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

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Abstract:

The results of past studies on parabens effect on fertility have proven conflicting. Currently, there is little research to come to a complete conclusion and propose regulations of parabens by the FDA. Most of the research regarding the results of EDC exposure on the human body focuses on chemicals such as phthalates and bisphenols, the more common disrupting chemicals. Because of this, more research is needed to discover the adverse effects of parabens as endocrine disrupting chemicals. To test the effect of paraben exposure on sea urchin fertility, male gametes were extracted and exposed to methyl- and propylparaben, and used to fertilize female gametes. Sea urchins were used because they are manageable in aquaria, spawn on command following an injection of KCl solution, gamete collection is relatively easy, and fertilization occurs outside of the body. Success of fertilization and development were analyzed for exposed sperm and embryos in four replicates. Results show that for all concentrations of paraben exposure, infertility and death are inevitable for sperm and embryos.

Introduction:

Endocrine disrupting chemicals (EDCs) are chemicals or mixtures of chemicals that have a disrupting effect on the amount of hormones released in the body. In turn, this can lead to birth defects and developmental complications in infants that will manifest into diseases later in life (Berger, Haeger, Potouridis, Puttmann & Wagner 2015).

Parabens are chemicals used as preservatives in pharmaceutical, cosmetic, and infant care products as well as some foods (Boberg, Christiansen, Hass & Taxvig 2010). While highly effective, these substances have been known to have many negative effects and are becoming a

rising concern for their possible endocrine disrupting effects at high exposure levels. Recently, researchers have also found that EDCs can possibly cause cancerous tumors as they were seen in breast cancer tissue due to their oestrogenic effect (Aljarrah, Coldham, Darbre, Miller, Poper & Sauer, 2004). In addition to being found in breast cancer tissue, a recent study found that parabens were being leached from baby teethers. However, the amount being leached was not quantified and needs to be further researched.

In a study conducted at Oregon Health Sciences University and Stanford University, researchers found that environmental exposure to EDCs also has a correlation to sporadic miscarriage, the loss of an embryo or fetus before 20 weeks of pregnancy. This complication of pregnancy is the most common among women, affecting approximately 15% of all pregnancies documented clinically. This can be a result of the sensitivity of embryonic tissue or fetal tissue. When introduced at an early stage of development, EDCs can have deleterious effects on development.

Endocrine Disrupting Chemicals and their Reproductive Effects

Table 1: Endocrine Disrupting Chemicals and their Reproductive Outcomes

Endocrine disrupting chemicals and their reproductive outcomes.

Compound	Mechanism	Oocyte effect	Endometrial effect	Miscarriage	Reference
DDT	Estrogen receptor agonist, androgen receptor antagonist	Decrease in mature oocytes (zebrafish), increased granulosa aromatase activity (human)	Decreased proliferation and increased apoptosis	OR, 1.4 (1.1–1.6) per 60 μg/L increase (prospective, n = 1,717); OR, 1.13 (1.02–1.26) (case control study); OR, 1.17 (1.05–1.29) prospective (n = 372)	(18–23)
BPA	Estrogen agonist	Meiotic errors, epigenetic changes, early apoptosis	Altered gene expression of HOXA10, Era, ERb, B3integrin, and ITGB3	Sporadic: OR, 1.97; recurrent: OR, 3.33 in highest quartile (n = 115); highest concentration: recurrent spontaneous abortion OR, 9.34 (3.06–28.44; n = 264)	(24–27)
PCBs and dioxin	Mixed estrogen agonist/antagonist, androgen receptor agonist	Inhibit meiotic spindle assembly, prevent normal oocyte maturation	Altered expression of estrogen responsive genes	OR, 1.6–2.52 depending on type of PCB; Agent Orange relative risk, 0.99 (0.85–1.16)	(28–30)
Phthalates	P receptor agonist in silico, interacts with PPAR with decrease in aromatase activity	Anovulation, abnormal granulosa steroidogenesis	Abnormal expression of Era, PR, and E-cadherin	Increased miscarriage OR, 2.87 (1.09–7.57)	(31–33)

Krieg. Environmental toxins and miscarriage. Fertil Steril 2016.

This table shows the reproductive outcomes of various endocrine disrupting chemicals. The table also summarizes how the chemicals work, their effect in the body and the amount of miscarriages they can cause. While these results seen in the table above show that women of reproductive age should actively seek caution in exposure to these EDCs, their presence in the environment is ubiquitous and often unavoidable (Krieg, Lathi & Shahine, 2016).

Not only can EDCs cause miscarriages, researchers have found that these substances can have adverse affects on the male reproductive system. These effects include degeneration of increase in relative weight of epididymis, decrease in sperm count and quality of sperm and decrease in level of male hormone testosterone (Goswami & Kalita, 2012).

Effects of Endocrine Disrupting Chemical Exposure to Male Reproductive System

Table 2: Sperm Characteristics in Controls and Cases

Table 1
Sperm characteristics in controls and cases.

	Controls, n = 80	Cases, n = 40	p-Value
1st sperm sample			
Period of abstinence (days)	3.2 (2, 4)	3.1 (2, 4)	0.50
Sperm concentration (million per mL)	58.6 (39.0, 104.4)	4.0 (1.9, 11.3)	<0.001
Motility (%A + %B)	57.5 (49.5, 70.0)	14.3 (15.0, 34.5)	<0.001
Normal motility: %A + %B ≥ 50	60 (75.0%)	2 (5.0%)	<0.001
Morphology (% normal)	3.3 (5.0, 12.0)	0.2 (0.8, 4.0)	<0.001
TMC (million)	110.4 (57.5, 204.5)	1.1 (1.0, 7.0)	<0.001
Normal TMC: TMC ≥ 20	80 (100%)	0 (0%)	<0.001
2nd sperm sample			
Period of abstinence (days)	2.7 (3, 4)	3.6 (3, 5)	0.68
Sperm concentration (million per mL)	65.1 (38.8, 106.0)	4.0 (1.6, 12.8)	<0.001
Motility (%A + %B)	54.6 (47.5, 68.0)	24.0 (17.5, 36.0)	<0.001
Normal motility: %A + %B ≥ 50	52 (65.0%)	6 (15.0%)	<0.001
Morphology (% normal)	4.1 (3.5, 7.5)	0.1 (0.5, 2.5)	<0.001
TMC (million)	113.8 (59.5, 189.6)	1.5 (1.3, 7.1)	<0.001
Normal TMC: TMC ≥ 20	80 (100%)	0 (0%)	<0.001

TMC: total motile count.
Data are geometric means (P25, P75) for continuous variables and numbers (%) for categorical variables.

