Abstract
The potential for human pharmaceuticals to bioaccumulate in aquatic organisms is rapidly becoming an area of scientific and regulatory interest. Historically, bioaccumulation assessments are conducted after consideration of a chemical’s hydrophobicity (e.g., Kow). The current strategy for determining the bioaccumulation potential of a human pharmaceutical is not different than that in science. Scientists are required to conduct an OECD 303 fish study if the Kow value of a given pharmaceutical is greater than a prescribed regulatory trigger value (e.g., Log Kow > 4). The physical-chemical knowledge (e.g., pKa) of the compound should be incorporated into this initial Kow assessment to better guide the need for a full “B” assessment. In many instances, the current strategy does not utilize the wealth of non-clinical and clinical data available on the absorption, disposition, metabolism and elimination (ADME) of the pharmaceutical of interest, which could be used to better inform scientists on important characteristics and physiological processes associated with that human pharmaceutical. It is important to recognize that fish have the ability to perform many of the same physiological processes that mammals perform, hence a pharmaceutical’s ADME characteristics could be similar in fish. These data can be used to understand the ability of a fish to absorb, distribute, biotransform and eliminate a human pharmaceutical. If needed, studies can be conducted using methodologies widely used on the drug development process to better guide the testing strategy (e.g., in vitro metabolism assays), as well as determining the overall need for an OECD 305 study. By utilizing all the available information collected during the drug development process on a human pharmaceutical, scientists can make a more informed decision regarding the need for bioaccumulation testing. This strategy can lead to a reduced number of vertebrate animals used in laboratory studies, as well as an overall cost savings.

Introduction
Bioconcentration, a process by which a chemical is absorbed from water, is a surrogate measure used by regulatory agencies to describe the bioaccumulation potential of a chemical. Unfortunately, the methodology by which bioconcentration tests are triggered are almost entirely based on the chemical’s Log Kow. The pharmaceutical development process and data developed represent an opportunity to use additional data and methodologies to assess a drug bioaccumulation potential.

Review of Existing Information
- Physical-Chemical Characteristics, QSARs (if applicable)
  - Kow, Molecular Size, Ionization
  - Clinical & Non-Clinical Metabolism
  - Rate of Metabolism, Metabolites Formed, Phase I vs. Phase II

Consideration of Short Term Assays
- Understand ADME “B” Characteristics
  - In Vitro (Microsomal, SR and Hepatocyte)
  - In Vivo-Metabolic Chamber
  - In Vivo-Reduced Time Assay (e.g., 7d uptake/7d depuration)

Standard Regulatory Guideline Study
- OECD 305 (aqueous or dietary)
  - Consider metabolite profile

Field Monitoring & Risk Management
- Consider bioavailability and fate parameters

Figure 1. Draft Framework For “B” Analysis and Testing

Carbamazepine: A Case Study

Background
- Commonly prescribed anticonvulsant
- Mode of action: Inhibition of Na+ channels
- Mentally used for treatment of epilepsy and trigeminal neuralgia
- Also used as mood stabilizer
- Has been available in the US for over 30 years

Mammalian Metabolism
- >70% orally administered CBZ is absorbed
- Undergoes extensive metabolism in liver
- 33 metabolites identified in human and rat urine
- Two important metabolites:
  - 10,11-dihydro-10,11-epoxy-carbamazepine (CBZ EP) (CYP3A4 and CYP2C9)
  - 10,11-dihydro-11-epi-carbamazepine (CBZ OHE) (Microsomal epoxide hydroxylase)

Is CBZ Readily Biotransformed in the Fish Liver?

Untransformed Assays
- Juvenile catfish were sampled for liver tissue
- 90% of liver tissue obtained
- Each reaction vessel for the 39 assays consisted of 50 liver fraction, test compound, a NADPH regeneration system consisting of nicotinamide and nicotinamide dehydrogenase, and NADPH.
- 3D matrix controls and solvent controls were run with each assay.
- Time points: 0.5, 15, 45, and 60 minutes.

Induced Assays
- Juvenile catfish were exposed to CBZ for 3 days in aquaculture solution prior to harvesting of the liver
- Assays were run with similar procedures as above.

Analysis of CBZ by LC-MS/MS

Figure 2. Mean loss of carbamazepine a SD (n=3) over 60 min study time period in untransformed assay.

Figure 3. Mean loss of carbamazepine a SD (n=3) over 60 min study time period in induced assay.

Conclusion
- All available clinical and non-clinical data can help guide the testing and decision making process with regards to a bioaccumulation testing strategy.
- A more intelligent testing and assessment strategy can be developed when considering all available data.
- Read-Across of rodent and human metabolism data can be used as a guide to understand what may occur in fish.
- Metabolite profiling and identification can be helpful in a Weight of Evidence (WoE) discussion, as well as analysis of pharmacologically vs. non-active metabolites
- These data can also be helpful in developing the environmental toxicology testing program.
- Will the fish rapidly eliminate or biotransform the drug which could influence the type of exposure scenario (i.e. static vs. static renewal vs. flow-through).
- Are there target tissues that should be more closely evaluated.
- For carbamazepine, mammalian data suggested that active and inactive metabolites are rapidly formed suggesting a that bioaccumulation of CBZ (BCF > 2000) in fish may not occur. Both Phase I and II metabolic processes are potentially important.
- Fish 59 studies suggested that CBZ is biotransformed.
- Fish exposed to CBZ for 3 days prior to sacrifice had a greater turnover of CBZ, suggesting that induction is an important factor to consider with CBZ biotransformation and accumulation.
- Short and long term fish BCF studies resulted in a lack of appreciable bioconcentration (BCF < 1000).
- In the catfish study (Figure 3), CBZ accumulation in the tissues increased until Day3, where subsequent samples illustrated a decrease in CBZ levels suggestive of an internal metabolic mechanism.
- No single target organ for CBZ in fish as all tissues sampled had similar CBZ levels
- Fish exposed continually to drug residues in a WWTP showed a low BAF for CBZ thereby supporting the rest of the data package

Table 1. Partition coefficients (K) for CBZ in different tissue compartments

<table>
<thead>
<tr>
<th>Tissue</th>
<th>K (L/kg)</th>
<th>Vm (L/kg)</th>
<th>Vc (L/kg)</th>
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<tr>
<td>Muscle</td>
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<td>1.0</td>
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<tr>
<td>Liver</td>
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<td>1.0</td>
</tr>
<tr>
<td>Plasma</td>
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<td>1.0</td>
</tr>
<tr>
<td>Brain</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Carbamazepine: A Case Study Continued

Does CBZ Bioaccumulate in Fish Collected From the Field?
- Test Organisms
  - Oncorhynchus mykiss (Linné, 1758): -Tilapia
  - Pecan Creek Wastewater Reclamation Plant (PCWRP)
  - Activated sludge WWTP w/ UV disinfection
  - Aug. surface of 2.5 mg/L in Pecan Creek - Lake Lewisville
- Tilapia kept in the seed filters of the facility were sampled

Table 3. Partition coefficients (K) for CBZ in different tissue compartments

<table>
<thead>
<tr>
<th>Tissue</th>
<th>K (L/kg)</th>
<th>Vm (L/kg)</th>
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References and Acknowledgements

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Footnotes
1Waterborne Environmental Inc., Leesburg, VA, USA; 2 Wildlife International, Easton, MD, USA