)	Total length (mm)	Lipid (w/w)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
1	2.35	50		<0.010	<0.010	<0.050	<0.050	<0.050
1	2.34	51		-	-	-	-	-
1	2.40	51		-	-	-	-	-
1	2.46	51		-	-	-	-	-
3	2.39	52		<0.050	<0.25	0.045#	<0.25	<0.10
3	3.48	60		<0.050	<0.25	<0.10	<0.25	0.67#
3	2.73	53		<0.050	<0.10	<0.050	<0.25	<0.10
3	2.16	50		<0.050	<0.10	<0.050	<0.25	<0.10
3	2.74	54		-	-	-	-	-
7	3.92	62		<0.050	<0.25	<0.050	<0.25	<0.10
7	3.86	61		<0.050	<0.25	<0.050	<0.25	<0.10
7	2.65	53		-	-	-	-	-
7	4.54	63		-	-	-	-	-
7	2.06	50		-	-	-	-	-
14	4.88	64		<0.050	<0.050	<0.050	<0.050	<0.050
14	4.93	63		-	-	-	-	-
14	6.36	72		<0.050	<0.050	<0.050	<0.050	<0.050
14	5.49	69		-	-	-	-	-
14	4.21	62		-	-	-	-	-
21	5.59	69		-	-	-	-	-
21	7.35	76		-	-	-	-	-

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (w/w)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
	21	6.78	74		-	-	-	-	-
	21	7.25	73		<0.050	<0.10	<0.050	<0.25	<0.10
	21	6.63	72		<0.050	<0.10	<0.050	<0.25	<0.10
	28	16.74	100		-	-	-	-	-
	28	9.90	80		-	-	-	-	-
	28	9.5511	83	0.10638	-	-	-	-	-
	28	10.15	82		<0.25	<0.25	<0.10	<0.50	<0.10
	28	8.60	82		<0.25	<0.25	<0.10	<0.50	<0.10
*	28	10.8578	84	0.11903					
*	28	8.6480	82	0.08866					
*	28	10.9750	89	0.09997					
*	28	4.2212	60	0.07615					
*	28								

Note: #Contamination further 2 samples analysed.

Further samples taken to investigate uptake of compounds from the gut to fish tissue

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (w/w)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	3	0.276			<0.10	<0.25	<0.25	<0.10	<0.10
		1.17			<0.10	<0.25	<0.25	<0.10	<0.10

Test stage					Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (w/w)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
		0.261			<0.10	<0.25	<0.25	<0.10	<0.10
		1.04			<0.10	<0.25	<0.25	<0.10	<0.10
Depuration	1	0.534			<0.010	<0.010	<0.050	<0.050	<0.050
		2.14			<0.010	<0.010	<0.050	<0.050	<0.050
		0.408			<0.010	<0.010	<0.050	<0.050	<0.050
		1.95			<0.010	<0.010	<0.050	<0.050	<0.050

Test

Test stage					Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (w/w)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
Uptake	0	1.14	39						
	0	1.61	43						
	0	1.19	43						
	0	1.10	40						
	0	1.29	40						
	3	1.38	46		1.28	2.41	1.99	1.86	0.36
	3	1.23	42		1.51	2.77	2.06	1.77	0.48
	3	1.00	40		1.22	2.56	1.83	1.01	0.50
	3	1.55	45		1.33	2.28	1.89	1.07	0.49
	3	1.74	47		1.70	2.80	2.54	2.61	0.45

	Test stage				Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (w/w)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
	13	2.8508	55	0.06752					
	13	2.0569	50	0.04544					
	13	2.4901	50	0.07561					
	13	2.1563	50	0.05931					
	13	2.0109	49	0.05008					
Depuration	1	3.45	56		3.41	5.68	3.71	3.23	1.11
	1	2.58	52		2.95	4.62	4.46	4.02	0.63
	1	2.13	50		2.31	3.62	1.88	1.71	0.28
	1	2.74	54		2.97	5.60	2.99	1.61	0.59
	1	2.34	52		2.90	5.68	4.87	6.37	0.35
	3	2.70	55		4.11	4.60	5.13	3.08	0.05
	3	2.62	54		3.31	4.09	3.76	1.75	0.05
	3	2.52	52		3.88	4.69	3.39	4.54	0.05
	3	2.67	55		3.53	3.87	3.55	3.79	0.05
	3	2.55	51		3.46	5.20	2.13	1.34	0.05
	7*	3.35	60						
	7	3.78	61		3.33	3.59	2.83	2.37	0.05
	7	3.22	57		3.11	3.44	3.02	2.75	0.05
	7	4.30	63		2.77	2.97	1.81	0.85	0.05
	7	2.95	54		3.33	3.33	2.10	3.17	0.72#
	14	4.22	63		1.83	1.98	0.81	0.44	0.03

	Test stage				Concentration (µg/g)				
	Day	Weight (g)	Total length (mm)	Lipid (w/w)	HCB	Musk xylene	o-Terphenyl	Methoxy chlor	B[a]P
	14	5.44	69		2.08	2.09	2.27	0.94	0.03
	14	4.22	63		1.55	1.23	0.83	0.36	0.03
	14	3.82	62		1.19	1.26	0.34	0.03	0.03
	14	5.08	68		1.13	0.84	0.31	0.03	0.22#
	21	6.34	72		1.24	0.95	1.12	0.44	0.05
	21	6.01	72		1.37	1.09	0.26	0.125	0.05
	21	6.77	74		1.04	0.69	0.40	0.125	0.05
	21	7.42	75		1.16	0.92	0.79	0.125	0.05
	21	4.13	64		1.09	0.86	0.31	0.125	0.05
	28	9.04	79		0.81	0.44	0.60	0.59	0.05
	28	9.72	82		0.71	0.33	0.050	0.250	0.05
	28	8.97	80		0.79	0.47	0.47	0.250	0.05
	28	9.15	85		0.68	0.42	0.21	0.250	0.05
	28	8.10	77		0.65	0.30	0.050	0.250	0.05
*	28	7.5522	75	0.11290					
*	28	9.9948	83	0.09017					
*	28	8.0455	74	0.12739					
*	28	7.9703	79	0.09367					
*	28	7.1631	75	0.09469					

Notes: #Outliers not used.
*Sample lost.

Further samples taken to investigate uptake of compounds from the gut to fish tissue

Test stage					Concentration (µg/g)				
	Day	**Weight (g)**	**Total length (mm)**	**Lipid (w/w)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxy chlor**	**B[a]P**
Uptake	3	0.25			0.00	9.19	6.19	5.34	16.64
		1.65			1.21	2.18	1.66	1.72	0.29
		0.287			0.00	8.21	5.82	3.22	1.66
		1.36			1.23	2.43	1.92	1.01	0.35
Depuration	1	0.514			7.259	14.678	10.131	7.989	4.255
		2.16			2.046	3.763	2.558	1.269	<0.050
		0.342			6.551	14.525	9.798	17.75	<0.050
		1.6			2.09	4.166	2.953	4.644	<0.050

Control – Average results lipid and concentrations

Test stage			Concentration (µg/g)				
	Day	**Lipid (w/w)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxychlor**	**B[a]P**
Uptake	0	0.03590					
	3		<LOD	<LOD	<LOD	<LOD	<LOD
	13	0.05831					
Depuration	1		<LOD	<LOD	<LOD	<LOD	<LOD
	3		0.05	0.25	<LOD	0.25	0.1
	7		0.05	<LOD	<LOD	<LOD	0.1
	14		0.05	0.05	0.05	0.05	0.05
	21		<LOD	<LOD	<LOD	0.25	0.1
	28		<LOD	<LOD	<LOD	<LOD	<LOD
*	28	0.09804					

Test – Average results lipid and concentrations

Test stage			Concentration (µg/g)				
	Day	**Lipid (w/w)**	**HCB**	**Musk xylene**	**o-Terphenyl**	**Methoxychlor**	**B[a]P**
Uptake	0						
	3		1.409957575	2.56	2.060576661	1.664033382	0.456027655

Test stage			Concentration (µg/g)				
	Day	Lipid (w/w)	HCB	Musk xylene	o-Terphenyl	Methoxychlor	B[a]P
	13	0.05959					
Depuration	1		2.909117357	5.040833497	3.584742981	3.388218807	0.593333845
	3		3.657653008	4.490054682	3.588769019	2.898962346	0.05
	7		3.13449826	3.330753073	2.441148544	2.283100748	0.05
	14		1.55658626	1.481063646	0.912063618	0.35920264	0.03
	21		1.178379396	0.901560506	0.576723274	0.187253519	0.05
	28		0.729167788	0.391709606	0.276204455	0.317623368	0.05
*	28	0.10376					

[Note given in spreadsheet: Where analysis indicates levels at LOQ a value of half the LOQ has been used to calculate mean values.]

Feed and test observations

Feed analysis [No units given in the spreadsheet – assumed to be fraction (w/w).]

Lipid content	Grab #1 (w/w)	Grab #2 (w/w)	Grab #3 (w/w)	Average (w/w)
Clean food (study start)	0.128	0.172	0.177	0.159
Solvent control food (study start)	0.178	0.184	0.154	0.172
Dosed food (study start)	0.177	0.179	0.179	0.178
Clean food (end of uptake)	0.185	0.151	0.177	0.171
Solvent control (end of uptake)	0.162	0.177	0.133	0.157
Dosed food (end of uptake)	0.187	0.177	0.168	0.177

Method used for lipid determination

ASE method was used.

Chemical concentrations – End of uptake

Chemical	Target concentration (µg/g)	Conc. 1 end uptake (µg/g)	Conc. 2 end uptake (µg/g)	Conc. 3 end uptake (µg/g)	Average
HCB	25	25.2	21.9	22.4	23.2
Musk xylene	50	45.5	46.0	41.1	44.2
o-Terphenyl	50	47.3	46.0	45.2	46.2
Methoxychlor	100	89.0	102.9	84.8	92.2
B[a]P	150	137.1	135.5	129.3	134.0

Chemical recoveries – Pre-study

Chemical	Target concentration (µg/g)	Sample 1 (µg/g or %) pre-study	Sample 2 (µg/g or %) pre-study	Sample 3 (µg/g or %) pre-study	Average
HCB	25	23.0	22.9	23.6	23.2
Musk xylene	50	45.8	44.9	46.6	45.8
o-Terphenyl	50	48.6	46.6	48.7	47.9
Methoxychlor	100	95.4	94.6	100.4	96.8

B[a]P	150	137.2	138.1	143.3	139.5

Feed quantities (for 3% 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	1.24	1.24		3% wet wt for each treatment
	3	1.38	1.72		3% wet wt for each treatment
	13	2.37	1.95		3% wet wt for each treatment
Depuration	1	2.65	2.53		3% wet wt for each treatment
	3	2.61	2.70		3% wet wt for each treatment
	7	3.52	3.41		3% wet wt for each treatment
	14	4.56	5.17		3% wet wt for each treatment
	21	6.13	6.72		3% wet wt for each treatment
	28	9.00	11.05		3% wet wt for each treatment

Notes: Average mass of test and control fish column has been left blank in the spreadsheet.
*Food adjusted to 3% at each sampling point based on the weight of sampled fish.

Daily observations

Feeding behaviour was normal in both the test group and the control group throughout the uptake and depuration phases.

In the control group, two dark discoloured fish were seen on days 8, 9 and 10 of the uptake phase. In the test group, one dark discoloured fish was seen on depuration days 8, 9, 10, 14, 15, 16, 17, 21, 22, 23. On depuration day 23 the dark discoloured fish was removed and culled.

Cumulative mortality is summarised in the table below.

		Cumulative mortality	
Phase	**Day**	**Test group**	**Control group**
Uptake	0	0	0
	1	0	0
	2	0	0
	3	0	1

Phase	Day	Cumulative mortality	
		Test group	Control group
	4	0	1
	5	1*	1
	6	1	1
	7	1	1
	8	2*	3*
	9	2	3
	10	2	3
	11	2	3
	12	2	3
	13	2	3
Depuration	1	2	3
	2	2	3
	3	2	3
	4	2	3
	5	2	3
	6	2	3
	7	2	3
	8	2	3
	9	2	3
	10	2	3
	11	2	3
	12	2	3
	13	2	3
	14	2	3
	15	2	3
	16	2	3
	17	2	3
	18	2	3
	19	2	3
	20	2	3
	21	2	3
	22	2	3
	23	3	3
	24	3	3
	25	3	3

		Cumulative mortality	
Phase	**Day**	**Test group**	**Control group**
	26	3	3
	27	3	3
	28	3	3

Note: * Fish culled due to damage while siphoning/bullying.

ANNEX 3 – ESTIMATES FOR THE GROWTH CORRECTED BCF FOR THE SUBSTANCES USED IN THE RING TEST

Table 64 Summary of estimated growth corrected BCF from the ring test using Method 1

Lab	**Fish weight (g)**		**Estimated k_1 (l kg day^{-1})**		**Estimated BCF (growth corrected)**									
	a	**b**	**a**	**b**	**HCB**		**MX**		**oTP**		**MC**		**BaP**	
					a	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
Lab 1	1.25	2.1	484	410	35,600	30,155	8,999	7,623	9,568	8,105	4,270	3,616	510	432
Lab 2a – trout	8.41	9.78	263	251	11,242	10,712	4,623	4,405	5,001	4,766	2,644	2,519	127	121
Lab 2b – carp (level 1)	5.42	7.11	303	278	11,256	10,319	2,840	2,604	1,180	1,082	1,095	1,004		
Lab 2b – carp (level 2)	5.42	7.11	303	278	13,338	12,229	3,102	2,844	953	874	1,162	1,065		
Lab 2b – carp (level 3)	5.42	7.11	303	278	19,920	18,263	3,902	3,577	1,149	1,053	1,313	1,204		
Lab 3	1.95	3.64	420	344	43,293	35,455	10,768	8,818	6,999	5,732	7,240	5,930		
Lab 4	1.17	2.32	495	397	37,750	30,323	17,413	13,987	12,878	10,345	9,226	7,411		
Lab 5	6.77	7.84	282	269	11,324	10,805	447	426	9,825	9,374	-25,177	-24,022	138	131
Lab 6	0.72	1.61	578	446	22,389	17,306	8,457	6,537	5,998	4,637	3,068	2,371	351	271
Lab 7	1.2	1.78	491	432	26,659	23,499	6,602	5,819	10,481	9,239	4,182	3,686		
Lab 8	1.24	2.08	485	411	50,564	42,850	10,439	8,846	7,502	6,358	7,867	6,667	429	364
Mean BCF all data					25,758	21,992	7,054	5,953	6,503	5,597	1,535	1,041	311	264
Standard deviation					14,119	11,289	4,810	3,791	4,104	3,492	9,294	8,613	173	138

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Mean BCF for trout (minus Lab 5 data)					32,500	27,186	9,614	8,005	8,347	7,026	5,500	4,600	354	297
Standard deviation					13,343	10,916	4,052	3,087	2,763	2,237	2,577	2,043	165	135
Mean BCF for carp					14,838	13,604	3,281	3,008	1,094	1,003	1,190	1,091		
Standard deviation					4,522	4,146	553	507	123	113	112	103		

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/day 14).

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

Table 65 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 (growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Lab 1	1.25	2.1	c	c	27,742	24,368	6,422	5,641	7,383	6,485	3,153	2,769	401	352
Lab 2a – trout	8.41	9.78	c	c	10,011	9,641	3,770	3,631	4,410	4,247	2,231	2,148	114	110
Lab 2b – carp (level 1)	5.42	7.11	c	c	9,720	9,082	2,246	2,099	1,009	943	896	837		
Lab 2b – carp (level 2)	5.42	7.11	c	c	11,518	10,763	2,453	2,292	815	762	951	888		
Lab 2b – carp (level 3)	5.42	7.11	c	c	17,202	16,073	3,085	2,883	982	918	1,074	1,004		
Lab 3	1.95	3.64	c	c	34,804	29,776	7,927	6,782	5,571	4,766	5,515	4,718		
Lab 4	1.17	2.32	c	c	29,282	24,676	12,368	10,423	9,891	8,335	6,781	5,714		
Lab 5	6.77	7.84	c	c	9,933	9,575	359	346	8,533	8,225	-20,924	-20,170	122	117
Lab 6	0.72	1.61	c	c	16,786	13,727	5,807	4,748	4,453	3,642	2,179	1,782	265	217
Lab 7	1.2	1.78	c	c	20,716	18,771	4,698	4,257	8,065	7,307	3,079	2,790		
Lab 8	1.24	2.08	c	c	39,381	34,604	7,445	6,542	5,786	5,084	5,806	5,102	338	297
Mean BCF all data					20,645	18,278	5,144	4,513	5,173	4,610	976	689	248	219
Standard deviation					10,648	8,910	3,349	2,777	3,199	2,845	7,546	7,129	128	107

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Mean BCF for trout (minus Lab 5 data)					25,532	22,223	6,920	6,003	6,508	5,695	4,106	3,575	280	244
Standard deviation					10,306	8,788	2,808	2,266	2,027	1,720	1,881	1,567	124	105
Mean BCF for carp					12,813	11,973	2,595	2,425	935	874	974	910		
Standard deviation					3,905	3,649	437	409	105	98	92	86		

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/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, 214 to 410 l kg^{-1} for Methoxychlor and 228 to 437 l kg^{-1} day^{-1} for benzo[a]pyrene.

