UNIVERSITY OF MARYLAND
COLLEGE PARK CAMPUS

Aquatic Pathobiology Center

VIRGINIA-MARYLAND REGIONAL COLLEGE OF VETERINARY MEDICINE

Facilitating Needed Drug Approval for Aquaculture:

In Vitro Metabolic Profiles to Characterize and Predict Drug Residues in Cultured Finfish

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UMD **JIFSAN** **US FDA-CVM**

CVM Mission

Facilitate drug approvals for minor species

while

protecting the food supply from harmful residues

Disease Impact

- **Infectious and parasitic diseases losses in millions of dollars: major obstacles for aquaculture growth** *(Georgiadis et al., 2001)***.**
- **Salmon industry in New Brunswick: $20 million/year in losses due to sea lice**

  (Davies and Rodger**,** 2000**).**
- **Japan estimates $125 million annual losses due to diseases of aquacultured species**

  (Schnick et al., 1999**).** **(40 drugs APPROVED in Japan)**

Drugs approved for aquaculture

Drug	Species	Indication
Formalin	Finfish, finfish eggs, shrimp	Ectoparasites
Sulfamerazine	Trouts	Furunculosis
Oxytetracycline	Salmonids, catfish, lobsters	Bacterial septicemia
Chorionic gonadotropin	Broodfish	Improvement of spawning
Sulfa-ormetroprim	Salmonids, catfish	Furunculosis
MS-222	Fish, other aquatic poikiloterms	Sedation/ anesthesia

General Objectives

Develop species groupings

- **Metabolic profiles**
- **Residue profiles**
- ***In vivo - in vitro* correlations**

Specific Objectives

- To contrast phase I (ECOD, EROD, PROD, BROD) and phase II (GST, GT, SULF) biotransformation kinetics in relevant aquacultured species.

- Baseline kinetics of farm-raised vs. lab-acclimated specimens of 3 species (rainbow trout, catfish, tilapia).

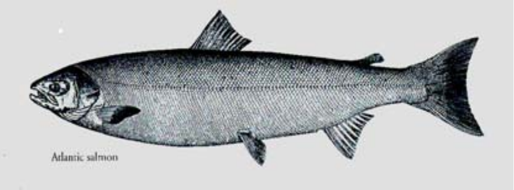
- *In vitro* metabolism of albendazole as a model drug in representative fish species.

Species

Channel catfish

Rainbow trout

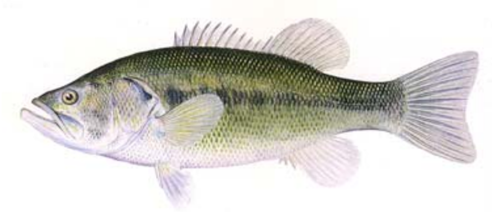
Tilapia



Atlantic salmon

Hybrid striped bass

Striped bass



Largemouth bass

Bluegill

Phase I and phase II pathways

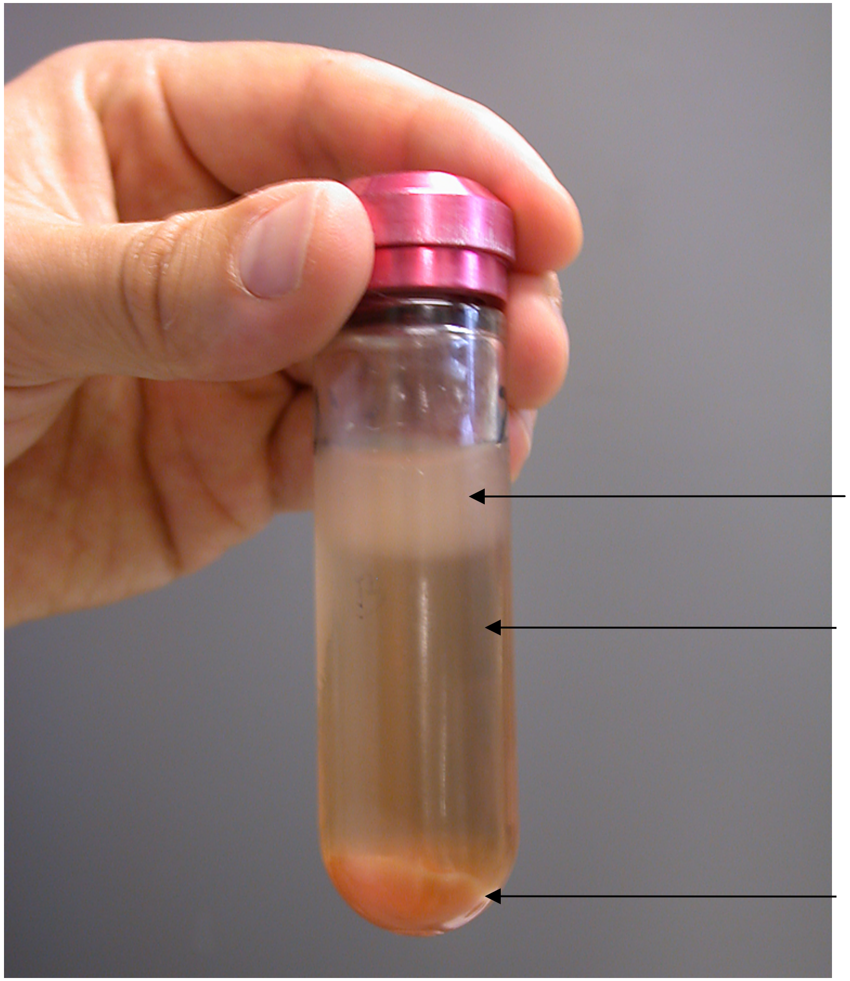
Use model substrates for comparative studies:

- ECOD - Ethoxycoumarin: phase I
- EROD - Ethoxyresorufin: phase I (1AI)
- PROD - Pentoxyresorufin: phase I
- BROD - Benzyloxyresorufin: phase I
- Resorufin: phase II GT & ST
- CDNB - Chlorodinitrobenzene: phase II GST

Tissue & assay preparation

- Market size fish
- Harvest livers
- Homogenization & centrifugation
- Microsomes and cytosol
- Optimization assays

