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Connections Between the Effects of Various Chemicals on the Development of Drosophila melanogaster and Homo sapiens

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CONNECTIONS BETWEEN THE EFFECTS OF VARIOUS CHEMICALS ON THE DEVELOPMENT OF *DROSOPHILA MELANOGASTER* AND *HOMO SAPIENS*

By

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ABSTRACT

This study, funded by the Elbert Pence and Fanny Boyce grant, attempts to draw conclusions between the effects of selected chemicals on *Drosophila melanogaster* and their potential effects on humans. It explores the effects of theobromine, caffeine, ethylene glycol, and ammonia on adult *D. melanogaster* and its developmental stages. Effects of these four chemicals on *D. melanogaster* are expected to provide insight into possible effects on humans.

The study was run in triplicate with vials containing different concentrations of each chemical being tested, with a control group vial containing no added chemicals. Observation of each vial was documented daily, noting the progression of each developmental stage. A chi-square test was completed comparing the four chemicals to their overall toxicity, and to overall viability of the F_1 generation. Correlations were analyzed for overall toxicity of each chemical and F_1 viability. A one-way between subjects analysis of variance (ANOVA) was used to analyze the effects of each chemical on *D. melanogaster* development. A Tukey post hoc procedure was implemented to determine which of the four chemicals had a significant effect on the number of days in each developmental stage. Correlations were made between concentration of all chemicals combined and their overall effect on developmental stages, along with each individual chemical's effect.

Inferences were formed based on the results of this study and of previous studies. Further research is necessary to reach a more definite conclusion about the effects of various chemicals on the development of *D. melanogaster* and the connection to humans.

Keywords: chemicals, development, *D. melanogaster*

INTRODUCTION

Previous studies on *Drosophila melanogaster* show susceptibility to chemicals that have specific effects on their lifespan and development (Devineni and Heberlein, 2013; Ho, Y.K., Koehn, D.J., Sobieski, R.J., Clifford, A.J., Clifford, C.K., 1984; Matsagas et al., 2009; Petersen, 1990). Few of these studies have connected the results from research on *D. melanogaster* to humans. Inferences have been drawn based on the work of Mumford et al. (1994), Smith (2002), Nawrot et al. (2003), Jacobsen and McMartin (1986), and Visek (1984), with continued work on questions raised by the Matsagas et al. study in 2009.

This project, funded by a grant from Elbert Pence and Fanny Boyce, consisted of testing the effects of four chemicals on adult *D. melanogaster* and the developmental stages. The chemicals that were chosen are readily available to both humans and *D. melanogaster.* It was hypothesized that chemicals already known to have adverse effects on humans would also have adverse effects on *D. melanogaster*. Theobromine was expected to have no significant effect on reproduction or development (Matsagas et al., 2009); caffeine was expected to increase biological function and reproduction rate (Matsagas et al., 2009); ethylene glycol was expected to be mildly toxic (Tyler, 1984); and ammonia was expected to be highly toxic to all stages of development (Roney et al., 2004).

The effects of these four chemicals on *D. melanogaster* were expected to provide insight into possible effects of these chemicals on humans and their development. Human subjects were not actually tested in this study; only inferences based on literary research and experimental results from *D. melanogaster* will be discussed.

REVIEW OF LITERATURE

D. melanogaster has been used as a model for both medical and scientific research for over a century (Stephenson, 2013). Research on *D. melanogaster* led to discoveries in genetic research, including sex-linked genetic inheritance and genetic mutations caused by radiation. Because *D. melanogaster* has a complex nervous system, discoveries have also been made in neuroscience and neurodevelopment. Human disease-related loci in *D. melanogaster* genes make it a model organism for studies on neurodegenerative diseases in humans. Studies conducted on the similarities between humans and *D. melanogaster* could produce results that are applicable to humans. Okray and Hassan's 2013 study on genetic approaches in *D. melanogaster* for the study of neurodevelopmental disorders indicate that *D. melanogaster* genetics can be directly applied and are relevant to human health, specifically in neurodevelopmental disorders.

