# The Role of Aryl Hydrocarbon Receptor in Mono-Substituted Isopropylated Triaryl Phosphate-Induced Cardiac Toxicity in Zebrafish

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# What are flame retardants?

- Flame retardants are intended to *prevent* fires, not extinguish them
- Flame retardants have been added to furniture foam since the 1970s

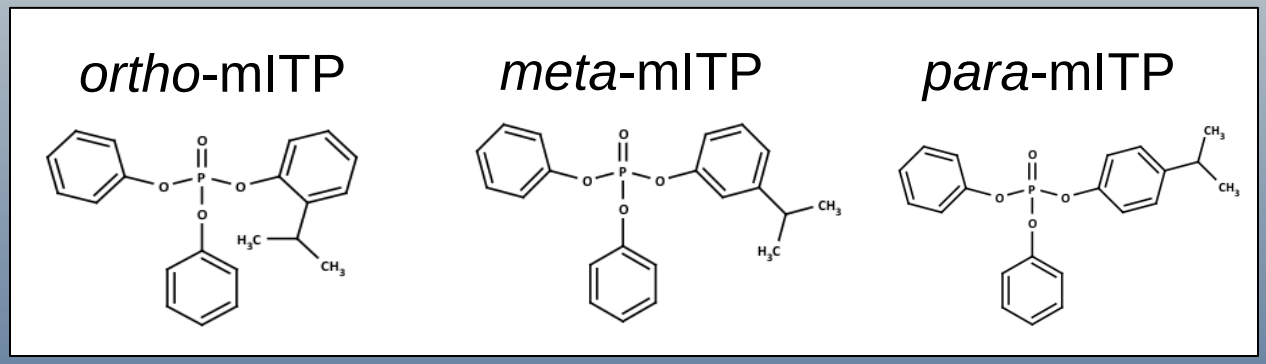
- California Technical Bulletin 117 required polyurethane foam in furniture to resist candle flame for 12s before igniting
- TB117 was finally discontinued this year, but legacy issues will likely continue for decades to come

# Why study flame retardants?

- In the U.S., most chemicals do not receive thorough toxicity testing – safety of nearly 100,000 chemicals is unknown
- Previous flame retardants separate from foam and attach to indoor house dust, leading to widespread exposure
- PBDEs have been shown to cause cancer, developmental defects, cognitive impairments & persist in the environment
- Replacement flame retardants have yet to be assessed thoroughly for toxicity

# Firemaster 550

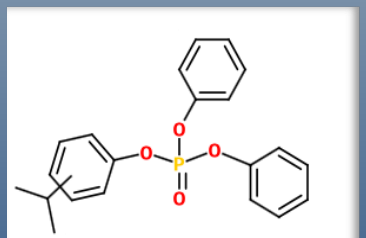
- Firemaster 550 replaced group of PBDEs in 2004 – only about five studies have investigated its toxicity
- Mono-substituted isopropylated triaryl phosphate (mITP) makes of ~34% of Firemaster 550



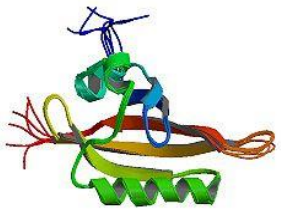

- McGee *et al.* (2013) showed
    1. mITP causes developmental toxicity in zebrafish
    2. mITP activates the aryl hydrocarbon receptor (AHR)
    3. an AHR antagonist rescues this toxicity

