National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

PBT workshop: bioaccumulation

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Bioaccumulation criteria (Annex XIII)

Framework	Legislation	B / vB criteria	Criteria / Technical Guidance on PBT assessment
Former EU new and existing substances legislations	EU Directive 93/67/EEC and EU Regulation793/93/EEC and EC 1488/94; both now replaced by REACH	B: BCF > **2,000** vB: BCF > **5,000**	TGD/TGD (EC, 2003)
REACH	EU Regulation EC/1907/2006 (EC, 2006)	B: BCF > **2,000** vB: BCF > **5,000**	Annex XIII (EC, 2011) / Guidance on information requirements and Chemical Safety Assessment (ECHA, 2008)
UNEP	Stockholm Convention (UNEP, 2001)	BCF > **5,000** or log K_{ow} > **5** Other, e.g. very toxic Monitoring data	Annex D (Stockholm Convention, 2001) / none
LRTAP (UN-ECE)	UNECE POP Protocol under the Convention on Long-range Transboundary air Pollution(UNECE, 1998a)	BCF > **5000** or log K_{ow} > **5** Other, e.g. very toxic Monitoring data	Executive Body Decision 1998/2 (UNECE, 1998b) / none
IMO Ballast Water Convention	International Convention for the Control and Management of Ships' Ballast Water and Sediments (IMO, 2004)	BCF > **2,000** or log $P_{ow} \geq$ **3**	Guideline G9 (MEPC, 2008) / None
Biocides	EU Directive 98/8/EG (EC, 1998)	Reference to revised TGD[d]	Technical Notes for Guidance on Annex I Inclusion ECB, 2002) / 'revised' TGD[d]
Plant protection products	EU Regulation 1107/2009 (EC, 2009)	POP: BCF > **5,000** or log K_{ow} > **5** Other, e.g. very toxic or bioaccumulation in non-target species PBT; B: BCF > **2,000** PBT; vB: BCF> **5,000**	Annex II (EC, 2009) / None
Human pharmaceuticals	EU Directive 2004 /27/EC (EC, 2004b)	Reference to Annex XIII[b]	Reference to Annex XIII[b] / Guidance on information requirements and Chemical Safety Assessment (ECHA, 2008)
Veterinary pharmaceuticals	EU Directive 2004/28/EC (EC, 2004c)	Reference to TGD	EMA/CVMP Revised guideline (EMA, 2008) / TGD[b]
OSPAR	OSPAR Convention for the protection of the marine environment of the North-East Atlantic (OSPAR, 2003)	log $K_{ow} \geq$ **4** or BCF ≥ **500**	OSPAR Cut-off values for the selection criteria of the OSPAR dynamec selection and prioritisation mechanism for hazardous substances (OSPAR, 2005) / none

Conclusions on Annex XIII

- Clear description of criteria
  - Bioconcentration in aquatic species
    - BCF for fish and invertebrates (mussels)

- Sufficient explanation on what information to consider
  - Terrestrial bioaccumulation
  - Monitoring data in humans and wildlife species
  - Chronic toxicity
  - Kinetics (k_1/k_2)
  - Biomagnification and trophic magnification (BMF, TMF)

- Lack of detail how to interpret this additional information

Higher trophic level marine predator

Lower trophic level predator

BMF2

TMF

Large fish

Human fish consumption

BMF1

Small fish

BCF

Water

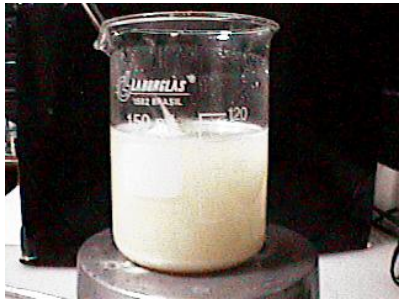
Aquatic bioconcentration (BCF) tests

- OECD 305 test (fish)

- ASTM E1022-94 (mussels)
  - Mussels might give completely different BCFs
- normalization to 5% lipids (if applicable)

- direct comparison with B and vB criteria

- difficult to maintain exposure concentrations



OECD Guideline No. 305

- Flow through test with fish
- Most valid applied to stable organic chemicals with 1.5 < log K_{ow} <6.0
- Radio labeled chemicals may be used (overestimation of BCF-parent compound, metabolites and assimilated carbon)
- Recommended species: OECD fish species

