Toxicological Effects of Dibutyl Phthalate on the Fecundity of *Drosophila melanogaster*

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Abstract

As the field of Endocrine Toxicology grows, more and more research is being done on the effects of various endocrine disrupting chemicals (EDCs) on the health and fertility of humans. Phthalates, a plasticizer added to synthetic resins to make them more flexible, is one of the most common and well-known EDCs today. Even though Dibutyl Phthalate is one of the most common forms of phthalate in everyday products, there are very few tests done to show its effects on the health of humans, specifically its effects on the endocrine system. Because the endocrine system is so complex and works so closely with other body systems, it is very sensitive to outside factors that have the potential to disrupt its balance. Research pertaining to these effects is very important given the importance of the endocrine system in all of the normal functions of the human body. However, testing the effects of various chemicals on humans brings about a morality issue. This is why most tests of this nature are performed on some type of model. *Drosophila melanogaster* (the fruit fly) is a common model in human endocrine, toxicological, and epidemiological studies because of the similarities its genome shares with that of humans. This study aims to aid in the research and understanding of how Dibutyl Phthalate (DBP) potentially affects the fertility of humans by observing how it affects the fertility of *D. melanogaster.* The results show that exposure to the female only and the male only significantly affects the number of eggs laid by the fruit fly. However, exposure to both parents does not significantly affect the number of eggs laid.

Introduction

Research in the field of Endocrine Toxicology has become more prevalent in recent decades. According to the U.S. Environmental Protection Agency (EPA), an EDC is "an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process" [7].

Endocrine disruptors are found throughout the environment. Some of the most common of these chemicals are found in plastics (bisphenol A [BPA] and phthalates), pesticides (DDT), and cosmetics (phthalates, parabens, and fragrances). Some chemicals in certain foods are considered EDCs because of their similar structure to estrogen and their ability to mimic the hormone within the body. These substances are called phytoestrogens. The most common phytoestrogen is isoflavone which is found in soy [9].

The endocrine system consists of various glands throughout the body that secrete hormones, as well as receptors that are located in various organs that recognize and respond to these hormones. Hormones act as chemical messengers throughout the body and perform many functions such as controlling blood sugar levels, regulating growth and development, producing energy, and regulating the function and growth of male and female reproductive organs. The endocrine system is important in development and survival because it regulates almost all of the body's different processes from the time of conception to the time of death. Many of the organs that produce and secrete hormones are controlled by other hormones. Therefore, slight changes in the level of one hormone can have impacts on the endocrine system as a whole. Because of this, the endocrine system is very sensitive to outside factors and chemicals [7].

The effects of endocrine disruptors on humans varies widely among the different types of chemicals and among different people. One of the biggest causes of varying effects is age at the time of exposure. An EDC will have a greater impact on an organism during its developmental stages than when that organism is physically mature. For example, during their fetal stages, mammals are very sensitive to external agents that have the capability of causing malformation, inhibiting or interfering with proper development, causing disease, or creating long-term effects. This phenomenon has been given the name "fetal basis of adult disease" which describes the effects of the environment of a developing organism on their future health and propensity for disease in adulthood [2].

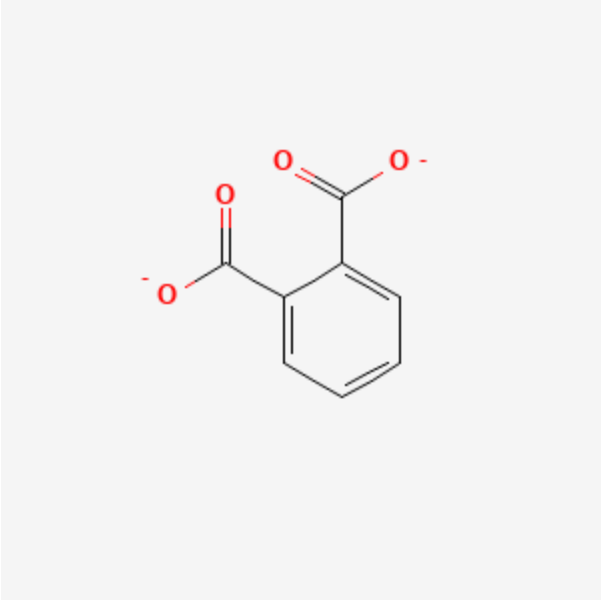

Figure 1. The two-dimensional structure of the Phthalate(2-) compound ($C_8H_4O_4$). It has a molecular weight of 164.11 g/mol and a formal charge of -2. Other common names of the compound include benzene-1,2-dicarboxylate and 1,2-Benzenecarboxylic acid. It is created by the deprotonation of both carboxy groups of phthalic acid [13].

Phthalates are one of the most common classes of endocrine disrupting chemicals. Phthalates are plasticizers that are added to synthetic resins to increase flexibility in plastic materials. Their structures can vary depending on how many side chains they possess and on the lengths of those side chains. They are separated into two groups based on their molecular weight:

high-molecular-weight phthalates (also called long-chain phthalates) and low-molecular-weight phthalates (also called short-chain phthalates). High-molecular-weight phthalates have an ester side-chain with five or more carbons and low-molecular-weight phthalates have an ester side-chain with one to four carbons. Some high molecular weight phthalates include Di(2-ethylhexyl) phthalate (DEHP), Di-isononyl phthalate (DINP), and Dioctyl phthalate (DOP) which are used in plastic tubing, food packaging and processing materials, vinyl toys, vinyl floor coverings, and building materials. Some low molecular weight phthalates include Dimethyl phthalate (DMP), Diethyl phthalate (DEP), and Dibutyl phthalate (DBP) which are used in personal-hygiene products and cosmetics [14].