Enzyme source for *in vitro* analyses



Lipids (discarded)

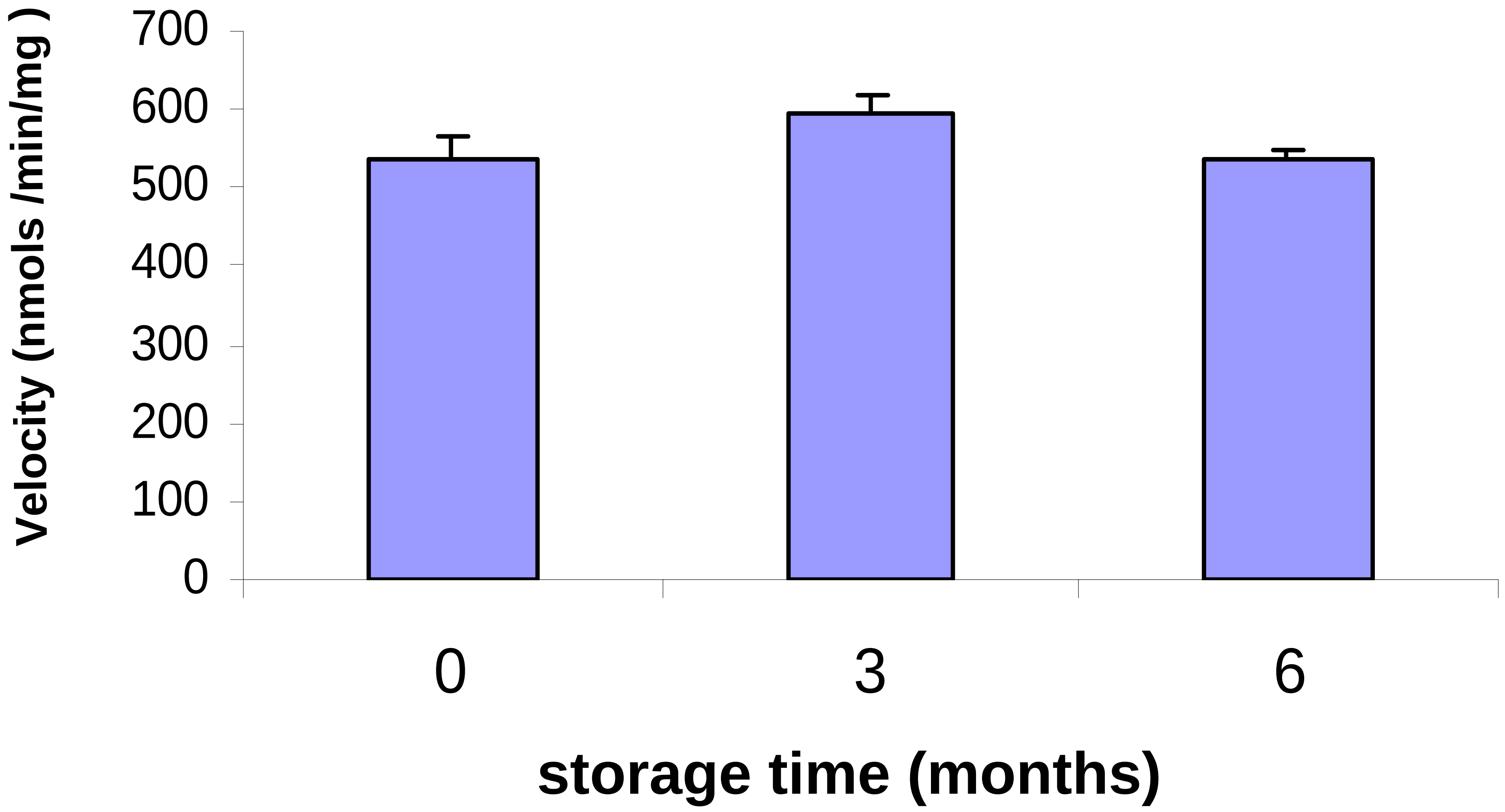
Cytosol

Microsomal fraction

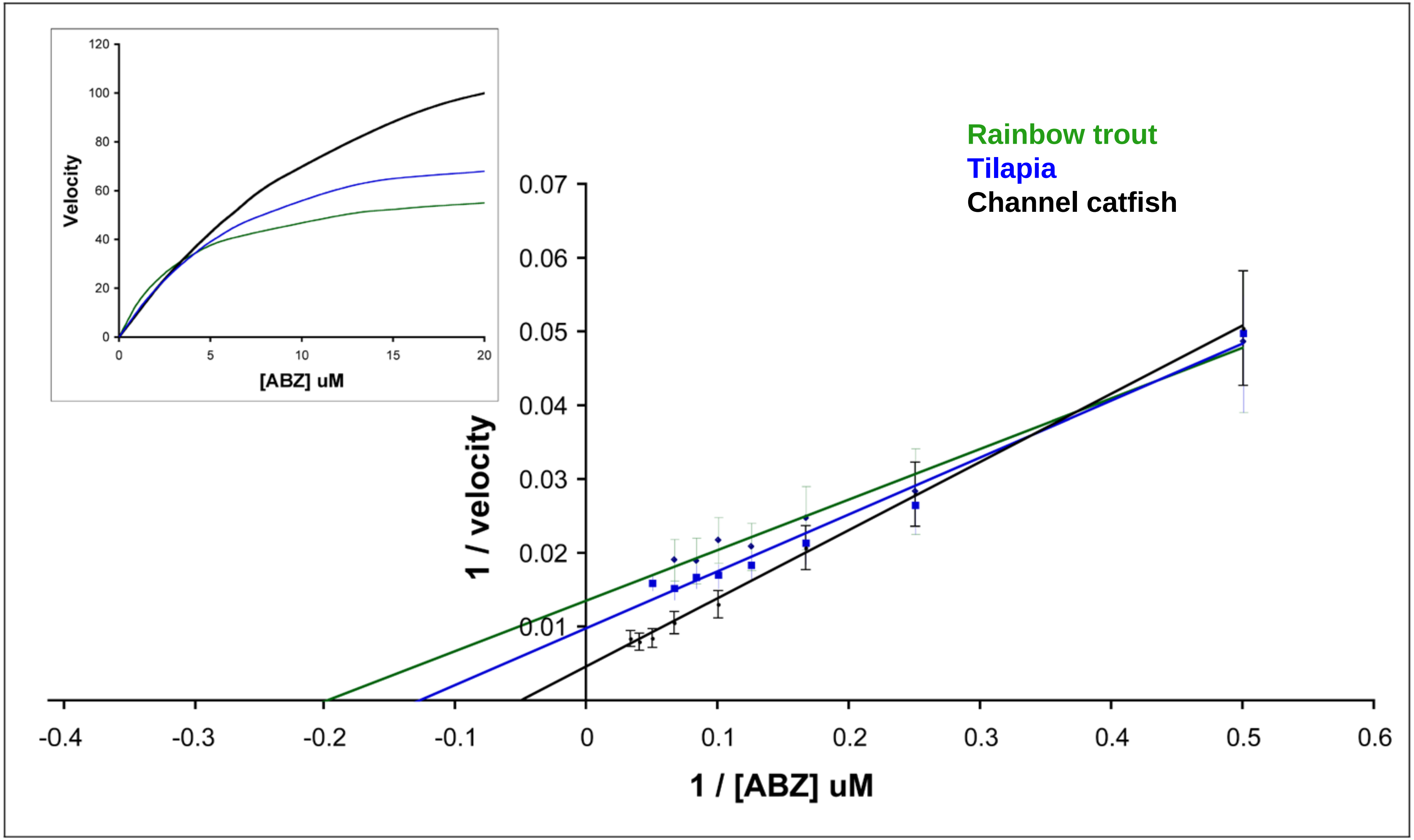
Enzyme activities at different substrate concentrations discerned using an absorbance-fluorescence microplate reader.