Results from BCF test

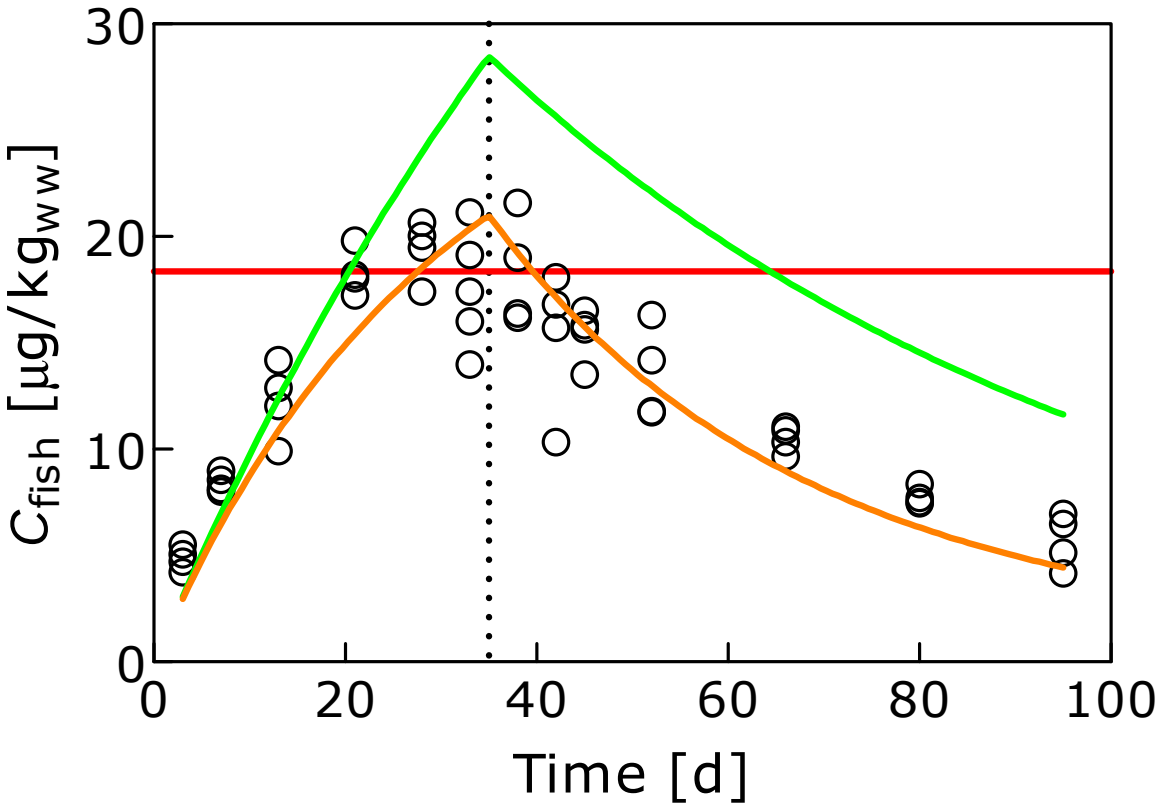
- Parameters determined
  - uptake rate constant (k_1) (size of organism)
  - depuration rate constant (k_2) (size of organism, lipid content, metabolism, growth)
  - C_f at steady state conditions
  - C_w at steady state conditions
  - bioconcentration factor (BCF)
  - lipid normalised BCF (allows comparison between species or specific organs)
- Calculation of BCF
  - BCF = Cf/Cw at steady state = k1/k2 (assuming first order kinetics), growth correction