Since phthalates are used ubiquitously in plastics, humans are likely exposed to some amount of phthalates on a daily basis. "Global plastic use consumes more than 3 million tons of phthalates per year… In the USA, more than 340 million pounds of phthalates are consumed every year and cause potential health and environmental risks" [16]. Phthalates have a relatively short half-life in the human body of approximately 12 hours, so their bio-metabolism is very rapid. After absorption into the cells, the chemical is hydrolyzed and then conjugated to form the water-soluble glucuronide. The effects the glucuronide conjugate causes on the body before it is excreted are determined by the type of phthalate. Low molecular weight phthalates are generally hydrolyzed to form monoester phthalates and are then excreted via urine, while high molecular weight phthalates usually undergo a number of bio-transformations before they are excreted via urine as phase two conjugates [16]. Studies have shown that low molecular weight phthalates can cause irritations to the skin, conjunctiva, and mucous membranes of the oral and nasal cavities, and exposure to high molecular weight phthalates is found to cause methylation of imprinted genes, which can be directly related to androgen and estrogen responses, protein secretion, and

spermatogenesis in humans [15]. Human epidemiological studies have found an association between phthalate exposure and adverse reproductive outcomes like Type II Diabetes and insulin resistance, obesity, allergies, and asthma [15]. Many epidemiological studies have focused on the effects of phthalates on pregnancy outcomes as well as reproductive health and success. Through these studies, it has been shown that phthalate exposure is associated with adverse effects on reproductive hormone levels, anogenital distance, and thyroid function [10], and phthalates have been found to be linked to social impairment of children [3].

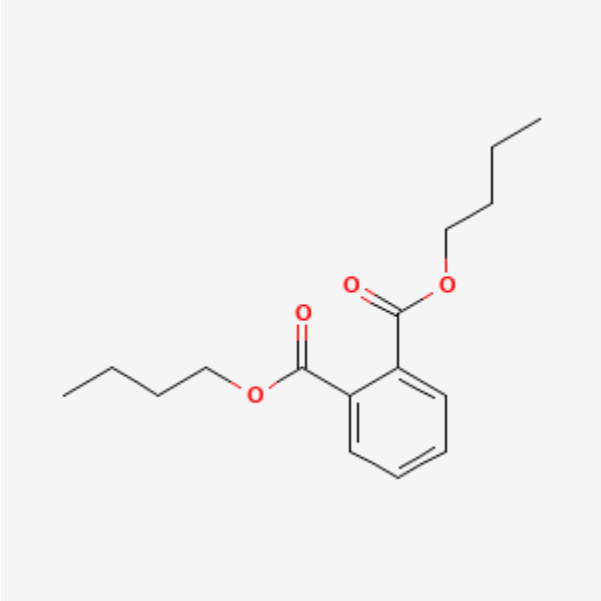

Figure 2. The two-dimensional structure of the Dibutyl Phthalate compound ($C_{16}H_{22}O_4$). It has a molecular weight of 278.34 g/mol and a formal charge of 0. Other common names of the compound include Di-n-butyl phthalate and Butyl phthalate. It is created by the condensation of the carboxy groups of phthalic acid with two molecules of butan-1-ol [12].

Even though dibutyl phthalate (DBP) is one of the most common phthalates found in the environment, there seem to be very few studies done on how it affects human health compared to other forms of the compound. According to PubChem, no information is available on the effects of DBP on humans from inhalation or oral exposure, and no studies are available on the reproductive, developmental, or carcinogenic effects of the chemical on humans [12]. It is a low

molecular weight phthalate diester formed through the condensation of the carboxyl groups of phthalic acid with two molecules of butan-1-ol. DBP is odorless and may be colorless or faint yellow depending on purity. Its chemical formula is $C_{16}H_{22}O_4$ and its molecular weight is 278.35 g/mol. It is found in food (particularly fish and seafood), the air, drinking water, perfumes, garden hoses, gloves, shoes, and nail polishes [5, 8].

Drosophila melanogaster (the fruit fly) has been a helpful model for studying various human diseases and human genetics over the years because of their easy maintenance, early maturity, fast reproduction, and high fecundity. Up to 75% of genes that play a role in human disease have a parallel in the fruit fly genome, and there are also numerous paralogues throughout the human genome that correspond to single genes in the fruit fly genome making them a good model organism for comparison studies across the two species [4].

Several studies have been conducted in regards to the effect of phthalates and other EDCs on the fruit fly.. In one study conducted by Liu, et al., it was found that exposure to DBP throughout development caused a significant change in the transcript number of 171 genes in adult male fruit flies, and it was confirmed through behavioral assays that exposure caused disruptions in their circadian rhythm. [11]. According to a study conducted by Cao, et al., exposure to low concentrations of di-(2-ethylhexyl) phthalate (DEHP) results in changing circulating carbohydrate levels, total lipid content, and metabolic gene expression in fruit fly males [6]. Several other studies were done in which the effects of known endocrine disruptors on the fruit fly were studied, but none of them were done with phthalates. One study showed that exposure to Bisphenol A, 4-nonylphenol, and 4-*tert*-octylphenol resulted in a reduction in the number of eggs produced by females [1].