Storage Data

Enzyme (GST) activity in rainbow trout
Effects of -80^{0}C storage time (means ± S.E., n=6)



Sample kinetic data for three species of fish



EROD

Species (sample size)	**V_{max}** (pmols resorufin/min/mg prot)	**K_m** (µM)	**V_{max} / K_m**
Rainbow trout (aquacultured) (n = 3)	28 ± 8	0.6 ± 0.07	49 ± 14
Rainbow trout (acclimated) (n = 7)	30 ± 5	0.1 ± 0.01	323 ± 43
Catfish (aquacultured) (n = 4)	39 ± 7	1.8 ± 0.5	24 ± 4
Tilapia (aquacultured) (n = 7)	74 ± 15	2.1 ± 0.3	33 ± 4
Tilapia (acclimated) (n = 8)	32 ± 2	0.2 ± 0.04	226 ± 36
Atlantic salmon (n = 5)	66 ± 7	0.2 ± 0.02	300 ± 28
Largemouth bass (n = 5)	27 ± 8	0.9 ± 0.1	30 ± 5

Sulfotransferase

Species (sample size)	**V_{max}** (pmols resorufin /min/mg prot)	**K_m** (µM)	**V_{max} / K_m**
Rainbow trout (aquacultured) (n = 8)	190 ± 20	0.7 ± 0.1	287 ± 18
Rainbow trout (acclimated) (n = 8)	239 ± 19	0.9 ± 0.1	298 ± 45
Catfish (aquacultured) (n = 5)	265 ± 27	0.8 ± 0.1	388 ± 63
Catfish (acclimated) (n = 3)	49 ± 10	0.1 ± 0.0	487 ± 97
Tilapia (aquacultured) (n = 5)	328 ± 17	1.0 ± 0.2	354 ± 64
Tilapia (acclimated) (n = 5)	86 ± 9	0.6 ± 0.1	164 ± 39
Atlantic salmon (n = 5)	215 ± 14	0.5 ± 0.1	436 ± 63
Largemouth bass (n = 4)	147 ± 10	0.6 ± 0.1	300 ± 83
Striped bass (n = 4)	45 ± 5	0.1 ± 0.03	394 ± 77
Hybrid striped bass (n = 3)	46 ± 4	0.3 ± 0.1	309 ± 89
Bluegill (n = 4)	107 ± 23	0.7 ± 0.1	167 ± 35

