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Sex-Dependent Electrophysiological Responses of Lucilia sericata to Concentration Gradient of Indole

Erin T. Filbrandt

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Honors Thesis

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Abstract

I investigated the responses to specific concentrations of indole in the adult stage of *Lucilia sericata*, the common green bottle fly. Indole is a commonly used attractant at concentrations between 0 and .04% that has a floral scent at low concentrations and a fecal odor at high concentrations (Cragg, 1950). There were three treatment cages: one all male, one all female, and one mixed male and female. The purpose of these conditions was to observe the difference between sensory responses of males, non-mated females, and mated females. All cages were placed in an incubator to maintain uniform temperatures and light cycles. All treatment cages also received the identical diet to reduce extraneous variables. I measured the response to a concentration gradient of indole through the use of the electroantennogram, an instrument that records electrical depolarizations occurring in the antennae of the specimen being studied when exposed to

specific volatiles. Positive controls and negative controls were used to establish a baseline for responses; the results using the treatment volatile indole were compared to these controls. I hypothesized that the responses of males, non-mated females, and mated females would differ from one other due to their different dietary needs. The males were expected to respond to the lower concentrations of indole; the non-mated females to lower and higher concentrations almost equally; and the mated females to lower concentrations and strongly to higher concentrations. Although responses were not as distinct as expected, the data follows the general trend of the responses predicted.

Acknowledgements

I would like to thank the University of Dayton Honors Program for presenting me with multiple unique opportunities and providing funds for my research. Thank you to my outstanding advisor Dr. Karolyn Hansen who has endured this project with me and continues to guide me in and out of the lab. Casey Walk, TJ Lee, John Riordan, and George Iannantuono, thank you for your essential assistance in the lab. Last but not least, huge thanks to my mentor and friend, Allissa Blystone whose research and support made this thesis possible.

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Introduction

Lucilia sericata is a primary colonizer of carrion in a natural environment. Females are attracted by putrefying substances, including volatile organic compounds given off by deceased human bodies. For this reason, this species is extremely important in forensic entomology. I studied the adult stage of the blowflies and examined the responses of males, mated females, and non-mated females to different concentrations of indole, a compound that is commonly used as bait for *L. sericata* in experiments (Ashworth and Wall, 1994). Responses to gradients of indole were measured through use of an electroantennogram (EAG), an instrument that records electrophysiological responses of the antennal receptor cells. Observing the responses to different concentrations of indole will help elucidate any differences in attraction between males, mated-females, and non-mated females. This information will give insight into what attracts *L. sericata* to carrion and will ultimately aid in tightening the post-mortem interval.

Literature Review

Lucilia sericata, commonly referred to as the green bottle fly, belongs to Class Insecta, Order Diptera, and Family Calliphoridae. They are located throughout the world but are mainly distributed throughout the United States, southern Canada, Australia, and South and Central American countries (Foster, et al., 2013). Their preferred climates are warm and humid. *Lucilia sericata* progresses through an extremely time sensitive series of developmental stages (Figure 1). This life cycle consists of eggs (18-21 hours), first instar (31-53 hours), second instar (12-42 hours), third instar (40-92 hours), pupae (7-10 days), and finally they emerge as a young adult and develop into sexually mature adults. The instar stages comprise the larval state of their lives and the pupal stage is the equivalent to a butterfly's cocoon stage. Development varies with diet, temperature, humidity, and other factors and the ranges listed above are based on the fastest and slowest developments observed.

http://digitalinsectcollection.wikispaces.com/Common+Green+B

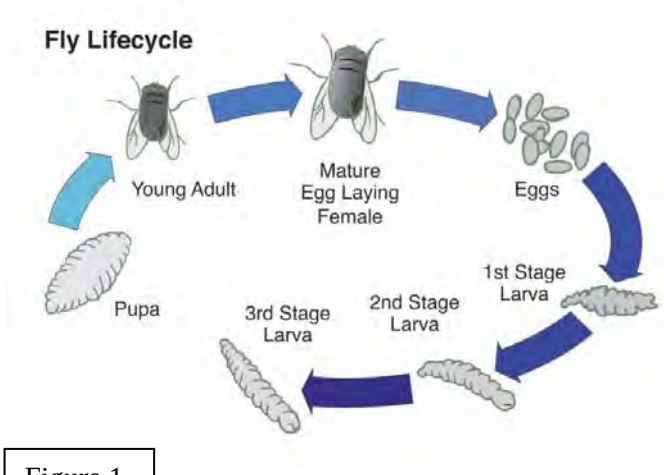


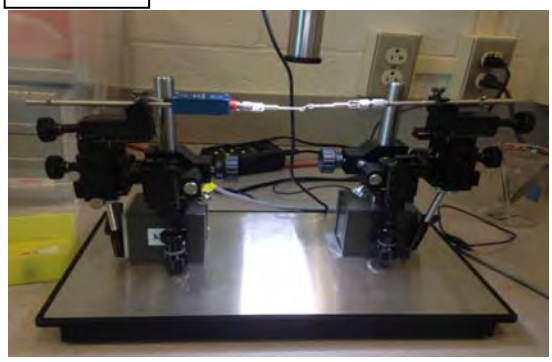
Figure 1

This time sensitive development is one of the reasons *L. sericata* is one of the most important species to the field of forensic entomology (Anderson, 2000). *L. sericata* is a primary colonizer of carrion. Organic matter begins to decay moments after death due to anaerobic bacteria, causing the release of volatile organic compounds. These volatile organic compounds (VOCs) attract females and can do so within two to four

hours of elemental exposure on the dead body (Reibe, 2010). Females lay eggs on meat, animal corpses, infected human or animal wounds, and excrement and can oviposit clusters of up to 200 eggs at a time. Carrion is an ideal place for oviposition to occur because larvae need a high protein source to complete the three molts of the larval stages and such protein can be found in decaying or necrotic tissue. A deficiency of protein at any point in the life cycle has been observed to result in death or deformation in a later developmental stage. Forensic teams take advantage of *L. sericata* because upon finding a carcass, they can observe the prevalence of each developmental stage (considering environmental conditions) and establish a post mortem interval, which is basically an estimated time of death.

Responses to volatiles can be measured on a machine called the electroantennogram (EAG). Flies have two types of electrophysiological responses to airborne substances and they occur in the olfactory neurons of the antennal cells. These two responses are slow receptor potentials and fast action potentials. The EAG measures the slow receptor potentials which are a manifestation of the interaction of the volatile odor with dendritic endings of the receptor cells. The responses are measured in millivolts per second and appear as a peak. Because the fly's "nervous system" is separate from the rest of its body, the fly can be decapitated, mounted on the EAG, and will continue to respond for 30-60 minutes. The head is mounted onto the machine using ultrasound gel with the base of the head on one electrode tube and the antennae on the other electrode tube, completing the circuit

Figure 2



(Figure 2). A cannon like apparatus is placed on the table pointing to the specimen and humidified air is constantly flowing through the apparatus. The volatile of choice is taken up with a syringe and injected into a small hole in this apparatus and is then carried to the specimen. Upon exposure to the volatile, the specimen's electrical response is recorded.

The volatile I chose to study is indole. As mentioned earlier, females are attracted to putrefying substances and this attraction seems to be largely due to the presence of indole, skatole, and ammonium carbonate. Also, more decomposed carrion, and consequently a stronger volatile profile, presents a greater attraction to colonizers. Indole can be produced by bacterial degradation of tryptophan and naturally occurs in feces, both of which are present on carrion. Indole is unique because it has a strong fecal odor at high concentrations but a flowery odor at low concentrations. It has also been experimentally proven to be an attractant at concentrations between 0 and .04% (Cragg, 1950). I tested a concentration gradient of indole by creating a series of tenfold dilutions beginning with a stock solution. The stock solution was made with ~99% solid indole dissolved in methanol and was ~10% based on solubility of indole in the solvent. This stock solution was then used to create dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}.

The significance of the different odors is relevant to a dietary dimorphism between males and females. Males and females have different dietary needs and therefore seek different food sources. Males tend to feed on more sugary substances such as flower nectar. Females, especially gravid females, need more protein because they must prepare their bodies for the eggs they are about to host in order to have viable offspring. Males do not need as much protein as they do not produce eggs. Due to these

different dietary needs, females tend to gravitate toward decaying organic matter indicative of protein and males tend to search sugary substances like flower nectar.

Hypothesis

With an observed dietary dimorphism, I expected the number and intensity of female responses to higher concentrations of indole to be higher than male responses, with mated female responses slightly stronger than non-mated female responses. I also expected males to have a greater number and intensity of responses than females to lower concentrations of indole.

Methods

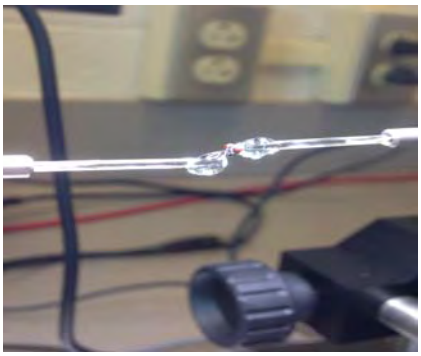
Three Bug Dorms were constructed each housing a different set of flies from the same cohort. The "Male Only" cage held 20 males: 0 females, the "Female-Mated" cage held 5 males: 15 females, and the "Female Only" cage held 20 females: 0 males. These flies were all selected from the same egging event of the same fly colony tent. The eggs were obtained by placing a piece of liver in the tent and waiting for 24 hours for females to lay eggs on it. The egg-covered liver was then placed in a jar containing vermiculite and the jar was given several pieces of liver every other day until pupation occurred (Figure 1). After pupation, the pupae were placed individually into small cups until emergence. Once emerged, the flies were separated by sex and assigned to each cage accordingly. The flies from the Female-Mated cage all emerged on the same day and all of the flies from the Female Only and Male Only cages emerged on the same day. The day of emergence is marked as Day 1.

Each cage was maintained under identical conditions including humidity, temperature, light cycle, and diet to eliminate confounding variables. The average humidity was 40%, the average temperature was 28°C, and the light cycle was 12 hours light: 12 hours dark, alternating at 8:00 in the morning and night. The diet consisted of honey water and liver given every other day with honey water given for the first time on the first day and liver given for the first time on the third day. Water delivery apparatus was refilled as needed and was available *ad libitum*.

For each round of testing, three flies were selected from each cage. With the times of emergence, three Female-Mated flies were tested on one day and the next day

six flies (three Female Only and three Male Only) were tested. The experiment consisted of an alternation between these testing groups. Tests were conducted on Day 3, Day 5, Day 7, and Day 9. Flies were selected using scintillation vials. Once the EAG was equilibrated (machine and software turned on, electrode tubing rinsed with DI water and subsequently KCL, and ultrasound gel placed on electrode tubing), the fly to be tested was placed in the freezer for approximately five minutes to impart temporary paralysis. After the fly is paralyzed, it is removed from the freezer and the head was removed using a razor blade. Then, using needle nose tweezers, the head was carefully placed onto the electrode tubing with the base of the head mounted onto the right electrode and the antennae onto the left electrode (Figure 3).

Figure 3



For VOC delivery, a cannon-like apparatus is connected to a bottle of bubbling water by a tube and constantly pushes humidified air toward the mounted specimen. The quality of the mount was tested by running the EAG software and measuring the mounted fly's response to humidified air. If the reading is typical of a humidified air response (see images in Results) then the testing may continue. First, the humidified air was pushed through the cannon to act as a negative control and baseline. Then the second negative control, ambient air, was injected into a small hole in the cannon, which directs whatever substance is injected onto the specimen, using a gas syringe. Methanol, the last negative control, was projected in the same manner. The variable volatiles, Indole 10^{-6}, Indole 10^{-5}, Indole 10^{-4}, Indole 10^{-3}, Indole 10^{-2}, Indole10^{-1}, and Indole stock solution, were then projected in the order as listed (lowest concentration first, then sequential exposures

through the highest concentration). Next, the positive control volatiles, Dimethyl Disulfide 10^{-3} and Dimethyl Disulfide 10^{-2}, were injected in the order as listed. Finally, humidified air was projected to ensure that the fly was not erroneously responding. Each volatile was injected for a total of three trials before moving on to the next volatile.

When each fly has been exposed to all of the volatiles in the series, it is removed from the machine and discarded. Between each trial, the electrode tubes must be rinsed with DI water and KCl and new ultrasound gel must be applied before the start of the next trial.

Antennal depolarizations were recorded using a Syntech EAG system equipped with a 4-channel IDAC integration unit. EAG Pro Software was used to record the antennal depolarization spectrum for the volatile exposures. Results were interpreted using the maximal depolarization for each volatile exposure using Image J software to determine peak height.

Results

Results were not as distinct as expected, however, some trends emerged that both supported my hypothesis and proposed questions for further research. The results were quantified by intensity of responses. Using a program called Image-J, the baseline and peak height of the depolarization could be measured. If the reading only showed a baseline, it was not considered a response; only visible peaks created by depolarizations were considered responses. Images of readings from the EAG can be found in Appendix A. Because each fly has a different baseline, it would be statistically inaccurate to calculate the mean responses of all flies, however, individual and mean response values for each fly to each volatile can be found in Appendix B. Mean responses were calculated by subtracting the mean of the baseline response to methanol from the mean of the response to the specific volatile because methanol was the solvent. The intensities for only those flies that had true peak responses can be found in Appendix C, along with graphs demonstrating relationship between intensity of response and dilution of indole.

The results can also be quantified by number of responses of each cage to each volatile, disregarding age. For Indole 10^{-6} five mated females, three non-mated females, and four males responded for a total of twelve responses. For Indole 10^{-5} four mated females, two non-mated females, and four males responded for a total of ten responses. For Indole 10^{-4} four mated females, three non-mated females, and three males responded for a total of ten responses. For Indole 10^{-3} three mated females, three non-mated females, and three males responded for a total of nine responses. For Indole 10^{-2} two mated females, two non-mated females, and one male responded for a total of five responses. For Indole 10^{-1} two mated females, two non-mated females, and zero males

responded for a total of four responses. For Indole Stock one mated female and one non-mated female responded for a total of two responses. No peaks were seen for humidified air, ambient air, Dimethyl Disulfide 10^{-3}, or Dimethyl Disulfide 10^{-2}. This information is summarized in Table 1 in Appendix E.

Discussion

As suggested in my hypothesis, more females responded to higher concentrations of indole than males and males responded more to lower concentrations of indole than higher concentrations, however, results were not consistent enough to confidently confirm it. Overall, females were more responsive than males, mated females being more responsive than non-mated females. This was expected because the females, especially mated females, are in need of food. An unexpected trend that was noticed was that more flies responded to lower concentrations, however, the intensity of the responses increased with increasing concentrations. For females, it is reasonable for them to respond to lower concentrations because although they need large portions of protein, they also feed on sugary substances if present. The same concept applies to the males responding to higher concentrations: although they do not need as much protein, they will still feed on protein if it is presented to them.

Another trend that was noticed that has been seen in previous literature was a significant change in response at Day 5 and Day 7. As seen in Figure 4 and Figure 5, there is a tremendous change in response Day 3 to Day 5 and Day 5 to Day 7. Because our flies were only checked for emergence once every 24 hours, the flies could have been 1 hour old or 23 hours old. The possibility of flies having this age range being put in the same experimental group paired with the developmental sensitivity to time may explain any large discrepancies in responses during the crucial Day 5 and Day 7. With implied importance on Day 5 and Day 7, it would be interesting to conduct more experiments to try and get a grasp on what is occurring in that time. Ideally, an experiment would be conducted in which fly responses could be recorded on the hour but with the nature of the

EAG, realistically, an experiment testing fly responses each day could provide insight into what is happening during this time period. Also, a longer timeline could provide more information concerning large changes of response between days. For example, in extending the testing past Day 9 responses may prove to be cyclically increasing and decreasing or other patterns may emerge.

An unexpected result was a lack of responses to Dimethyl Disulfide. Previous experiments have demonstrated a positive response to Dimethyl Disulfide and none of the flies in this experiment responded (Blystone, 2014). This may be due to olfactory fatigue because Dimethyl Disulfide was the last volatile to be projected. Also, there seemed to be a general drop in response at approximately Indole 10^{-3}. According to the literature, concentrations around 0.04% are used as an attractant, which is close to the Indole 10^{-3} solution. Being exposed to a known attractant at such a close proximity is likely to overwhelm the olfactory receptors, causing them to be less responsive until they return to their baseline. A method of preventing olfactory fatigue is to allow more time between each exposure to each volatile. By increasing time between exposures, the olfactory neurons are allowed to repolarize to their normal level of responsiveness. The only complication of increased rest period for the fly is that the severed fly head only responds for approximately 40 minutes after decapitation so fitting in all volatile exposures before diminished response is difficult. Now that results show more flies responding to lower concentrations and imply fatigue being reached around Indole 10^{-3}, changing the spectrum of dilutions may provide interesting results. With all of these factors, research using lower concentrations of indole has great potential to be very fruitful.

References

Anderson GS. 2000. Minimum and maximum development rates of some forensically important Callophoridae (Diptera). Journal of Forensic Science 45: 824-832

Ashworth, J. R. and Wall, R. (1994), Responses of the sheep blowflies Lucilia sericata and Lcuprina to odour and the development of semiochemical baits. Medical and Veterinary Entomology, 8: 303–309. doi: 10.1111/j.1365-2915.1994.tb00093.x

Blystone, Allissa M. “Effects of Age, Diet, and Sex on the Gustatory and Olfactory Sensing Capabilities of the Forensically Important Blow Fly, *Lucilia sericata* (Diptera: Calliphoridae). Diss. University of Dayton, 2014. Print.

Cragg, J.B. (1950). The reactions of Lucilia sericata (Mg.) to various substances placed on sheep. Parasitology, 40, pp 179-186. doi:10.1017/S0031182000018011.

Foster, John E. et al. "A Molecular Key For The Identification Of Blow Flies In Southeastern Nebraska * A Molecular Key For The Identification Of Blow Flies In Southeastern Nebraska." *Journal Of Forensic Sciences (Blackwell Publishing Limited)* 58.1 (2013): 173-178. *Academic Search Complete*. Web. 29 Mar.

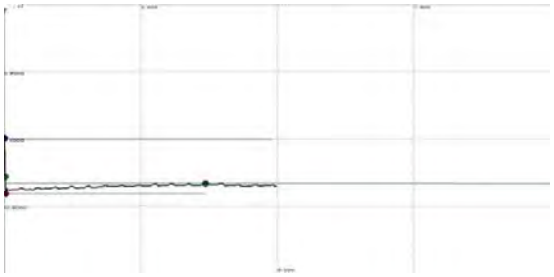
Hobson, E. P. (1936), Sheep Blow-Fly Investigations III. Observations on the Chemotropism of Lucilia Sericata mg. Annals of Applied Biology, 23: 845–851. doi: 10.1111/j.1744-7348.1936.tb06130.x

Reibe, S., and B. Madea. "How promptly do blowflies colonise fresh carcasses? A study comparing indoor with outdoor locations." *Forensic science international* 195.1 (2010): 52-57.