Inman's 1984 research completed on the effects of various drugs, including caffeine, on the development and DNA replication of *D. melanogaster* describes the methodology used to study the effects on *D. melanogaster* eggs. The results indicate that eggs can be exposed to chemical-infused medium for up to 30 minutes without measurable effects on development. Other studies have focused on the effects of caffeine on *D. melanogaster* reproduction. A study completed by Matsagas et al. in 2009 focused on the long-term functional side effects of stimulants and sedatives in *D. melanogaster*. Researchers predicted that stimulants such as caffeine and theobromine would increase reproduction, as they are known to increase biological activity (Matsagas et al., 2009). The study revealed that caffeine dramatically decreases fertility, and theobromine is rather benign. The researchers explicitly state that their findings do not necessarily apply to humans, but their study raises questions as to whether their conclusions could be applied to human fertility with further testing.

Herrero (2012) found that *D. melanogaster* respond to chemosensory signals, as they use chemicals to perceive their environment and make behavioral choices. It was found that both taste and odor of chemicals affect certain behaviors, including feeding, learning, memory, and navigation behavior. The study, however, did not relate the findings to possible explanations for human behavior in olfactory or gustatory responses to the chemicals used.

METHODS

Chemicals or compounds readily available to humans and *D. melanogaster* were chosen to work with. The four chemicals used in this project were theobromine, caffeine, ethylene glycol, and ammonia. Theobromine is a stimulant found in chocolate, which is readily available to both organisms, and was chosen because few studies have been done on its specific effects on *D. melanogaster*. Caffeine was chosen because of its prevalence in the human diet, and because it has been used in previous studies on *D. melanogaster* (Matsagas et al., 2009). Ethylene glycol, more commonly known as antifreeze, was chosen because it is a common household chemical available to both humans and *D. melanogaster*. Ammonia, the fourth chemical, was chosen for this project because it is known to have detrimental effects on humans and other organisms (Visek, 1984), but also because it is found in many household cleaners.

A small-scale experiment was begun in the spring of 2014 using methodology found in a study by Inman (1984). Few studies have utilized a similar methodology, so it was chosen as a starting point. As this research project progressed, new techniques and methods were developed based on trial and error.

Wild type *D. melanogaster* adults were acquired for the experiment. The food media was prepared using a recipe shared by Dr. Janna McLean. It contains unsulfured molasses, corn meal, water, brewer's yeast, agar, tegosept, and proprionic acid. After the medium was fully cooked it was infused with different concentrations of each chemical and poured into individual vials. Ten different concentrations of each chemical being tested were used, including one control group vial that contained only the food media. Three vials of each chemical concentration totalled 33 vials for each chemical. There was a total of 12 control vials, three for each chemical. Caffeine, theobromine, and ethylene glycol each had concentrations spanning from 1.0g to 0.0g per 100mL of food media, separated by increments of 0.1g. Ammonia is a more potent chemical (Roney et al., 2004), so the highest concentration used was 0.3mL per 100 mL of food media. It was determined through literary research that the amount of ammonia used in regular household chemicals is 5-10% (Roney et al., 2004), but the concentrations of ammonia used in this study spanned from 0.3mL to 0.0mL per 100mL of food media, with increments of 0.025mL. The

lowest concentration of ammonia used was 0.075mL per 100mL of normal food, while the other chemicals' lowest concentration was 0.1g per 100mL of normal food.

Five male and five female *D. melanogaster* adults were placed in each vial. After ten days, all adult *D. melanogaster* individuals were removed and placed in a morgue. A time period of ten days was chosen because the average time required for immature pupae to develop from eggs is 10 days, as observed in the control vials. Removal of the adult individuals ensured no mixing of the parental (P) and filial (F_1) generations. Observation of each vial was documented daily, noting the development of larvae, immature pupae, mature pupae, and the number of F_1 generation individuals emerging. The timeline observed in the control group vials was considered the standard for each developmental stage. All vials were maintained at 21°C in an incubator. The experiment was conducted in triplicate to gather a set of data that more accurately represented the effects of each chemical on *D. melanogaster* development.

