Lipid normalisation in the OECD 305 dietary test

Chief Scientist's Group report

April 2023

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Dr Robert Bradburne
Chief Scientist

Contents

Executive summary

One of the main environmental priorities under the UK REACH Regulation is the assessment and identification of persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) substances. Criteria for identification of PBT are set out in Annex 13 of the Regulation. A key test method used to investigate bioaccumulation “B or vB” is the OECD 305 Test Guideline (Bioaccumulation in Fish: Aqueous and Dietary Exposure). This test guideline was updated in 2012 to include tests using dietary exposure. To support the update to the test guideline, the OECD together with three lead countries (UK, Germany and the Netherlands) prepared guidance on specific aspects of the test. Guidance Document on Aspects of OECD TG 305 on Fish Bioaccumulation (Series on Testing & Assessment No. 264) was published in July 2017 and covers, amongst other aspects, the lipid normalisation of the dietary biomagnification factor (BMF) to both the lipid content of the fish and the lipid content of the food used.

At a late stage during the development of the guidance, the Chemicals Evaluation and Research Institute, Japan (CERI) presented new research investigating the effect of different foods on the dietary test. CERI suggested that the dietary BMF should only be standardised for the fish lipid content, not the food lipid content. CERI also suggested the BMF should be standardised to 5% fish lipid content. The CERI data were still under consideration at the time of publication of the OECD guidance document No. 264, and consequently the guidance document suggests reporting BMF values standardised to 5% fish lipid in addition to the lipid-normalised values.

This report reviews the CERI research and considers the results in relation to other experimental and theoretical evidence, with the aim of providing further guidance for the interpretation of the results of the OECD 305 dietary accumulation test. It will also inform a future update of the OECD guidance document for the OECD 305 test guideline.

The available evidence from dietary accumulation studies suggests strongly that the growth-corrected and lipid-normalised kinetic BMF value ($BMF_{kgL,}$ as defined in the OECD 305 Test Guideline) varies depending on the lipid content of the diet/food used in the study. This is demonstrated both theoretically and experimentally by comparing studies with differing food lipid contents, and can be explained by

- differences in the fugacity capacity between diets of different lipid contents,
- differences in the apparent feeding rate when expressed on a lipid basis.

The implication of this is that the lipid normalisation method currently recommended in the OECD 305 test guidance will lead to differences in the BMF_{kgL} obtained using different diets. In particular, the BMF_{kgL} will increase as the lipid content of the diet increases. This may have regulatory consequences for the use of the BMF_{kgL} in the assessment of bioaccumulation potential.

Variability resulting from differences in lipid content in diet can be reduced by standardising the growth corrected BMF to a standard 5% lipid content in fish and not

normalising to the lipid content in the diet, i.e. the $BMF_{kg5\%}$, as proposed by Hashizume *et al*. (2018). This will allow for better comparability of results from different tests. This is similar to standardising bioconcentration factor (BCF) values to a standard 5% fish lipid content to allow comparison across different studies.

The BMF_{kgL} is however a relevant and important metric obtained from the OECD 305 dietary test as it expresses the true potential for biomagnification of a chemical that accumulates primarily in lipids (and is numerically equivalent to the fugacity ratio of the chemical in the fish lipids compared with the diet lipids).

This presents a dilemma when interpreting the results of the OECD 305 dietary test; whether to use the $BMF_{kg5\%,}$ as it is less dependent upon different lipid contents of diet, or to use the $BMF_{kgL,}$ which better represents the true biomagnification potential of a substance.

We propose that the following approach is taken to facilitate the interpretation of data:

- Whenever results from OECD 305 dietary tests are reported, they should always be reported along with the lipid content of the food.
- Both the $BMF_{kg5\%}$ and the BMF_{kgL} should be reported from the study for the following reasons:
    - The $BMF_{kg5\%}$ allows for better comparison between different studies. This will be particularly relevant if a study is carried out using a reference substance or is intended to be compared to reference or marker substances.
    - The BMF_{kgL} provides a better indication of the potential for biomagnification of the substance, as it represents the fugacity ratio between the fish and diet. However, the result should always be considered alongside the lipid content of the food used.

Although the focus of this report is on the growth-corrected BMF values, similar conclusions would also apply to the equivalent non-growth-corrected BMF values.

The fact that the BMF value obtained in the dietary accumulation study depends upon both the feeding rate used and the dietary lipid content used, potentially causes issues for interpretation. This is as the value obtained will depend upon the study design and measured parameters. This could be addressed by recommending that tests are carried out using a standard diet lipid content and feeding rate or by basing their regulatory decisions regarding bioaccumulation on endpoints from the test that are not dependent on these factors, such as the growth-corrected depuration rate constant (k_{2g}).

1 Introduction

One of the main environmental priorities under both the UK and EU REACH Regulations[1] is the assessment and identification of persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) substances, the criteria for which are set out in Annex 13 of the UK Regulations.

Accordingly, a substance fulfils the bioaccumulative criterion (B) when the bioconcentration factor in aquatic species is higher than 2000 L/kg and fulfils the very bioaccumulative criterion (vB) when the bioconcentration factor in aquatic species is higher than 5000 L/kg. The assessment of B and vB also includes 'information on the ability of the substance to biomagnify in the food chain, where possible expressed by biomagnification factors or trophic magnification factors'.

A key test method to investigate bioaccumulation is the OECD 305 Test Guideline (Bioaccumulation in Fish: Aqueous and Dietary Exposure), as revised by the OECD in 2012. To support the 2012 update, OECD together with three lead countries (UK, Germany and the Netherlands) prepared guidance on specific aspects of the test. This guidance was published in July 2017 (Series on Testing & Assessment No. 264, OECD, 2017). The guidance covers, amongst other aspects, the lipid normalisation of the dietary biomagnification factor (BMF) to both the lipid content of the fish and lipid content of the food used.

During the latter stages of development of the guidance, the Chemicals Evaluation and Research Institute, Japan (CERI) presented new research investigating the effect of different spiked foods in the dietary test. CERI suggested that, based on their research findings, the dietary BMF should only be corrected for fish lipid content, not food lipid content. CERI also suggested that BMF should be standardised to 5% fish lipid. A further outcome from the CERI research was that, if standardisation to 5% fish lipid is performed, the addition of lipid to the food does not appear to affect the test results. Subsequently a paper of the study was published in an academic journal (Hashizume *et al*., 2018). This

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

The EU REACH Regulation was brought into UK law, as amended by the REACH (EU Exit) Regulations 2019 (Statutory Instrument 2019 No 758), on 1st January 2021.

work is acknowledged in the published OECD guidance (footnote 13, page 62 of OECD, 2017).

This report reviews the CERI/Hashizume *et al*. (2018) study and considers the results in relation to other experimental and theoretical evidence.

Please note that the terms “diet” and “food” are used interchangeably in this report.

2 Basis of lipid normalisation in the OECD 305 test

The terms 'lipid correction' and 'lipid normalisation' tend to be used interchangeably in the OECD 305 test guideline, and this can lead to confusion. For clarity, the term 'lipid normalisation' is used in this report when describing the method currently used in the guideline:

- **Lipid normalisation**: This is correction of the BMF to the lipid content of **both** the fish **and** food used.

The current method described in the OECD 305 test guideline for lipid normalisation of a BMF value is via a lipid correction factor (Lc) as shown below (Equation **1**; taken from OECD, 2012a). This results in a lipid-normalised BMF where both the concentration in fish and concentration in food are expressed on a lipid weight basis Equation **2**).

$$\boldsymbol{Lc = \frac{L_{fish}}{L_{food}}}$$

Equation 1

$$\boldsymbol{BMF_{kgL} = \frac{BMF_{kg}}{Lc} = BMF \times \frac{L_{food}}{L_{fish}}}$$

Equation 2

Where Lc = lipid correction factor.

L_{fish} = weight fraction of lipid in fish.

L_{food} = weight fraction of lipid in food.

BMF_{kg} = Growth-corrected kinetic dietary BMF (value not lipid-normalised).

BMF_{kgL} = Growth-corrected and lipid-normalised kinetic dietary BMF.

- **Lipid standardisation**: This is correction of the BMF to a standard lipid content of the **fish only**.

The alternative method described by Hashizume *et al*. (2018) for lipid standardisation of a BMF value is to standardise the BMF to a 5% lipid content as shown below (Equation **3**; taken from Hashizume *et al*. (2018)). This results in a BMF where the concentration in fish is expressed on a lipid weight basis and the concentration in food is not lipid-corrected.

$$\boldsymbol{BMF_{kg5\%} = \frac{BMF_{kg}}{L_{fish}} \times 0.05}$$

Equation 3

Where $BMF_{kg5\%}$ = Growth-corrected and lipid-standardised kinetic dietary BMF (standardised to a 5% fish lipid content).

The $BMF_{kg5\%}$ is equivalent to expressing the concentrations in fish on a lipid weight basis and the concentrations in food on a whole food weight basis.

3 CERI study (Hashizume *et al.*, 2018)

Details of the CERI study are published in Hashizume *et al.* (2018). The study was a ring test of the OECD 305 dietary accumulation test guideline using common carp (*Cyprinus carpio*). Nine studies were carried out within six laboratories, operating to good laboratory practice. All tests were carried out using hexachlorobenzene (HCB) which is a substance that is known to bioaccumulate and is often used as a reference substance in the dietary accumulation test.

The key parameters of the test are summarised in Table 3.1.

Table 3.1 Test details used in the CERI study (Hashizume *et al.*, 2018)

Parameter	Value	Comment
Concentration of HCB	100 µg/g food	The measured concentrations[#] used in the nine tests were between 85.2 and 99.0 µg/g food.
Daily feeding rate	0.02 or 0.03 g food/g live fish/d	Fed at 2% or 3% body weight daily, provided in two feedings spaced 30 minutes apart. Fish were allowed to feed naturally. The amount of feed was stated to have been adjusted at each sampling point to account for growth. However, there does not appear to have been a sampling point during the uptake phase (see Section 3.4). It was not stated whether uneaten food or faecal matter was removed from the test system during the test.
Fish body weight at the start of test	4.95 ± 0.83 g	Mean ± standard deviation; n = 20
Uptake phase	10 – 13 days	
Depuration phase	15 – 29 days	Depuration was carried out until at least one depuration half-life had passed.

Parameter	Value	Comment
Temperature	25°C ± 2°C	
pH of test water	7.5 – 8.1	
Dissolved oxygen content of test water	7.6 – 8.0 mg/L	Maintained by aeration

[#]Quantification of HCB was by gas chromatography-mass spectrometry (GC-MS)

3.1 Food preparation methods

Each of the nine tests were carried out under different conditions with respect to the food, food preparation method, or feeding rate. In all, a total of four fish pellet feeds (feeds A – D), five methods to prepare the test food (methods I – V), and two feeding rates (2% or 3% body weight/day) were investigated. The food preparation methods are summarised below.

