

Evaluation of The Effect of Paraben Exposure on Sea Urchin (*Strongylocentrotus Purpuratus*) Fertility



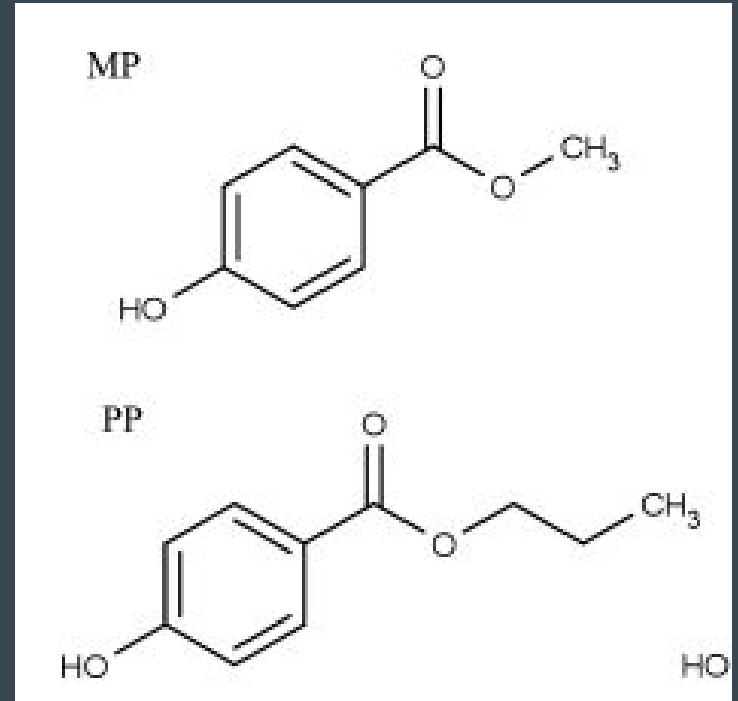
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AP Research

Background

Current Problems, Previous Studies
and Purpose

Parabens

- Preservatives in pharmaceutical, cosmetic, and infant care products as well as some foods
- Highly effective
- Many negative effects
- rising concern for their possible endocrine disrupting effects



Endocrine Disrupting Chemicals (EDCs)

- Chemicals or mixtures of chemicals that have a disrupting effect on the amount of hormones released in the body
 - birth defects
 - developmental complications in infants
 - manifest into diseases later in life
 - Male reproductive system
 - Sporadic miscarriages

Exposure to Parabens

Table 5

Sources, pathways of human exposure and environmental levels of Parabens.

Sources	Pathway	Level	References
Personal care products	Dermal absorption	8000 µg/g Total PB	CIR (2008)
Foodstuff	Ingestion	39.3 ng/g	Liao et al. (2013)
Tap water	Ingestion	28 ng/L ⁻¹ ButP	Carmona et al., (2014)
Indoor dust	Inhalation	468 ng/g MetP	Canosa et al. (2007)
Sludge	Environment	44.1 ng/g ⁻¹ dw ProP	Albero et al. (2012)
Soil	Environment	1.21–8.04 ng/g dw MetP	Pérez et al. (2012)
Sediment	Environment	152 ng/g ⁻¹ MetP	Carmona et al. (2014)
Fish	Environment/ Ingestion	0.05–3600 ng/g MetP	Ramaswamy et al. (2011)

Purpose

- FDA does not have any restrictions on the use of parabens in cosmetics and food products in the United States
- European Union (EU) restricts the use to 0.4% for individual compounds and 0.8% for a total of them. Researchers have suggested decreasing the amount to 1.9%.
- Instil regulations and human testing

**Does exposure to Parabens have
an effect on sea urchin fertility?**

Hypthesis

If the Sea Urchin sperm are exposed to over 10 μ l of paraben for one hour, the success of their fertilization with female gametes will be decreased from amount of embryos produced in the control group. In addition, the sperm exposed to the two different parabens will face a decrease in motility.

The Experiment

Materials, Gamete Extraction,
Gamete Exposure, Fertilization and
Analysis

Materials

- Sea Urchins from Gulf Specimen Marine Lab (Panacca, Florida)
- Instant Ocean (Tat Tropical Illusion Fish Store)
- 10 M NaOH for pH increase
- 0.5 M KCl
- Methylparaben (Sigma Aldrich)
- Propylparaben (Sigma Aldrich)
- Microscope

Why Sea Urchins?