# Hypothesis:

mITP causes cardiac toxicity through the AHR




mITP

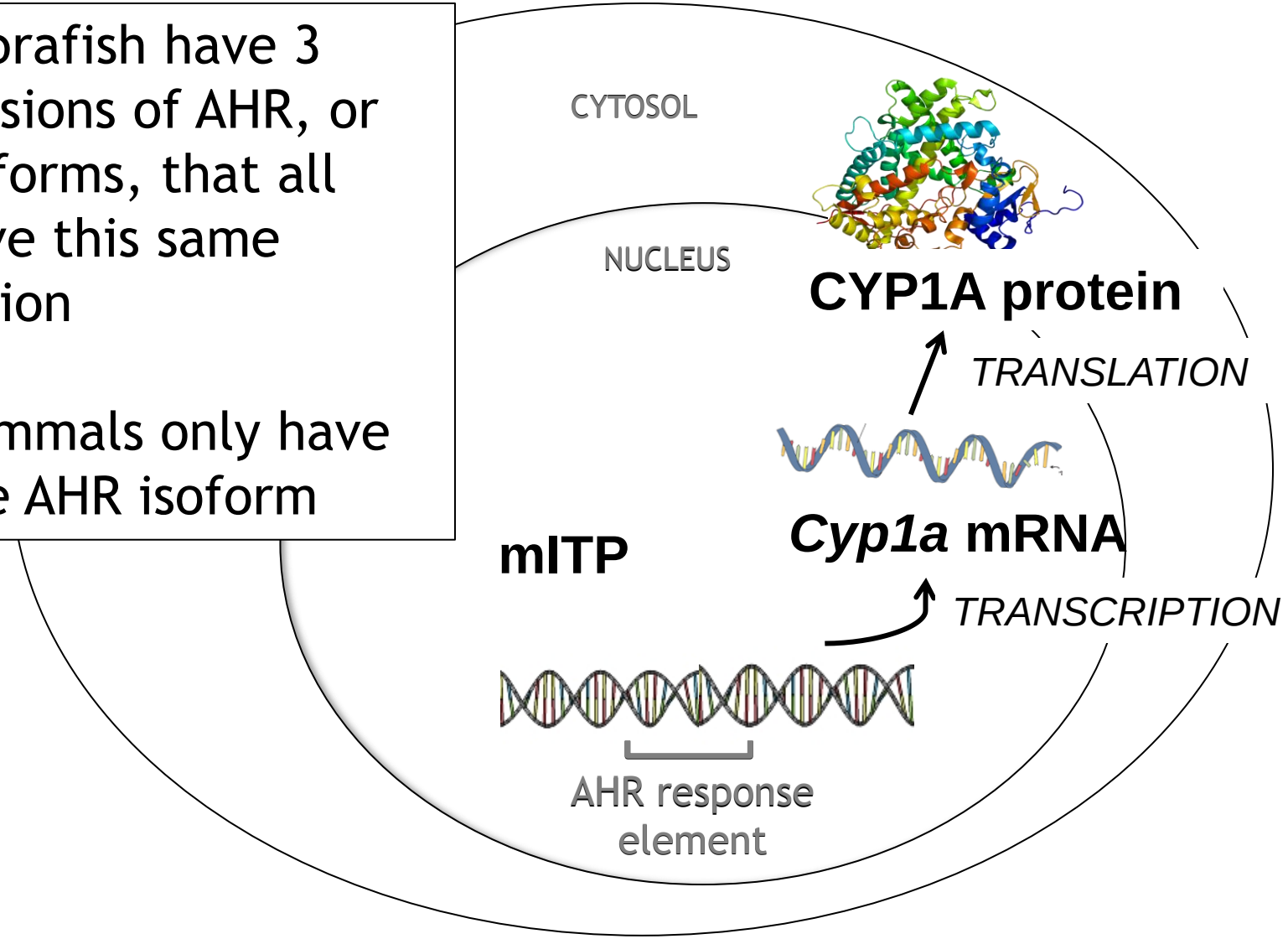


Aryl hydrocarbon receptor

toxicity

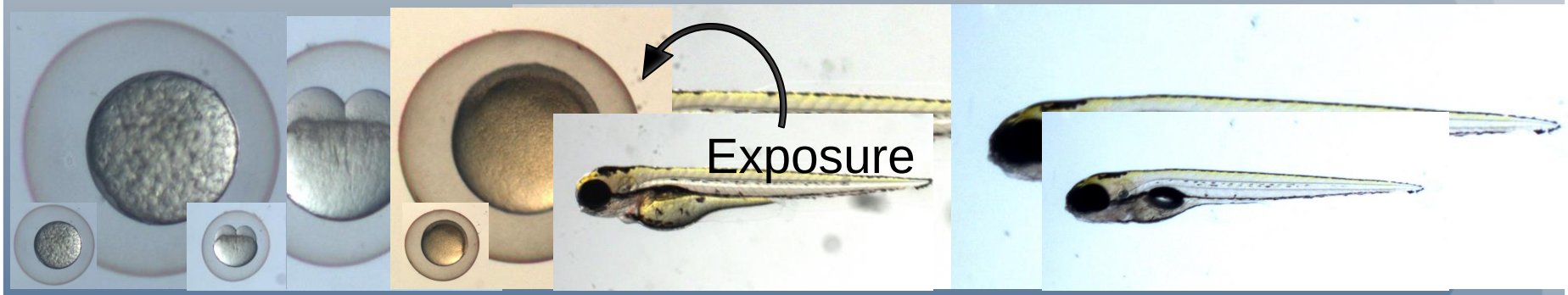
# Aryl Hydrocarbon Receptor (AHR)

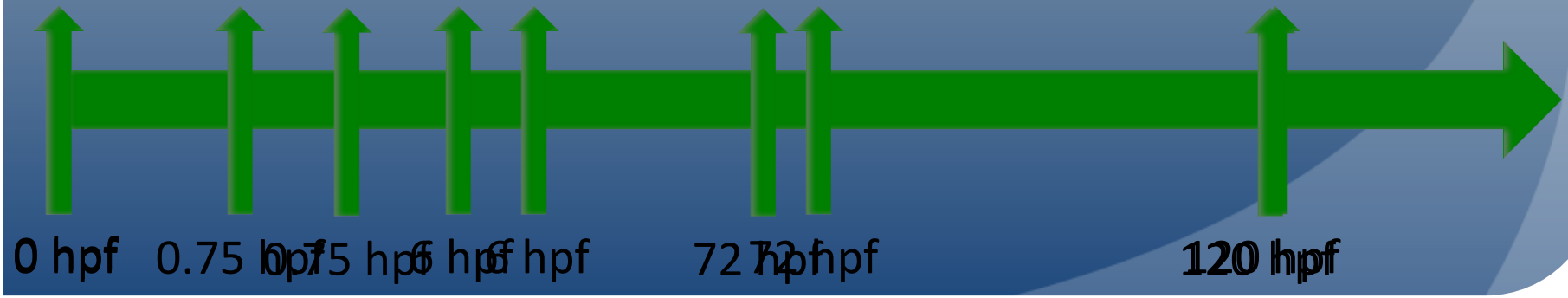
- Zebrafish have 3 versions of AHR, or isoforms, that all have this same action
- Mammals only have one AHR isoform