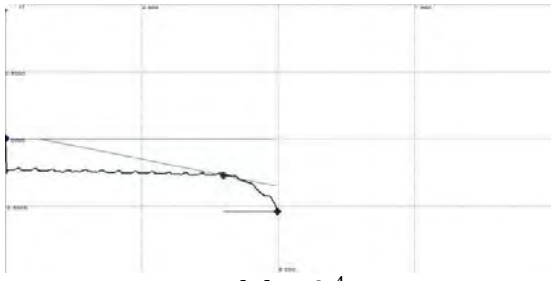
Appendix A

Day 3 Female-Mate Cage, Female 1

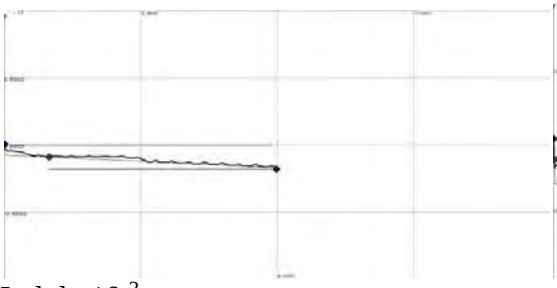
Humidified Air



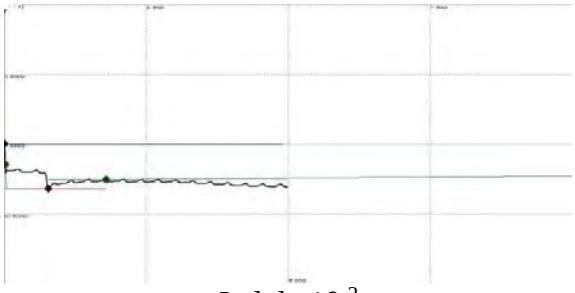
Ambient Air



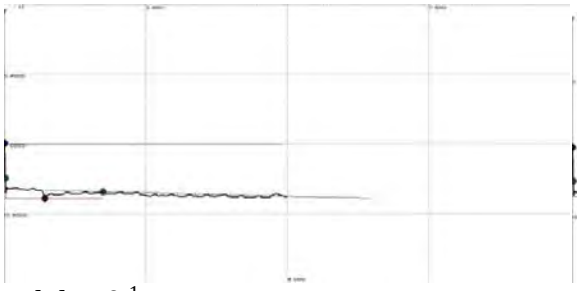
Methanol



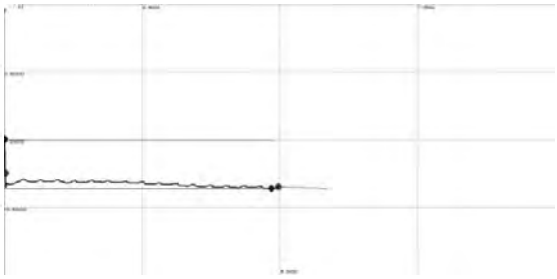
Indole 10^{-4}



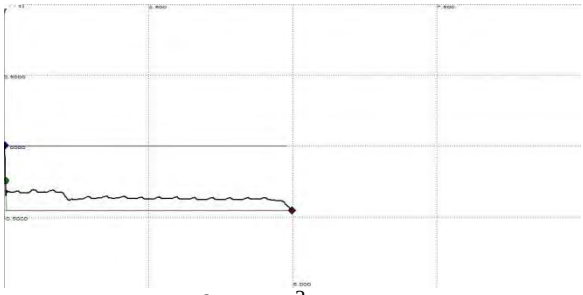
Indole 10^{-3}



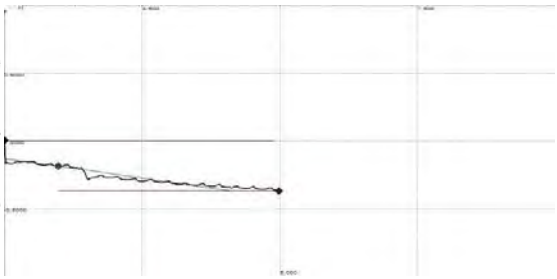
Indole 10^{-2}



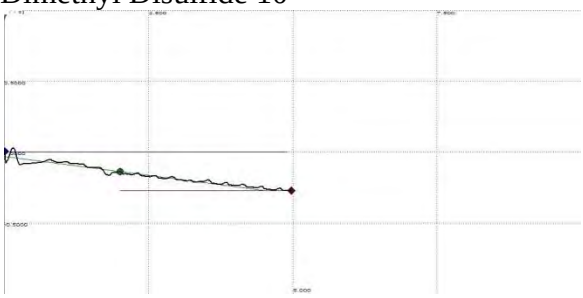
Indole 10^{-1}



Indole Stock

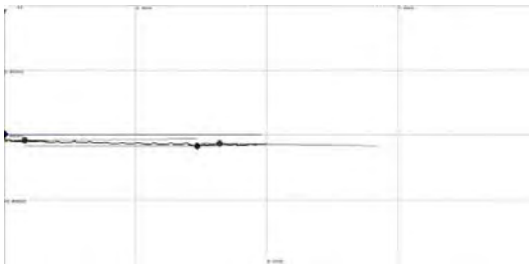


Dimethyl Disulfide 10^{-3}

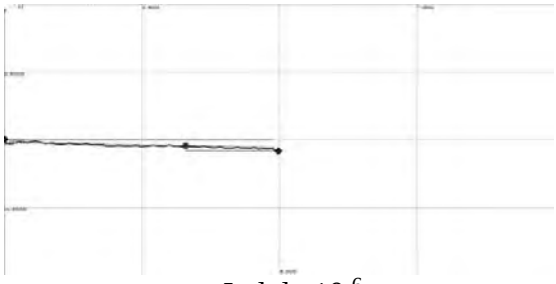


Day 3 Female-Only Cage, Female 3

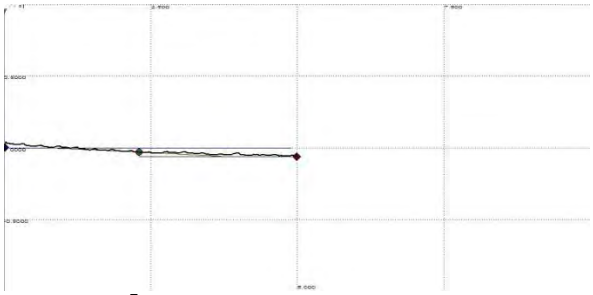
Humidified Air



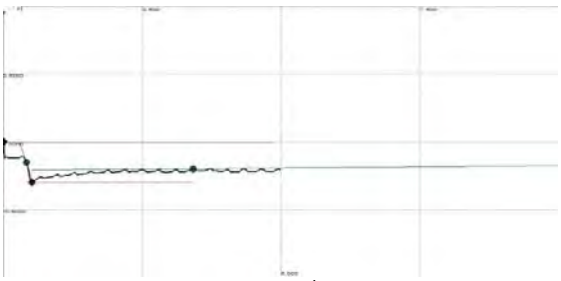
Ambient Air



Methanol



Indole 10^{-6}



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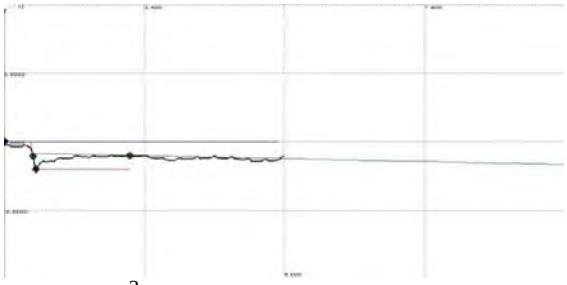
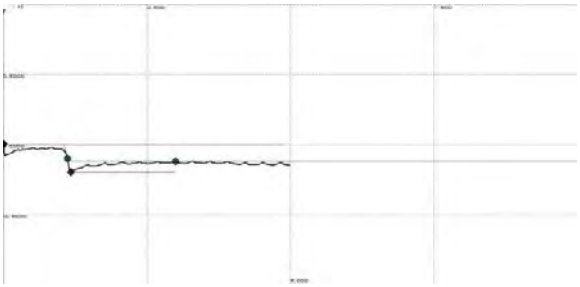
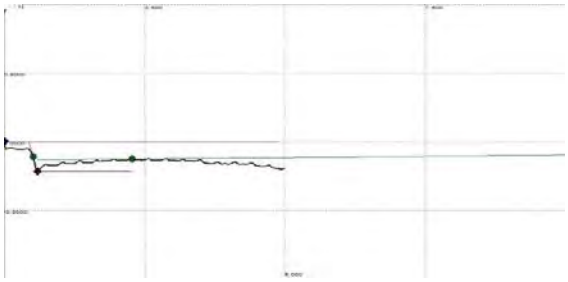


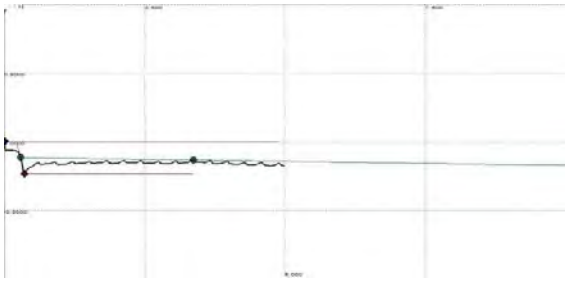
Indole 10^{-4}



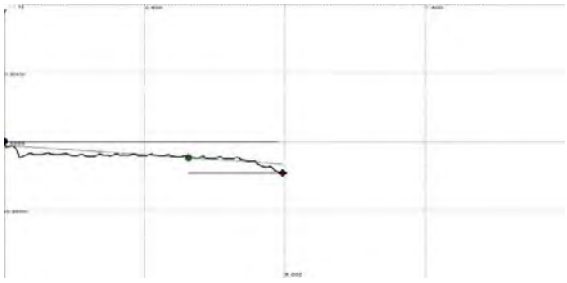
Indole 10^{-3}



Indole 10^{-2}



Indole 10^{-1}



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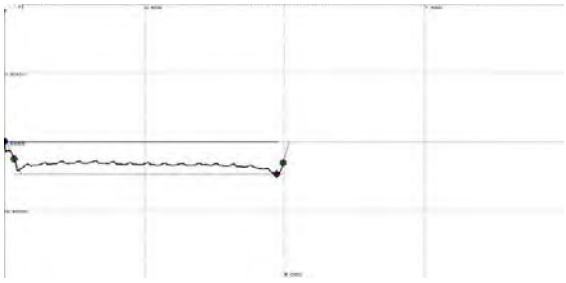
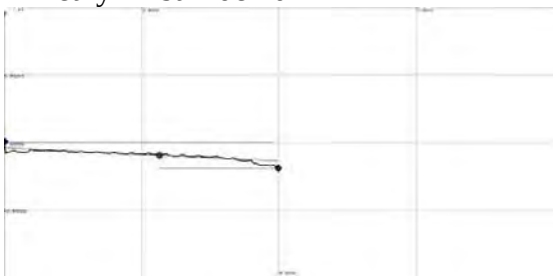
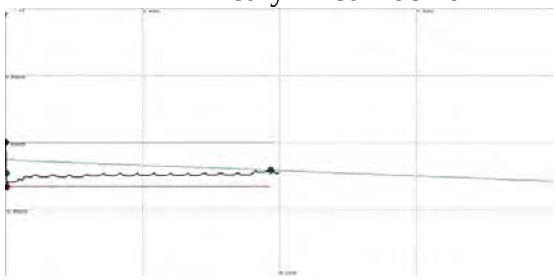