RESULTS

A chi-square test was performed comparing the four chemicals for toxicity, or the number of P generation adults that were killed after 10 days. This type of test was used because the data contained one categorical variable (the chemical) with two or more categories (theobromine, caffeine, ethylene glycol, and ammonia). The null hypothesis was that toxicity to the P generation does not vary between groups. The alternative hypothesis was that toxicity varies between the different chemicals. Based on the results from the chi-square test (Table 1.1), the null hypothesis was rejected and the alternative hypothesis was accepted.

Table 1.1: Chi-Square test for total toxicity by chemical.

Chemical	Observed N	Expected N	Residual
Theobromine	140	226.3	-86.3
Caffeine	529	226.3	302.8
Ethylene glycol	198	226.3	-28.3
Ammonia	38	226.3	-188.3
Total	905		

Test Statistics	Chemical
Chi-Square (a)	598.156
df	3
Asymp. Sig.	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 226.3.

Correlations were then analyzed for all the chemicals combined and their general toxicity, as well as for each chemical and its individual toxicity. Overall, there was a moderate correlation between the concentrations of each chemical and their toxicity (Table 1.2). Theobromine, caffeine, and ethylene glycol all showed significant correlation between their concentration and toxicity (Tables 1.3, 1.4, and 1.5), while ammonia did not show significant correlation between its concentration and toxicity (Table 1.6). Positive Pearson Correlation values indicate that as concentration of the chemical increased, toxicity of the chemical also increased.

Table 1.2: Correlation between all chemicals' concentrations and toxicity.

All chemicals		Concentration	Toxicity
Concentration	Pearson Correlation	1	.349(**)
	Sig. (2-tailed)		.000
	N	927	927
Toxicity	Pearson Correlation	.349(**)	1
	Sig. (2-tailed)	.000	
	N	927	927

** Correlation is significant at the 0.01 level (2-tailed).

Table 1.3: Correlation between theobromine's concentration and toxicity.

Theobromine		Concentration	Toxicity
Concentration	Pearson Correlation	1	.273(**)
	Sig. (2-tailed)		.001
	N	140	140
Toxicity	Pearson Correlation	.273(**)	1
	Sig. (2-tailed)	.001	
	N	140	140

** Correlation is significant at the 0.01 level (2-tailed).

Table 1.4: Correlation between caffeine's concentration and toxicity.

Caffeine		Concentration	Toxicity
Concentration	Pearson Correlation	1	.416(**)
	Sig. (2-tailed)		.000
	N	529	529
Toxicity	Pearson Correlation	.416(**)	1
	Sig. (2-tailed)	.000	
	N	529	529

** Correlation is significant at the 0.01 level (2-tailed).

Table 1.5: Correlation between ethylene glycol's concentration and toxicity.

Ethylene Glycol		Concentration	Toxicity
Concentration	Pearson Correlation	1	.445(**)
	Sig. (2-tailed)		.000
	N	198	198
Toxicity	Pearson Correlation	.445(**)	1
	Sig. (2-tailed)	.000	
	N	198	198

** Correlation is significant at the 0.01 level (2-tailed).

Table 1.6: Correlation between ammonia's concentration and toxicity.

Ammonia		Concentration	Toxicity
Concentration	Pearson Correlation	1	-.243(***)
	Sig. (2-tailed)		.142
	N	38	38
Toxicity	Pearson Correlation	-.243(***)	1
	Sig. (2-tailed)	.142	
	N	38	38

*** Correlation is not significant at the 0.01 level (2-tailed).

A one-way between subjects analysis of variance (ANOVA) test was performed on the four chemicals' effects on *D. melanogaster* developmental stages. ANOVA was selected because the independent variable (the chemical) was a between subjects factor with two or more separate groups (theobromine, caffeine, ethylene glycol, and ammonia), while the dependent variable (time between each stage of development) was continuous. The null hypothesis was that the mean number of days between each stage of *D. melanogaster* development for the four chemicals was equal. The alternative hypothesis was that the mean number of days between each developmental stage is different for at least one of the chemicals used. The null hypothesis was rejected and the alternative hypothesis was accepted since more than one of the groups differed significantly; this is indicated by one or more Sig. value of <.05 in Table 2.1.