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

Table 66 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 (growth corrected)**									
	a	**b**	**a**	**b**	**HCB**		**MX**		**oTP**		**MC**		**BaP**	
					a	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
Lab 1	1.25	2.1	757	665	55,632	48,865	14,063	12,352	14,952	13,134	6,672	5,860	797	700
Lab 2a – trout	8.41	9.78	470	452	20,076	19,333	8,256	7,950	8,931	8,600	4,721	4,547	226	218
Lab 2b – carp (level 1)	5.42	7.11	524	490	19,491	18,213	4,919	4,596	2,043	1,909	1,896	1,771		
Lab 2b – carp (level 2)	5.42	7.11	524	490	23,097	21,582	5,372	5,020	1,651	1,543	2,012	1,880		
Lab 2b – carp (level 3)	5.42	7.11	524	490	34,494	32,231	6,757	6,313	1,989	1,859	2,274	2,125		
Lab 3	1.95	3.64	677	579	69,793	59,709	17,359	14,851	11,283	9,653	11,672	9,986		
Lab 4	1.17	2.32	769	648	58,718	49,482	27,085	22,824	20,031	16,881	14,351	12,094		
Lab 5	6.77	7.84	496	478	19,918	19,200	786	757	17,281	16,658	-44,282	-42,687	242	233
Lab 6	0.72	1.61	868	710	33,662	27,527	12,716	10,398	9,018	7,375	4,612	3,772	527	431
Lab 7	1.2	1.78	764	693	41,541	37,642	10,287	9,322	16,332	14,799	6,516	5,905		
Lab 8	1.24	2.08	758	666	78,970	69,391	16,304	14,326	11,717	10,296	12,287	10,797	670	589
Mean BCF all data					41,399	36,652	11,264	9,883	10,475	9,337	2,066	1,459	493	434
Standard deviation							7,334	6,082	6,478	5,762	15,970	15,088	255	213
					21,352	17,868								

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Mean BCF for trout (minus Lab 5 data)					51,199	44,564	15,153	13,146	13,181	11,534	8,690	7,566	555	485
Standard deviation					20,667	17,622	6,150	4,963	4,105	3,483	3,980	3,316	245	209
Mean BCF for carp					25,694	24,009	5,682	5,310	1,894	1,770	2,060	1,925		
Standard deviation					7,831	7,318	958	895	213	199	194	181		

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/day 14).

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

Table 67 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 (growth corrected)**									
	a	**b**	**a**	**b**	**HCB**		**MX**		**oTP**		**MC**		**BaP**	
					a	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
Lab 1	1.25	2.1	669	615	49,156	45,217	12,426	11,430	13,212	12,153	5,895	5,423	704	648
Lab 2a – trout	8.41	9.78	492	480	21,019	20,514	8,644	8,437	9,351	9,126	4,943	4,825	237	231
Lab 2b – carp (level 1)	5.42	7.11	528	505	19,624	18,785	4,952	4,740	2,057	1,969	1,909	1,827		
Lab 2b – carp (level 2)	5.42	7.11	528	505	23,255	22,261	5,409	5,177	1,662	1,591	2,026	1,939		
Lab 2b – carp (level 3)	5.42	7.11	528	505	34,730	33,245	6,803	6,512	2,003	1,917	2,289	2,191		
Lab 3	1.95	3.64	622	563	64,158	58,024	15,957	14,432	10,372	9,381	10,730	9,704		
Lab 4	1.17	2.32	676	605	51,579	46,196	23,792	21,309	17,596	15,760	12,606	11,290		
Lab 5	6.77	7.84	509	497	20,455	19,977	807	788	17,747	17,332	-45,475	-44,414	248	243
Lab 6	0.72	1.61	731	642	28,318	24,877	10,697	9,397	7,587	6,665	3,880	3,409	444	390
Lab 7	1.2	1.78	673	632	36,572	34,323	9,057	8,500	14,379	13,494	5,737	5,384		
Lab 8	1.24	2.08	669	616	69,728	64,157	14,395	13,245	10,346	9,519	10,849	9,982	592	545
Mean BCF all data					38,054	35,234	10,267	9,452	9,665	8,992	1,399	1,051	445	411
Standard deviation					18,048	16,050	6,282	5,566	5,908	5,528	15,995	15,450	207	184

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Mean BCF for trout (minus Lab 5 data)					45,790	41,901	13,567	12,393	11,835	10,871	7,806	7,145	494	453
Standard deviation					18,070	16,275	5,245	4,566	3,419	3,088	3,473	3,088	202	182
Mean BCF for carp					25,870	24,764	5,721	5,477	1,907	1,826	2,074	1,986		
Standard deviation					7,885	7,548	964	923	214	205	195	187		

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/day 14).

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

Table 68 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 (growth corrected)**									
	a	**b**	**a**	**b**	**HCB**		**MX**		**oTP**		**MC**		**BaP**	
					a	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
Lab 1	1.25	2.1	654	595	48,054	43,724	12,147	11,053	12,916	11,752	5,763	5,244	688	626
Lab 2a – trout	8.41	9.78	462	449	19,741	19,206	8,119	7,899	8,782	8,544	4,643	4,517	222	216
Lab 2b – carp (level 1)	5.42	7.11	500	476	18,602	17,706	4,694	4,468	1,950	1,856	1,809	1,722		
Lab 2b – carp (level 2)	5.42	7.11	500	476	22,044	20,982	5,127	4,880	1,576	1,500	1,920	1,828		
Lab 2b – carp (level 3)	5.42	7.11	500	476	32,921	31,334	6,448	6,138	1,898	1,807	2,170	2,065		
Lab 3	1.95	3.64	603	538	62,136	55,464	15,454	13,795	10,045	8,967	10,392	9,276		
Lab 4	1.17	2.32	661	584	50,492	44,577	23,290	20,562	17,225	15,207	12,340	10,895		
Lab 5	6.77	7.84	481	468	19,299	18,790	761	741	16,744	16,303	-42,906	-41,775	234	228
Lab 6	0.72	1.61	723	624	28,006	24,190	10,579	9,138	7,503	6,481	3,837	3,314	439	379
Lab 7	1.2	1.78	658	613	35,783	33,305	8,861	8,248	14,068	13,094	5,613	5,224		
Lab 8	1.24	2.08	654	596	68,176	62,050	14,075	12,810	10,116	9,207	10,608	9,655	579	527
Mean BCF all data					36,841	33,757	9,960	9,066	9,348	8,611	1,472	1,088	433	395
Standard deviation					17,833	15,628	6,186	5,395	5,720	5,293	15,174	14,584	206	181

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Mean BCF for trout (minus Lab 5 data)					44,627	40,360	13,218	11,929	11,522	10,465	7,599	6,875	482	437
Standard deviation					17,706	15,740	5,169	4,419	3,385	3,012	3,404	2,980	201	179
Mean BCF for carp					24,522	23,340	5,423	5,162	1,808	1,721	1,966	1,872		
Standard deviation					7,474	7,114	914	870	203	193	185	176		

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/day 14).

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

Table 69 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 (growth corrected)**									
	a	**b**	**a**	**b**	**HCB**		**MX**		**oTP**		**MC**		**BaP**	
					a	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
Lab 1	1.25	2.1	649	599	47,746	44,011	12,070	11,126	12,833	11,829	5,726	5,278	684	630
Lab 2a – trout	8.41	9.78	481	470	20,572	20,091	8,460	8,262	9,152	8,938	4,838	4,725	232	226
Lab 2b – carp (level 1)	5.42	7.11	516	494	19,173	18,374	4,838	4,637	2,010	1,926	1,865	1,787		
Lab 2b – carp (level 2)	5.42	7.11	516	494	22,721	21,773	5,284	5,064	1,624	1,556	1,979	1,897		
Lab 2b – carp (level 3)	5.42	7.11	516	494	33,932	32,517	6,646	6,369	1,957	1,875	2,237	2,143		
Lab 3	1.95	3.64	606	549	62,429	56,601	15,527	14,078	10,093	9,151	10,441	9,466		
Lab 4	1.17	2.32	656	589	50,086	44,982	23,103	20,749	17,087	15,345	12,241	10,994		
Lab 5	6.77	7.84	498	487	20,003	19,547	789	771	17,354	16,959	-44,470	-43,458	243	237
Lab 6	0.72	1.61	708	624	27,445	24,188	10,367	9,137	7,353	6,480	3,760	3,314	430	379
Lab 7	1.2	1.78	654	614	35,517	33,385	8,796	8,268	13,964	13,126	5,571	5,237		
Lab 8	1.24	2.08	650	599	67,726	62,443	13,982	12,891	10,049	9,265	10,538	9,716	575	530
Mean BCF all data					37,032	34,356	9,988	9,214	9,407	8,768	1,339	1,009	433	401
Standard deviation					17,495	15,602	6,093	5,415	5,750	5,392	15,626	15,107	200	178

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Mean BCF for trout (minus Lab 5 data)					44,503	40,815	13,186	12,073	11,504	10,591	7,588	6,961	480	441
Standard deviation					17,544	15,841	5,087	4,444	3,313	3,001	3,372	3,006	196	177
Mean BCF for carp					25,276	24,221	5,590	5,357	1,864	1,786	2,027	1,942		
Standard deviation					7,704	7,382	942	903	209	200	191	183		

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/day 14).

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

Table 70 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 (growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Lab 1	1.25	2.1	541	481	39,807	35,367	10,063	8,940	10,699	9,506	4,774	4,241	570	507
Lab 2a – trout	8.41	9.78	351	339	14,981	14,474	6,161	5,952	6,664	6,439	3,523	3,404	169	163
Lab 2b – carp (level 1)	5.42	7.11	387	364	14,404	13,540	3,635	3,417	1,510	1,419	1,401	1,317		
Lab 2b – carp (level 2)	5.42	7.11	387	364	17,069	16,045	3,970	3,732	1,220	1,147	1,487	1,398		
Lab 2b – carp (level 3)	5.42	7.11	387	364	25,492	23,962	4,993	4,694	1,470	1,382	1,680	1,579		
Lab 3	1.95	3.64	489	424	50,431	43,742	12,543	10,879	8,153	7,072	8,434	7,315		
Lab 4	1.17	2.32	550	470	41,955	35,892	19,352	16,556	14,313	12,244	10,254	8,772		
Lab 5	6.77	7.84	368	356	14,792	14,305	584	564	12,833	12,411	-32,886	-31,804	180	174
Lab 6	0.72	1.61	614	511	23,796	19,807	8,989	7,482	6,375	5,307	3,260	2,714	373	310
Lab 7	1.2	1.78	546	499	29,698	27,145	7,355	6,722	11,676	10,672	4,659	4,258		
Lab 8	1.24	2.08	542	482	56,497	50,212	11,664	10,366	8,383	7,450	8,790	7,813	480	426
Mean BCF all data					29,902	26,772	8,119	7,210	7,572	6,823	1,398	1,001	354	316
Standard deviation					15,115	12,837	5,207	4,389	4,662	4,202	11,782	11,195	179	152

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Mean BCF for trout (minus Lab 5 data)					36,738	32,377	10,875	9,557	9,466	8,384	6,242	5,502	398	352
Standard deviation					14,748	12,744	4,361	3,585	2,892	2,491	2,838	2,403	173	149
Mean BCF for carp					18,989	17,849	4,199	3,947	1,400	1,316	1,523	1,431		
Standard deviation					5,788	5,440	708	665	157	148	143	135		

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/day 14).

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

Table 71 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 (growth corrected)**									
	a	**b**	**a**	**b**	**HCB**		**MX**		**oTP**		**MC**		**BaP**	
					a	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
Lab 1	1.25	2.1	482	436	35,453	32,025	8,962	8,095	9,529	8,607	4,252	3,841	508	459
Lab 2a – trout	8.41	9.78	332	322	14,181	13,768	5,832	5,662	6,309	6,125	3,335	3,238	160	155
Lab 2b – carp (level 1)	5.42	7.11	362	343	13,445	12,749	3,393	3,217	1,409	1,336	1,308	1,240		
Lab 2b – carp (level 2)	5.42	7.11	362	343	15,933	15,107	3,706	3,514	1,139	1,080	1,388	1,316		
Lab 2b – carp (level 3)	5.42	7.11	362	343	23,794	22,562	4,661	4,419	1,372	1,301	1,568	1,487		
Lab 3	1.95	3.64	442	391	45,558	40,312	11,331	10,026	7,365	6,517	7,619	6,742		
Lab 4	1.17	2.32	488	427	37,286	32,604	17,199	15,039	12,720	11,123	9,113	7,969		
Lab 5	6.77	7.84	346	336	13,905	13,511	549	533	12,064	11,722	-30,915	-30,038	169	164
Lab 6	0.72	1.61	537	459	20,822	17,784	7,865	6,718	5,578	4,764	2,853	2,437	326	279
Lab 7	1.2	1.78	486	450	26,415	24,450	6,541	6,055	10,385	9,613	4,143	3,835		
Lab 8	1.24	2.08	483	436	50,304	45,454	10,385	9,384	7,464	6,744	7,827	7,072	427	386
Mean BCF all data					27,009	24,575	7,311	6,606	6,849	6,267	1,136	831	318	288
Standard deviation					13,249	11,500	4,586	3,958	4,196	3,853	10,975	10,512	154	134

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Mean BCF for trout (minus Lab 5 data)					32,860	29,485	9,731	8,711	8,479	7,642	5,592	5,019	355	320
Standard deviation					13,084	11,530	3,834	3,239	2,520	2,221	2,516	2,180	150	132
Mean BCF for carp					17,724	16,806	3,920	3,717	1,307	1,239	1,421	1,348		
Standard deviation					5,402	5,122	661	626	147	139	134	127		

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/day 14).

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

Table 72 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 (growth corrected)**									
	a	**b**	**a**	**b**	**HCB**		**MX**		**oTP**		**MC**		**BaP**	
					a	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
Lab 1	1.25	2.1	394	363	28,953	26,674	7,319	6,743	7,782	7,169	3,472	3,199	415	382
Lab 2a – trout	8.41	9.78	291	284	12,451	12,158	5,121	5,000	5,539	5,409	2,928	2,859	140	137
Lab 2b – carp (level 1)	5.42	7.11	312	299	11,610	11,122	2,930	2,807	1,217	1,166	1,129	1,082		
Lab 2b – carp (level 2)	5.42	7.11	312	299	13,758	13,180	3,200	3,065	983	942	1,198	1,148		
Lab 2b – carp (level 3)	5.42	7.11	312	299	20,546	19,684	4,024	3,856	1,185	1,135	1,354	1,297		
Lab 3	1.95	3.64	367	333	37,840	34,286	9,411	8,528	6,117	5,543	6,328	5,734		
Lab 4	1.17	2.32	398	357	30,374	27,260	14,010	12,574	10,362	9,300	7,423	6,662		
Lab 5	6.77	7.84	302	295	12,109	11,832	478	467	10,506	10,265	-26,921	-26,304	147	144
Lab 6	0.72	1.61	430	378	16,652	14,664	6,290	5,539	4,461	3,929	2,282	2,009	261	230
Lab 7	1.2	1.78	396	372	21,539	20,238	5,334	5,012	8,468	7,957	3,379	3,175		
Lab 8	1.24	2.08	394	363	41,069	37,846	8,479	7,813	6,094	5,615	6,390	5,889	349	321
Mean BCF all data					22,445	20,813	6,054	5,582	5,701	5,312	815	614	262	243
Standard deviation					10,614	9,459	3,696	3,282	3,485	3,266	9,462	9,146	121	108

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Mean BCF for trout (minus Lab 5 data)					26,982	24,732	7,995	7,316	6,975	6,417	4,600	4,218	291	268
Standard deviation					10,640	9,601	3,086	2,693	2,010	1,819	2,045	1,822	119	107
Mean BCF for carp					15,304	14,662	3,385	3,243	1,128	1,081	1,227	1,176		
Standard deviation					4,665	4,469	570	546	127	121	115	111		

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/day 14).

