# ILSI-HESI SETAC *In-Vivo* Bioaccumulation Database Workshop

***Annie Weisbrod***

**The Hague, 5 May 2006**

**Contributions from: Tom Parkerton (ExxonMobil), Mark Bonnell (Env Canada), Larry Burkhard (US EPA), Kent Woodburn (Dow Chemicals), Jon Arnot (Trent University)**

# Workshop basics

- Background & Purpose
- Participants
- Session topics & overviews
- Next steps & recommendations

- Review of in vivo bioaccumulation protocols

# Background

- **The advance of PBT programs is challenged by reality.**
  - **Few empirical bioaccumulation (B) data are available for the 10,000s of commercial substances that require evaluation.**
  - **Most preliminary B assessments rely on computer model predictions and *in vivo* data extrapolation.**
- **<u>January 2005</u>: ILSI-HESI agrees to coordinate workshops to gain scientific & global consensus for advancement of B assessment techniques.**
- **<u>April 2005</u>: Techniques & methods to improve B assessments (e.g. reduce animal testing & cost of in vivo tests, meet regulatory timing needs). Outcomes:**
  - **<u>Partnerships</u> to develop *in vitro* tests & translations, generate data on representative chemicals, communicate how to use tiers of information from B and ADME models, *in vitro* and *in vivo* lab and field studies.**
  - **<u>Three more workshops needed</u>: Review existing B data, Use of ADME methods to improve B assessment, Needs in the EU.**
    - **4th topic for Future: How to use & collect field data**

# Purpose of Workshop

- **November 2005: Experts from governments, industry, and academia examined the availability and quality of *in vivo* fish bioaccumulation data, and proposed steps to improve bioaccumulation predictions.**
  - **Focus on fish data because regulatory assessments & legislation tend to be on bioconcentration of substances from water into fish.**
  - **Workshop goals were to understand where the data are, overlap among databases, the chemical diversity of data, and how to improve access for assessors and modelers.**
    - **A majority of the available data appear to have been used in the development and testing of QSARs and computer models used today.**

- **March 2005: In vitro ADME Workshop (Sue & Birgit)**

# Participants: 4 sectors, 3 continents

| | |
|---|---|
| J Arnot | *Trent University* |
| F Gobas | *Simon Frasier University* |
| O Mekenyan | *University of Bourgas* |
| B Boethling | *US EPA – Off. Pollut. Prev. & Toxics* |
| M Bonnell | *Env Canada - New Substances* |
| T Bridges | *US Army Corps of Engineers* |
| L Burkhard | *US EPA - Env Effects Research Lab* |
| P Cureton | *Env Canada - Existing Substances* |
| M Lin | *Env Canada - Existing Substances* |
| C Russom | *US EPA - Env Effects Research Lab* |
| Y Sakuratani | *METI-NITE, Japan* |
| T Traas | *RIVM, Netherlands* |
| P Howard | *Syracuse Research Corporation* |
| J Tunkel | *Syracuse Research Corporation* |

| | |
|---|---|
| L Lucas | *Leadscope* |
| C Yang | *Leadscope* |
| C Sachse-Zaquez | *Res. Inst. for Fragrance Materials* |
| D Salvito | *Res. Inst for Fragrance Materials* |
| T Springer | *Wildlife International* |
| K Thomas | *ILSI-HESI* |
| W DeWolf | *DuPont* |
| B Hoke | *DuPont* |
| S Jackson | *BASF* |
| T Parkerton | *ExxonMobil Biomedical* |
| D Powell | *Dow Corning* |
| A Weisbrod | *Procter & Gamble* |
| K Woodburn | *Dow Chemical* |

# Workshop Sessions

1. **Session 1 - Overview of existing data sources on fish *in vivo* bioaccumulation**
   - BCFWIN BCF Database – Syracuse Research Corp & US EPA
   - Environment Canada BCF & BAF Database
   - Japan METI-NITE BCF Database
   - US EPA OPPT (PMN) BCF Database
   - US EPA ORD Aquire database
   - RIVM Database
   - CONCAWE (Hydrocarbons) Database
   - US ACE ERDC BSAF Database
   - US EPA ORD Superfund BSAF data
   - Review of BCF data for modelling - Mekenyan POPs