# Benefits of zebrafish model

- Adult zebrafish is only 1” long, weighs 1g
- Genome is sequenced & 1000s of labs utilize them as model
- Fast life cycles, development occurs externally & similar signaling pathways to humans – namely during development

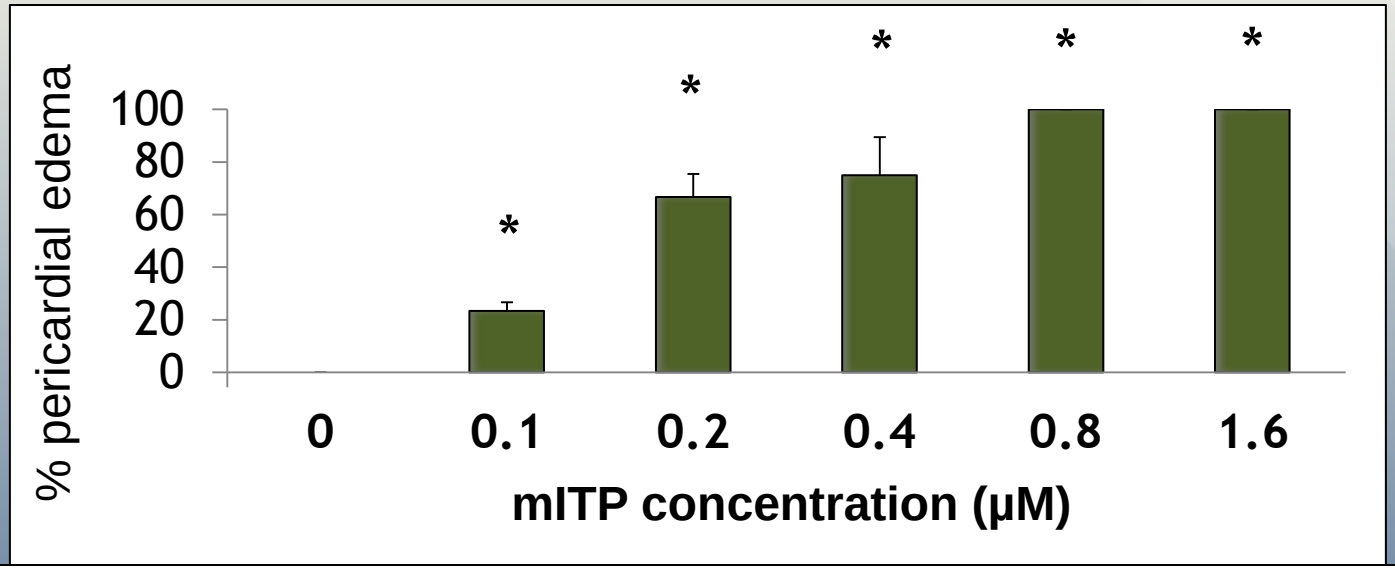


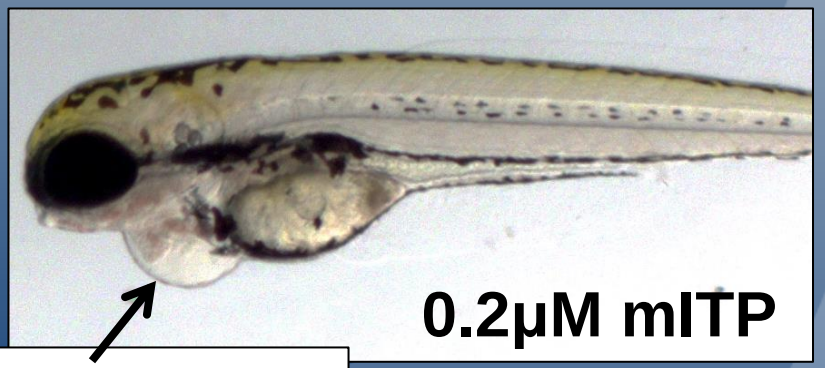
# Goals of this study:

1. Determine mITP concentration-response in developing zebrafish
2. Confirm AHR antagonist rescues toxicity as shown in McGee *et al.* (2013)
3. Predict which AHR isoform (AHR2, AHR1A or AHR1B) is activated by mITP using *in silico* AHR homology model
4. Determine whether AHR knockdown rescues toxicity
5. Identify which AHR isoform in zebrafish is activated by mITP (were the *in silico* predictions correct?)