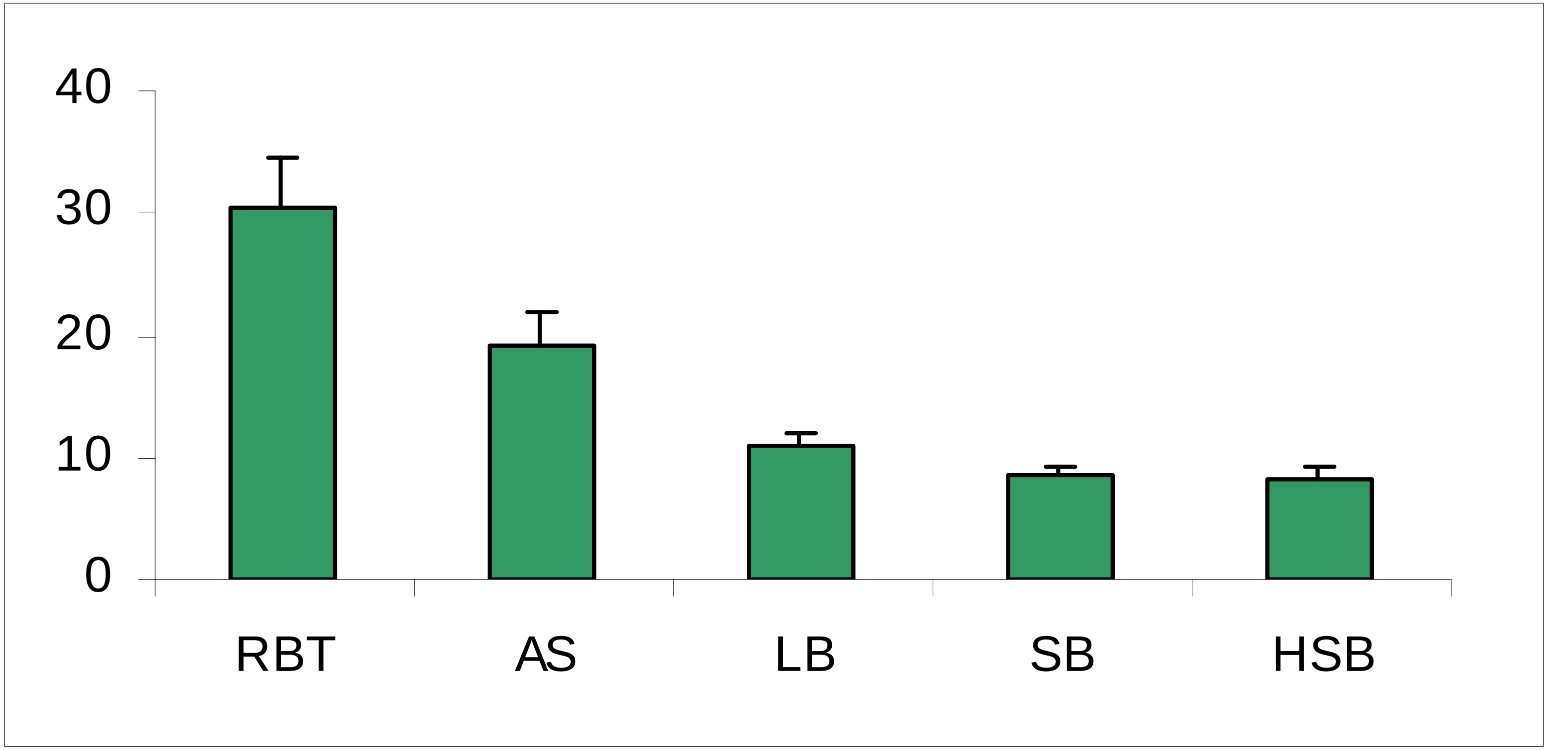
Glutathione-s-transferase

Species (sample size)	**V_{max}** (nmols CDNB/min/mg prot)	**K_m** (mM)	**V_{max} / K_m**
Rainbow trout (aquacultured) (n = 8)	929 ± 65	0.4 ± 0.05	2260 ± 200
Rainbow trout (acclimated) (n = 7)	419 ± 32	0.1 ± 0.01	4690 ± 254
Catfish (aquacultured) (n = 8)	657 ± 39	0.1 ± 0.02	5568 ± 413
Catfish (acclimated) (n = 6)	1972 ± 125	1.1 ± 0.1	1891 ± 74
Tilapia (aquacultured) (n = 8)	1508 ± 70	0.3 ± 0.01	5005 ± 23
Tilapia (acclimated) (n = 7)	1474 ± 109	0.6 ± 0.05	2434 ± 70
Atlantic salmon (n = 5)	1349 ± 107	0.5 ± 0.1	2816 ± 329
Largemouth bass (n = 8)	589 ± 52	0.4 ± 0.06	1491 ± 117
Striped bass (n = 7)	334 ± 30	0.2 ± 0.02	1525 ± 99
Hybrid striped bass (n = 7)	471 ± 39	0.4 ± 0.04	1395 ± 105
Yellow perch (n = 5)	490 ± 91	0.6 ± 0.1	859 ± 75
Bluegill (n = 8)	354 ± 26	0.3 ± 0.04	1394 ± 145

UDP-glucuronosyltransferase

Species (sample size)	**V_{max}** (pmols resorufin/min/mg prot)	**K_m** (µM)	**V_{max} / K_m**
Rainbow trout (aquacultured) (n = 4)	930 ± 258	32.3 ± 8.7	30.4 ± 4.4
Rainbow trout (acclimated) (n = 8)	834 ± 208	25.0 ± 6.0	34.0 ± 3.0
Tilapia (aquacultured) (n = 6)	368 ± 89	29.0 ± 9.0	15.4 ± 2.7
Tilapia (acclimated) (n = 6)	400 ± 88	29.0 ± 9.0	16.0 ± 3.0
Atlantic salmon (n = 5)	410 ± 86	24.0 ± 6.0	19.0 ± 3.0
Largemouth bass (n = 7)	273 ± 16	27.0 ± 3.0	11.0 ± 1.0
Striped bass (n = 5)	231 ± 29	29.0 ± 5.0	8.4 ± 1.0
Hybrid striped bass (n = 6)	271 ± 37	36.0 ± 7.0	8.2 ± 0.9
Bluegill (n = 6)	263 ± 31	17.5 ± 2.3	16.3 ± 3.1