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

Table 73 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 (growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Lab 1	1.25	2.1	426	384	31,311	28,269	7,915	7,146	8,416	7,598	3,755	3,390	449	405
Lab 2a – trout	8.41	9.78	293	284	12,500	12,134	5,141	4,990	5,561	5,398	2,940	2,854	141	137
Lab 2b – carp (level 1)	5.42	7.11	319	302	11,857	11,240	2,992	2,836	1,243	1,178	1,153	1,093		
Lab 2b – carp (level 2)	5.42	7.11	319	302	14,051	13,319	3,268	3,098	1,004	952	1,224	1,160		
Lab 2b – carp (level 3)	5.42	7.11	319	302	20,984	19,891	4,110	3,896	1,210	1,147	1,383	1,311		
Lab 3	1.95	3.64	390	345	40,218	35,565	10,003	8,846	6,502	5,750	6,726	5,948		
Lab 4	1.17	2.32	431	377	32,932	28,778	15,191	13,274	11,235	9,817	8,049	7,033		
Lab 5	6.77	7.84	305	297	12,260	11,911	484	470	10,637	10,334	-27,257	-26,481	149	145
Lab 6	0.72	1.61	475	405	18,400	15,702	6,950	5,931	4,930	4,207	2,521	2,151	288	246
Lab 7	1.2	1.78	429	397	23,330	21,586	5,778	5,346	9,172	8,487	3,660	3,386		
Lab 8	1.24	2.08	427	385	44,428	40,123	9,172	8,284	6,592	5,953	6,913	6,243	377	341
Mean BCF all data					23,843	21,684	6,455	5,829	6,046	5,529	1,006	735	281	255
Standard deviation					11,708	10,154	4,052	3,494	3,705	3,400	9,679	9,269	136	118

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Mean BCF for trout (minus Lab 5 data)					29,017	26,023	8,593	7,688	7,487	6,744	4,938	4,429	314	282
Standard deviation					11,557	10,178	3,388	2,859	2,227	1,961	2,223	1,924	133	117
Mean BCF for carp					15,631	14,817	3,457	3,277	1,152	1,092	1,253	1,188		
Standard deviation					4,764	4,516	583	552	129	123	118	112		

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/day 14).

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

Table 74 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 (growth corrected)**									
	a	**b**	**a**	**b**	**HCB**		**MX**		**oTP**		**MC**		**BaP**	
					a	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
Lab 1	1.25	2.1	614	496	45,141	36,492	11,399	9,215	12,131	9,807	5,410	4,374	647	523
Lab 2a – trout	8.41	9.78	281	264	12,008	11,287	4,933	4,637	5,341	5,021	2,822	2,653	135	127
Lab 2b – carp (level 1)	5.42	7.11	336	301	12,507	11,190	3,153	2,821	1,311	1,173	1,216	1,088		
Lab 2b – carp (level 2)	5.42	7.11	336	301	14,821	13,260	3,443	3,081	1,059	948	1,290	1,154		
Lab 2b – carp (level 3)	5.42	7.11	336	301	22,134	19,803	4,331	3,875	1,276	1,142	1,458	1,304		
Lab 3	1.95	3.64	512	396	52,742	40,834	13,103	10,145	8,526	6,601	8,815	6,825		
Lab 4	1.17	2.32	631	476	48,152	36,368	22,187	16,757	16,425	12,405	11,761	8,883		
Lab 5	6.77	7.84	307	289	12,334	11,614	486	458	10,700	10,075	-27,403	-25,803	150	141
Lab 6	0.72	1.61	770	553	29,834	21,450	11,257	8,094	7,992	5,746	4,085	2,937	467	336
Lab 7	1.2	1.78	624	531	33,928	28,864	8,393	7,140	13,338	11,347	5,319	4,525		
Lab 8	1.24	2.08	616	498	64,161	51,900	13,232	10,703	9,519	7,700	9,976	8,070	545	441
Mean BCF all data					31,615	25,733	8,720	6,993	7,965	6,542	2,250	1,455	389	314
Standard deviation					18,611	14,071	6,284	4,647	5,211	4,174	10,480	9,448	234	177

Lab	Fish weight (g)		Estimated k_1 (l kg day^{-1})		Estimated BCF (growth corrected)									
	a	b	a	b	HCB		MX		oTP		MC		BaP	
					a	b	a	b	a	b	a	b	a	b
Mean BCF for trout (minus Lab 5 data)					40,852	32,456	12,072	9,527	10,467	8,375	6,884	5,466	449	357
Standard deviation					17,116	13,304	5,328	3,782	3,732	2,855	3,317	2,473	221	171
Mean BCF for carp					16,487	14,751	3,642	3,259	1,215	1,087	1,321	1,182		
Standard deviation					5,025	4,496	614	549	136	122	124	111		

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/day 14).

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

Table 75 Summary of estimated growth corrected BCF from the ring test using Method 21

Lab	Estimated k_1 (l kg day^{-1})[a]					Estimated BCF (growth corrected)[a]				
	HCB	**MX**	**oTP**	**MC**	**BaP**	**HCB**	**MX**	**oTP**	**MC**	**BaP**
Lab 1	664	502	619	533	761	48,839	9,322	12,226	4,701	801
Lab 2a – trout	664	502	619	533	761	28,385	8,814	11,761	5,357	366
Lab 2b – carp (level 1)	664	502	619	533	761	24,692	4,705	2,411	1,927	
Lab 2b – carp (level 2)	664	502	619	533	761	29,261	5,139	1,948	2,045	
Lab 2b – carp (level 3)	664	502	619	533	761	43,698	6,463	2,347	2,312	
Lab 3	664	502	619	533	761	68,476	12,860	10,311	9,190	
Lab 4	664	502	619	533	761	50,704	17,660	16,110	9,945	
Lab 5	664	502	619	533	761	26,675	795	21,556	-47,593	371
Lab 6	664	502	619	533	761	25,745	7,343	6,424	2,831	462
Lab 7	664	502	619	533	761	36,099	6,750	13,219	4,544	
Lab 8	664	502	619	533	761	69,189	10,786	9,562	8,639	673
Mean BCF all data						41,069	8,240	9,807	354	534
Standard deviation						16,545	4,492	6,188	16,169	194
Mean BCF for trout (minus Lab 5 data)						46,777	10,505	11,373	6,458	575
Standard deviation						17,736	3,768	3,045	2,754	198
Mean BCF for carp						32,550	5,436	2,235	2,095	
Standard deviation						9,921	916	251	197	

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.

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

Table 76 Summary of estimated growth corrected BCF from the ring test using Method 22

Lab	Estimated k_1 (l kg day^{-1})[a]					Estimated BCF (growth corrected)[a]				
	HCB	**MX**	**oTP**	**MC**	**BaP**	**HCB**	**MX**	**oTP**	**MC**	**BaP**
Lab 1	778	616	734	648	871	57,216	11,456	14,497	5,717	917
Lab 2a – trout	778	616	734	648	871	33,254	10,831	13,946	6,515	419
Lab 2b – carp (level 1)	778	616	734	648	871	28,927	5,782	2,859	2,344	
Lab 2b – carp (level 2)	778	616	734	648	871	34,279	6,315	2,310	2,488	
Lab 2b – carp (level 3)	778	616	734	648	871	51,194	7,942	2,783	2,811	
Lab 3	778	616	734	648	871	80,221	15,803	12,226	11,177	
Lab 4	778	616	734	648	871	59,400	21,701	19,103	12,095	
Lab 5	778	616	734	648	871	31,251	976	25,560	-57,882	425
Lab 6	778	616	734	648	871	30,161	9,024	7,618	3,443	529
Lab 7	778	616	734	648	871	42,290	8,295	15,675	5,527	
Lab 8	778	616	734	648	871	81,057	13,254	11,338	10,507	770
Mean BCF all data						48,114	10,125	11,629	431	612
Standard deviation						19,382	5,520	7,338	19,665	222
Mean BCF for trout (minus Lab 5 data)						54,800	12,909	13,486	7,854	659
Standard deviation						20,778	4,630	3,611	3,349	226
Mean BCF for carp						38,133	6,679	2,650	2,548	
Standard deviation						11,623	1,126	298	239	

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.

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

ANNEX 4: STANDARD OPERATING PROCEDURE FOR THE RING TEST

- PROTOCOL -

Study Title: Fish, Dietary Bioaccumulation Study

Study Number:

Test Substance:

Date:

Proposed Key Dates:

Experimental Start ....
Experimental Completion ....
Draft Report Completion ....
Final Report Completion ....

Approved By:

_____ _____

SAFETY FIRST

INTRODUCTION

Objective

This study will be conducted to determine the elimination rate constant after chemical analysis of incurred fish for the five test compounds from rainbow trout (*Oncorhynchus mykiss*) tissue. The test substances will be administered to the test system via the diet.
The data collected in this study will be used subsequently to derive the half-life ($t_{1/2}$, from the elimination rate constant, $k_{depuration}$), the assimilation efficiency (α), the biomagnification factor (BMF) and the lipid-normalised biomagnification factor (BMF_L) for the individual substances. Calculation methods will be made available separately.

Testing Facility

[to be completed by laboratory]

Compliance

This study will be performed in compliance with the draft OECD Guideline 305[1]. Additionally, general OECD Guidelines[2] regarding fish handling and husbandry procedures shall be observed.

Justification for Selection of Test System

Oncorhynchus mykiss is a common test species for freshwater toxicity studies. This study will form part of the ring test activities for the draft additional method, biomagnification in fish, being considered for inclusion in the OECD 305 test guideline.

Justification of Dosing Route

Potential environmental exposure of the test compounds is via feeding on lower trophic levels. Dietary bioaccumulation in fish is a meaningful measure of the potential for hydrophobic chemicals to undergo dietary biomagnification in the environment.

MATERIALS and METHODS

Test Substance Identification

Five individual test compounds will be tested:

Compound Name	CAS
hexachlorobenzene	118-74-1
musk xylene (2,4,6-trinitro-5-tert-butyl-1,3-xylene)	81-15-2
o-terphenyl	84-15-1
methoxychlor	72-43-5
benzo(a)pyrene	50-32-8

Carrier

Finfish Starter - Zeigler Bros., Inc., Gardners, PA or similar.

Dilution Water

Natural water or reconstituted moderately hard water can be used in the test, and should be obtained from an uncontaminated and uniform quality source in accordance with the existing OECD 305 test guideline. Test water should be characterized as far as possible, and total organic carbon and natural particle content remain as low as possible during the test. Source of the test water should be documented. The dilution water will be aerated prior to use. Concentrations of trace elements as recommended by OECD are attached at the end of this document.

Storage Conditions

Refrigeration (test feed)
Freezer (-80^{o}C) for sacrificed fish

Characterization of Test Material

The test substances' identity (chemical name and CAS number), purity and known impurities (along with concentrations) shall be documented. As a minimum, this may be based on information obtained from the manufacturer and incorporated into the study report.

Analysis of Substances in Feed

Samples of the test and control diets will be extracted and analyzed for the test compounds prior to initiation of the study and at the conclusion of the uptake phase. The methods of analysis will be included in the raw data and described in the final

report. The method that was used on the EMBSI-supplied feed samples in the pre-study recovery work should be followed (see document "dietary ring test analytical (v2.1).doc" for EMBSI's protocol). 3 samples should be randomly taken from the ca. 100g spiked batch of feed at test initiation, and 3 samples at the end of the uptake phase. Each sample must be analysed for all five test substances. Concentration results should not vary by more than 15% between the 3 samples taken at test start, and between the 3 samples taken at the end of the uptake phase. If variation is >15% between the 3 samples at test start, the feed batch should be remixed and reanalyzed before the test is begun. Mean measured concentrations for each of the five test substances should not vary more than 20% between test start and the end of uptake phase.

Sampling of Fish for Test Substance Analysis

Sampling intervals for test substance analysis includes one optional sampling point on uptake phase day 3 (used to estimate uptake rate) and are depuration phase days 1, 3, 7, 14, 21 and 28. To save analytical resources, it is possible to analyse only 2 of the 5 control fish at each sampling point for chemical concentrations; however the remaining 3 control fish must still be removed and their weight and length measured before storing (frozen). If any test substance is measured in the control fish, then it is necessary to analyse all five control fish for the test compounds. The method that was used on the EMBSI-supplied fish samples in the pre-study recovery work should be followed (see document "dietary ring test analytical (v2.1).doc" for EMBSI's protocol).

Fish Lipid Analysis

Lipid content will be measured individually on 5 fish taken from the stock population at the beginning of the uptake phase, 5 fish from each of the treatment tanks at the end of the uptake phase and 5 fish taken at the end of the depuration phase. The method used to quantify the lipid will be documented in the raw data and the results included in the final report.

Solubility

Not applicable within the confines of the study design.

Test System

Juvenile *Oncorhynchus mykiss*

Supplier

The fish supplier will be documented in the raw data and final report.

Husbandry and Acclimation

The stock population will be acclimated in dilution water for at least one week at test temperature and fed throughout on a sufficient diet and of the same type to be used during the

test. Any remaining fish from the stock population will be maintained for a reasonable time, so that they may be used for method development, training purposes, etc. Following that they will be euthanized according to the most appropriate and humane technique. An example of such a method is to use a tricaine methane sulfonate (MS 222) solution, prepared in laboratory dilution water (an MS222 concentration of 500 mg/L in laboratory dilution water pH buffered to 7.0-7.5 could be used).

Fish are held under static conditions using biological and mechanical filtration and are fed daily with Finfish Starter or similar.

The contents of the "clean" fish feed, especially lipid content, will be recorded and included in the test report.

Number and Sex

Proposed number: 118 (10 for initial weight assessment on arrival, 90 for study, 8 for spike recovery analysis, 10 for analytical method development).

Sex: Not Applicable

Age at Initiation of Exposure

Juveniles, actual age will be noted in the raw data and final report.

Test System Identification

Organisms will not be individually identified prior to or while in the test chambers.. All test chambers will be labeled to show study number and concentration.

Selection

Organisms will be randomly selected from the stock population and transferred directly to the test chambers. A printout of the randomization schedule will be included in the raw data. Organisms should be within a weight range of 1-8 g at test start. Select fish of similar weight such that the smallest are no smaller than two-thirds of the weight of the largest. All should be of the same year-class and come from the same source. Since weight and age of a fish appear sometimes to have a significant effect on BCF values record these details accurately. Optionally, a sub-sample of the stock of fish can be weighed before the test in order to estimate the mean weight. NB no fish should be less than 1g in weight.

To ensure that quality organisms are used for the study, fish will be selected from a pool of organisms larger than that needed for the study. The study director or his designee determines organism suitability.

Feeding Rate

Fish will be fed at a level of approximately 3% of wet body weight per day. The amount of feed may be adjusted at each sampling point based on the weights of sacrificed fish to account for growth during the experiment to maintain a level of 3%.

When adjusting the amount of feed required, the individual weights from test and control fish should be combined and averaged so that both test and control groups are fed the same quantity of feed. Initial feeding will be based on weight measurements of the stock population prior to the start of the test.

Test Feed

Feed containing each of the five test compounds spiked via a corn oil suspension of the test substances, solvent-based spiking of the test substances, or a combination of the two methods. It is fed to the treatment fish during the uptake phase of the study.

Control Feed

Feed treated in exactly the same manner as the test feed (ie including corn or fish oil and/or treated with a solvent), but containing no test substances should be fed to the control fish during the uptake phase of the study.

Clean Feed

Feed as supplied by the manufacturer, containing no test substance and not treated with solvent or corn/fish oil for spiking and fed to all fish (test and control groups) during the depuration phase of the study.

Contaminants

No known contaminants should be present in the test water or the feed at levels high enough to interfere with this study. The dilution water may be prepared from UV-sterilized, deionized well water.

EXPERIMENTAL PROCEDURE

Definitive Test Design

GROUP	NOMINAL CONCENTRATION (µg/g)	NUMBER OF ORGANISMS
Control	0	45 (40**)
Treatment	375*	45 (40**)

* Total dietary concentration minimized to mitigate cumulative effects on fish and provide sufficient analytical sensitivity. The concentrations used are based on previous successful testing. ** If the test lab opts out of the sampling time point during uptake, fewer fish are required.

TEST SUBSTANCES	NOMINAL CONCENTRATION[1] (µg/g)
hexachlorobenzene	25
musk xylene (2,4,6-trinitro-5-tert-butyl-1,3-xylene)	50
o-terphenyl	50
methoxychlor	100
benzo(a)pyrene	150
Total Diet Concentration	**375**

[1] feed as supplied in used in spiking; concentrations therefore include the minimal quantity of moisture associated with supplied feed.

Preparation and Administration of Test Material

A flow-through system will be used to provide a sufficient volume of dilution water to the test tanks. The actual flow rate should be sufficient to supply at least five water replacements over a 24 hour period, and will be recorded in the raw data.

The appropriate amounts of the test substances will be added to the test feed to achieve nominal concentrations as per the definitive test design. A single diet will be prepared containing each of the five test compounds. It is important to note that the lipid content of the diet should not be artificially increased by the addition of large quantities of fish or corn oil. (the draft guideline says that "ideally the lipid content of feed should be 15 – 20% w/w"; however slightly lower lipid concentrations are not ruled out). The test compounds will be dissolved or suspended in:
i) corn oil at a concentration that yields the approximate individual diet concentrations when 0.5 mL of corn oil suspension is added to a total of 100 g fish feed (0.5% corn oil in feed). The exact methods and procedures for dosing the feed will be documented and included in the raw data. Control feed will be prepared by adding 0.5 mL of corn oil containing no test compounds to 100 g of clean feed. (the method for preparing the

spiked and control diets by this method is given in document "dietary ring test analytical (v2.1).doc")
ii) an appropriate quantity of a suitable organic solvent (eg cyclohexane or acetone; 10 – 40 mL). Either an aliquot, or all (added in portions), of this solution is mixed with the appropriate mass of fish food to achieve the required nominal dose level. The food/test substance can be mixed in a stainless steel mixing bowl and the freshly-dosed fish food left in the bowl in a laboratory hood for two days (stirred occasionally) to allow the excess acetone to evaporate, or mixed in a rotary evaporator bulb with continuous rotation. The excess solvent can be "blown" off under a stream of air or nitrogen if necessary. Care must be taken to ensure that the test substance does not crystallise as the solvent is removed. The spiked diet should be stored under conditions that maintain stability of the test chemical within the feed mix (eg refrigeration) until use.