Table 2.1: ANOVA for all chemicals and all developmental stages of *D. melanogaster*.

ANOVA for all chemicals		Sum of Squares	df	Mean Square	F	Sig.
Egg to larvae	Between Groups	51.789	4	12.947	26.801	.000
	Within Groups	58.936	122	.483		
	Total	110.724	126			
Larvae to immature pupae	Between Groups	205.528	4	51.382	43.334	.000
	Within Groups	116.200	98	1.186		
	Total	321.728	102			
Immature to mature pupae	Between Groups	1.713	4	.428	1.200	.316
	Within Groups	34.611	97	.357		
	Total	36.324	101			
Mature pupae to F1 generation	Between Groups	1.050	4	.262	1.240	.299
	Within Groups	20.529	97	.212		
	Total	21.578	101			

A Tukey post hoc procedure was then implemented to determine which of the four chemicals had a significant effect on the number of days in each stage of *D. melanogaster* development. Chemicals with values sharing the same column are not significantly different from each other. Chemicals with values that do not share a column in Tables 2.2, 2.3, 2.4, and 2.5 are significantly different from other chemicals. Between the egg and larval stages of development, theobromine was the only chemical to have a significant difference in number of days between stages (Table 2.2). During the stage of development from larvae to immature pupae, both caffeine and theobromine showed significant

differences in number of days between the stages (Table 2.3). From the immature pupae to mature pupae stage, as well as the mature pupae to F_1 generation stage, none of the chemicals were significantly different in the number of days between the developmental stages (Tables 2.4 and 2.5).

Table 2.2: ANOVA Tukey post hoc homogeneous subsets for the egg to larvae stage of development.

	N	Subset for alpha = .05	
Chemical	1	2	1
Ethylene glycol	30	1.4667	
Caffeine	29	1.4828	
Ammonia	30	1.5333	
Controls	11	1.9091	
Theobromine	27		3.0741
Sig.		.226	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 21.825. b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Table 2.3: ANOVA Tukey post hoc homogeneous subsets for the larvae to immature pupae stage of development.

	N	Subset for alpha = .05		
Chemical	1	2	3	1
Ammonia	30	6.2667		
Controls	11	6.2727		
Ethylene glycol	30	7.3667		
Theobromine	27		8.7407	
Caffeine	5			12.0000
Sig.		.090	1.000	1.000

a. Uses Harmonic Mean Sample Size = 12.671. b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Table 2.4: ANOVA Tukey post hoc homogeneous subsets for the immature pupae to mature pupae stage of development.

	N	Subset for alpha = .05
Chemical	1	1
Caffeine	4	4.2500
Controls	11	4.5455
Ammonia	30	4.6333
Theobromine	27	4.6667
Ethylene glycol	30	4.8333
Sig.		.149

a. Uses Harmonic Mean Sample Size = 11.246. b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Table 2.5: ANOVA Tukey post hoc homogeneous subsets for the mature pupae to F_1 generation stage of development.

	N	Subset for alpha = .05
Chemical	1	1
Ammonia	30	1.2333
Ethylene glycol	30	1.2667
Theobromine	27	1.3333
Controls	11	1.3636
Caffeine	4	1.7500
Sig.		.067

a. Uses Harmonic Mean Sample Size = 11.246. b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Correlations were made between the concentrations of all chemicals, as well as each chemical individually, and their effect on days between each stage of development. Positive Pearson Correlation values indicate that as the concentration of the chemical increased, the number of days between stages of development also increased. Overall, there was a significant positive correlation between the concentration of the chemicals and the number of days between the larvae to immature pupae and immature to mature pupae stages of *D. melanogaster* development (Table 3.1). The concentration of theobromine and the stage from larvae to immature pupae showed a significant positive correlation (Table 3.2); caffeine's concentration showed a significant positive correlation with the immature pupae to mature pupae stage (Table 3.3); ethylene glycol concentration showed a significant positive correlation with the larvae to immature pupae stage (Table 3.4); ammonia showed no significant correlation between its concentration and the stages of *D. melanogaster* development (Table 3.5).