- Role in biology as a model organism.
 - artificial spawning,
 - fertilization and rearing
 - embryo optical transparency
- Manageable in aquaria
- Spawn on command following an injection of KCl solution
- Fertilization occurs outside of the body

Methods

Sea Urchin Maintenance

- Twelve sea urchins were kept in a ten gallon saltwater tank.
- Saltwater was made from an instant ocean kit and filtered
- Sea urchins were fed everyday with 1.5 grams of seaweed.
- pH of the tank needed to be between 8.0-8.1 pH.
 - To regulate the pH of the tank, a pH meter probe was used.
 - When the pH dropped, 10 M NaOH was added until 8.1 pH was reached again.
- The tank was cleaned every two weeks

Gamete Extraction

- Before gamete removal, a 0.5 M potassium chloride solution was made.
- Urchins were placed mouth side up in a petri dish and 0.1 mL KCl was injected into each side of the sea urchin.
- If male, milky white sperm would become visible.
 - After collection, a sperm dilution of 10 microliters in 100 ml chilled seawater was made.
- If the sea urchins were female, red strands of eggs would be released on the bottom of the petri dish
 - 1 ml of settled eggs was transferred to a new beaker with 100 ml chilled seawater and stored at 4 C.

Gamete Exposure to Parabens

- Propylparaben at concentrations of 10 $\mu\text{l}/1\text{ mL}$ seawater, 50 $\mu\text{l}/1\text{ mL}$ seawater, and 100 $\mu\text{l}/1\text{ mL}$ seawater.
- Methylparaben at concentrations of 2.66 $\mu\text{l}/1\text{ mL}$ seawater, 13.3 $\mu\text{l}/1\text{ mL}$ seawater and 26.6 $\mu\text{l}/1\text{ mL}$ seawater.
- 5 mL of sperm dilution was placed in six different falcon tubes
 - 30 minutes, 1 hour, 2 hours, and 24 hours.

In Vitro Fertilization

- 1 mL of sperm was drawn and expelled onto 100 μ l of eggs.
- egg and sperm were gently mixed and placed on a plate shaker for consistent agitation and equal distribution.
 - This process was repeated for each exposure time
- Embryos were left alone for two hours post-fertilization before the amount of fertile embryos were counted and recorded.
- Embryos were incubated at 25 C on a constant shaker for a week.

Fertile Embryo Success Analysis

- Survival and development of the fertilized and unfertilized embryos were analyzed and recorded in comparison to the control group embryos.
- Living versus dead embryos were determined by analyzing motility and the stages of development.
- Observations were made for all embryos based on the following table which shows the development of two common types of sea urchins.

Stages of Development in Sea Urchin Embryos

species	1st division	2 nd division	blastula	gastrula	pluteus
L. pictus 18C	90'	2.5 hrs	24 hrs	2 days	5 days
S. purp 12C	120'	3 hrs	24 hrs	2 days	5 days

Results

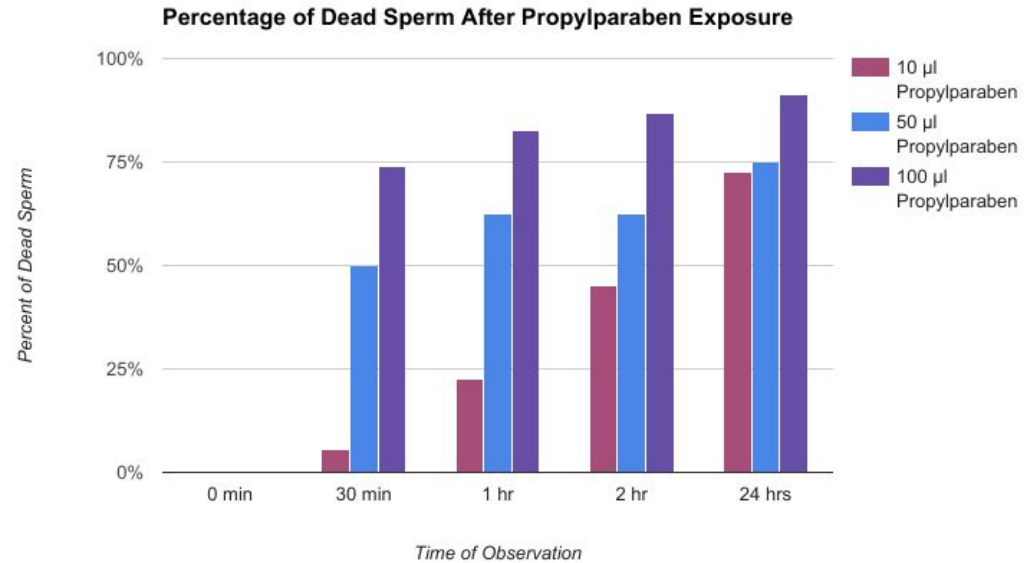
Physical Appearance, Experimental
Results



