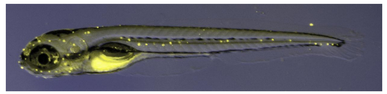
Does BPA Cause Hearing Loss? Assessing the Potential Ototoxicity Induced by Bisphenol-A in *Danio rerio* (Zebrafish) Lateral Line

Meghal Sheth

Overview

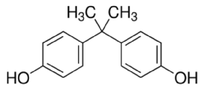
The zebrafish (*Danio rerio*) lateral line consists of a system of sensory organs that are optimal for *in vivo* studies of hair cell death and protection. Zebrafish hair cells are structurally and functionally similar to those in the human inner ear. In zebrafish these hair cells are arrayed in clusters called neuromasts along the head and trunk of the animal, making these cells easily accessible.



5 day-old zebrafish labeled with the vital dye DASPEI. Neuromasts are yellow dots.

From Coffin et al. 2010, Zebrafish

I used this system for multiple hair cell death and protection studies (reviewed in Coffin et al. 2010, Zebrafish). Here I examined the potential for bisphenol-A (BPA), the common monomer used in the production of polycarbonate plastics and epoxy resins, to kill hair cells. I have shown that BPA kills hair cells in a dose- and time-dependent manner. I have then asked what cell death signaling pathways are activated in BPA-damaged hair cells, and have identified oxidative stress as a potential cell death signaling pathway and believe that antioxidants have the capability to provide hair cell protection from BPA.



Chemical structure of BPA

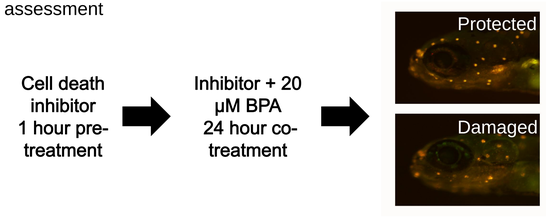
From Sigma Aldrich

Methods

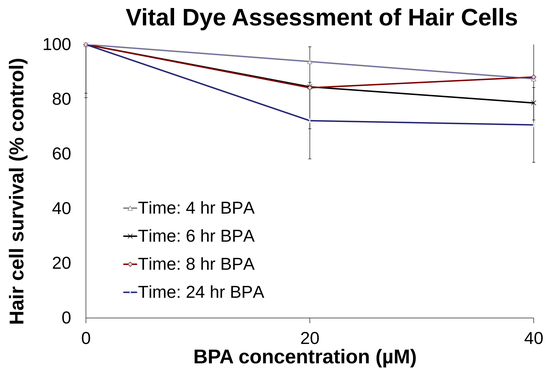
5 day post fertilization (DPF) zebrafish were exposed to different concentrations of BPA (0, 20, 40, 60, and 80μM) for 1, 3, 6 and 24 hour time increments. Treated fish were immediately assessed after treatment, using the DASPEI scoring method.

Hair cells were assessed through quantitative DASPEI scoring. 0 neuromasts around the head of the fish were scored per fish, and scores varied from 0-2, 0 (no labeling), 1 (moderate labeling), and 2 (bright labeling). Scores were added up for each neuromast, giving each fish a total score from 0-20.

I screened a custom cell death inhibitor library to identify compounds that protect hair cells from BPA damage (Coffin et al. 2013, Apoptosis). A sample size of N=8-12 fish per compound was used for the initial screening process. For this process, a different assessment was used. Each fish was given a score of either 0, 3, or 5, representing significant damage at scores of either 0 or 3. DASPEI labeling was consistent for all fluorescent assessment

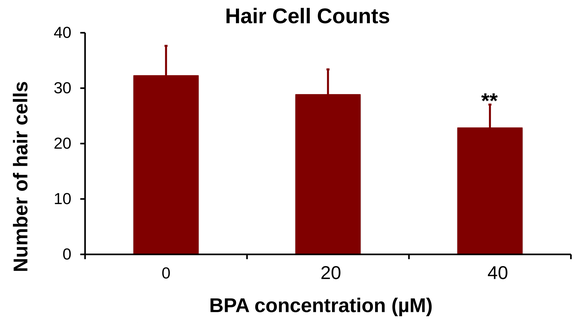


BPA Induces Hair Cell Death

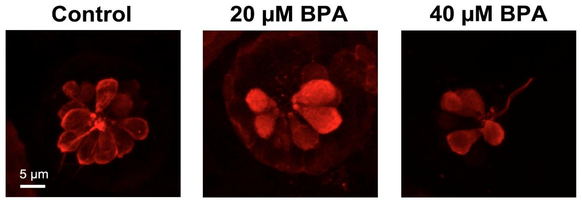


As both time and concentration increased, hair cell survival decreased. Between 1 and 6 hours, hair cell damage was not significant, however, through a 2-Way ANOVA statistical analysis, the increase of time was significant ($p = 0.002$). N=10-14 fish. This experiment shows that **BPA can kill hair cells.**