1. iii) a combination of the two methods described above: test substances in solution (hexane or acetone) are prepared and added to corn/fish oil and then the organic solvent is evaporated before mixing the oil with the feed. The feed is dried overnight to ensure no solvent remains. Concentrations of solutions of the test substances in solvent should be chosen such that the target concentrations in final feed are met by the addition of the solvent solution to the minimum quantity of fish/corn oil (ca 0.5 mL).

The lipid content of the spiked feed must be recorded (NB lipid content of test and control diets should be the same). The amount of treated (Test) and untreated (Control) diets will be measured daily and fed to the fish *ad lib* as one feeding. The daily dose of feed may be split into two feedings if the feed is not all eaten (ie still remaining when tanks are cleaned 30 minutes after feeding) or not taken up rapidly enough (NB this may have consequences for when analysis is carried out).

Test Chamber and Volume of Solution

A suitable set up of test and control chambers will be used, in accordance with the OECD 305 TG, ensuring appropriate fish loading rates. An example laboratory set up is as follows:
test chambers may be 40 L glass aquaria with stainless steel standpipes that allow approximately 31 L of solution in the test chamber. Fresh dilution water will flow through the test chambers at a target rate of 160 - 200 mL/minute, resulting in 7 - 9 water changes in a 24 hour period. The test chambers will be aerated throughout the study to ensure that adequate dissolved oxygen levels are maintained. Chambers will be covered to minimize contamination and/or evaporation.

During fish feeding, the flow will be turned off (and, if used, the standpipes capped) to prevent loss of feed. Once the fish are observed to have completed their feeding, the water flow is resumed (and, if used, the standpipes uncapped).

Exposure Duration

Uptake phase: 13 days
Depuration phase: 28 days

Physical Measurements

Range of acceptable test water temperatures: 13-17°C. Variation should be less than ± 2°C in any one tank (eg 15 ±. 2°C)). Temperature should be measured daily, and preferably continuously in one tank.

Dissolved Oxygen (≥ 60% of air saturation) and pH. As a minimum these should be measured at test start and in the middle and at the end of the uptake phase, and once a week during the depuration phase.

Total organic carbon. This should be measured before the test start as part of the routine water quality assessment, but TOC measurement is not required during or after the test. (If laboratories routinely measure TOC during studies, then they can do so).

Diurnal light: ~16 hours light, ~8 hours dark.

A record of daily observations for any mortality, adverse effects and feeding behaviour in both control and test group should be kept.

Environmental conditions should be monitored to provide a record of the continuous measurements for temperature and lighting in the test area.

Fish Sampling Procedure

Treatment fish will be fed for 13 days with feed containing the test compounds. Control fish will be fed for the same duration with feed treated in the same manner as the test feed, but containing no test compounds. In the event that signs of stress are observed, the uptake phase may be shortened; the ring test coordinators should be made aware and a memo will be added to the study file documenting the details. Fish samples will be collected from each tank according to the sampling schedule listed in this protocol. Sampling intervals include uptake phase day 3 (used to estimate uptake rate) and depuration phase days 1, 3, 7, 14, 21 and 28.

At each sampling period, 5 fish from each tank (control and treatment) will be sacrificed. Sampling should occur at roughly the same time of day for each sample during the depuration phase. Optionally, the guts may be removed from the test fish before analysis; **if this is done, fish must still be treated in the same way as detailed below to ensure that each laboratory samples fish at the same time and that each fish receives a similar period of "clearing" prior to analysis in the case of sampling on day 3 of uptake and day 1 of depuration.**

In order to ensure that gastro-intestinal tract is cleared of test or control diet, fish sampled on uptake day 3 will be treated as follows. Shortly after being fed their respective diets, 5 test and control fish will be transferred to separate smaller tanks containing clean water. Approximately 5 hours after being fed their day 3 diets, they will be fed clean feed. These fish will be sacrificed the following morning.

On uptake day 13, the remaining fish will also be fed clean food approximately 5 hours after their last treatment or control feeding. Five test and control fish will be sacrificed the following morning and correspond to day 1 depuration samples. On days when fish sampling is scheduled during the depuration phase, it shall occur just prior to the daily feeding.

All fish will be euthanized according to the most appropriate and humane method. An example is the use of a tricaine methane sulphonate (MS 222) solution, prepared in laboratory dilution water (an MS222 concentration of 500 mg/L in laboratory dilution water pH buffered to 7.0-7.5 could be used).

All fish will be treated humanely in accordance with National and OECD guidance. The study design and personnel training must be sufficient to minimize animal pain within the confines of the study objective.

Fish Sampling Schedule

Phase	Day	Analysis	Number of Fish Sampled
Uptake	**0**	**Lipid**	**5 control [a]**
Uptake	**3 [b,c]**	**Test Substance Concentrations**	**5 control + 5 test**
Uptake	**13 [d]**	**Lipid**	**5 control + 5 test**
Depuration	**1**	**Test Substance Concentrations**	**5 control + 5 test**
Depuration	**3**	**Test Substance Concentrations**	**5 control + 5 test**
Depuration	**7**	**Test Substance Concentrations**	**5 control + 5 test**
Depuration	**14**	**Test Substance Concentrations**	**5 control + 5 test**
Depuration	**21**	**Test Substance Concentrations**	**5 control + 5 test**
Depuration	**28**	**Test Substance Concentrations**	**5 control + 5 test**
Depuration	**28**	**Lipid**	**5 control + 5 test**

All fish should be individually weighed and length (total) measured prior to analysis.

a. Fish taken from same lot just prior to initiation. Not included as study fish.

b. OPTIONAL - Used to estimate uptake rate.

c. Test fish fed spiked diet in the morning of the designated day. Approximately 5 hours after test diet feeding, fed clean feed. Fish sacrificed the following morning.

d. Test fish fed spiked diet in the morning of the designated day. Approximately 5 hours after test diet feeding, fed clean feed. Fish sacrificed the following morning corresponding to depuration day 1.

Experimental Evaluation

Observations for mortality, any adverse effects and feeding behaviour will be performed and recorded daily. Additional observations may be performed. Fish are considered dead if there is no respiratory movement and no reaction to a slight mechanical stimulus can be detected. During observations, organisms will be examined for abnormal behavior or coloration. In the event that signs of stress are observed, the uptake phase may be shortened; the ring test coordinators should be made aware and a memo will be added to the study file documenting the details. Any dead fish will be removed.
After completion of the study, the monitoring of environmental conditions will be discontinued. All remaining fish will be euthanized, weighed and measured (total length).

Cleaning

To maintain good hygiene, uneaten food (if uneaten food is observed it will be documented) and feces will be siphoned from the test chambers shortly after feeding (within 30 minutes to 1 hour). Care will be taken not to injure the test organisms.

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Organism Loading

Loading will be in accordance with the OECD 305 TG (0.1 - 1.0 g of fish (wet weight) per liter of solution per day based on the flow through conditions).

Length / Weight of Test System

Length and weight measurements of a sub-sample of the stock fish will be recorded prior to the start of the study in order to estimate a mean weight. The 5 fish used for lipid determination can be used for this purpose as part of the ten stock fish. Individual length and weight measurements will also be recorded on fish removed at each sampling period. Total length will be measured.

Conditions for Validity

The test is acceptable if:

- Temperature variation is less than ± 2 ºC in treatment or control groups
- Concentration of dissolved oxygen does not fall below 60% of the air saturation value
- The concentration of the test substance in fish food is kept constant over the feeding period within a range of ± 20%
- Concentrations of test chemical are not detected, or are present only at typical trace levels, in un-spiked food or control fish tissues relative to treated samples
- A high degree of homogeneity of substance in feed must be demonstrated in preliminary analytical work on the spiked diet; concentrations for the same substance between the 3 samples must not vary more than +/- 15%

- Mortality or other adverse effects/disease in both control and test group fish should be ≤10% at the end of the test Average growth in both test and control groups should to be similar.

Calculations

Test results are used to derive the elimination rate constant as a function of the total wet weight of the fish. The Excel spreadsheet for the ring test allows tabulation of test data, and automates the calculations described below. In addition, the assimilation efficiency (α), the biomagnification factor (BMF) and its lipid-normalised value (BMF_L) will be calculated. A memorandum outlining the calculations will be provided at a later date.

Basic data analysis and calculations are as follows:

Weight data

1. Individual fish weights and lengths are tabulated separately for test and control groups during the uptake and depuration phases.

2. Weight and length data are converted to natural logs and plotted vs. day, separately for test and control.

3. A linear least squares correlation is calculated for the ln(fish weight) vs. day for both test and control (individual data not daily means) using standard statistical procedures.

4. The variances in the slopes of the test and control lines are calculated and used to evaluate the statistical significance of the difference in the two slopes (growth rates) using the student t-test. If there is no significant difference, the test and control data are pooled and an overall fish growth rate for the study (k_{growth}) calculated as the overall slope of the linear correlation. If statistical differences are observed growth rates for control and treated fish are reported separately, and the growth rate for the treated fish is used in subsequent calculations.

Calculations (Cont'd)

TEST CHEMICAL CONCENTRATION IN FISH DATA

1. Individual fish test substance residue measurements expressed in terms of concentration (w/w) are tabulated for test and control fish for individual sample times.
2. The individual fish concentration data for the depuration period are converted to their natural logarithms and plotted versus time (day). If a visual inspection of the plot shows obvious outliers, a statistically valid outlier test may be applied to remove spurious data points.
3. A linear least squares correlation is calculated for the ln(concentration) vs. depuration day data. The slope and intercept (day 0 of depuration) of the line are reported as the overall elimination rate ($k_{overall}$) and time zero concentration ($C_{0,depuration}$).
4. The variances in the slope and intercept of the line are calculated using standard statistical procedures and the 90% confidence intervals around these results evaluated.

Lipid normalisation

1. The mean lipid fraction (w/w) in the fish and the food are calculated.

REPORTS

After termination of the study, a final report will be prepared containing select information acquired during the study. As a minimum the report shall contain:

- In-life / feeding observations
- Test substance concentrations measurements in spiked diets for each analysis
- Food type employed and feeding rates during uptake phase, any deviations from once-a-day feeding
- Tabulated fish individual weight and length data and calculations for test and control groups, derived growth rate(s) and 95% confidence interval(s), plots of growth data
- Indication of any treatment related effects on fish growth
- Complete description of all chemical and lipid analysis procedures employed including quantitation limits, variability and recovery.

RECORDS

All appropriate materials, methods and experimental measurements required in this protocol will be recorded and documented in the raw data. Any changes, additions or revisions of this protocol must be approved by the ring test coordinator and OECD drafting team. These changes will be documented in writing, including the date and the justification for the change.

The protocol, final report, raw data or computer generated listings of raw data, and supporting documentation will be maintained in the Archives of the testing facility.

REFERENCES

[1]. Bioconcentration and Bioaccumulation if Fish: Aqueous and Dietary Exposure. OECD Guidelines for Testing of Chemicals. Guideline 305, Draft: 22.03.2010

2. Bioconcentration: Flow-through Fish Test. OECD Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems, Guideline 305, adopted June 14, 1996.

SOME CHEMICAL CHARACTERISTICS OF AN ACCEPTABLE DILUTION/TEST WATER

Substance	Limit concentration
Particulate matter	5 mg/L
Total organic carbon	2 mg/L
Un-ionised ammonia	1 µg/L
Residual chlorine	10 µg/L
Total organophosphorous pesticides	50 ng/L
Total organochlorine pesticides plus polychlorinated biphenyls	50 ng/L
Total organic chlorine	25 ng/L
Aluminium	1 µg/L
Arsenic	1 µg/L
Chromium	1 µg/L
Cobalt	1 µg/L
Copper	1 µg/L
Iron	1 µg/L
Lead	1 µg/L
Nickel	1 µg/L
Zinc	1 µg/L
Cadmium	100 ng/L
Mercury	100 ng/L
Silver	100 ng/L

PART II

ADDITIONAL REPORT INCLUDING COMPARATIVE ANALYSIS OF TROUT AND CARP RESULTS

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Introduction

The current Organisation for Economic Co-ordination and Development (OECD) Test Guideline 305, "Bioconcentration in fish, flow-through test", [1] for bioaccumulation testing outlines a method to measure a chemical's bioconcentration factor (BCF) in fish. Technical issues and analytical challenges exist in the test method, particularly for poorly water soluble substances, resulting in uncertain or erroneous BCF determinations [2, 3]. The OECD 305 TG is being revised in an effort to reduce animal usage in bioconcentration tests and to include the new dietary bioaccumulation testing method for poorly water soluble substances. A method has been proposed for bioaccumulation testing of poorly water soluble chemicals in which the fish are exposed to test chemical in the diet rather than the water [4]. This method builds on various reported bioaccumulation studies in the literature [5-8]. The proposed revised testing methods require evaluation to demonstrate reproducibility of results and to determine the degree of inter-laboratory variation that can be expected if the test is adopted and used broadly. The "validation" of the testing methods is required for the acceptance of the TG for use by the OECD or member countries of the OECD.

As part of this effort, the UK, the Netherlands and Germany, have coordinated a ring test of the new dietary bioaccumulation testing method. A total of ten laboratories have volunteered to conduct studies for this ring test. To date eight studies have been completed using rainbow trout (*Onchorhynchus mykiss*) and one laboratory has conducted three studies using carp (*Cyprinus carpio*). The carp testing included three different dietary exposure concentrations. Five test substances were used in each study. A report summarizing the testing completed and the data has been drafted [9]. The general objective of that report is to evaluate the overall results and the degree of inter-laboratory variation.

The current report focusses on a comparative analysis of the results from the two test species (carp and trout). This analysis includes an intra-laboratory comparison (key testing results for carp and trout from the same laboratory) and an inter-laboratory comparison (key testing results based on average values for trout from the other laboratories). The present analysis draws upon previous studies that examine species-specific differences in bioaccumulation in fish, including plausible explanations for any observed differences.

Dietary Test Method

A brief overview is provided of the dietary testing method and the primary results used for bioaccumulation assessments are described. Fish are first exposed to chemicals added to their food ("spiked") only for a defined "uptake" phase. Fish are not intentionally exposed to chemical in the water during the uptake phase. Fish are then placed in clean water and are fed "unspiked", uncontaminated food for a defined "depuration" phase. In the ring test, five test substances were tested in each study. Fish food was "spiked" and test fish are fed daily during the uptake (or exposure) phase. The test records include:

- chemical concentrations in fish tissue during the depuration phase;
- test substance concentrations in the food;
- feeding rate;
- fish weights and lengths at each sampling point;
- fish and food lipid contents.

These data allow the calculation of the following parameters that are used in assessing bioaccumulation:

- a depuration rate constant (k_2; the overall rate constant for the removal of the test substance from the test organism including possible contributions to chemical elimination from fecal egestion k_e, metabolic biotransformation k_m, respiration at the surface of the gills k_v, and growth dilution k_g) for each substance;
- the assimilation efficiency (α; the relative amount of substance that is taken up from the gut) for each substance;
- the fish growth rate constant (k_g; a potentially important contributing parameter for k_2);
- the growth corrected depuration (elimination) rate constant (k_{2g}) for each substance;
- the lipid contents ratio of fish:food;
- the dietary biomagnification factor (BMF, growth corrected, and corrected for the lipid content of the fish and the food, *i.e.* noted in this document as BMF_{Lg}) for each substance.

The calculation of these parameters can vary depending on how the data are selected and treated. The project manager has provided results derived from the complementary validation project [9] to ensure that unnecessary variability in the data analysis and comparisons can be addressed. Although a standard operating procedure exists, some of the testing protocols were manipulated to evaluate possible changes in testing as a result of changes in these selected test methods. These variables include exposure temperature, food spiking techniques, and other factors [9].

It is also possible to estimate kinetic BCFs from the dietary test data by using a model calculation of the chemical uptake rate constant from water (k_1) and the depuration rate constants, either overall depuration (k_2) or growth corrected overall depuration (k_{2g}). The present report includes estimates of the BCF using selected uptake rate constants.

Format of the Report

The raw data provided in Excel™ spread sheets were reviewed and calculations were compared to values provided in the main report ("Final Draft – November 2011"[9]). The calculations in the report were deemed to be correct. One objective of the present report is to facilitate integration of the current evaluations into the main report (*i.e.* as an Addendum); therefore, the values used in the subsequent calculations and comparisons are cited from the tables in the main report [9], except where otherwise noted.