- **OUTPUT = Publication for Environmental Health Perspectives**

# Main bioaccumulation data sources

| Data Source | # chemicals | Public access? |
|---|---|---|
| Japan METI-NITE database | **~800 "existing"<br>~3100 "new"** | www.safe.nite.go.jp/english/index.html<br>"New chem." data are proprietary |
| U.S. EPA AQUIRE database | **~700** | www.epa.gov/ecotox |
| U.S. National Library of Medicine Hazardous Substances (HSDB) | **hundreds** | http://toxnet.nlm.nih.gov. |
| Env Canada BCF and BAF database | **950** | Request from Env Can. |
| RIVM database | **~700** | No. |
| SRC Fish BCF & Environmental Fate | **694** | http://esc-plaza.syrres.com/efdb.htm |
| CONCAWE Hydrocarbons BCF | **84** | No. |
| U.S. Army ERDC BSAF database | **205** | http://el.erdc.usace.army.mil/dots/database.html |
| U.S. EPA Superfund BSAF data | **In progress** | Expected 2007 |
| "Handbook of Physical Chemical Properties and Environmental Fate" | **hundreds** | Mackay et al (2006). CRC Press LLC |
| "Comparative QSAR" | **209** | J Devillers (1998). Taylor & Francis Publishers |

# Workshop Sessions

1. Session 1 – Data Sources
2. **Session 2 – Data base content and fields**
   - **Prioritize needs for a BCF database combining data from all sources, e.g. data quality assessment**
   - **Identify database fields most useful for BCF evaluation and use**
     - **Organism properties (species, weight)**
     - **Chemical attributes (CAS #, SMILES)**
     - **Study considerations (lab or field, static or flow-thru, duration)**
     - **Analytical methods (reported, parent-specific, all media & tissues)**
     - **Data analysis (steady state, first-order kinetics, growth & lipid corrections)**
   - **OUTPUT= Recommended fields for OECD BCF template, CEFIC-EURAS database, AQUIRE BCF database**

# Workshop Sessions

1. Session 1 – Data Sources
2. Session 2 – Data base content and fields
3. **Session 3 - Critical evaluation of published BCF studies**
4. Session 4 & 5 – Working groups

# Why evaluate B data? Motivation

- **Increasing use of *in-vivo* B data in decision-making, e.g.**
  - **PBT evaluation in EU under REACH and Water Framework Dir.**
  - **PBT categorization of Canadian Domestic Substance List**
- **Experimental data may not follow a specific guideline, e.g. OECD 305, but can still yield useful information.**
- **There is no harmonized / accepted guidance for evaluating in vivo B data**
- **The lack of systematic B data evaluation & compilation impedes:**
  - **Consistency in decision-making**
  - **Development of improved predictive models**
  - **Prioritization for collection of new B data**

# Goals of Session - evaluating data

- **To develop consensus on the guidance to evaluate exiting, and reporting future, in-vivo lab bioaccumulation data ... Suggest use of Klimisch approach:**
  - **Reliable**
  - **Reliable with restrictions**
  - **Not reliable (invalid for use)**
  - **Not assignable (not enough study detail)**
- **To test the practicality of the guidance**
  - **Develop an initial check list of study considerations**
  - **Conduct break-out exercise in which small groups (3-4 persons) apply the check list in assessing published fish BCF studies**
- **To identify additional considerations relevant for field bioaccumulation studies**

# What we did - Checklist

- **Proposed check list for lab B data evaluation & reporting (not all here…selected fields)**
  - Test substance identity, purity & water solubility
  - Fish size & lipid content
  - Validity & nature of test substance analysis
    - Parent-specific vs. non-specific, e.g. total radioactivity
  - Exposure conditions of test
    - Static vs. flow-through
    - Constant vs. variable concentrations (e.g. Banerjee method)
    - Magnitude of test concentration relative to solubility limit / toxicity
  - Organism health, water quality conditions during test
  - Details of reported BCF endpoint
    - Steady-state value
    - Unambiguous units (wet, dry, lipid basis)
    - Correction for growth-dilution and /or bioavailability