Dimethyl Disulfide 10^{-3}

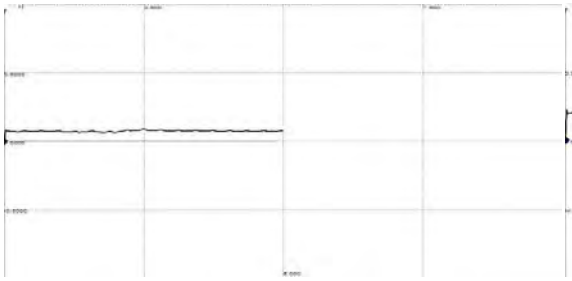


Dimethyl Disulfide 10^{-2}

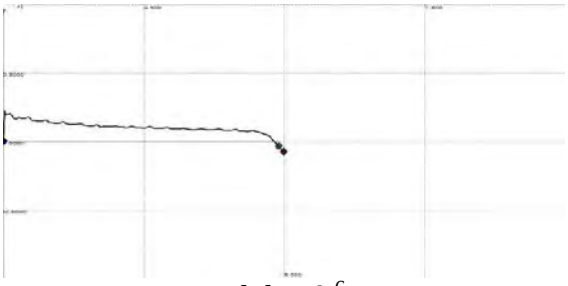


Day 3 Male Only Cage, Male 1

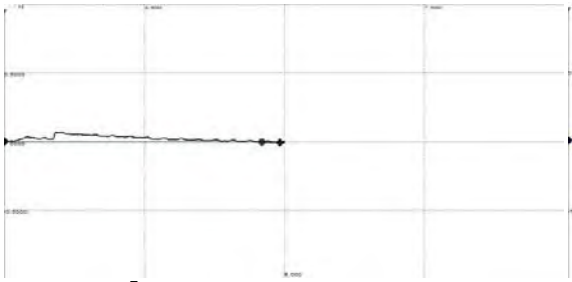
Humidified Air



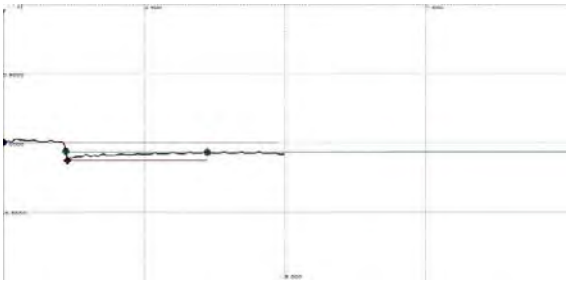
Ambient Air



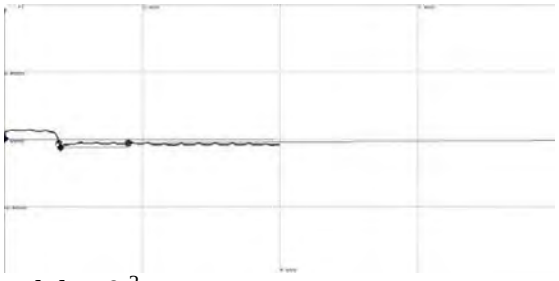
Methanol



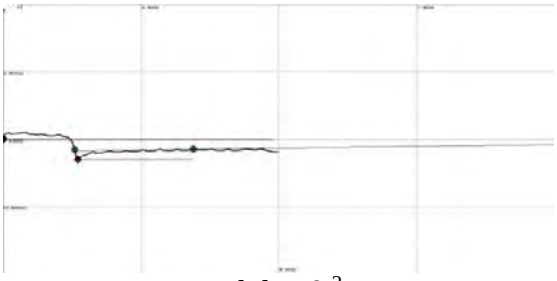
Indole 10^{-6}



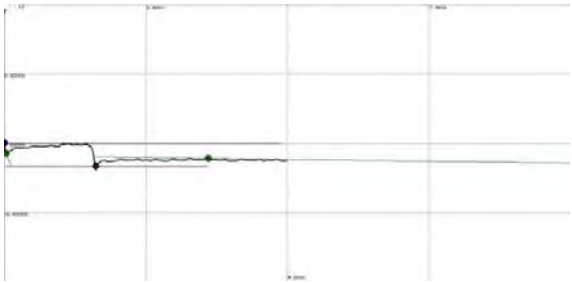
Indole 10^{-5}



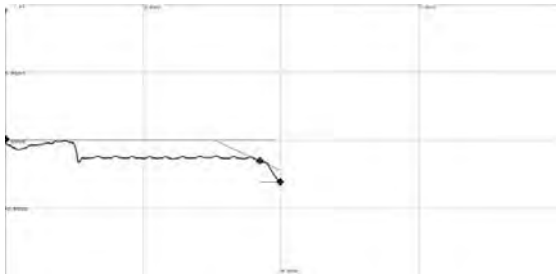
Indole 10^{-4}



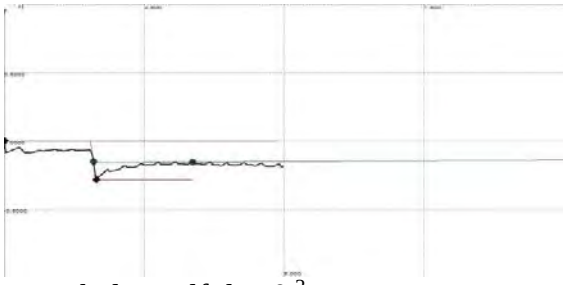
Indole 10^{-3}



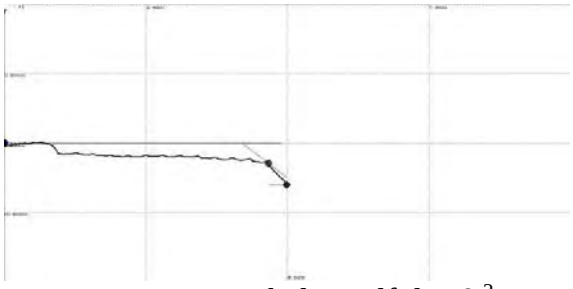
Indole 10^{-2}



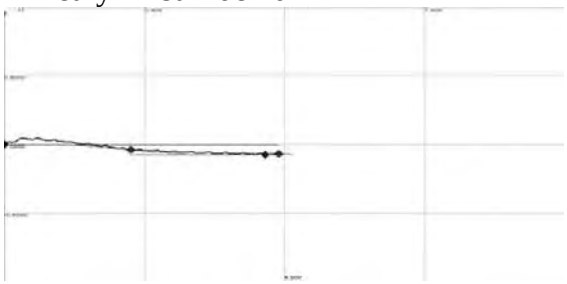
Indole 10^{-1}



Indole Stock



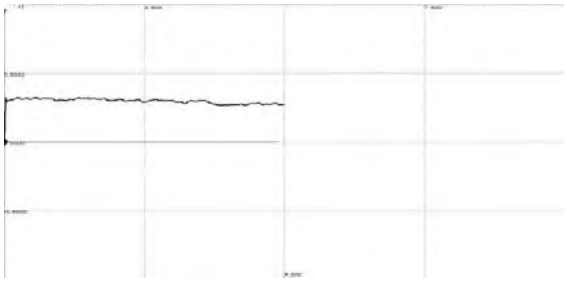
Dimethyl Disulfide 10^{-3}



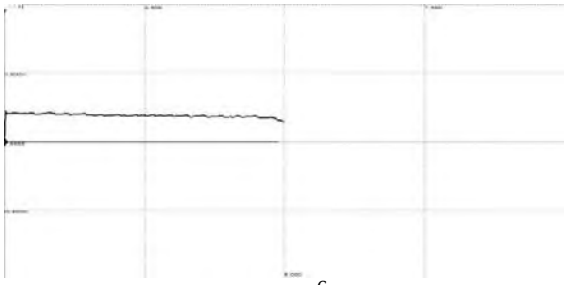
Dimethyl Disulfide 10^{-2}

Day 5 Female-Mated Cage, Female 1

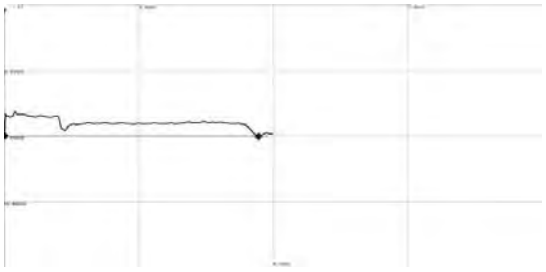
Humidified Air



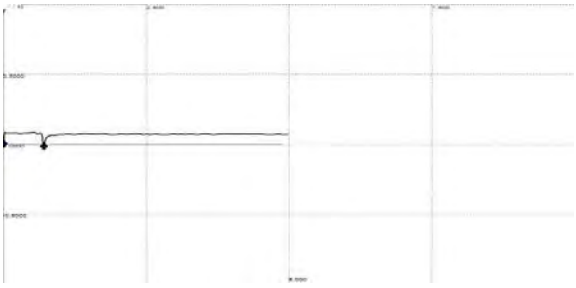
Ambient Air



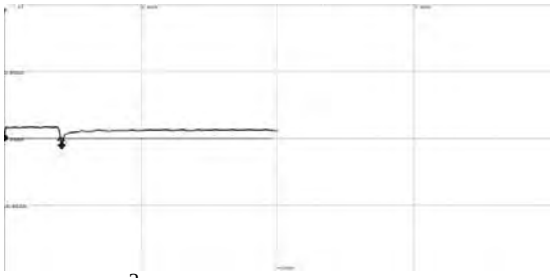
Methanol



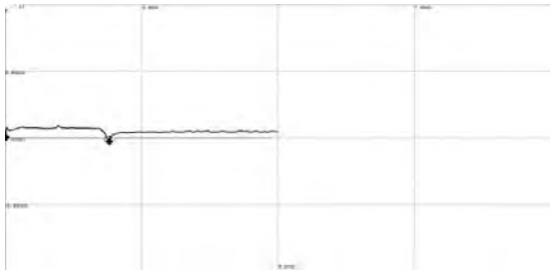
Indole 10^{-6}



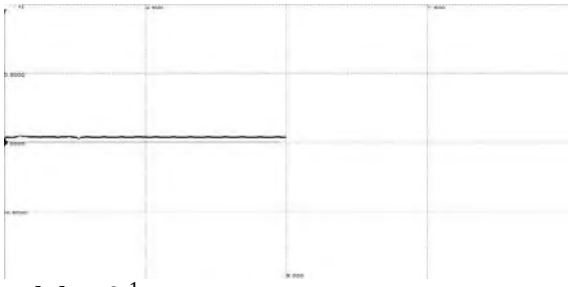
Indole 10^{-5}



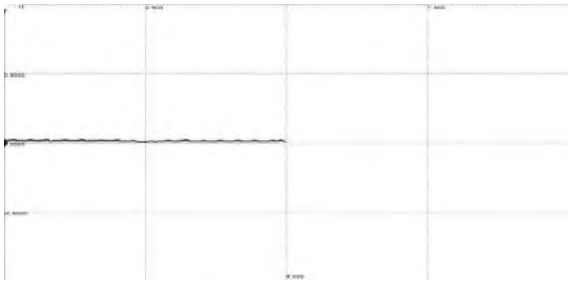
Indole 10^{-4}



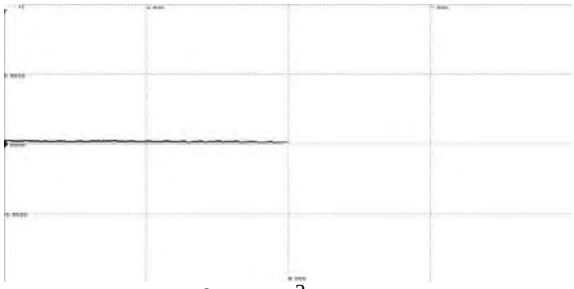
Indole 10^{-3}



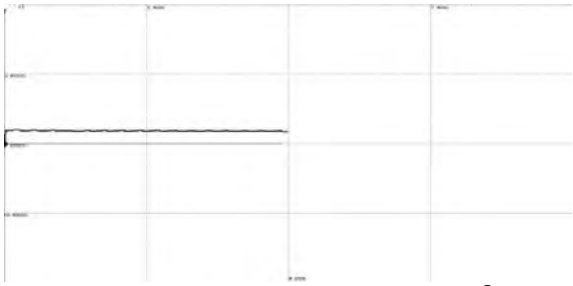
Indole 10^{-2}



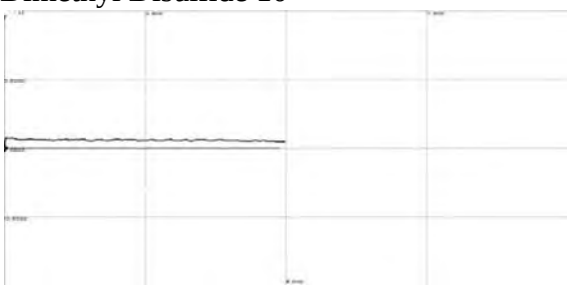
Indole 10^{-1}



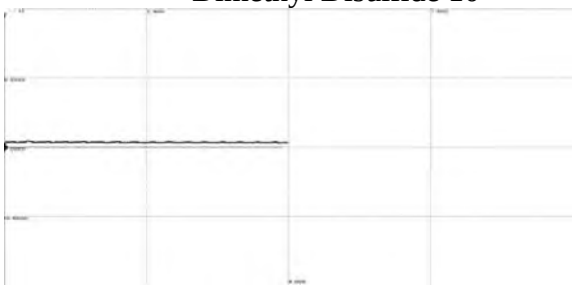
Indole Stock



Dimethyl Disulfide 10^{-3}

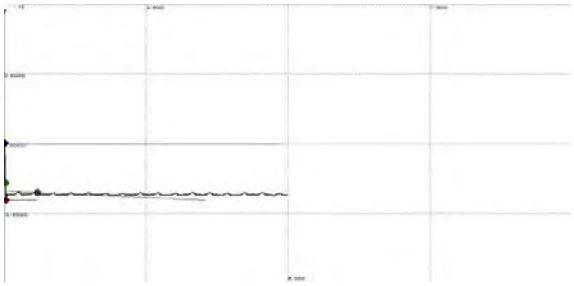


Dimethyl Disulfide 10^{-2}

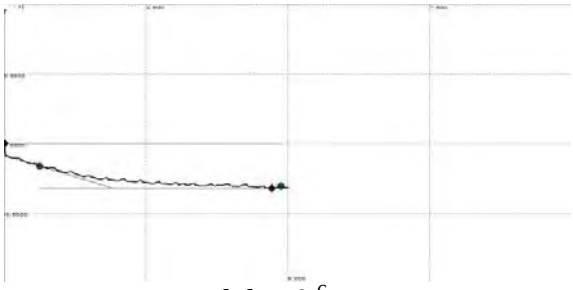


Day 5 Female Only Cage, Female 1

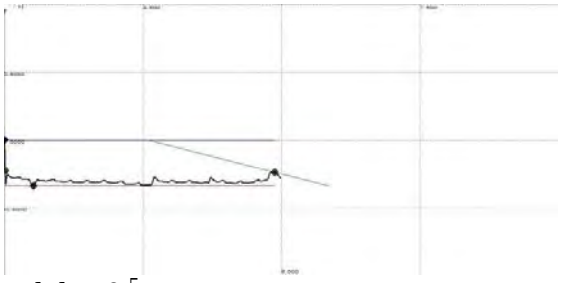
Humidified Air



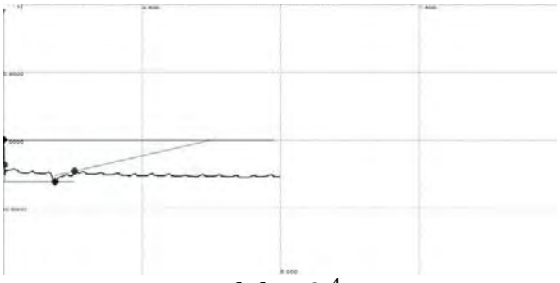
Ambient Air



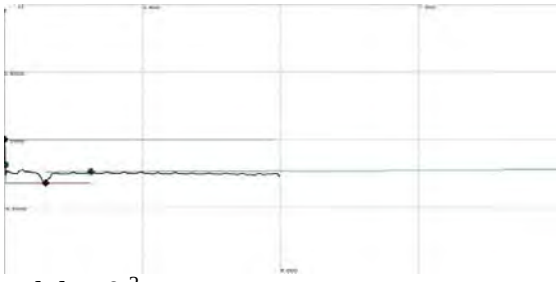
Methanol



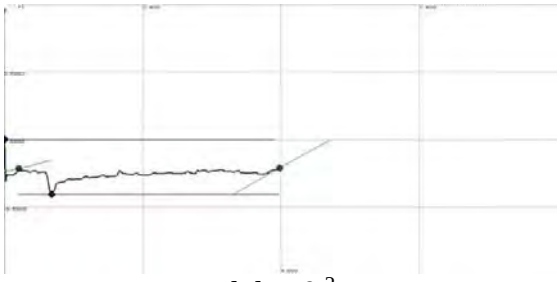
Indole 10^{-6}



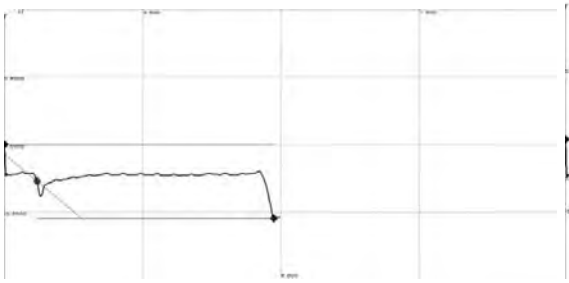
Indole 10^{-5}



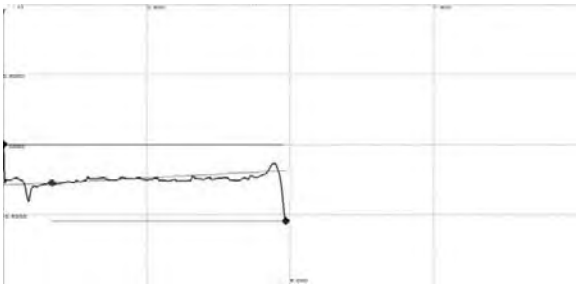
Indole 10^{-4}



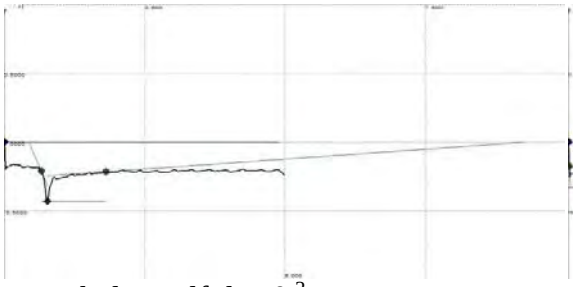
Indole 10^{-3}



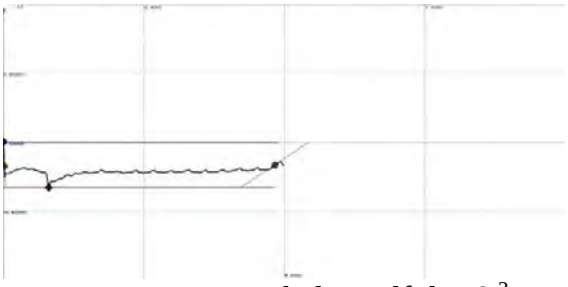
Indole 10^{-2}



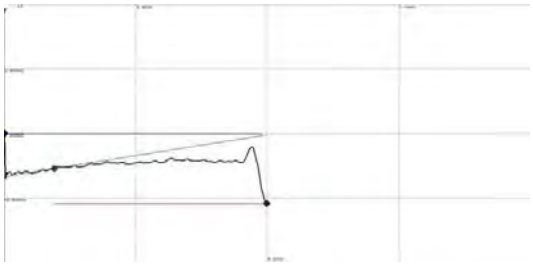
Indole 10^{-1}



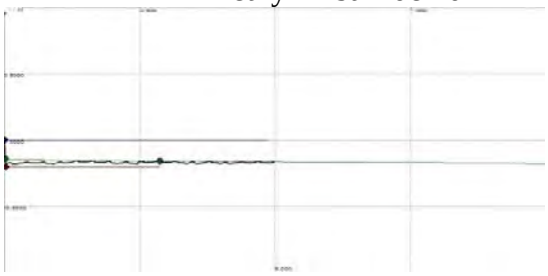
Indole Stock



Dimethyl Disulfide 10^{-3}

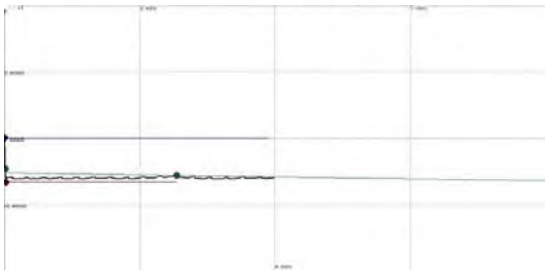


Dimethyl Disulfide 10^{-2}

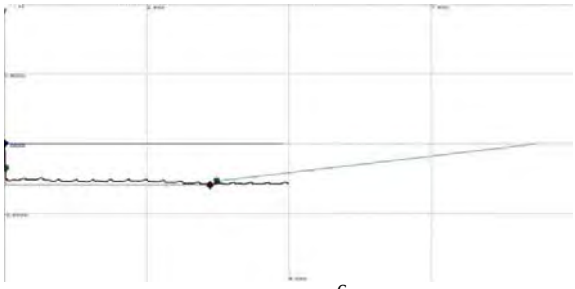


Day 5 Male Only

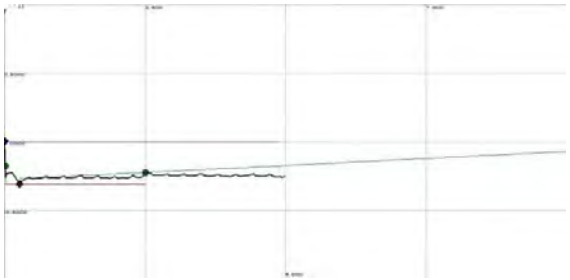
Humidified Air



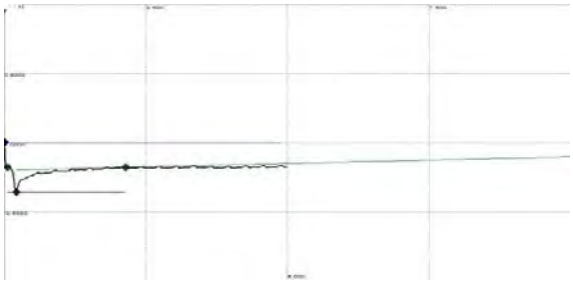
Ambient Air



