The evolution of detoxification resistance in the cactophilic *Drosophila mojavensis* and *Drosophila arizonae*

by

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Abstract

Understanding the evolution of detoxification resistance in organisms has broad implications to many aspects of biology for human health, from biological control to understanding the origins of biodiversity. *Drosophila mojavensis* are cactophilic *Drosophila* that live in Catalina Island, Baja California, the Mojave Desert, and the Sonora Desert. *Drosophila arizonae* are cactus generalists that live in Baja California and the Sonora Desert. In this experiment, the detoxification ability of two different populations of *D. mojavensis* will be tested. Different concentrations of the compounds Hexanoic acid, Dichlorodiphenyltrichloroethane (DDT), Malathion and Diazinon will be placed in the fly media. The time to first pupation, time to first eclosion, larval viability, and sex ratio of adults will be recorded for each assay. This information will be used to determine the concentration that the two populations show variation in detoxification ability for each compound.

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Introduction

Drosophila mojavensis is a species of cactophilic *Drosophila* that is composed of four host races that are geographically and genetically isolated. *Drosophila arizonae* is a sister species of *Drosophila mojavensis* that diverged about 1.5 million years ago (Matzkin 2008). Each of the *Drosophila mojavensis* host races, which occupy mainland Sonora Desert, Baja California, Catalina Island, and Mojave Desert, utilizes a different species of cactus: organpipe (*Stenocereus thurberi*), agria (*S. gummosus*), prickly pear (*Opuntia spp*.), and barrel (*Ferocactus cylindraceus*), respectively (Fellows and Heed 1972; Ruiz and Heed 1988). *Drosophila mojavensis* is thought to have originated in Baja California, utilizing a Stenocereus cactus before migrating up the peninsula and colonizing Catalina Island and the Mojave Desert, shifting cactus hosts along the way (Ruiz *et al.* 1990). Later, the Sonora Desert population was colonized from the Baja California population. This colonization also resulted in a cactus host shift (Matzkin 2008).

Because each of the cactus hosts is different, the four *Drosophila mojavensis* populations are provided with distinct chemical environments with differences including compounds such as alcohols, alkaloids, triterpenes, and glycosides. Previous studies dealing with *Drosophila mojavensis* have shown that this chemical variation can drive the molecular and functional evolution of metabolic genes (Matzkin 2008). Some of the compounds that *Drosophila mojavensis* utilizes in its cactus host are toxic. This leads to constant selection pressure to evolve resistance to these compounds, which is especially true in the presence of a cactus host shift. Therefore, it can be predicted that as a consequence of a cactus host shift, detoxification enzymes might be under selection at both the transcriptional and the coding sequence level (Matzkin *et al.* 2006). Since each population feeds on a particular cactus, it is very likely that a different set of

loci is associated with the detoxification of compounds specific to each cactus (Matzkin *et al.* 2006). In this experiment, variation in detoxification ability between two populations of *Drosophila mojavensis* is tested.

Materials and Methods

All fly samples used in this experiment were collected from the field and maintained as isofemale lines on Banana media. Two populations of *Drosophila mojavensis* were used in this experiment: CI 1007-8-3 and OPMN 0407.002. CI 1007-8-3 is from Catalina Island and OPNM 0407.002 is from Organ Pipe National Monument, which is in the Sonora Desert. Four different compounds were tested: Hexanoic acid, Dichlorodiphenyltrichloroethane (DDT), Diazinon, and Malathion. These compounds were added to the Banana media in specific concentrations. 0.1%, 0.5%, and 1% Hexanoic acid was tested. 0.1 µg/mL, 1 µg/mL, and 10 µg/mL DDT was tested. 0.000001%, 0.000005%, 0.00001%, 0.0001%, 0.0005%, and 0.001% Diazinon was tested. 0.1 µg/ml, 0.5 µg/ml, and 1.0 µg/ml Malathion was tested.

To do this, the Banana media was prepared and then microwaved until it became a liquid. A specific concentration of the compound being tested was added to the media. 5-10 mL of the media was then dispensed into glass vials and allowed to cool. A control group that only contained the Banana media was also made. 8-10 vials were made for each concentration that was tested. After the media had cooled, 40 first instar larvae were picked and placed into each vial. Half of the vials from each concentration contained CI 1007-8-3 larvae and the other half contained OPNM 0407.002 larvae. The vials were stored at room temperature. The time to first pupation, time to first eclosion, viability, and male/female ratio were recorded.