Growth in bioconcentration tests

Juvenile fish

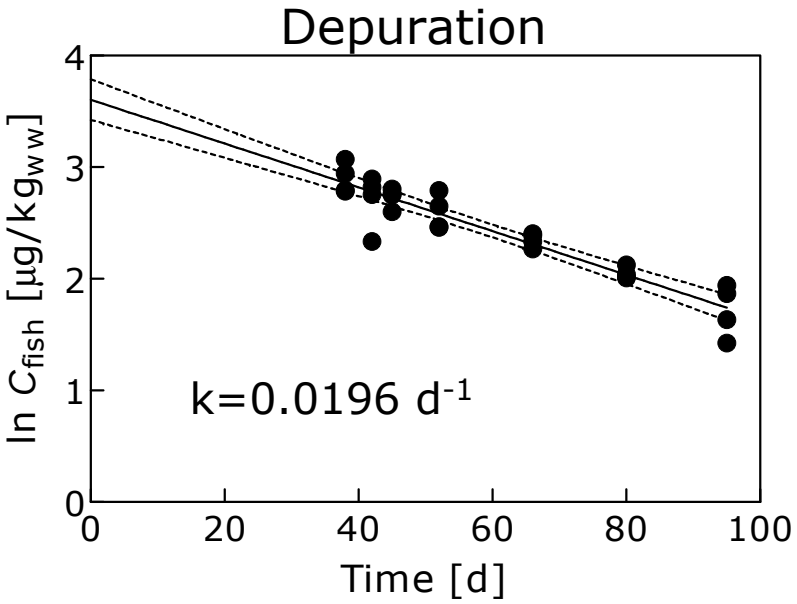
- Consume up to 3% of their body weight per day
- Grow fast, body weight increase can be 3% per day as well !
  - High caloric content of diet
- Grow fat, doubling of lipid content in 28 days not unusual
  - 1 to 3% of body weight per day (depending on the species used and calorific value of the food) keeps the lipid concentration in most species of fish at a relatively constant level during the test

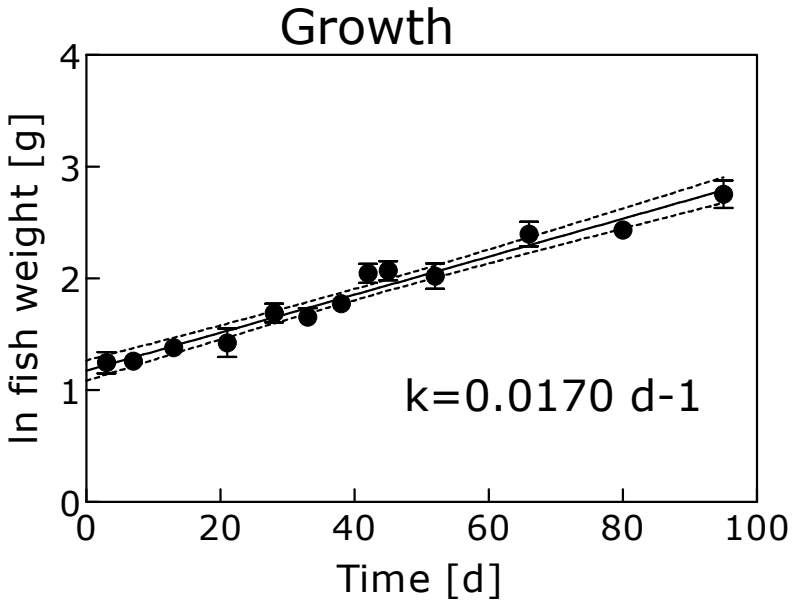
Example



Steady-state is not reached
Kinetic fitting leads to higher BCF
Growth correction results in even higher BCF

What is reason of plateau level?





Almost equal amount of substance in increasing mass of fish

› Little excretion, mostly dilution

- Why growth correction?
  - Not all fish grow, especially prey fish remain small

- Steady-state BCF is useless, if fish are growing fast

Dietary accumulation test

- Draft to be included in OECD 305 test (fish)
- Biomagnification study
- Clearance rate measured, uptake rate estimated from weight
- Normalization to 5% lipids
- Calculated BCF directly compared with B and vB criteria

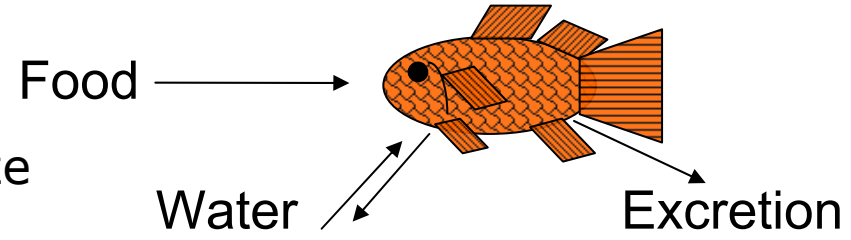
- Regular OECD 305 preferred
  - Constant exposure concentration,
  - difficult to maintain exposure concentrations
    - Solubility < 0.01-0.1 mg/L
    - log K_{ow} > 5
- Alternatively, dietary test

Kinetics and BMF from dietary test

- BMF from dietary test is not comparable with field BMFs

- Only dietary exposure
  - Quotient of sum uptake rates and sum elimination rates
  - Gill uptake and dietary uptake simultaneous
  - Depuration similar in all cases (passive diffusion, metabolism)

- Small fish instead of full food web
  - Depuration kinetics change with size
  - Dietary BMF is size dependent



- BMF from dietary test is not good metric for field biomagnification

Field data

- Bioaccumulation factor (BAF)
  - Ratio between organisms and water
  - Exposure both from water and food
    - Important: Non-steady state, reduced bioavailability (SPM, DOC)

- Biomagnification factor (BMF)
  - Ratio between organisms and food (prey)
  - Exposure both from water and food
    - Important: Non-steady state, depends on couple prey-predator

- Normalization to (5%) lipids

- Definitive assignment of B status possible?