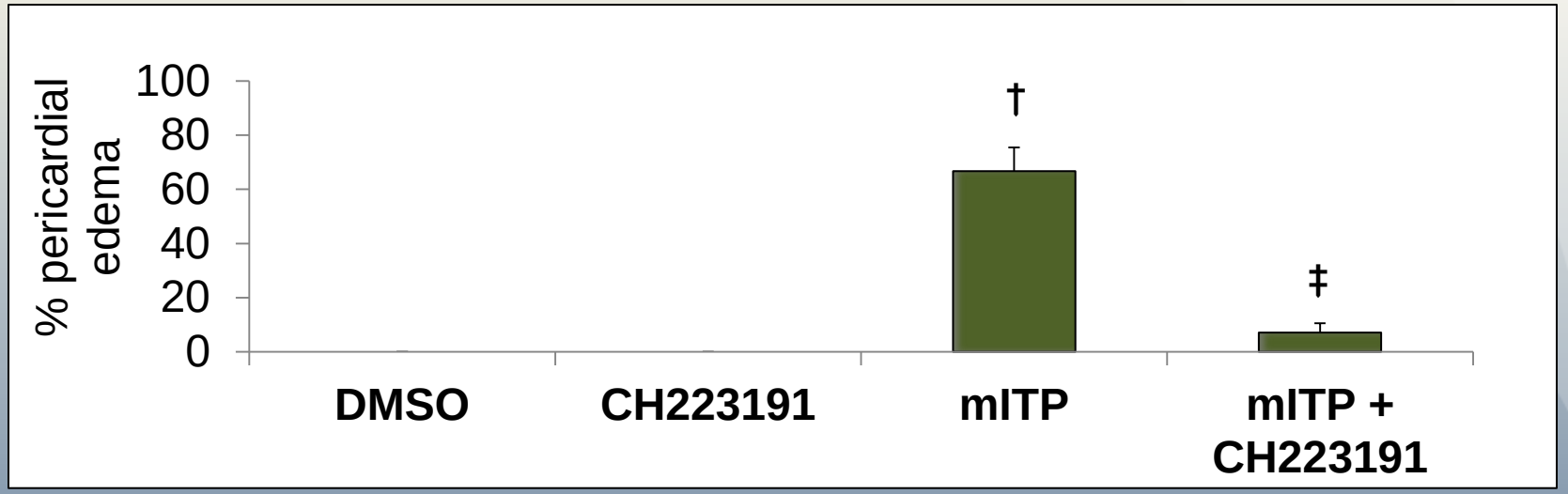
# 1. mITP causes concentration-dependent increase in cardiac toxicity

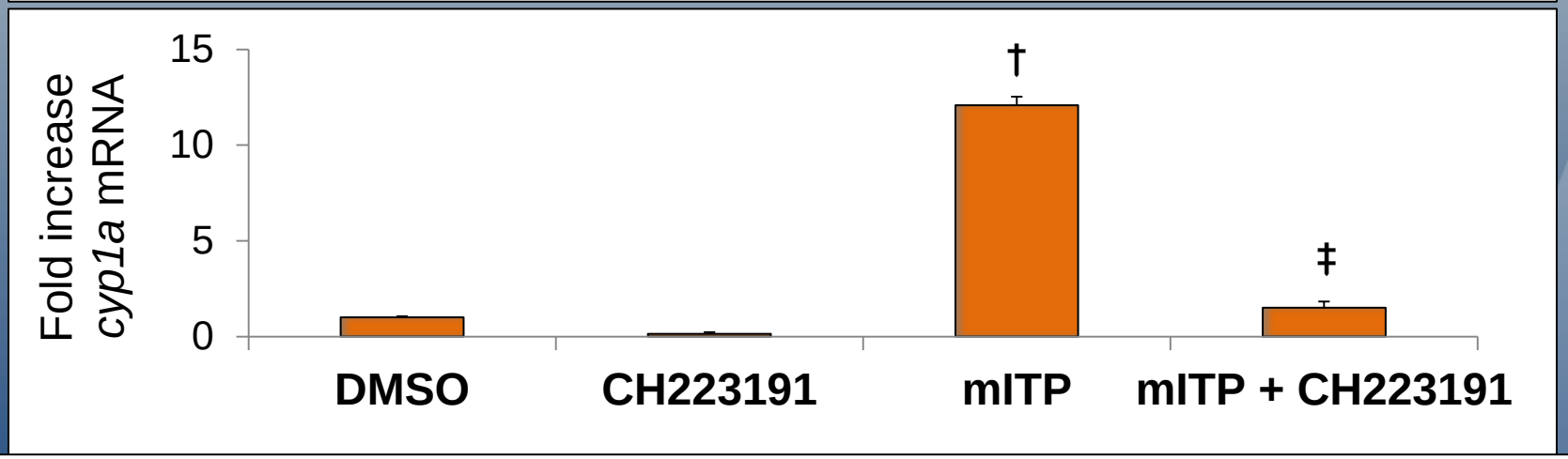



Asterisk (*) means significant increase relative to vehicle control ($p < 0.05$)