Results

Figure 1 shows the average time to first pupation for CI 1007-8-3 and OPNM 0407.002 in different concentrations of Hexanoic acid. In 0.5% Hexanoic acid, only two OPNM 0407.002 larvae pupated. Figure 2 shows the average time to first eclosion for CI 1007-8-3 and OPNM 0407.002 for each concentration of Hexanoic acid. Only one OPNM 0407.002 fly eclosed in 0.5% Hexanoic acid. Figure 3 shows the average viability for CI 1007-8-3 and OPNM 0407.002 in different concentrations of Hexanoic acid. Only one OPNM 0407.002 fly survived in 0.5% Hexanoic acid. None of the larvae surived in 1% Hexanoic acid. Paired t-Tests with showed that the difference in average viability was marginally significant for 0.5% Hexanoic acid ($t=7.057$, $P=0.089$). The difference in the average time to first eclosion was significant in the 0.5% Hexanoic acid group ($t=22.312$, $P=0.029$).

Figure 4 shows the average time to first pupation for CI 1007-8-3 and OPNM 0407.002 in different concentrations of DDT. None of the OPNM 0407.002 larvae survived in 1 μg/mL of DDT. Figure 5 shows the average time to first eclosion for CI 1007-8-3 and OPNM 0407.002 for each concentration of DDT. No flies eclosed in the vials with 1 μg/mL of DDT. Figure 6 shows the average viability for CI 1007-8-3 and OPNM 0407.002 in different concentrations of DDT. There were no adult flies in 1 μg/mL of DDT and none of the larvae survived in 10 μg/mL of DDT. Paired t-Tests showed that the difference between CI 1007-8-3 and OPNM 0407.002 was not statistically significant for the average time to first pupation, average time to first eclosion, or average viability.

None of the larvae survived in 0.0001%, 0.0005%, or 0.001% Diazinon. The experiment was repeated twice with 0.000001%, 0.000005%, and 0.00001% Diazinon. The survival rate of

the Diazinon larvae was similar to the survival rate of the control group larvae. None of the larvae from either population survived in 0.1µg/ml, 0.5µg/ml, or 1.0 µg/ml of Malathion.

Discussion

In the Hexanoic acid and DDT trials, CI 1007-8-3 performed better than OPNM 0407.002. This shows that there is a variation in detoxification ability between the two populations. The difference in the average time to first eclosion was significant in the control group for the Hexanoic acid concentration testing. Since the average time to first pupation and average viability were not statistically different, this result could be an error due to small sample size; only four replicates were done for Hexanoic acid. Based on the data obtained from this study, the concentration where the variation in detoxification ability occurs in Hexanoic acid is 0.5%.

In the DDT experiment, there was not a statistical difference between CI 1007-8-3 and OPNM 0407.002 for the average time to first pupation, average time to first eclosion, or average viability in 0.1 µg/mL of DDT. No flies survived in 1 µg/mL of DDT, but the CI 1007-8-3 larvae pupated. Because of this, it is very likely that if concentrations of DDT between 0.1 µg/mL and 1 µg/mL are tested, a statistically significant variation in detoxification ability between the two populations will be found.

Future Research

This experiment will be repeated using concentrations of Diazinon that were lower than the first trial, but higher than the second two trials. This experiment will also be repeated using lower concentrations of Malathion. Once the concentration where CI 1007-8-3 and OPNM 0407.002 show variation in detoxification ability have been determined for all four compounds, larvae from several lines of all four populations of *Drosophila mojavensis*, as well as *Drosophila arizonae*, will be tested to determine variation in detoxification ability across populations.

Acknowledgements

I would like to thank Dr. Luciano Matzkin for his guidance and assistance with this project. I would also like to thank Beth Wilson and Dr. Harry Delugach for their help in answering any questions I had during this project.

References

Fellows D.F., Heed W.B. (1972) Factors affecting host plant selection in desert-adapted cactiphilic *Drosophila*. *Ecology*, **53**, 850–858.

