The waterways near where Diamond Alkali factory in Newark was located are contaminated with a carcinogen (*2,3,7,8-Tetrachlorodibenzo-P-Dioxin*). This factory made many chemicals however, the intended products are not the focus of this proposal. The "by-product of these manufacturing processes was 2,3,7,8-TCDD (dioxin)" (*Diamond Alkali Co. Newark, NJ*). It effects organisms via the aryl hydrocarbon receptor pathway. If this pathway were not present it seems that TCDD would not be harmful. It is because of this TCDD that there is upregulation in certain transcript production (Denison & Nagy). Overtime this "is linked to impairment of the immune system, the developing nervous system, the endocrine system and reproductive functions" (*Dioxins and Their Effects on Human Health*). However, TCDD is not the only chemical that is found in waterways.

A waterway near this factory, the Hackensack River has "world-record levels of Hg" [1]. Mercury is known to cause "[...n]eurological and behavioural disorders may be observed after inhalation, ingestion or dermal exposure " (*Mercury and Health*).Given the proximity of these waterways to each other it suggests that organisms in these waterways were exposed to both chemicals. In fact, in many cases when dealing with sites near cities and factories organisms in waterways are exposed to many chemicals.Given that the effects of TCDD and mercury in humans are well known perhaps the next step would be to see the effects of these chemical in another organism particularly one in the waterways. However, it might be prudent to study the effects of TCDD and Mercury alone and in combination. In one article it was suggested that "insulin resistance increased with serum dioxins [...] and blood mercury" (Chang *et. al.*). This makes it all more vital that the effects of TCDD and mercury be properly understood as we can

[1] This quote is from the proposal of Isaac Wirgin as it is dealing with his current research. This is what I am working on. I could not find the original source he used.

see how it affects organisms in waterways. As is sometimes the case, some chemicals affect organisms differently at low doses versus high doses and therefore this should also be taken into account.

In the case of this experiment the organism uses was *Microgadus tomcod*. This common name of this organism is Atlantic tomcod. This organism seems to be a bit more resistant to exposure to certain harmful chemicals and as a result it seems that it might be an interesting organism to study. It was decided that there would be three replicates per experimental group. One group gets a low dose of TCDD. Another group gets a high dose of TCDD. A low dose and high dose group for mercury as $HgCl_2$ is made. For the controls, it would be best to expose one group to just water as this would stimulate the ideal normal conditions and another with a chemical that would simulate the condition in which there would be a contaminant. For the latter, it was suggested that acetone be used. Therefore, the results for RNA sequencing of each of the samples would be compared to the group exposed to acetone. This acetone group would then be compared to the group exposed only to water. It may be prudent to take an average of the RNA reads for each experimental group and then compare them to each other. The hope is to see a difference in expression of RNA between groups particularly for genes associated with the Atlantic tomcod heart. Knowing this may help us understand the effect of that these chemicals on Atlantic tomcod when they are young may give a better understanding of how these effects may be compounded when these organisms grow older. The next step would be to explore options dealing with what software or programs would be most useful.

Interestingly, "*Salmon* [is a]software for quantifying transcript abundance" (Love *et. al.*) and it is a software I am planning to use for this project. It has also been suggested that I used

edgeR and DESeq2 to see how RNA expression changes with each treatment. Given that this experiment is based largely on a paper called "Identification of a unique gene expression signature in mercury and 2,3,7,8-tetrachlorodibenzo-p-dioxin co-exposed cells" by Lakshmanan Jagannathan *et. al.* the procedures used in this paper will be used as a reference with slight changes. For example, they used an arbitrary cut off for fold change (of 1.5) which might be useful. Another statistically significant cut off may be useful. They used a cut off of 0.05 in some cases for significance. Perhaps, using 0.01 would make it more stringent. They used Java TreeView 3.0 to make a heat map to see the data and this might be useful. They used ClustVis to cluster the data and this software may be useful. The researchers also used t-tests as well as z-scores when comparing different experimental groups, this may be a good step to use. Bar graphs and pie charts may help visualize the difference between which genes seem to have more RNA expression and which have significantly less expression. Perhaps, the most useful method to visualize the genes that are expressed in common between to treatments would be to create a Venn diagram that shows what genes are expressed more or less in each condition compared to another. However, all of these methods seem like they would be good to use in the beginning.

This brings about certain obstacles. For example, the cut off for fold change used in the paper by Jagannathan *et. al.* is 1.5 however, there should be a better way to deal see if there is a significant difference in RNA levels for each treatment than allotting a value as a cut off. In this case, a biostatistician may need to be consulted in this case. Another small obstacle that needs to be overcome is one dealing with gene alignment. The genes cannot be aligned easily as the reference genome for Atlantic tomcod to check what genes are does not seem to be of a high enough quality to use. Therefore, perhaps the genome of a related fish may need to be used as a

reference to see what each of the RNA sequences are for. This would be two interesting conundrums that would need to be solved yet might help me understand the effects of mercury and TCDD on the organism in this experiment.

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