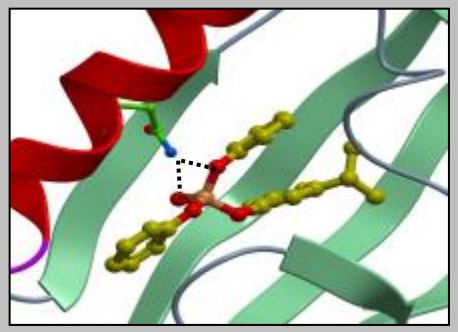
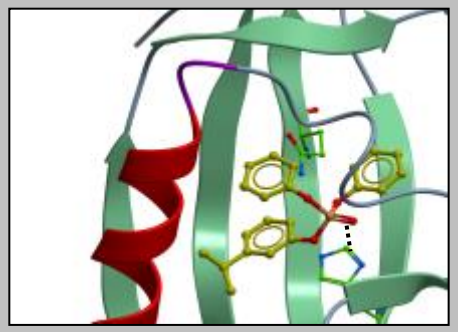
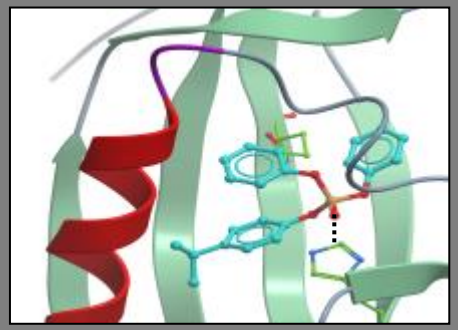
# 2. AHR antagonist (CH223191) rescues cardiac toxicity & *cyp1a* induction



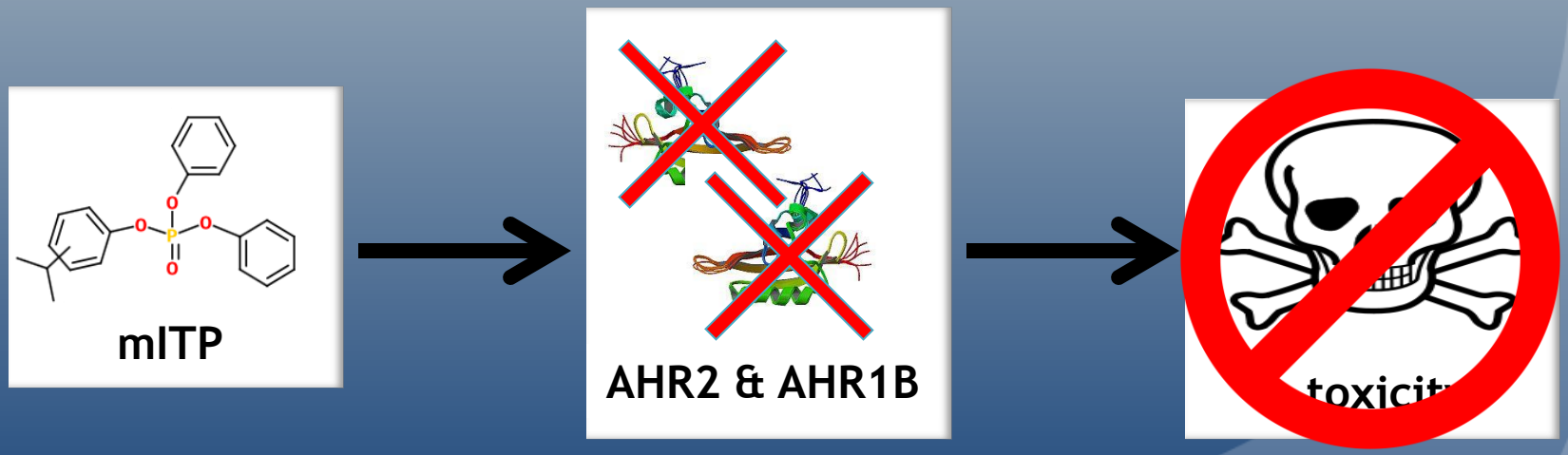

Dagger (†) means significant increase relative to vehicle controls & double-dagger (‡) means significant decrease relative to mITP alone ($p < 0.05$)

# 3. mITP is predicted to dock in AHR2 & AHR1B – but not AHR1A

| | AHR2 | AHR1B | AHR1A |
|---|---|---|---|
| *ortho*-mITP | ✗ | ✗ | ✗ |
| *meta*-mITP |  |  | ✗ |
| *para*-mITP | ✗ |  | ✗ |

# New Hypothesis:

mITP causes cardiac toxicity through AHR2 & AHR1B



# Individual AHR Isoform Knockdown

**AHR2 mutant**

(Goodale *et al*. 2012)