In an experiment conducted at the Division of Reproductive Medicine, University Hospital, researchers also found that prenatal exposure to EDCs may be associated with abnormalities of the testicular dysgenesis syndrome later in life, which includes reduced semen quality and quantity, increased incidence of cryptorchidism, a condition in which one or both of the testes fail to descend from the abdomen into the scrotum, and hypospadias, a condition in males in which the opening of the urethra is on the underside of the penis, and increased incidence of testicular cancer (Hond et al., 2015). The table above shows the amount of dead sperm from a study conducted on sperm exposure to endocrine disrupting chemicals.

Sources of Human Exposure and Environmental Levels of Parabens

Table 3: Sources, Pathways of Human Exposure and Environmental Levels of 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)

The table above shows the sources and levels of human and environmental exposure to various types of Parabens ranging from personal care products to soil. (Barcelo, Capri, Giulivo & Lopez de Alda, 2016). Due to their common appearance in the environment and in daily cosmetic products, parabens are not to be ignored and should be studied more thoroughly

In order to test paraben effect on the reproductive system, sea urchins were used in this experiment due to their role in biology as a model organism. This species has been deemed as an ideal organism due to its many important features. These can include their artificial spawning, fertilization and rearing, and embryo optical transparency (Shiel 2011). In addition, sea urchins are manageable in aquaria, spawn on command following an injection of KCl solution, gamete collection is relatively easy, and fertilization occurs outside of the body (Rast 2006). The KCl solution stimulated the gonad wall to contract which caused ripe gametes to emerge from the gonopores surrounding the anus on the aboral side of the animal (Sea Urchin Embryology).

Currently, the FDA does not currently have any restrictions on the use of parabens in cosmetics and food products in the United States (Center for Food Safety and Applied Nutrition). However, the European Union (EU) restricts the use to 0.4% for individual compounds and 0.8% for a total of them (European Scientific Committee) . Researchers, however, have suggested decreasing the amount to 1.9%. Because their is no regulated amount of paraben usage in the United States, the regulated amount instilled by the EU provided a basis for the various concentrations in the experiment. However, because the regulated amount is meant for human use, calculations were made using average body mass in order to determine the correct concentration for sea urchins. In addition, the varying solubilities of the two different parabens was used to make the correct stock solutions and dilutions from that amount for exposure.

Structures of Methylparaben (MP) and Propylparaben (PP)

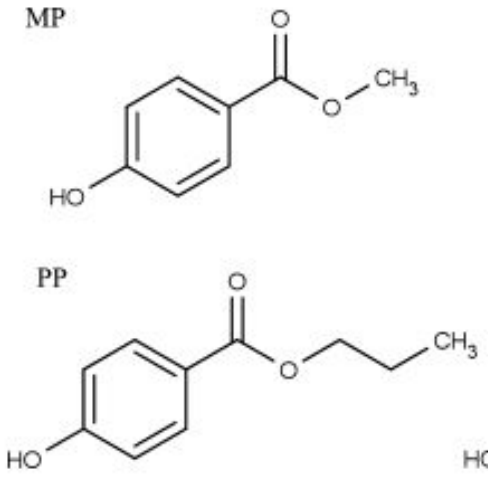

Figure 1. Shows the chemical structures of methylparaben and propylparaben, the two types of parabens that were used in this experiment.

(Boberg, et al., 2010)

Purpose:

The purpose of this experiment was to determine if parabens have an effect on sea urchin fertility. If the results concluded that these preservatives can have harmful effects like infertility, the argument against the use of parabens will have more strength and validity. In addition, because there is still little known about the complete effects of parabens, more research should be conducted on human test subjects in order to prove their adverse effects. From this, the FDA will be able to put regulations in place.

Safety Issues:

Sea urchins should be handled with care because physical injuries from the spines of most sea urchins are possible, however, only a few urchins are venomous. Eye protection, gloves, and a lab coat are necessary whenever handling any of the chemicals used in this experiment. Excessive levels of potassium chloride (KCl) can poison the kidneys which in turn can cause renal failure and blood toxicity. This can result in cardiac arrest if not treated. Skin that comes in contact with potassium chloride may also experience irritation that causes redness or tingling of the skin. In more severe cases, a rash can occur. Exposure to sodium hydroxide (NaOH) can cause severe burns and permanent damage to any tissue that it comes in contact with. Needles used in this experiment should also be handled with caution to prevent any injuries from occurring.

Hypothesis:

If the Sea Urchin sperm are exposed to over 10 µl 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.

Materials:

- Sea Urchins from Gulf Specimen Marine Lab (Panacca, Florida)
- Instant Ocean solution (Tat Tropical Illusion Fish Store)
- Deionized water (located in E8)
- 10 gallon fish tank (Tat Tropical Illusion fish store)
- Salt water filter with aeration system (Tat Tropical Illusion fish Store)
- Seaweed, food for bottom feeders (Tat Tropical Illusion fish store)
- 10 M NaOH for pH regulation (located in E8)
- Pure Paraben Powders (Sigma Aldrich)
    - Methylparaben 1g
    - Propylparaben 1g
- Sterile Petri dishes (located in E8)
- pH meter probe (located in E8)
- pH buffer (located in E8)
- Serological pipette (located in E8)
- Pipette aid (located in E8)

- Plate Shaker (located in E8)
- For Gamete Removal:
    - 0.5 M KCL solution (located in E8)
    - Sea water (located in E8)
    - 1-5cc syringe with needle (located in E8)
    - Micro-centrifuge tubes (located in E8)
    - 150 mL Beaker (located in E8)
    - 100 mL Beaker (located in E8)
    - Microscopes (located in E8)
- 20 µl Micropipette (located in E8)
- 20 µl Micropipette tips (located in E8)
- 200 µl Micropipette (located in E8)
- 200 µl Micropipette tips (located in E8)
- Laboratory Refrigerator (located in E8)
- Analytical Balance (located in E8)
- Funnel (located in E8)
- 15 mL falcon tubes (located in E8)

Methods:

All experiments took place at Thousand Oaks High School, in classroom E8. Before beginning any experimentation, acquired sea urchins were made accustomed to their new environment. In addition, all solutions were prepared before gamete removal and exposure.