Physical Appearance of Sperm

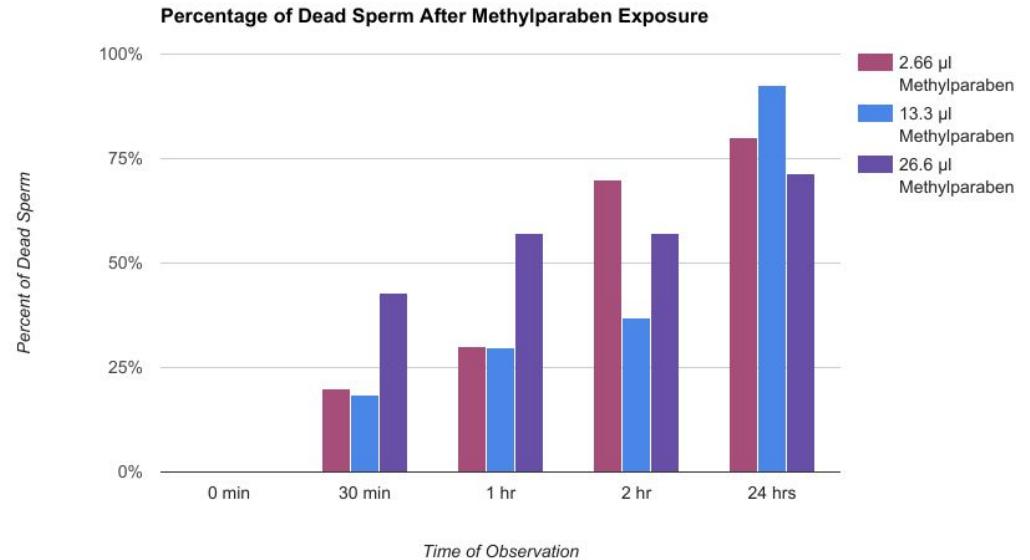
Propylparaben Exposure

- Highest concentration after the longest amount of exposure had the largest amount of deaths.
- Smallest amount of propylparaben exposure after the shortest amount of time had the smallest effect