V_{max} / K_m ratios for UDPGT



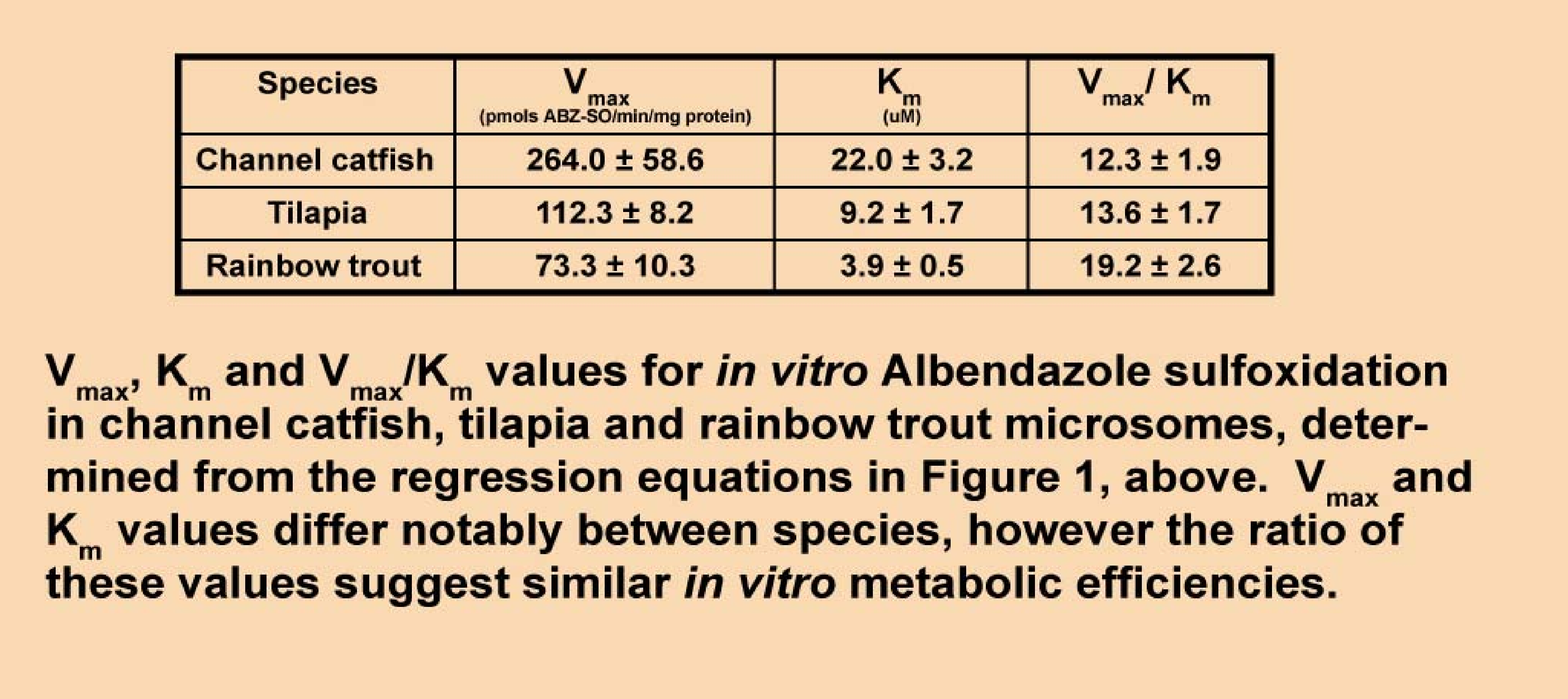
Move from baseline data to working with

Microsomes from albendazole exposed fish

or

Microsomes exposed to albendazole *in vitro*

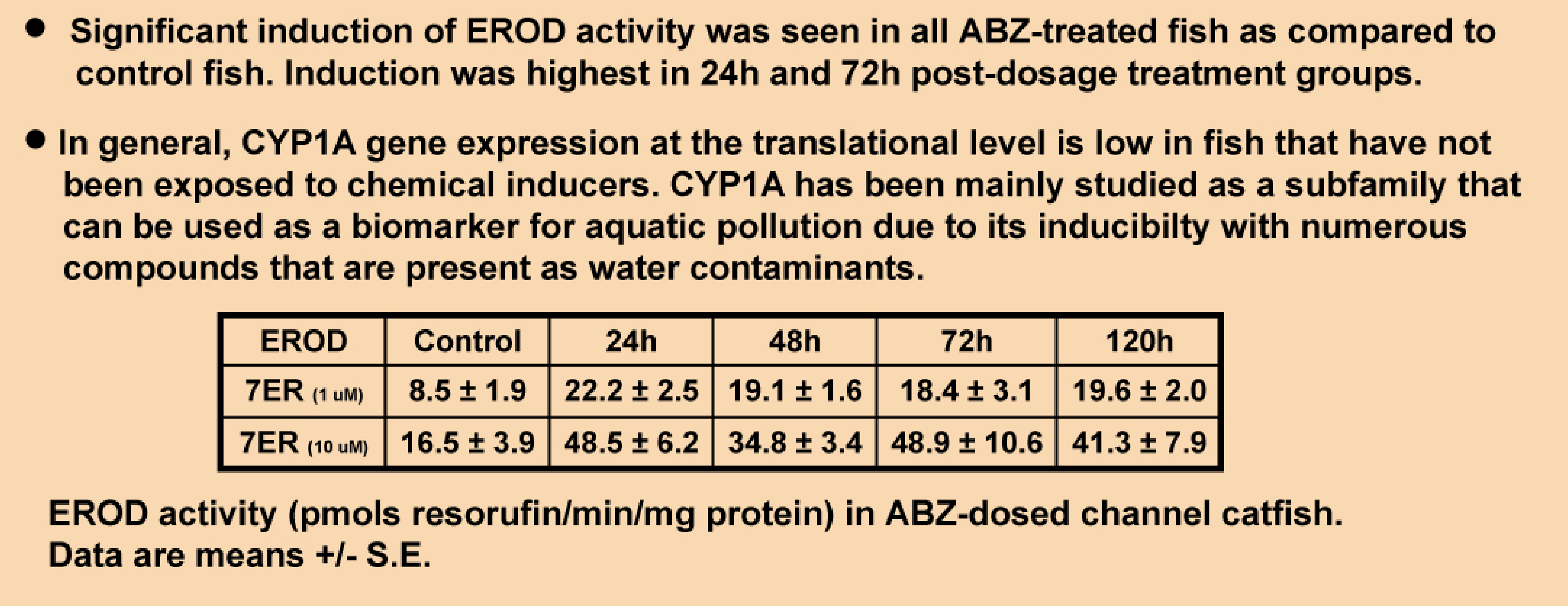
in vitro Albendazole metabolism in 3 species



Species	V_{max} (pmols ABZ-SO/min/mg protein)	K_m (uM)	V_{max}/ K_m
Channel catfish	264.0 ± 58.6	22.0 ± 3.2	12.3 ± 1.9
Tilapia	112.3 ± 8.2	9.2 ± 1.7	13.6 ± 1.7
Rainbow trout	73.3 ± 10.3	3.9 ± 0.5	19.2 ± 2.6

V_{max}, K_m and V_{max}/K_m values for *in vitro* Albendazole sulfoxidation in channel catfish, tilapia and rainbow trout microsomes, determined from the regression equations in Figure 1, above. V_{max} and K_m values differ notably between species, however the ratio of these values suggest similar *in vitro* metabolic efficiencies.

Does Albendazole induce EROD, PROD, BROD or GST after in vivo dosing?



- Significant induction of EROD activity was seen in all ABZ-treated fish as compared to control fish. Induction was highest in 24h and 72h post-dosage treatment groups.
- In general, CYP1A gene expression at the translational level is low in fish that have not been exposed to chemical inducers. CYP1A has been mainly studied as a subfamily that can be used as a biomarker for aquatic pollution due to its inducibilty with numerous compounds that are present as water contaminants.

