

OECD 305 Dietary Uptake Method for Assessing Bioaccumulation in Fish

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Introduction

- Background
- Study Design
- Calculations
- Discussion/outlook
- Questions

Why run a dietary test?

- If it is possible to maintain stable aqueous test item concentrations at levels that are within the aqueous solubility of the test item, then it is recommended that the current OECD 305 aqueous exposure method is used
- However, for many test items it is not possible to do this
- Examples of such materials are:
 - Superlipophilic substances
 - Log Pow >6
 - Solubility below 0.01 – 0.1 mg/L
- Complex mixtures
- Surface active substances

Why run a dietary test?

- For these materials the proposed dietary uptake method provides an alternative
- Exposure via food removes problems associated with aqueous dosing
- Note that the dietary approach gives a ***Biomagnification Factor (BMF)*** ie the concentration of test item in the fish relative to its food source, as the endpoint
- And not a Bioconcentration Factor (BCF) which is determined in the aqueous exposure method

Development of the method

- Bioconcentration studies in fish: OECD Guideline 305 (and OPPTS 850.1730)
- Documents related to fish feeding studies:
 - Fish, Dietary Bioaccumulation Study. A protocol developed and used by Exxonmobil Biomedical Sciences, Inc (EMBSI) (RIVM/Netherlands document, January 20, 2004).
 - Background Document to the Fish Dietary Study Protocol (RIVM/Netherlands document, January 20, 2004).
- OECD Guidelines for Testing Chemicals, **Proposal** for Updating Guideline 305

Study design – uptake phase

- **Uptake phase**
 - Fish fed a diet of fish food spiked with the test item
 - Commercial fish food
 - Test item added via solution in fish/vegetable oil, an organic solvent spike, or direct addition to the food
 - Fed at 1 – 2 % body weight
 - Test concentration of 1 – 1000 ug/g
 - Typically 7 – 14 days



Study design – depuration phase

- **Depuration phase**
 - Typically 28 days
 - Fish fed non-treated fish food
 - Monitor the decline in test item concentrations within the fish



Study design – sampling during depuration phase

Day	Parent Test Item Equivalent	Lipid	Metabolite Identification	Parent Test Item
1	5	3	4	
3	5			4
7	5			4
14	5			4
21	5			
28	5	3		
Total	30	9	4	12

Calculations

$$\text{BMF} = \frac{I \cdot \alpha}{k_2}$$

where:

- I = food ingestion rate (g food/g fish/day);
- α = assimilation efficiency (absorption of test item across the gut);
- k_2 = depuration rate constant (day⁻¹)

Calculations

$$\alpha = \frac{C_{0,d} \cdot k_2}{I \cdot C_{food}} \cdot \left(\frac{1}{1 - e^{(-k_2 \cdot t)}} \right)$$

where:

α = assimilation efficiency;

$C_{0,d}$ = concentration in fish at time zero of the depuration phase (mg/kg);