Materials and Methods

The Oregon R (OR) wild type strain of fruit flies was used in the experiments conducted in this study. Dibutyl phthalate (DBP) obtained from Sigma-Aldrich was used in the study. There were four treatment groups within the experiment with various mating pairs regarding parental exposure to DBP.

Control Group

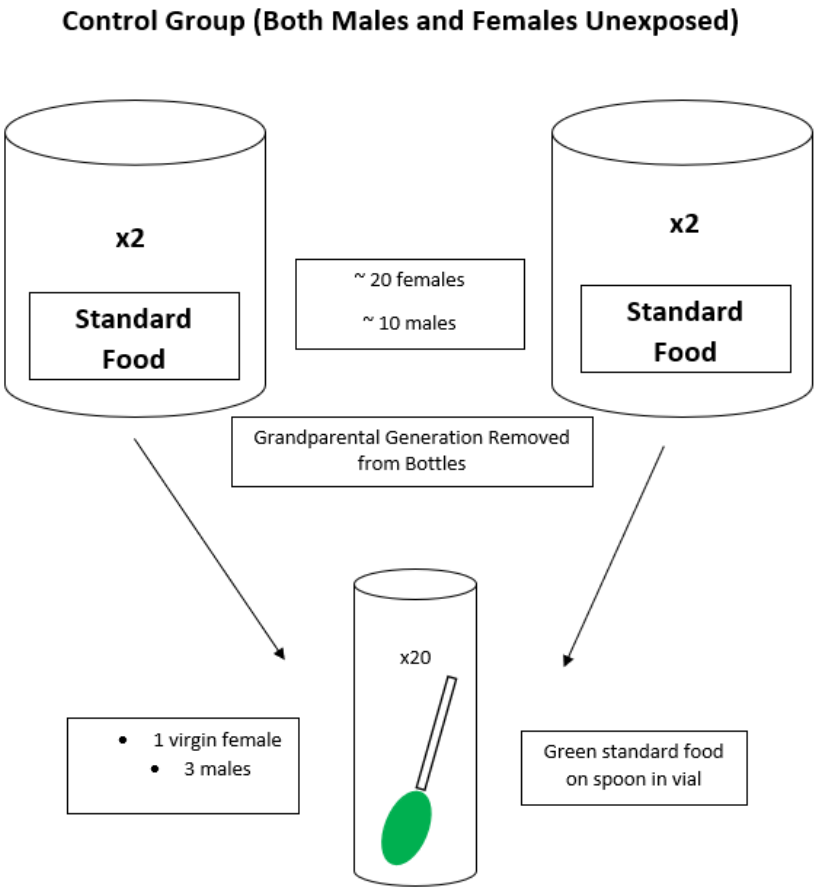

Figure 3. Four bottles were started with the grandparental generation, each containing standard food. Once the larvae (the parental generation) developed, the grandparental generation was removed from the bottles. Approximately seven days later when the parental generation larvae had grown into mature adults and their sexes were distinguishable, three males and one virgin female were placed into twenty vials with spoons containing green standard food. The eggs laid in these vials were counted every twenty-four hours for six days, and the spoons were replaced.

The first treatment group of the experiment was the control group. In this group, both males and females used were unexposed to DBP. Four bottles of OR Strain *D. melanogaster* were started containing approximately 20 females and 10 males. These bottles all contained standard food (Table 1).

Water (mL)	850
Agar (g)	4.5
Yeast 500 (g)	15.5
D-(+)-Glucose (g)	51.6
Sucrose (g)	25.8
Cornmeal (g)	85.8
Acid A Mix (mL)	10
Tegosept (mL)	7

Table 1. Ingredients of standard fly food.

After mating occurred and larvae developed, the original adult flies were removed from the bottles to allow the larvae adequate space and food, and to ensure a clear distinction between the original grandparental adult flies and the new parental adult progeny. When the progeny became mature and their sexes were distinguishable, virgin females and males were separated out. One virgin female and three males were put into twenty vials. Within each vial was a plastic spoon with approximately 5g of green standard food (Table 2).

Water (mL)	425
Agar (g)	2.25
Yeast 500 (g)	7.75
D-(+)-Glucose (g)	25.8
Sucrose (g)	12.9
Cornmeal (g)	42.9
Acid A Mix (mL)	5
Tegosept (mL)	3.5
Blue Food Coloring (mL)	0.25

Table 2. Ingredients for green standard food.

Adding a small amount of blue food coloring to the food created more contrast between the normally-yellow food and the eggs, making it easier to count accurately. Every 24 hours for six days the spoon was removed and replaced with another spoon containing green standard food and the eggs laid were counted and recorded.