The present report is organized into four main sections. The first section (1) addresses intra-laboratory comparisons. The second section (2) addresses inter-laboratory comparisons. The third section (3) reviews and discusses inter-species variability in key bioaccumulation assessment metrics (*e.g.* bioconcentration factors, biomagnification factors, biotransformation rate constants) with a focus on differences between trout and carp. A fourth section (4) includes some general comments and summary of the report. It is envisaged that these sections will become incorporated as an appendix into the main report for the project. Efforts were made to maintain general consistency with the main report with respect to the formatting of text, tables and figures in these sections. Sections 1, 2 and 3 of this report were produced in accordance with the relevant sections of the OECD's guidance on the validation and international

acceptance of new or updated test methods for hazard assessments[1], taking into account how previous validation studies for OECD test guidelines have been conducted and reported[2].

A brief rationale for some of the decisions made in this report is first provided. Unless otherwise stated in this report, it was assumed that all of the testing and summary data are normally distributed to provide consistency with the general approach taken in the main report [9]. The testing results for benzo[a]pyrene (BaP) were generally deemed unreliable in the main report. Therefore, the focus of this comparative analysis is on the other four chemicals in the ring test; namely, hexachlorobenzene (HCB), musk xylene (MX), o-terphenyl (oTP), and methoxychlor (MC). There are stated concerns regarding the consistency of the results from Lab 5 as outlined in the main report [9]; therefore, the data from Lab 5 were not included in the inter-laboratory comparisons in this report. This approach is intended to reduce additional potential uncertainty when investigating possible inter-species variability in the reported bioaccumulation information. The comparisons below are based on the assumption that the exposure duration is for 13 days. The default, assumed nominal feeding rate of 3% body weight per day was used for all of the comparisons. Bioaccumulation data that include day 1 of the depuration phase were included in the comparisons below. The Lab 2 carp data were presented as single, mean, concentration values at each time point; therefore, some relevant parameters were derived from these mean concentrations rather than the individual concentrations. The selected set of data for the analyses conducted herein are thus consistent with the recommendations and methods used in the main report [9].

[1]Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment. OECD Series on Testing and Assessment, No. 34, 2005. Available from: http://www.oecd.org/document/30/0,3746,en_2649_34377_1916638_1_1_1_1,00.html.

[2] For example: Report of the Test Method Validation of Avian Acute Oral Toxicity Test (OECD test guideline 223). OECD Series on Testing and Assessment, No. 131, 2010.

Report of the Validation of an Enhancement of OECD TG 211: *Daphnia magna* Reproduction Test. OECD Series on Testing and Assessment, No. 93, 2008.

Available from http://www.oecd.org/document/30/0,3746,en_2649_34377_1916638_1_1_1_1,00.html.

Intra-Laboratory Comparison

1. The dietary bioaccumulation test result parameters for the carp and the trout studies from the same laboratory are compared. The focus for comparisons is on the similar chemical exposure concentrations in the tests; however, the other two chemical exposure concentration tests conducted with the carp only are also included. These comparisons include the following parameters:

- Concentrations of each test chemical in fish at the start of depuration estimated from depuration curves ($C_{0,depuration}$; μg/g);
- Depuration rate constants (k_2; 1/day);
- Depuration rate constants, growth corrected (k_{2g}; 1/day);
- Chemical assimilation efficiencies calculated from depuration data (α; unitless);
- Kinetic biomagnification factors, lipid corrected (BMF_L; kg/kg);
- Kinetic biomagnification factors, growth and lipid corrected (BMF_{Lg}; kg/kg);
- Estimated kinetic bioconcentration factors (BCF; L/kg).

2. Table 1 summarizes the concentrations of each test chemical in trout and carp at the start of depuration as estimated from depuration regressions ($C_{0,depuration}$) from Lab 2 (from Tables 21 to 25 in the main report). The 95% confidence intervals (CI) for the $C_{0,depuration}$ estimates (from Tables 21 to 25 in the main report) and the average measured day 1 depuration concentrations $C_{1,depuration}$ (from Table 20 in the main report) are also shown for comparisons. $C_{1,depuration}$ values are obtained from fish that were fed clean food approximately 5 hours after being fed their day 13 diet (last exposure diet) and were sacrificed the following morning. The $C_{1,depuration}$values are within the range of the 95% CIs for all treatments except for the carp level 1[3] o-terphenyl and the trout benzo[a]pyrene test results. This indicates that fish concentration estimation methods may be relatively consistent during the transition from the exposure phase to the depuration phase.

3. The carp data include three different dietary exposure concentrations. The nominal dietary concentrations tested as described in the ring test standard operating procedure are referred to here as "level 3" data or "treatment 1x". The "level 3" nominal dietary concentrations are the same in the other studies using trout. In addition to the standard "level 3" approach, nominal chemical concentrations added to the fish food were five times and ten times higher than those specified in the standard operating procedure. These are referred to as "level 2" or "treatment 5x" and "level 1" or "treatment 10x", respectively. Benzo[a]pyrene was not tested at higher dietary concentrations. As expected, the $C_{0,depuration}$ and $C_{1,depuration}$ values for carp increased with increased dietary exposure concentrations. The most appropriate treatments for direct inter-species comparisons are when the nominal dietary exposure concentrations are the same (*i.e.* level 3, 1x). The level 3 carp $C_{0,depuration}$ values are lower than the trout $C_{0,depuration}$ values for all chemicals even though there are no significant differences in dietary exposure concentrations in Lab 2 level 3 tests. Table 15 in the main report indicates that, in general, dietary exposure concentrations for carp (level 3) are higher than trout. Thus, all factors being equal, it would be expected that $C_{0,depuration}$ values in carp would be higher than $C_{0,depuration}$ values in trout. However, in

[3] See paragraph 3 for a description of the different carp study treatments.

general, comparisons of the 95% CIs indicates the $C_{0,depuration}$ values between trout and carp from Lab 2 are notably different. The 95% CIs only overlap for methoxychlor. For methoxychlor, the 95% CIs for carp (level 3) are large reflecting substantial uncertainty in this $C_{0,depuration}$ estimate. For benzo[a]pyrene, $C_{0,depuration}$ estimates could not be derived for carp due to insufficient data during the depuration phase as a result of rapid elimination; however, $C_{0,depuration}$ estimates could be derived for trout. Collectively these data indicate that chemical absorption efficiencies may be lower in carp compared to trout or that carp may have a greater capacity to eliminate (*e.g.* biotransform) these chemicals compared to trout or a combination of both factors.

4. Table 2 summarizes the overall depuration rate constants (k_2) calculated from Lab 2 data for trout and carp (from Tables 21 to 25 in the main report). The coefficient of determination from the depuration regression analysis (r^2) and the 95% confidence intervals (CI) for the k_2 estimates are also shown. The k_2 values are larger for carp than for trout suggesting faster elimination for carp, including the higher dietary exposure concentrations for carp. For benzo[a]pyrene, k_2 estimates could not be derived for carp due to insufficient data during the depuration phase (rapid elimination), whereas k_2 estimates could be derived for trout.

5. In some cases, the 95% CIs for the k_2 estimates are different indicating that the overall depuration rates in carp are faster than the overall depuration rates in trout. The exceptions are for hexachlorobenzene and methoxychlor (all treatments) and for musk xylene (level 3). It is recognized that gill elimination rates, biotransformation rates and growth rates, and thus k_2 are a function of organism size, lipid content, and system temperature [10-14]; therefore, these apparent differences in k_2 may not explicitly be the result of inter-species differences related to physiological, anatomical or metabolic factors.

6. Table 3 summarizes the growth corrected depuration rate constants (k_{2g}) calculated from Lab 2 data for trout and carp (from Tables 40 to 44 in the main report). Geometric means and 95% CIs were calculated from the three carp treatments and compared against the trout values. The 95% CI calculations for the carp data used 2 degrees of freedom (*i.e.* critical value of 4.303 for the *t* distribution, [15]). The k_{2g} values for musk xylene, o-terphenyl and methoxychlor are larger for carp than for trout which again indicates faster overall elimination rates in carp for these chemicals under these conditions. For musk xylene, o-terphenyl and methoxychlor, the k_{2g} values from trout are less than the estimates of the lower 95% CIs from the carp data. For hexachlorobenzene, a highly persistent chemical, the k_{2g} values are not significantly different in trout and carp based on the Lab 2 dataset. For benzo[a]pyrene, k_{2g} estimates could not be derived for carp due to insufficient data during the depuration phase as a result of rapid elimination, whereas k_{2g} estimates could be derived for trout.

Table 1 **Comparisons of the chemical concentration data (µg/g) for trout and carp (3 different dietary concentrations) from Lab 2. $C_{0,depuration}$ values are calculated as the intercept from the depuration phase linear regression analysis. $C_{1,depuration}$ values are averages of measured fish concentrations from fish sacrificed on day 1 of the depuration phase.**

Chemical	$C_{0,depuration}$	$C_{0,depuration}$	$C_{1,depuration}$
Species (treatment)	**intercept**	**95% CI**	**average**
HCB			
Trout (1x)	5.68	5.19 to 6.22	5.48
Carp (level 1 – 10x)	23.81	16.10 to 35.20	20.47
Carp (level 2 – 5x)	12.54	11.08 to 14.20	12.3
Carp (level 3 – 1x)	2.31	1.25 to 4.27	2.45
MX			
Trout (1x)	10.38	9.19 to 11.73	9.5
Carp (level 1 – 10x)	38.63	25.30 to 58.97	29.84
Carp (level 2 – 5x)	19.97	16.07 to 24.80	19.97
Carp (level 3 – 1x)	3.08	1.99 to 4.75	2.76
oTP			
Trout (1x)	5.97	4.45 to 8.00	5.51
Carp (level 1 – 10x)	31.32	22.31 to 43.95	19.52
Carp (level 2 – 5x)	11.5	4.99 to 26.55	9.63
Carp (level 3 – 1x)	0.81	0.54 to 1.20	0.62
MC			
Trout (1x)	11.16	7.37 to 16.89	7.68
Carp (level 1 – 10x)	9.48	3.31 to 27.14	6.85
Carp (level 2 – 5x)	4.29	1.70 to 10.86	3.86
Carp (level 3 – 1x)	0.63	0.0029 to 133.62	0.6
BaP			
Trout (1x)	4.8	1.64 to 14.00	0.61
Carp (level 1 – 10x)	a	a	-
Carp (level 2 – 5x)	a	a	-
Carp (level 3 – 1x)	a	a	<0.004

a) Insufficient data points available to derive the depuration curve.

Table 2 **Comparisons of the overall depuration rate constant (k_2; day^{-1}) data for trout and carp from Lab 2.**

Chemical Species (treatment)	k_2 from slope	95% CI	r^2
HCB			
Trout (1x)	0.0399	0.034 to 0.046	0.88
Carp (level 1 – 10x)	0.0603	0.024 to 0.097	0.90
Carp (level 2 – 5x)	0.0561	0.045 to 0.068	0.99
Carp (level 3 – 1x)	0.0486	-0.008 to 0.105	0.71
MX			
Trout (1x)	0.0734	0.066 to 0.081	0.93
Carp (level 1 – 10x)	0.14	0.100 to 0.179	0.98
Carp (level 2 – 5x)	0.131	0.110 to 0.151	0.99
Carp (level 3 – 1x)	0.111	0.071 to 0.151	0.96
oTP			
Trout (1x)	0.0691	0.051 to 0.088	0.68
Carp (level 1 – 10x)	0.29	0.259 to 0.322	0.99
Carp (level 2 – 5x)	0.351	0.209 to 0.492	0.98
Carp (level 3 – 1x)	0.297	0.229 to 0.365	0.99
MC			
Trout (1x)	0.116	0.089 to 0.142	0.74
Carp (level 1 – 10x)	0.31	0.132 to 0.488	0.97
Carp (level 2 – 5x)	0.294	0.137 to 0.451	0.97
Carp (level 3 – 1x)	0.264	-0.945 to 1.473	0.89
BaP			
Trout (1x)	2.094	1.615 to 2.572	0.93
Carp (level 1 – 10x)	a	a	a
Carp (level 2 – 5x)	a	a	a
Carp (level 3 – 1x)	a	a	a

a) Insufficient data points available to derive the depuration curve.

Table 3 Comparisons of the growth rate corrected depuration rate constant (k_{2g}; day^{-1}) data for trout and carp from Lab 2.

	Chemical			
Species (treatment)	**HCB**	**MX**	**oTP**	**MC**
Trout (1x)	0.023	0.057	0.053	0.10
Carp (level 1 – 10x)	0.027	0.11	0.26	0.28
Carp (level 2 – 5x)	0.023	0.098	0.32	0.26
Carp (level 3 – 1x)	0.015	0.078	0.26	0.23
Geometric mean - carp	0.021	0.094	0.28	0.26
95% CI	0.0099 to 0.045	0.061 to 0.15	0.21 to 0.38	0.20 to 0.33

7. Table 4 summarizes the chemical dietary assimilation efficiency (α) data from Lab 2. These values were calculated using 13 days for the exposure duration value. The α values are generally larger in trout than in carp. Except for o-terphenyl, the trout data are greater than the upper 95% CIs for carp. In carp, the α values are higher at higher exposure concentrations. There is a high degree of variability in the data for o-terphenyl. As shown in paragraph 79 of the main report, the calculation of α includes estimates of $C_{0,depuration}$, k_2, chemical concentration in the diet, and the ingestion rate (estimated from the nominal feeding rate). The uncertainty from the different parameters may be substantial and these uncertainties will therefore propagate into the α estimate. The underlying uncertainties in the calculation of α are not considered in the current analysis. Chemical concentrations in carp were not measured in the uptake phase so it is not possible to compare the differences in α measurement methods (i.e. uptake phase vs. depuration phase) between carp and trout.

Table 4 Comparisons of the chemical dietary assimilation efficiency (α; unitless) data for trout and carp from Lab 2.

	Chemical			
Species / Treatment	**HCB**	**MX**	**oTP**	**MC**
Trout (1x)	0.79	0.95	0.55	0.65
Carp (level 1 – 10x)	0.41	0.54	0.74	0.13
Carp (level 2 – 5x)	0.4	0.48	0.59	0.1
Carp (level 3 – 1x)	0.33	0.31	0.17	0.06
Geometric mean - carp	0.38	0.43	0.42	0.092
95% CI	0.28 to 0.51	0.21 to 0.89	0.059 to 3.0	0.035 to 0.24

8. Table 5 summarizes kinetic, lipid normalized biomagnification factors (BMF_L) from Lab 2. These BMF_L values were calculated using the equation outlined in paragraph 79 of the main report and selecting 13 days for the exposure duration value, the overall depuration rate constant (k_2) and the average lipid content during the test. The average lipid content used in this calculation was for the test organisms only; control fish lipid content measurements were not included. The nominal daily ingestion rate of 0.03 g-

food / g-fish was assumed in the calculations. Only the BMF_L for hexachlorobenzene is greater than the generally accepted threshold of concern of 1. For each chemical, the BMF_Ls in trout are larger than the BMF_Ls in carp. For carp, the BMF_Ls are higher at higher exposure concentrations. The uncertainties in the input and assumed parameters used to calculate BMF_L will propagate uncertainty in the BMF_L calculation estimate; however, these uncertainties are not recognized in the current analysis.

Table 5 Comparisons of the kinetic, lipid normalized biomagnification factor (BMF_L; kg/kg) data for trout and carp from Lab 2.

	Chemical			
Species / Treatment	**HCB**	**MX**	**oTP**	**MC**
Trout (1x)	1.11	0.73	0.45	0.32
Carp (level 1 – 10x)	0.58	0.33	0.22	0.04
Carp (level 2 – 5x)	0.56	0.29	0.13	0.03
Carp (level 3 – 1x)	0.53	0.22	0.04	0.02
Geometric mean – carp	0.56	0.27	0.11	0.026
95% CI	0.50 to 0.62	0.16 to 0.46	0.015 to 0.81	0.011 to 0.061

9. Table 6 summarizes kinetic, growth corrected and lipid normalized biomagnification factors (BMF_{Lg}) from Lab 2. These BMF values were calculated using 13 days for the exposure duration value, the growth corrected depuration rate constant (k_{2g}) and the average lipid content during the test (test organisms only, not including control fish lipid measurements). The nominal daily ingestion rate of 0.03 g-food / g-fish was assumed in the calculations. All of the BMF_{Lg} values for hexachlorobenzene are greater than the generally accepted threshold of concern of 1. For each chemical, the BMF_{Lg}s in trout are higher than the BMF_{Lg}s in carp. For carp, the BMF_{Lg}s are higher at higher exposure concentrations, except for hexachlorobenzene. Except for hexachlorobenzene, this trend may again reflect the higher α values obtained at higher exposure concentrations (see Table 4).