Trophic magnification factors

- Trophic magnification factor (TMF) or food web magnification factor (FWMF)
- Average BMF per trophic level
- Pellston workshop 2008:
  - Information from field studies provides the most conclusive evidence of the ability of chemicals to biomagnify in food-webs.
  - The most relevant measure of biomagnification in food-webs is the Trophic Magnification Factor (TMF) or Food-Web Magnification Factor (FWMF)
- Indicates trophic magnification, no magnification or trophic dilution

Other data

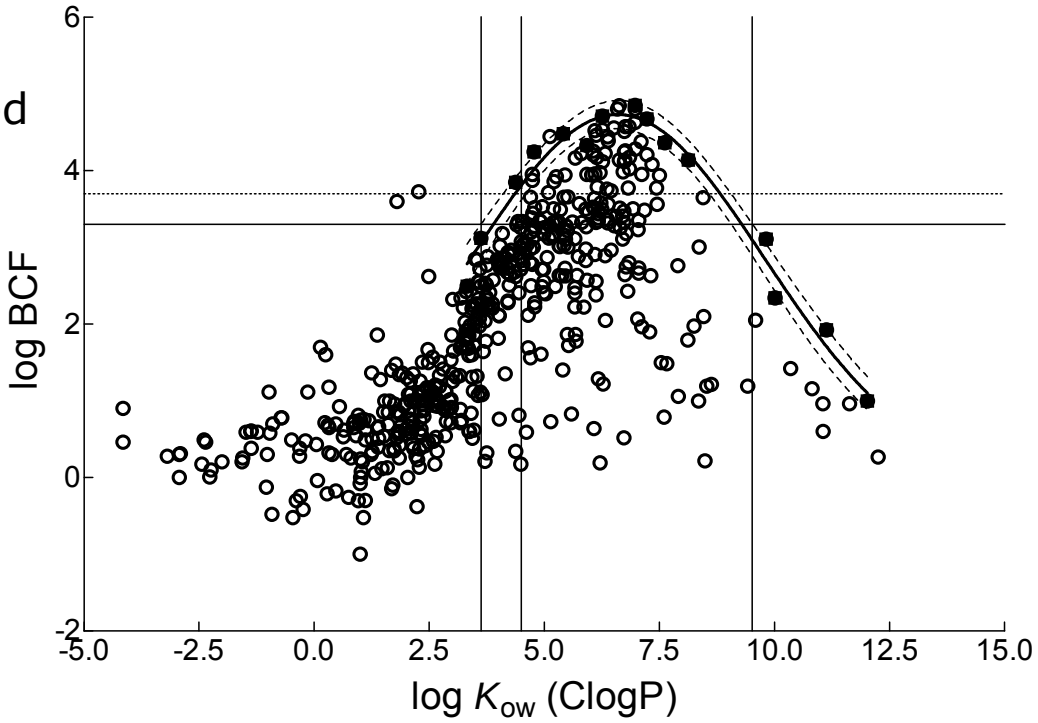
- Terrestrial and benthic bioaccumulation studies
  - Normalized values (lipid, organic carbon) usually are ~1 kg/kg
    - Important: Reduced bioavailability (e.g. black carbon), reduced uptake
- Mammalian studies
  - Indirect: Absence of toxicity in long-term toxicity studies
  - Direct: Toxicokinetic study
    - Important: Differences in metabolism between fish and mammals
- Fish toxicity studies
  - Low chronic systemic toxicity
- Biotransformation, e.g. *in vitro* rate constants
- Read–across
  - Experimental data for other very similar substances

Use in weight of evidence approach

Octanol-water partition coefficient

- log K_{ow} > 9.6 → BCF < 2000 based on calculated log K_{ow} and both aquatic and dietary BCF