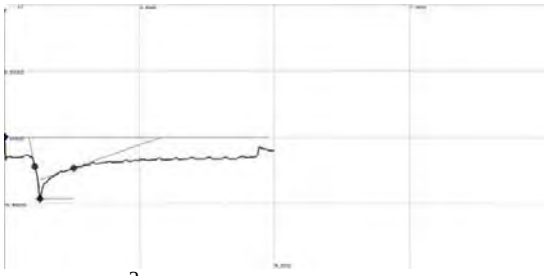
Methanol



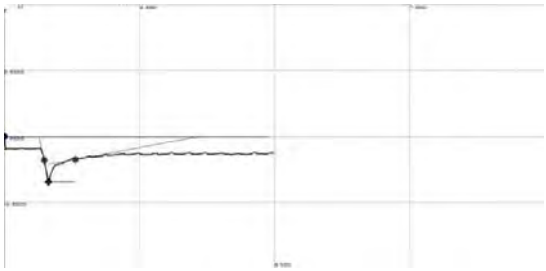
Indole 10^{-6}



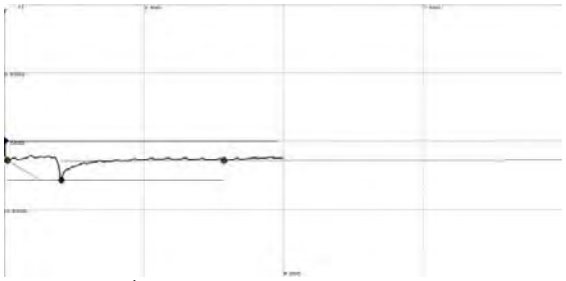
Indole 10^{-5}



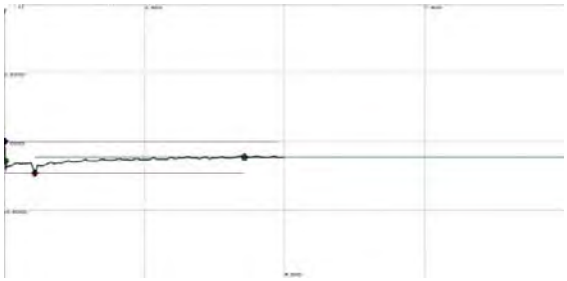
Indole 10^{-4}



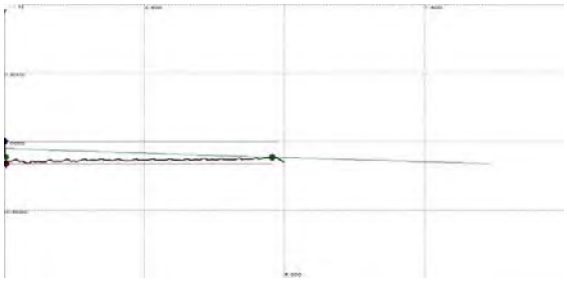
Indole 10^{-3}



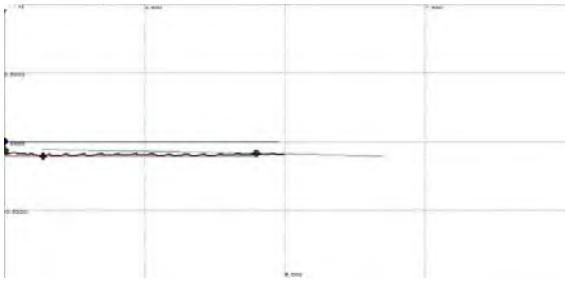
Indole 10^{-2}



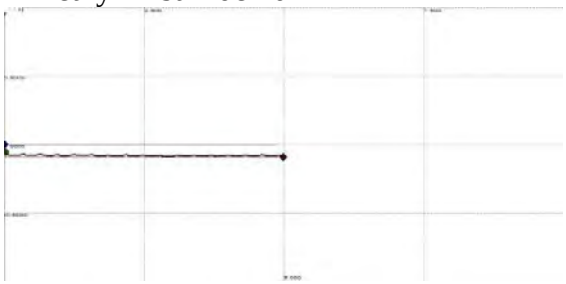
Indole 10^{-1}



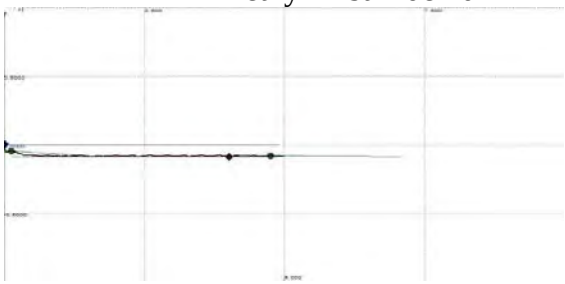
Indole Stock



Dimethyl Disulfide 10^{-3}

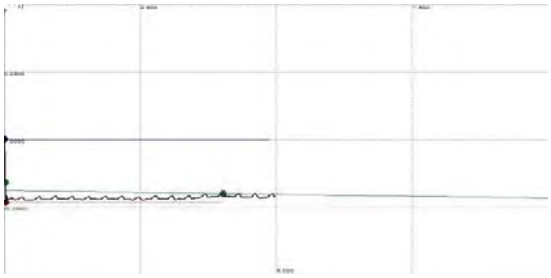


Dimethyl Disulfide 10^{-2}

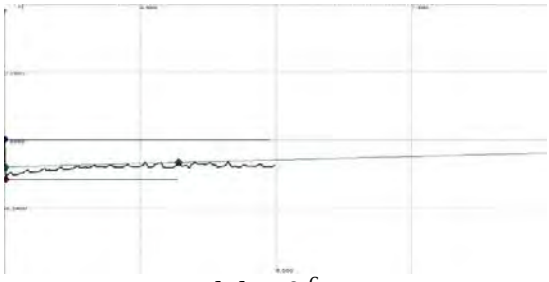


Day 7 Female-Mated Cage, Female 2

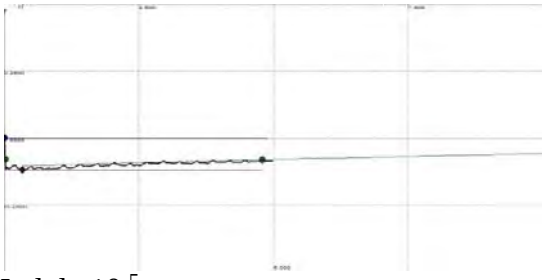
Humidified Air



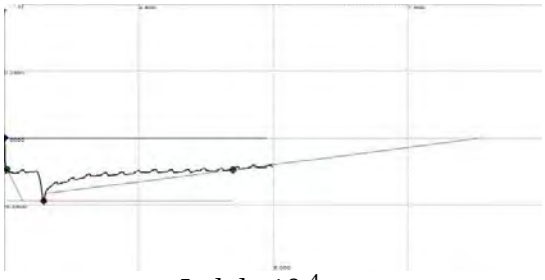
Ambient Air



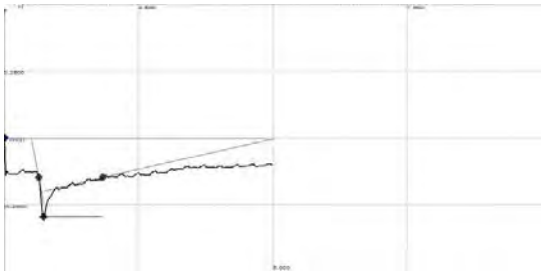
Methanol



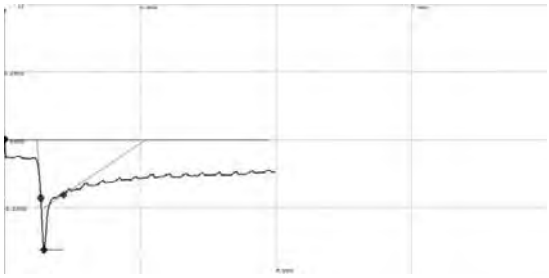
Indole 10^{-6}



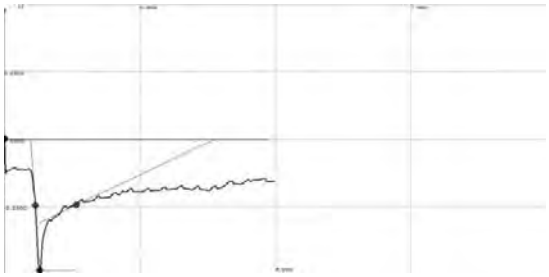
Indole 10^{-5}



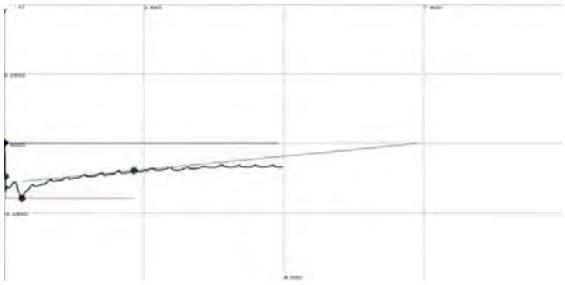
Indole 10^{-4}



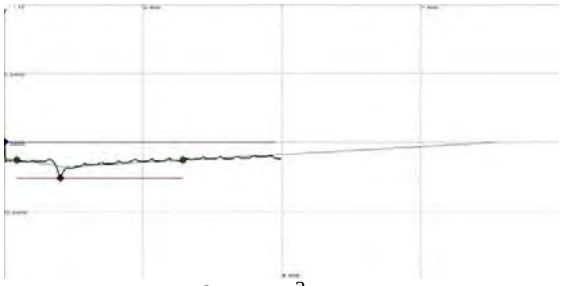
Indole 10^{-3}



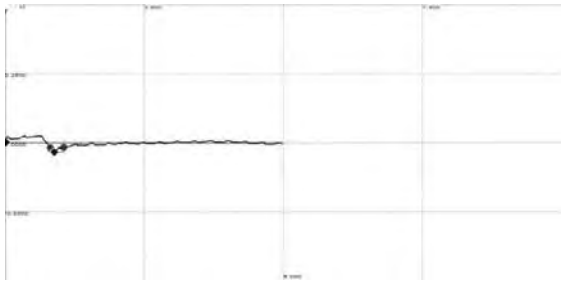
Indole 10^{-2}



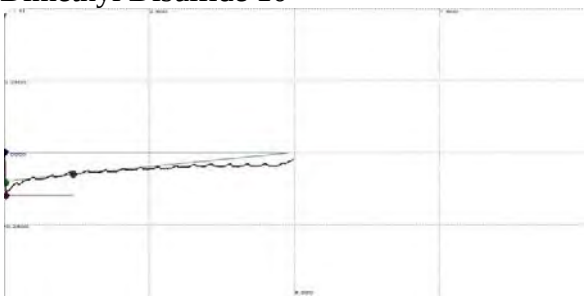
Indole 10^{-1}



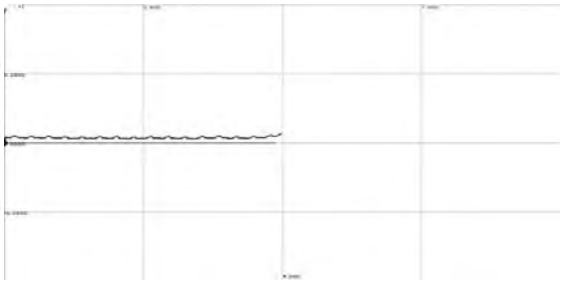
Indole Stock



Dimethyl Disulfide 10^{-3}

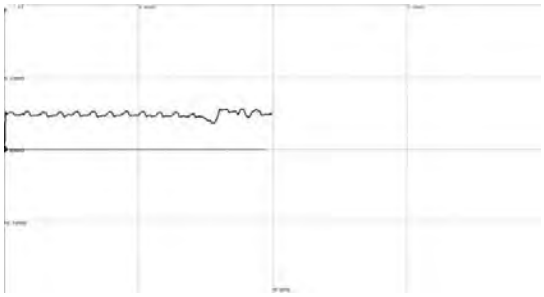


Dimethyl Disulfide 10^{-2}

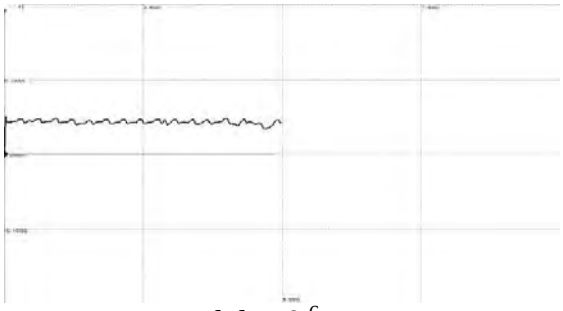


Day 7 Female Only Cage, Female 1

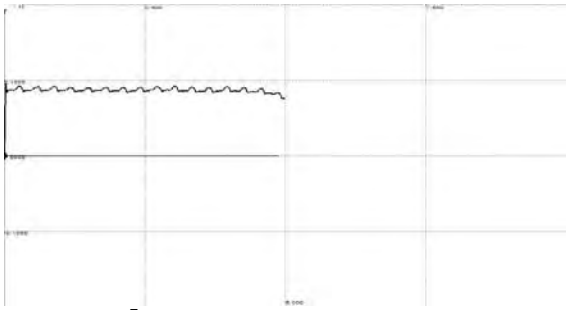
Humidified Air



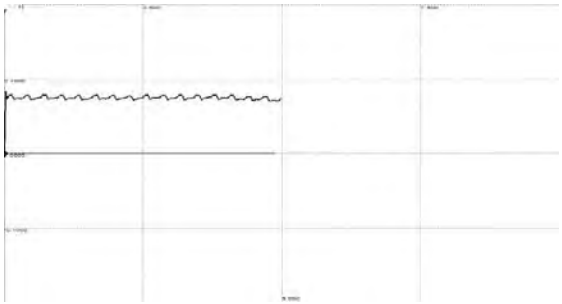
Ambient Air



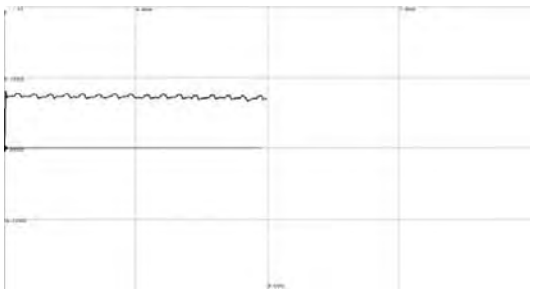
Methanol



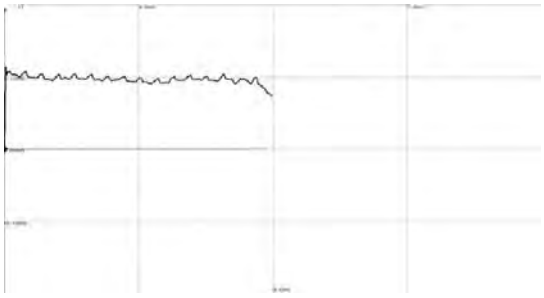
Indole 10^{-6}



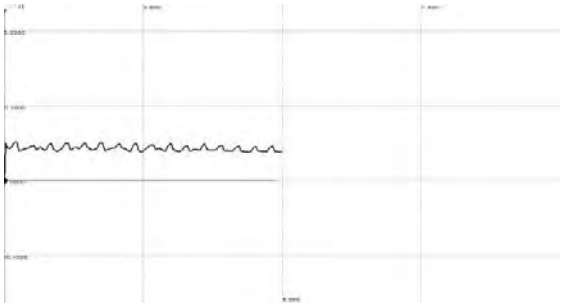
Indole 10^{-5}



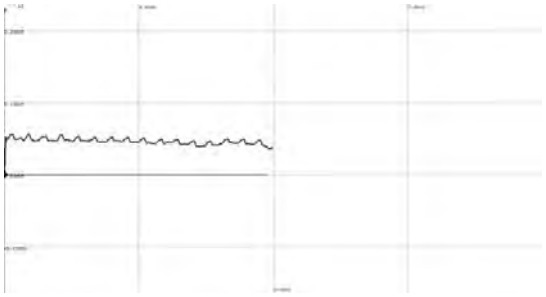
Indole 10^{-4}



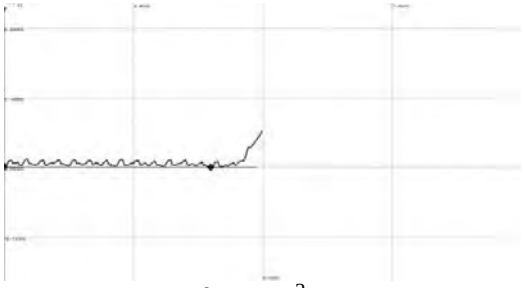
Indole 10^{-3}



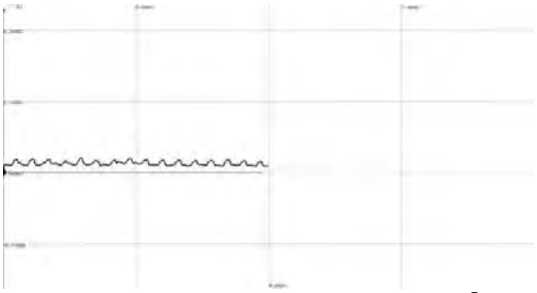
Indole 10^{-2}



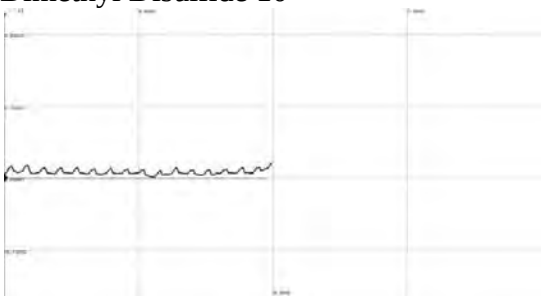
Indole 10^{-1}



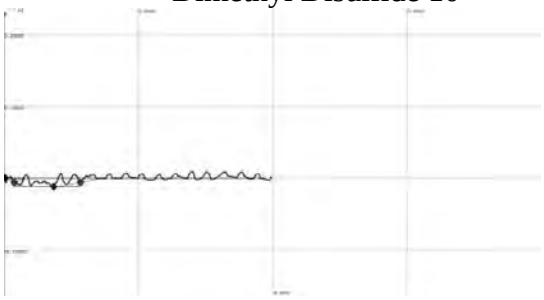
Indole Stock



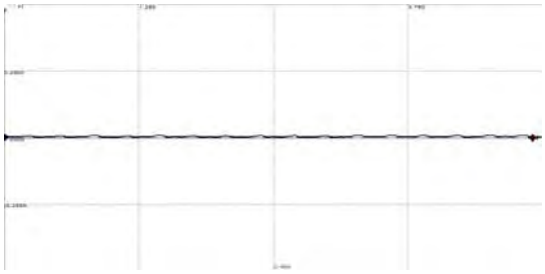
Dimethyl Disulfide 10^{-3}



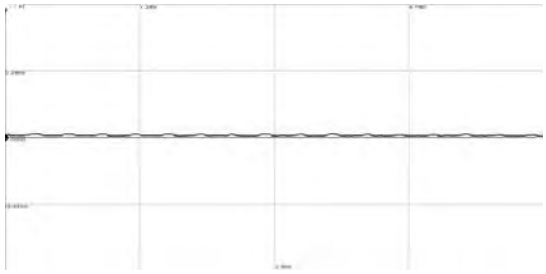
Dimethyl Disulfide 10^{-2}



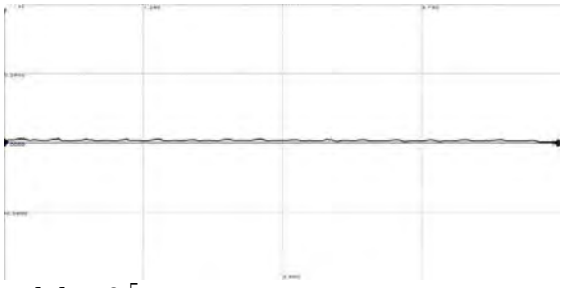
Day 7 Male Only, Male Humidified Air



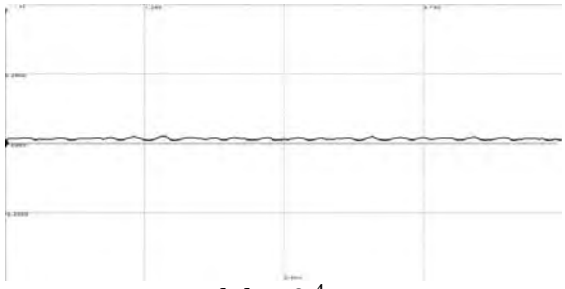
Ambient Air



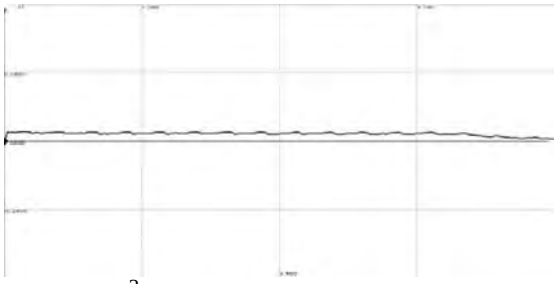
Methanol



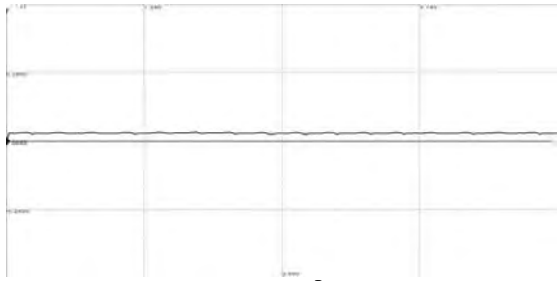
Indole 10^{-6}



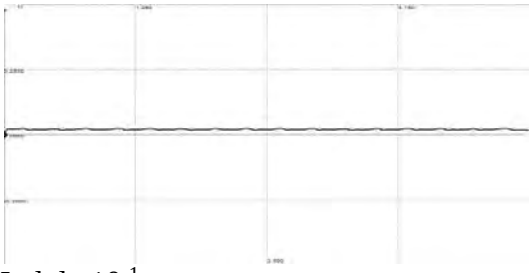
Indole 10^{-5}



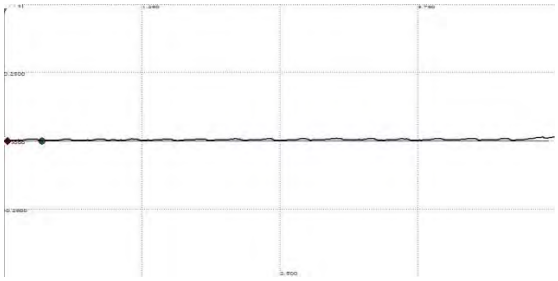
Indole 10^{-4}



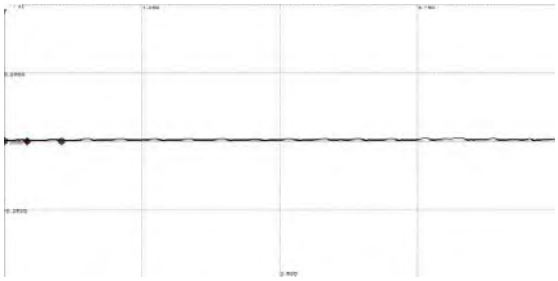