- Methods I to IV started by dissolving HCB in acetone at a concentration of 1000 mg/L:
  - Method I. A 20 mL sample of the HCB in acetone solution was added to 200 g of fish pellet feed, mixed well, and the acetone was removed via a nitrogen purge. The mixture was homogenized and dried under ambient conditions for approximately 24 hours.
  - Method II. A 20 mL sample of the acetone solution was added to fish feed oil (20 g, stated to be Riken Feed Oil Omega; Eiken Shoji) and thoroughly mixed. The acetone was removed by rotary evaporation at 40°C for 20 minutes. The fish pellet feed (180 g) was added to the oil, and the food was homogenized and dried under ambient conditions for approximately 24 hours.
  - Method III. This was the same as for method II, but corn oil was used in place of fish feed oil.
  - Method IV. A 20 mL sample of the acetone solution was added to 180 g of fish pellet feed and thoroughly mixed. The acetone was removed by rotary evaporation at 40°C for 20 minutes. Twenty grams of fish feed oil was added, and the food was homogenized and dried under ambient conditions for approximately 24 hours.
- Method V. A 10 g/L suspension of HCB in fish feed oil was prepared. A 2 mL sample of this was added to 200 g of fish pellet feed and mixed thoroughly.

The lipid contents in the prepared foods ranged between 5.23% and 17.1% w/w. The concentration of HCB in the test foods was analysed in triplicate at the beginning and end

of the uptake phase. The measured concentration in the food ranged between 85.2 µg/g to 99.0 µg/g and varied by no more than ± 10%.

3.2 BMF values for HCB obtained in the CERI study

During the test, fish were sampled at the end of the uptake phase and five to six times during the depuration phase.

The BMF and other parameters were calculated using the standard equations in the OECD 305 Test Guideline. The BMF values obtained in the study are summarised in Table 3.2.

Table 3.2 Summary of the BMF values for HCB obtained in the CERI study (Hashizume *et al*., 2018)

Parameter	Test								
	A	B	C	D	E	F	G	H	I
Feeding rate (% body weight/day)	2	2	3	3	3	3	3	3	3
Assimilation efficiency (α)	1.09	0.618	0.667	0.502	0.441	0.610	0.881	0.587	0.669
Growth-corrected kinetic BMF (BMF_{kg})	0.121	0.289	0.150	0.437	0.162	0.199	0.315	0.552	0.530
Growth-corrected and lipid-normalised BMF (BMF_{kgL})	0.267	0.998	0.285	1.31	0.569	0.912	1.23	1.40	1.36
Growth-corrected kinetic BMF standardised to 5% fish lipid content ($BMF_{kg5\%}$)	0.235	0.344	0.272	0.451	0.205	0.283	0.359	0.463	0.409

The raw data from the tests was kindly provided for the current project by the authors of the Hashizume *et al*. (2018) paper. Re-analysis of the data confirmed the depuration rate

constants, growth rate constants, assimilation efficiencies, and BMF values reported in the paper. In addition, there were no obvious deviations from the expected first order kinetics during the depuration phase. The BMF values quoted in Hashizume *et al*. (2018) were determined using the derived time zero concentration in the fish at the start of the depuration phase. The analysis was also performed using the measured time zero concentration in fish at the end of the uptake phase; this did not result in any major differences in final results. For Test A, the assimilation efficiency determined by Hashizume *et al*. (2018) was just over 1.09 (109%) which is theoretically impossible. This high value was confirmed in the re-analysis, where the derived time zero concentration in fish at the start of the depuration phase was used. However, when the measured time zero concentration in fish at the end of the uptake phase was used, the assimilation efficiency was calculated to be 0.81 (81%). This had only a minor effect on the derived growth-corrected BMF_{kg}, reducing it from 0.12 to 0.091, and would not significantly affect the overall conclusions of the study.

In general, the raw data showed that the lipid contents of the fish were reasonably constant over the entire study period (measurements were taken as a minimum at the start of the test, and at the end of the uptake and depuration phases; in some tests measurements were taken at each sampling time). The average lipid contents over the entire test period were used in the analysis by Hashizume *et al*. (2018) and again in the re-analysis undertaken for this report.

Based on this review of the raw data, it is concluded that the tests were generally well conducted, followed the OECD 305 test guideline closely, and are considered reliable.

3.3 Comparison of lipid-normalised vs. lipid-standardised BMF values

Hashizume *et al*. (2018) reported kinetic, growth-corrected and lipid-normalised BMFs (termed BMF_{kgL} in the paper) that were estimated using the equations from the OECD 305 test guideline. It was noted by Hashizume *et al*. (2018) that the growth-corrected lipid-normalised biomagnification factor BMF_{kgL} differed markedly between tests using diets with different lipid contents. For example, in Tests A and C, which used diets with approximately 5% lipid contents, the BMF_{kgL} values were 0.267 and 0.285, compared with values between 0.569 and 1.40 that were determined in the tests using higher lipid content diets. The overall mean and 95% confidence interval for the BMF_{kgL} values across all studies was 0.925 and 0.587 – 1.27.

Hashizume *et al*. (2018) found that there was much better agreement between the data if the BMF values were standardised only to a standard fish lipid content of 5%. When this was done, the 5% lipid-standardised growth-corrected BMF ($BMF_{kg5\%}$) values varied only by a factor of 2 between the tests (range 0.205 – 0.463; average 0.336; 95% confidence interval 0.264-0.407). It was concluded that lipid-standardisation to a standard fish lipid content alone may be a better way of analysing dietary toxicity test results than normalising the data to both fish and food lipid. In light of this, the authors recommended

that dietary BMF values are reported both as lipid-normalised values and as values standardised to 5% fish lipid only.

3.4 Uncertainties in the CERI study

Although the Hashizume *et al*. (2018) is considered a well conducted and reliable study, there remain a few, relatively minor, areas where the paper is not clear:

- It is not clear how long after the last feeding the fish samples were taken, i.e., whether the fish were allowed empty their stomach contents prior to analysis.
- It was not stated whether uneaten food or faecal matter were removed from the test system during the test.
- It is stated that the feeding rate was corrected for growth at each sampling point, but according to the analytical methodology and raw data, fish were first sampled at the end of the uptake phase. This means that it is likely that the feeding rates were not adjusted for growth during the uptake phase. This is not uncommon in this type of study, but it means that the actual feeding rate may have declined slightly during the uptake phase. The paper states that there was no marked difference between the two different feeding rates, based on the results of Test A (BMF_{kgL} = 0.267 at a 2% feeding rate) and Test C (BMF_{kgL} = 0.285 at a 3% feeding rate) or Test B (BMF_{kgL} = 0.998 at a 2% feeding rate) and Test D (BMF_{kgL} = 1.31 for a 3% feeding rate). While this is technically true based on these results, it should be noted that the theory (Section 5 of this report) indicates that the BMF at a 3% feeding rate should only be a factor of 1.5 times that at a 2% feeding rate (i.e., 3/2 = 1.5) with all other things being equal; therefore, given the other uncertainties in the determination of a BMF, it is unlikely that such a difference would be detectable in reality.

However, these points are not considered to affect the overall findings of the study.

4 Other relevant studies

4.1 Papers cited in the CERI study as support for lack of influence of dietary lipid content

The CERI study cites three main papers as support for the lack of influence of dietary lipid content on dietary uptake. These papers, namely Gobas *et al*. (1993), Sharifi *et al*. (1997) and Liu *et al*. (2010), are briefly reviewed below.

4.1.1 Effect of lipid on uptake of organochlorines in goldfish - Gobas *et al*. (1993)

The dietary uptake of several organochlorine substances in goldfish (*Carassius auratus)* has been investigated using diets with different lipid contents (Gobas *et al*., 1993). The study investigated the mechanism of intestinal absorption and biomagnification of organic chemicals, focussing on whether uptake is controlled predominantly by chemical diffusion or lipid co-transport. Theoretical fugacity-based models/equations were constructed for both possible mechanisms of uptake. Based on these equations, Gobas *et al*. (1993) hypothesised that if intestinal absorption is predominantly through diffusion, then a relatively large increase in the food lipid content should result in only a small decrease in the dietary uptake efficiency and uptake rate. However, if intestinal absorption is predominantly through lipid co-assimilation, the dietary uptake efficiency should be low in a low lipid diet and increase as the lipid content of the diet increases.

A series of experiments were undertaken to investigate the effect of food lipid on the intestinal absorption in goldfish, to try and distinguish between the two possible processes. The fish were exposed to a number of organochlorines (1,2,4,5-tetrachlorobenzene, pentachlorobenzene, hexachlorobenzene, 2,2',5,5'-tetrachlorobiphenyl, 2,2',4,4',6,6'-hexachlorobiphenyl, 2,2',3,3',4,4',5,5'-octachlorobiphenyl, decachlorobiphenyl and octachlorostyrene) in diets with lipid contents ranging from close to zero (<0.2%) to 13.5%. The concentrations of the chemicals used ranged between 7.69 µg/g food to 43.7 µg/g food.

The fish were fed diets of either low lipid (<0.2% lipid), medium lipid (6.3% lipid) or high lipid (13.5% lipid) food containing the substances for 21 days. The medium lipid food was a standard dried fish food. The low lipid food was prepared by extraction of the lipid from the dried fish food using petroleum ether. The high lipid food was prepared by adding the extracted lipids from the preparation of the low lipid food to the standard dried fish food and evaporating the petroleum ether. The mean fish weight in each of the three treatment groups was 1.28 to 1.30 g (range of individual means), and the fish had mean lipid contents of 1.00 to 1.01% (range of individual means). The lipid contents and weights of the fish did not increase over time. The feeding rate used was around 13 mg food per fish per day (equivalent to a feeding rate of around 1% of body weight, based on the quoted fish weights).

Faecal egestion rate and food digestibility[2] were also measured during the study. The faecal egestion rates were 3.1 mg faeces per fish per day for the low lipid diet, 3.9 mg faeces per fish per day for the medium lipid diet, and 5.2 mg faeces per fish per day for the high lipid diet. Food digestibility was 76% for the low lipid diet, 70% for the medium lipid diet and 60% for the high lipid diet.

The study found no significant differences in the dietary uptake efficiency between the three treatment groups for the substances with lower log K_{ow} values (log K_{ow} 4.51 – 6.10). However, for substances with higher log K_{ow} values (hexa- (log K_{ow} 7.0), octa- (log K_{ow} 7.8) and deca-chlorobiphenyl (log K_{ow} 8.26) and octachlorostyrene (log K_{ow} 6.29)), the dietary uptake efficiency was significantly higher ($p < 0.05$) for the low lipid food than the high lipid food. Gobas *et al*. (1993) concluded that these results suggested that intestinal absorption is predominantly controlled by chemical diffusion rather than lipid co-transport. The higher dietary uptake efficiencies from the low lipid foods were thought to be a result of a higher digestibility of the low-lipid food, leading to a lower faecal egestion rate and hence higher dietary uptake efficiency than the high lipid food.

4.1.2 Effect of dietary lipid on uptake of DDT and chlorobenzenes in goldfish – Sharifi *et al*. (1997)

Sharifi *et al*. (1997) investigated the effect of dietary lipid on the uptake of a mixture of p,p'-DDT[3] and four chlorobenzenes (1,3,5-trichlorobenzene, 1,2,3,5-tetrachlorobenzene, pentachlorobenzene and hexachlorobenzene) in goldfish (*Carassius auratus*). The aim of the study was to investigate the mechanism of intestinal absorption, specifically if the uptake was governed by passive diffusion or by lipid co-assimilation.

If lipid co-assimilation is the predominant mechanism, then:

- uptake of chemicals would be expected to be unrelated to their lipophilicity, but should be dependent upon the lipid content of the food; and
- the uptake efficiency would be predicted to increase as the lipid content of the food increased.

If passive diffusion is the predominant mechanism of uptake, then Sharifi *et al.* (1997) showed that the BMF and body concentration would be independent of the lipid content of the food.