Table 3.1: Correlation between all chemicals' concentrations and developmental stages.

All chemicals		Concentration	Egg to larvae	Larvae to immature pupae	Immature to mature pupae	Mature pupae to F1 generation
Concentration	Pearson Correlation	1	.113	.415(**)	.239(*)	-.062
	Sig. (2-tailed)		.208	.000	.016	.539
	N	128	127	103	102	102
Egg to larvae	Pearson Correlation	.113	1	-.022	-.010	.097
	Sig. (2-tailed)	.208		.828	.919	.332
	N	127	127	103	102	102
Larvae to immature pupae	Pearson Correlation	.415(**)	-.022	1	-.092	.118
	Sig. (2-tailed)	.000	.828		.357	.239
	N	103	103	103	102	102
Immature to mature pupae	Pearson Correlation	.239(*)	-.010	-.092	1	-.535(**)
	Sig. (2-tailed)	.016	.919	.357		.000
	N	102	102	102	102	102
Mature pupae to F1 generation	Pearson Correlation	-.062	.097	.118	-.535(**)	1
	Sig. (2-tailed)	.539	.332	.239	.000	
	N	102	102	102	102	102

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 3.2: Correlation between theobromine's concentration and developmental stages.

Theobromine		Concentration	Egg to larvae	Larvae to immature pupae	Immature to mature pupae	Mature pupae to F1 generation
Concentration	Pearson Correlation	1	-.065	.559(**)	.213	-.184
	Sig. (2-tailed)		.746	.002	.286	.359
	N	27	27	27	27	27
Egg to larvae	Pearson Correlation	-.065	1	-.520(**)	.361	-.052
	Sig. (2-tailed)	.746		.005	.064	.798
	N	27	27	27	27	27
Larvae to immature pupae	Pearson Correlation	.559(**)	-.520(**)	1	-.226	.017
	Sig. (2-tailed)	.002	.005		.257	.931
	N	27	27	27	27	27
Immature to mature pupae	Pearson Correlation	.213	.361	-.226	1	-.667(**)
	Sig. (2-tailed)	.286	.064	.257		.000
	N	27	27	27	27	27
Mature pupae to F1 generation	Pearson Correlation	-.184	-.052	.017	-.667(**)	1
	Sig. (2-tailed)	.359	.798	.931	.000	
	N	27	27	27	27	27

** Correlation is significant at the 0.01 level (2-tailed).

Table 3.3: Correlation between caffeine's concentration and developmental stages.

Caffeine		Concentration	Egg to larvae	Larvae to immature pupae	Immature to mature pupae	Mature pupae to F1 generation
Concentration	Pearson Correlation	1	.101	.778	1.000(**)	.333
	Sig. (2-tailed)		.603	.121	.000	.667
	N	30	29	5	4	4
Egg to larvae	Pearson Correlation	.101	1	-.477	-.333	.333
	Sig. (2-tailed)	.603		.417	.667	.667
	N	29	29	5	4	4
Larvae to immature pupae	Pearson Correlation	.778	-.477	1	.816	.000
	Sig. (2-tailed)	.121	.417		.184	1.000
	N	5	5	5	4	4
Immature to mature pupae	Pearson Correlation	1.000(**)	-.333	.816	1	.333
	Sig. (2-tailed)	.000	.667	.184		.667
	N	4	4	4	4	4
Mature pupae to F1 generation	Pearson Correlation	.333	.333	.000	.333	1
	Sig. (2-tailed)	.667	.667	1.000	.667	
	N	4	4	4	4	4

** Correlation is significant at the 0.01 level (2-tailed).

Table 3.4: Correlation between ethylene glycol's concentration and developmental stages.