Indole 10^{-3}



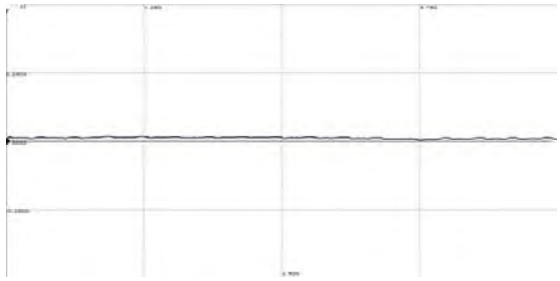
Indole 10^{-2}



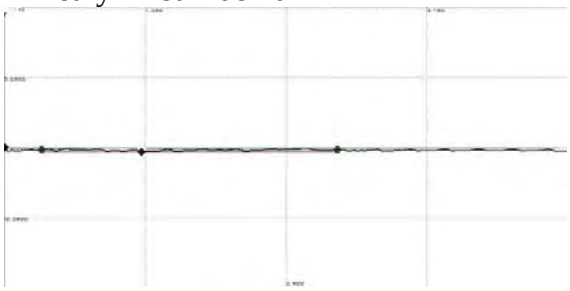
Indole 10^{-1}



Indole Stock



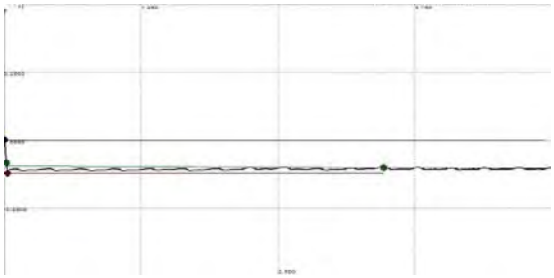
Dimethyl Disulfide 10^{-3}



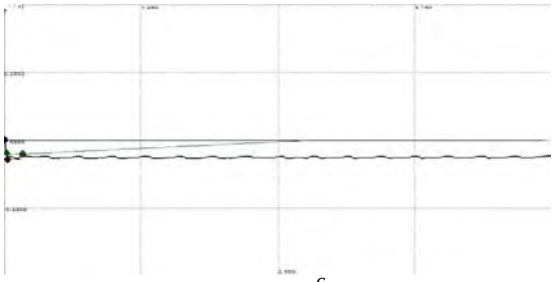
Dimethyl Disulfide 10^{-2}

Day 9 Female-Mated Cage, Female 1

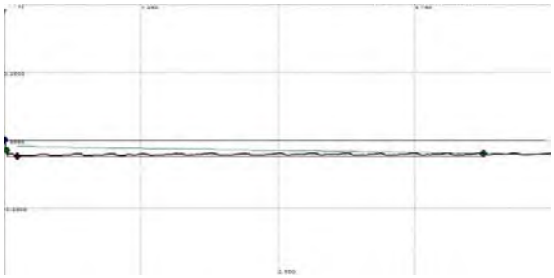
Humidified Air



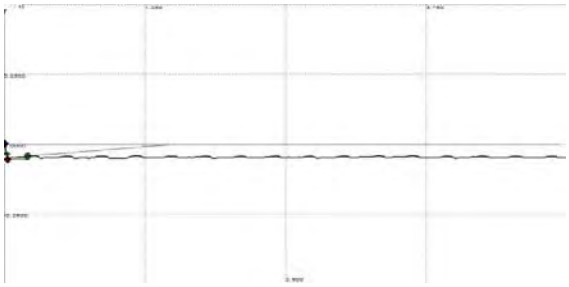
Ambient Air



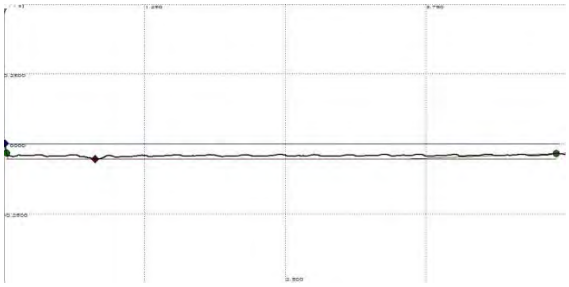
Methanol



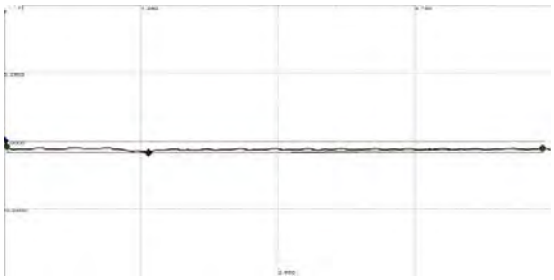
Indole 10^{-6}



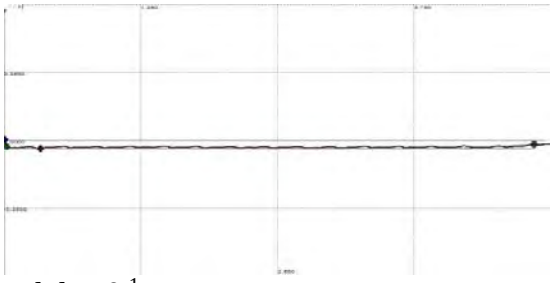
Indole 10^{-5}



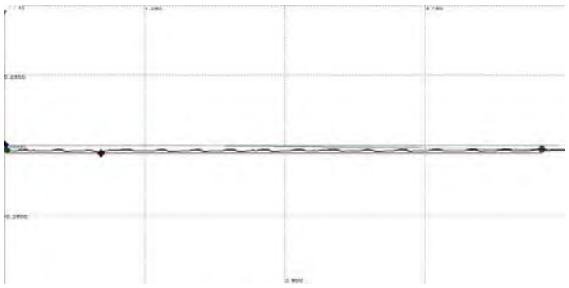
Indole 10^{-4}



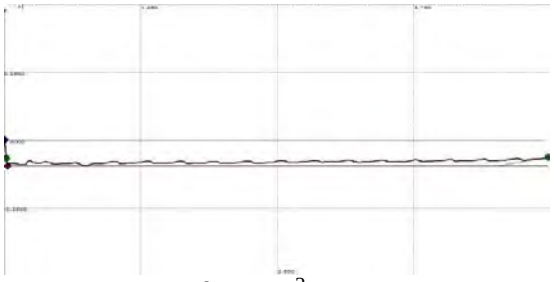
Indole 10^{-3}



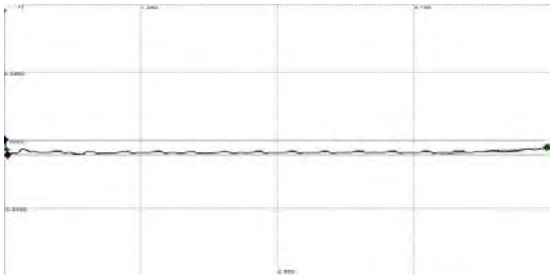
Indole 10^{-2}



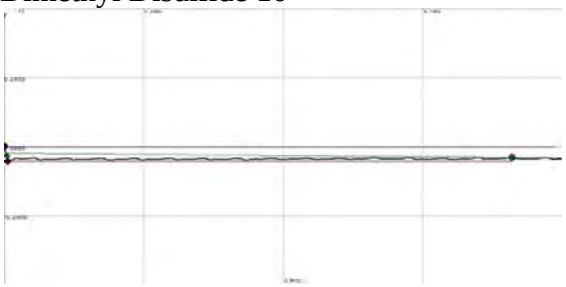
Indole 10^{-1}



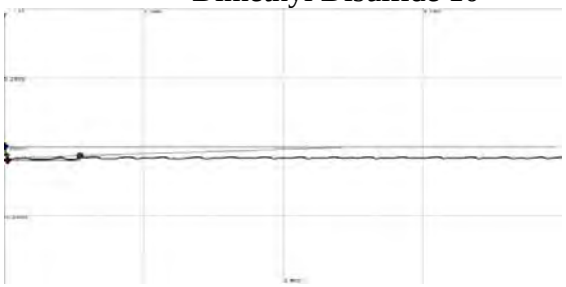
Indole Stock



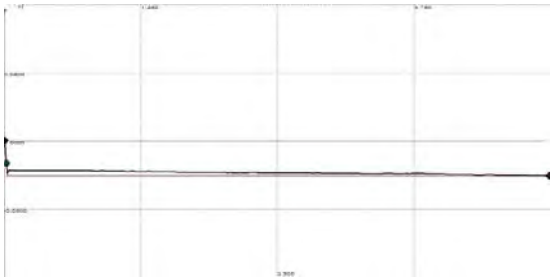
Dimethyl Disulfide 10^{-3}



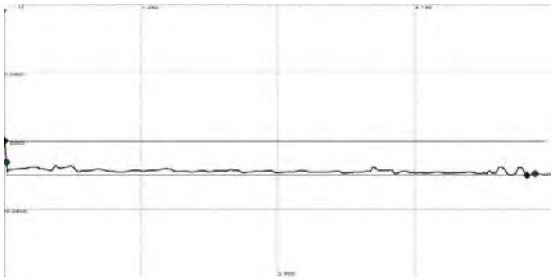
Dimethyl Disulfide 10^{-2}



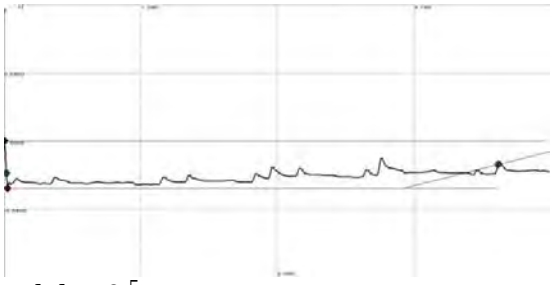
Day 9 Female Only Cage, Female Humidified Air



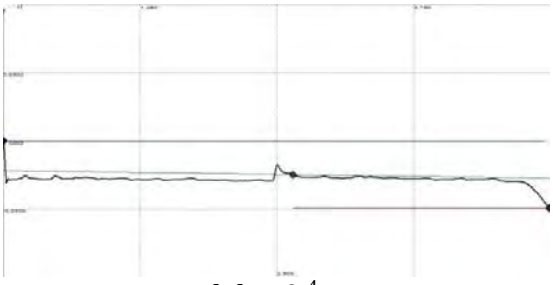
Ambient Air



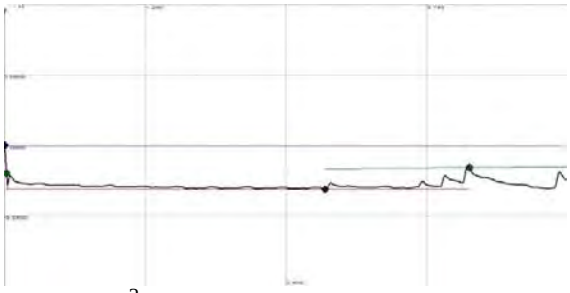
Methanol



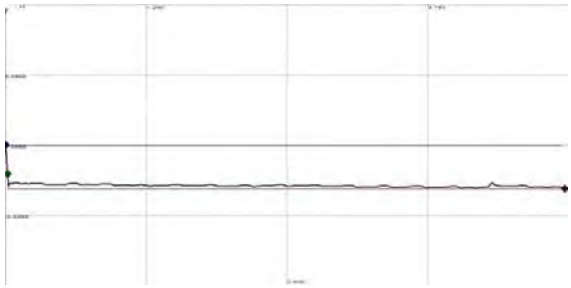
Indole 10^{-6}



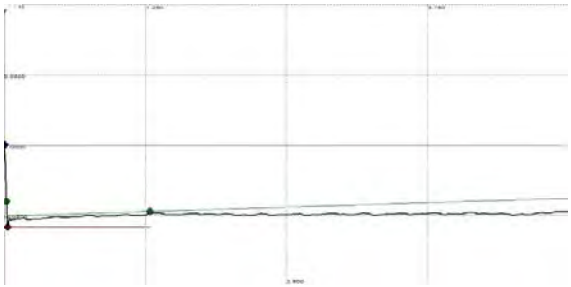
Indole 10^{-5}



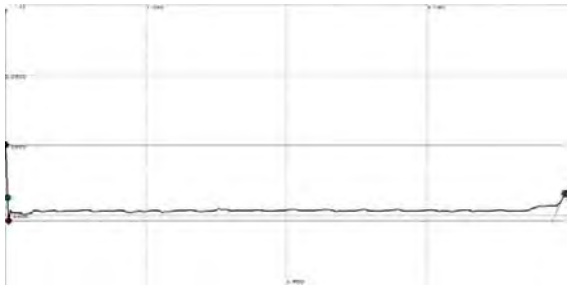
Indole 10^{-4}



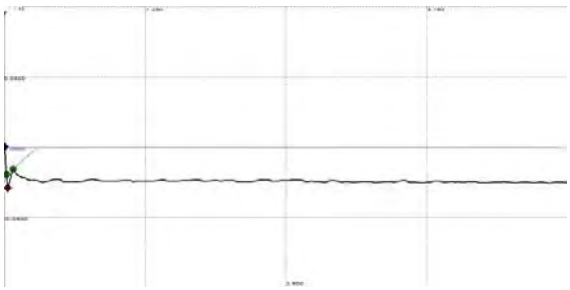
Indole 10^{-3}



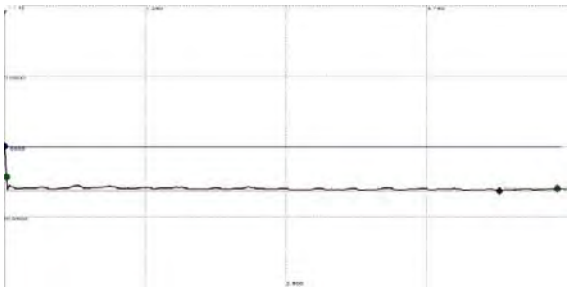
Indole 10^{-2}



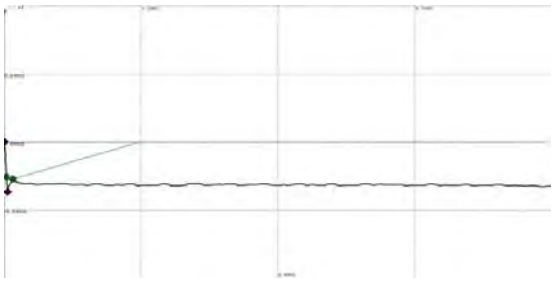
Indole 10^{-1}



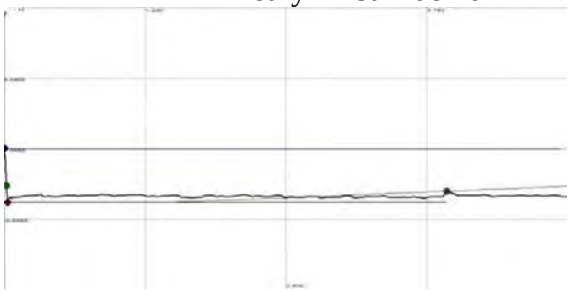
Indole Stock



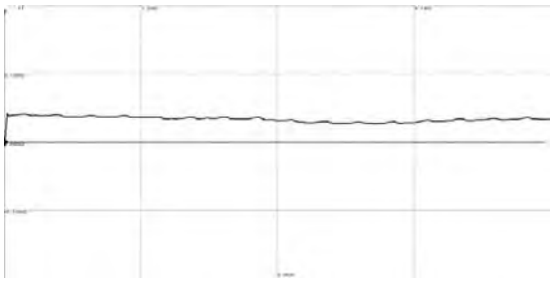
Dimethyl Disulfide 10^{-3}



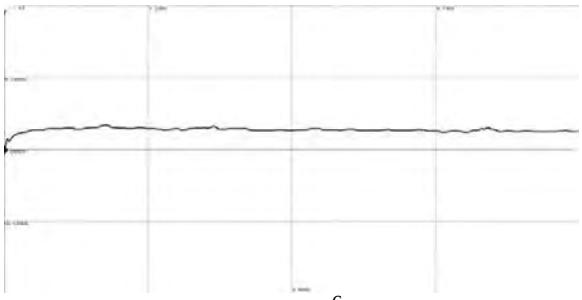
Dimethyl Disulfide 10^{-2}



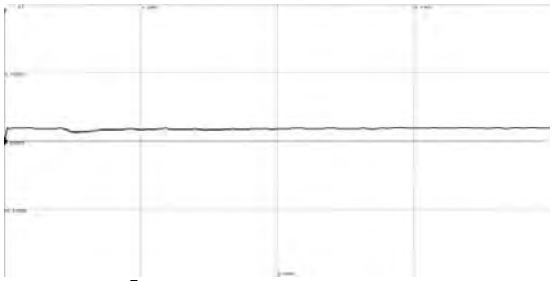
Day 9 Male Only Cage, Male Humidified Air



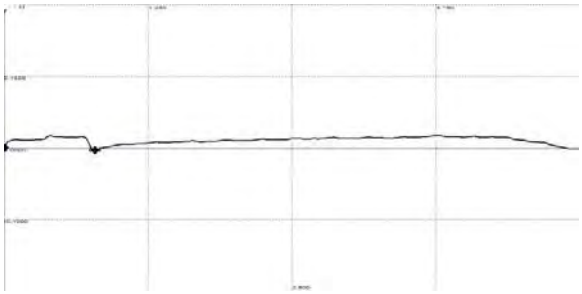
Ambient Air



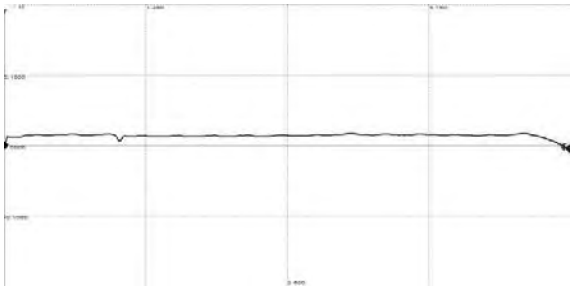
Methanol



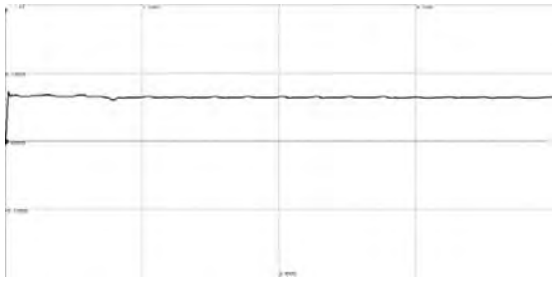
Indole 10^{-6}



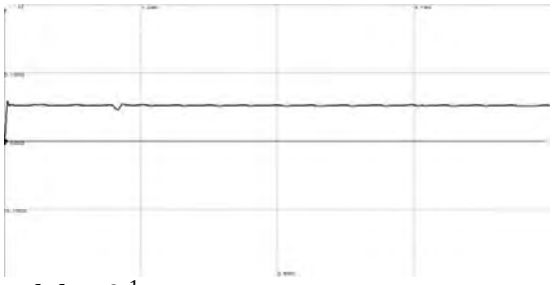
Indole 10^{-5}



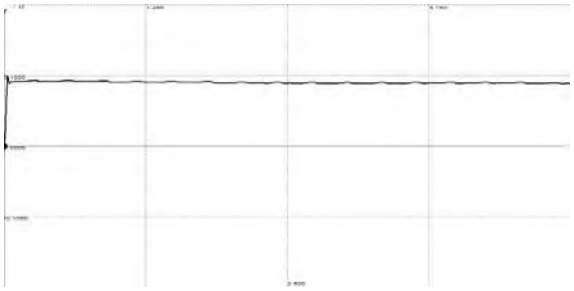
Indole 10^{-4}



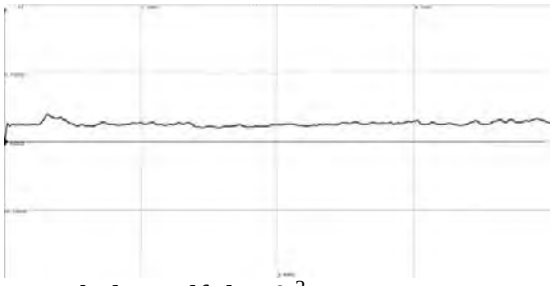
Indole 10^{-3}



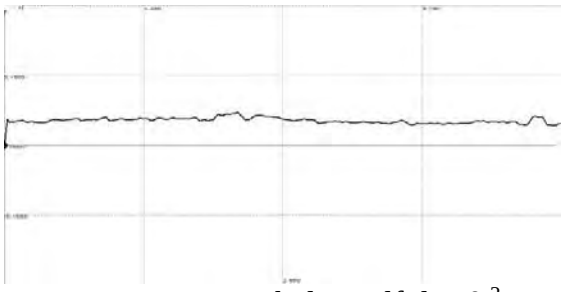
Indole 10^{-2}



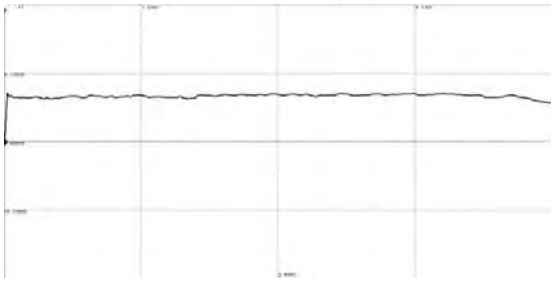
Indole 10^{-1}



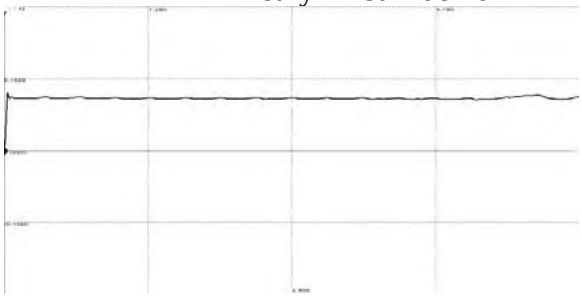
Indole Stock



Dimethyl Disulfide 10^{-3}



Dimethyl Disulfide 10^{-2}



Appendix B

Key:

HA: humidified air AA: ambient air I-6: indole 10^{-6} I-5: indole 10^{-5}

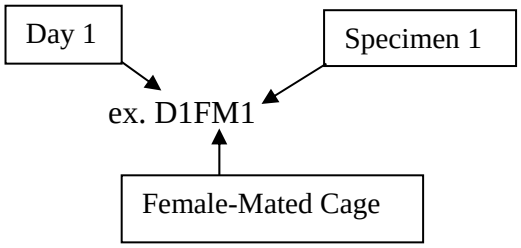
I-4: indole 10^{-4} I-3: indole 10^{-3} I-2: indole 10^{-2} I-1: indole 10^{-1}

IS: indole stock DMDS-3: Dimethyl Disulfide 10^{-3} DMDS-2: Dimethyl Disulfide 10^{-2}

FM: female-mated FO: female-only MO: male only

length: peak height avg.: mean s.d.: standard deviation

Flies abbreviated as Day (x), Cage, Number of Specimen



D3FM1	trial	length	avg.	s.d.
HA	1	0.323	0.3345	0.023
	2	0.346		
AA	1	0.252	0.2243333	0.025775
	2	0.201		
	3	0.22		
MeOH	1	0.102	0.1453333	0.071619
	2	0.106		
	3	0.228		
I-4	1	0.374	0.315	0.054009
	2	0.303		
	3	0.268		
I-3	1	0.362	0.3016667	0.057292
	2	0.248		
	3	0.295		
I-2	1	0.193	0.277	0.072856
	2	0.323		
	3	0.315		
I-1	1	0.374	0.2846667	0.147856
	2	0.114		
	3	0.366		
IS	1	0.299	0.29	0.012288
	2	0.276		
	3	0.295		
DMDS-3	1	0.166	0.193	0.037643
	2	0.177		
	3	0.236		

D3FM2	trial	length	avg.	s.d.
HA	1	0.346	0.2846667	0.113231
	2	0.354		
	3	0.154		
AA	1	0.118	0.202	0.096234
	2	0.181		
	3	0.307		
MeOH	1	0.248	0.248	-
I-4	1	0.327	0.2166667	0.118745
	2	0.091		
	3	0.232		
I-3	1	0.28	0.2063333	0.07115
	2	0.138		
	3	0.201		
I-2	1	0.193	0.1496667	0.047427
	2	0.157		
	3	0.099		
I-1	1	0.189	0.109	0.069311
	2	0.067		
	3	0.071		
IS	1	0.02	0.0226667	0.004619
	2	0.028		
	3	0.02		
DMDS-3	1	0.11	0.1116667	0.017559
	2	0.13		
	3	0.095		

D3FM3	trial	length	avg.	s.d.
HA	1	0.327	0.3425	0.031
	2	0.358		
AA	1	0.043	0.0866667	0.041669
	2	0.126		
	3	0.091		
MeOH	1	0.047	0.0366667	0.014572
	2	0.043		
	3	0.02		
I-4	1	0.024	0.042	0.022338
	2	0.035		
	3	0.067		
I-3	1	0.051	0.0356667	0.015503
	2	0.036		
	3	0.02		
I-2	1	0.106	0.0733333	0.033531
	2	0.039		
	3	0.075		
I-1	1	0.024	0.041	0.029445
	2	0.024		
	3	0.075		
IS	1	0.028	0.0276667	0.003512
	2	0.031		
	3	0.024		
DMDS-3	1	0.039	0.0656667	0.02444
	2	0.071		
	3	0.087		

D3FO1	trial	length	avg.	s.d.
HA	1	0.382	0.382	0
	2	0.382		
AA	1	0.343	0.3373333	0.016258
	2	0.319		
	3	0.35		
MeOH	1	0.307	0.336	0.037242
	2	0.323		
	3	0.378		
I-6	1	0.358	0.3476667	0.014572
	2	0.331		
	3	0.354		
I-5	1	0.339	0.323	0.031241
	2	0.343		
	3	0.287		
I-4	1	0.327	0.3203333	0.018903
	2	0.299		
	3	0.335		
I-3	1	0.323	0.3056667	0.018037
	2	0.307		
	3	0.287		
I-2	1	0.346	0.3373333	0.007767
	2	0.335		
	3	0.331		
I-1	1	0.287	0.2963333	0.016166
	2	0.287		
	3	0.315		
IS	1	0.291	0.303	0.012
	2	0.315		
	3	0.303		
DMDS-3	1	0.28	0.2756667	0.017898
	2	0.256		
	3	0.291		
DMDS-2	1	0.28	0.298	0.016093
	2	0.311		
	3	0.303		

D3FO2	trial	length	avg.	s.d.
HA	1	0.252	0.246	0.012
	2	0.24		
AA	1	0.047	0.088	0.048816
	2	0.075		
	3	0.142		
MeOH	1	0.098	0.0733333	0.021455
	2	0.063		
	3	0.059		
I-6	1	0.114	0.1036667	0.021455
	2	0.118		
	3	0.079		
I-5	1	0.067	0.0526667	0.035726
	2	0.012		
	3	0.079		
I-4	1	0.087	0.0856667	0.014048
	2	0.099		
	3	0.071		
I-3	1	0.049	0.0396667	0.009018
	2	0.039		
	3	0.031		
I-2	1	0.059	0.0446667	0.028361
	2	0.012		
	3	0.063		
I-1	1	0.13	0.164	0.030512
	2	0.189		
	3	0.173		
IS	1	0.012	0.0616667	0.047226
	2	0.067		
	3	0.106		
DMDS-3	1	0.071	0.05	0.023896
	2	0.024		
	3	0.055		
DMDS-2	1	0.13	0.0773333	0.046307
	2	0.059		
	3	0.043		

D3FO3	trial	length	avg.	s.d.
HA	1	0.063	0.1103333	0.081984
	2	0.063		
	3	0.205		
AA	1	0.079	0.0813333	0.043547
	2	0.126		
	3	0.039		
MeOH	1	0.161	0.101	0.067446
	2	0.114		
	3	0.028		
I-6	1	0.24	0.1703333	0.067159
	2	0.106		
	3	0.165		
I-5	1	0.288	0.188	0.107093
	2	0.075		
	3	0.201		
I-4	1	0.138	0.147	0.038301
	2	0.189		
	3	0.114		
I-3	1	0.209	0.0893333	0.103645
	2	0.028		
	3	0.031		
I-2	1	0.11	0.193	0.07398
	2	0.217		
	3	0.252		
I-1	1	0.039	0.1033333	0.061273
	2	0.161		
	3	0.11		
IS	1	0.169	0.1046667	0.059719
	2	0.051		
	3	0.094		
DMDS-3	1	0.075	0.087	0.010583
	2	0.091		
	3	0.095		
DMDS-2	1	0.114	0.164	0.066903
	2	0.138		
	3	0.24		

D3MO1	trial	length	avg.	s.d.
HA	1	0.122	0.0893333	0.031565
	2	0.087		
	3	0.059		
AA	1	0.118	0.1023333	0.015503
	2	0.087		
	3	0.102		
MeOH	1	0.055	0.0536667	0.00611
	2	0.059		
	3	0.047		
I-6	1	0.047	0.105	0.050229
	2	0.134		
	3	0.134		
I-5	1	0.138	0.1446667	0.008327
	2	0.154		
	3	0.142		
I-4	1	0.165	0.0993333	0.06501
	2	0.035		
	3	0.098		
I-3	1	0.157	0.119	0.065818
	2	0.157		
	3	0.043		
I-2	1	0.13	0.068	0.053731
	2	0.039		
	3	0.035		
I-1	1	0.256	0.1693333	0.078799
	2	0.15		
	3	0.102		
IS	1	0.071	0.0826667	0.013868
	2	0.079		
	3	0.098		
DMDS-3	1	0.031	0.051	0.028
	2	0.039		
	3	0.083		
DMDS-2	1	0.051	0.0483333	0.016166
	2	0.063		
	3	0.031		

D3MO2	trial	length	avg.	s.d.
HA	1	0.039	0.043	0.006928
	2	0.039		
	3	0.051		
AA	1	0.063	0.0356667	0.023756
	2	0.02		
	3	0.024		
MeOH	1	0.028	0.0316667	0.010017
	2	0.024		
	3	0.043		
I-6	1	0.024	0.0476667	0.031182
	2	0.036		
	3	0.083		
I-5	1	0.051	0.047	0.006928
	2	0.039		
	3	0.051		
I-4	1	0.024	0.0263333	0.007767
	2	0.02		
	3	0.035		
I-3	1	0.028	0.0356667	0.020599
	2	0.059		
	3	0.02		
I-2	1	0.035	0.0446667	0.023072
	2	0.071		
	3	0.028		
I-1	1	0.071	0.0513333	0.02442
	2	0.059		
	3	0.024		
IS	1	0.047	0.0603333	0.01222
	2	0.071		
	3	0.063		
DMDS-3	1	0.067	0.079	0.012
	2	0.079		
	3	0.091		
DMDS-2	1	0.059	0.066	0.008888
	2	0.063		
	3	0.076		
HA	1	0.059	0.059	

D3MO3	trial	length	avg.	s.d.
HA	1	0.378	0.3753333	0.008327
	2	0.366		
	3	0.382		
AA	1	0.421	0.421	0.004
	2	0.425		
	3	0.417		
MeOH	1	0.394	0.3793333	0.012858
	2	0.37		
	3	0.374		
I-6	1	0.374	0.3596667	0.015631
	2	0.362		
	3	0.343		
I-5	1	0.362	0.3546667	0.010214
	2	0.359		
	3	0.343		
I-4	1	0.366	0.3606667	0.004619
	2	0.358		
	3	0.358		
I-3	1	0.335	0.3376667	0.004619
	2	0.335		
	3	0.343		
I-2	1	0.35	0.332	0.022338
	2	0.307		
	3	0.339		
I-1	1	0.339	0.3376667	0.00611
	2	0.343		
	3	0.331		
IS	1	0.351	0.3376667	0.01222
	2	0.335		
	3	0.327		
DMDS-3	1	0.335	0.3466667	0.013868
	2	0.362		
	3	0.343		
DMDS-2	1	0.339	0.288	0.072125
	2	0.237		

D5FM1	trial	length	avg.	s.d.
HA	1	0.465	0.321667	0.130726
	2	0.291		
	3	0.209		
AA	1	0.193	0.190333	0.002309
	2	0.189		
	3	0.189		
MeOH	1	0.115	0.097333	0.016258
	2	0.094		
	3	0.083		
I-6	1	0.114	0.099667	0.013577
	2	0.098		
	3	0.087		
I-5	1	0.067	0.082667	0.023756
	2	0.11		
	3	0.071		
I-4	1	0.067	0.076333	0.010066
	2	0.087		
	3	0.075		
I-3	1	0.047	0.037667	0.008327
	2	0.035		
	3	0.031		
I-2	1	0.134	0.058	0.066302
	2	0.012		
	3	0.028		
I-1	1	0.02	0.02	0
	2	0.02		
	3	0.02		
IS	1	0.154	0.118333	0.03371
	2	0.114		
	3	0.087		
DMDS-3	1	0.051	0.041667	0.010066
	2	0.043		
	3	0.031		
DMDS-2	1	0.043	0.047	0.014422
	2	0.063		
	3	0.035		

D5FM2	trial	length	avg.	s.d.
HA	1	0.346	0.396333	0.061582
	2	0.378		
	3	0.465		
AA	1	0.075	0.05	0.021794
	2	0.035		
	3	0.04		
MeOH	1	0.043	0.096	0.055669
	2	0.154		
	3	0.091		
I-6	1	0.024	0.047667	0.044501
	2	0.099		
	3	0.02		
I-5	1	0.024	0.034333	0.017898
	2	0.024		
	3	0.055		
I-4	1	0.051	0.037667	0.011547
	2	0.031		
	3	0.031		
I-3	1	0.154	0.081333	0.063217
	2	0.051		
	3	0.039		
I-2	1	0.126	0.112667	0.017739
	2	0.11		
	3	0.102		
	4	0.142		
I-1	1	0.032	0.040667	0.007767
	2	0.047		
	3	0.043		
IS	1	0.252	0.120667	0.115483
	2	0.075		
	3	0.035		
DMDS-3	1	0.197	0.151	0.063151
	2	0.177		
	3	0.079		
DMDS-2	1	0.288	0.220667	0.059969
	2	0.201		
	3	0.173		
HA	1	0.213	0.213	

D5FM3	trial	length	avg.	s.d.
HA	1	0.114	0.085333	0.03188
	2	0.051		
	3	0.091		
AA	1	0.016	0.038333	0.035275
	2	0.02		
	3	0.079		
MeOH	1	0.035	0.041667	0.015144
	2	0.031		
	3	0.059		
I-6	1	0.161	0.199333	0.124989
	2	0.098		
	3	0.339		
I-5	1	0.079	0.113	0.088068
	2	0.213		
	3	0.047		
I-4	1	0.122	0.199333	0.151596
	2	0.374		
	3	0.102		
I-3	1	0.094	0.066667	0.02442
	2	0.059		
	3	0.047		
I-2	1	0.106	0.081	0.036592
	2	0.039		
	3	0.098		
I-1	1	0.142	0.109	0.031607
	2	0.106		
	3	0.079		
IS	1	0.043	0.040667	0.027574
	2	0.067		
	3	0.012		
DMDS-3	1	0.098	0.081333	0.020108
	2	0.087		
	3	0.059		
DMDS-2	1	0.26	0.145667	0.1145
	2	0.146		
	3	0.031		
HA	1	0.059	0.059	

D5FO1	trial	length	avg.	s.d.
HA	1	0.35	0.322667	0.025697
	2	0.319		
	3	0.299		
AA	1	0.287	0.288667	0.025541
	2	0.315		
	3	0.264		
MeOH	1	0.402	0.44	0.083738
	2	0.536		
	3	0.382		
I-6	1	0.528	0.531667	0.006351
	2	0.528		
	3	0.539		
I-5	1	0.539	0.541667	0.004619
	2	0.539		
	3	0.547		
I-4	1	0.539	0.539333	0.004509
	2	0.535		
	3	0.544		
I-3	1	0.323	0.307333	0.240882
	2	0.54		
	3	0.059		
I-2	1	0.094	0.146667	0.045786
	2	0.177		
	3	0.169		
I-1	1	0.13	0.101333	0.024826
	2	0.087		
	3	0.087		
IS	1	0.291	0.171667	0.10429
	2	0.098		
	3	0.126		
DMDS-3	1	0.11	0.115333	0.016653
	2	0.134		
	3	0.102		
DMDS-2	1	0.098	0.112667	0.014048
	2	0.126		
	3	0.114		
HA	1	0.154	0.154	

D5FO2	trial	length	avg.	s.d.
HA	1	0.339	0.332	0.034044
	2	0.362		
	3	0.295		
AA	1	0.323	0.273	0.047697
	2	0.228		
	3	0.268		
MeOH	1	0.232	0.271667	0.052348
	2	0.252		
	3	0.331		
I-6	1	0.307	0.278	0.034395
	2	0.24		
	3	0.287		
I-5	1	0.307	0.294	0.041073
	2	0.327		
	3	0.248		
I-4	1	0.374	0.362333	0.03499
	2	0.323		
	3	0.39		
I-3	1	0.366	0.334667	0.045081
	2	0.355		
	3	0.283		
I-2	1	0.256	0.208667	0.042712
	2	0.197		
	3	0.173		
I-1	1	0.256	0.293	0.118899
	2	0.426		
	3	0.197		
IS	1	0.327	0.233667	0.081445
	2	0.177		
	3	0.197		
DMDS-3	1	0.185	0.191333	0.030006
	2	0.224		
	3	0.165		
DMDS-2	1	0.193	0.171667	0.018475
	2	0.161		
	3	0.161		
HA	1	0.169	0.169	

D5FO3	trial	length	avg.	s.d.
HA	1	0.143	0.149667	0.008327
	2	0.147		
	3	0.159		
AA	1	0.175	0.164	0.036756
	2	0.123		
	3	0.194		
MeOH	1	0.202	0.214	0.017436
	2	0.234		
	3	0.206		
I-6	1	0.524	0.411333	0.100022
	2	0.333		
	3	0.377		
I-5	1	0.381	0.366333	0.012858
	2	0.357		
	3	0.361		
I-4	1	0.389	0.329333	0.083429
	2	0.365		
	3	0.234		
I-3	1	0.31	0.328333	0.028361
	2	0.361		
	3	0.314		
I-2	1	0.302	0.274	0.02498
	2	0.254		
	3	0.266		
I-1	1	0.468	0.502667	0.03107
	2	0.512		
	3	0.528		
IS	1	0.234	0.275	0.078045
	2	0.365		
	3	0.226		
DMDS-3	1	0.242	0.242333	0.011504
	2	0.231		
	3	0.254		
DMDS-2	1	0.202	0.25	0.041569
	2	0.274		
	3	0.274		
HA	1	0.139	0.139	