Test Group #1

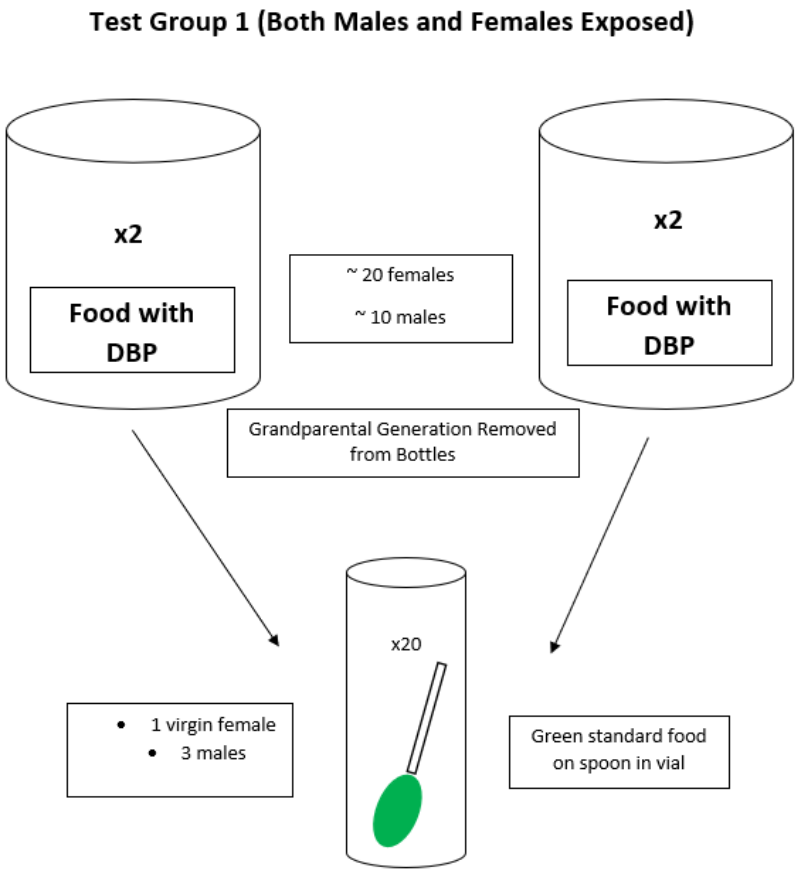

Figure 4. Four bottles were started with the grandparental generation, each containing contaminated food. Once the larvae (the parental generation) developed, the grandparental generation was removed from the bottles. Approximately seven days later when the parental generation larvae had grown into mature adults and their sexes were distinguishable, three males and one virgin female were placed into twenty vials with spoons containing green standard food. The eggs laid in these vials were counted every twenty-four hours for six days, and the spoons were replaced.

The second treatment group was Test Group 1. In this group, both males and females were exposed to DBP during development. Four bottles of OR Strain *D. melanogaster* were started containing approximately 20 females and 10 males. These bottles all contained food contaminated with 10 mg/L of Dibutyl Phthalate (Table 3).

Water (mL)	850
Agar (g)	4.5
Yeast 500 (g)	15.5
D-(+)-Glucose (g)	51.6
Sucrose (g)	25.8
Cornmeal (g)	85.8
Acid A Mix (mL)	10
Tegosept (mL)	7
Dibutyl Phthalate (mg/L)	10

Table 3. Ingredients for contaminated food.

After mating occurred and larvae developed, the original adult flies were removed from the bottles to allow the larvae adequate space and food, and to ensure a clear distinction between the original grandparental adult flies and the new parental adult progeny. When the progeny became mature and their sexes were distinguishable, virgin females and males were separated out. One virgin female and three males were put into twenty vials. Within each vial was a plastic spoon with approximately 5g of green standard food (Table 2). Every 24 hours for six days the spoon was removed and replaced with another spoon containing green standard food and the eggs laid were counted and recorded.

Test Group #2

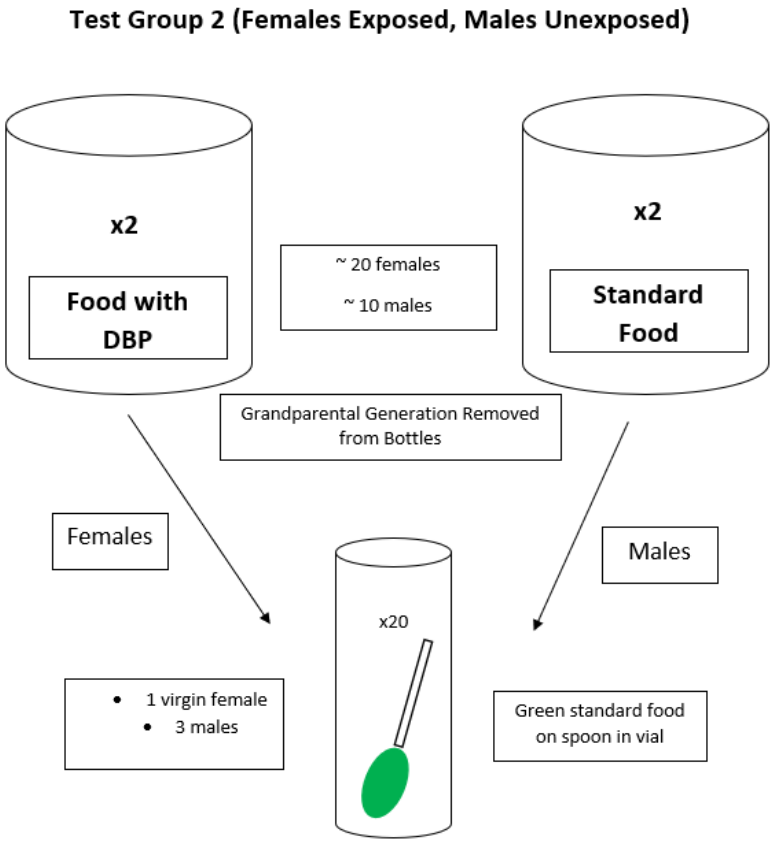

Figure 5. Four bottles were started with the grandparental generation, two containing standard food and two containing contaminated food. Once the larvae (the parental generation) developed, the grandparental generation was removed from the bottles. Approximately seven days later when the parental generation larvae had grown into mature adults and their sexes were distinguishable, three males and one virgin female were placed into twenty vials with spoons containing green standard food. The males were taken from bottles containing normal food and the females were taken from bottles containing contaminated food. The eggs laid in these vials were counted every twenty-four hours for six days, and the spoons were replaced.