- **Evaluated ~15 published manuscripts in teams of 2-3.**

# Findings from study examination

- **Having a 'consensus' check list is helpful.**
  - **Consistent basis to evaluate BCF data across studies and to guide the publication of future studies.**
  - **From this exercise, we added more considerations for:**
    - **Sample design**
    - **Spatial & temporal variability**
    - **Site-specific assumptions (e.g. sediment-water equilibrium)**
    - **Approach used to address undetected test substances**
- **The specification of rigid criteria that define reliability classes for fish BCF data is not straight-forward.**
  - **Existing literature often omit key study details (next slide)**
    - **Weight of evidence / professional judgment required**
  - **Schemes to assess BCF reliability depends on user needs**
    - **Can vary, but must be transparent (next slide)**
- **Further work to optimize and standardize the design of field bioaccumulation assessments given their resource intensity**

# Recommendations

1. **The disparate B datasets should be combined into one easily accessible database to improve data accessibility and eliminate duplicate records.**
2. **Reliability of existing *in vivo* B data should be evaluated.**
   - **Predictive models cannot be improved using unreliable data; few current databases have assessed quality.**
3. **Current assessments need to go beyond $K_{OW}$-based decisions**
   - **BCFs estimated using only Kow neglect the effects of biotransformation and differential uptake process efficiencies of different chemicals.**
   - **New in-vivo and in-vitro approaches are needed to focus resources and minimize unnecessary animal use for rational chemicals management.**
4. **Given the potential importance of dietary pathways in chemical uptake, and of chemical biotransformation, there is a need to understand real world mechanisms.**
   - **BCF values do not include the trophic magnification or dilution processes observed in nature.**
   - **BAF and BSAF can be important to understand.**

# Next Steps

- **Two publications summarizing the data sources and guidance on study quality is needed**
  - **Manuscripts submitted to EHP & IEAM in May/June 2006.**
- **Combine data from various sources to eliminate duplicates and improve access for modellers & assessors.**
  - **Env Canada, RIVM, Mekenyan (with METI?) working to compile full set into US EPA Aquire.**
  - **CEFIC LRI for EURAS for "gold standard database"**
- **A review of the chemical domains of existing models would be helpful to identify areas for expansion (i.e. which chemical classes would benefit from having *in vivo* B data?).**
- **Communicate the complexity of B in nature.**

