Lipid Normalisation in the OECD 305 Dietary Test

Mike Crookes[1], Claire Gibson[1], **Emma Jack**[2], Steph Jones[3] and Pippa Curtis Jackson[3]

[1] Vitis Regulatory Limited, Whitstable, Kent, CT5 3BJ, United Kingdom [2] Vitis Regulatory Srl, 1040 Brussels, Belgium [3] Environment Agency, Bristol, BS1 5AH, United Kingdom

Background

The OECD 305 Test Guideline[1] is the key test method used to investigate bioaccumulation potential. The Guideline was updated in 2012 to include tests using dietary exposure and, to support this update, guidance on specific aspects of the test were prepared. This guidance was published in 2017[2] 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 in the development of the guidance, The Chemicals Evaluation and Research Institute, Japan (CERI) presented new information (later published by Hashizume *et al.* (2018)[3]) that suggested that the BMF should only be standardised for the fish lipid content, not the food lipid content.

This study considers lipid normalisation versus standardisation, taking into account other experimental and theoretical evidence obtained via targeted literature review, with the aim of providing recommendations on interpretation and reporting of dietary bioaccumulation tests for a future update to the OECD guidance.

Terminology

- **Lipid normalisation:** This is correction of the BMF to the lipid content of **both** the fish **and** food used, as outlined in the OECD 305 Test Guideline.

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

Where: BMF_{kgL} is the growth-corrected and lipid-normalised kinetic dietary BMF.
BMG_{kg} is the growth-corrected kinetic dietary BMF.
L_{food} is the weight fraction of lipid in food.
L_{fish} is the weight fraction of lipid in fish.

- **Lipid standardisation:** This is correction of the BMF to a standard lipid content of the **fish only**, as suggested by Hashizume *et al.* (2018)[3].

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

Where: $BMG_{kg5\%}$ is the growth-corrected and lipid standardised kinetic dietary BMF standardised to a 5% fish lipid content.

Data evaluation

Further datasets covering different dietary lipid contents and fish species were obtained from the OECD 305 ring test[4] and ECHA dissemination database for hexachlorobenzene ((HCB); CAS No. 118-74-1), 2,2',4,4',5,5'-hexachloro-1,1'-biphenyl ((PCB-153); CAS No. 35065-27-1), and o-terphenyl (CAS No. 84-15-1).

- The relationship between the BMF_{kgL} and $BMF_{kg5\%}$ versus dietary lipid content was evaluated for each substance; data for HCB are shown in Figures 1 and 2.
- 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 within each species.
- There is no apparent trend in the $BMF_{kg5\%}$ values with food lipid content.

The majority of the data were obtained using a feeding rate of 3% body weight. However a number of studies used a lower feeding rates; therefore a similar analysis was also carried out using BMF values that have been adjusted to a feeding rate of 3% body weight as follows:

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

- Adjusted BMF data for HCB are shown in Figures 3 and 4.
- The trends in the adjusted BMF_{kgL} and adjusted $BMF_{kg5\%}$ are the same as for the non-adjusted versions.