EROD	Control	24h	48h	72h	120h
7ER (1 uM)	8.5 ± 1.9	22.2 ± 2.5	19.1 ± 1.6	18.4 ± 3.1	19.6 ± 2.0
7ER (10 uM)	16.5 ± 3.9	48.5 ± 6.2	34.8 ± 3.4	48.9 ± 10.6	41.3 ± 7.9

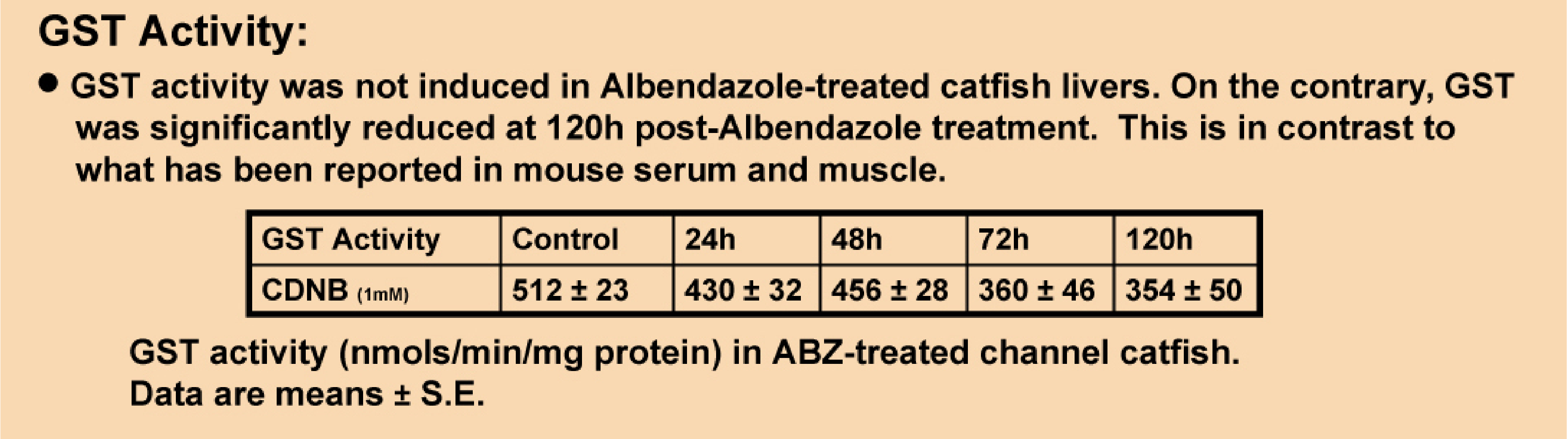
EROD activity (pmols resorufin/min/mg protein) in ABZ-dosed channel catfish.
Data are means +/- S.E.

PROD and BROD activity after *in vivo* albendazole exposure

No induction due to albendazole treatment in vivo.

Baseline activities have not been observed in any of the fish species tested in our previous experiments.

GST activity after *in vivo* albendazole exposure.



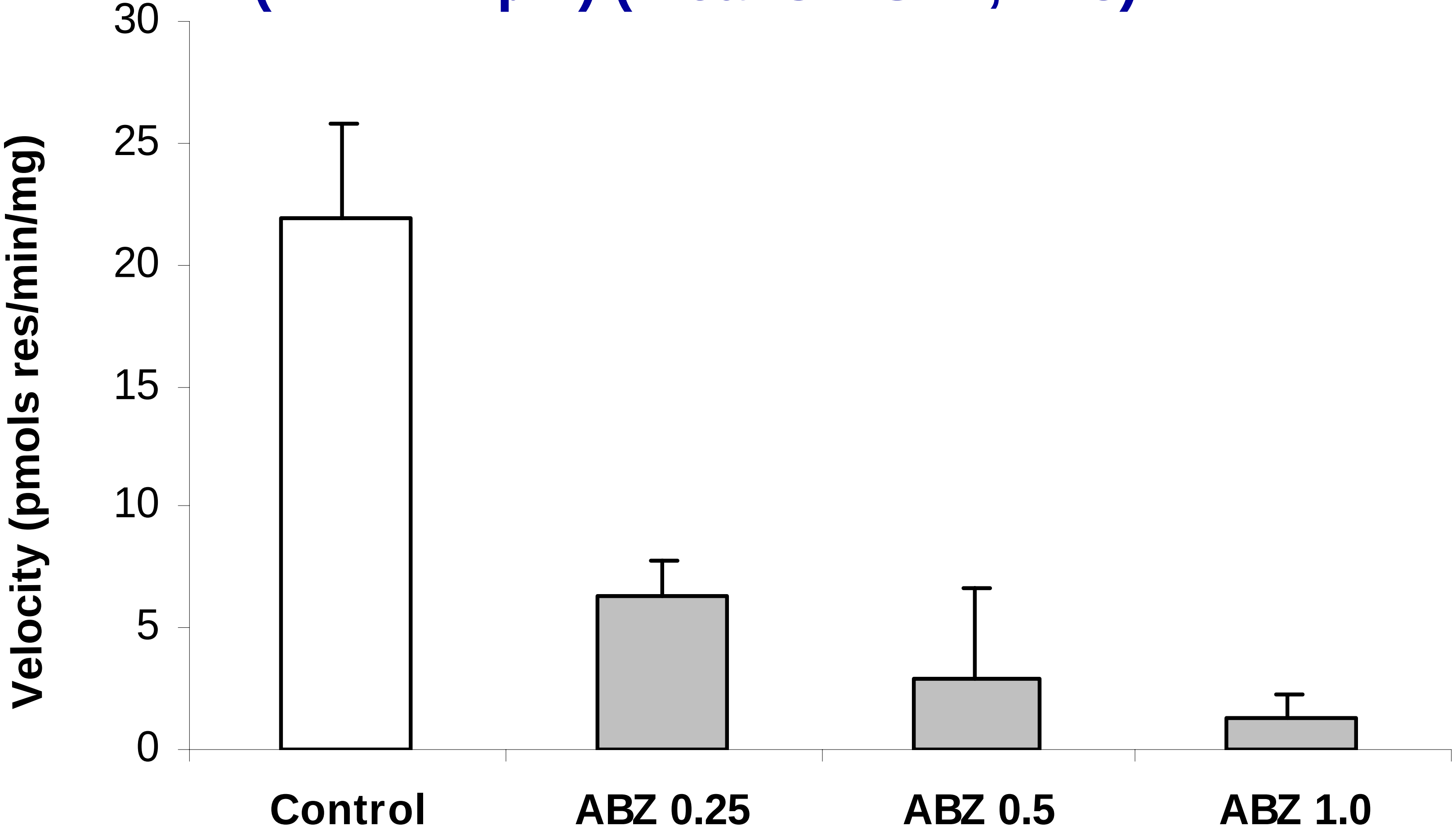
GST Activity:

- GST activity was not induced in Albendazole-treated catfish livers. On the contrary, GST was significantly reduced at 120h post-Albendazole treatment. This is in contrast to what has been reported in mouse serum and muscle.