# Summary of *in vivo* fish bioaccumulation protocols

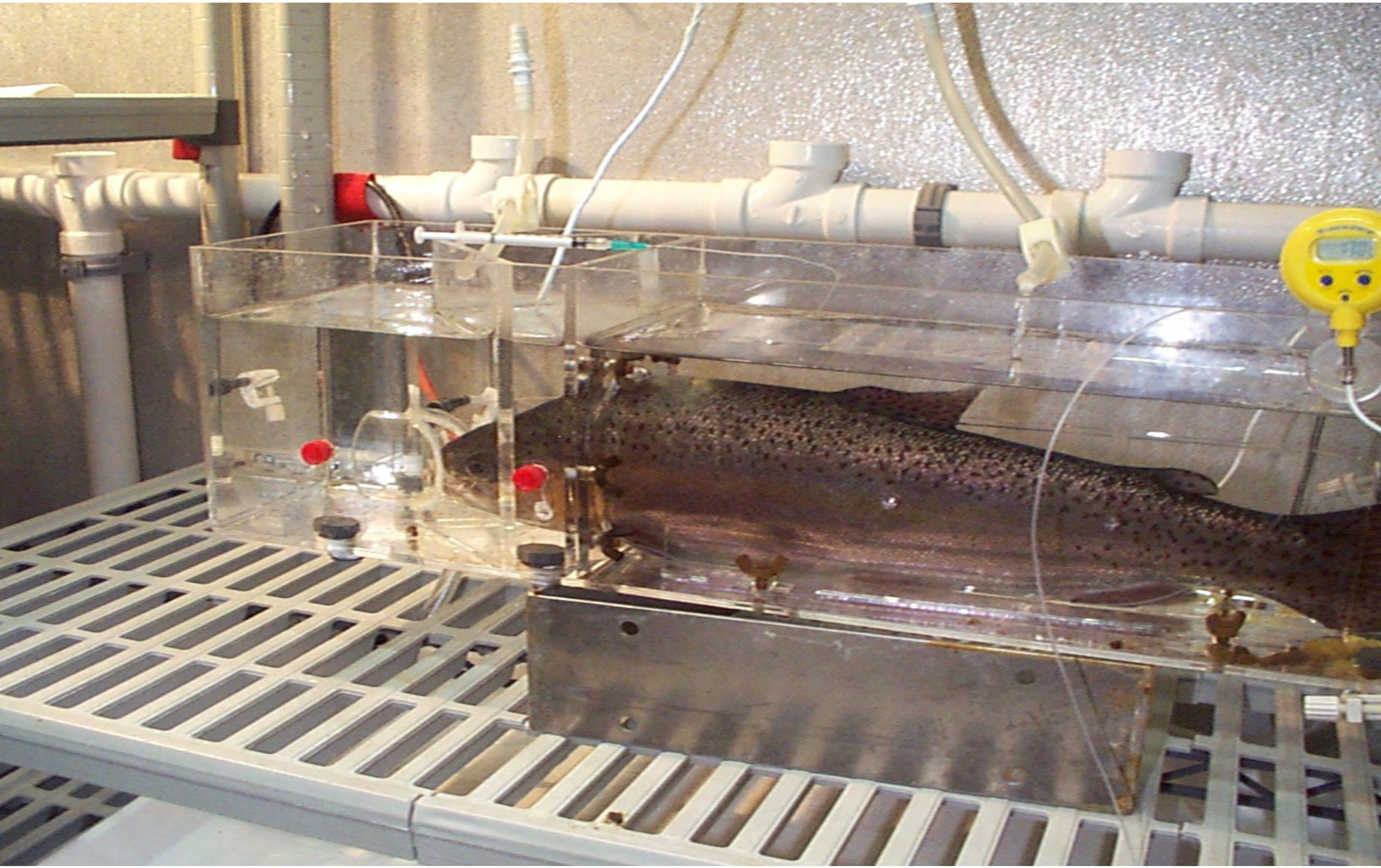
**OECD 305 BCF test**

**Dietary exposure**

**Short term BCF kinetic tests**

# Respirometer-Metabolism Chamber
# 96-hr *In Vivo* **ADME** Screen (1 Kg trout)

**D Huggett, Pfizer**



# Fish Bioconcentration Test

- **STANDARD Fish BCF study: OECD 305E/EPA 1730**
  - $^{14}C$ radiolabeled material
  - two dose levels
  - 28-day water exposure; 14-day clearance
  - Rainbow trout, bluegill, fathead minnow, zebrafish
  - expensive ($125,000), animal intensive

- **Modified OECD 305E test, kinetic modeling:**
  - Measure uptake rates; calculate depuration rate
  - shortens experimental time frame (5-14 days)
  - reduces animal usage and cost (analytical)
  - equivalent data quality output (i.e., BCF value)
  - requires more complex data analysis

K Woodburn, Dow

# BCF comparison: OECD / EPA method vs. short-term, exposure-only analysis

- OECD 305: 42-day total exposure and clearance (28-d uptake + 14-d clearance
- 7-day exposure only, no clearance, with samples generally taken on days 1, 2, 4, 7