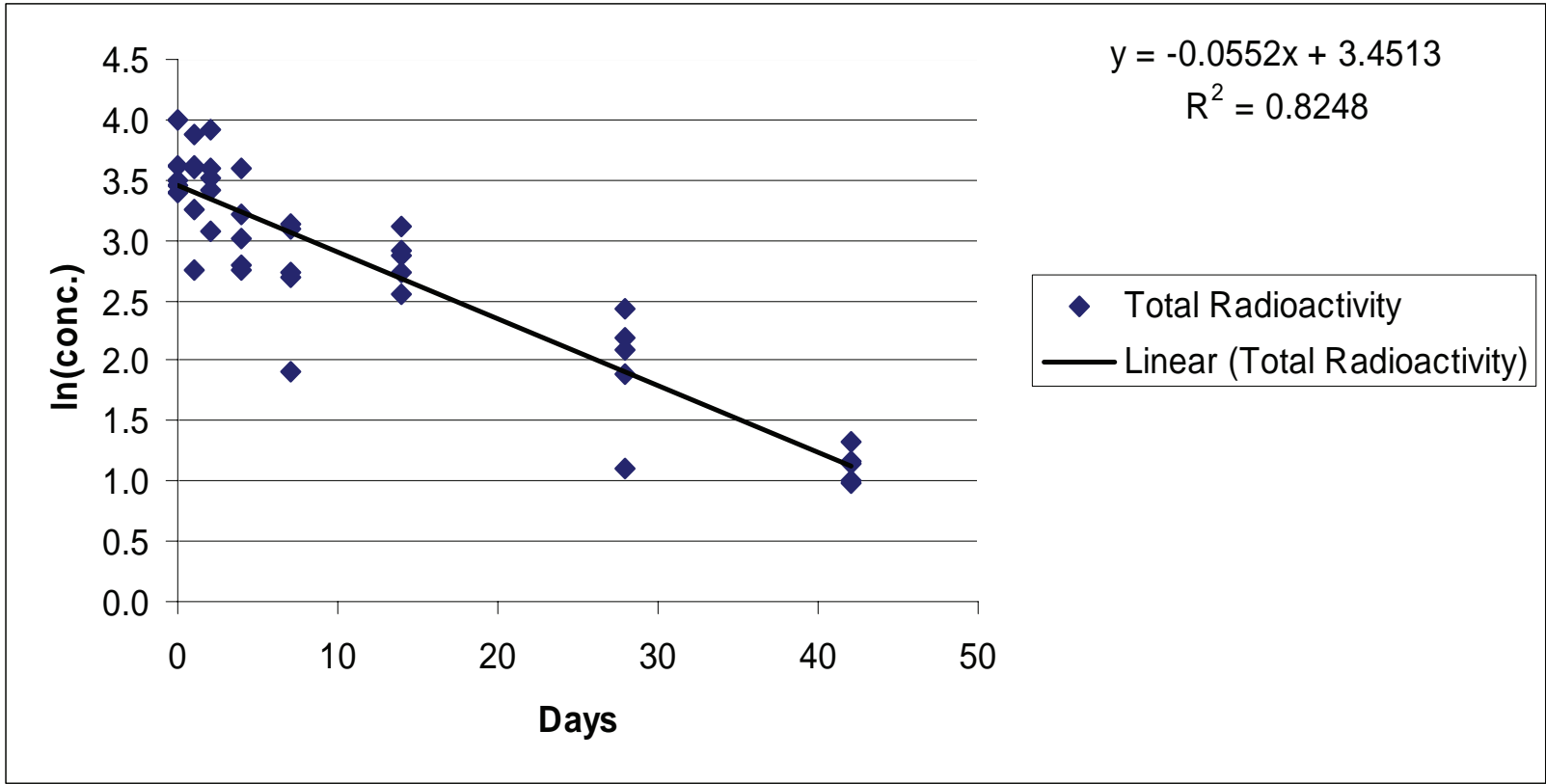
k_2 = depuration rate constant (day⁻¹);