**AHR1A/1B morpholino injection**

## AHR1A KO

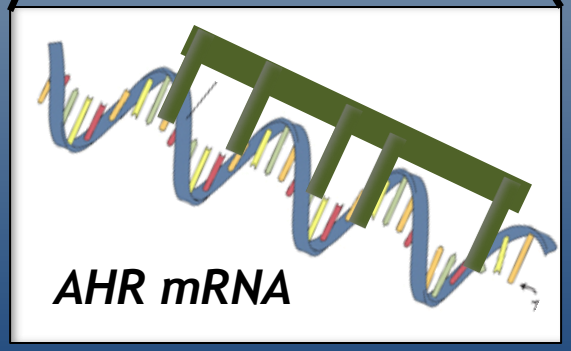
Control AHR1A MO

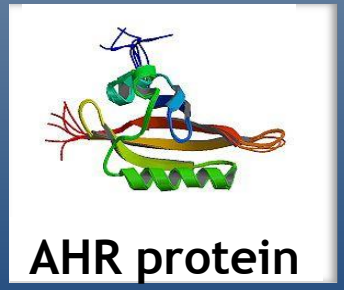
WT deletion

## AHR1B KO

Control AHR1B MO

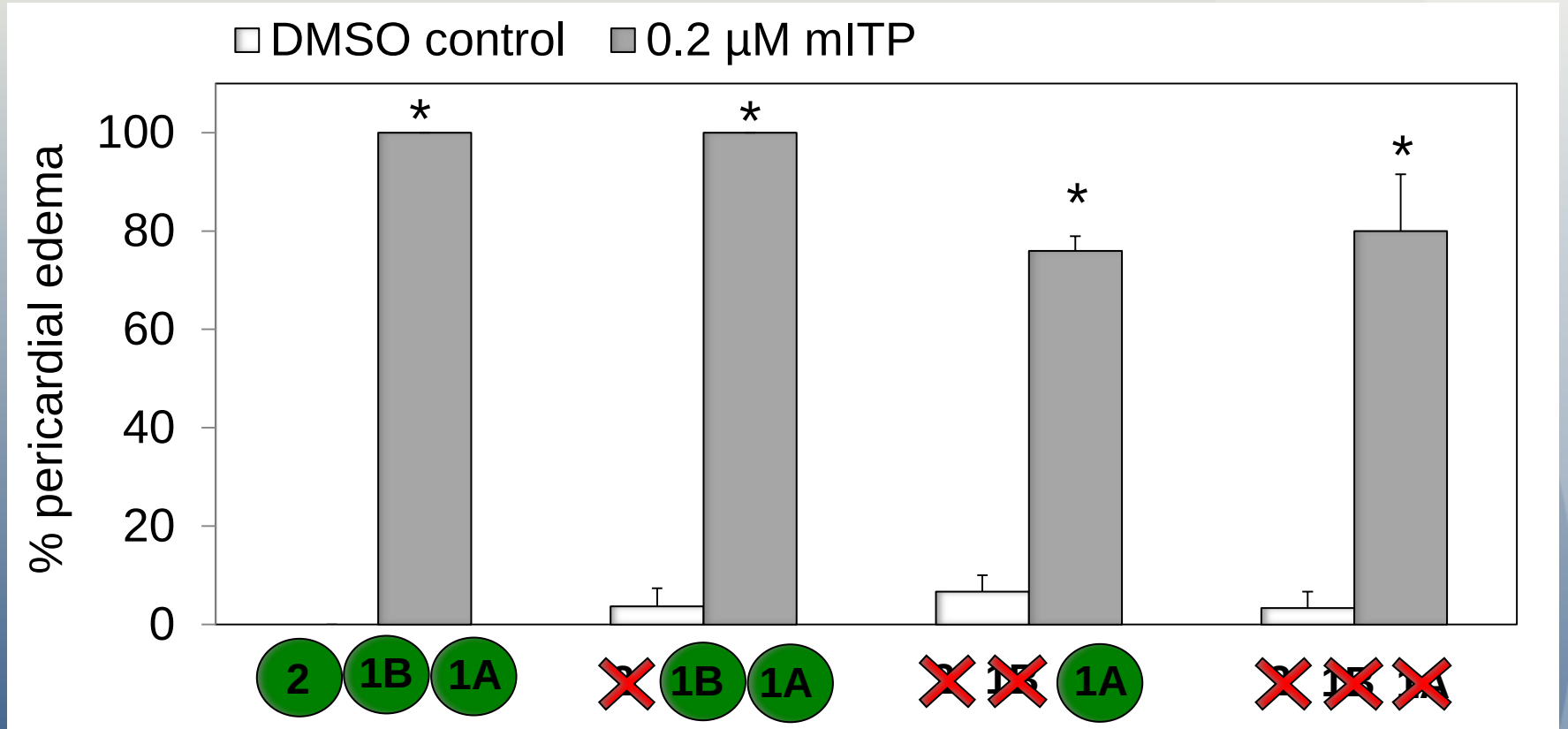
WT insertion




**CYP1A mRNA & protein**

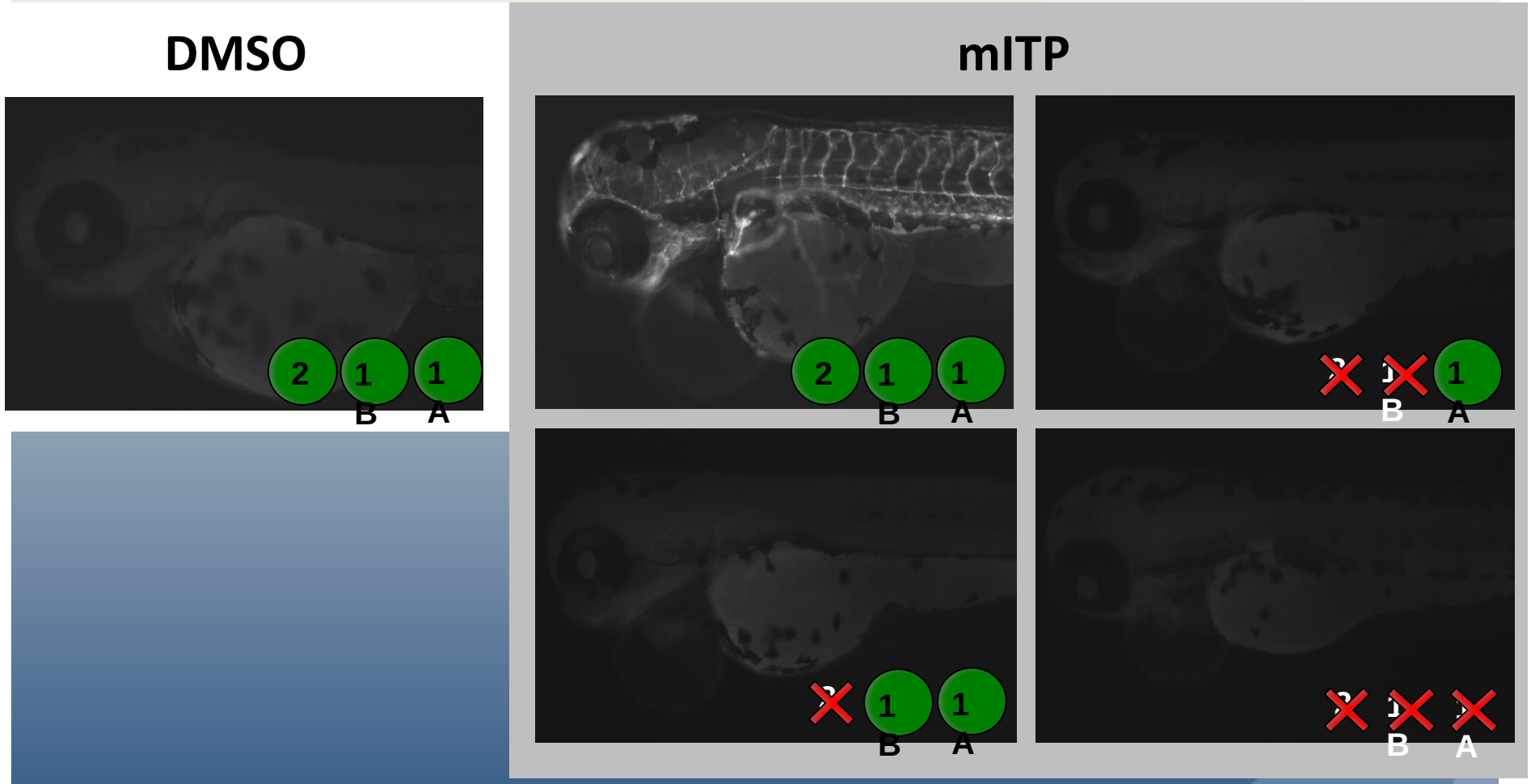
# 3. mITP-induced cardiac toxicity is AHR-independent