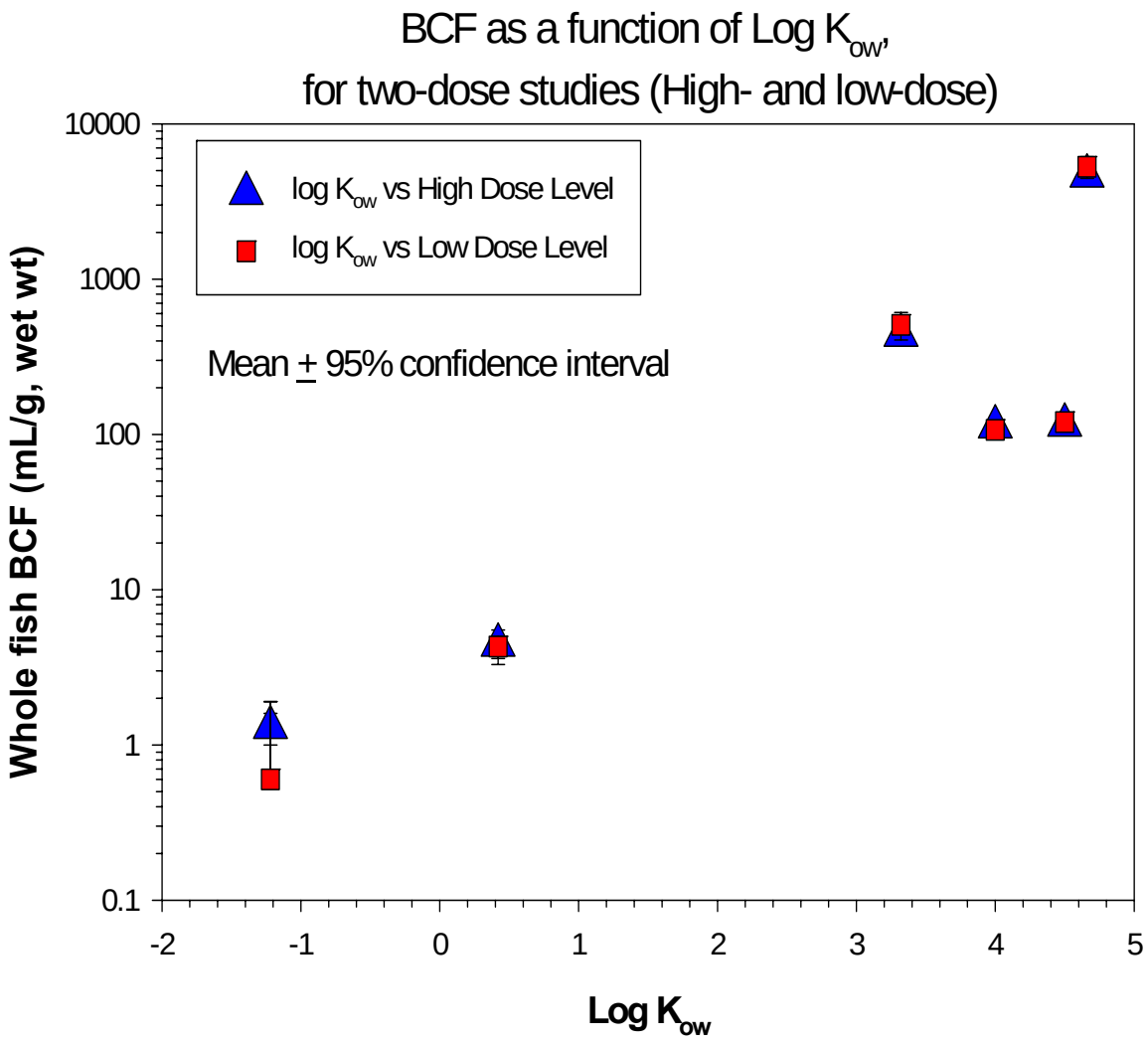
| | | OECD: 42-d Study | | | 7-d Exposure | | |
|---|---|---|---|---|---|---|---|
| **Chemical** | **Log $K_{ow}$** | **k1** | **k2 (km)** | BCF | **k1** | **k2*** | BCF |
| **EL-161** | **5.1** | **365** | **0.27** | 1350 | **260** | **0.25** | 1040 |
| **XDE-105** | **4.5** | **16** | **(0.42)** | 40 | **15** | **(0.30** | 50 |
| **butyl ester** | **3.3** | **10** | **(20)** | 0.5 | **36** | **(63)** | 0.6 |
| **methyl ester** | **3.8** | **195** | **(85)** | 2.2 | **154** | **(0.84)** | 1.8 |
| **Dowco 233** | **-0.5** | **0.7** | **0.8** | ~1 | **2.3** | **0.4** | 6 |
| **XDE-795** | **4.7** | **1440** | **0.31** | 4650 | **1560** | **0.36** | 4342 |
| **XDE-179** | **4.7** | **480** | **(0.28)** | 1730 | **520** | **(0.65)** | 800 |