Sea Urchin Maintenance

Twelve sea urchins were kept in a ten gallon saltwater tank. Saltwater was made from an instant ocean kit in which 145 g of instant ocean salt was mixed with 1 gallon of water. Once the tank was filled with 5 gallons of the saltwater, temperature was maintained at 25 C and filtered through a filter from the pet store with rainfall aeration. Sea urchins were then placed in the tank while remaining in their original transportation bags to prevent salinity and temperature shock. After 30 minutes, the sea urchins and the seawater they were transported in were transferred into the tank. Sea urchins were fed everyday with 1.5 grams of seaweed. Seaweed was broken into small bits and submerged in water before being added to the tank to prevent the pieces of food from floating. In ideal conditions, the 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 as well as a pH buffer to calibrate the probe. When the pH dropped, 10 M NaOH was added until 8.1 pH was reached again. The tank was cleaned every two weeks to maintain optimal conditions for the sea urchins.

Gamete Collection

Before gamete removal, a 0.5 M potassium chloride solution was made. After the KCl stock solution was made, urchins were placed mouth side up in a petri dish and 0.1 mL of the solution was injected into each side of the sea urchin with a syringe with a needle. After injection, the needle was cleaned with 70% isopropyl alcohol followed by seawater. Sea urchins were gently shaken for a few seconds in order for the KCl solution to mix inside of the animal. Gender of the sea urchins was determined by the type of gametes that appeared after injection. If

male, milky white sperm would become visible. Contrastingly, if the sea urchins were female, red strands of eggs would be released on the bottom of the petri dish in pools of water.

Males: Urchins were placed mouth side down on top of a petri dish after KCl injection. After a minute or two, white sperm began to appear on the surface of the urchin. Sperm was then collected with a 20 µl micropipet and placed in a small microcentrifuge tube. Following collection, test tubes were stored at 4 C and lasted up to a week. Viability of the sperm was checked everyday by removing 10 µl of sperm from the concentrated sample and observing motility under a microscope. While motility faced a small decrease as the week progressed, the amount of dead sperm in the end compared to the beginning amount was not affected enough to collect new samples. After collection, a sperm dilution of 10 microliters in 100 ml chilled seawater was made. Before fertilizing the eggs, sperm motility was examined to observe the amount of living sperm vs dead. To do so, 100 µl of sperm suspension was placed on a depression slide without a coverslip, and examined with a 20x lens then a 40x lens.

Females: Urchins were placed mouth side up onto a beaker full of sea water after injection. The eggs were shed into the sea water and collected at the bottom of the beaker. This process took 10-30 minutes to finish shedding the eggs. Eggs and sea urchins were kept at the same temperature [13]. Like the sperm, an egg dilution was also made after collection. 1 ml of settled eggs was transferred to a new beaker with 100 ml chilled seawater and stored at 4 C. Before being used for fertilization, eggs were gently mixed to prevent large masses of eggs from forming.

Sea Urchin Exposure to Parabens

Sea Urchin sperm were exposed to two chemicals separately. Propylparaben at concentrations of 10 µl/1 mL seawater, 50 µl/1 mL seawater, and 100 µl/ 1 mL seawater. Methylparaben at concentrations of 2.66 µl/ 1 mL seawater, 13.3 µl/ 1 mL seawater and 26.6 µl/ 1 mL seawater. Concentrations have a direct correlation to those mentioned in introduction and were calculated through paraben solubility. Each stock solution for the two different parabens was prepared in an 100 mL volumetric flask and stored at 4 C. 5 mL of sperm dilution was placed in six different falcon tubes that would be used for each different paraben concentration. Sperm were then exposed to the respective concentrations of parabens for 30 minutes, 1 hour, 2 hours, and 24 hours.

In Vitro Fertilization

After extracting both the male and female gametes from the control group and exposing the sperm, 1 mL of sperm was drawn from the collected amount and expelled onto 100 µl of eggs. Once fully expelled, the egg and sperm were gently mixed and placed on a plate shaker for consistent agitation and equal distribution. This process was repeated after exposure to parabens for 30 minutes, 1 hour, 2 hours and 24 hours. Embryos were left alone for approximately two hours after fertilization before the amount of fertile embryos were counted and recorded. This process was repeated for each concentration of paraben exposure. Embryos were incubated at 25 C on a constant shaker for a week. In order to prevent the petri dishes from drying out, 10 mL of seawater from the sea urchin tank was added to each petri dish with a serological pipette.

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. The purple sea urchin, the organism used in this experiment, can be seen as S. purp, an abbreviation for its scientific name, Strongylocentrotus purpuratus, in the chart given below. Observations were made for all embryos based on the following table which shows the development of two common types of sea urchins. However, the information regarding Strongylocentrotus purpuratus was the only data used from the table.