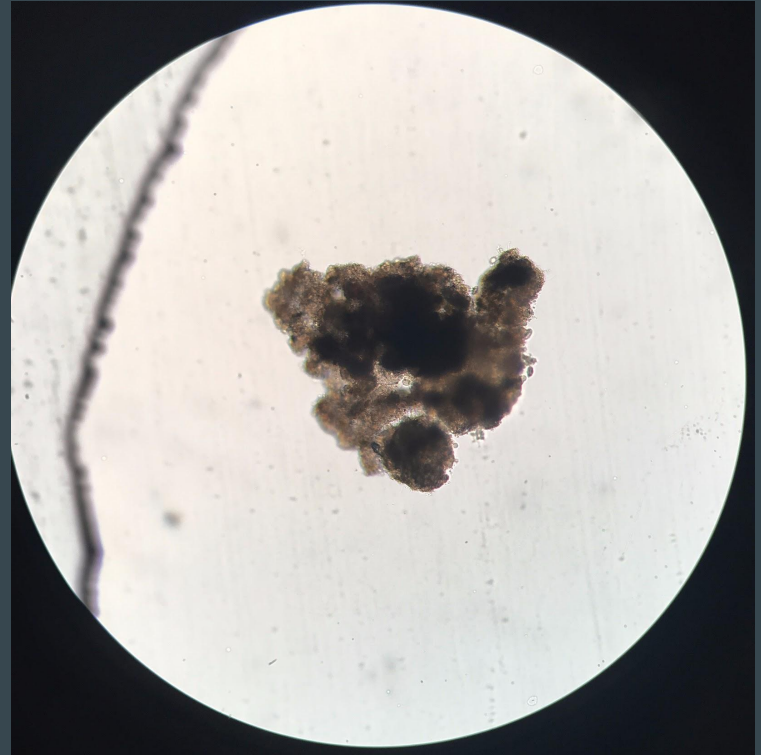
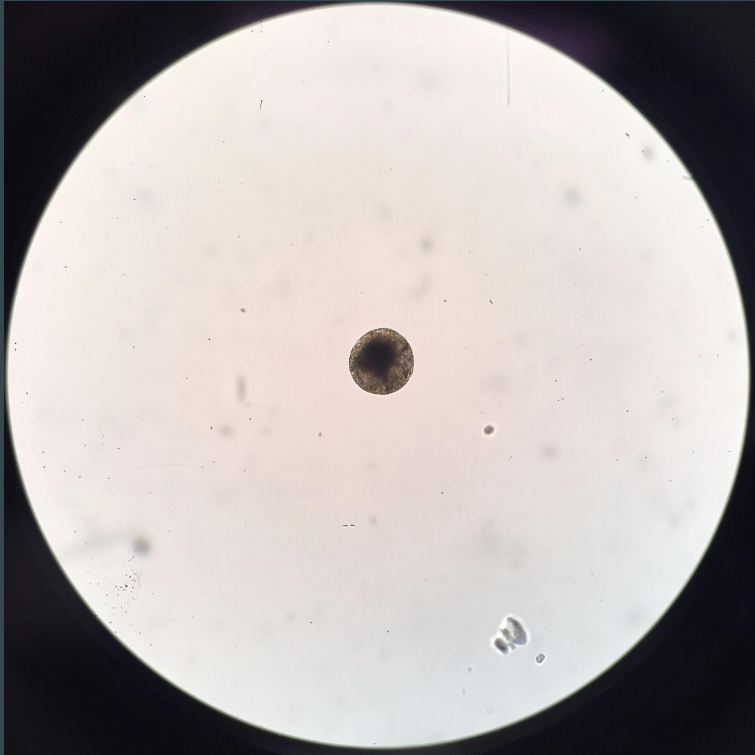
Methylparaben Exposure

- Middle concentration of methylparaben at the longest exposure time had the highest percentage of deaths
- Middle concentration at the shortest amount of exposure had the lowest percentage of deaths.



92.59%

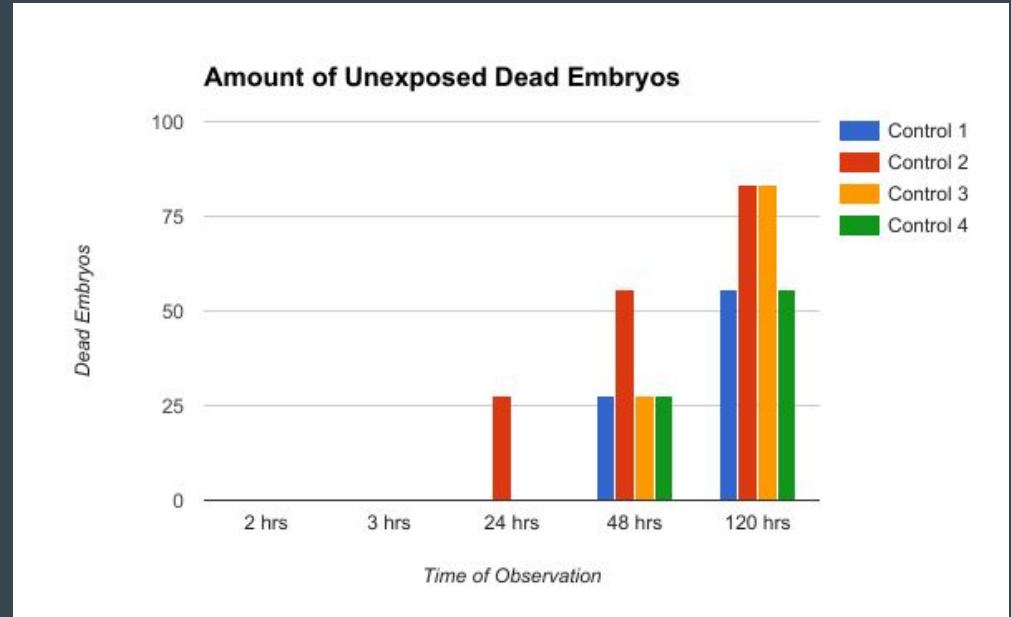
Percentage of dead sperm after exposure to 26.6 μl methylparaben/ 1 mL seawater for 24 hours.