The third treatment group was Test Group 2. In this group, females were exposed and males were unexposed to DBP during development. Four bottles of OR Strain *D. melanogaster* were started containing approximately 20 females and 10 males. Two of these bottles contained the contaminated food (Table 3) and two bottles contained the standard food (Table 1). After mating occurred and larvae developed, the original adult flies were removed from the bottles to allow the larvae adequate space and food, and to ensure a clear distinction between the original

grandparental adult flies and the new parental adult progeny. When the progeny became mature and their sexes were distinguishable, virgin females and males were separated out. Virgin females were taken from the two bottles containing contaminated food and males were taken from the two bottles containing standard food. One virgin female and three males were put into twenty vials. Within each vial was a plastic spoon with approximately 5g of green standard food (Table 2). Every 24 hours for six days the spoon was removed and replaced with another spoon containing green standard food and the eggs laid were counted and recorded.

Test Group #3

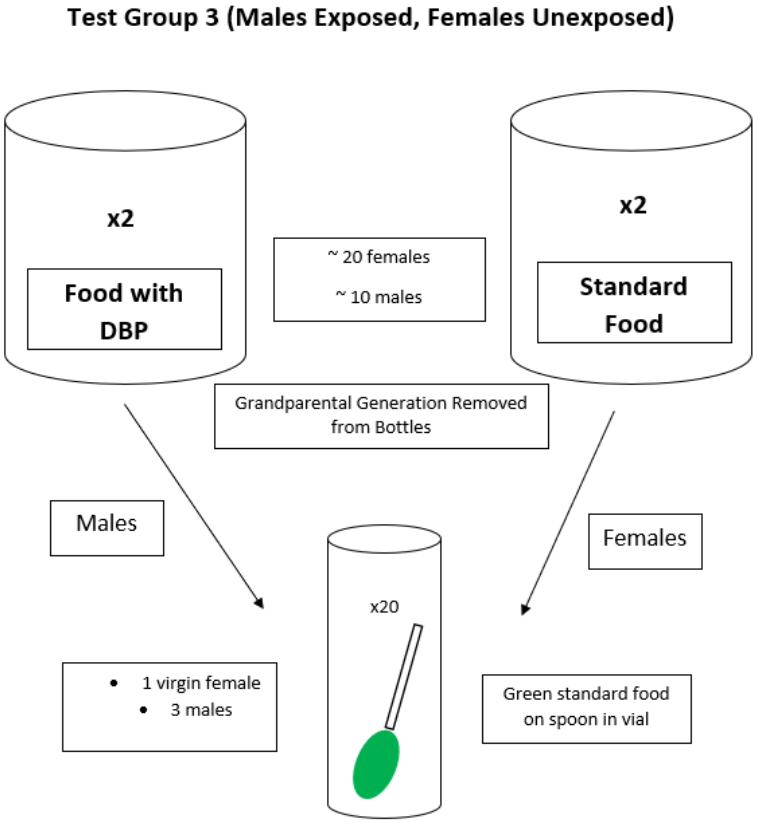

Figure 6. Four bottles were started with the grandparental generation, two containing standard food and two containing contaminated food. Once the larvae (the parental generation) developed, the grandparental generation was removed from the bottles. Approximately seven days later when the parental generation larvae had grown into mature adults and their sexes were distinguishable, three males and one virgin female were placed into twenty vials with spoons containing green standard food. The males were taken from bottles containing contaminated food and the females were taken from bottles containing standard food. The eggs laid in these vials were counted every twenty-four hours for six days, and the spoons were replaced.

The fourth treatment group was Test Group 3. In this group, the males used were exposed to DBP during development and the females used were unexposed. Four bottles of OR Strain *D. melanogaster* were started containing approximately 20 females and 10 males. Two of these bottles contained the contaminated food (Table 3) and two bottles contained standard food (Table 1). After mating occurred and larvae developed, the original adult flies were removed from the bottles to allow the larvae adequate space and food, and to ensure a clear distinction between the original grandparental adult flies and the new parental adult progeny. When the progeny became mature and their sexes were distinguishable, virgin females and males were separated out. The virgin females were taken from the two bottles containing standard food and the males were taken from the two bottles containing contaminated food. One virgin female and three males were put into twenty vials. Within each vial was a plastic spoon with approximately 5g of green standard food (Table 2). Every 24 hours for six days the spoon was removed and replaced with another spoon containing green standard food and the eggs laid were counted and recorded.

Results

Test Group 1

Eggs in all twenty vials were counted every twenty-four hours for each part of the experiment. The twenty vials were set with one exposed female and three exposed males and the spoon with food was switched out and the eggs laid on it were counted every twenty four hours.