Table 4: Developmental Stages of 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:

Fertilization of Unexposed Gametes

Table 5: Amount of Dead Unexposed Embryos

time	Control 1	Control 2	Control 3	Control 4
2 hrs	0	0	0	0
3 hrs	0	0	0	0
24 hrs	0	27.75	0	0
48 hrs	27.75	55.5	27.75	27.75
120 hrs	55.5	83.25	83.25	55.5

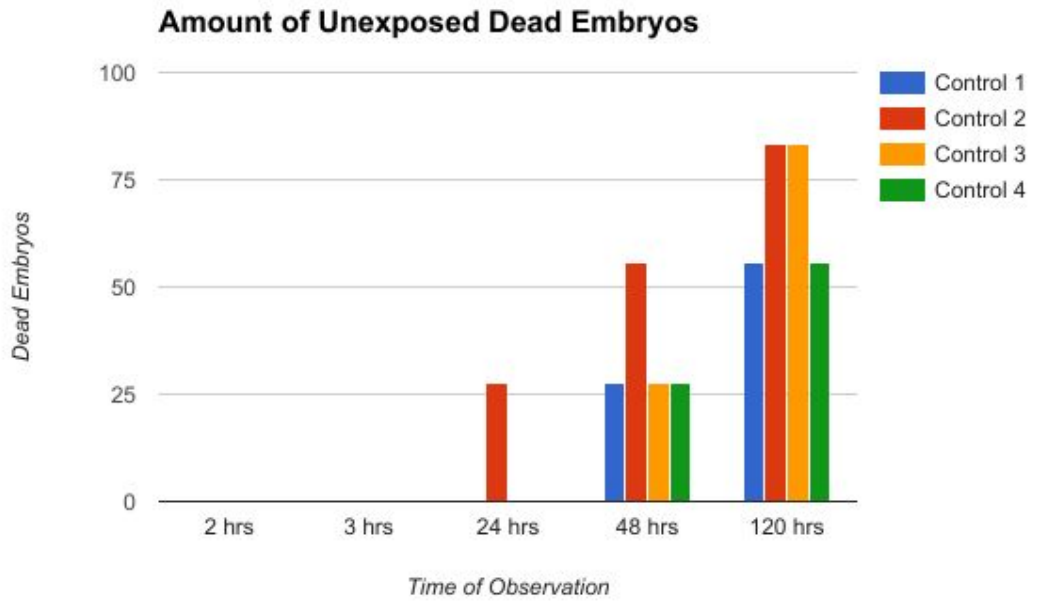

Figure 2: Represents the amount of dead embryos that were not exposed to any amount of Parabens.

The data shown above summarizes the success of fertilization of unexposed gametes and their deaths. The unexposed embryos served as a control in this experiment and while the amount of deaths increased after time of exposure and with each time of observation, embryos of the control lived much longer and developed more successfully than the embryos exposed to various

paraben concentrations. The amounts given in the table and the graph are an average of four replicates.

Effect of Paraben Exposure on Sea Urchin Fertility

Table 6: Amount of Dead Embryos after 30 Minutes of Paraben Exposure

time	10 µl Propylparaben	50 µl Propylparaben	100 µl Propylparaben	2.66 µl Methylparaben	13.3 µl Methylparaben	26.6 µl Methylparaben
2 hrs	0	0	0	0	0	0
3 hrs	0	0	27.75	0	0	0
24 hrs	0	27.75	27.75	0	27.75	27.75
48 hrs	55.5	55.5	55.5	27.75	55.5	55.5
120 hrs	83.25	55.5	83.25	55.5	55.5	83.25

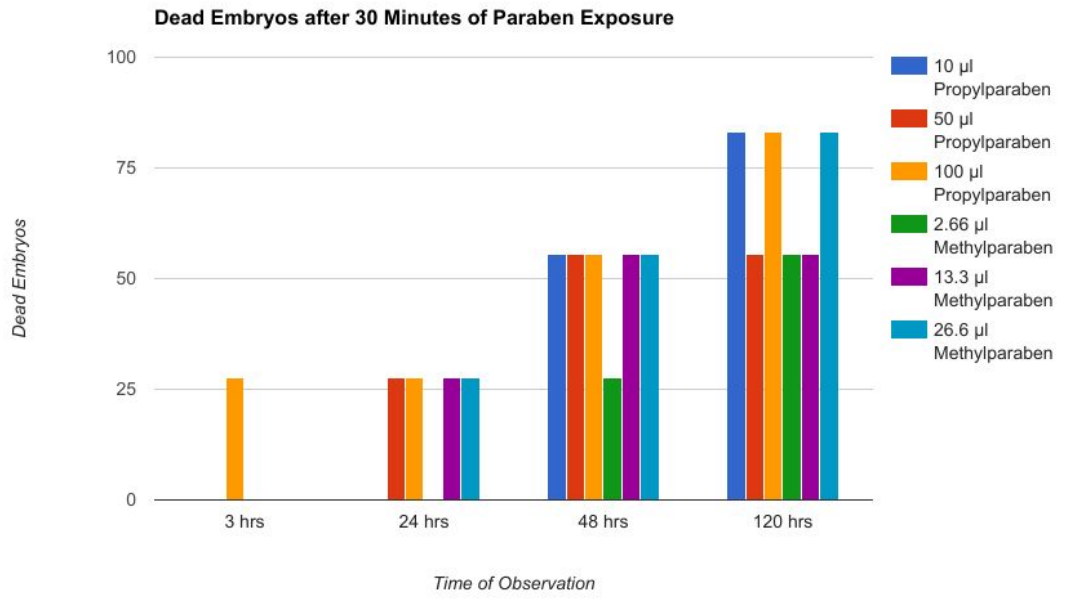

Figure 3. Represents the amount of dead embryos after 30 minutes of exposure to various concentrations of Propyl- and Methylparaben

The data shown above summarizes the averages of four replicates of dead embryos after 30 minutes of exposure to all concentrations of both propylparaben and methylparaben. An increase in the amount of dead embryos can be seen for each concentration at each time of observation. It can also be seen that methylparaben at a concentration of 2.66 µl had the smallest effect on sea urchin embryo mortality. This may be a result of the parabens decreased effects to that of propylparaben.