Individual Hair Cell Damage

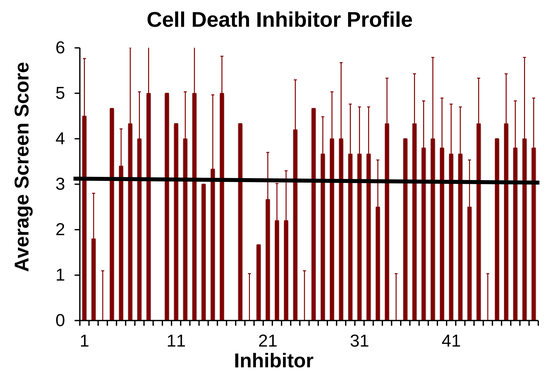


Here immunofluorescence was used to validate the DASPEI scoring results. Control fish had an average of 33 hair cells total in the four neuromasts examined. At 40μM BPA, the total number of hair cells dropped about 10 hair cells to 23 hair cells total. **These results correlate directly to the time course experiment and dose response curve associated with the DASPEI scoring. **p<0.01**



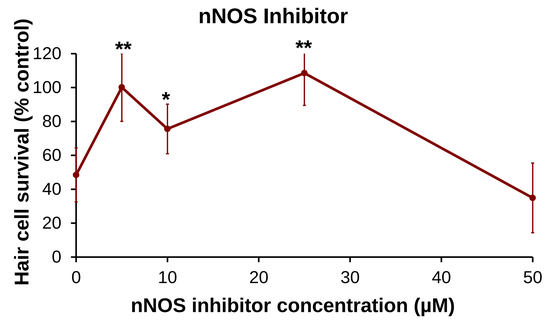
Confocal images of representative anti-parvalbumin-labeled hair cells.

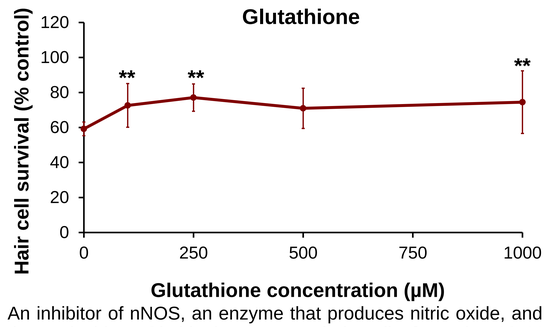
Identifying Cell Death Inhibitors



Several cell death inhibitors appear to confer protection from BPA ototoxicity (examples below). The black line shows the average fluorescent intensity score of fish treated with 20 µM BPA only. N=5 fish per treatment, scores of "0" represent dead fish. Black line indicates BPA induced damage.

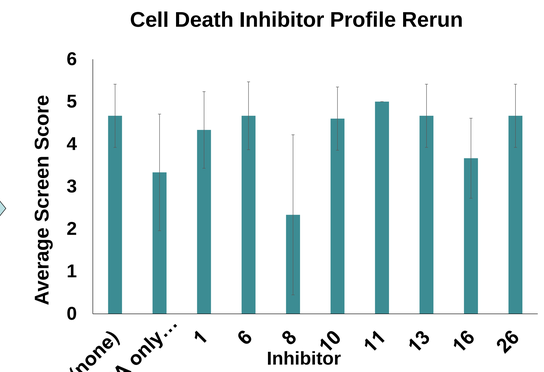
Antioxidants Protect Hair Cells





An inhibitor of nNOS, an enzyme that produces nitric oxide, and the antioxidant Gluthionine, protect hair cells from the 20μM BPA. 100% represents the control condition. BPA may cause the production of reactive oxygen species, leading to hair cell death. **(1-way ANOVA, p<0.001). *p<0.05, **p<0.01.**

Reassessing Potential Inhibitors



Conclusions

- BPA induces hair cell death in zebrafish in a dose- and time-dependent manner.
- BPA induces formation of reactive oxygen and nitrogen species in other zebrafish cell types, suggesting BPA acts similarly across tissues (Xu et al. 2013 Env. Tox. Chem.).
- This study provides additional evidence about the damaging effects of BPA on both aquatic organisms and human health.

Future Directions

- Determine whether or not BPA actually enters cells directly or if there is extracellular signaling occurring.
- Look at estrogen as a potential mechanism for hair cell protection, as BPA is a known endocrine disruptor.
- Educate the public about the current regulations on BPA and some of the effects it has.

A CamelBak BPA-Free Water bottle.

Works Cited

Coffin, Allison B., Henry Ou, Kelly N. Owens, Felipe Santos, Julian A. Simon, Edwin W Rubel, and David W. Raible. "Chemical Screening For Hair Cell Loss And Protection In The Zebrafish Lateral Line." *Zebrafish* 7.1 (2010): 3-11. Print.

Coffin, Allison B., Katherine E. Reinhart, Kelly N. Owens, David W. Raible, and Edwin W Rubel. "Extracellular Divalent Cations Modulate Aminoglycoside-induced Hair Cell Death In The Zebrafish Lateral Line." *Hearing Research* 253.1-2 (2009): 42-51. Print.

I'd like to thank my mentor, teachers, and parents for supporting me through the ups and downs of this research design and process.