D5MO1	trial	length	avg.	s.d.
HA	1	0.119	0.127	0.02498
	2	0.107		
	3	0.155		
AA	1	0.127	0.099	0.024331
	2	0.087		
	3	0.083		
MeOH	1	0.071	0.069	0.004359
	2	0.072		
	3	0.064		
I-6	1	0.087	0.088333	0.00611
	2	0.083		
	3	0.095		
I-5	1	0.072	0.062667	0.008327
	2	0.06		
	3	0.056		
I-4	1	0.079	0.061	0.015588
	2	0.052		
	3	0.052		
I-3	1	0.067	0.072667	0.03188
	2	0.107		
	3	0.044		
I-2	1	0.056	0.052	0.004
	2	0.048		
	3	0.052		
I-1	1	0.063	0.081667	0.022745
	2	0.075		
	3	0.107		
IS	1	0.063	0.209	0.12644
	2	0.282		
	3	0.282		
DMDS-3	1	0.222	0.184	0.033451
	2	0.171		
	3	0.159		
DMDS-2	1	0.159	0.148333	0.01222
	2	0.135		
	3	0.151		
HA	1	0.075	0.075	

D5MO2	trial	length	avg.	s.d.
HA	1	0.032	0.04	0.010583
	2	0.036		
	3	0.052		
AA	1	0.063	0.035667	0.02442
	2	0.016		
	3	0.028		
MeOH	1	0.044	0.033333	0.010066
	2	0.024		
	3	0.032		
I-6	1	0.028	0.041	0.019157
	2	0.032		
	3	0.063		
I-5	1	0.036	0.037333	0.018037
	2	0.02		
	3	0.056		
I-4	1	0.032	0.029333	0.008327
	2	0.02		
	3	0.036		
I-3	1	0.016	0.032	0.01833
	2	0.052		
	3	0.028		
I-2	1	0.024	0.043667	0.034064
	2	0.083		
	3	0.024		
I-1	1	0.067	0.049333	0.024786
	2	0.06		
	3	0.021		
IS	1	0.032	0.030667	0.00611
	2	0.036		
	3	0.024		
DMDS-3	1	0.048	0.054667	0.008327
	2	0.052		
	3	0.064		
DMDS-2	1	0.056	0.066	0.00866
	2	0.071		
	3	0.071		

D5MO3	trial	length	avg.	s.d.
HA	1	0.378	0.348	0.026665
	2	0.339		
	3	0.327		
AA	1	0.331	0.319	0.014422
	2	0.323		
	3	0.303		
MeOH	1	0.256	0.270667	0.012858
	2	0.28		
	3	0.276		
I-6	1	0.26	0.237667	0.032005
	2	0.252		
	3	0.201		
I-5	1	0.264	0.270667	0.00611
	2	0.276		
	3	0.272		
I-4	1	0.248	0.256	0.008
	2	0.264		
	3	0.256		
I-3	1	0.299	0.304333	0.00611
	2	0.311		
	3	0.303		
I-2	1	0.315	0.307	0.013856
	2	0.315		
	3	0.291		
I-1	1	0.197	0.189	0.017436
	2	0.169		
	3	0.201		
IS	1	0.166	0.166667	0.002082
	2	0.165		
	3	0.169		
DMDS-3	1	0.177	0.174333	0.004619
	2	0.177		
	3	0.169		
DMDS-2	1	0.169	0.161667	0.006351
	2	0.158		
	3	0.158		
HA	1	0.154	0.154	

D7FM1	trial	length	avg.	s.d.
HA	1	0.346	0.3783333	0.0399541
	2	0.366		
	3	0.423		
AA	1	0.421	0.402	0.0247588
	2	0.411		
	3	0.374		
MeOH	1	0.392	0.398	0.0052915
	2	0.4		
	3	0.402		
I-6	1	0.459	0.473	0.0157162
	2	0.49		
	3	0.47		
I-5	1	0.537	0.5323333	0.0045092
	2	0.528		
	3	0.532		
I-4	1	0.535	0.535	0.001
	2	0.534		
	3	0.536		
I-3	1	0.52	0.5323333	0.0109697
	2	0.536		
	3	0.541		
I-2	1	0.136	0.1686667	0.0289194
	2	0.179		
	3	0.191		
I-1	1	0.207	0.2023333	0.0064291
	2	0.195		
	3	0.205		
IS	1	0.211	0.221	0.0095394
	2	0.23		
	3	0.222		
DMDS-3	1	0.24	0.248	0.0138564
	2	0.24		
	3	0.264		
DMDS-2	1	0.234	0.2426667	0.0102632
	2	0.254		
	3	0.24		
HA	1	0.193	0.193	

D7FM2	trial	length	avg.	s.d.
HA	1	0.305	0.2586667	0.0450592
	2	0.256		
	3	0.215		
AA	1	0.116	0.105	0.0105357
	2	0.104		
	3	0.095		
MeOH	1	0.13	0.1253333	0.0213854
	2	0.102		
	3	0.144		
I-6	1	0.232	0.2236667	0.0180093
	2	0.203		
	3	0.236		
I-5	1	0.177	0.2553333	0.0683691
	2	0.303		
	3	0.286		
I-4	1	0.311	0.3513333	0.0490951
	2	0.337		
	3	0.406		
I-3	1	0.427	0.4386667	0.0369098
	2	0.48		
	3	0.409		
I-2	1	0.142	0.0936667	0.0542433
	2	0.104		
	3	0.035		
I-1	1	0.128	0.112	0.016
	2	0.096		
	3	0.112		
IS	1	0.047	0.0416667	0.0050332
	2	0.041		
	3	0.037		
DMDS-3	1	0.022	0.0276667	0.0172143
	2	0.014		
	3	0.047		
DMDS-2	1	0.014	0.0233333	0.0083267
	2	0.03		
	3	0.026		
HA	1	0.093	0.093	

D7FM3	trial	length	avg.	s.d.
HA	1	0.049	0.2053333	0.1353969
	2	0.285		
	3	0.282		
AA	1	0.28	0.2076667	0.0751421
	2	0.213		
	3	0.13		
MeOH	1	0.209	0.195	0.0208806
	2	0.171		
	3	0.205		
I-6	1	0.331	0.3683333	0.038553
	2	0.408		
	3	0.366		
I-5	1	0.451	0.3626667	0.0819898
	2	0.289		
	3	0.348		
I-4	1	0.508	0.4976667	0.0308275
	2	0.463		
	3	0.522		
I-3	1	0.406	0.5036667	0.0906109
	2	0.52		
	3	0.585		
I-2	1	0.394	0.367	0.0433474
	2	0.39		
	3	0.317		
I-1	1	0.416	0.401	0.1500633
	2	0.244		
	3	0.543		
IS	1	0.581	0.527	0.0478226
	2	0.51		
	3	0.49		
DMDS-3	1	0.057	0.112	0.0631744
	2	0.181		
	3	0.098		
DMDS-2	1	0.043	0.065	0.0210713
	2	0.067		
	3	0.085		
HA	1	0.055	0.055	

D7FO1	trial	length	avg.	s.d.
HA	1	0.048	0.048	0
	2	0.048		
	3	0.048		
AA	1	0.025	0.0373333	0.0106927
	2	0.043		
	3	0.044		
MeOH	1	0.083	0.0833333	0.0015275
	2	0.085		
	3	0.082		
I-6	1	0.078	0.0766667	0.0011547
	2	0.076		
	3	0.076		
I-5	1	0.072	0.0713333	0.0030551
	2	0.074		
	3	0.068		
I-4	1	0.1	0.097	0.0036056
	2	0.093		
	3	0.098		
I-3	1	0.063	0.0476667	0.0136137
	2	0.037		
	3	0.043		
I-2	1	0.045	0.0513333	0.0070946
	2	0.059		
	3	0.05		
I-1	1	0.03	0.015	0.013
	2	0.007		
	3	0.008		
IS	1	0.006	0.0076667	0.0037859
	2	0.012		
	3	0.005		
DMDS-3	1	0.004	0.007	0.0026458
	2	0.008		
	3	0.009		
DMDS-2	1	0.006	0.008	0.0034641
	2	0.006		
	3	0.012		
HA	1	0.035	0.035	

D7F02	trial	length	avg.	s.d.
HA	1	0.141	0.1193333	0.0205994
	2	0.117		
	3	0.1		
AA	1	0.06	0.0636667	0.0063509
	2	0.06		
	3	0.071		
MeOH	1	0.068	0.0773333	0.0136504
	2	0.071		
	3	0.093		
I-6	1	0.086	0.0726667	0.0146401
	2	0.075		
	3	0.057		
I-5	1	0.058	0.0566667	0.0015275
	2	0.055		
	3	0.057		
I-4	1	0.179	0.1593333	0.01861
	2	0.142		
	3	0.157		
I-3	1	0.091	0.0983333	0.0066583
	2	0.104		
	3	0.1		
I-2	1	0.064	0.0726667	0.0117189
	2	0.086		
	3	0.068		
I-1	1	0.239	0.108	0.1136354
	2	0.049		
	3	0.036		
IS	1	0.047	0.0403333	0.0058595
	2	0.036		
	3	0.038		
DMDS-3	1	0.038	0.042	0.0034641
	2	0.044		
	3	0.044		
DMDS-2	1	0.049	0.045	0.0036056
	2	0.044		
	3	0.042		
HA	1	0.035	0.035	

D7FO3	trial	length	avg.	s.d.
HA	1	0.112	0.1106667	0.0023094
	2	0.112		
	3	0.108		
AA	1	0.167	0.151	0.0155242
	2	0.136		
	3	0.15		
MeOH	1	0.12	0.1436667	0.0269506
	2	0.138		
	3	0.173		
I-6	1	0.13	0.1103333	0.0306649
	2	0.075		
	3	0.126		
I-5	1	0.051	0.0943333	0.0377403
	2	0.12		
	3	0.112		
I-4	1	0.146	0.128	0.0245764
	2	0.1		
	3	0.138		
I-3	1	0.098	0.1173333	0.0190088
	2	0.118		
	3	0.136		
I-2	1	0.122	0.0933333	0.0933333
	2	0.075		
	3	0.083		
I-1	1	0.026	0.0576667	0.0576667
	2	0.035		
	3	0.112		
IS	1	0.018	0.0433333	0.0221435
	2	0.053		
	3	0.059		
DMDS-3	1	0.065	0.056	0.0266646
	2	0.026		
	3	0.077		
DMDS-2	1	0.071	0.0536667	0.0161658
	2	0.039		
	3	0.051		
HA	1	0.03	0.03	

D7MO1	trial	length	avg.	s.d.
HA	1	0.022	0.0263333	0.0045092
	2	0.031		
	3	0.026		
AA	1	0.265	0.2816667	0.0425245
	2	0.33		
	3	0.25		
MeOH	1	0.104	0.0803333	0.0215019
	2	0.075		
	3	0.062		
I-6	1	0.046	0.0653333	0.0181475
	2	0.068		
	3	0.082		
I-5	1	0.115	0.1023333	0.0600111
	2	0.155		
	3	0.037		
I-4	1	0.051	0.045	0.0087178
	2	0.049		
	3	0.035		
I-3	1	0.04	0.039	0.0075498
	2	0.046		
	3	0.031		
I-2	1	0.031	0.028	0.0088882
	2	0.035		
	3	0.018		
I-1	1	0.018	0.0176667	0.0025166
	2	0.02		
	3	0.015		
IS	1	0.022	0.0206667	0.0041633
	2	0.016		
	3	0.024		
DMDS-3	1	0.02	0.0213333	0.0041633
	2	0.018		
	3	0.026		
DMDS-2	1	0.013	0.0166667	0.0063509
	2	0.013		
	3	0.024		
HA	1	0.035	0.035	

D7MO2	trial	length	avg.	s.d.
HA	1	0.21	0.2113333	0.0090738
	2	0.221		
	3	0.203		
AA	1	0.088	0.098	0.0117898
	2	0.111		
	3	0.095		
MeOH	1	0.135	0.1276667	0.0162891
	2	0.139		
	3	0.109		
I-6	1	0.13	0.134	0.0144222
	2	0.122		
	3	0.15		
I-5	1	0.141	0.131	0.0086603
	2	0.126		
	3	0.126		
I-4	1	0.276	0.218	0.0530283
	2	0.206		
	3	0.172		
I-3	1	0.177	0.2096667	0.0311341
	2	0.213		
	3	0.239		
I-2	1	0.201	0.2226667	0.0548118
	2	0.182		
	3	0.285		
I-1	1	0.206	0.205	0.0055678
	2	0.21		
	3	0.199		
IS	1	0.214	0.2113333	0.0030551
	2	0.208		
	3	0.212		
DMDS-3	1	0.226	0.2173333	0.022301
	2	0.192		
	3	0.234		
DMDS-2	1	0.219	0.205	0.0131149
	2	0.193		
	3	0.203		
HA	1	0.208	0.208	

D7MO3	trial	length	avg.	s.d.
HA	1	0.013	0.009	0.0034641
	2	0.007		
	3	0.007		
AA	1	0.011	0.0133333	0.0040415
	2	0.018		
	3	0.011		
MeOH	1	0.013	0.0133333	0.0045092
	2	0.009		
	3	0.018		
I-6	1	0.018	0.0163333	0.0015275
	2	0.015		
	3	0.016		
I-5	1	0.026	0.0286667	0.0025166
	2	0.029		
	3	0.031		
I-4	1	0.033	0.025	0.007
	2	0.02		
	3	0.022		
I-3	1	0.022	0.0233333	0.0023094
	2	0.022		
	3	0.026		
I-2	1	0.015	0.0123333	0.0023094
	2	0.011		
	3	0.011		
I-1	1	0.013	0.0103333	0.0023094
	2	0.009		
	3	0.009		
IS	1	0.02	0.0163333	0.0047258
	2	0.011		
	3	0.018		
DMDS-3	1	0.011	0.0123333	0.0011547
	2	0.013		
	3	0.013		
DMDS-2	1	0.011	0.0116667	0.0030551
	2	0.009		
	3	0.015		
HA	1	0.011	0.011	

D9FM1	trial	length	avg.	s.d.
HA	1	0.117	0.107	0.0111355
	2	0.109		
	3	0.095		
AA	1	0.126	0.0833	0.0369504
	2	0.062		
	3	0.062		
MeOH	1	0.06	0.0523	0.0070946
	2	0.051		
	3	0.046		
I-6	1	0.042	0.0403	0.0086217
	2	0.031		
	3	0.048		
I-5	1	0.038	0.0437	0.009815
	2	0.055		
	3	0.038		
I-4	1	0.049	0.048	0.0017321
	2	0.046		
	3	0.049		
I-3	1	0.044	0.0273	0.0147422
	2	0.022		
	3	0.016		
I-2	1	0.016	0.072	0.0575587
	2	0.131		
	3	0.069		
I-1	1	0.077	0.0573	0.0173877
	2	0.051		
	3	0.044		
IS	1	0.051	0.0507	0.0045092
	2	0.046		
	3	0.055		
DMDS-3	1	0.044	0.042	0.002
	2	0.042		
	3	0.04		
DMDS-2	1	0.046	0.042	0.0052915
	2	0.044		
	3	0.036		
HA	1	0.036	0.036	

D9FO1	trial	length	avg.	s.d.
HA	1	0.015	0.0193	0.0037859
	2	0.021		
	3	0.022		
AA	1	0.022	0.0223	0.0015275
	2	0.021		
	3	0.024		
MeOH	1	0.027	0.027	0.001
	2	0.026		
	3	0.028		
I-6	1	0.023	0.025	0.002
	2	0.027		
	3	0.025		
I-5	1	0.025	0.0283	0.0030551
	2	0.029		
	3	0.031		
I-4	1	0.027	0.0263	0.0011547
	2	0.027		
	3	0.025		
I-3	1	0.049	0.0477	0.0011547
	2	0.047		
	3	0.047		
I-2	1	0.043	0.0367	0.0118462
	2	0.044		
	3	0.023		
I-1	1	0.023	0.0243	0.0015275
	2	0.024		
	3	0.026		
IS	1	0.027	0.0297	0.0025166
	2	0.03		
	3	0.032		
DMDS-3	1	0.029	0.03	0.0017321
	2	0.029		
	3	0.032		
DMDS-2	1	0.03	0.0613	0.0015275
	2	0.033		
	3	0.031		
HA	1	0.03	0.03	

D9MO1	trial	length	avg.	s.d.
HA	1	0.172	0.168	0.004
	2	0.164		
	3	0.168		
AA	1	0.129	0.112	0.0260576
	2	0.125		
	3	0.082		
MeOH	1	0.082	0.0833	0.0061101
	2	0.09		
	3	0.078		
I-6	1	0.082	0.09	0.0069282
	2	0.094		
	3	0.094		
I-5	1	0.055	0.1433	0.1635003
	2	0.043		
	3	0.332		
I-4	1	0.32	0.3087	0.020502
	2	0.321		
	3	0.285		
I-3	1	0.266	0.2747	0.0077675
	2	0.281		
	3	0.277		
I-2	1	0.449	0.405	0.0636632
	2	0.434		
	3	0.332		
I-1	1	0.125	0.1863	0.0750355
	2	0.164		
	3	0.27		
IS	1	0.129	0.1587	0.0335012
	2	0.152		
	3	0.195		
DMDS-3	1	0.344	0.323	0.0185203
	2	0.316		
	3	0.309		
DMDS-2	1	0.359	0.345	0.0185203
	2	0.352		
	3	0.324		
HA	1	0.316		

Day 3 Mean Response Intensity

	D3FM1	D3FM2	D3FM3	D3FO1	D3FO2	D3FO3	D3MO1	D3MO2	D3MO3
HA	0.1892	0.0367	0.30583	0.046	0.17267	0.0093	0.03566	0.01133	-0.004
AA	0.079	-0.046	0.05	0.0013	0.01467	-0.0197	0.04863	0.004	0.0417
MeOH	0	0	0	0	0	0	0	0	0
I-6	-0.1453	-0.248	-0.03667	0.0117	0.03037	0.0693	0.05133	0.015997	-0.02
I-5	-0.1453	-0.248	-0.03667	-0.013	-0.02063	0.087	0.09103	-0.00534	-0.0196
I-4	0.1697	-0.0313	0.00533	-0.0157	0.01237	0.046	0.04566	0.004	-0.0246
I-3	0.1564	-0.0417	-0.001	-0.0303	-0.03366	-0.01167	0.06533	0.013	-0.0186
I-2	0.1317	-0.0983	0.03666	0.0013	-0.02866	0.092	0.01433	0.01966	-0.0416
I-1	0.1414	-0.139	0.00433	-0.0397	0.09067	0.0023	0.11563	0.02863	-0.0473
IS	0.1447	-0.22533	-0.009	-0.033	-0.01166	0.0037	0.02903	0.04733	-0.0416
DMDS-3	0.0477	-0.1363	0.02903	-0.0603	-0.02333	-0.014	-0.00267	0.03433	-0.0326
DMDS-2				-0.038	0.004	0.063	-0.00537	0.02733	-0.0913
HA				-0.336	-0.07333	-0.101	-0.05367	-0.03167	-0.3793