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[2] This was measured in the Gobas *et al*. (1993) study as the ratio of the weights of the consumed food (administered food – dried faecal matter) to the administered food.

[3] 1,1'-(2,2,2-trichloroethylidene)bis(4-chlorobenzene)

The fish food used in the study had a lipid content of 2.9%. Olive oil was added to produce two further foods with higher lipid contents of 6.9% and 10.9%. The concentration of the substances added to the diet were between 3.2 – 3.6 mg/kg dry weight for DDT and 44.2 – 63.6 mg/kg dry weight for each of the chlorobenzenes. Groups of goldfish were fed a diet containing the substances over a ten-week period. The fish were fed at a rate of 3.5% of bodyweight on Monday-Saturday each week and samples of fish for analysis were collected every Monday. The fish weights and lipid contents of fish in the three treatment groups (food lipid contents 2.9%, 6.9% and 10.9%) were similar (average fish weights were 3.33 – 3.57 g at the start of the test and average lipid contents of the sampled fish throughout the study were 3.95 – 4.42%). No significant differences in the uptake rates or BMFs of the individual substances were observed between the three diets. Sharifi *et al*. (1997) concluded that the results were consistent with intestinal absorption being controlled by chemical diffusion rather than lipid co-assimilation.

4.1.3 Influence on assimilation efficiency of naturally contaminated and spiked diets on uptake of PCBs in koi carp - Liu *et al.* (2010)

Liu *et al*. (2010) investigated the influence of diet on the assimilation efficiency for 47 PCB congeners in juvenile koi carp (*Cyrpinus carpio*). Five different diets were used. Two diets were naturally contaminated; benthic invertebrates consisting of mayflies (*Hexagenia sp*.) collected from contaminated areas of Lake Erie, Canada, and forage fish consisting of emerald shiners (*Notropis atherinoides*) collected from contaminated areas of the Detroit River, United States. The natural diets were minced, and the food particles were well mixed prior to use. The remaining three diets were commercial fish pellets spiked with PCBs. Two sources of fish pellets were used: a koi-specific feed containing 6% lipid and a trout chow containing 11% lipid. The trout chow was amended with olive oil to produce diets of 16% and 22% lipid. The commercial diets were spiked with PCB mixtures giving a congener pattern similar to that previously found in biota from the Great Lakes. The total PCB concentration in the spiked diet was around 0.5 – 0.7 µg/g dry weight. The dry weight lipid contents measured in the diets were 4.1% for the mayfly diet, 14.3% for the emerald shiner diet, and 6.74%, 18.4% and 24.0% for the three commercial diets.

The fish (body weight ~10 g) were exposed individually so that the amount of food consumed per fish could be determined. The food was given at 0, 3 and 6 hours of the experiment. Excess food was removed from each tank and the amount of uneaten food was determined. The assimilation efficiency was estimated by mass balance following a 48-hour fasting period after the last feeding. The measured amounts of food consumed were around 0.045 – 0.055 g dry food/g fish for the natural food and 0.10 – 0.12 g dry food/g fish for the commercial feeds.

The dietary assimilation efficiencies determined ranged between 26% and 101% across all substances and diets. Principal component analysis identified that the assimilation efficiencies were significantly higher across the individual PCB congeners in the natural (invertebrate) diets than other diets. No significant differences were found in the assimilation efficiencies of individual PCB congeners in the other diets. Liu *et al*. (2010)

concluded that assimilation efficiency was not correlated to dietary lipid content and hypothesised that the higher assimilation efficiencies seen using the invertebrate diet than in other diets may be due to the higher digestibility of that diet compared with the other diet. However, Liu *et al*. (2010) were not able to test this further and noted that further work would be needed to confirm on this aspect. A significant relationship was evident in all diets between the assimilation efficiency and the log K_{ow} of the PCB congener, with the assimilation efficiency generally decreasing as the log K_{ow} increased.

4.1.4 Summary and comparison of the results from the Gobas *et al*. (1993), Sharifi *et al*. (1997) and Liu *et al*. (2010) studies

The studies by Gobas *et al*. (1993), Sharifi *et al*. (1997) and Liu *et al*. (2010) all investigated the effect of dietary lipid content on the assimilation efficiencies or BMF of lipophilic organochlorine compounds that are known to have a relatively high bioaccumulation potential. The Gobas *et al.* (1993) and Sharifi *et al.* (1997) studies used laboratory diets whereas the Liu *et al.* (2010) used both laboratory and natural diets. All three studies suggested that the assimilation efficiency, and hence BMF, was not correlated to dietary lipid content, and the results were consistent with intestinal absorption being controlled by chemical diffusion rather than lipid co-assimilation. The Gobas *et al.* (1993) study and the Liu et al. (2010) study both found that, in some cases, the food digestibility may affect the assimilation efficiency.

4.2 Other literature not considered in the CERI study

Two additional papers that support the lack of influence of food lipid on dietary accumulation in laboratory studies are summarised below. It is important to note that a fully comprehensive literature search was beyond the scope of the current project and other papers (either supportive or non-supportive) may be available. A search of the published literature was undertaken to identify any further relevant papers published between January 2018 and July 2022, but no additional supporting experimental studies were found (Annex 1). The publication by Gobas *et al.* (2021) describing the theoretical background to lipid normalisation and lipid standardisation is covered in Section 5.4 of this report.

Dabrowska *et al*. (1999) investigated the influence of dietary and body lipids on the dietary uptake efficiency of 2,2’,4,4’,5,5’-hexachlorobiphenyl (PCB-153) in two species of fish, yellow perch (*Perca flavescens*) and rainbow trout (*Oncorhynchus mykiss*). Groups of fish with different body lipid contents were fed with either a low-lipid diet (5.75% lipid w/w) or a high-lipid diet (14.1% lipid w/w) spiked with ^{14}C-PCB153 for 32 days, followed by a depuration period. The fish were sampled at 10 – 12 day intervals during the uptake period and after 12, 23 and 34 days (yellow perch) or 17 and 27 days (rainbow trout) of the depuration period.

The food used in the study was a mixture of fish, krill, and wheat meal, along with various other components, and had a lipid content of either 5.75% by weight (low-lipid diet) or 14.1% by weight (high-lipid diet). The diets were spiked with ^{14}C-PCB153 at either 5 or 50 µg/kg. The feeding rates used in the tests were in the range 0.0051 – 0.0060 g food/g fish/day for yellow perch and 0.0165 g food/g fish/day for rainbow trout. The fish were maintained on either a low-lipid or high-lipid diet for 10 weeks prior to the start of the test to establish groups of fish with different total body lipids before exposure to ^{14}C-PCB153. This was measured to be 3.04% and 5.49% lipid for yellow perch and 5.58% and 7.71% for rainbow trout at the start of the exposure, for the low and high-lipid diets, respectively.

The assimilation efficiency was determined from the slope of the linear relationship between the ^{14}C-PCB153 concentration in fish with exposure time and the concentration of ^{14}C-PCB153 in food. The study reports a bioaccumulation factor (BAF), determined from the assimilation efficiency, feeding rate and depuration rate constant. In addition, the dietary assimilation efficiency was determined from kinetic analysis of the data. The methods used are not identical to but are broadly comparable to the general approach currently used in the OECD 305 Test Guideline.

The rate constant for growth dilution was also determined in the study and all concentrations of ^{14}C-PCB153 were corrected for growth before calculation of the bioaccumulation parameters. However, full details of how this was done are not provided.

The study found no significant difference in the assimilation efficiency between fish fed a low-lipid diet and those fed a high lipid diet. Dabrowska *et al*. (1999) concluded that effects of both body and dietary lipids on the elimination rate constant were more significant in terms of the overall bioaccumulation potential of PCB153 than any effects of dietary lipids on the uptake efficiency.

In contrast to this, Vetter *et al*. (1985) found that benzo(a)pyrene was co-adsorbed with dietary lipid when fed in a single dose to killifish (*Fundulus heteroclitus*). In the study the benzo(a)pyrene (35 µg) was fed to the fish in a single piece of gelatin (35 mg) containing 18% triolein (a triglyceride). Microscopic analysis of the fish intestines showed that benzo(a)pyrene was co-digested and co-transported with the dietary lipid.

4.3 Reconsideration of the OECD Ring Test data

The data for the OECD 305 dietary study ring test were originally analysed by lipid-normalising the results to both the fish and food lipid contents. The relevant statistics around the BMF_{kgL} (lipid-normalised and growth-corrected) values from the original ring test (OECD, 2012b) are summarised in **Table 4.1**. The test results were all obtained using a 3% feeding rate.

The lipid contents of the diets used in the rainbow trout (*Oncorhynchus mykiss*) studies ranged between 6.38% and 21.1%; for the carp (*Cyrpinus carpio*) studies they were 16.8% in all cases.

The lipid normalised and growth-corrected BMF_{kgL} values quoted in OECD (2012b) have been recalculated for this report to a standard fish lipid content of 5% to give lipid standardised and growth corrected $BMF_{kg5\%}$ values, as suggested in the Hashizume *et al*. (2018) paper. The resulting statistics are summarised in Table 4.1.

Table 4.1 Summary of OECD Ring Test (OECD, 2012b) Results

	Experiments with rainbow trout				Experiments with carp			
	Mean BMFkgL	Relative standard deviation	Mean BMFkg5%	Relative standard deviation	Mean BMFkgL	Relative standard deviation	Mean BMFkg5%	Relative standard deviation
Hexachlorobenzene	3.10	37%	1.08	38%	1.45	14%	0.43	15%
Musk xylene	0.77	39%	0.26	32%	0.38	16%	0.11	15%
o-Terphenyl	0.50	20%	0.17	22%	0.15	67%	0.05	66%
Methoxychlor	0.16	63%	0.05	54%	0.03	33%	0.01	5%

As can be seen, standardisation of the data to 5% fish lipid alone results in generally similar relative standard deviations[4] for these data, as was originally obtained for the lipid-normalised values (BMF_{kgL} values normalised to both the lipid content of food and fish) in the ring test. Therefore, these data are inconclusive as to which method is more appropriate. However, it should be noted that the carp data for HCB, when standardised to a 5% fish lipid content (mean 0.43; relative standard deviation 15%), are more consistent with those reported by Hashizume *et al*. (2018) (mean 0.336; range 0.235 – 0.463). The equivalent comparison for the BMF_{kgL} is mean 1.45 (relative standard deviation 14%) from the ring test data and mean 0.925 (range 0.267-1.40) from Hashizume *et al.* (2018).

4.4 Information from the ECHA dissemination database

The ECHA dissemination database[5] contains robust study summaries of information submitted under the EU REACH regulation. This database has been screened for results of OECD 305 dietary accumulation studies. An initial search of the database was made using the OECD eChemPortal[6] for aquatic bioaccumulation studies where the exposure route was “feed”. This identified 184 study endpoints. These studies were manually screened by checking the corresponding entry in the ECHA database for the results of dietary accumulation studies where the same substance had been tested using diets with differing lipid contents. Studies with inorganic substances were not included. Most of the relevant studies were carried out using a single diet and so are of limited value in investigating the effects of diet lipid content on the results obtained. However, a known bioaccumulative reference substance (for example, hexachlorobenzene (HCB)) was used in several of the studies; these did show some difference in the lipid content of the diet used between studies. The reference substance data are summarised in Annex 2. Data from the ECHA dissemination database were analysed alongside the data from other sources (notably the OECD (2012b) ring test results and the Hashizume *et al*. (2018)

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[4] The apparent lower relative standard deviation for methoxychlor for carp may be due to the data in OECD (2012b) that are presented to one significant figure only. This may result in rounding errors when recalculated to 5% fish lipid.