** From* $dC_{fish}/d_t = k_1 * C_{water} - k_2 * C_{fish}$ *. To solve simultaneously for k1 and k2, fish and water residue data are analyzed with software designed for modeling dynamic systems, such as SimuSolv and Berkeley Madonna. Assumes first order kinetics.*

K Woodburn, Dow

# OECD 305/EPA 1730: Are Two Separate Exposure Experiments Really Necessary?

- **OECD 305/EPA 1730 currently requires <u>two</u> separate exposure levels (1x, 10X)**
- **Data collected over a range of $K_{ow}$ (N=6) suggest dual exposures are not necessary to validate BCF and represent an unnecessary use of animals, time, $.**




K Woodburn, Dow

# Dietary Bioaccumulation Test

- **Advantages vs. OECD BCF test:**
  - Allows higher / constant test exposures for poor water soluble compounds
  - Improves analytical detection of parent compound
  - Allows characterization of biomagnification
  - Reduces fish required & cost (50-75%)
- **Basic Method**
  - Spike substance into fish diet (14% lipid); measure.
  - Feed 3% bwd spiked diet to trout for 7-10 days (uptake)
  - Transfer exposed fish to clean food (depuration)
  - Analyze fish at different depuration times (0, 1, 2, 4, 7, 14 days)

$$BMF = \frac{C_{Fish_{ss}}}{C_{Diet}} = \frac{\alpha I}{K_e}$$

T Parkerton, ExxonMobil

# What is relevant?

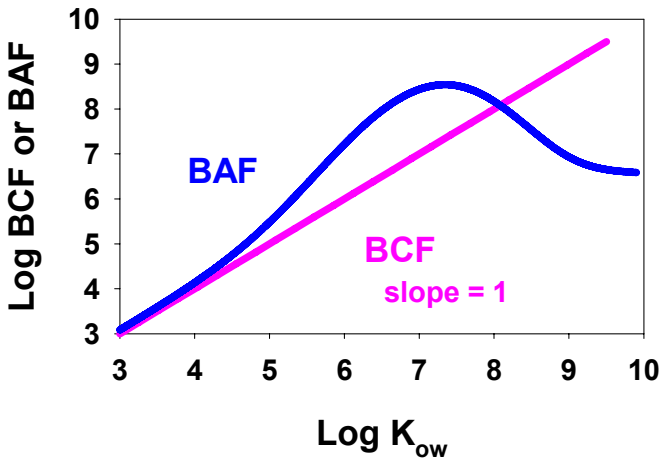
- **Field data are complex, and not well predicted by lab tests.**

- **Models that appropriately incorporate all inputs and outputs might be OK....work remains...**

# Why Lab BCF ≠ Field BAF

- Lab-derived BCF
  - Water based exposure
  - No growth
  - Consistent % lipid
  - Constant Conditions
    - Chemical exposure
    - Temperature
  - Measures uptake & depuration kinetics
  - May or may not include metabolism processes
  - Steady-state conditions

**Steady-State BCF and BAF**
**(lipid normalized & freely dissolved)**
**(not metabolized)**




- Field-derived BAF
  - Water & food exposures
  - Organism life history
    - Diet, Growth, Food web structure, Trophic level, Metabolism, and Migration
  - Ecosystem conditions vary
    - Temporal and spatial variability in exposure
    - Sediment-water column chemical relationships
    - Temperature
  - Simultaneous exposure to all substances
  - Conditions: Depends upon past and current loadings
    - Commonly pseudo-steady-state conditions
  - **Provides ultimate "Truth"**

L Burkhardt, U.S. EPA


Thanks for listening. Questions?