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

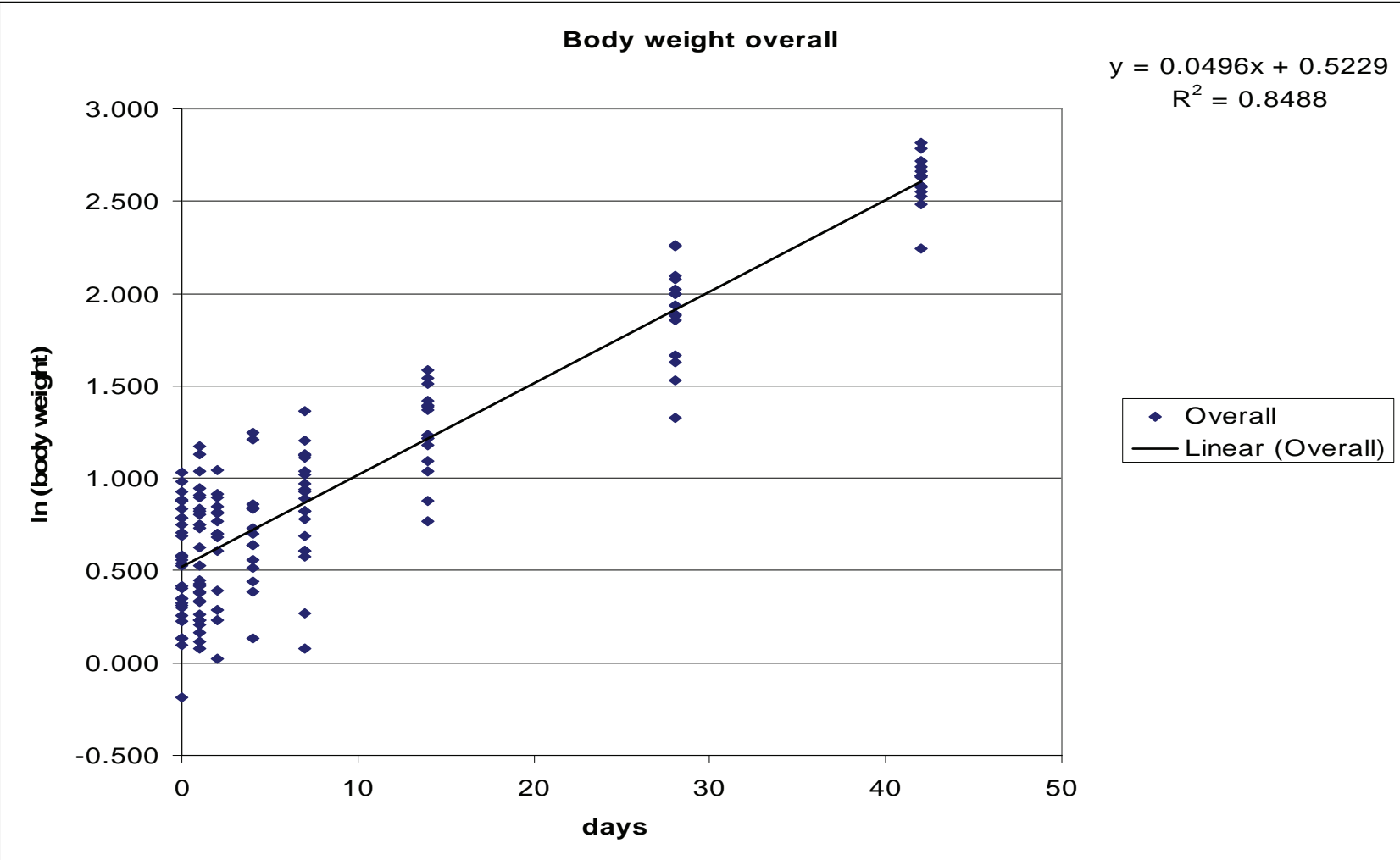
C_{food} = concentration in food (mg/kg food);

t = duration of the feeding period (d)