This process was repeated for six days. Once the six days of mating were completed, the data (Table 4 and Table 5) were analyzed with a Two-Way ANOVA with Replication. This test was performed because there were two groups with various sublevels that needed to be analyzed. The two groups were the Control and Test Group 1, and the sublevels were the twenty vials within each group. Days labeled with "N/A" in the data tables (Table 4 and Table 5) represent a death of the female within that vial. These days were counted as a "0" in the ANOVA test since zero eggs were laid. The results of this test (Table 6) showed that the difference between the number of eggs laid between the control group and the exposed group was not significantly different, revealing that exposure to DBP does not affect the number of eggs laid by fruit flies, at least at the concentration level of 10 mg/L achieved in this experiment.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Vial 1	0	18	34	63	79	N/A
Vial 2	0	12	21	12	55	25
Vial 3	0	20	36	64	67	51
Vial 4	0	0	N/A	N/A	N/A	N/A
Vial 5	0	21	25	34	22	N/A
Vial 6	0	25	31	24	28	35
Vial 7	0	27	31	40	30	8
Vial 8	0	13	12	37	78	29
Vial 9	0	9	29	20	45	24
Vial 10	0	19	N/A	N/A	N/A	N/A
Vial 11	0	0	0	0	11	N/A
Vial 12	0	0	0	N/A	N/A	N/A
Vial 13	0	0	0	N/A	N/A	N/A
Vial 14	0	0	N/A	N/A	N/A	N/A
Vial 15	0	10	20	36	58	51
Vial 16	0	20	50	38	42	0
Vial 17	2	30	68	39	69	59
Vial 18	3	74	76	54	6	23
Vial 19	7	20	9	18	18	43
Vial 20	21	48	43	78	95	6

Table 4. Data from the Control Group in which males and females were unexposed. Numbers in the table are the number of eggs laid within the corresponding twenty-four hour period. Days labeled with “N/A” represent a death of the female within that vial.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Vial 1	0	0	14	0	0	N/A
Vial 2	0	0	N/A	N/A	N/A	N/A
Vial 3	17	13	81	25	0	0
Vial 4	0	0	0	0	N/A	N/A
Vial 5	0	0	0	0	N/A	N/A
Vial 6	8	37	96	30	N/A	N/A
Vial 7	19	13	18	3	2	N/A
Vial 8	11	24	48	30	N/A	N/A
Vial 9	38	23	81	96	90	56
Vial 10	0	0	1	0	N/A	N/A
Vial 11	32	50	0	0	N/A	N/A
Vial 12	2	23	34	32	N/A	N/A
Vial 13	6	56	46	67	50	29
Vial 14	27	45	66	22	2	0
Vial 15	35	38	81	63	1	0
Vial 16	7	1	0	0	N/A	N/A
Vial 17	25	35	70	77	63	29
Vial 18	4	1	0	0	N/A	N/A
Vial 19	20	1	55	0	N/A	N/A
Vial 20	38	25	82	55	N/A	N/A

Table 5. Data from Test Group 1 in which both males and females were exposed. Numbers in the table are the number of eggs laid within the corresponding twenty-four hour period. Days labeled with “N/A” represent a death of the female within that vial.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	297.04	1	297.038	0.57	0.45	3.89
Columns	15303.27	5	3060.65	5.9	0.000038	2.25
Interaction	11232.64	5	2246.53	4.33	0.00087	2.25
Within	118231.35	228	518.56			
Total	145064.3	239				

Table 6. Results from the Two-Way ANOVA test with Replication. A p-value less than 0.05 represents a statistically significant difference between the two groups analyzed within an ANOVA test. The p-value of the test was 0.45. This shows that there is no statistically significant difference between the number of eggs laid by the Control Group flies and the Test Group 1 flies.

Test Group 2

Eggs in all twenty vials were counted every twenty-four hours for five days. A six day duration identical to that of the first round of tests was the plan, however with a lack of available time, the duration of the test had to be cut back one day. The twenty vials were set with one exposed female and three unexposed males, and the spoon with food was switched out and the eggs laid on it were counted every twenty-four hours. Once the five days of mating were completed, the data from Test Group 2 (Table 7) and the Control Group (Table 5) were analyzed with a Two-Way ANOVA with Replication. Days labeled with "N/A" in the data tables (Table 5 and Table 7) represent a death of the female within that vial. These days were counted as a "0" in the ANOVA test since zero eggs were laid. The results of this test (Table 8) showed that the difference between the number of eggs laid between the two groups was statistically significant. This result reveals that female exposure to DBP significantly affects the number of eggs laid by fruit flies.