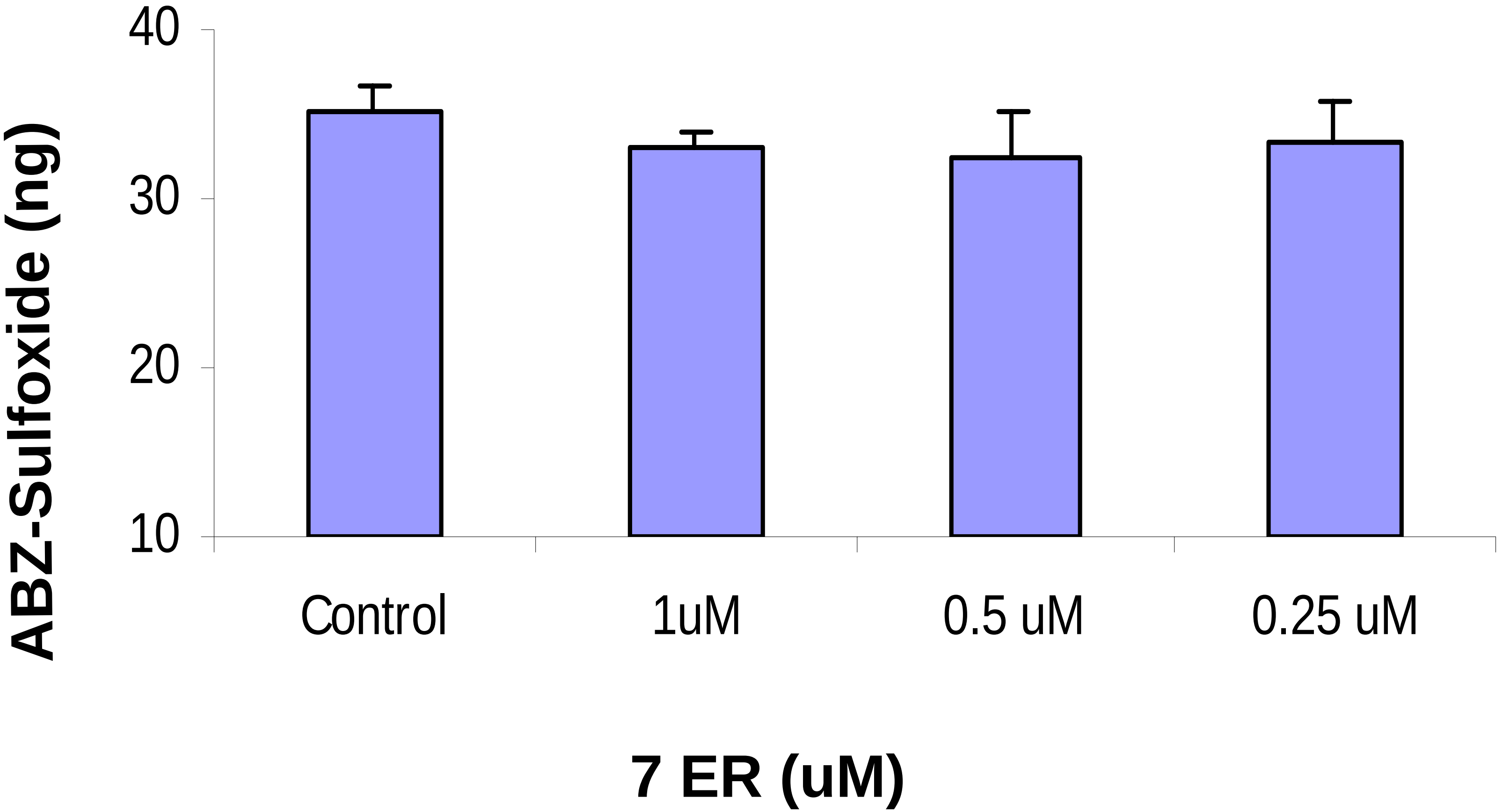
GST Activity	Control	24h	48h	72h	120h
CDNB (1mM)	512 ± 23	430 ± 32	456 ± 28	360 ± 46	354 ± 50

GST activity (nmols/min/mg protein) in ABZ-treated channel catfish. Data are means ± S.E.

ABZ treatment *in vitro* suppresses EROD activity in rainbow trout microsomes (7ER = 1 µM) (means ± S.E., n=8)



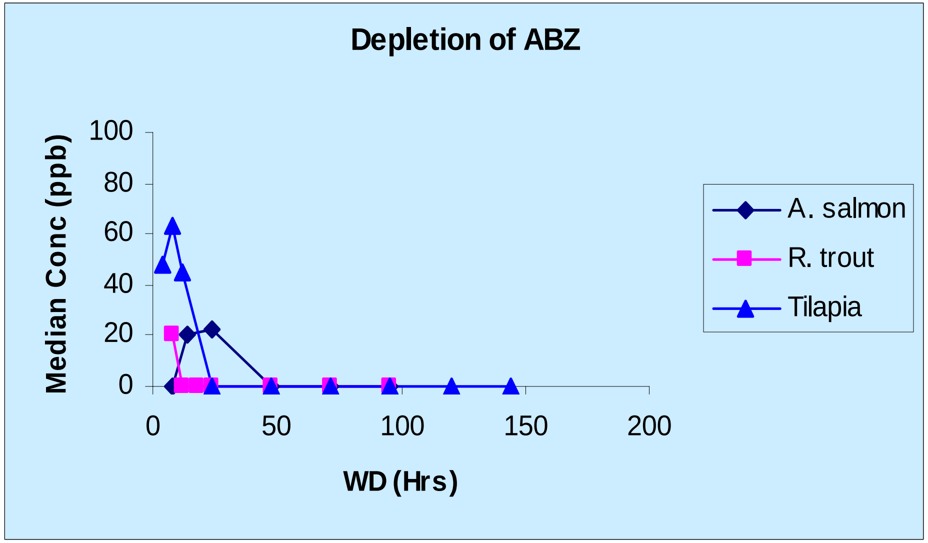
Effects of 7ER on ABZ sulfoxidation *in vitro* rainbow trout (ABZ = 1 uM) (means ± S.E., n=4)