Physical Appearance of Embryos

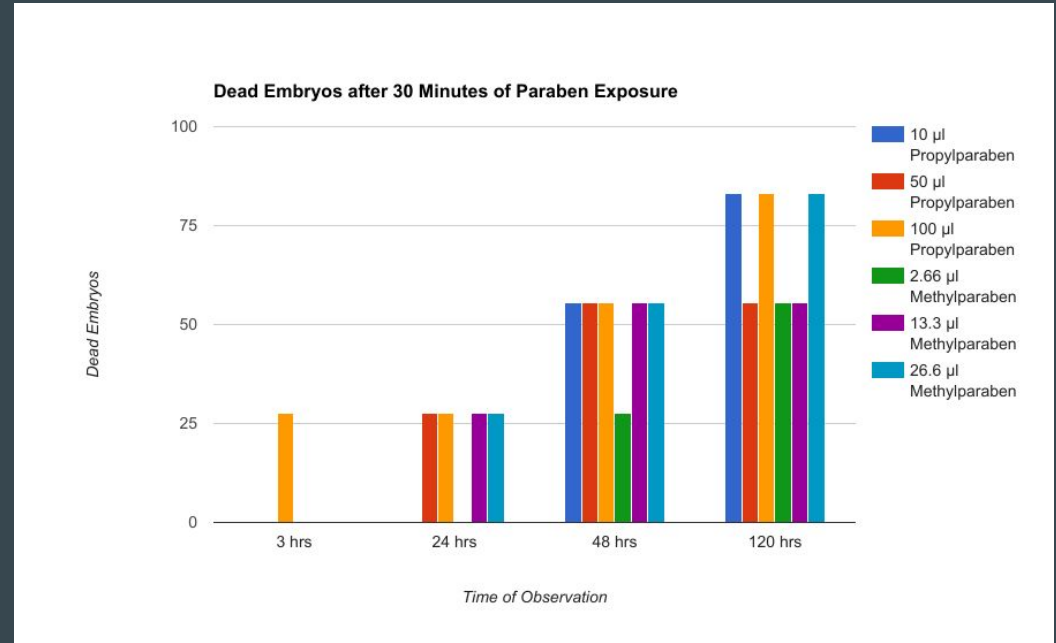
Control - Unexposed Embryos

- Amount of death increased after time of exposure and with each time of observation
- Embryos of the control lived much longer and developed more successfully