	Day 1	Day 2	Day 3	Day 4	Day 5
Vial 1	0	N/A	N/A	N/A	N/A
Vial 2	0	2	0	0	50
Vial 3	0	N/A	N/A	N/A	N/A
Vial 4	3	0	15	20	21
Vial 5	11	0	N/A	N/A	N/A
Vial 6	6	1	30	49	51
Vial 7	0	4	27	32	41
Vial 8	2	1	N/A	N/A	N/A
Vial 9	8	2	84	58	60
Vial 10	4	0	N/A	N/A	N/A
Vial 11	15	0	12	15	26
Vial 12	0	6	N/A	N/A	N/A
Vial 13	0	0	8	0	1
Vial 14	10	1	14	0	0
Vial 15	4	5	12	70	26
Vial 16	0	3	1	31	0
Vial 17	3	2	24	64	59
Vial 18	0	3	21	39	0
Vial 19	0	0	N/A	N/A	N/A
Vial 20	0	0	2	0	0

Table 7. Data from Test Group 2 in which females were exposed and males were unexposed to DBP. Numbers in the table are the number of eggs laid within the corresponding twenty-four hour period. Days labeled with "N/A" represent a death of the female within that vial.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	5886.13	1	5886.13	14.68	0.00017	3.89
Columns	15166.73	4	3791.68	9.46	5.43E-07	2.42
Interaction	2530.75	4	632.69	1.58	0.18	2.42
Within	76193.35	190	401.02			
Total	99776.96	199				

Table 8. Results from the Two-Way ANOVA test with Replication. A p-value less than 0.05 represents a statistically significant difference between the two groups analyzed within an ANOVA test. The p-value of the test was 1.73×10^{-4}. This shows that there is a statistically significant difference between the number of eggs laid by the Control Group flies and the Test Group 2 flies.

Test Group 3

Eggs in all twenty vials were counted every twenty-four hours for five days. The twenty vials were set with one unexposed female and three exposed males, the spoon with food was switched out and the eggs laid on it were counted every twenty-four hours. Once the five days of mating were completed, the data from Test Group 2 (Table 9) and the Control Group (Table 5) were analyzed with a Two-Way ANOVA with Replication. Days labeled with "N/A" in the data tables (Table 5 and Table 9) represent a death of the female within that vial. These days were counted as a "0" in the ANOVA test since zero eggs were laid. The results of this test (Table 10) showed that the difference between the number of eggs laid between the two groups was statistically significant. This result reveals that male exposure to DBP significantly affects the number of eggs laid by fruit flies.

Vial 1	0	0	N/A	N/A	N/A
Vial 2	0	4	3	15	0
Vial 3	1	0	N/A	N/A	N/A
Vial 4	0	0	N/A	N/A	N/A
Vial 5	0	4	15	32	0
Vial 6	0	5	42	3	55
Vial 7	1	7	46	17	0
Vial 8	3	4	38	10	31
Vial 9	0	4	20	50	52
Vial 10	1	15	28	57	37
Vial 11	0	9	48	38	0
Vial 12	0	4	N/A	N/A	N/A
Vial 13	10	6	0	N/A	N/A
Vial 14	4	4	0	N/A	N/A
Vial 15	2	4	N/A	N/A	N/A
Vial 16	0	N/A	N/A	N/A	N/A
Vial 17	0	0	N/A	N/A	N/A
Vial 18	4	7	13	16	43
Vial 19	7	4	22	18	0
Vial 20	2	0	0	5	0

Table 9. Data from Test Group 3 in which males were exposed and females were unexposed to DBP. Numbers in the table are the number of eggs laid within the corresponding twenty-four hour period. Days labeled with “N/A” represent a death of the female within that vial.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	7750.12	1	7750.12	22.09	4.98E-06	3.89
Columns	12542.18	4	3135.54	8.94	1.24E-06	2.42
Interaction	2771.9	4	692.98	1.98	0.1	2.42
Within	66657.55	190	350.83			
Total	89721.75	199				

Table 10. Results from the Two-Way ANOVA test with Replication. A p-value less than 0.05 represents a statistically significant difference between the two groups analyzed within an ANOVA test. The p-value of the test was 4.98×10^{-6}. This shows that there is a statistically significant difference between the number of eggs laid by the Control Group flies and the Test Group 3 flies.

Discussion

The results of the first treatment group with Test Group 1 were surprising since all other published journal articles with similar studies had results that showed a significant effect of the EDC on the fruit fly [1, 6, 11]. Further studies could be done in a similar and more extensive manner to provide new results that could be compared to the results of this study. These studies could have a longer duration to provide more data over a longer time span, a greater concentration and/or various concentrations of DBP within the food, or a greater number of samples. I did notice two things throughout the first part of the experiment that could provide areas for further research. The first was that the flies in Test Group 1 generally produced more eggs earlier on within the six days than the Control Group. Their peak day with the greatest amount of eggs was on day 3, whereas the peak day for the Control Group was on day 5. Further studies could be done to determine whether or not exposure to DBP significantly affects maturation rates and/or life cycles of *Drosophila melanogaster*. The second thing I noticed was that more females died within Test Group 1 than within the Control Group. Fourteen flies died in Test Group 1, whereas only 10 died in the Control Group. Further tests could be done to see how

exposure to DBP affects death rates of *Drosophila melanogaster*. This observation could simply be attributed to chance though since it was such a small difference between the two groups. If a further study was done on this question, a greater sample number would be helpful to provide more data in order to be more accurate in the analysis.

It is also surprising that there is a significant effect on fecundity when only one sex is exposed and not when both sexes had been exposed. Again, further studies could be done to determine why this is the case. Assays observing mating behaviors could determine if one sex having been exposed to DBP and not the other affects their ability or desire to mate with one another. Performing tests to determine whether or not exposure to DBP affects maturation rates in flies could also provide a possible explanation for the significant results in Test Groups 2 and 3. If exposure to DBP causes the flies to mature at a faster rate than the flies raised on standard food, then the exposed and unexposed flies may not be able to mate with each other as well as if both had been either exposed or unexposed.