[5] https://www.echa.europa.eu/information-on-chemicals

[6] https://www.echemportal.org/echemportal/. This allows the ECHA database to be searched for specific test endpoints.

study; Annex 2). Figure 4.1 shows the BMF_{kgL} values obtained for HCB in the various studies. The data are for various species but, although there is considerable scatter in the data, there does appear to be a general trend for the BMF_{kgL} increasing with increasing dietary lipid content; this is particularly apparent in the data for the Eurasian carp (*Cyprinus carpio*). There also appear to be differences in the BMF_{kgL} obtained between different species. The same data normalised to a 5% fish lipid content ($BMF_{kg5\%}$) are shown in Figure 4.2.

There is no apparent trend in the $BMF_{kg5\%}$ values with food lipid content, similar to the findings of the original Hashizume *et al.* (2018) study (it should be noted that the analysis here includes the Hashizume *et al.* (2018) data as part of the dataset used; the data considered from other studies generally show a similar trend to that from the Hashizume *et al.* (2018) study).

As discussed earlier and in Section 5, the BMF obtained theoretically depends on the feeding rate used in the study. The HCB data used in the above analysis are from studies with differing feeding rates (between 1.5% and 3% of body weight). To control for this in the analysis, the BMF values have been adjusted to a feeding rate of 3% body weight (see Annex 2 for details) in Figure 4.3 for the adjusted BMF_{kgL} and Figure 4.4 for the adjusted $BMF_{kg5\%}$. The trends in the adjusted BMF_{kgL} and adjusted $BMF_{kg5\%}$ are the same as for the non-adjusted versions.

Figure 4.1 BMF_{kgL} values for HCB for different diet lipid contents

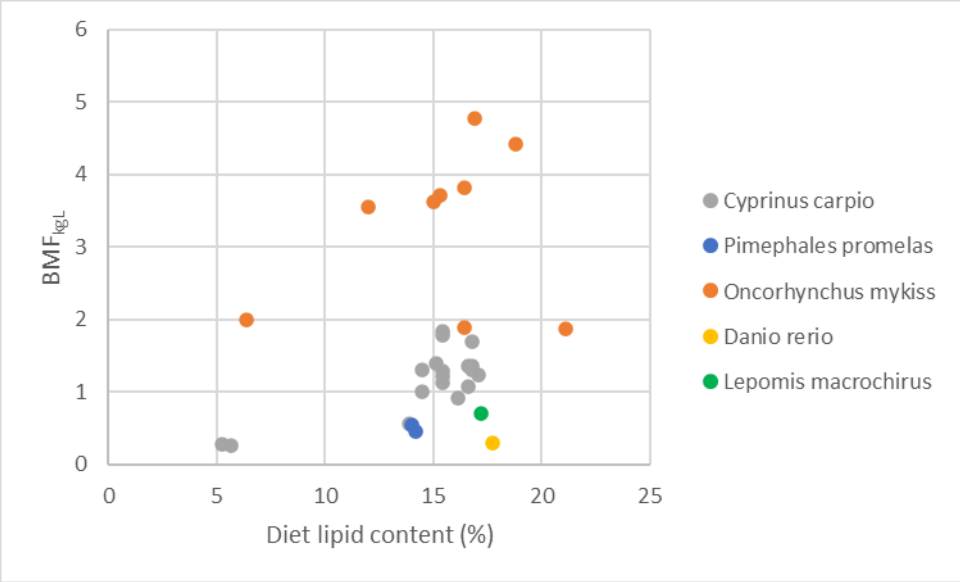


Figure 4.2 $BMF_{kg5\%}$ values for HCB for different diet lipid contents

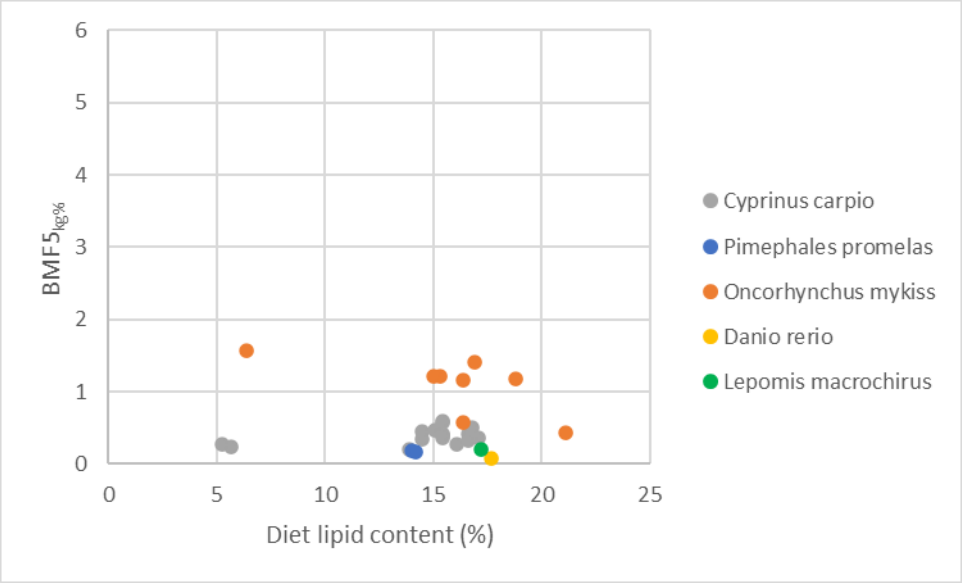


Figure 4.3 Adjusted BMF_{kgL} values for HCB for different diet lipid contents

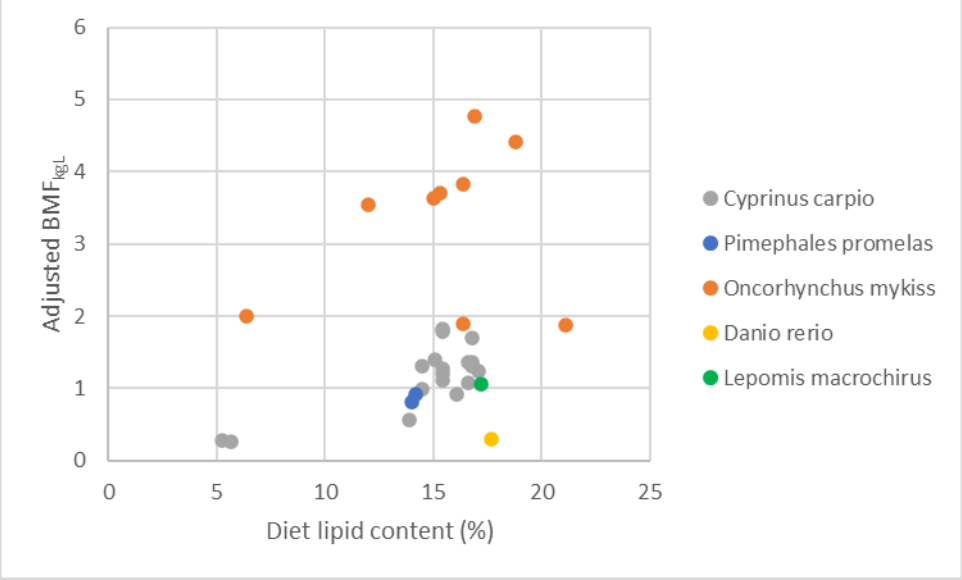
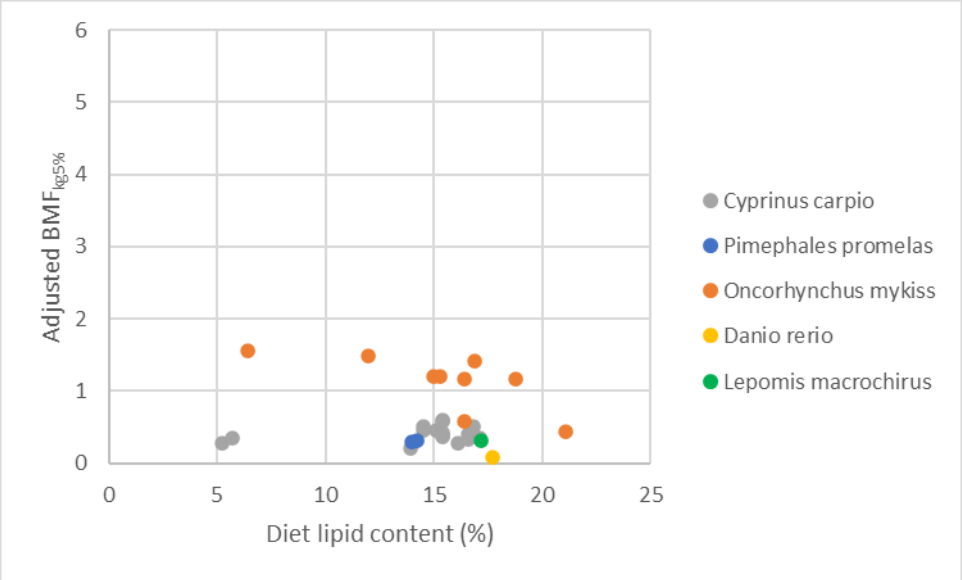


Figure 4.4 Adjusted $BMF_{kg5\%}$ values for HCB for different diet lipid contents



The ECHA dissemination database also contains a small amount of dietary accumulation data on o-terphenyl and PCB-153[7]. These data are summarised in Annex 2 alongside data for the same substances from other sources.

The variation of the BMF_{kgL} and $BMF_{kg5\%}$ with dietary lipid content for o-terphenyl are shown in Figure 4.5 and Figure 4.6. All of the studies were carried out using a 3% body weight feeding rate, except for the single datapoint for blue gill sunfish (*Lepomis macrochirus*) and so the BMF values are not adjusted for differences in feeding rate in these plots. Again, the data for rainbow trout (*Oncorhynchus*. *mykiss*) suggest that the BMF_{kgL} increases with increasing dietary lipid content and that the $BMF_{kg5\%}$ is independent of the lipid content of the diet. There are insufficient data points for the other two species to discern any trends.

The data for PCB153 are summarised in Figure 4.7 for the BMF_{kgL} values. Unfortunately, in this case the range of lipid contents in the diet covered by the data is relatively small and so it was not possible to discern any trends in the data.

[7] 2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl [CAS No. 35065-27-1].