Table 6 Comparisons of the kinetic, lipid normalized, growth corrected biomagnification factor (BMF_{Lg}; kg/kg) data for trout and carp from Lab 2.

	Chemical			
Species / Treatment	**HCB**	**MX**	**oTP**	**MC**
Trout (1x)	1.9	0.94	0.59	0.37
Carp (level 1 – 10x)	1.3	0.43	0.25	0.04
Carp (level 2 – 5x)	1.4	0.38	0.15	0.03
Carp (level 3 – 1x)	1.7	0.31	0.05	0.02
Geometric mean - carp	1.5	0.37	0.12	0.029
95% CI	1.0 to 2.1	0.25 to 0.56	0.016 to 0.90	0.013 to 0.067

10. The bioconcentration factor (BCF; L/kg) was calculated from the two types of depuration rate constant data (k_2 and k_{2g}) using selected published gill uptake rate constant (k_1; L/kg/d) models. The k_1 models considered here are: (i) Barber 2001 - standard [16], (ii) Erickson and McKim 1990 – standard

[17], (iii) Sijm *et al.*1995 [14], (iv) Hendriks *et al.* 2001 [11], (v) Barber 2003 – calibrated [18], (vi) Arnot and Gobas 2004 [19], and (vii) Arnot *et al.* 2008 [20]. For input parameters, models i, ii and iii require fish mass; models iv and v require fish mass and chemical K_{OW}; model vi requires fish mass, chemical K_{OW}, and dissolved oxygen concentration (or system temperature); and model vii requires fish mass, chemical K_{OW}, and system temperature.

11. The k_1 models were parameterized using log K_{OW} values of 5.73, 4.45, 5.53, 5.08 and 6.13 for hexachlorobenzene, musk xylene, o-terphenyl, methoxychlor and benzo[a]pyrene, respectively (refer to Table 21 and related discussion on K_{OW} and K_{OW} uncertainty in paragraphs 46 and 47). The k_1 models were parameterized using the average mass during the depuration phase (g) and overall average lipid content during the test and average system temperatures during the entire test. When applicable, these values were further averaged for trout (inter-laboratory, n=6) and carp from Lab 2 (n=3). The bioavailable chemical concentration in the water was assumed to be 100% of the total water concentration. Refer to Table 17 and paragraphs 28 and 29 for further summary information on fish mass, lipid content and temperature.

12. Since the large majority of published BCF data are derived as ratios of the chemical concentration in the fish and the chemical concentration in the water assuming steady state conditions or kinetically using k_1 and the overall total depuration rate data (k_2) [2], the BCFs calculated from the dietary exposure data using k_2 are considered more relevant for comparisons here. The majority of published BCF data are based on wet weight organism concentrations (*i.e.* lipid content data were not typically provided) [2]; therefore, the BCFs calculated and presented for comparisons here are on a wet weight basis.

13. Table 7 summarizes the BCF calculations using k_2 and Table 8 summarizes the BCF calculations using k_{2g} for Lab 2 trout and carp data. Two sets of carp BCFs are calculated. The first uses the level 3 test data (*i.e.* consistent with the exposure concentration in the trout test) and the second uses the average k_2 and k_{2g} values from levels 1, 2, and 3. The BCFs calculated with the k_{2g} data are higher than the BCF calculated with the k_2 data reflecting the lower values in the denominator of the kinetic BCF equation, *i.e.* k_1 / k_2 or k_1 / k_{2g}. Comparative BCFs range by about a factor of 2 between the lowest and highest values for these chemicals reflecting the differences in k_1 model estimates.

Table 7 Comparisons of bioconcentration factor (BCF; L/kg) data for trout and carp from Lab 2 calculated using measured k_2.

Species / Chemical	Modelled BCF								
	Barber 2001 (16)	**Erickson & McKim 1990 (17)**	**Sijm *et al.*1995 (14)**	**Hendriks *et al.* 2001 (11)**	**Barber 2003 calibrated (18)**	**Arnot and Gobas 2004 (19)**	**Arnot *et al.* 2008 (20)**	**Mean**	**SD**
Trout									
HCB	11,513	7,976	5,756	9,390	5,918	10,241	7,121	8,274	2,195
MX	6,259	4,336	3,129	3,962	3,206	5,551	3,860	4,329	1,174
oTP	6,648	4,605	3,324	5,372	3,417	5,913	4,111	4,770	1,264
MC	3,960	2,743	1,980	3,060	2,034	3,521	2,448	2,821	743
BaP	219	152	110	181	113	195	136	158	42
Carp (level 3, *n*=1)									
HCB	10,119	7,236	5,455	8,609	5,853	10,951	10,355	8,368	2,230
MX	4,507	3,223	2,430	2,976	2,598	4,865	4,600	3,600	1,027
oTP	1,653	1,182	891	1,393	956	1,789	1,691	1,365	364
MC	1,907	1,364	1,028	1,537	1,102	2,063	1,950	1,565	420
BaP	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Carp (*n*=3)									
HCB	8,918	6,349	4,757	7,542	5,073	9,530	9,094	7,323	1,966
MX	3,773	2,686	2,013	2,477	2,139	4,021	3,837	2,992	859
oTP	1,582	1,126	844	1,326	900	1,691	1,613	1,297	349
MC	1,691	1,204	902	1,355	962	1,807	1,724	1,378	372
BaP	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 8 **Comparisons of bioconcentration factor (BCF; L/kg) data for trout and carp from Lab 2 calculated using measured k_{2g}.**

Species	Modelled BCF								
Chemical	**Barber 2001 (16)**	**Erickson and McKim 1990 (17)**	**Sijm *et al.* 1995 (14)**	**Hendriks *et al.* 2001 (11)**	**Barber 2003 calibrated (18)**	**Arnot and Gobas 2004 (19)**	**Arnot *et al.* 2008 (20)**	**Mean**	**SD**
Trout									
HCB	19,973	13,836	9,986	16,290	10,266	17,766	12,353	14,353	3,808
MX	8,059	5,583	4,029	5,102	4,128	7,149	4,971	5,574	1,512
oTP	8,667	6,004	4,334	7,004	4,455	7,709	5,360	6,219	1,647
MC	4,594	3,182	2,297	3,550	2,360	4,084	2,840	3,272	862
BaP	219	152	110	181	113	195	136	158	42
Carp (level 3, *n*=1)									
HCB	33,055	23,638	17,821	28,122	19,120	35,774	33,826	27,336	7,284
MX	6,357	4,546	3,427	4,198	3,664	6,860	6,487	5,077	1,448
oTP	1,907	1,364	1,028	1,607	1,103	2,064	1,951	1,575	420
MC	2,156	1,542	1,162	1,738	1,246	2,332	2,205	1,769	474
BaP	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Carp (*n*=3)									
HCB	22,295	15,872	11,893	18,856	12,683	23,826	22,735	18,309	4,915
MX	5,218	3,715	2,783	3,425	2,958	5,561	5,306	4,138	1,188
oTP	1,752	1,247	934	1,468	996	1,872	1,786	1,437	386
MC	1,887	1,343	1,006	1,512	1,072	2,015	1,923	1,537	415
BaP	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Inter-Laboratory Comparison

14. The dietary bioaccumulation test result parameters for the carp (Lab 2) and the trout from the different laboratories were compared using the mean results from all of the other trout studies (*i.e.* Labs 1, 3, 4, 6, 7 and 8; n = 6). Lab 2 and Lab 5 data for trout were not included in these analyses. These comparisons primarily include the following parameters:

- Concentrations of each test chemical in fish at the start of depuration estimated from depuration curves ($C_{0,depuration}$; μg/g);
- Depuration rate constants (k_2; 1/day);
- Depuration rate constants, growth corrected (k_{2g}; 1/day);
- Chemical assimilation efficiencies calculated from depuration data (α; unitless);
- Kinetic biomagnification factors, lipid corrected (BMF_L; kg/kg);
- Kinetic biomagnification factors, growth and lipid corrected (BMF_{Lg}; kg/kg);
- Estimated kinetic bioconcentration factors (BCF; L/kg).

15. Table 9 summarizes the concentrations of each test chemical in trout and carp at the start of depuration as estimated from depuration regressions ($C_{0,depuration}$ data from Tables 21 to 25 in the main report). The geometric means for trout are from six different labs. The 95% CI calculations used 5 degrees of freedom (*i.e.* critical value of 2.571 for the t distribution, [15]). For all chemicals, except o-terphenyl, there are no significant differences in the $C_{0,depuration}$ estimates for carp (level 3 treatment from Lab 2) and the mean $C_{0,depuration}$ estimates for trout (same target dietary exposure concentrations) from the other laboratories in the ring test. For o-terphenyl, the $C_{0,depuration}$ in carp from Lab 2 is lower than the mean value in trout from the six other labs. There is substantial uncertainty in the $C_{0,depuration}$ estimate in carp for methoxychlor (presumably the result of a missing data point in the depuration phase, *i.e.* n = 3 instead of n = 4). For benzo[a]pyrene, $C_{0,depuration}$ estimates could not be derived for carp due to insufficient data during the depuration phase as a result of rapid elimination; however, $C_{0,depuration}$ estimates could be derived for trout in some of the other laboratories. Measured dietary concentrations among labs were not always consistent and could deviate from the target concentrations (see Table 14 in the main report).

Table 9 Comparisons of the chemical concentration data (μg/g) for trout (inter-laboratory, *n*=6) and carp from Lab 2 (level 3) estimated at the start of depuration.

	Chemical			
Species / Treatment	**HCB**	**MX**	**oTP**	**MC**
Geometric mean - trout	3.72	4.85	3.57	2.59
95% CI	2.66 to 5.21	2.98 to 7.91	2.69 to 4.73	1.89 to 3.53
Carp (level 3 – 1x)	2.31	3.08	0.81	0.63
95% CI	1.25 to 4.27	1.99 to 4.75	0.54 to 1.20	0.0029 to 133.62

16. Table 10 lists means, standard deviations (SD), relative standard deviations (RSD) and 95% CIs for the overall depuration rate constants (k_2) calculated from Lab 2 data for carp (mean of three treatments) and six other laboratories for trout (k_2 data from Tables 21 to 25 in the main report). Considering variability and uncertainty, the k_2 values are comparable for hexachlorobenzene and musk xylene; however, the k_2 for o-terphenyl and methoxychlor for carp are notably larger (faster elimination) than the k_2 for trout. The relative variability in the k_2 data for o-terphenyl and methoxychlor for trout is also noticeably greater. For benzo[a]pyrene, k_2 estimates could not be derived for carp due to insufficient data during the depuration phase (rapid elimination), whereas k_2 estimates could be derived for some of the tests using trout.

Table 10 Comparisons of the overall depuration rate constant (k_2, day^{-1}) data for trout (inter-laboratory, *n*=6) and carp from Lab 2 (*n*=3).

	Chemical			
Species	**HCB**	**MX**	**oTP**	**MC**
Mean - trout	0.054	0.091	0.099	0.14
SD	0.0051	0.015	0.022	0.049
RSD	9%	16%	23%	36%
95% CI	0.049 to 0.060	0.076 to 0.11	0.075 to 0.12	0.086 to 0.19
Mean - carp	0.055	0.13	0.31	0.29
SD	0.0059	0.015	0.033	0.023
RSD	11%	12%	11%	8%
95% CI	0.045 to 0.065	0.10 to 0.15	0.25 to 0.37	0.25 to 0.33

17. Table 11 lists means, standard deviations (SD), relative standard deviations (RSD) and 95% CIs for the growth corrected depuration rate constants (k_{2g}) calculated from Lab 2 data for carp (mean of three treatments) and six other laboratories for trout (k_{2g} data from Tables 40 to 44 in the main report). Considering variability and uncertainty, the k_{2g} values are comparable for hexachlorobenzene and musk xylene; however, the k_{2g} for o-terphenyl and methoxychlor for carp are notably larger (indicating faster depuration) than the k_{2g} for trout. In general terms, the relative variability from inter-laboratory testing for trout is greater than the intra-laboratory testing for carp.

Table 11 Comparisons of the growth corrected depuration rate constant (k_{2g}, day^{-1}) data for trout (inter-laboratory, *n*=6) and carp from Lab 2 (*n*=3).

	Chemical			
Species	**HCB**	**MX**	**oTP**	**MC**
Mean - trout	0.015	0.052	0.060	0.099
SD	0.0062	0.017	0.020	0.052
RSD	41%	34%	34%	53%
95% CI	0.0085 to 0.022	0.033 to 0.070	0.038 to 0.080	0.044 to 0.15
Mean - carp	0.022	0.094	0.28	0.26
SD	0.0059	0.015	0.033	0.023
RSD	27%	16%	12%	9%
95% CI	0.0069 to 0.036	0.057 to 0.13	0.20 to 0.36	0.20 to 0.31

18. Table 12 lists means, standard deviations (SD), relative standard deviations (RSD) and 95% CIs for the chemical dietary assimilation efficiency (α) data for carp from Lab 2 (mean of three treatments) and for trout from six different laboratories. These α data were calculated using 13 days for the exposure duration value (from Table 29 in the main report). The α values in the intra-laboratory comparison were generally larger in trout than in carp (see Table 4); however, in the inter-laboratory comparison the mean and the 95% CI values for trout and carp are similar for all chemicals. As noted earlier, the value for o-terphenyl from the carp level 3 treatment is highly uncertain. Chemical concentrations in carp were not measured in the uptake phase so it is not possible to compare the differences in α measurement methods (*i.e.* uptake phase vs. depuration phase) between carp and trout.

19. The plausible range of values for α is from 0 (no absorption of the chemical) to 1 (complete absorption of the chemical). The variability in α is notably larger in carp (intra-laboratory) for o-terphenyl and methoxychlor and notably larger in trout (inter-laboratory) for hexachlorobenzene and musk xylene. In general terms, the uncertainty in estimating α from the test data can be considerable. This parameter is subsequently used to calculate the BMF, and uncertainties and errors in its estimation will be propagated into errors when calculating the BMF.

20. Estimates for α that are less than 0 or greater than 1 are not considered plausible or reliable. The uncertainty in the α data for carp for o-terphenyl is large enough that these data should be log-transformed (*i.e.* to avoid the calculation of negative values in the 95% CI); however, for general consistency with other comparisons in the main report, the data were not transformed here. For comparisons in the treatment of the α data for carp, the values in Table 12 can be compared with the values in Table 4 (log-transformed).

Table 12 Comparisons of the chemical dietary assimilation efficiency (α; unitless) data for trout (inter-laboratory, *n*=6) and carp from Lab 2 (*n*=3).

	Chemical			
Species	**HCB**	**MX**	**oTP**	**MC**
Mean - trout	0.57	0.44	0.35	0.13
SD	0.16	0.16	0.08	0.02
RSD	28%	36%	24%	14%
95% CI	0.40 to 0.74	0.27 to 0.60	0.26 to 0.43	0.11 to 0.15
Mean - carp	0.38	0.44	0.50	0.10
SD	0.04	0.12	0.30	0.04
RSD	11%	27%	59%	36%
95% CI	0.27 to 0.49	0.15 to 0.74	-0.23 to 1.23	0.01 to 0.18

21. Table 13 summarizes kinetic, lipid normalized biomagnification factors (BMF_L) for carp from Lab 2 (mean of three treatments) and for trout from six different laboratories. These values were calculated using 13 days for the exposure duration value, the overall depuration rate constant (k_2), and the average lipid content during the test (test organisms only, not including control fish lipid measurements). The nominal daily ingestion rate of 0.03 g-food / g-fish was assumed in the calculations. All of the mean BMF_L data are less than the generally accepted threshold of concern of 1. For each chemical, the mean BMF_L in trout (inter-laboratory) is larger than the mean BMF_L in carp (intra-laboratory). For methoxychlor, the 95% CIs for the BMF_L are larger for trout than for carp. The variability in BMF_L is larger in carp (intra-laboratory) for o-terphenyl and methoxychlor and larger in trout (inter-laboratory) for hexachlorobenzene and musk xylene. In certain cases, the uncertainty in estimating BMF_L from the test data can be considerable. BMFs less than 0 are not plausible. The high RSD and negative value in the 95% CI for o-terphenyl suggest that these data should probably be transformed; however, this transformation in the data was not done here so that the treatment of these data is consistent with the treatment of similar data in the main report.

Table 13 Comparisons of the kinetic, lipid normalized biomagnification factor (BMF_L; kg/kg) data for trout (inter-laboratory, *n*=6) and carp from Lab 2 (*n*=3).