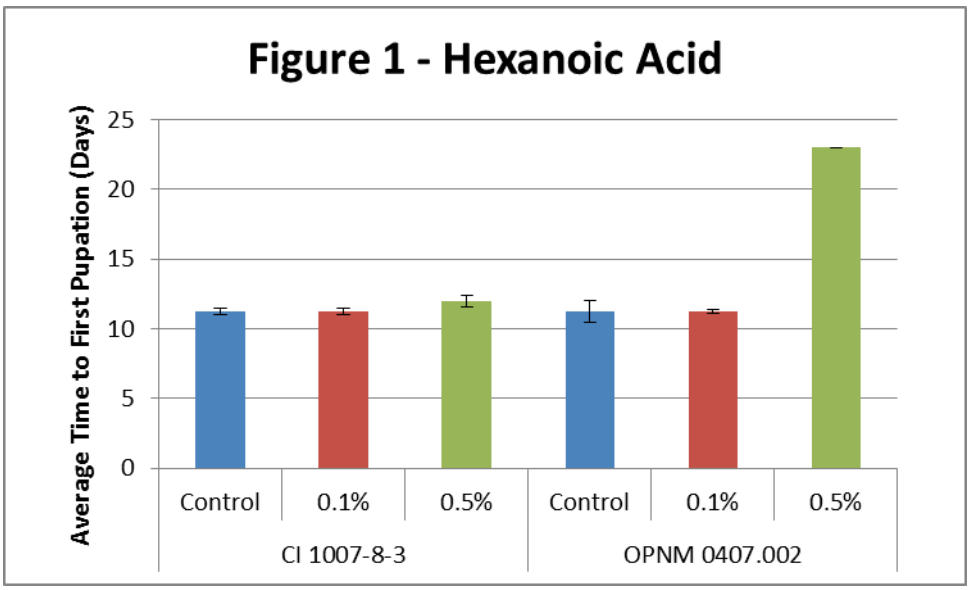
Matzkin L.M., Watts T.D., Bitler B.G., Machado C.A., Markow T.A. (2006) Functional genomics of cactus host shifts in *Drosophila mojavensis*. *Molecular Ecology*, **15**, 4635–4643.

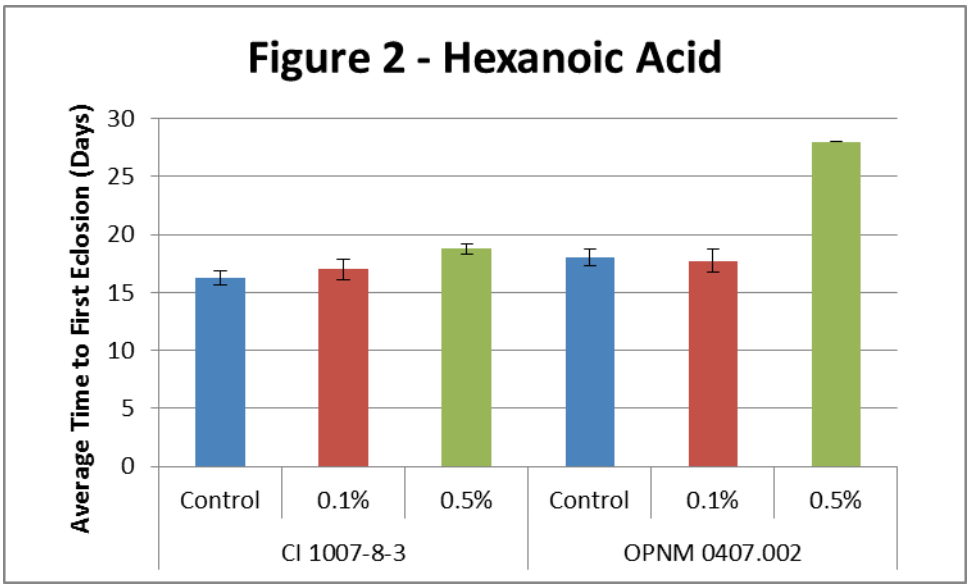
Matzkin L.M. (2008) The molecular basis of host adaptation in cactophilic *Drosophila*: molecular evolution of a glutathione s-transferase gene (*GstD1*) in *Drosophila mojavensis*. *Genetics*, **178**, 1073–1083.

Ruiz A., Heed W.B. (1988) Host-plant specificity in the cactophilic *Drosophila mulleri* species complex. *Journal of Animal Ecology*, **57**, 237–249.

Ruiz A., Heed W.B., Wasserman M. (1990) Evolution of the mojavensis cluster of cactophilic *Drosophila* with descriptions of two new species. *Journal of Heredity*, **81**, 30–42.