Figure 4.5 BMF_{kgL} values for o-terphenyl for different diet lipid contents

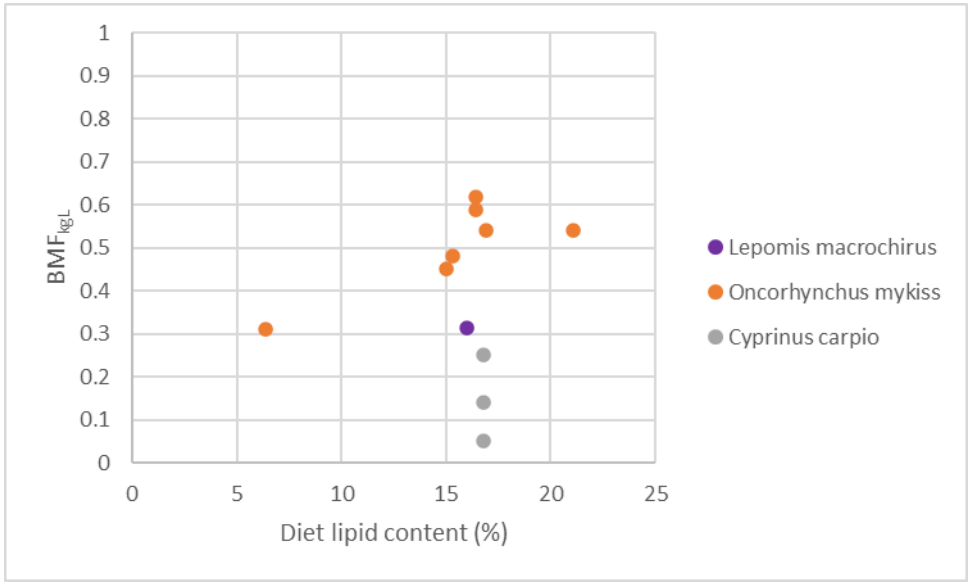


Figure 4.6 $BMF_{kg5\%}$ values for o-terphenyl for different diet lipid contents

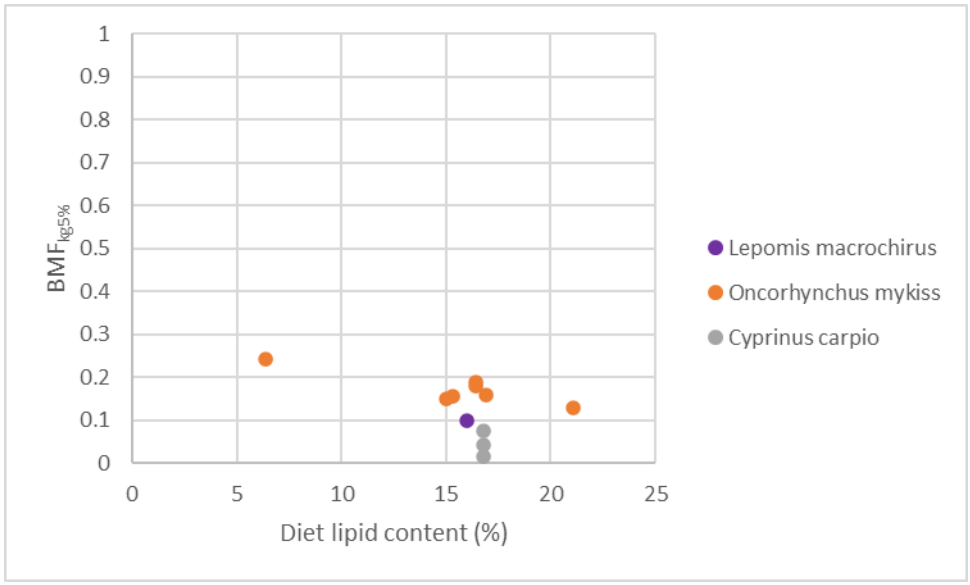
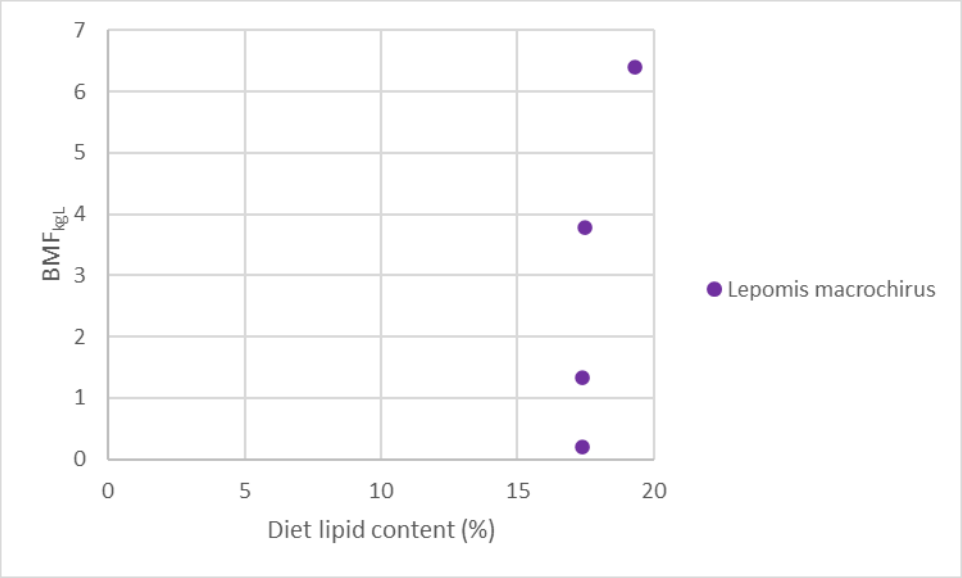


Figure 4.7 BMF_{kgL} values for PCB-153 for different diet lipid contents



Gobas *et al*. (2021) carried out a similar analysis using a more extensive database (which included the results from OECD (2012b) and Hashizume *et al.* (2018) of BMF values for various substances and reported similar findings to the above. The Gobas *et al*. (2021) study is considered further in Section 5.

5 Theoretical background to lipid correction

There are several published papers that have considered the theoretical background to bioaccumulation through dietary exposure. The most relevant to the theoretical background on lipid normalisation and lipid standardisation are discussed below.

5.1 Mathematical background to parameters - Mackay *et al*. (2013)

The mathematical background to the relationships between various bioaccumulation parameters has been summarised by Mackay *et al*. (2013). The relevant theory relating to lipid normalisation and standardisation is summarised below; the full paper should be consulted for details of how this is derived. Mackay *et al*. (2013) considered the various bioaccumulation metrics, including BCF and BMF, using both mass-balance considerations and fugacity considerations; similar conclusions were reached with both approaches.

Mackay *et al*. (2013) indicated that the BMF is preferably defined as the lipid-normalised ratio of the concentration in a predator to that of diet. Although this is defined in terms of concentrations in mol m^{-3} lipid, it is numerically equivalent to concentrations defined in terms of g/g lipid, assuming the density of lipid is the same in both predator and diet (Equation 4; taken from Mackay *et al*. (2013)).

$$\boldsymbol{BMF = \frac{Cpredator\ (mol\ m^{-3} lipid)}{Cdiet\ (mol\ m^{-3}\ lipid)}}$$

Equation 4

Mackay *et al*. (2013) showed that the BMF for a simple food chain (a predator consuming a prey species) can be described by the ratios of two bioaccumulation factors (BAFs) as follows (Equation 5; adapted from Mackay *et al*. (2013)).

$$BMF = \frac{Cpredator}{Cprey} = \frac{BAFpredator}{BAFprey}$$

$$= \boldsymbol{BCFpredator \times \left[\left(\frac{kDpred}{kRpred}\right) + \left(\frac{Cwater}{Cprey}\right)\right]}$$

Equation 5

where: $BCF_{predator}$ = bioconcentration factor for the predator

$k_{Rpredator}$ = rate constant for chemical uptake from water by gill respiration (m^3 water d^{-1})

$k_{Dpredator}$ = rate constant for dietary uptake (m^3 food d^{-1})

For the dietary test, C_{water} = 0 and so this simplifies to Equation 6:

$$BMF = BCFpredator \times \frac{kDpred}{kRpred}$$

Equation 6

Thus, even though the dietary test should not involve uptake from water, it is theoretically related to the BCF by the ratio of the rate constant for dietary uptake and the rate constant for uptake from water by gill respiration. If this equation holds, then it has important consequences for the lipid normalisation and standardisation of dietary tests. In particular, although it is known that the $BCF_{predator}$ will depend on the lipid content of the predator fish[8], the rate constants for uptake from both water (Crookes and Brooke, 2011) and diet do not necessarily depend on the lipid contents of the fish or food, respectively (the possible effect of dietary lipid on the uptake rate constant from diet is considered further in Section 6). Therefore, from this simple perspective, there appears to be some theoretical justification for standardising the results of the dietary accumulation test to the fish lipid alone.

It is important to note that in the full equation above, where C_{water} is not zero, the BMF also depends on the ratio of the C_{water}:C_{prey}. This term is equivalent to the reciprocal of the BCF for the prey. The BMF in this case, **as would be the case in field studies**, should be dependent upon the lipid contents in both predator and prey (although the relationship may not be straight forward) and lipid correction to the lipid contents of both predators and prey would be appropriate.

The equations derived by Mackay *et al*. (2013) can also be derived using fugacity, by essentially replacing the concentrations (in units of mol m^{-3}) by the term Z×f, where Z (in units of mol m^{-3} Pa^{-1}) is the fugacity capacity of the substance in the compartment of interest (e.g. water, whole fish, specific tissues etc.) and f (in units of Pa) is the fugacity of the substance in that compartment. When expressed in this way, essentially the same conclusions can be reached, as the fugacity capacity of the prey is dependent upon the lipid content of the prey, and this is only a significant term in the equation when uptake from water occurs.

As an illustration, Mackay *et al*. (2013) indicated that, for a small fish, the value of k_D is typically around 0.01 d^{-1} and k_R may be around 200 d^{-1} and so the ratio of k_D/k_R is typically around 5×10^{-5} m^3 food/m^3 water. Thus, for the dietary study, the BMF would be expected to be around 5×10^{-5} times the BCF. The BCF for hexachlorobenzene (HCB) is around 5000 or higher (OECD, 2012b) and so the BMF in the dietary test would be expected to be

[8] It is generally recognised that fish bioconcentration factors for substances are dependent upon the lipid content of the fish for substances that accumulate primarily in lipid (OECD, 2017).

around 0.25. This is in good agreement with the lipid-standardised $BMF_{kg5\%}$ values from the CERI study of 0.336 (mean value; range 0.205-0.463) (Hashizume *et al.* 2018).

5.2 Mechanism of biomagnification in fish - Gobas *et al.* (1999)

Gobas *et al*. (1999) considered the mechanism of biomagnification in fish under both laboratory and field conditions and developed a simple fugacity model for the gastrointestinal absorption of chemicals. The theoretical basis of the model was that gastrointestinal uptake and magnification of a chemical occurs because:

- digestion of the food results in a lowering of the lipid content of the partially digested food (reducing the fugacity capacity) compared with that of the undigested food; and
- the faecal egestion rate is lower than the feeding rate.

These two factors mean that, at steady state, the fugacity of a chemical in the gastrointestinal tract will be higher than the fugacity of the chemical in diet. Gobas *et al*. (1999) also indicated that a similar increase in chemical fugacity in the gastrointestinal tract over that in diet would be expected to occur under non-steady state conditions (e.g., under laboratory conditions), if the absorption rate of a chemical is smaller than the absorption rate of food lipid (which is responsible for much of the fugacity capacity in the food).

Gobas *et al.* (1999) tested their model using data from a 73-day laboratory gastrointestinal magnification study using 2,2',4,4',6,6'-hexachlorobiphenyl with adult rainbow trout (*Oncorhynchus mykiss*) and a field study investigating the gastrointestinal magnification of PCB congeners in rock bass (*Ambloplites rupestris*). Both studies showed that the fugacity of the chemicals in the gastrointestinal tract increased above that of the food; the fugacity increase in the gastrointestinal tract was a result of the decrease in the fugacity capacity of the partially digested food and an increase in chemical concentration, resulting from food absorption in the gastrointestinal tract. Gobas *et al*. (1999) concluded that food digestibility and absorption are important factors in the dietary uptake efficiency and biomagnification factor. The increase in fugacity that occurs drives passive diffusion of the substance across the intestinal wall, such that the concentration in a predator exceeds that of its prey.