Table 7: Amount of Dead Embryos After 1 Hour of Paraben Exposure

time	10 µl Propylparaben	50 µl Propylparaben	100 µl Propylparaben	2.66 µl Methylparaben	13.3 µl Methylparaben	26.6 µl Methylparaben
2 hrs	0	0	0	0	0	0
3 hrs	0	0	27.75	0	0	27.75
24 hrs	27.75	27.75	27.75	27.75	27.75	27.75
48 hrs	55.5	55.5	83.25	27.75	55.5	55.5
120 hrs	111	138.75	138.75	55.5	83.25	83.35

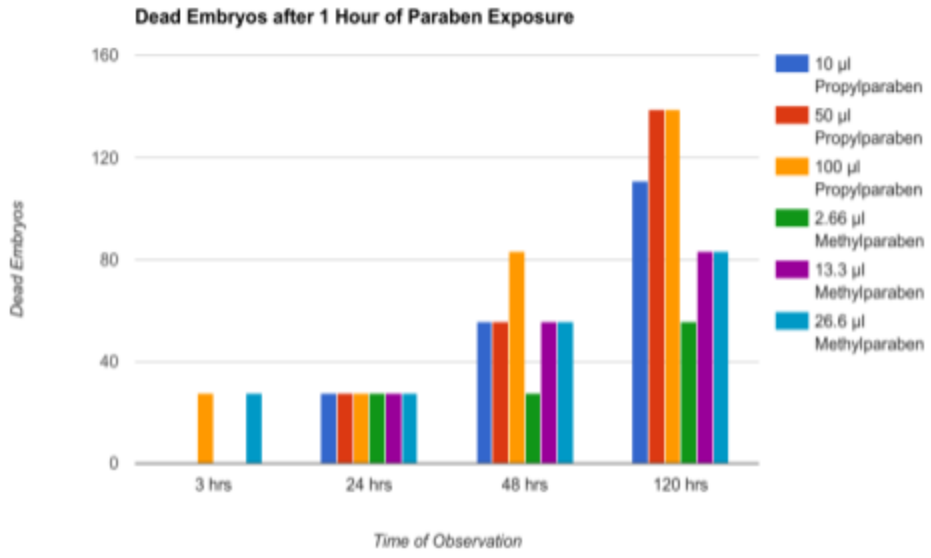

Figure 4. represents the amount of dead embryos after 1 hour of exposure to various concentrations of Propyl- and Methylparaben

While more deaths after 1 hour of exposure to each type of paraben at various concentrations did not occur, the data shows a more accurate correlation to the increasing amount of deaths with the increasing concentrations. The data also shows that Propylparaben has a more negative effect on sea urchin fertility than methylparaben. The amounts given were an average of four replicates.

Table 8: Amount of Dead Embryos After 2 Hours of Paraben Exposure

time	10 µl Propylparaben	50 µl Propylparaben	100 µl Propylparaben	2.66 µl Methylparaben	13.3 µl Methylparaben	26.6 µl Methylparaben
2 hrs	0	0	0	0	0	0
3 hrs	0	0	27.75	0	0	27.75
24 hrs	27.75	27.75	55.5	27.75	27.75	55.5
48 hrs	27.75	55.5	83.25	55.5	55.5	83.25
120 hrs	55.5	55.5	111	55.5	83.25	111

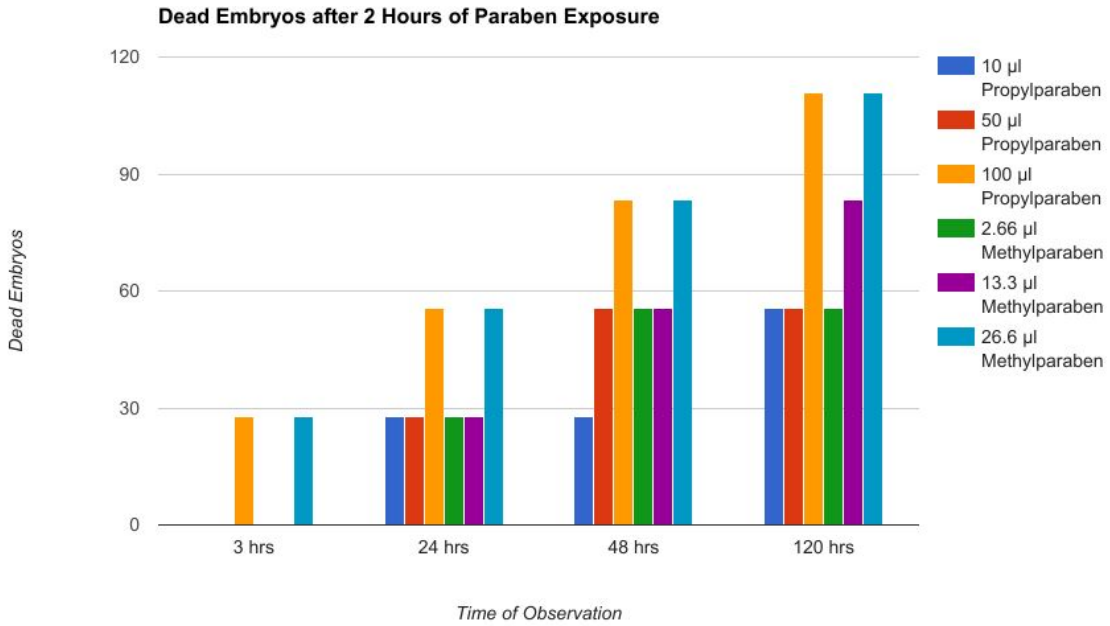

Figure 5. represents the amount of dead embryos after 2 hours of exposure to various concentrations of Propyl- and Methylparaben.

The data shows that after 2 hours of exposure to the parabens and their respective concentrations, the amount of deaths of the embryos did increase from that of exposure for only 1 hour. In addition, the data also shows that the higher concentrations for each paraben result in more deaths. The amounts shown in the table and the graph are an average of four replicates.