- Lower values for log K_{ow} derived with:
  - Only dietary BCF without bioavailability correction
  - Different log K_{ow} estimates (KOWWIN)
  - Experimental log K_{ow} values



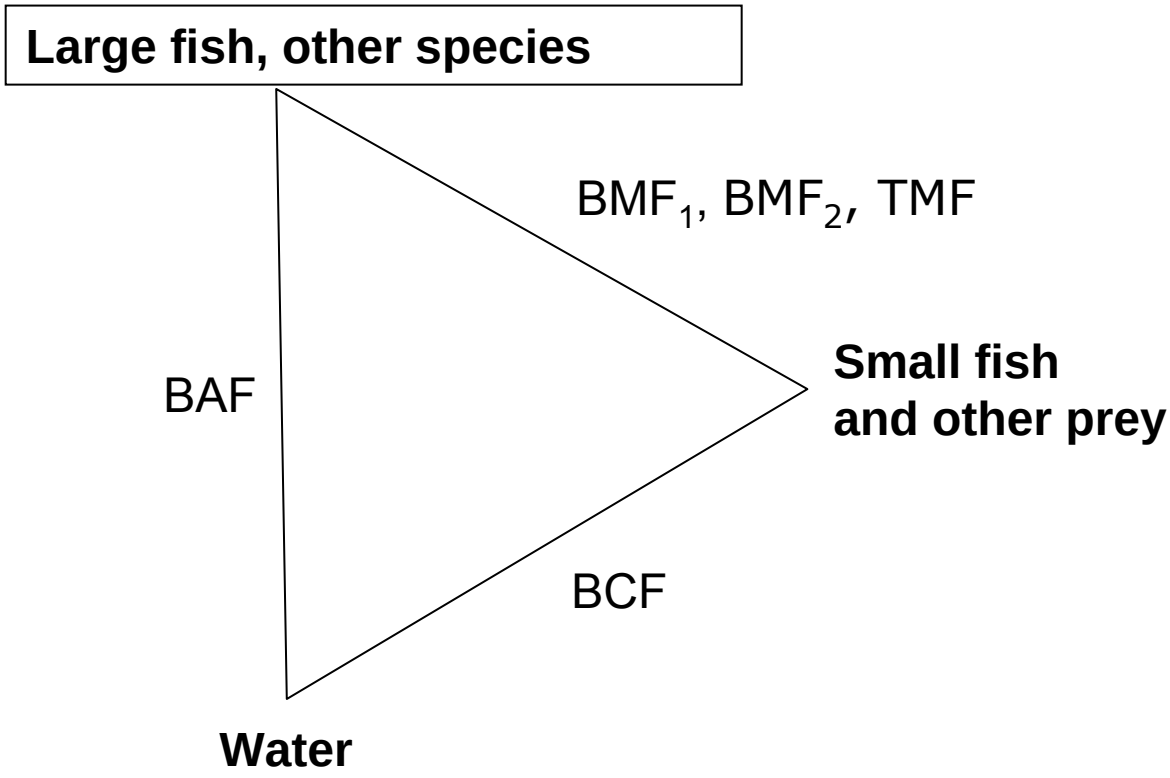
Indicators for a limited bioaccumulation potential

Indicator	Probably not B	Probably not vB
Maximum molecular length	> 43 Å	> 43 Å
$D_{max\ ave}$ in combination with molecular weight	> 17 Å **and** > 700 g/mol	> 17 Å **and** > 1100 g/mol
Octanol solubility ($mg/l_{octanol}$) in combination with other indicators (absence of chronic toxicity)	<0.002 mmol/l * MW (g/mol)	<0.002 mmol/l * MW (g/mol)
log K_{ow} (calculated)	>10	>10

Use of indicators

- To be used in a weight-of-evidence approach, indicators are not used as such, only in combination with other data
  - In combination with
    - Chronic mammalian toxicity data
    - Mammalian toxicokinetics
    - Bioaccumulation in invertebrates
    - Read across with other substances
    - Chronic fish studies

Weight of evidence approach



Non-testing information

Terrestrial and benthic accumulation

Mammalian toxicity and toxicokinetics

Monitoring data in humans

Conclusions

- BCF testing is complicated for PBTs
- Dietary test is very useful experimental alternative
- Indicators are valuable non-testing methods
  - Indicators are not criteria
  - Not to be used on their own
- Interpretational issues remain with regard to
  - Vertebrates (fish) versus invertebrates (mussels, crustaceans)
  - Biomagnification (trophic magnification) versus bioconcentration from water