	Chemical			
Species	**HCB**	**MX**	**oTP**	**MC**
Mean - trout	0.86	0.39	0.28	0.079
SD	0.33	0.13	0.052	0.020
RSD	38%	34%	18%	25%
95% CI	0.51 to 1.2	0.25 to 0.53	0.23 to 0.34	0.058 to 0.099
Mean - carp	0.56	0.28	0.13	0.027
SD	0.023	0.055	0.086	0.0090
RSD	4%	20%	66%	34%
95% CI	0.50 to 0.62	0.14 to 0.42	-0.083 to 0.35	0.0045 to 0.049

22. Table 14 summarizes kinetic, lipid normalized and growth corrected biomagnification factors (BMF_{Lg}) for carp from Lab 2 (mean of three treatments) and for trout from six different laboratories. These values were calculated using 13 days for the exposure duration value, the growth corrected depuration rate constant (k_{2g}), and the average lipid content during the test (test organisms only, not including control fish lipid measurements). The nominal daily ingestion rate of 0.03 g-food / g-fish was assumed in the calculations. For hexachlorobenzene, the mean BMF_{Lg} data for trout and carp are greater than 1. For musk xylene, the upper 95% confidence limit for the mean based on inter-laboratory data for trout is greater than 1; however, for carp the upper 95% confidence limit is less than 1. For all other chemicals and tests the upper 95% confidence limits are less than 1.

23. For each chemical, the mean BMF_{Lg} in trout (inter-laboratory) is larger than the mean BMF_{Lg} in carp (intra-laboratory). The mean BMF_{Lg} values in trout range from about a factor of 2 to a factor of 4 times higher than the mean BMF_{Lg} values in carp. For methoxychlor, the 95% CIs for BMF_{Lg} are larger for trout than for carp. The variability in BMF_{Lg} is larger in carp (intra-laboratory) for o-terphenyl and larger in trout (inter-laboratory) for hexachlorobenzene and musk xylene. The inter- and intra-laboratory variability for the estimate of BMF_{Lg} for methoxychlor is comparable. The 95% CIs for BMF_{Lg} range from less than a factor of 2 to about a factor of 10. In certain cases, the uncertainty in estimating BMF_{Lg} from the test data can be considerable.

Table 14 Comparisons of the kinetic, lipid normalized, growth corrected biomagnification factor (BMF_{Lg}; kg/kg) data for trout (inter-laboratory, *n*=6) and carp from Lab 2 (*n*=3).

	Chemical			
Species	**HCB**	**MX**	**oTP**	**MC**
Mean - trout	3.30	0.74	0.48	0.12
SD	1.13	0.32	0.10	0.039
RSD	34%	44%	21%	33%
95% CI	2.1 to 4.5	0.40 to 1.1	0.38 to 0.59	0.078 to 0.16
Mean - carp	1.46	0.38	0.15	0.030
SD	0.21	0.06	0.10	0.010
RSD	15%	16%	66%	33%
95% CI	0.93 to 2.0	0.23 to 0.52	-0.10 to 0.39	0.0058 to 0.055

24. The bioconcentration factor (BCF; L/kg) was calculated from the depuration rate constant data (k_2 and k_{2g}) using selected published gill uptake rate constant (k_1; L/kg/d) models. The approach here follows the approach outlined in paragraphs 10, 11 and 12. Since the large majority of published BCF data are derived as ratios of the chemical concentration in the fish and the chemical concentration in the water assuming steady state conditions or kinetically using k_1 and the overall total depuration rate data (k_2) [2], the BCFs calculated from the dietary exposure data using k_2 are considered more relevant for comparisons here. The majority of published BCF data are based on wet weight organism concentrations (*i.e.* lipid content information was not typically provided) [2]; therefore, the BCFs calculated and presented for comparisons here are on a wet weight basis.

25. Table 15 summarizes the BCF calculations using k_2 and Table 16 summarizes the BCF calculations using k_{2g}. The BCF values for carp (levels 1, 2, and 3) were calculated using the mean k_2 and k_{2g} and are compared against the mean trout values from six different labs. The BCFs calculated with k_{2g} are higher than BCFs calculated with k_2 reflecting the lower values in the denominator. Comparable BCFs range by about a factor of 2 between the lowest and highest values for these chemicals reflecting the difference in the k_1 model estimates.

Table 15 **Comparisons of bioconcentration factor (BCF; L/kg) data for trout (inter-laboratory) and carp from Lab 2 calculated using measured k_2.**

Species	**Modelled BCF**								
Chemical	**Barber 2001 (16)**	**Erickson and McKim 1990 (17)**	**Sijm *et al.* 1995 (14)**	**Hendriks *et al.* 2001 (11)**	**Barber 2003 calibrated (18)**	**Arnot and Gobas 2004 (19)**	**Arnot *et al.* 2008 (20)**	**Mean**	**SD**
Trout (*n*=6)									
HCB	10,211	7,632	6,114	9,212	6,961	10,869	6,233	8,176	1,927
MX	6,059	4,529	3,628	4,243	4,116	6,432	3,688	4,671	1,125
oTP	5,569	4,163	3,335	4,978	3,796	5,928	3,400	4,453	1,047
MC	3,938	2,944	2,358	3,367	2,683	4,190	2,403	3,126	729
BaP	430	321	257	392	293	458	262	345	81
Carp (*n*=3)									
HCB	8,918	6,349	4,757	7,542	5,073	9,530	9,094	7,323	1,966
MX	3,773	2,686	2,013	2,477	2,139	4,021	3,837	2,992	859
oTP	1,582	1,126	844	1,326	900	1,691	1,613	1,297	349
MC	1,691	1,204	902	1,355	962	1,807	1,724	1,378	372
BaP	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 16 **Comparisons of bioconcentration factor (BCF; L/kg) data for trout (inter-laboratory) and carp from Lab 2 calculated using measured k_{2g}.**

Species	**Modelled BCF**								
Chemical	**Barber 2001 (16)**	**Erickson and McKim 1990 (17)**	**Sijm *et al.* 1995 (14)**	**Hendriks *et al.* 2001 (11)**	**Barber 2003 calibrated (18)**	**Arnot and Gobas 2004 (19)**	**Arnot *et al.* 2008 (20)**	**Mean**	**SD**
Trout (*n*=6)									
HCB	36,758	27,474	22,009	33,164	25,058	39,130	22,439	29,433	6,936
MX	10,603	7,925	6,349	7,425	7,203	11,256	6,455	8,174	1,969
oTP	9,190	6,869	5,502	8,214	6,264	9,782	5,609	7,347	1,727
MC	5,569	4,163	3,335	4,761	3,794	5,926	3,398	4,421	1,032
BaP	444	332	266	404	303	472	271	356	84
Carp (*n*=3)									
HCB	22,295	15,872	11,893	18,856	12,683	23,826	22,735	18,309	4,915
MX	5,218	3,715	2,783	3,425	2,958	5,561	5,306	4,138	1,188
oTP	1,752	1,247	934	1,468	996	1,872	1,786	1,437	386
MC	1,887	1,343	1,006	1,512	1,072	2,015	1,923	1,537	415
BaP	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Inter-Species Variability

26. The general objective of this section is to draw upon previous knowledge regarding possible reasons for species-specific differences in the ring test results. As discussed earlier, some evidence from the ring test suggests that carp may have greater capacity to biotransform certain organic chemicals compared to trout. Thus, exploring possible species differences in biotransformation capacity will be an important aspect of this analysis. It is however recognized that variability in the ring test datasets can be significant.

27. Rainbow trout (*Onchorhynchus mykiss*) is representative of most salmonids (family Salmonidae) and the Common carp (*Cyprinus carpio*) is representative of many cyprinids (family Cyprinidae). These species are often considered for chemical testing. There are some general physiological and anatomical differences between these two families that may contribute to differences in dietary bioaccumulation testing results, such as:

- Carp are omnivorous (in nature they are predominantly benthic and primary consumers), whereas trout are largely carnivorous (in nature they are predominantly pelagic);
- Carp have pharyngeal teeth (some maceration of food), whereas trout have simple teeth, they do not have pharyngeal teeth (prey is typically ingested whole);
- Carp do not have stomachs, whereas trout have stomachs;
- The gastrointestinal tract of carp is long and exists throughout the visceral cavity, the gastrointestinal tract of trout is comparatively short;
- Carp liver has no defined shape and is integrated throughout and around the intestinal tract – the liver fills much of the visceral space, whereas trout liver has a defined shape, size and location in the visceral cavity.

28. The scientific literature shows that body size and lipid content influence chemical elimination (depuration) rates in fish and other species (*e.g.* [11]). The general observed relationships are that depuration rates decrease with increasing body size and increasing lipid contents. Table 17 compares the body size (from Table 26 in the main report) and lipid contents (from Table 19 in the main report) from the different labs for trout and carp. For the inter-laboratory comparisons, carp were slightly larger and fatter than trout and for the intra-laboratory comparisons trout were slightly larger and fatter than carp. These small differences are not considered to be significant for explaining possible differences in measured bioaccumulation parameters during the ring test. If differences in body size were several fold different and if differences in lipid contents were greater, then the ring test bioaccumulation parameters may be more noticeably different.

29. The water temperature in the Lab 2 trout study was 15.2°C, the mean temperature for the three carp studies in Lab 2 was 24.7 °C, and from trout data used for inter-laboratory (n=6) comparisons the mean temperature was 14.2 °C (from Table 18 in the main report). Temperature effects on bioaccumulation parameters have been observed in other studies [10, 13]; however, there do not appear to be any noticeable inter-laboratory or intra-laboratory differences in bioaccumulation test parameters as a result of differences in water temperature.

Table 17 Comparisons of the average mass during depuration phase (g) and overall average lipid content during the test (% w/w) for trout (inter-laboratory, *n*=6) and carp from Lab 2 (*n*=3).

Lab - species (treatment)	Average mass during depuration phase (g)	Overall average lipid content during test (% w/w)
1	3.8	3.5
2 - trout	12.9	8.7
2 - carp (level 1)	8.7	5.9
2 - carp (level 2)	8.9	6.4
2 - carp (level 3)	8.0	6.4
3	7.1	6.9
4	4.2	6.9
5	11.2	9.9
6	2.5	3.8
7	2.6	5.8
8	4.7	6.6
Mean – trout (*n*=6)[a]	4.1	5.6
Mean – carp (*n*=3)	8.6	6.3

[a]studies from inter-laboratory comparisons only

30. The scientific literature indicates that dietary lipid content may influence dietary biomagnification [21-24]. Whole body (wet weight) and lipid normalized BMFs are shown to be a function of lipid contents in the organism and the diet (*e.g.* [25, 26]). For the intra-laboratory comparisons, there were no significant differences in average dietary lipid contents for trout and carp (*i.e.* Lab 2). There were differences in the lipid contents of the diet during the ring test, particularly for Labs 1 and 7 (see Table 16 in the main report); however, these differences did not seem to contribute significantly to the variability in the inter-laboratory comparisons (*i.e.* carp vs. trout) because the average values were comparable (*i.e.* 16.8% for carp vs. 15.2% for trout, *n*=6). The differences in lipid content may be of greater importance for inter-laboratory comparisons for trout species only (*i.e.* data from Labs 1 and 7).

Historical biotransformation rate data: Intra-laboratory and inter-laboratory variability

31. A method has been developed by Arnot *et al.* to estimate biotransformation rate constants (k_M; /day) in fish for organic chemicals from laboratory bioaccumulation data [20]. The mass balance method accounts for possible differences in body size, lipid content, temperature, oxygen concentration, and other parameters that may influence the elimination and biotransformation rate constants. The estimation method includes a screening-level uncertainty analysis to estimate the general confidence (uncertainty) in the k_M estimates. The estimation method was applied to a large set of bioaccumulation data that was evaluated for data quality resulting in a k_M database for approximately 700 organic chemicals [27]. The k_M estimates were normalized to a 10 g fish at 15 °C to account for possible differences in body size and temperature (noted as $k_{M,N}$ estimates). In the published study, $k_{M,N}$ values for carp and trout were

compared for 18 chemicals. There was general agreement in the rate constants between the species (slope of 1.08 from a log-log regression) and the general trend in the data suggested that trout may have a slightly greater capacity to biotransform these chemicals than carp [27]. However, it must be recognized that the data for those 18 chemicals are from different laboratories using different exposure regimes for a limited number of chemicals.

32. Table 18 lists the data used in the previous carp-trout $k_{M,N}$ analysis (*i.e.* Figure 2B in *ref.* [27]) and includes the screening-level uncertainty estimate for $k_{M,N}$ expressed as a confidence factor (*Cf*). A *Cf* of 2 implies there is a 95% probability that the median value lies within a factor of 2 of the estimated value in a log-normal distribution (assumed here). The list of chemicals includes hexachlorobenzene and o-terphenyl that were also included in the ring test set. For hexachlorobenzene, there are no recognizable differences in the $k_{M,N}$ estimates between the two species. For o-terphenyl, the absence of overlap in the 95% CIs suggests that the $k_{M,N}$ in trout is faster than the $k_{M,N}$ in carp. These results for o-terphenyl are the opposite of the results found in the ring test. It is noted that the data in Table 18 for hexachlorobenzene and o-terphenyl are from the same laboratory which should minimize possible inter-laboratory variation.

Table 18 Comparisons of metabolic biotransformation rate constant ($k_{M,N}$; day^{-1}) estimates for trout and carp from previous laboratory studies [27]. The $k_{M,N}$ calculations include uncertainty estimates expressed as confidence factors (*Cf*).

		Trout		Carp	
CAS RN	**Chemical name**	$k_{M,N}$	*Cf*	$k_{M,N}$	*Cf*
50-29-3	1,1-(2,2,2-Trichloroethylidene)bis(4-chlorobenzene)	3.45E-02	2.1	6.91E-03	9.9
208-96-8	acenaphthylene	1.09E+00	2.7	1.15E-01	9.7
333-41-5	phosphorothioic acid, o,o-diethyl o-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] ester	5.04E+00	3.7	1.78E+01	3.7
634-66-2	1,2,3,4-tetrachlorobenzene	5.06E-01	4.0	2.13E-01	9.1
1746-01-6	2,3,7,8-tetrachlorodibenzo[b,e][1,4]dioxin	2.43E-02	5.1	1.79E-02	5.6
5103-74-2	1,2,4,5,6,7,8,8-Octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene	5.95E-03	4.2	2.30E-02	2.5
84-15-1	o-terphenyl	4.34E-02	4.1	1.98E-03	3.4
118-74-1	hexachlorobenzene	9.50E-03	8.2	3.70E-03	10.5
120-12-7	anthracene	9.97E-01	3.4	7.04E-01	4.4
206-44-0	flouranthene	1.13E+00	3.1	9.57E-01	3.2
207-08-9	benzo[k]fluoranthene	7.76E-01	3.0	8.22E-01	3.0
217-59-4	triphenylene	9.08E-01	3.0	8.15E-01	3.1
243-17-4	benzo[b]fluorene	1.14E+00	3.0	2.50E+00	3.0
2189-60-8	*n*-octyl benzene	4.59E-01	3.0	3.78E-01	3.0
2541-69-7	7-methylbenz[a]anthracene	7.64E-01	3.0	8.15E-01	3.0
5707-44-8	4-ethylbiphenyl	1.07E+00	3.2	4.24E-01	4.0
40458-98-8	2,7-diisopropyl naphthalene	9.25E-01	3.0	1.14E-01	3.2
N/A	2-isopropyl decalin	1.29E-01	3.5	7.58E-02	5.2

New biotransformation rate data: Intra-laboratory and inter-laboratory variability

33. The mass balance, biotransformation rate constant k_M estimation method [20] was applied to the ring test data to explore possible inter-species differences in k_M between carp and trout. The method was parameterized using ring test-specific information (*i.e.* body size, lipid content, temperature, oxygen concentration). The growth corrected depuration rate constants k_{2g} were used and uncertainty (confidence factors) in these model input parameters were derived from the coefficient of variation in the k_{2g} estimates [28].

Table 19 Comparisons of metabolic biotransformation rate constant ($k_{M,N}$; day^{-1}) estimates for trout and carp from the ring test. The $k_{M,N}$ calculations include uncertainty estimates expressed as 95% confidence intervals (CI). When *n*>1 the mean is the geometric mean.

Chemical	**$k_{M,N}$**		
Species	**Mean**	**Lower 95% CI**	**Upper 95% CI**
HCB			
trout (*n*=6)	0.00088	0.00052	0.0015
trout (*n*=1)	0.0050	0.0014	0.017
carp (*n*=3)	0.0027	0.0019	0.0038
MX			
trout (*n*=6)	N/A	N/A	N/A
trout (*n*=1)	0.0013	0.000070	0.022
carp (*n*=3)	0.0016	0.0010	0.0026
oTP			
trout (*n*=6)	0.0090	0.0057	0.014
trout (*n*=1)	0.014	0.0051	0.037
carp (*n*=3)	0.064	0.047	0.087
MC			
trout (*n*=6)	0.010	0.0061	0.017
trout (*n*=1)	0.024	0.011	0.051
carp (*n*=3)	0.049	0.029	0.083
BaP			
trout (*n*=6)	0.28	0.17	0.45
trout (*n*=1)	0.68	0.42	1.1
carp (*n*=3)	N/A	N/A	N/A

34. For hexachlorobenzene, o-terphenyl, and methoxychlor, the mean $k_{M,N}$ estimates for carp are apparently faster than the mean $k_{M,N}$ estimates for trout based on inter-laboratory comparisons. Based on intra-laboratory comparisons, there are no notable differences in $k_{M,N}$ estimates for carp and trout for hexachlorobenzene and methoxychlor; however, Lab 2 carp appear to biotransform o-terphenyl faster

than Lab 2 trout. The previous inter-species (trout vs. carp), intra-laboratory comparisons showed no significant differences in $k_{M,N}$ for hexachlorobenzene, whereas trout appeared to biotransform o-terphenyl faster than carp [27]. The new and previous $k_{M,N}$ comparisons for trout and carp for o-terphenyl appear to be contradictory; however, these apparent differences may simply reflect natural variability and/or the limited sample sizes in the tests.