Appendix 1: Figures





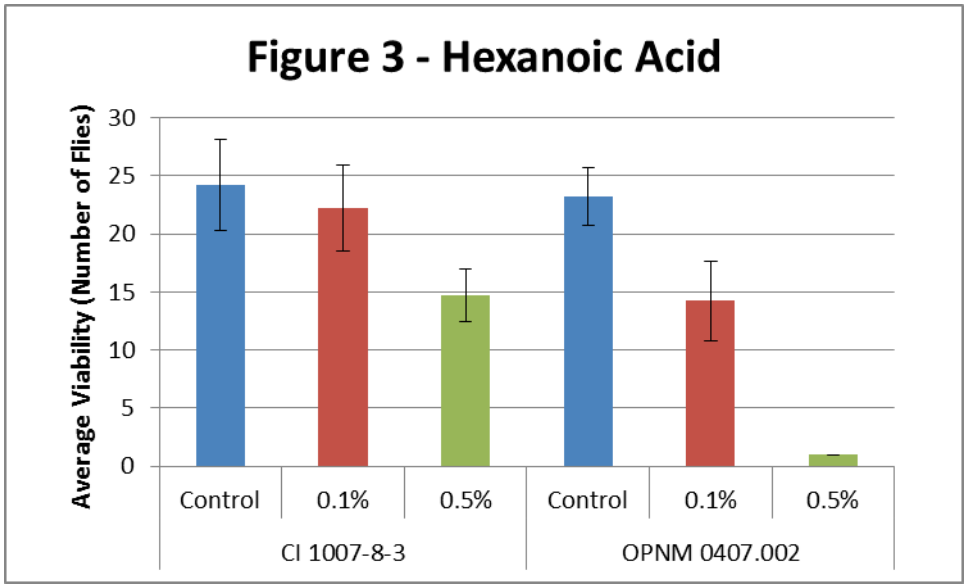


Figure 3 - Hexanoic Acid
Average Viability (Number of Flies)
30
25
20
15
10
5
0
Control
0.1%
0.5%
Control
0.1%
0.5%
CI 1007-8-3
OPNM 0407.002

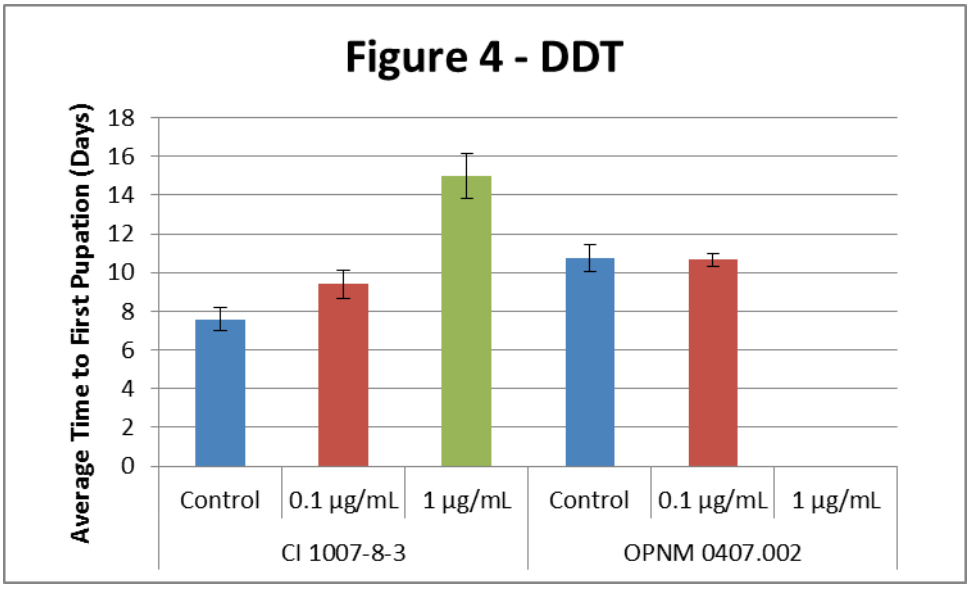


Figure 4 - DDT
Average Time to First Pupation (Days)
18
16
14
12
10
8
6
4
2
0
Control
0.1 µg/mL
1 µg/mL
Control
0.1 µg/mL
1 µg/mL
CI 1007-8-3
OPNM 0407.002