A second round of ANOVA tests were conducted on each set of data with only sixteen of the vials rather than twenty. The four that were not included were the four with the most female deaths to observe how the removal of these vials affected the results. Similar results were found and are recorded in the appendix.

Appendix

Test Group 1

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Control	0	18	34	63	79	0
Control	0	12	21	12	55	25
Control	0	20	36	64	67	51
Control	0	25	31	24	28	35
Control	0	27	31	40	30	8
Control	0	13	12	37	78	29
Control	0	9	29	20	45	24
Control	0	19	0	0	0	0
Control	0	0	0	0	11	0
Control	0	10	20	36	58	51
Control	7	20	9	18	18	43
Control	21	48	43	78	95	6
Test 1	0	0	14	0	0	0
Test 1	17	13	81	25	0	0
Test 1	8	37	96	30	0	0
Test 1	19	13	18	3	2	0
Test 1	11	24	48	30		0
Test 1	32	50	0	0	0	0
Test 1	27	45	66	22	2	0
Test 1	35	38	81	63	1	0
Test 1	7	1	3	15	0	0
Test 1	25	35	70	77	63	29
Test 1	20	1	55	0	0	0
Test 1	38	25	82	55	0	0

Table 11. The data used for Test Group 1 for the second ANOVA test with only 16 data sets per sample.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	374.08	1	374.08	0.76	0.38	3.89
Columns	19099.5	5	3819.9	7.78	0.0000012	2.26
Interaction	14019.85	5	2803.97	5.71	0.000065	2.26
Within	88382.88	180	491.02			
Total	121876.313	191				

Table 12. The results of the second ANOVA test for Test Group 1. The p-value was still greater than 0.05 as it was in the first ANOVA test. This test gave the same results of no significant difference between the number of eggs laid between the Control and Test Group 1.

Test Group 2

Test	Day 1	Day 2	Day 3	Day 4	Day 5
Test 2	0	2	0	0	50
Test 2	3	0	15	20	21
Test 2	11	0	0	0	0
Test 2	6	1	30	49	51
Test 2	0	4	27	32	41
Test 2	2	1	0	0	0
Test 2	8	2	84	58	60
Test 2	4	0	0	0	0
Test 2	15	0	12	15	26
Test 2	0	6	0	0	0
Test 2	0	0	8	0	1
Test 2	10	1	14	0	0
Test 2	4	5	12	70	26
Test 2	0	3	1	31	0
Test 2	3	2	24	64	59
Test 2	0	3	21	39	0
Control	0	18	34	63	79
Control	0	12	21	12	55
Control	0	20	36	64	67
Control	0	21	25	34	22
Control	0	25	31	24	28
Control	0	27	31	40	30
Control	0	13	12	37	78
Control	0	9	29	20	45
Control	0	19	0	0	0
Control	0	0	0	0	11
Control	0	10	20	36	58
Control	0	20	50	38	42
Control	2	30	68	39	69
Control	3	74	76	54	6
Control	7	20	9	18	18
Control	21	48	43	78	95

Table 13. The data used for Test Group 2 for the second ANOVA test with only 16 data sets per sample.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	7384.81	1	7384.81	19.25	0.000021	3.9
Columns	18946.71	4	4736.68	12.35	1.05E-08	2.43
Interaction	3165.79	4	791.45	2.064	0.088	2.43
Within	57531.69	150	383.54			
Total	87028.99	159				

Table 14. The results of the second ANOVA test for Test Group 2. The p-value was still greater than 0.05 as it was in the first ANOVA test. This test gave the same results of a significant difference between the number of eggs laid between the Control and Test Group 2.

Test Group 3

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
Control	0	18	34	63	79
Control	0	12	21	12	55
Control	0	20	36	64	67
Control	0	21	25	34	22
Control	0	25	31	24	28
Control	0	27	31	40	30
Control	0	13	12	37	78
Control	0	9	29	20	45
Control	0	19	0	0	0
Control	0	0	0	0	11
Control	0	10	20	36	58
Control	0	20	50	38	42
Control	2	30	68	39	69
Control	3	74	76	54	6
Control	7	20	9	18	18
Control	21	48	43	78	95
Test 3	0	4	3	15	0
Test 3	1	0	0	0	0
Test 3	0	4	15	32	0
Test 3	0	5	42	3	0
Test 3	1	7	46	17	55
Test 3	3	4	38	10	0
Test 3	0	4	20	50	31
Test 3	1	15	28	57	52
Test 3	0	9	48	38	37
Test 3	0	4	0	0	0
Test 3	10	6	0	0	0
Test 3	4	4	0	0	0
Test 3	2	4	0	0	0
Test 3	4	7	13	16	29
Test 3	7	4	22	18	43
Test 3	2	0	0	5	0

Table 15. The data used for Test Group 2 for the second ANOVA test with only 16 data sets per sample.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	9687.66	1	9687.66	29.47	2.24E-07	3.9
Columns	15677.72	4	3919.43	11.92	1.95E-08	2.43
Interaction	3464.88	4	866.22	2.63	0.036	2.43
Within	49316.69	150	328.78			
Total	78146.94	159				

Table 16. The results of the second ANOVA test for Test Group 3. The p-value was still greater than 0.05 as it was in the first ANOVA test. This test gave the same results of a significant difference between the number of eggs laid between the Control and Test Group 3.

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