35. For musk xylene, reliable $k_{M,N}$ estimates could not be calculated for trout from Labs 1, 3, 4, 6, 7, or 8; therefore, no inter-laboratory comparisons between carp and trout could be made. When the value for the biotransformation rate constant is very similar or less than the value for the overall elimination rate constant, k_M cannot be quantified using the mass balance estimation method [20]. In particular, for lower K_{OW} chemicals, the gill elimination rate constant typically dominates the overall elimination rate constant. The k_M estimate for trout for musk xylene from Lab 2 is highly uncertain, further reflecting the difficulty in obtaining reliable estimates for $k_{M,N}$ for trout using the mass balance method and the ring test data for musk xylene. There do not appear to be any notable differences in $k_{M,N}$ for trout and carp based on the intra-laboratory comparison.

36. For benzo[a]pyrene, the $k_{M,N}$ estimate for trout from Lab 2 is faster than the mean $k_{M,N}$ estimate for trout from Labs 1, 6 and 8 (geometric mean). This is particularly noteworthy because for most ring test chemicals there are no apparent intra-laboratory differences between trout and carp, but there are some apparent inter-laboratory differences between trout and carp. Perhaps the trout in Lab 2 are from a strain that has a slightly enhanced capacity to biotransform some of the ring test chemicals compared to other strains of trout used in other laboratories. There could be other possible factors as well. Notably $k_{M,N}$ could not be estimated for trout from Labs 3, 4, and 7 and carp from Lab 2 because the depuration rate constants could not be determined due to rapid depuration as discussed earlier. Lab 5 data were not considered here for reasons discussed earlier.

37. For future testing, it is recommended that predictions of k_M be considered for estimating appropriate sampling regimes during the depuration phase. A k_M–QSAR model is freely and publicly available and requires only chemical structure information for k_M predictions [29-31]. Other QSARs are in development and should also be available in the future. The *in silico* predictions for k_M can be used independently or incorporated into mass balance models to determine the half-life of the chemical in fish. A general rule of thumb for the uncertainty in the k_M–QSAR predictions is a factor of about 5.5. For example, the k_M–QSAR estimate for benzo[a]pyrene for a 10 g fish at 15 °C is 0.77 /day. Considering the screening-level uncertainty in this estimate the actual rate is expected to be between 0.14 and 4.2 /day. This estimate is in strong agreement with the *in vivo* estimates from the ring test data. However, it is emphasized that this is a screening-level model and errors beyond these uncertainty estimates will exist.

Historical BMF data: Intra-laboratory and inter-laboratory variability

38. The BMF_L and BMF_{Lg} data from the ring test are compared with existing measured BMF data for trout and carp using a dietary bioaccumulation database [32]. The database includes BMF_{Lg} data for hexachlorobenzene (n=11) and o-terphenyl (n=2), only. All data are from trout, except for one hexachlorobenzene value from catfish. There are no published BMF_{Lg} data for carp for the ring test chemicals; however, some data were provided in the ring test as discussed in the main report. The historical BMF_{Lg} data were derived following similar methods as the ring test; however, there are some differences in exposure duration, exposure temperature, exposure concentrations and tissue analysis. In two of the 10 data points from trout for hexachlorobenzene, the gastro-intestinal tract and liver were not included in the tissue analysis.

39. Based on historical data, the geometric mean for hexachlorobenzene for BMF_{Lg} in trout is 1.73 (n=10, 95% CI = 1.18 – 2.54) and the BMF_{Lg} in catfish is 1.7 [32]. The geometric mean for BMF_{Lg} in trout from the ring test (not including Lab 5 data) is 2.90 (n=7, 95% CI = 2.04 – 4.13). The geometric

mean for BMF_{Lg} in carp from the Lab 2 data is 1.45 (n=3, 95% CI = 1.02 – 2.07). The values are comparable and are consistently greater than the BMF threshold of concern of 1.

40. Based on historical data, the BMF_{Lg} values for o-terphenyl in trout are 0.034 and 0.19. The geometric mean for BMF_{Lg} in trout from the ring test (not including Lab 5 data) is 0.49 (n=7, 95% CI = 0.40 – 0.60). The geometric mean for BMF_{Lg} in carp from the Lab 2 data is 0.12 (n=3, 95% CI = 0.016 – 0.90). The values are comparable and are consistently less than the BMF threshold of concern of 1.

Historical BCF data: Intra-laboratory and inter-laboratory variability

41. A database of measured BCF values [2] and the recent literature were reviewed to obtain acceptable quality measured BCF data to explore possible inter-species differences for comparisons with the BCFs estimated from the ring test data. There are some notable differences in the BCF data selected in this report compared to the main report. The BCF data were evaluated for general data quality following the methods outlined by Arnot and Gobas [2]. The general objective of the quality assessment is to reduce uncertainty in the measured data compilation, recognizing that uncertainty in the measured data still remains. Furthermore, for the BCF data originating from the Japanese testing program [33], the average BCF values were calculated and used in this report. In the main report, the BCF values from the Japanese testing program used the maximum reported BCF during the test. According to OECD 305E guidance, the BCF should be calculated as the average of the measured BCF values after the system has reached steady state [1]. The selection of maximum BCF values may explain why some of the BCF predictions calculated using the k_1 models in the main report are lower than the reported measured values in some cases.

42. The focus in this report is to compare BCFs for the ring test chemicals, primarily in carp and trout; however, acceptable quality measured BCFs in other species were also included. Table 20 summarizes the measured BCFs and compares these values with the BCFs calculated in this report for the ring test chemicals. The modelled values are detailed in Tables 7, 8, 15 and 16. It is noted that the mean BCFs are lower in carp than in trout. The comparatively lower BCFs for carp primarily reflect the larger k_2 or k_{2g} values for carp.

43. Table 20 shows that mean measured BCFs for hexachlorobenzene, for all species, and for trout and carp are comparable. Acceptable quality BCFs for musk xylene and o-terphenyl have only been measured in carp. Acceptable quality BCFs for benzo[a]pyrene have not been measured in trout or carp. There are no acceptable quality BCF data available in any fish species for methoxychlor. The measured BCFs follow the general trend observed in the depuration rate constants from the ring test, *i.e.* faster depuration rate constants result in lower BCFs for these chemicals.

44. In some cases, BCFs modelled using the overall depuration rate constant k_2 are in better agreement with measured BCFs and in other cases BCFs modelled using growth rate corrected depuration rate constants k_{2g} are in better agreement with measured BCFs. The modelled BCFs agree better with the measured BCFs for hexachlorobenzene when k_{2g} is used, *i.e.* growth corrected. Since hexachlorobenzene is the most persistent chemical in the ring test, this suggests that perhaps the growth rates in the ring test were generally faster than in the BCF tests. For musk xylene and o-terphenyl, the model agreement with measured data is generally better using the overall depuration rate constants suggesting that processes other than growth are the key contributing factors to the overall elimination in the dietary ring tests and in the literature BCF data. Therefore, growth correcting depuration rate constants for estimating BCFs may only be necessary or relevant for slowly biotransformed chemicals. For benzo[a]pyrene, the BCF model predictions are in general agreement with the measured BCFs regardless of the selected depuration rate constant. This is because the biotransformation rate for benzo[a]pyrene is relatively high so this process controls the total elimination rate constant in all cases for this substance.

Table 20 Comparisons of bioconcentration factors (BCF; L/kg) derived from various methods.

	Chemical				
	HCB	**MX**	**oTP**	**MC**	**BaP**
All species (measured)					
n	17	3	2		3
Mean	20,202	4,117	1,776		586
SD	9,190	900	1,729		236
RSD	45%	22%	97%		40%
Trout (measured)					
n	3				
Mean	17,233				
SD	4,535				
RSD	26%				
Trout (modelled, k_2)					
n	7	7	7	7	7
Mean	8,176	4,671	4,453	3,126	345
SD	1,927	1,125	1,047	729	81
RSD	24%	24%	24%	23%	24%
Trout (modelled, k_{2g})					
n	7	7	7	7	7
Mean	29,433	8,174	7,347	4,421	356
SD	6,936	1,969	1,727	1,032	84
RSD	24%	24%	24%	23%	24%
Carp (measured)					
n	2	3	2		
Mean	18,556	4,117	1,776		
SD	628	900	1,729		
RSD	3%	22%	97%		
Carp (modelled, k_2)					
n	7	7	7	7	
Mean	7,323	2,992	1,297	1,378	
SD	1,966	859	349	372	
RSD	27%	29%	27%	27%	
Carp (modelled, k_{2g})					
n	7	7	7	7	
Mean	18,309	4,138	1,437	1,537	
SD	4,915	1,188	386	415	
RSD	27%	29%	27%	27%	

45. It is noted that the comparisons made here are limited due to a general paucity of measured BCF data and because the BCFs are reported on a wet weight basis and variability in lipid contents is known to contribute to the variability in BCFs.

46. The octanol-water partition coefficient (K_{OW}) is a key chemical property often associated with bioaccumulation assessment. Chemicals typically requiring evaluation for bioaccumulation potential have K_{OW} values that span about 10 orders of magnitude (*i.e.* log K_{OW} values ranging from ~ 2 to 12, or even higher, see *e.g.* [34]). Table 21 provides a brief overview of K_{OW} values for the five chemicals included in the ring test. In comparison to the universe of chemicals requiring evaluation, the five chemicals selected for the ring test encompass a relatively small representation of chemical hydrophobicity (*i.e.* K_{OW}s only range about 1.5 orders of magnitude). Of particular mention is the apparent log K_{OW} for musk xylene because if the log K_{OW} of musk xylene is less than 5, then it is not expected to have dietary biomagnification potential in fish [26]. Furthermore, as shown in Table 21 the uncertainty in K_{OW} can also be substantial and this has implications for data interpretation and subsequent modelling.

47. In general, a broader range of chemical structures (*i.e.* biotransformation capacity) and partitioning properties (*i.e.* more chemicals with higher K_{OW}s) may have provided a better opportunity to identify possible issues with the proposed test guidelines and possible inter-species differences. Existing data show that $k_{M,N}$ values in fish span at least 6 orders of magnitude and the estimates for the selected ring test chemicals span less than 3 orders of magnitude [27]. In particular, any further "validation" should include more hydrophobic chemicals since these are the types of chemicals of greatest potential for bioaccumulation and for which the aquatic based BCF test results are likely to be most uncertain. These are also the types of chemicals that are generally well-suited for obtaining *in vivo* biotransformation rate constant estimates using mass balance methods [20, 27].

Table 21 The octanol-water partition coefficients (K_{OW}) for the ring test chemicals.

	Chemical				
	HCB	**MX**	**o-TP**	**MC**	**BaP**
Measured values	5.73	N/A	N/A	5.08	6.13
KOWWIN model estimates [30]	5.86	4.45	5.52	5.67	6.11
Model estimates (mean, *n*=6) [35]	5.68	3.85	5.53	5.12	5.93
Model estimates (SD)	0.22	0.44	0.43	0.45	0.56
Values used in BCF modelling	5.73	4.45	5.53	5.08	6.13

Summary

In general, the ring test has provided valuable information and some insight on issues that may arise when conducting the dietary bioaccumulation test broadly. It is possible that unforeseen complicating factors that were not identified in the ring test may arise for more hydrophobic chemicals because of the relatively limited range in chemical hydrophobicity in the selected ring test chemicals.

Fish growth rates in the field (natural environment) will typically be slower than the growth rates in the ring test because of lower natural feeding rates and higher activity levels (*i.e.* less energy available for growth). The main report includes data analyses for (i) growth corrected and (ii) lipid normalized, growth corrected BMFs, whereas this analysis includes comparisons of BMF data for trout and carp using (i) lipid normalized and (ii) lipid normalized, growth corrected BMFs. Hexachlorobenzene is observed to biomagnify in aquatic food webs as recognized by lipid normalized, field BMFs greater than 1. Thus, hexachlorobenzene could be viewed as a positive control for laboratory BMF testing and values greater than 1 should provide confidence in test data quality. In this context, only the lipid normalized, growth corrected BMFs for hexachlorobenzene consistently show BMFs greater than 1 in all cases (trout and carp). Growth corrected bioaccumulation parameters, *i.e.*, BMF_{Lg}, are therefore perhaps more ecologically relevant than non-growth corrected values, *i.e.*, BMF_{L}, particularly for hydrophobic chemicals that are poorly biotransformed such as hexachlorobenzene. This also suggests that for comparisons and interpretations of dietary bioaccumulation test data, the BMF results are best expressed on a lipid normalized, growth corrected basis. There may be some further considerations to reduce the feeding rate from the current proposed OECD TG feeding rate to minimize possible complications for measuring and interpreting the data derived from higher feeding rates [9].

The general findings of the ring test data analysis show that there is considerable inter-species variability in bioaccumulation parameters based on inter-laboratory and intra-laboratory comparisons. This key finding is consistent with existing inter-species comparisons of bioaccumulation and toxicity data using aquatic organisms. Primary reasons for the inter-species variability in test results are attributable to natural variability and individual differences in metabolic properties, lipid contents and to a lesser degree differences in body size and temperature. Physiological and anatomical differences may also play a contributing role in inter-species variability. Determining possible differences in bioaccumulation for different fish species is exacerbated by the paucity of measured data under controlled conditions. In relative terms, this ring test provides a consistent framework for exploring possible inter-laboratory, intra-laboratory and inter-species differences in bioaccumulation testing and test results. It has been recognized that even though substantial efforts were made to obtain consistency in the testing in different labs in this ring test, the prescribed protocol was not consistently followed in some aspects [9]. These inconsistencies may contribute to some of the variability observed in the ring test results.

The uncertainty in the estimated species-specific bioaccumulation parameters (including true measurement uncertainty and organism variability within the test system) is generally greater than any consistent, detectable inter-species variability. A primary candidate parameter for potential inter-species variability is the biotransformation rate. There is some evidence from the ring test suggesting carp may have a greater capacity to biotransform some organic chemicals at rates faster than trout. Available historical data provides conflicting evidence [27, 36-38]. Inter-species biotransformation variability may not exhibit universal trends. If differences exist, they may be chemical-, species-, and condition-specific. The physiological differences in species may also play a general role in differences in biotransformation of many organic chemicals (*e.g.* greater blood flow and surface area of the liver in carp). From a chemical screening point of view, the preliminary evidence from the ring test suggests that more conservative estimates of bioaccumulation potential (particularly through dietary exposures) may be obtained using trout rather than carp; however, due to the limited sample size, chemical domain and the conflicting

historical evidence these inferences are tenuous [27]. Due to a general lack of available comparable bioaccumulation data, it is unclear what the possible implications of selecting species other than trout and carp from the list of candidate test species included in the OECD 305 TG would have on test results. In other words, inter-species differences in bioaccumulation potential may exist due to differences in biotransformation capacity or other factors, but generalizations cannot be made based on the available data. *In vitro* testing for biotransformation capacity for a range of species may provide some better insights into bioaccumulation differences between fish species.

From a regulatory perspective the inherent variability and uncertainty in measured and modelled bioaccumulation data should be considered when evaluating chemicals for potential hazard and risk. To-date, this issue has not been adequately addressed. As highlighted in the ring test data analysis, these uncertainties can be substantial. It is recommended that the testing guidance consider methods that can better estimate uncertainties in key bioaccumulation parameters that contribute to regulatory decision making. In particular, if the BCF or BMF are calculated from other estimated bioaccumulation test parameters, the uncertainties in these test parameters will propagate into the BCF and BMF estimates.

The testing guidance provides the opportunity to obtain relatively consistent measured bioaccumulation data. In the future, these data can be further exploited by applying methods to estimate biotransformation rate constants [20]. Such data may provide more opportunities to explore possible inter-species variability and to develop k_M databases that can be used to develop and evaluate new and improved QSARs for this important bioaccumulation parameter since k_M is often also required for exposure and risk assessment [20, 39-41]. The revised OECD 305 draft protocol [42] recognizes the value in *a priori* estimation of k_M using QSAR models (*e.g.* [29]) because these data can be incorporated into mass balance models to estimate the time required for steady state to be approached in aquatic and dietary based tests (*i.e.* exposure duration) and this information can also provide guidance on sampling periods in the depuration phase. The current guidance for incorporating estimates of biotransformation rates in the OECD 305 test design in the revised draft protocol (i.e. Annex 5 in *ref.* [42]) could be improved.

Acknowledgement

I thank Don Mackay for a review of the draft report and for providing helpful comments.

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