Table 9: Amount of Dead Embryos After 24 Hours of Paraben Exposure

time	10 µl Propylparaben	50 µl Propylparaben	100 µl Propylparaben	2.66 µl Methylparaben	13.3 µl Methylparaben	26.6 µl Methylparaben
2 hrs	0	0	0	0	0	0
3 hrs	0	27.75	27.75	0	0	27.75
24 hrs	27.75	27.75	27.75	27.75	27.75	55.5
48 hrs	55.5	55.5	83.25	55.5	55.5	83.25
120 hrs	55.5	55.5	83.25	83.25	111	138.75

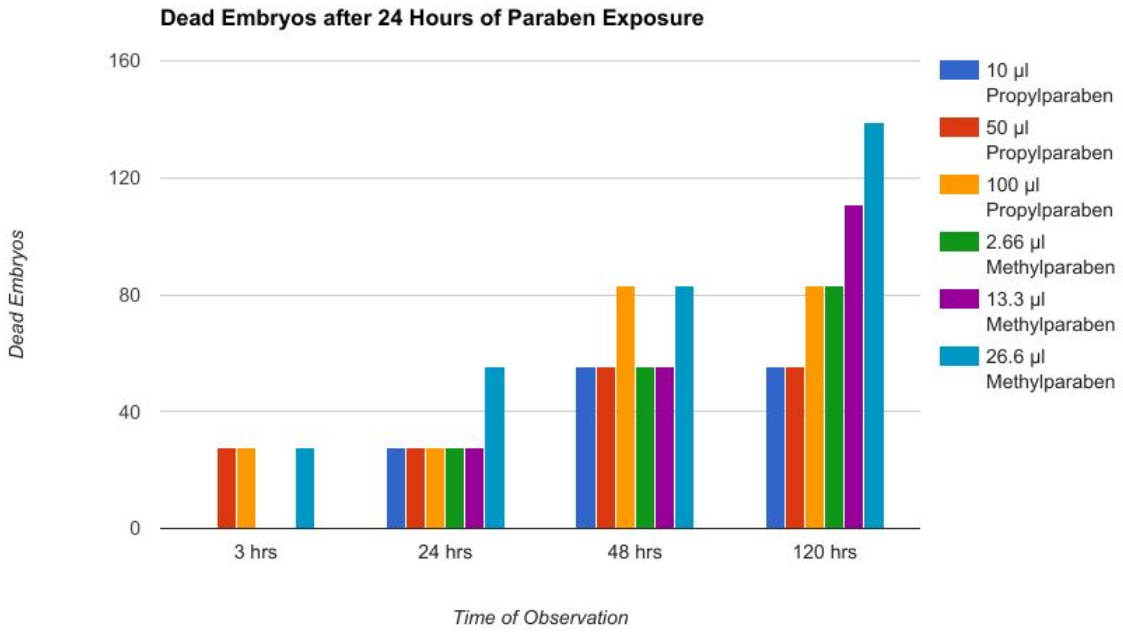

Figure 6. represents the amount of dead embryos after 24 hours of exposure to various concentrations of Propyl- and Methylparaben

The data in figure 6 is average of four replicates, showing the increase in death after each time of observation for the embryos exposed to parabens for 24 hours. While this amount of 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. Despite this, results show that there were more deaths after only 3 hours post-fertilization, an occurrence that was unique to this specific amount of exposure.

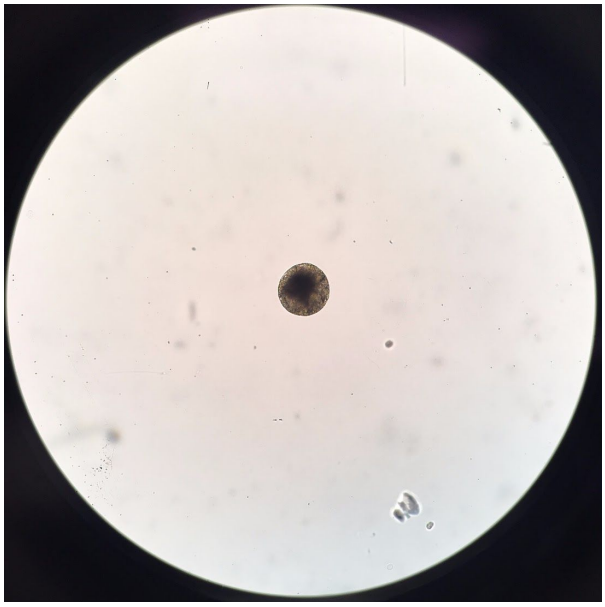

Figure 7. Shows an unexposed embryo 20x

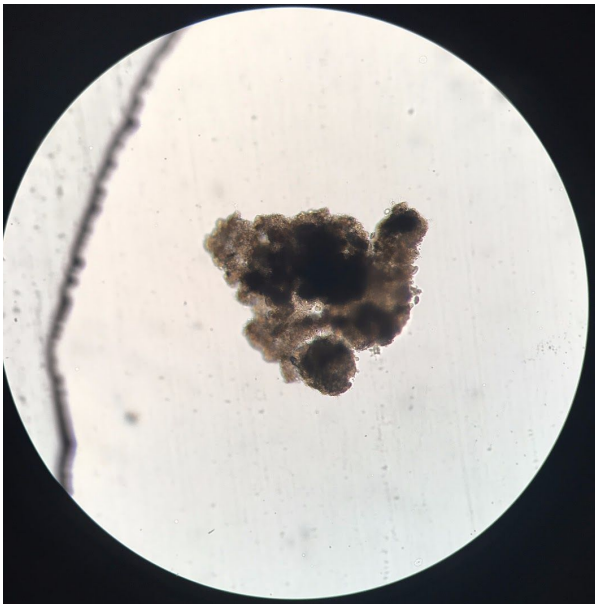

Figure 8. Shows a denatured embryo after 2 hours of 13.3 µl Methylparaben exposure 40x

In Figure 7, an unexposed embryo is pictured with 20x lens. In the picture, the sea urchin embryo is still intact however, its membrane did expand after contact and fertilization with

sperm. In contrast, Figure 8 shows a denatured embryo after 2 hours of 13.3 µl Methylparaben exposure taken with a 40x lens. The image shows the broken apart embryo, however, this is not the result of a developmental first division that sea urchin embryos undergo after 2 hours. The denaturing can be a result of the paraben exposure to the sperm.