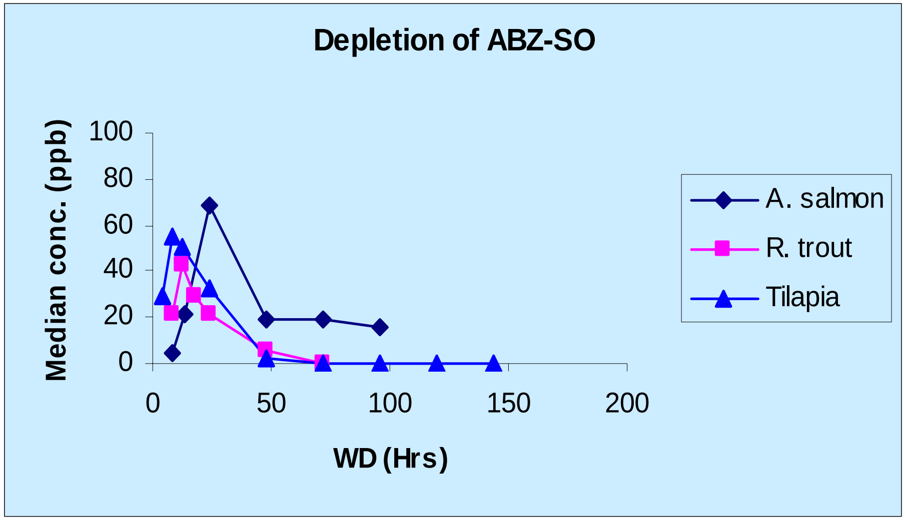


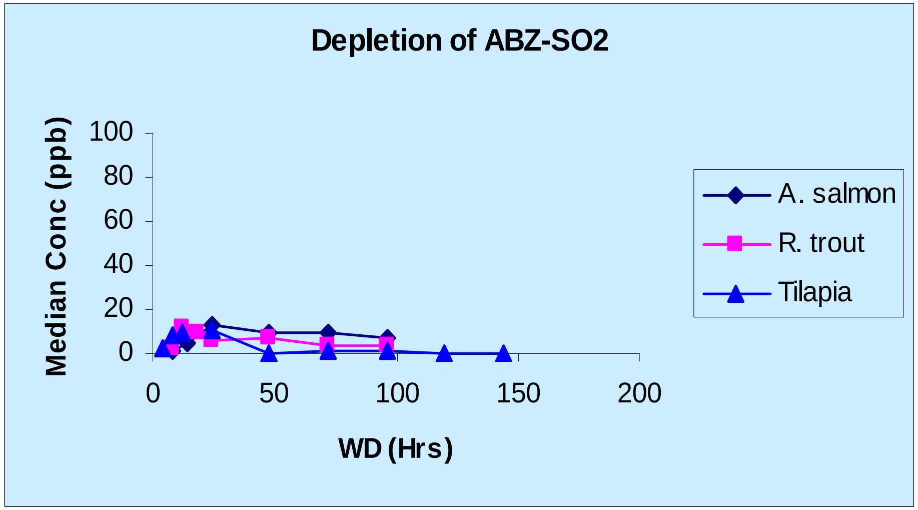
No significant changes in ABZ-sulfox, suggests that CYP1A does not play a critical role in this reaction

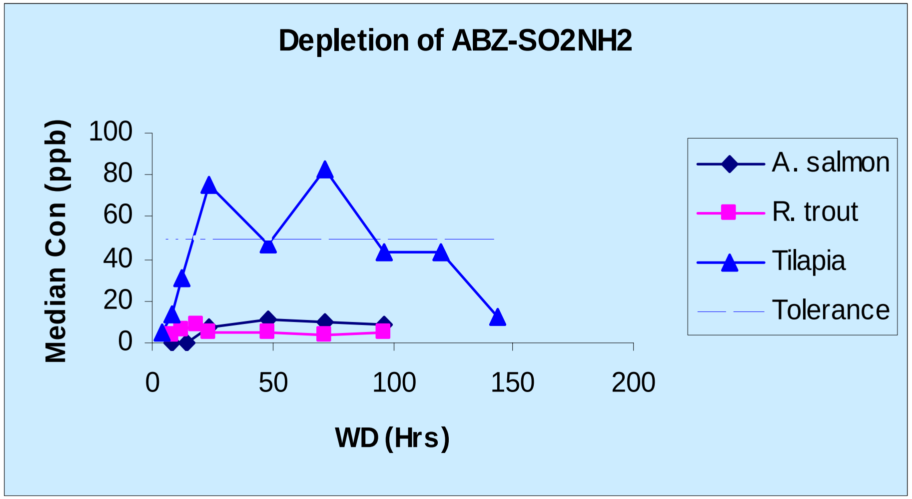
In vivo efforts: Albendazole

(FDA-CVM metabolite/residue analyses)


Depletion of ABZ


Depletion of ABZ-SO


Depletion of ABZ-SO2


Depletion of ABZ-SO2NH2

Working

- Complete *in vitro* species comparisons
- Data analysis
- CVM *in vivo* albendazole exposures – residue analysis – additional fish species –
    catfish, LMB
- Compare and contrast the *in vivo* and *in vitro* data to screen for correlations which could be used in a regulatory setting.

Anticipated benefits

- **Accelerate the drug approval process for multiple fish species, based on modeling drug metabolic profiles and tissue residues.**
- **Reduce cost of approval process**
- **Effective disease control**
- **Improved production and profits**
- **Controlled drug use**

Fin