Ethylene glycol		Concentration	Egg to larvae	Larvae to immature pupae	Immature to mature pupae	Mature pupae to F1 generation
Concentration	Pearson Correlation	1	-.131	.493(**)	.279	.000
	Sig. (2-tailed)		.489	.006	.136	1.000
	N	30	30	30	30	30
Egg to larvae	Pearson Correlation	-.131	1	-.718(**)	-.052	.154
	Sig. (2-tailed)	.489		.000	.784	.415
	N	30	30	30	30	30
Larvae to immature pupae	Pearson Correlation	.493(**)	-.718(**)	1	-.010	.006
	Sig. (2-tailed)	.006	.000		.960	.975
	N	30	30	30	30	30
Immature to mature pupae	Pearson Correlation	.279	-.052	-.010	1	-.512(**)
	Sig. (2-tailed)	.136	.784	.960		.004
	N	30	30	30	30	30
Mature pupae to F1 generation	Pearson Correlation	.000	.154	.006	-.512(**)	1
	Sig. (2-tailed)	1.000	.415	.975	.004	
	N	30	30	30	30	30

** Correlation is significant at the 0.01 level (2-tailed).

Table 3.5: Correlation between ammonia's concentration and developmental stages.

Ammonia		Concentration	Egg to larvae	Larvae to immature pupae	Immature to mature pupae	Mature pupae to F1 generation
Concentration	Pearson Correlation	1	.070	-.182	.032	.041
	Sig. (2-tailed)		.714	.335	.867	.829
	N	30	30	30	30	30
Egg to larvae	Pearson Correlation	.070	1	-.730(**)	-.139	.200
	Sig. (2-tailed)	.714		.000	.465	.289
	N	30	30	30	30	30
Larvae to immature pupae	Pearson Correlation	-.182	-.730(**)	1	-.113	-.119
	Sig. (2-tailed)	.335	.000		.551	.531
	N	30	30	30	30	30
Immature to mature pupae	Pearson Correlation	.032	-.139	-.113	1	-.495(**)
	Sig. (2-tailed)	.867	.465	.551		.005
	N	30	30	30	30	30
Mature pupae to F1 generation	Pearson Correlation	.041	.200	-.119	-.495(**)	1
	Sig. (2-tailed)	.829	.289	.531	.005	
	N	30	30	30	30	30

** Correlation is significant at the 0.01 level (2-tailed).

A chi-square test was completed comparing the four chemicals and overall viability of the F_1 generation, or number of individuals that emerged after 21 days. This type of test was used because the data contained one categorical variable (the chemical) with two or more categories (theobromine, caffeine, ethylene glycol, and ammonia). The null hypothesis was that viability of the F_1 generation does not vary between groups. The alternative hypothesis was that F_1 viability does vary between the different chemicals. Based on the results from the chi-square test (Table 4.1), the null hypothesis was rejected and the alternative hypothesis was accepted.

Table 4.1: Chi-Square test for overall viability of F_1 generation by chemical.

Chemical	Observed N	Expected N	Residual
Theobromine	709	1756.5	-1047.5
Caffeine	148	1756.5	-1608.5
Ethylene glycol	2685	1756.5	928.5
Ammonia	3484	1756.5	1727.5
Total	7026		

Test Statistics	Chemical
Chi-Square (a)	4287.445
df	3
Asymp. Sig.	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 1756.5.

Correlations were then analyzed for all the chemicals combined and the general F_1 viability, as well as for each chemical and its individual F_1 viability. Overall, there was a significant correlation between concentration of each chemical and F_1 viability (Table 4.2). Theobromine, caffeine, and ethylene glycol showed significant correlation between concentration and F_1 viability (Tables 4.3, 4.4, and 4.5), while ammonia did not show significant correlation between concentration and F_1 viability (Table 4.6). Negative Pearson Correlation values indicate that as concentration of the chemical increased, F_1 viability decreased.

Table 4.2: Correlation between all chemicals' concentrations and F_1 generation viability.

All chemicals		Concentration	F_1 generation
Concentration	Pearson Correlation	1	-.586(**)
	Sig. (2-tailed)		.000
	N	128	128
F_1 generation	Pearson Correlation	-.586(**)	1
	Sig. (2-tailed)	.000	
	N	128	128

** Correlation is significant at the 0.01 level (2-tailed).

Table 4.3: Correlation between theobromine's concentration and F_1 generation viability.