Although in this model, the fugacity capacity of food is dependent, in part, upon the lipid content of the food, there are two important aspects that may need to be considered in relation to lipid normalisation and standardisation of dietary accumulation data. Firstly, it is not necessarily the food lipid content that is important, but rather the relative changes in food lipid content resulting from digestion of the food and the faecal egestion rate. Secondly, in non-steady state conditions, the rate of chemical absorption compared with the rate of lipid absorption may be important. This means that factors other than food lipid itself, for example, the overall digestibility of the food are also important. Therefore, based

on this simple model, the effects of food lipid on the uptake in the gastrointestinal tract may not be straight forward (e.g., the lipid content of the food may also affect the overall digestibility) and may vary from chemical to chemical.

5.3 Deriving bioconcentration factors Gobas and Lo (2016)

In simple terms, the non-lipid-normalised and non-growth-corrected dietary BMF can be defined in terms of the rate of uptake and the rate of depuration using Equation 7 from Gobas and Lo (2016) (this is consistent with the equations used in the OECD 305 Test Guideline).

$$BMF = \frac{k_1}{k_2} = \frac{\alpha \times I}{k_2}$$

Equation 7

Where k_1 = uptake rate constant (g g^{-1} d^{-1}).

k_2 = overall depuration rate constant (d^{-1}).

α = assimilation efficiency. This is the fraction of the chemical fed that is taken up into fish tissues each day.

I = feeding rate (g g^{-1}).

If, as the experimental evidence suggests, the assimilation efficiency is independent of the dietary lipid content, then the only parameter that is lipid dependent is the overall depuration rate constant, which depends on the lipid content of the fish. Therefore, this suggests standardisation only to the fish lipid content is important for the dietary study. The possible effect of dietary lipid on the uptake rate constant from diet is considered further in Section 6.

5.4 Normalizing the biomagnification factor - Gobas *et al.* (2021)

Through fugacity considerations of the biomagnification process, Gobas *et al.* (2021) recently demonstrated that the gastrointestinal magnification factor, when expressed in terms of a fugacity ratio, is directly related to the fugacity capacity of the diet and the lipid content of the diet.

Using the fugacity format, Gobas *et al.* (2021) demonstrated that the lipid-normalised BMF can be expressed as follows (Equation 8).

$$BMF_L = \frac{C_{Fish,L}}{C_{Diet,L}} = \frac{f_B}{f_D} = \frac{f_G}{f_D} \times \frac{f_B}{f_G} = F_{GD} \times F_{BG}$$

Equation 8

Where: BMF_L= lipid-normalised BMF.

$C_{Fish,L}$ = concentration in fish on a lipid weight basis.

$C_{Diet,L}$ = concentration in diet on a lipid weight basis.

f_B = fugacity in the fish body (Pa).

f_D = fugacity in the diet (Pa).

f_G = fugacity in the digesta[9] (Pa).

F_{GD} = ratio of the fugacity in digesta/gastrointestinal tract to the fugacity in diet. This is effectively the gastrointestinal magnification factor.

F_{BD}= ratio of the fugacity in the fish body to the fugacity in digesta/gastrointestinal tract.

Gobas *et al. (*2021) showed that the value of F_{GD} is controlled by a) the degree to which diet is assimilated into the body, and b) the ratio of the fugacity capacities of the diet and digesta (this is related to the change in sorptive capacity as the diet is digested). Based on this, Gobas *et al.* (2021) concluded that both food absorption and food digestion are important factors driving the biomagnification process.

In terms of analysis of the results of the OECD 305 dietary test, an important conclusion reached by Gobas et al. (2021) was that the value of F_{GD} is directly related to the fugacity capacity and lipid content of the diet and that, as the lipid content of the diet increases, the lipid-normalised biomagnification factor (BMF_L) is predicted to increase. The same conclusions would also apply to the growth-corrected and lipid-normalised BMF_{kgL}.

This is an important finding as it demonstrates that, at least based on current theories, different BMF_L's and BMF_{kgL}'s would be expected for the same substance when using

[9] Digesta in this model represent the digested diet passing through gastrointestinal tract. This is eventually excreted by the fish through faeces. According to Gobas et al. (2020) the composition of digesta is usually not known and difficult to measure for fish but can be approximated from the dietary composition using estimates of dietary assimilation efficiencies of lipids, protein, non-digestible organic matter and water.

diets with different lipid contents, and that the BMF_L and BMF_{kgL} should increase as the lipid content of the diet increases.

5.5 Summary

The Gobas *et al.* (2021) paper provides the theoretical background as to why it would be expected that the lipid-normalised BMF_L and BMF_{kgL} should increase with increasing lipid content of the diet. The result of this is that the $BMF_{kg5\%}$ would be expected to be relatively independent of the lipid content of the diet. This is in line with the CERI study (Hashizume *et al.*, 2018) and further analysis on the data carried out in this report, and the Gobas *et al.* (2021) paper. At first sight, this conclusion appears to be in contradiction with some of the other theories outlined above. This apparent contradiction is discussed further in Section 6.

It is also relevant to note that the overall digestibility[10] of the diet, as discussed in *Gobas et al*. (2021), is important in the dietary accumulation study and so there may be dietary factors other than lipid content that could affect the result of the study.

[10] Often taken to be the ratio of the weights of the consumed food (administered food – dried faecal matter) to the administered food.

6 Discussion and consideration of laboratory vs. field biomagnification studies

If it is true that only fish lipid content is important for the dietary study, then there is an apparent conflict between the dietary test and the general theory/models for biomagnification, where comparison of concentrations in predators and prey (diet) on a lipid basis is appropriate. This is considered further below.

It is useful to consider a simplified food chain in which a predatory fish consumes a prey fish. Both fish will be exposed through their respective diets and through water. Using a simple mass-balance consideration, the concentration in the prey fish ($Fish_1$; whose diet is primarily alga/zooplankton) and predatory fish ($Fish_2$) can be written as follows (Equation 10 and Equation 11 respectively; equations derived for this report; this is effectively an extension of the expression derived by Mackay *et al*. (2013) discussed in Section 5.1, but adapted by the author of this report to exemplify a simple food chain):

$$[\boldsymbol{Conc_{alga-zoo}}] = [\boldsymbol{Conc_{water}}] \times \boldsymbol{BCF_{alg}}$$

Equation 9

Where: $Conc_{alga\text{-}zoo}$ = concentration in alga/zooplankton.

C_{water} = concentration in water.

$BCF_{alga\text{-}zoo}$ = bioconcentration factor for alga/zooplankton.

Uptake in the prey fish ($Fish_1$) is through both water exposure (related to the BCF) and dietary exposure (related to the BMF for food uptake) from consumption of alga/zooplankton.

$$[Conc_{Fish1}] = ([Conc_{water}] \times BCF_{Fish1}) + \left([Conc_{alga\_zoo}] \times BMF_{Fish1}\right)$$

$$= [\boldsymbol{Conc_{water}}] \times \left(\boldsymbol{BCF_{Fish1}} + (\boldsymbol{BCF_{alga\_zoo}} \times \boldsymbol{BMF_{Fish1}})\right)$$

Equation 10

Where: $Conc_{Fish1}$ = concentration in prey fish.

BCF_{Fish1} = bioconcentration factor for prey fish.

BMF_{Fish1} = biomagnification factor for prey fish.

Uptake in the predatory fish ($Fish_2$) is through water exposure and dietary exposure from consumption of fish in trophic level 2.

$$[Conc_{Fish2}] = ([Conc_{water}] \times BCF_{Fish2}) + ([Conc_{Fish1}] \times BMF_{Fish2})$$

$$= [\boldsymbol{Conc_{water}}] \times \left(\boldsymbol{BCF_{Fish2}} + \left(\boldsymbol{BMF_{Fish2}} \times \left(\boldsymbol{BCF_{Fish1}} + \left(\boldsymbol{BCF_{alga_{zoo}}} \times \boldsymbol{BMF_{Fish1}} \right) \right) \right) \right)$$

Equation 11

Where: $Conc_{Fish2}$ = concentration in predatory fish.

BCF_{Fish2} = bioconcentration factor for predatory fish.

BMF_{Fish2} = biomagnification factor for predatory fish.

The overall field BMF for these two fish is shown in Equation 12:

$$\boldsymbol{BMF_{Field}} = \frac{[\boldsymbol{Conc_{Fish2}}]}{[\boldsymbol{Conc_{Fish1}}]} = \frac{\boldsymbol{BCF_{Fish2}} + \left(\boldsymbol{BMF_{Fish2}} \times (\boldsymbol{BCF_{Fish1}} + \left(\boldsymbol{BCF_{alga\_zoo}} \times \boldsymbol{BMF_{Fish1}} \right) \right)}{\boldsymbol{BCF_{Fish1}} + (\boldsymbol{BCF_{alga\_zoo}} \times \boldsymbol{BMF_{Fish1}})}$$

Equation 12

Where: BMF_{Field} = field biomagnification factor.

Similar equations can be developed for further trophic levels or where the initial trophic level is for exposure through sediment pore water. This indicates that the field BMF is a complex combination of the BCFs and BMFs for the species in the food chain (see Equation 12). Where the dietary BMF is included in the equation, it is always as a product of a BCF. As the BCFs are generally recognised to be dependent upon the lipid contents of the fish (or in this case also alga/zooplankton) the field BMF is still effectively the ratio of two terms that are lipid dependent, even if the dietary BMF terms are not lipid dependent. Therefore, the fact that the dietary BMF may not be dependent upon the lipid content of the diet used is not necessarily inconsistent with the fact that field BMF data for aquatic food chains should be expressed on a lipid-normalised basis.

It is also important to note that this equation suggests that a dietary BMF of much less than one (<< 1) can also lead to a field BMF greater than one (> 1), as the numerator term in the equation will always be greater than the denominator term in the equation, provided the BMF_{Fish2} is non-zero and the BCFs for fish 2 and fish 1 are similar. Similarly, apparent field BMFs >1 can result from differences in the BCF values for the fish in different trophic levels and are not necessarily related to the dietary uptake.

The above simplified food chain relates to exposure to fish. The situation with respect to lipid correction may be more complicated in other food chains where air-breathing species are included. This has not been considered further under the current project.

The final aspect that needs to be considered is that some current theories for the mechanism of biomagnification in fish (e.g., Gobas *et al.*, 1999) consider that it is dependent, in part, on the lipid content of the diet. However, as discussed above, the overall digestibility of the food may also be important. Therefore, there are factors other than lipid content of the food that may drive biomagnification. In addition, under non-steady state conditions, kinetic aspects may also be important (e.g., the rate of uptake of lipids compared with the rate of uptake of the substance itself). Thus, the effects of the

dietary lipid on the overall BMF obtained in such studies are unclear, when considered from a fugacity-based mechanistic aspect.

Overall, considering the uncertainties inherent in carrying out and interpreting dietary tests, the Hashizume *et al*. (2018) study is suggestive that, to minimise variability between different test systems, the results of the OECD 305 dietary accumulation study may be best interpreted by standardisation to the fish lipid content, and a 5% lipid content would be a reasonable value to use. This is consistent with several other experimental results and simple mass balance considerations for dietary accumulation. However, at first sight, this is not necessarily consistent with some mechanistic models for biomagnification.