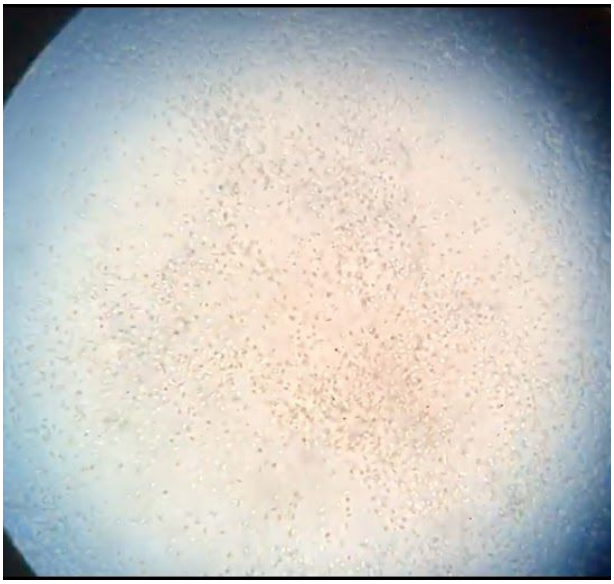

Figure 9. Shows the pre-exposed sperm 20x

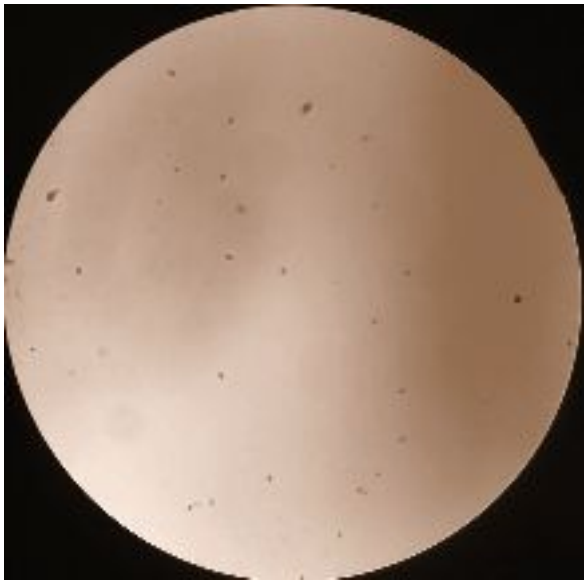

Figure 10. Shows sperm after 30 minute exposure to 50 µl Propylparaben 40x

In figure 9, sperm is shown that has not yet been exposed to parabens with 20x lens. The sample on the slide was 100 µl taken from the sperm dilution. Each grey dot seen in both figures 9 and 10 is an individual sperm. In contrast, figure 10 shows sperm that has been exposed to 50 µl Propylparaben at 40x. The sample taken was highly diluted, explaining why there is less sperm present than in the previous figure. While motility cannot be seen in this photo, the sperm began to denature and died.

Effect of Paraben Exposure on Sea Urchin Sperm

Table 10: Percentage of Dead Sperm After Propylparaben Exposure for 1 Hour

time	10 µl Propylparaben	50 µl Propylparaben	100 µl Propylparaben
0 min	0%	0%	0%
30 min	5.50%	50%	73.91%
1 hr	22.50%	62.50%	82.60%
2 hr	45%	62.50%	86.96%
24 hrs	72.50%	75%	91.30%

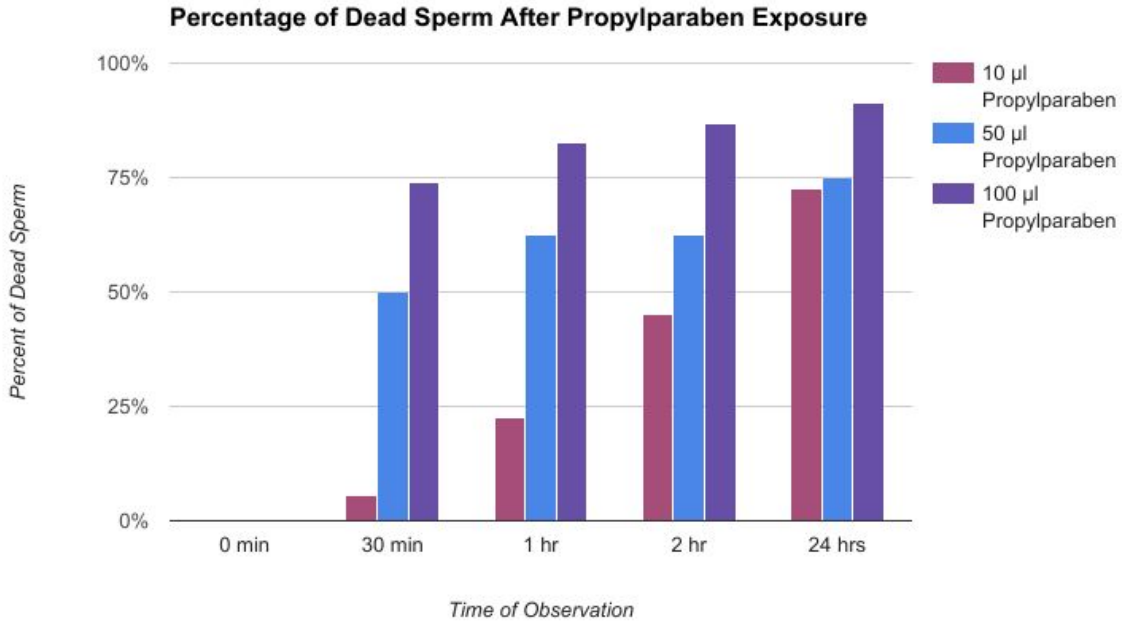

Figure 11. represents the percentage of dead sperm after Propylparaben exposure at various concentrations and time. At 0 minutes, sperm was not exposed to any amount of Propylparaben.

The percentages shown were an average of four replicates. The results in figure 11 show the increasing death of sperm exposed to 10 µl propylparaben/ 1 mL seawater, 50 µl propylparaben/ 1 mL seawater and 100 µl propylparaben/ 1 mL seawater after increasing increments of exposure time. The highest concentration after the longest amount of exposure had

the largest amount of deaths. Respectively, the smallest amount of propylparaben exposure after the shortest amount of time had the smallest effect on sperm survival. Death was analyzed by observing the motility of the sperm.

