Framework for Addressing Bioaccumulation Potential of Human Pharmaceuticals

Duane B. Huggett [1], and Nikki Maples-Reynolds[1], Santos Garcia [2]

[1] *Waterborne Environmental Inc., Leesburg, VA, USA;* [2] *Wildlife International, Easton, MD, USA*

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. K_{ow}). The current strategy for determining the bioaccumulation potential of a human pharmaceutical is not different in that scientists are required to conduct an OECD 305 fish study if the K_{ow} value of a given pharmaceutical is greater than a prescribed regulatory trigger value (e.g. Log K_{ow} > 3). The physical-chemical knowledge (e.g. pKa) of the compound should be incorporated into this initial K_{ow} 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 to 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 K_{ow}. The pharmaceutical development process and data developed represent an opportunity to use additional data and methodologies to assess a drugs 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

→ STOP

Consideration of Short Term Assays
- Understand ADME and "B" Characteristics
  - In Vitro (Microsomal, S9 and Hepatocyte)
  - In Vivo-Metabolic Chamber
  - In Vivo-Reduced Time Assay (e.g. 7d uptake/7d depuration)

→ STOP

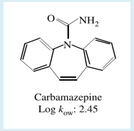
Standard Regulatory Guideline Study
- OECD 305 (aqueous or dietary)
  - Consider metabolite profile

→ STOP

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: Modulation of Na+ channels
  - Mostly 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
- 72% of 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-epoxycarbamazepine (CBZ-EP) (CYP3A4 and CYP2C8)
      - 10,11-dihydro-10,11-dihydroxycarbamazepine (CBZ-DiOH) (Microsomal epoxide hydrolase)

Is CBZ Readily Biotransformed in the Fish Liver?
- Uninduced Assays
  - Juvenile catfish were sampled for liver tissue
    - S9 fraction obtained
  - Each reaction vessel for the S9 assay consisted of S9 liver fraction, test compound, a NADPH regeneration system consisting of isocitrate and isocitrate dehydrogenase, and NADPH.
    - S9 matrix controls and solvent controls were run with each assay.
    - Time points: 0, 15, 45, and 60 minutes.
- Induced Assays
  - Juvenile catfish were exposed to CBZ for 3 days in aqueous solution prior to harvesting of the liver
  - S9 Assays were run with similar procedures as above.
- Analysis of CBZ by LC-MS/MS

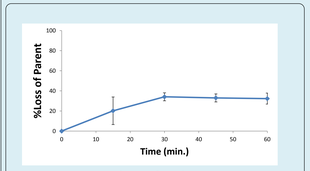


Figure 2. Mean loss of carbamazepine ± SD (n=3) over 60 min study time period in uninduced assay.

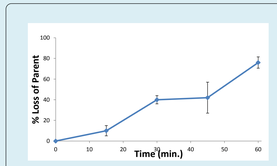


Figure 3. Mean loss of carbamazepine ± SD (n=3) over 60 min study time period in induced assay.

Does CBZ Bioaccumulate in Fish?
- Test Organisms
  - *Pimephales notatus* (Rafinesque, 1820) and *Ictalurus punctatus* (Rafinesque, 1818)
- Bioconcentration studies conducted according to OECD 305 guidelines
  - Fish were kept in standard rearing conditions of 25 ° C water and a 16:8 light/dark cycle throughout experiments.
  - Minnow exposures – 5 turnovers/day/tank
  - Catfish exposures – 2 turnovers/day/tank
  - A continuous flow-through diluter system was used for all laboratory experiments.
- 42d and 14d carbamazepine BCF studies
  - 42d study with minnows had a 300 µg/L exposure concentration
  - 14d study with catfish had a 125 µg/L exposure concentration
- Carrier solvent concentrations well below 0.01%
- Analysis of CBZ by LC-MS/MS

Table 1. Wet weight tissue-specific BCFs for *Pimephales* sp. and *I. punctatus derived* from CBZ laboratory exposures

Tissue	*P. notatus*	*I. punctatus*
	BCF_k	BCF_k
Muscle	1.9	1.8
Liver	4.6	1.5
Plasma	---	7.1
Brain	---	1.6

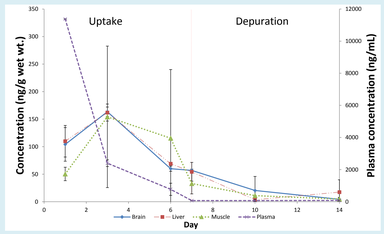


Figure 4. CBZ concentration (ng/g wet wt. or ng/mL, mean ± SD, n=3) in muscle, brain, liver, and plasma of channel catfish exposed to aqueous CBZ.

Carbamazepine: A Case Study Continued

Does CBZ Bioaccumulate in Fish Collected From the Field?
- Test Organisms
  - *Oreochromis niloticus* (Linnaeus, 1758)--Tilapia
- Pecan Creek Wastewater Reclamation Plant (PCWRP)
  - Activated sludge WWTP w/ UV disinfection
  - Avg. outflow of 12.5 mgd into Pecan Creek → Lake Lewisville
- Tilapia living in the sand filters of the facility were sampled

Table 2. Bioaccumulation factors (BAFs) for field collected *O. niloticus*.

Tissue	*O. niloticus*
	BAF
Muscle	2.8
Liver	3.8
Plasma	2.5
Brain	---

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

Tissue	Partition Coefficient	
	I. punctatus	*O. niloticus*
$P_{Blood:\ water}$	1.4	2.5
$P_{Blood:\ liver}$	2.2	0.7
$P_{Blood:\ muscle}$	3.5	0.9
$P_{Blood:\ brain}$	2.1	---

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 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 S9 studies suggested that CBZ is biotransformed.
    - Fish exposed to CBZ for 3 d 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 < 2000).
    - 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 physiological process to eliminate CBZ (e.g. induction)
    - Suggest 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

References and Acknowledgements

- Garcia, S., Foster, M., Constantine, L., Huggett, D.B. (2012). Field and Laboratory Fish Tissue Accumulation of Carbamazapine. Ecotoxicology and Environmental Safety. 84:207-211.

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