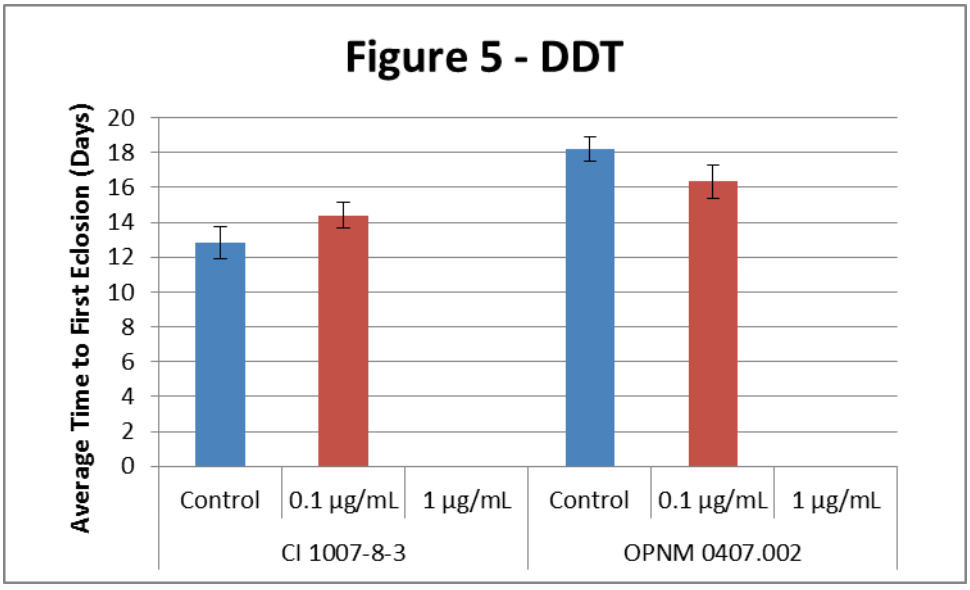


Figure 5 - DDT
Average Time to First Eclosion (Days)
20
18
16
14
12
10
8
6
4
2
0
Control
0.1 µg/mL
1 µg/mL
Control
0.1 µg/mL
1 µg/mL
CI 1007-8-3
OPNM 0407.002

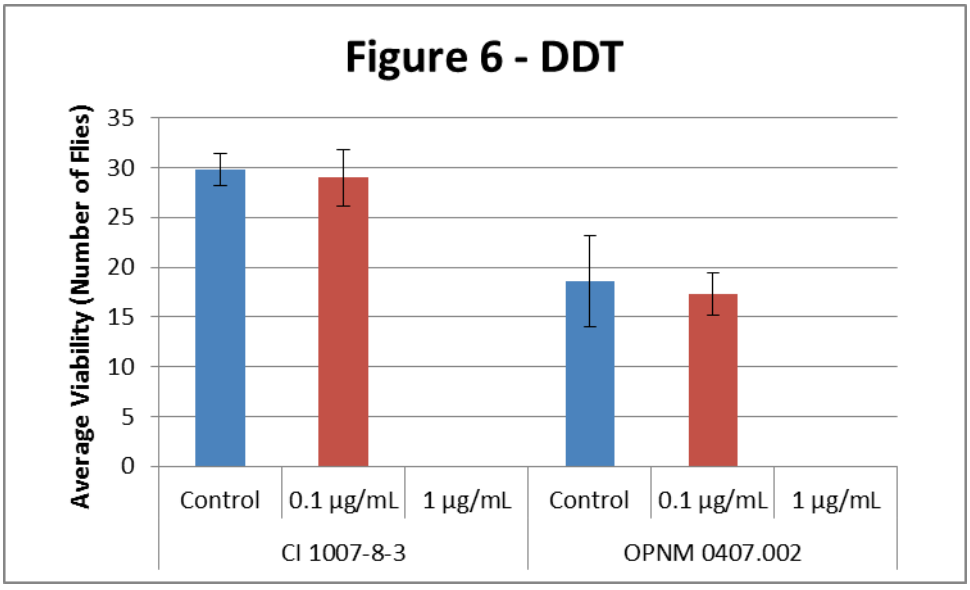


Figure 6 - DDT
Average Viability (Number of Flies)
35
30
25
20
15
10
5
0
Control
0.1 µg/mL
1 µg/mL
Control
0.1 µg/mL
1 µg/mL
CI 1007-8-3
OPNM 0407.002

Appendix 2: Raw Data

P: Time to first pupation

E: Time to first eclosion

V: Viability (number of living flies)

M/F: Males/Females

CI 1007-8-3 – Hexanoic Acid				
Control	P	E	V	M/F
Trial 1	11	16	19	6M/13F
Trial 2	12	16	17	10M/7F
Trial 3	11	18	27	13M/14F
Trial 4	11	15	34	15M/19F
Average	11.25	16.25	24.25	
Standard Error	0.25	0.629153	3.902456	
0.1% Hexanoic Acid	P	E	V	M/F
Trial 1	11	19	16	10M/6F
Trial 2	12	16	17	5M/12F
Trial 3	11	18	32	21M/11F
Trial 4	11	15	24	11M/13F
Average	11.25	17	22.25	
Standard Error	0.25	0.912871	3.705289	
1% Hexanoic Acid	P	E	V	M/F
Trial 1	-	-	-	-
Trial 2	-	-	-	-
Trial 3	-	-	-	-
Trial 4	-	-	-	-
Average	-	-	-	-
Standard Error	-	-	-	-