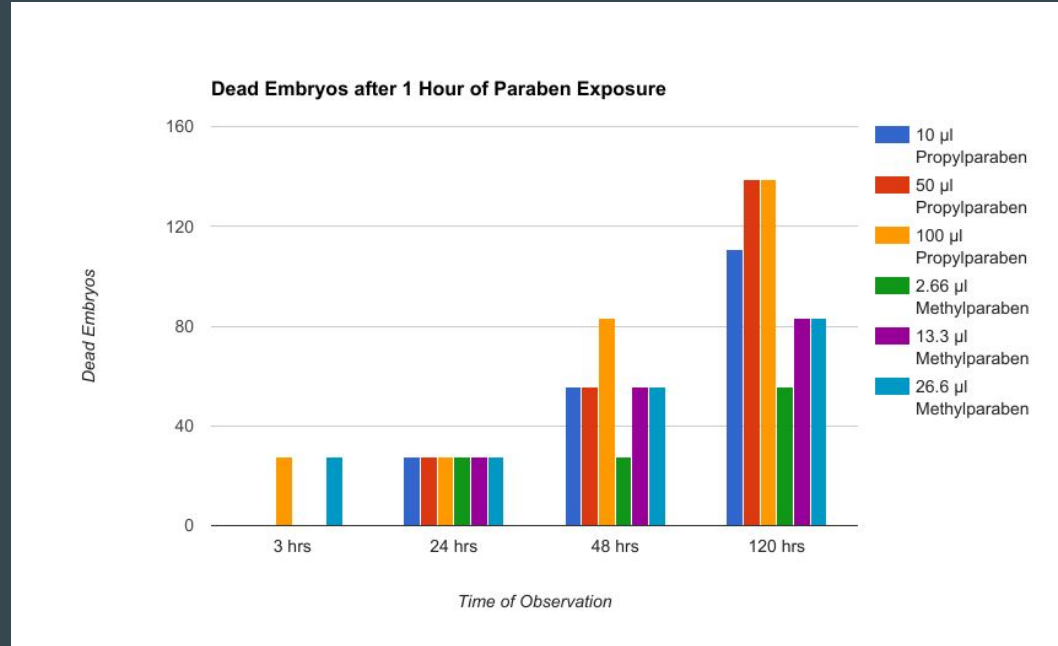
30 minutes of Paraben Exposure

- An increase in the amount of dead embryos can be seen for each concentration at each time of observation.
- Methylparaben at a concentration of 2.66 μl had the smallest effect on sea urchin embryo mortality.



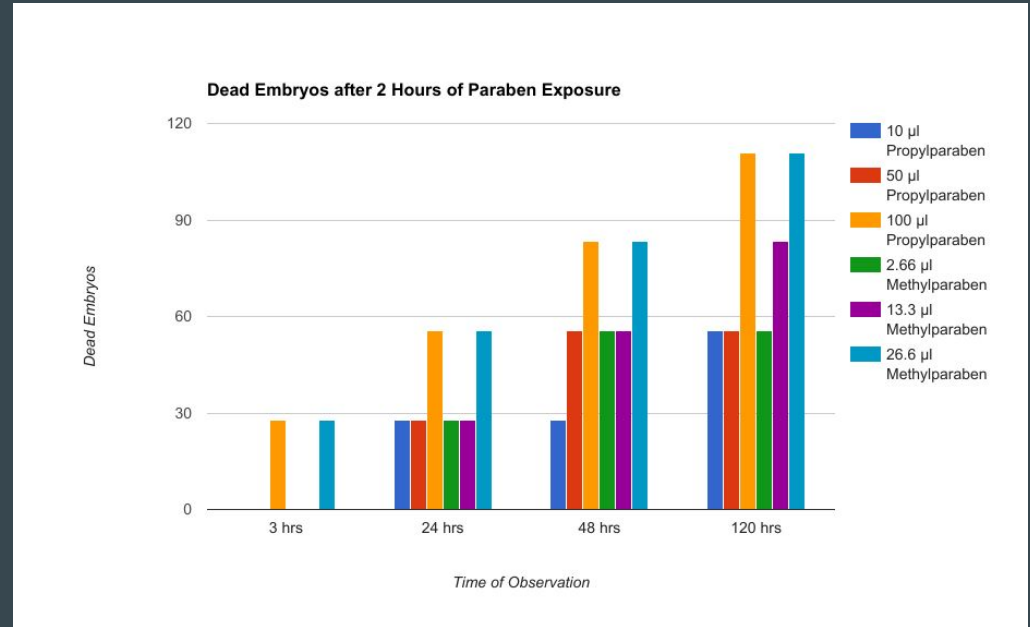
1 hour of Paraben Exposure

- More deaths after 1 hour of exposure to each type of paraben at various concentrations did not occur
- Data shows a more accurate correlation to the increasing amount of deaths with the increasing concentrations.
- Propylparaben has a more negative effect on sea urchin fertility