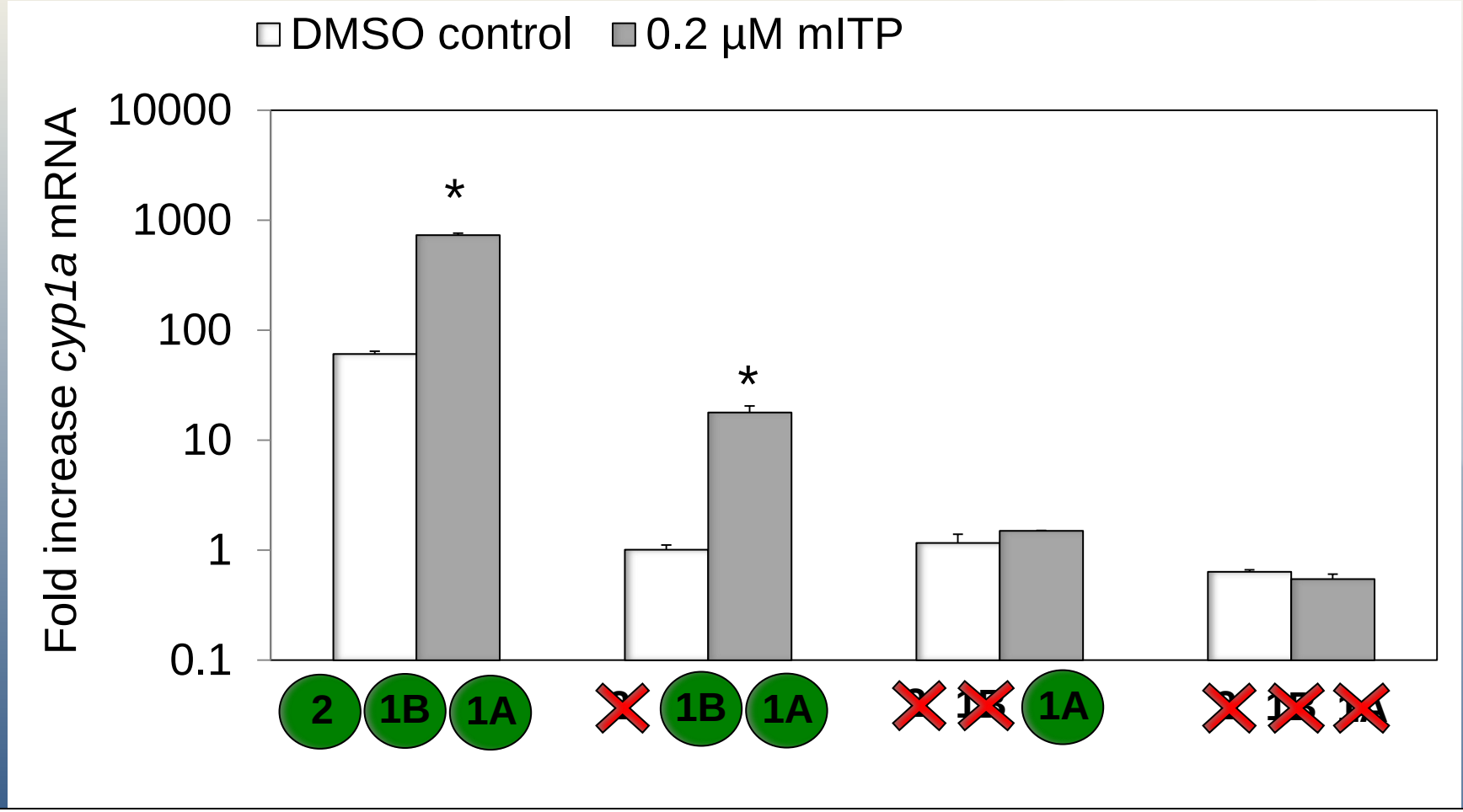

Asterisk (*) means significant increase relative to controls within the same group ($p < 0.05$)

# 3. mITP-induced cardiac toxicity is AHR-independent

# 4A. mITP activates AHR2



# 4B. mITP also activates AHR1B – but not AHR1A



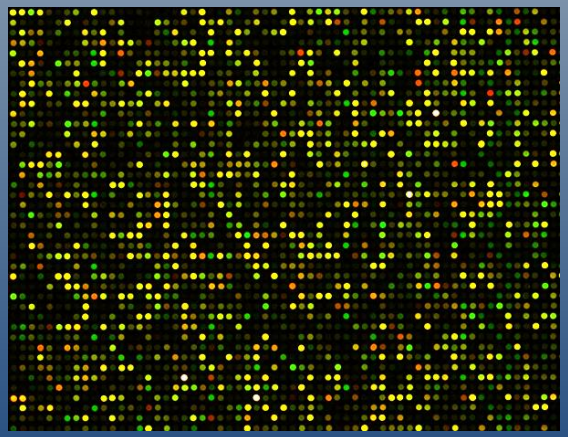

Asterisk (*) means significant increase relative to controls within the same group ($p < 0.05$)

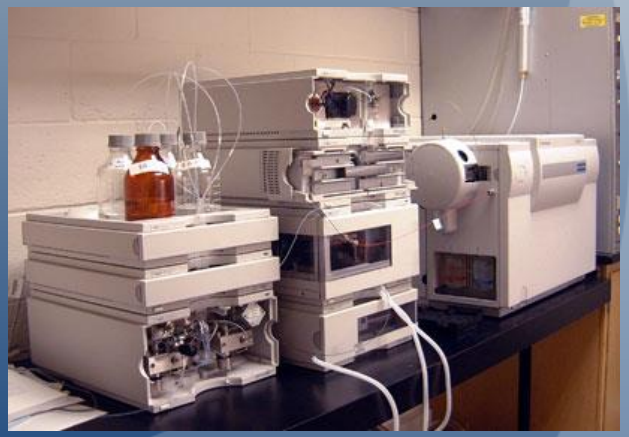
# Conclusions:

- mITP causes concentration-dependent increase in cardiac toxicity in developing zebrafish
- Cardiac toxicity is prevented by “AHR antagonist” (CH223191) as was shown in McGee *et al.* (2013)
- mITP *does not* cause cardiac toxicity through the AHR
- mITP causes cardiac toxicity through an unknown pathway that is also antagonized by CH223191
- mITP activates AHR2 and AHR1B isoforms *in vivo* as correctly predicted by the computational model

# Going forward:

- Analyze results from microarray in order to gain more clues on the mechanism of toxicity
- Synthesize analytical standards to test each congener separately (are some congeners toxic and not others?)
- Determine human dose in populations and study effects, if any, of mITP exposure on human development





# Acknowledgements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# Questions?