Another way to examine OECD 305 test results is in terms of lipid-normalised concentrations. In the test, it is possible to determine the key kinetic parameters using lipid-normalised concentrations in the fish and food by dividing the concentrations in fish and food by the respective lipid contents (expressed as a mass fraction) of the fish and food respectively. When this is done, the assimilation efficiency and overall depuration rate constants obtained are essentially identical to those using whole body weight and whole food concentrations. However, the feeding rate, when expressed on a g lipid food/g lipid fish basis, is dependent upon the ratio of the lipid fraction in food/lipid fraction in fish. For example when this was done for Test A in the Hashizume *et al*. (2018) paper, the concentration in fish at the start of depuration (estimated from the intercept of the ln [concentration] versus time curve for depuration), changed from 9.95 µg/g in the original study to 386 µg/g lipid, the feeding rate changed from 0.02 g food/g fish to 0.044 g lipid food/g lipid fish, and the concentration in food changed from 99 µg/g food to 1,746 µg/g lipid, but the assimilation efficiency estimated was the same in both cases.

This change in feeding rate when moving from whole body or food values to lipid-normalised values may explain the apparent contradictions between the different theories. Studies that nominally use the same feeding rate (on a g whole fish/g whole fish basis), but different dietary lipid contents, are actually using markedly different feeding rates when considered on g lipid/g fish basis. As noted above, the uptake rate in any one study is theoretically dependent on the product of the feeding rate and the assimilation efficiency. The higher the lipid content of the food, the higher the effective feeding rate on a g lipid food/g lipid fish basis. When expressed on this basis, the feeding rates used in the Hashizume *et al*. (2018) study range between 0.044 g lipid food/g lipid fish to 0.14 g lipid food/g lipid fish. This is consistent with the findings in the Hashizume *et al*. (2018).

The dietary lipid content may also be important for some types of substances, for example, those that are co-assimilated with dietary lipid; this would need to be considered on a case-by-case basis.

The overall digestibility of the diet is also important and may also vary with dietary lipid content. For example, Gobas *et al*. (1993) found that the assimilation efficiency of high log K_{ow} substances (log K_{ow} of 6.3 or higher) was higher in a low lipid content food than a higher lipid content food; this was thought to be a result of a higher digestibility of low-lipid

food, leading to a lower faecal egestion rate and hence higher dietary uptake efficiency, than with the high lipid food.

Gobas *et al*. (2021) has also carried out an analysis of the Hashizume *et al*. (2018) data from a theoretical point of view. Through fugacity considerations of the biomagnification process, Gobas *et al*. (2021) demonstrated that the gastrointestinal magnification factor when expressed in terms of a fugacity ratio is directly related to the fugacity capacity of the diet and the lipid content of the diet. Key details of the analysis carried out by Gobas *et al*. (2021) are given in Section 5.4; for full details of the derivation, the original paper should be consulted. In the current context, this means that, from a theoretical point of view, the lipid-normalised $BMF_{kgL,}$ rather than being a constant value, should actually increase as the lipid content of the diet increases. This is in line with Hashizume *et al*. (2018).

Therefore, the Hashizume *et al.* (2018) findings can be explained from a theoretical point of view, both in terms of fugacity theory and also in terms of a change in apparent feeding rate, when expressing feeding rates on a lipid basis.

Overall, the proposal by Hashizume *et al*. (2018) to express the results of the OECD 305 test in terms of both a lipid-normalised and growth-corrected value and also a growth-corrected value standardised only to a fish lipid content of 5% (i.e. the $BMF_{kg5\%}$) would appear to be appropriate **when comparing results from different studies**. Further, it is important that the feeding rate used in the study is clearly reported and we would recommend that when a lipid-normalised value is reported, the feeding rate (in g lipid food/g lipid fish) is also reported alongside the value. Similarly, where a value standardised to a 5% fish lipid content is reported, the corresponding feeding rate (in g whole food/g fish) should be reported. This should help ensure comparability of data across studies.

However, as noted by Gobas *et al*. (2021), the lipid-normalised and growth-corrected dietary BMF value (BMF_{kgL}) is a better indicator of **the overall biomagnification potential** of a substance as it gives an indication of the fugacity in the fish over that in the diet (and hence a measure of the biomagnification potential). Again, as the BMF_{kgL} is expected to increase with both the dietary lipid content and the feeding rate, it is important that the dietary lipid content and the feeding rate are reported alongside the BMF_{kgL} value.

It is important to note that the results from the dietary BMF study are not directly comparable with the results of field BMF studies, and a dietary BMF value <1 does not necessarily mean that the field BMF will be < 1. Similarly, substances with field BMF values > 1 will not necessarily give a dietary BMF > 1 in the dietary study. To use the dietary BMF to estimate a field BMF, it needs to be considered alongside the other uptake routes using a model such as that discussed earlier. When using the data, it is important that the feeding rate is “realistic” in relation to natural diets. Although nominally using a high lipid feed at a feeding rate of 0.02 g/g bw may appear to be environmentally relevant, when considered in terms of a lipid-based feeding rate, it may not be so environmentally relevant.

7 Conclusions and recommendations

Understanding the effects of food lipid in the dietary accumulation test is complex and the relatively large variability seen in the results of tests, as evidenced in the OECD 305 Test Guideline ring test (see Section 4.3 and OECD, 2012b), means that definitive conclusions on how such data should be corrected to the food lipid content are difficult to reach. The following points should be noted:

- Experiments to investigate the mechanism of dietary uptake of organic chemicals in fish suggest that intestinal absorption is controlled by chemical diffusion rather than lipid co-assimilation.
- Passive diffusion is thought to be driven by fugacity changes that occur during digestion of the food. This is thought to result from changes in the fugacity capacity in the partly digested food compared with that of the diet and the overall digestibility of the food. The fugacity capacity of the diet is thought to be dependent upon the lipid content of the diet, but it is the **change in lipid content** that may be important as the **food is digested**, rather than the dietary lipid content itself. The rate of absorption of dietary lipid compared with the rate of absorption of the substance itself may be important in non-steady state situations, as found in the OECD 305 dietary study.
- The lipid content of the food may affect the digestibility of the food. There is evidence that the higher the digestibility of the food, the higher the assimilation efficiency for a given substance. It is known that the amount and type of dietary lipids can affect the digestibility and utilisation of a diet (e.g., see Dabrowska *et al.*, 1999 and references quoted therein).
- There are several studies, including Hashizume *et al*. (2018), that suggest that the assimilation efficiency observed in laboratory dietary studies with fish is not dependent upon the lipid content of the diet. This is considered further below.

The available evidence from dietary accumulation studies suggests strongly that the growth-corrected and lipid-normalised BMF value (BMF_{kgL}) varies depending on the lipid content of the diet. This is demonstrated both experimentally (e.g., Hashizume *et al*., 2018) and theoretically (e.g., Gobas *et al*., 2021) and can be explained by both fugacity considerations and effective changes to the feeding rate when considered on a lipid basis.

The implication of this is that the lipid normalisation method currently recommended in the OECD 305 test guidance will lead to differences in the BMF_{kgL} obtained using different diets. In particular, the BMF_{kgL} will increase as the lipid content of the diet increases.

The variability resulting from differences in lipid content in the diet can be reduced by standardising the BMF_{kg} to a standard 5% lipid content in fish and not normalising to the lipid content in the diet, that is, the $BMF_{kg5\%}$ as proposed by Hashizume *et al*. (2018). This will allow for better comparability of results from different tests. In many ways, this is similar to standardising BCF values to a standard 5% lipid content to allow comparison across different studies.

The BMF_{kgL} is however a relevant and important metric obtained from the OECD 305 dietary test, as it expresses the true potential for biomagnification of a chemical and represents the fugacity ratio of the chemical in the fish compared with the diet.

This, therefore, presents a dilemma; whether to use the $BMF_{kg5\%}$ as it is less dependent upon different lipid contents of the diet, or whether to use the BMF_{kgL} that better represents the true biomagnification potential of a substance.

A possible option would be to recommend a standard lipid content of the diet to be used in all tests, or failing that, to recommend a standard feeding rate on a kg lipid/kg fish basis. This should help increase the reproducibility of results across different tests. However, this may prove to be impractical as it would require regulators to agree on the standard value to be used (agreement of such values would, in part, be a political decision beyond the scope of this report).

We propose that the following approach is taken to facilitate the interpretation of data:

- Results from OECD 305 dietary tests should always be reported with the lipid content of the food.
- Both the $BMF_{kg5\%}$ and the BMF_{kgL} should be reported from the study for the following reasons:
    - The $BMF_{kg5\%}$ allows for better comparison across different studies. This will be particularly relevant if a study is carried out using a reference substance or is intended to be compared to reference or marker substances.
    - The BMF_{kgL} provides a better indication of the potential for biomagnification of the substance, as it represents the fugacity ratio between the fish and diet. However, the result should always be considered alongside the lipid content of the food used.

There are also implications in comparing the BMF_{kgL} obtained in the OECD 305 dietary study with BMF or TMF values from field studies. Some of the difficulties are given below.

- The BMF_{kgL} obtained in an OECD 305 study is specific to the lipid content of the diet used in the study. This lipid content may differ from the lipid content of the diet in the field data.
- The BMF_{kgL} is growth-corrected. The data used to derive BMF or TMF values from field data are usually not growth-corrected, and field data are subject to many other factors such as migratory patterns and food availability etc. that may impact growth.
- Field data encompass all routes of exposure (e.g., exposure via the water phase as well as diet). Organisms in dietary studies are only exposed via diet (although it should be acknowledged that limited unintentional exposure via water may also occur from partitioning of the substance during feeding).

Although the focus of this report has been on the growth-corrected BMF values, similar conclusions would also apply to the equivalent non-growth-corrected BMF values.

The fact that the BMF value obtained in the dietary accumulation study depends upon both the feeding rate used and the dietary lipid content used potentially causes issues for regulators as the value obtained will depend upon the experimental set up used. This could potentially be addressed by:

- recommending tests are carried out using a standard diet lipid content and feeding rate; or
- basing regulatory decisions on bioaccumulation using endpoints from the test that are not dependent on these factors, such as the growth-corrected depuration rate constant (k_{2g}).

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9 List of abbreviations

Abbreviation	Meaning
α	Assimilation efficiency
BMF	Biomagnification factor
BMF_{kg}	Growth-corrected kinetic dietary biomagnification factor
BMF_{kgL}	Growth-corrected and lipid-normalised kinetic dietary biomagnification factor (normalised to both the lipid content of fish and the lipid content of diet)
$BMF_{kg5\%}$	Growth-corrected and lipid-standardised kinetic dietary biomagnification factor (standardised to a 5% fish lipid content).
BMF_{L}	Lipid-normalised dietary biomagnification factor
HCB	Hexachlorobenzene
L_{c}	Lipid correction factor.
L_{fish}	Weight fraction of lipid in fish.
L_{food}	Weight fraction of lipid in food.
TMF	Trophic magnification factor.

Annex 1 – Details of the literature search

Two specific literature searches were performed, targeted at finding information on dietary bioaccumulation studies. Searches were filtered to include only studies that were published after the year 2018 and were written in English.