Day 5 Mean Response Intensity

	D5FM1	D5FM2	D5FM3	D5FO1	D5FO2	D5FO3	D5MO1	D5MO2	D5MO3
HA	0.2244	0.3003	0.04366	-0.1173	0.0603	-0.0643	0.058	0.00667	0.1073
AA	0.093	-0.046	-0.00334	-0.1513	0.0013	-0.05	0.03	0.00234	0.0483
MeOH	0	0	0	0	0	0	0	0	0
I-6	0.00237	-0.04833	0.15763	0.09167	0.0063	0.1973	0.01933	0.00767	-0.033
I-5	-0.01463	-0.06167	0.07133	0.1017	0.0223	0.1523	-0.00633	0.004	0
I-4	-0.02097	-0.05833	0.15763	0.0993	0.0906	0.1153	-0.008	-0.004	-0.0147
I-3	-0.05963	-0.01467	0.025	-0.1327	0.063	0.1143	0.00367	-0.00133	0.0336
I-2	-0.0393	0.0167	0.03933	-0.2933	-0.063	0.06	-0.017	0.010337	0.0363
I-1	-0.0773	-0.05533	0.06733	-0.3387	0.0213	0.2887	0.01267	0.016	-0.0817
IS	0.021	0.0247	-0.00101	-0.2683	-0.038	0.061	0.14	-0.00266	-0.104
DMDS-3	-0.05563	0.055	0.03966	-0.3247	-0.0804	0.0283	0.115	0.02134	-0.0964
DMDS-2	-0.0503	0.1247	0.10403	-0.3273	-0.1	0.036	0.0793	0.03267	-0.109
HA	-0.0973	0.117	0.01733	-0.286	-0.1027	-0.075	0.006	-0.03333	-0.1167

Day 7 Mean Response Intensity

	D7FM1	D7FM2	D7FM3	D7FO1	D7FO2	D7FO3	D7MO1	D7MO2	D7MO3
HA	-0.0197	0.1334	0.0103	-0.03533	0.04197	-0.033	-0.054	0.0836	-0.00433
AA	0.004	-0.0203	0.0127	-0.046	-0.01366	0.0073	0.2014	-0.0297	0
MeOH	0	0	0	0	0	0	0	0	0
I-6	0.075	0.0984	0.1733	-0.00666	-0.00466	-0.0334	-0.01497	0.0063	0.003
I-5	0.1345	0.13	0.1677	-0.012	-0.02066	-0.04937	0.022	0.0033	0.01534
I-4	0.137	0.226	0.3027	0.01367	0.08197	-0.0157	-0.0353	0.0903	0.01167
I-3	0.1343	0.3134	0.3087	-0.03566	0.021	-0.0264	-0.0413	0.082	0.01
I-2	-0.2293	-0.0316	0.172	-0.032	-0.00466	-0.05037	-0.0523	0.095	-0.001
I-1	-0.1957	-0.0133	0.206	-0.06833	0.03067	-0.08603	-0.0626	0.0773	-0.003
IS	-0.177	-0.0836	0.332	-0.07566	-0.03703	-0.10037	-0.05963	0.0836	0.003
DMDS-3	-0.15	-0.09763	-0.083	-0.07633	-0.03533	-0.0877	-0.05897	0.0896	-0.001
DMDS-2	-0.1553	-0.10197	-0.13	-0.07533	-0.03233	-0.09003	-0.06363	0.0773	-0.00166
HA	-0.205	-0.0323	-0.14	-0.04833	-0.04233	-0.1137	-0.0453	0.0803	-0.00233

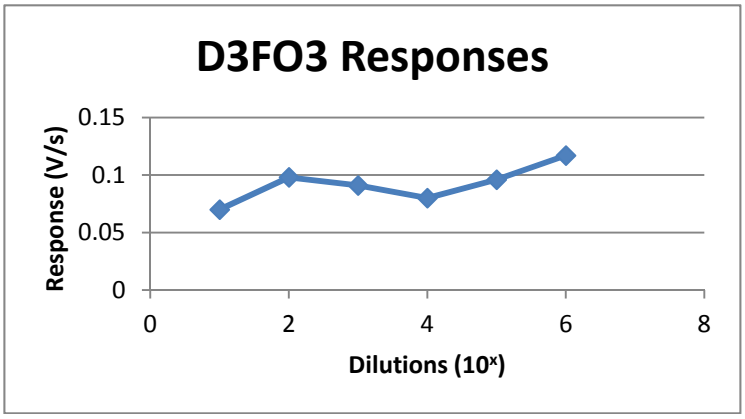
Day 9 Mean Response Intensity

	D9FM	D9FO	D9MO
HA	0.05467	-0.00767	0.0847
AA	0.031	-0.00467	0.0287
MeOH	0	0	0
I-6	-0.012	-0.002	0.0067
I-5	-0.00866	0.00133	0.06
I-4	-0.00433	-0.00067	0.2254
I-3	-0.025	0.02067	0.1914
I-2	0.01967	0.00967	0.3217
I-1	0.005	-0.00267	0.103
IS	-0.00166	0.00267	0.0754
DMDS-3	-0.01033	0.003	0.2397
DMDS-2	-0.01033	0.03433	0.2617
HA	-0.01633	0.003	0.2327

Appendix C

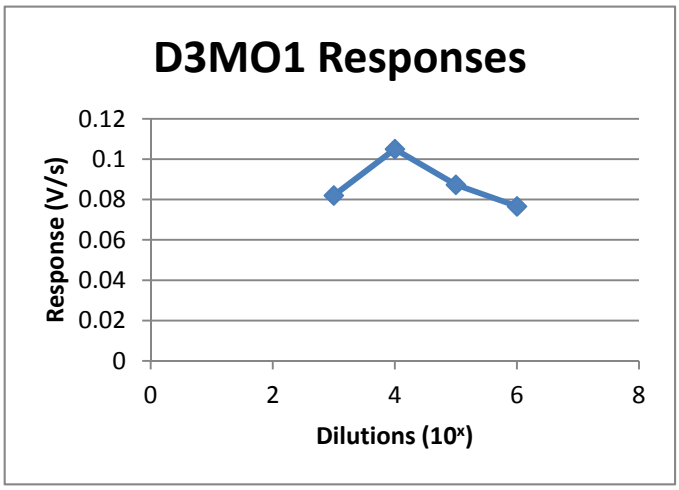
D3FO3

I-61	0.117
I-51	0.094
I-53	0.098
I-41	0.066
I-42	0.094
I-31	0.091
I-22	0.102
I-23	0.094
I-12	0.07



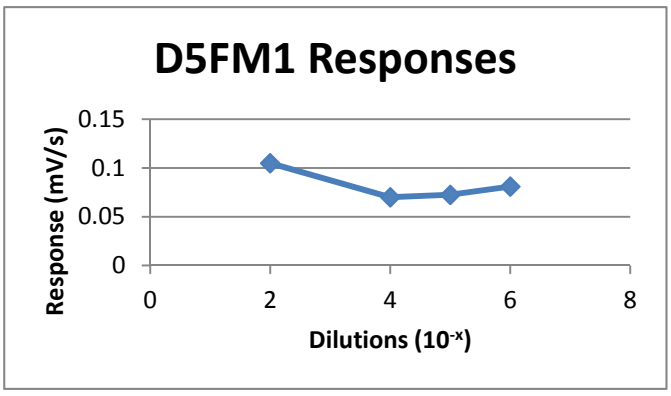
D3MO1

I-61	0.074
I-62	0.07
I-63	0.086
I-51	0.09
I-52	0.094
I-53	0.078
I-41	0.105
I-31	0.094
I-32	0.07



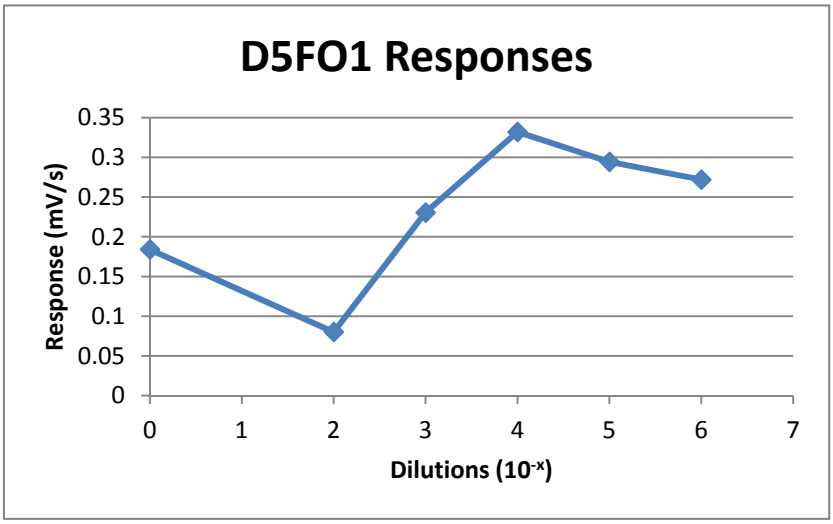
D5FM1

I-61	0.106
I-62	0.078
I-63	0.059
I-52	0.09
I-53	0.055
I-42	0.07
I-21	0.105



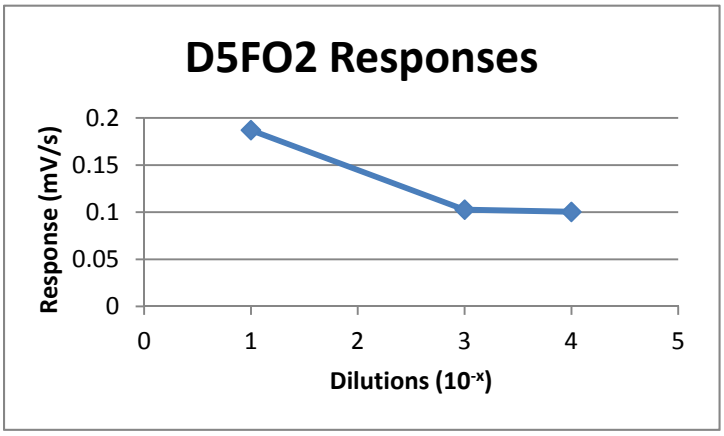
D5FO1

I-61	0.246
I-62	0.285
I-63	0.285
I-51	0.293
I-52	0.293
I-53	0.297
I-41	0.332
I-42	0.352
I-43	0.312
I-31	0.191
I-32	0.27
I-22	0.078
I-23	0.082
IS1	0.184



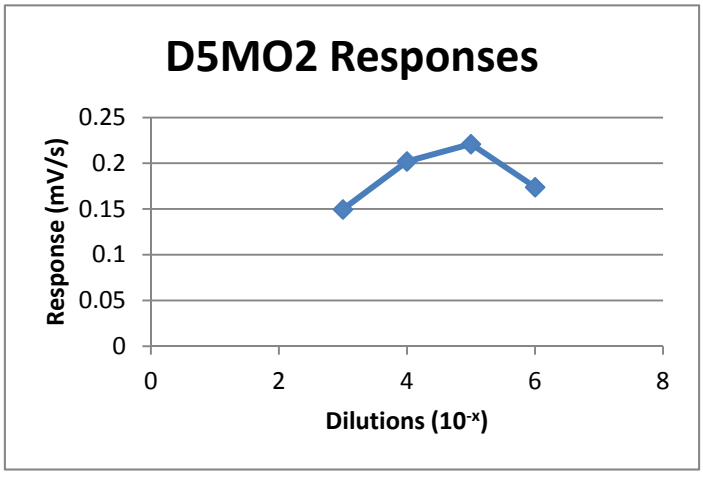
D5FO2

I-41	0.102
I-42	0.082
I-43	0.117
I-31	0.113
I-32	0.105
I-33	0.09
I-12	0.187



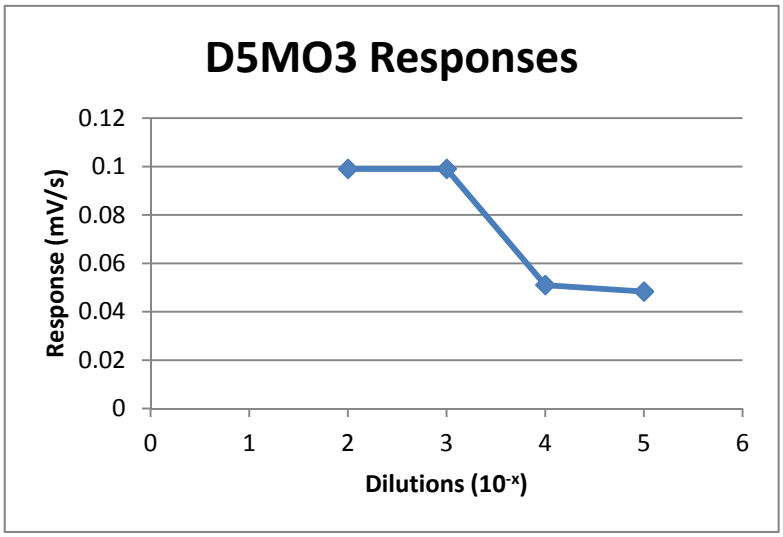
D5MO2

I-61	0.176
I-62	0.172
I-51	0.191
I-52	0.23
I-53	0.242
I-41	0.164
I-42	0.227
I-43	0.215
I-31	0.168
I-32	0.129
I-33	0.152



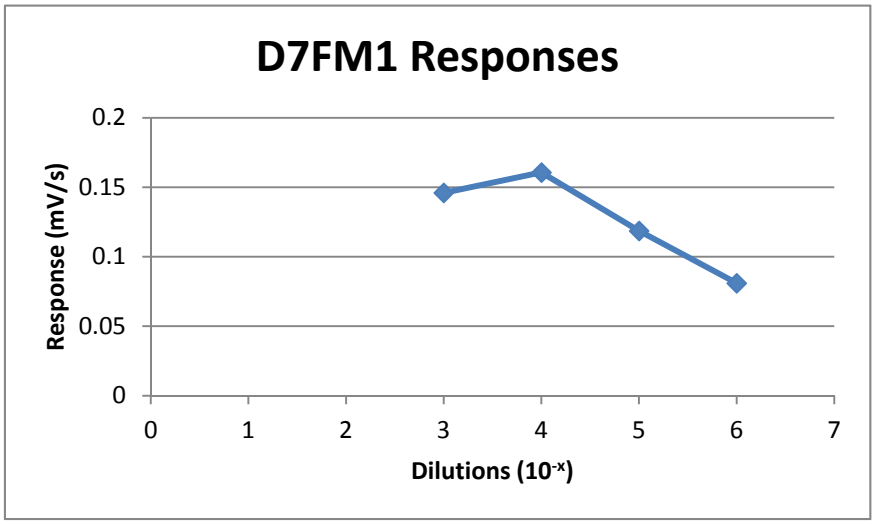
D5MO3

I-51	0.039
I-52	0.051
I-53	0.055
I-41	0.039
I-43	0.063
I-31	0.094
I-32	0.105
I-33	0.098
I-21	0.11
I-22	0.105
I-23	0.082



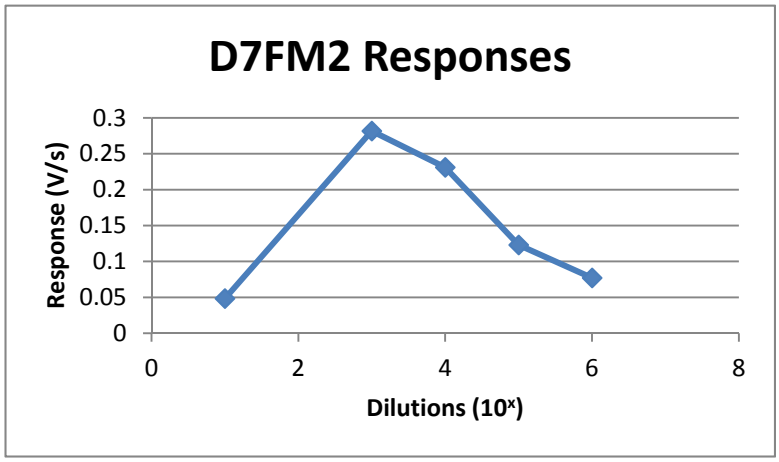
D7FM1

I-61	0.059
I-62	0.104
I-63	0.08
I-51	0.104
I-52	0.125
I-53	0.127
I-41	0.174
I-42	0.154
I-43	0.154
I-31	0.143
I-32	0.152
I-33	0.143



D7FM2

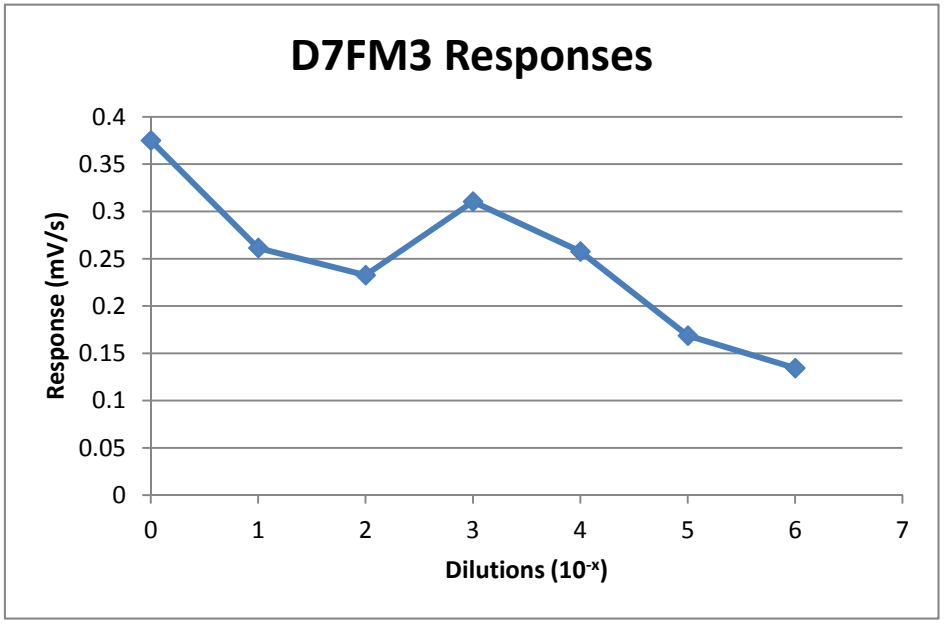
I-61	0.063
I-62	0.066
I-63	0.102
I-51	0.08
I-52	0.154
I-53	0.135
I-41	0.176
I-42	0.23
I-43	0.287
I-31	0.279
I-32	0.316



I-33	0.25
I-11	0.053
I-12	0.045
I-13	0.047

D7FM3

I-61	0.092
I-62	0.172
I-63	0.139
I-51	0.205
I-52	0.145
I-53	0.156
I-41	0.248
I-42	0.232
I-43	0.293
I-31	0.242
I-32	0.326
I-33	0.363
I-21	0.238
I-22	0.268
I-23	0.192
I-11	0.268
I-12	0.166
I-13	0.35
IS1	0.402
IS2	0.367
IS3	0.356



D7MO1

I-61	0.049
I-62	0.062
I-63	0.072
I-51	0.107
I-52	0.158