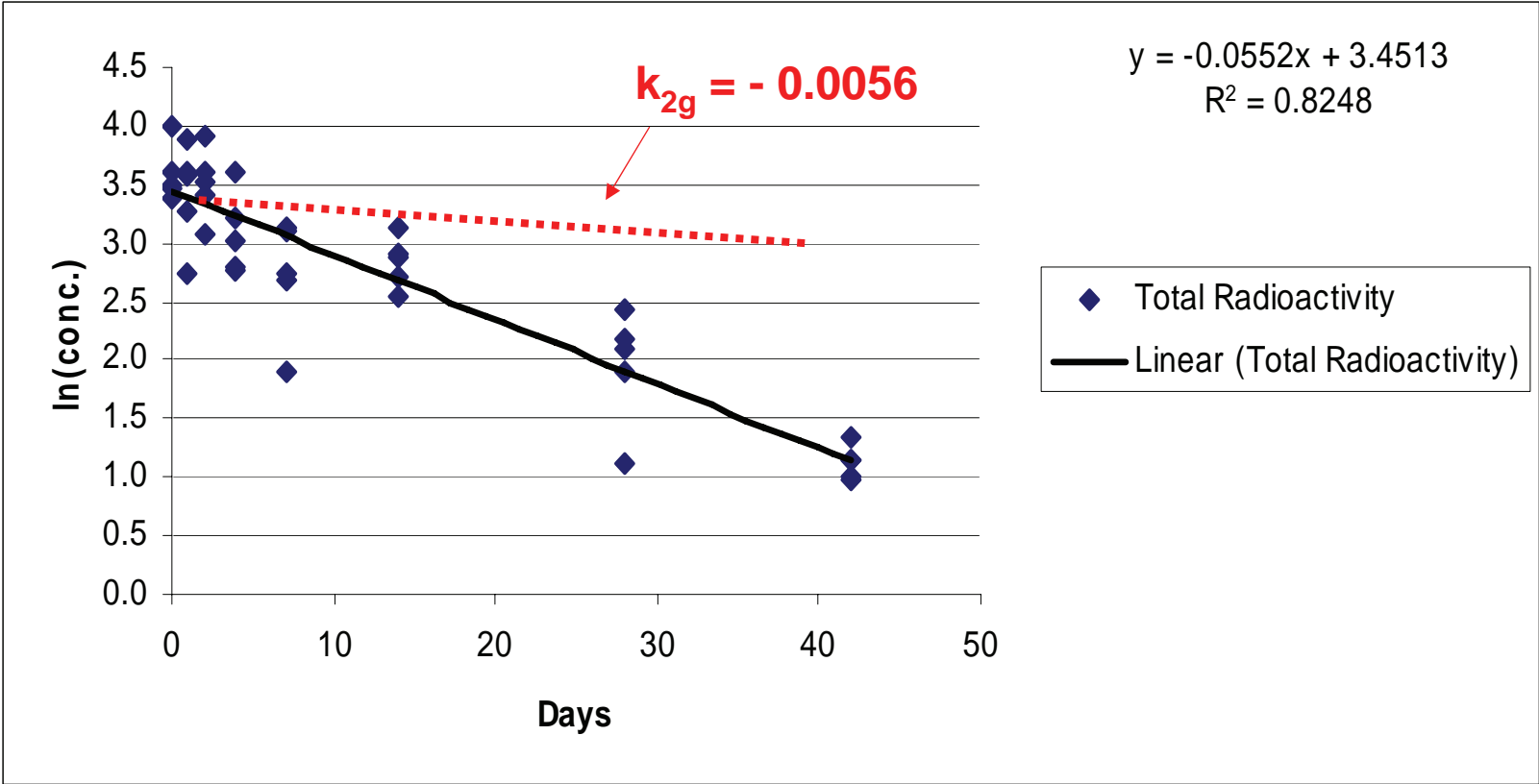
Example – parent equivalents in fish



Example – fish growth



Example: growth correction, k_{2g}



Calculations

We now have all the variables to enable the BMF to be calculated

$$\text{BMF} = \frac{I \cdot \alpha}{k_2}$$

$$\alpha = \frac{C_{0,d} \cdot k_2}{I \cdot C_{\text{food}}} \cdot \left(\frac{1}{1 - e^{(-k_2 \cdot t)}} \right)$$

Lipid correction

- BMF can also be corrected for lipid content

$$L = \frac{L_{\text{fish}}}{L_{\text{food}}} \qquad \text{BMF}_L = \frac{\text{BMF}}{L}$$

Where:

L = lipid correction factor;

L_{fish} = mean lipid fraction in fish

L_{food} = mean lipid fraction in food

Discussion/outlook

- What does a BMF value mean?
 - $\text{BMF}_L < 0.01$ implies a BCF < 2000
 - $\text{BMF}_L < 0.1$ implies a BCF < 5000
 - $\text{BMF}_L > 0.1, < 1$ implies a BCF around 5000
 - $\text{BMF}_L > 1$ implies a BCF > 5000
- Should growth correction of rate constant be performed?
- Lipid normalisation?

- A draft OECD 305 Guideline including the dietary uptake method is hoped to be issued for public comment in Spring 2012
- Finalisation of the method late 2012

Contract Research Services

Harlan Laboratories

Research Models and Services

Any questions please contact Chris Mead at cmead@harlan.com