Theobromine		Concentration	F_1 generation
Concentration	Pearson Correlation	1	-.476(*)
	Sig. (2-tailed)		.012
	N	27	27
F_1 generation	Pearson Correlation	-.476(*)	1
	Sig. (2-tailed)	.012	
	N	27	27

* Correlation is significant at the 0.05 level (2-tailed).

Table 4.4: Correlation between caffeine's concentration and F_1 generation viability.

Caffeine		Concentration	F_1 generation
Concentration	Pearson Correlation	1	-.516(**)
	Sig. (2-tailed)		.004
	N	30	30
F_1 generation	Pearson Correlation	-.516(**)	1
	Sig. (2-tailed)	.004	
	N	30	30

** Correlation is significant at the 0.01 level (2-tailed).

Table 4.5: Correlation between ethylene glycol's concentration and F_1 generation viability.

Ethylene glycol		Concentration	F_1 generation
Concentration	Pearson Correlation	1	-.812(**)
	Sig. (2-tailed)		.000
	N	30	30
F_1 generation	Pearson Correlation	-.812(**)	1
	Sig. (2-tailed)	.000	
	N	30	30

** Correlation is significant at the 0.01 level (2-tailed).

Table 4.6: Correlation between ammonia's concentration and F_1 generation viability.

Ammonia		Concentration	F_1 generation
Concentration	Pearson Correlation	1	.018(***)
	Sig. (2-tailed)		.925
	N	30	30
F_1 generation	Pearson Correlation	.018(***)	1
	Sig. (2-tailed)	.925	
	N	30	30

*** Correlation is not significant at the 0.01 level (2-tailed).

DISCUSSION

It was hypothesized that chemicals presenting toxicity or inhibition of development in *D. melanogaster* could have similar effects on humans. Theobromine was expected to be rather benign; caffeine was expected to increase biological function and reproduction rate; ethylene glycol was expected to be mildly toxic; and ammonia was expected to be highly toxic.

The results found in Tables 1.3-1.5 indicate a significant positive correlation between the concentration and toxicity of theobromine, caffeine, and ethylene glycol. This is interpreted as the three chemicals being toxic to *D. melanogaster*, which suggests that they would also be toxic to humans in high enough concentrations. The results in Table 1.6 indicate no significant correlation between the concentration and toxicity of ammonia, which is not what was expected.

The number of days between *D. melanogaster* developmental stages was significantly affected by theobromine and caffeine, but only during the earlier stages of development. In later stages, none of the chemicals had significant effects on the length of each developmental stage. The concentrations of each individual chemical, however, did show a significant effect on the number of days between stages of development. The most significant effects were seen in theobromine, caffeine, and ethylene glycol during the second and third developmental stages. No significant effects were found for the concentrations of ammonia used in this study. The results suggest that theobromine, caffeine, and ethylene glycol have serious effects on the development of *D. melanogaster*, while ammonia is rather benign at the concentrations used.

As seen in Tables 4.2-4.5, a significant negative correlation between concentrations of theobromine, caffeine, and ethylene glycol and the F_1 generation's viability existed. This means that with an increasing concentration of the three chemicals, F_1 viability decreased. It can be inferred from these results that theobromine, caffeine, and ethylene glycol decrease the reproductive success of *D. melanogaster*, which suggests that they would in turn decrease the reproductive success of humans. The concentrations of ammonia used were found to have no significant effect on the viability of the F_1 generation. This suggests that ammonia would also have no significant effect on human reproduction.

It has been determined that caffeine causes significant changes in human behavior, while theobromine does not cause significant behavioral changes (Mumford et al., 1994). It has been suggested that products commonly consumed by humans that contain cocoa also contain quantities of caffeine and theobromine that could potentially have a significant effect on human behavior (Mumford et al., 1994). Another study completed on the effects of caffeine in humans revealed that concentrations of caffeine consumed by the average person generally produce positive effects on the individual's behavior, but caffeine in excessive amounts may lead to negative effects, especially in individuals who are more sensitive to caffeine (Smith, 2002).