Fig. 1: BMF_{kgL} versus diet lipid content for HCB

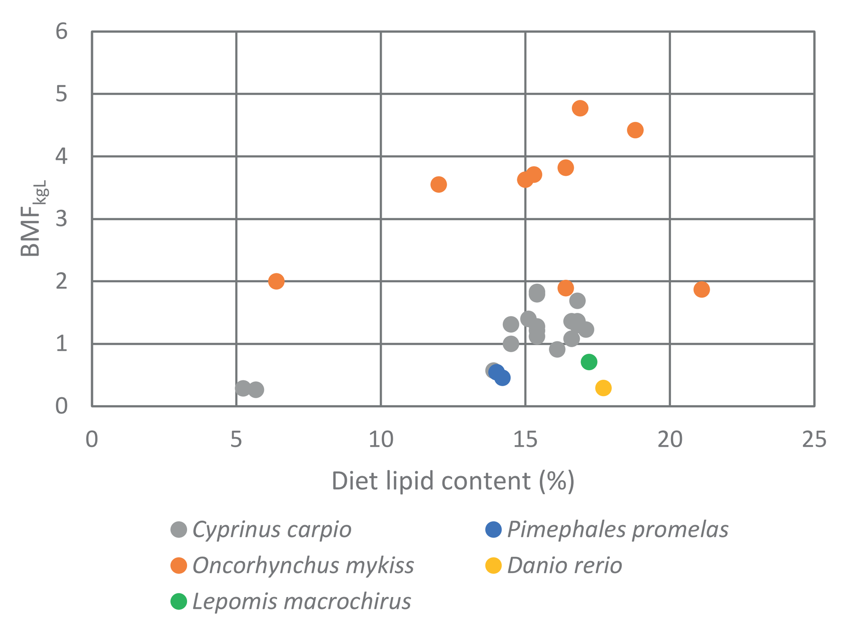


Fig. 2: $BMF_{kg5\%}$ versus diet lipid content for HCB

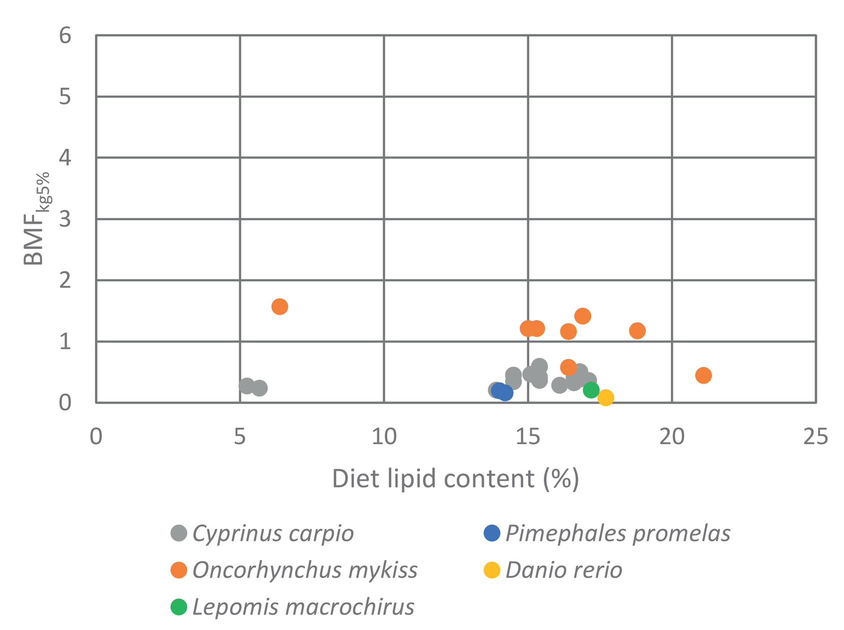


Fig. 3: Adjusted BMF_{kgL} versus diet lipid content for HCB

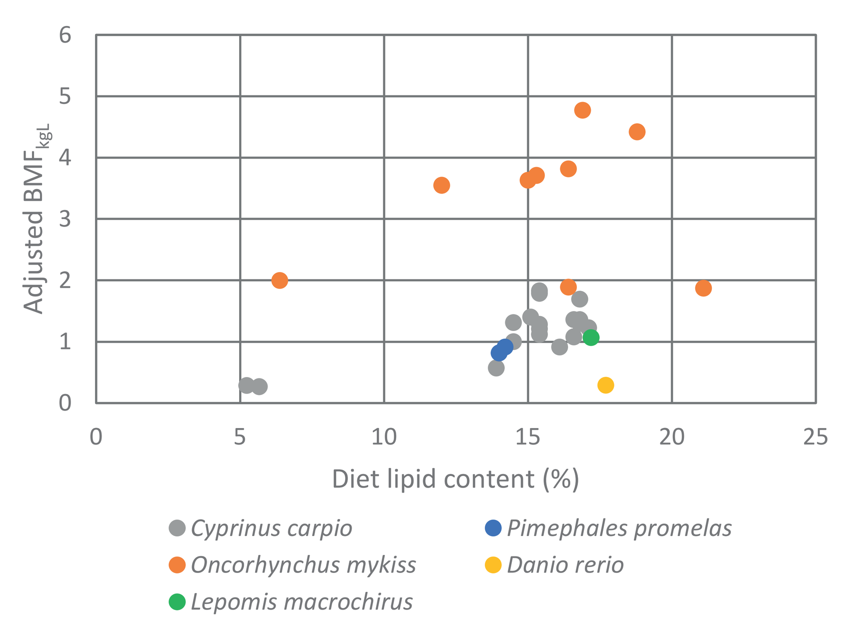
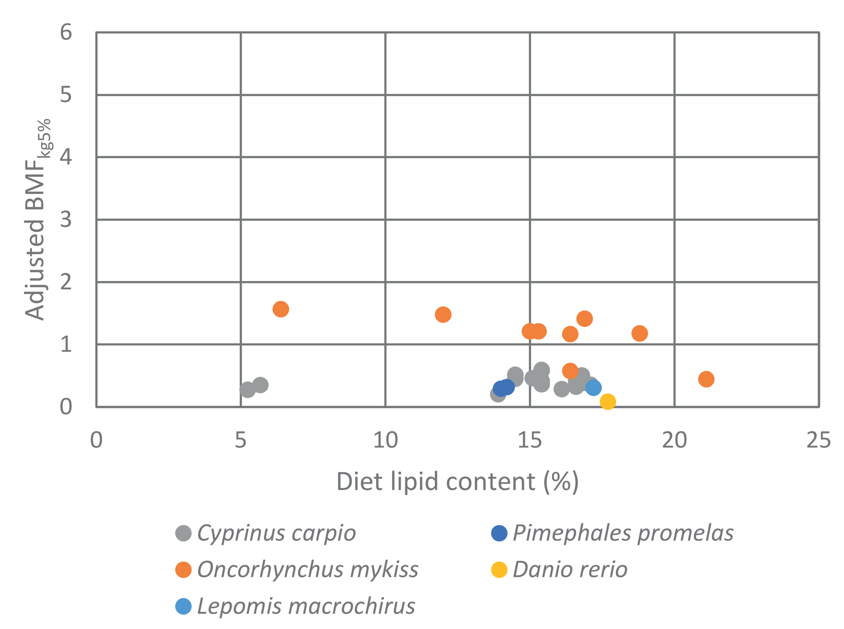


Fig. 4: Adjusted $BMF_{kg5\%}$ versus diet lipid content for HCB



Key findings

The available evidence from dietary accumulation studies suggests strongly that the BMF_{kgL} varies depending on the lipid content of the food used in the study. Conversely, the $BMF_{kg5\%}$ value is relatively independent of the lipid content of the food.

This can be explained by:

- Differences in the fugacity capacity between diets of different lipid contents (as demonstrated by Gobas *et al.* (2021)[5]).
- Differences in the apparent feeding rate when expressed on a lipid basis. The equations used in the OECD 305 Test Guideline show that the BMF is directly proportional to the feeding rate.

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

where k_1 = uptake rate constant ($g.g^{-1}.d^{-1}$).
k_2 = overall depuration rate constant (d^{-1}).
α = assimilation efficiency.
I = feeding rate ($g.g^{-1}$).

- The rate of uptake in any one study is dependent on the product of feeding rate and 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. This results in a higher effective uptake rate constant and hence BMF_{kgL}.

Recommendations

We propose the following approach should be taken to facilitate the interpretation of data from the OECD 305 dietary accumulation test:

- The lipid content of the food should always be reported alongside the BMF value.
- Both the $BMF_{kg5\%}$ and the BMF_{kgL} should be reported. The $BMF_{kg5\%}$ allows for better comparison of results between different studies, whereas the BMF_{kgL} provides a better indication of the potential biomagnification of the substance, as it represents the fugacity ratio between fish and the diet. However, the result should always be considered alongside the lipid content of the food used.

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 causes issues for interpretation, as the value obtained will depend upon the study design and measured parameters. This could potentially be addressed by recommending that:

- tests are carried out using a standard diet lipid content and feeding rate; and/or
- by basing regulatory decisions regarding bioaccumulation on endpoints from the test that are not dependent on these factors, such as the growth-corrected depuration rate constant.

References

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