In the first search, the following keywords were used:

"bioconcentration" OR "bioaccumulation" OR "biomagnification" OR "accumulation"

The search returned >1000 citations; only the first 1000 citations were screened as these were deemed to be the most relevant to the keywords searched.

In the second search, the following search terms were used:

"bioconcentration model" OR "bioaccumulation model" OR "bioaccumulation kinetics" OR "bioconcentration kinetics" OR "accumulation kinetics"

The search returned 100 citations; all citations underwent further screening.

The results obtained from the searches in PubMed contained studies that can be used for this project and studies that fall outside of the scope of the project. Therefore, all obtained literature has been through a multi-step inclusion/exclusion screening process.

An initial screening was performed based on the title, abstract and keywords of the obtained literature, to decide whether the study was relevant or not. Only studies reporting aquatic bioaccumulation data were included. Studies on specific substance types which do not follow classical lipid uptake (nanomaterials, metals, surfactants) were excluded.

The studies that were selected after the initial screening based on the title and abstract underwent a second screening whereby the methodology section of the study was reviewed. The following criteria were evaluated:

- Does the study contain detailed methodology for the biomagnification experimental study/modelling?

- Are biomagnification (BMF) or trophic magnification (TMF) values reported?

- Are there comparators of lipid normalised/lipid-standardised biomagnification values, or did the study include diets with different lipid content?

Based on the above criteria, the study by Gobas et al. (2021) was identified as relevant to the project. A number of field biomagnification studies were identified; however it was deemed that these studies did not contain significant new information for the present project. Several experimental laboratory biomagnification studies were also identified; following the second screening steps, it was concluded that these studies did not include relevant new data (study did not include more than one diet; no information on the use of a reference substance).

Annex 2 – Collation of data for reference substances

The available dietary accumulation data for substances that are commonly used as reference substances in the OECD 305 dietary accumulation tests (e.g. hexachlorobenzene, PCB-153 and o-terphenyl) are summarised in the Tables below. These data are taken from a number of published sources and complemented with data from the ECHA dissemination database where available.

The BMF_{kgL} values were taken directly from the referenced source. Along with the reported food lipid content and the feeding rate. The $BMF_{kg5\%}$ was estimated from these data by using the following approach.

$$BMF_{kg5\%} = \frac{0.05 \times BMF_{kgL}}{Fraction\ lipid\ in\ diet}$$

The feeding rate used in the majority of the studies was 3% body weight. However, a number of studies used lower feeding rates. As discussed in the main report, the BMF value obtained in the dietary accumulation study should theoretically be proportional to the feeding rate used. In order to investigate any influence of this on interpretation of the data, adjusted BMF_{kgL} and adjusted $BMF_{kg5\%}$, whereby the adjusted BMF value represents the equivalent or expected BMF value at a 3% body weight feeding rate, were estimated using the following approach.

$$Adjusted\ BMF_{kgL} = \frac{BMF_{kgL} \times 0.03}{Feeding\ rate\ used\ in\ study\ (fraction\ of\ body\ weight)}$$

$$Adjusted\ BMF_{kg5\%} = \frac{BMF_{kg5\%} \times 0.03}{Feeding\ rate\ used\ in\ study\ (fraction\ of\ body\ weight)}$$

Hexachlorobenzene (CAS No. 118-74-1)

Species	Lipid content of diet (%)	Feeding rate (% body weight/day)	Original value		Adjusted to 3% feeding rate		Source[1]
			BMG_{kgL}	$BMF_{kg5\%}$	BMG_{kgL}	$BMF_{kg5\%}$	
Cyprinus carpio	5.67	2	0.267	0.235	0.401	0.353	Hashizume *et al*. (2018)
Cyprinus carpio	14.5	2	0.998	0.344	1.497	0.516	Hashizume *et al*. (2018)
Cyprinus carpio	5.23	3	0.285	0.272	0.285	0.272	Hashizume *et al*. (2018)
Cyprinus carpio	14.5	3	1.31	0.452	1.31	0.452	Hashizume *et al*. (2018)
Cyprinus carpio	13.9	3	0.569	0.205	0.559	0.205	Hashizume *et al*. (2018)
Cyprinus carpio	16.1	3	0.912	0.283	0.912	0.283	Hashizume *et al*. (2018)
Cyprinus carpio	17.1	3	1.23	0.360	1.23	0.360	Hashizume *et al*. (2018)
Cyprinus carpio	15.1	3%	1.40	0.464	1.40	0.464	Hashizume *et al*. (2018)
Cyprinus carpio	16.6	3%	1.36	0.410	1.36	0.410	Hashizume *et al*. (2018)

Species	Lipid content of diet (%)	Feeding rate (% body weight/day)	Original value		Adjusted to 3% feeding rate		Source[1]
			BMG_{kgL}	$BMF_{kg5\%}$	BMG_{kgL}	$BMF_{kg5\%}$	
Pimephales promelas	14	2%	0.544	0.194	0.816	0.291	ECHA Dissemination Database
Oncorhynchus mykiss	18.8	3%	4.42	1.18	4.42	1.18	ECHA Dissemination Database; Brooke *et al*. (2009)/Scheebaum (2008)
Oncorhynchus mykiss	6.38	3%	2.00	1.57	2.00	1.57	OECD, 2012b
Oncorhynchus mykiss	16.4	3	1.89	0.576	1.89	0.576	OECD, 2012b
Cyprinus carpio	16.8	3	1.30	0.387	1.30	0.387	OECD, 2012b
Cyprinus carpio	16.8	3	1.36	0.405	1.36	0.405	OECD, 2012b
Cyprinus carpio	16.8	3	1.69	0.503	1.69	0.503	OECD, 2012b
Oncorhynchus mykiss	15.3	3	3.71	1.21	3.71	1.21	OECD, 2012b

Species	Lipid content of diet (%)	Feeding rate (% body weight/day)	Original value		Adjusted to 3% feeding rate		Source[1]
			BMG_{kgL}	$BMF_{kg5\%}$	BMG_{kgL}	$BMF_{kg5\%}$	
Oncorhynchus mykiss	16.4	3	3.82	1.16	3.82	1.16	OECD, 2012b
Oncorhynchus mykiss	15	3	3.63	1.21	3.63	1.21	OECD, 2012b
Oncorhynchus mykiss	21.1	3	1.87	0.443	1.87	0.443	OECD, 2012b
Oncorhynchus mykiss	16.9	3	4.77	1.41	4.77	1.41	OECD, 2012b
Cyprinus carpio	15.4	3	1.28	0.416	1.21	0.416	Inoue *et al*. (2012)
Cyprinus carpio	15.4	3	1.12	0.364	1.12	0.364	Inoue *et al*. (2012)
Cyprinus carpio	15.4	3	1.21	0.393	1.21	0.393	Inoue *et al*. (2012)
Cyprinus carpio	15.4	3	1.79	0.581	1.79	0.581	Inoue *et al*. (2012)
Cyprinus carpio	15.4	3	1.83	0.594	1.83	0.594	Inoue *et al*. (2012)

Species	Lipid content of diet (%)	Feeding rate (% body weight/day)	Original value		Adjusted to 3% feeding rate		Source[1]
			BMG_{kgL}	$BMF_{kg5\%}$	BMG_{kgL}	$BMF_{kg5\%}$	
Cyprinus carpio	16.6	3	1.08	0.325	1.08	0.325	Inoue *et al*. (2012)
Danio rerio	17.7	3	0.29	0.082	0.29	0.082	ECHA Dissemination Database
Lepomis macrochirus	17.2	2	0.710	0.206	1.07	0.310	ECHA Dissemination Database
Pimephales promelas	14.2	1.5	0.455	0.160	0.91	0.320	ECHA Dissemination Database
Oncorhychus mykiss	12	3	3.55	1.48	3.55	1.48	ECHA Dissemination Database

Notes: Information from ECHA Dissemination Database taken from https://www.echa.europa.eu/information-on-chemicals.

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PCB-153 (2,2',4,4',5,5'-Hexachloro-1,1'-biphenyl; CAS No. 35065-27-1)

Species	Lipid content of diet (%)	Feeding rate (% body weight/day)	Original value		Adjusted to 3% feeding rate		Source[1]
			BMG_{kgL}	$BMF_{kg5\%}$	BMG_{kgL}	$BMF_{kg5\%}$	
Lepomis macrochirus	17.5	1	3.78	1.08	11.3	3.24	ECHA Dissemination Database
Lepomis macrochirus	19.3	1	6.4	1.66	19.2	4.97	ECHA Dissemination Database
Lepomis macrochirus	17.4	1	0.201	0.058	0.603	0.173	ECHA Dissemination Database
Lepomis macrochirus	17.4	1	1.33	0.382	3.99	1.15	ECHA Dissemination Database

Notes: Information from ECHA Dissemination Database taken from https://www.echa.europa.eu/information-on-chemicals.

o-Terphenyl (CAS No. 84-15-1)

Species	Lipid content of diet (%)	Feeding rate (% body weight/day)	Original value		Adjusted to 3% feeding rate		Source[1]
			BMG_{kgL}	$BMF_{kg5\%}$	BMG_{kgL}	$BMF_{kg5\%}$	
Lepomis macrochirus	16	1	0.31	0.098	0.94	0.29	ECHA Dissemination Database
Oncorhynchus mykiss	6.38	3	0.31	0.24	0.31	0.24	OECD, 2012b
Oncorhynchus mykiss	16.4	3	0.59	0.18	0.59	0.18	OECD, 2012b
Cyprinus carpio	16.8	3	0.25	0.074	0.25	0.074	OECD, 2012b
Cyprinus carpio	16.8	3	0.14	0.042	0.14	0.042	OECD, 2012b
Cyprinus carpio	16.8	3	0.05	0.015	0.05	0.015	OECD, 2012b
Oncorhynchus mykiss	15.3	3	0.48	0.16	0.48	0.16	OECD, 2012b
Oncorhynchus mykiss	16.4	3	0.62	0.19	0.62	0.19	OECD, 2012b

Species	Lipid content of diet (%)	Feeding rate (% body weight/day)	Original value		Adjusted to 3% feeding rate		Source[1]
			BMG_{kgL}	$BMF_{kg5\%}$	BMG_{kgL}	$BMF_{kg5\%}$	
Oncorhynchus mykiss	15	3	0.45	0.15	0.45	0.15	OECD, 2012b
Oncorhynchus mykiss	21.1	3	0.54	0.13	0.54	0.13	OECD, 2012b
Oncorhynchus mykiss	16.9	3	0.54	0.16	0.54	0.16	OECD, 2012b
Cyprinus carpio	16.6	3	0.091	0.027	0.091	0.027	Inoue *et al*. (2012)

Notes: Information from ECHA Dissemination Database taken from https://www.echa.europa.eu/information-on-chemicals.

INOUE, Y., HASHIZUME, N., YOSHIDA, T., MURAKAMI, H., SUZUKI, Y., KOGA, Y., TAKESHIGE, R., KIKUSHIMA, E., YAKATA, N., AND OTSUKA, M., 2012. Comparison of bioconcentration and biomagnification factors for poorly water-soluble chemicals using common carp (*Cyprinus carpio* L.). Archives of Environmental Contamination and Toxicology, **63**, 241-248.

OECD, 2012b. 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, Organisation for Economic Co-operation and Development, ENV/JM/MONO(2012)20, July 2012.

Gobas *et al.* (2021) has carried out a similar analysis using a database of dietary accumulation studies (this includes some of the above data along with data from other studies and unpublished data).

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