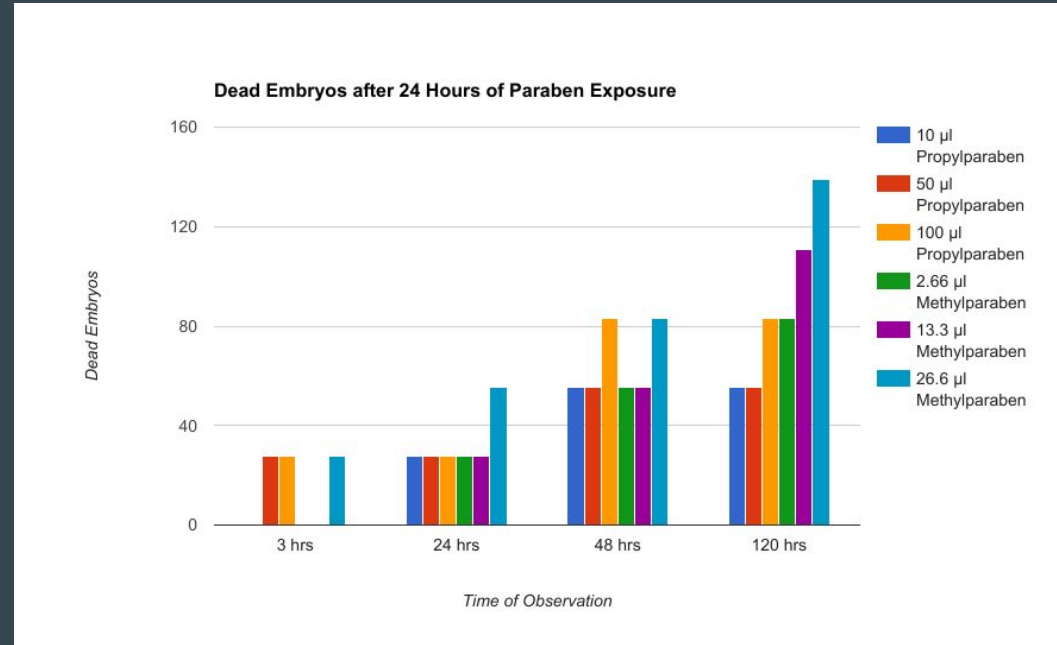
2 hours of Paraben Exposure

- Amount of deaths of the embryos did increase from that of exposure for only 1 hour.
- The higher concentrations for each paraben result in more deaths.



24 hours of Paraben Exposure

- Did not cause the largest amount of deaths, this can be attributed to the decreasing amount of embryos in the petri dishes after each sample is taken out.
- More deaths after only 3 hours post-fertilization, an occurrence that was unique to this specific amount of exposure.



159x

Embryos at the highest concentration for propylparaben at the longest amount of exposure time were 159 times more likely to die and not complete development.

Discussion

Explanation of findings, sources of error

Discussion

- Fertilization of the control group was successful and they were able to undergo the stages of development.
 - While some deaths did occur in the control group, this was not until the end of observation.
- Results for the exposed embryos show that all concentrations of Methyl- and Propylparaben exposure are detrimental to the sea urchin sperm and fertility.
- Results indicate that while the embryos of the control group eventually died, the success of their fertilization was greater than that of the embryos created from paraben exposed gametes.
- Embryos produced from Propylparaben exposed gametes were subjected to more adverse conditions than the Methylparaben exposed gametes.

Paraben Exposure to Male Gametes

- Paraben exposure was also detrimental to sperm survival
- Analyzed by observing the sperm motility, whereas lack of movement indicated death.
- Results show an increase in death of sperm from the control group to the experimental group.
- At the highest concentration for propylparaben at the longest amount of exposure time, 91.3% of the sperm had died.
- At the highest concentration for methylparaben at the longest amount of exposure, 92.59% of the sperm had died.

In Correspondence to Hypothesis

- Results prove the original proposition to be correct.
- Any amount of exposure to either paraben and for any increment of time will have a negative effect on sea urchin embryo and sperm survival.
- The greatest effect of negativity was seen in the embryos, fertilized from the group of sperm exposed to 26.6 μl of Methylparaben/1 mL of seawater after 120 hours.

Conclusion

Conclusions and Sources of Error

Conclusions

- Parabens can alter fertility rates when directly exposed to sea urchin gametes.
- Higher levels made a significant difference in the success of sea urchin fertilization.
- Need for further testing on human fertility is before the amount of parabens found in food and cosmetic could be limited by FDA restrictions.

Sources of Error

- Amounts given were averages and may not be accurate
- The age and original conditions of the sea urchins were not given.

Further Work

- Analyze the effects of all types of parabens on sea urchin fertility including ethyl- and butylparaben.
- Differences in effect of exposing the sperm versus exposing the eggs
- Paraben exposure on a sea urchins reproductive organs
- Research must be conducted on human test subjects in order to determine the effects of parabens on humans
 - Human gametes
 - The reproductive system.

References

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