Table 11: Percentage of Dead Sperm After Methylparaben Exposure for 1 Hour

time	2.66 µl Methylparaben	13.3 µl Methylparaben	26.6 µl Methylparaben
0 min	0%	0%	0%
30 min	20%	18.51%	18.51%
1 hr	30%	29.63%	29.63%
2 hr	70%	37.04%	37.04%
24 hrs	80%	92.59%	92.59%

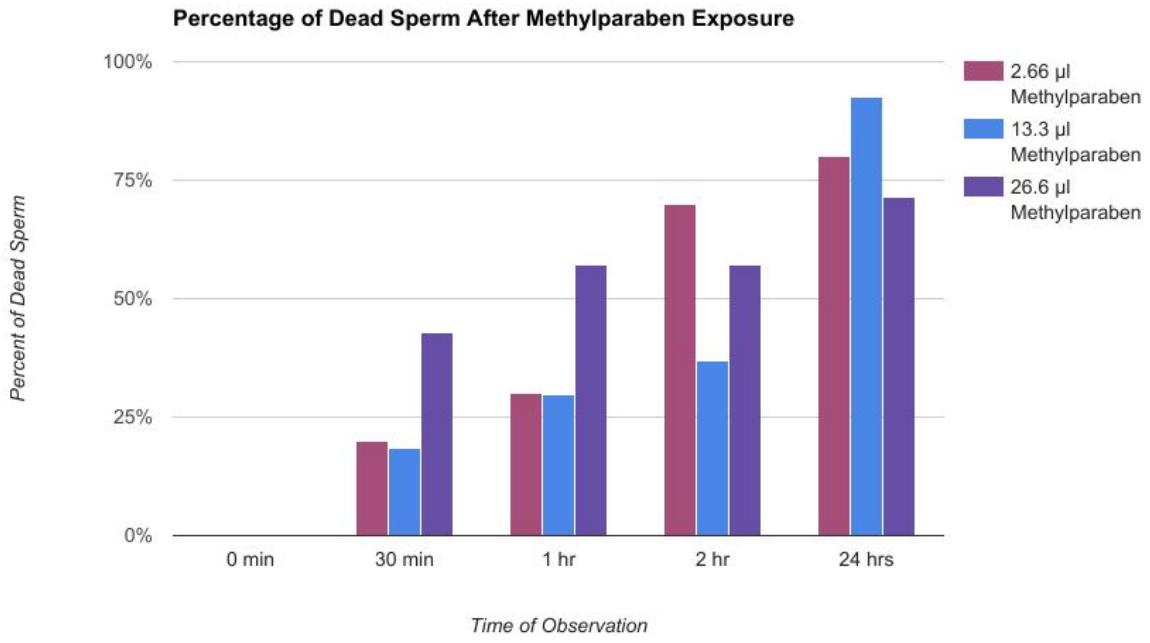

Figure 12. represents the percentage of dead sperm after Methylparaben exposure at various concentrations and time. At 0 minutes, sperm was not exposed to any amount of Methylparaben.

The results in figure 12 show the increasing death of sperm exposed to 2.66 µl methylparaben/ 1 mL seawater, 13.3 µl methylparaben/ 1 mL seawater and 26.6 µl methylparaben/ 1 mL seawater after increasing increments of exposure time. Death was analyzed by observing the motility of the sperm. Data shown was an average of four replicates and shows that the middle concentration of methylparaben at the longest exposure time had the highest percentage of deaths while the middle concentration at the shortest amount of exposure had the lowest percentage of deaths. Despite the highest percentage of deaths not belonging to the highest concentration of methylparaben, the results still show an increase in death with increasing exposure time.

Discussion:

The control group of sperm was not exposed to any amount of parabens for any amount of time. Instead gametes from the sperm and egg dilution were mixed together. Their fertilization was successful and they were able to undergo the stages of development for a sea urchin embryo. While some deaths did occur in the control group, this was not until the end of observation. In contrast, the results for the exposed embryos show that all concentrations of Methyl- and Propylparaben exposure are detrimental to the sea urchin sperm and fertility. The higher concentrations of paraben exposure had increasingly negative effect on the organisms, as opposed to the lower concentrations. Further, the 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. In fact, embryos exposed to the highest concentration of propylparaben, 100 µl propylparaben/ 1 mL seawater were 159 times more likely to die and denature than the embryos made from the control group.

In addition to their effects on developmental success, paraben exposure was also detrimental to sperm survival. This was analyzed by observing the sperm motility, whereas lack of movement indicated death. The results show an increase in death of sperm from the control group to the experimental group. At the highest concentration for propylparaben, 100 µl propylparaben/ 1 mL seawater, at the longest amount of exposure time, 24 hours, 91.3% of the sperm had died. In addition, at the highest concentration for methylparaben, 26.6 µl methylparaben/ 1 mL seawater, at the longest amount of exposure, 24 hours, 92.59% of the sperm had died. This shows that both parabens had deathly effects on the sperm.

In correspondence to the hypothesis, results prove the original proposition to be correct. However, data shows that 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:

Based on this research, it has been found that parabens can alter fertility rates when directly exposed to sea urchin gametes. While lower levels of paraben exposure had a smaller effect on fertility, higher levels made a significant difference in the success of sea urchin

fertilization. Therefore, a need for further testing on human fertility is necessary before the amount of parabens found in food and cosmetic could be limited by FDA restrictions.

Further Work:

The experiment should be extended to analyze the effects of all types of parabens on sea urchin fertility including ethyl and butyl paraben. Research can also be conducted on the differences in effect of exposing the sperm versus exposing the eggs. The effect of paraben exposure on a sea urchins reproductive organs may also be a point of interest as well. More importantly, research must be conducted on human test subjects in order to determine the effects of parabens on humans, not just sea urchins. These tests should be conducted not only on the gametes of humans but also the responsive system.

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