The toxicity of ethylene glycol is recognized to be rather complicated, but is primarily due to severe acidosis produced by the metabolism of glycolate, and to the calcium oxalate precipitation that results from metabolism of ethylene glycol (Jacobsen and McMartin, 1986). Ammonia may have both stimulatory effects at low concentrations and inhibitory effects at higher concentrations or longer exposure times (Visek, 1984).

The results of this study may be improved by using different concentrations of each chemical. This particular field of research could benefit if higher concentrations of theobromine, ethylene glycol, and ammonia were used in future studies. Increasing the concentrations of these chemicals would likely give more significant results on their effects on *D. melanogaster*'s life cycle and development. By using *D. melanogaster* as a model, effects of theobromine, caffeine, ethylene glycol, ammonia, and other chemicals on humans can be better predicted. *D. melanogaster* has been used in scientific research for a long time and has shown conclusive results that can be applied to human medicine (Stepehnson, 2013; Okray and Hassan, 2013). Further research would be necessary in order to reach a more definite conclusion about the effects of selected chemicals on the development of *D. melanogaster* and humans.

REFERENCES

Devineni, A.V., Heberlein, U. (2013). The Evolution of Drosophila melanogaster as a Model for Alcohol Research. *Annual Rev. Neuroscience* 36: 121-138.

Herrero, P. (2012). Fruit fly behavior in response to chemosensory signals. *Peptides* 38(2): 228-237.

Ho, Y.K., Koehn, D.J., Sobieski, R.J., Clifford, A.J., Clifford, C.K. (1984). Effects of purine amino groups on the development of Drosophila. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology* 79(2): 435-439.

Inman, R.B. (1984). Methodology for the study of the effect of drugs on development and DNA replication in Drosophila melanogaster embryonic tissue. *Biochimica Et Biophysica Acta (BBA) – Gene Structure and Expression* 783(3): 205-215.

Jacobsen, D., McMartin, K.E. (1986). Methanol and ethylene glycol poisonings. *Medical Toxicology* 1(5): 309-334.

Matsagas, K., Lim, D.B., Horwitz, M., Rizza, C.L., Mueller, L.D., et al. (2009) Long-Term Functional Side-Effects of Stimulants and Sedatives in *Drosophila melanogaster*. PLoS ONE 4(8): e6578.

Mumford, G.K., Evans, S.M., Kaminski, B.J., Preston, K.L., Sannerud, C.A., Silverman, K., Griffiths, R.R. (1994). Discriminative stimulus and subjective effects of theobromine and caffeine in humans. *Psychopharmacology* 115(1-2): 1-8.

Nawrot, P., Jordan, S., Eastwood, J., Rotstein, J., Hugenholtz, A., Feeley, M. (2003). Effects of caffeine on human health. *Food Additives & Contaminants* 20(1): 1-30.

Okray, Z., Hassan, B.A. (2013). Genetic approaches in Drosophila for the study neurodevelopmental disorders. *Neuropharmacology* 68: 150-156.

Petersen, N.S. (1990). Effects of heat and chemical stress on development. *Adv. Genet.* 28: 275-296.

Roney, N., Llados, F., Little, S.S., Knaebel, D. (2004). Toxicological profile for ammonia. *Toxicological Profiles*, *1*. Retrieved September 26, 2014, from http://www.atsdr.cdc.gov/toxprofiles/tp126.

Smith, A. (2002). Effects of caffeine on human behavior. *Food and Chemical Toxicology* 40(9): 1243-1255.

Stephenson, R. (2013). Drosophila melanogaster: a fly through its history and current use. *J. R. Coll. Physicians Edinb.* 43(1): 70-75.

Tyler, T. (1984). Acute and subchronic toxicity of ethylene glycol monobutyl ether. *Environmental Health Perspectives 57:* 185-191.

Visek, W.J. (1984). Ammonia: its effects on biological systems, metabolic hormones, and reproduction. *Journal of Dairy Science* 67(3): 481-498.

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