0.5% Hexanoic Acid	P	E	V	M/F
Trial 1	14	20	11	3M/8F
Trial 2	12	19	11	3M/8F
Trial 3	11	18	17	6M/11F
Trial 4	11	18	20	11M/9F
Average	12	18.75	14.75	
Standard Error	0.707107	0.478714	2.25	

OPNM 0407.002 – Hexanoic Acid				
Control	P	E	V	M/F
Trial 1	12	19	17	5M/12F
Trial 2	10	16	23	11M/12F
Trial 3	10	18	29	17M/12F
Trial 4	13	19	24	9M/15F
Average	11.25	18	23.25	
Standard Error	0.75	0.707107	2.462214	
0.1% Hexanoic Acid	P	E	V	M/F
Trial 1	13	19	6	1M/5F
Trial 2	8	15	17	8M/9F
Trial 3	11	18	12	8M/4F
Trial 4	13	19	22	9M/13F
Average	11.25	17.75	14.25	
Standard Error	1.181454	0.946485	3.424787	
1% Hexanoic Acid	P	E	V	M/F
Trial 1	-	-	-	-
Trial 2	-	-	-	-
Trial 3	-	-	-	-
Trial 4	-	-	-	-
Average				
Standard Error				

0.5% Hexanoic Acid	P	E	V	M/F
Trial 1	-	-	-	-
Trial 2	23	-	-	-
Trial 3	23	28	1	1M/0F
Trial 4	-	-	-	-
Average	23	28	1	
Standard Error	0	0	0	

CI 1007-8-3 - DDT				
Control	P	E	V	M/F
Trial 1	7	11	32	16M/16F
Trial 2	7	12	31	11M/18F
Trial 3	6	11	24	14M/10F
Trial 4	9	15	29	13M/16F
Trial 5	9	15	33	20M/13F
Average	7.6	12.8	29.8	
Standard Error	0.6	0.916515	1.593738	
0.1 µg/mL	P	E	V	M/F
Trial 1	7	12	28	13M/15F
Trial 2	9	14	27	10M/16F
Trial 3	9	14	25	8M/17F
Trial 4	11	16	25	12M/13F
Trial 5	11	16	40	24M/16F
Average	9.4	14.4	29	
Standard Error	0.748331	0.748331	2.810694	
1 µg/mL	P	E	V	M/F
Trial 1	15	-	-	-
Trial 2	18	-	-	-
Trial 3	11	-	-	-
Trial 4	15	-	-	-
Trial 5	16	-	-	-
Average	15			
Standard Error	1.140175			

10 µg/mL	P	E	V	M/F
Trial 1	-	-	-	-
Trial 2	-	-	-	-
Trial 3	-	-	-	-
Trial 4	-	-	-	-
Trial 5	-	-	-	-
Average				
Standard Error				

OPNM 0407.002 - DDT				
Control	P	E	V	M/F
Trial 1	-	26	3	1M/2F
Trial 2	10	16	16	6M/9F
Trial 3	10	16	20	6M/14F
Trial 4	10	16	23	15M/8F
Trial 5	13	17	31	8M/23F
Average	10.75	18.2	18.6	
Standard Error	0.67082	1.959592	4.610857	
0.1 µg/mL	P	E	V	M/F
Trial 1	-	-	-	-
Trial 2	10	16	13	6M/4F
Trial 3	11	17	19	10M/9F
Trial 4	11	16	20	8M/12F
Trial 5	-	-	-	-
Average	10.66667	16.33333	17.33333	
Standard Error	0.333333	0.333333	2.185813	
1 µg/mL	P	E	V	M/F
Trial 1	-	-	-	-
Trial 2	-	-	-	-
Trial 3	-	-	-	-
Trial 4	-	-	-	-
Trial 5	-	-	-	-
Average				
Standard Error				

10 µg/mL	P	E	V	M/F
Trial 1	-	-	-	-
Trial 2	-	-	-	-
Trial 3	-	-	-	-
Trial 4	-	-	-	-
Trial 5	-	-	-	-
Average				
Standard Error				