BIOLOGY EXTEND

ESSAY

What effect do the different concentrations of DDT and an AZO dye have on the opercular movement of *Cirrhinus cirrhosus*?

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BIOLOGY EXTENDED ESSAY

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ABSTRACT

I was inquisitive about the aquatic flora and fauna and their mass destruction due to the increase in chemical pollutants coming from industries, agricultura l farms and houses. Therefore I thought and pondered about the reasons behind this destruction and the question which I thought would be worthy of investigation is:

"**What effect do the different concentrations of DDT and an AZO dye have on the opercular movement of *Cirrhinus cirrhosus*?**"

For this research, I investigated the effects of DDT and an Azo -Dye on the opercular movement of Mrigal (*Cirrhinus cirrhosus)* a fresh water fish. This was attained by performing primary research, which was achieved by setting up 3 similar experiments, first using different concentrations of DDT, second using different concentration of the Azo-Dye and third using a mixture of these two toxins. All the experiments were carried out to find out the difference in opercular moveme nt in fish, as it would help answer my question. For the first and second experiment, I obtained 25 data for each concentration, and 125 data for all the concentrations of DDT including the controlled experiment. The same was repe ated in the third, howeve r data collected was 25 for the mixture and 50 including the controlled experiment.

From the results that I recorded, and observed I can say that DDT, the Azo -Dye and the mixture of these two toxins reduce the opercular movement of Mrigal. The graphs show a clear trend of decline in the opercular movement.

The results of the three investigations helped me conclude that DDT and the Azo -Dye both have a similar affect on the opercular movement of the fish and that the mixture of these two toxins is collectiv ely more dangerous. These toxins affect the fishes by reducing their opercular movement with an increase in concentration.

Word count: 295

INTRODUCTION

The aim of this extended essay is to find out the effects of industrial pollutants on the breathing rate of *Cirrhinus cirrhosus* (Mrigal) a fresh water fish. I cover the discipline of science called toxicology in the following experiments. Toxicology is the science of poisons; it deals with the study of economic poisons, their effects, mechanisms of action and metabolism of toxicants in test animals. The two pollutants for which the test will be conducted are DDT (Dichlorodiphenyltrichloroethane) and local textile dyes (Azo-Dyes). Both of which, are found in abundance in Rajasthan, India. The se toxins are often a cause of Biomagnification[1]. Mrigal, a fresh water fish which is widely cultured in India will help us replicate the effect of such pollutants in controlled laboratory conditions. Further, to support our results I would carry out a third investigation to see the results of a mixture of these two toxins on the fish. Through an analysis of the three investigations I would like to understand the real effect of these pollutants on our environment. Hence chosing the research question:

"What effect do the different concentrations of DDT and an AZO dye have on the opercular movement of *Cirrhinus cirrhosus*?"

THE OPERCULUM AND THE BREATHING PROCESS OF A FISH:-

The operculum of a fish is the hard bony flap covering and protecting the gills. The operculum is composed of four fused bones; the opercle, preopercle, interopercle, and subopercle. The posterior rim on the operculum is a flexible, ribbed structure which acts as a seal in order to stop reverse water flow during respiration.

For most fish, the operculum is critical in obtaining oxygen. It opens as the mouth closes, causing the pressure inside the fish to drop. Water then flows towards the

[1] http://www.tutorvista.com/content/biology/biology-ii/environment-and-environmental-problems/water-pollution.php

lower pressure across the fish's gill lamellae, allowing some oxygen to be absorbed from the water.[2]

The movements of the mouth floor and operculum are synchronized to produce a stream of water, in through the mouth, over the gills and out of the operculum.

The method of pumping water over the gills seems to differ in detail according to the type of fish but, in general, the pressure in the mouth cavity is reduced by the f loor of the mouth being lowered.

Thus, water enters through the mouth to even out the pressure. The increased pressure due to the reduced volume of the mouth cavity forces open the operculum and expels the water through the opercular opening, causing it to pass between the gill filaments as it leaves.[3]

AN INTRODUCTION TO *CIRRHINUS CIRRHOSUS*:-

The Mrigal is a freshwater fish species from the Carps family of the order Cypriniformes. The Mrigal is an introduced species and is actually native to large river systems of India[4]. The Mrigal, currently is the widest farmed species amongst the Indian floodplains of Bangladesh, India and Pakistan. It is found in the rivers of Myanmar that drain into the Bay of Beng al. It was introduced for the purpose of aquaculture, together with Catla (*Catla catla*) and Rohu *(Labeo rohita)* to other areas of India beyond its natural range in the early 1940's and in the 1950's and 1960's to

[2] http://en.wikipedia.org/wiki/Operculum_(fish)

[3] http://www.biology-resources.com/fish-01.html

[4] http://www.fishthailand.co.uk/species/mrigal.html

other Asian countries[5]. This particular species of fish will help us in calculating its opercular movement, thus determining the affects DDT and the Azo-Dye on the fish.

ABOUT DDT:-

DDT is probably the most infamous synthetic pesticide obtainable today. DDT is a constant organic pollutant that is exceedingly hydrophobic and greatly absorbed by soils. Its soil half life can range from 22 days to 30 years. Routes of loss and degradation include runoff, volatilization, photolysis and aerobic and anaerobic biodegradation. When added to the aquatic ecosystems it is quickly absorbed by organisms and by soil or it evaporates, leaving little DDT dissolved in the water itself[6]. In my experiments DDT will be added to the fish's environment and there direct response would be shown by the difference in their opercular movement.

THE TEXTILE INDUSTRY:-

The textile printing in Sanganer town, district Jaipur (Rajasthan, India), is famous worldwide for its dyeing and printing industries. There are about 400 industries involved in textile printing processes which require approximately 4000 kl/day of

[5] http://www.uaex.edu/pperschbacher/Fish/Mrigal2.htm

[6] http://en.wikipedia.org/wiki/DDT

good quality fresh water, which sometimes may go up to 5000 kl/day to meet market demands.[7]

WHAT AZO-DYES ARE:-

Azo compounds are compounds bearing the functional group R-N=N-R', in which R and R' can be either aryl or alkyl. The N=N group is called an Azo group. Azo compounds are generally seen to have vibrant colours, specially reds, oranges, and yellows. Therefore, they are used as dyes, and are commonly known as Azo-Dyes, an example of which is Disperse Orange 1. The production of Azo dyes was an important step in the development of the chemical industry.

Azo pigments are colourless, which are coloured using an Azo compound. Azo pigments are vital in a variety of paints including artist's paints. They have admirable colouring properties, again mainly in the yellow to red range, as well as light fastness.

Many Azo pigments non-toxic, although some, such as dinitroaniline orange, orthonitroaniline orange, or pigment orange 1, 2, and 5 have been found to be mutagenic[8]. Likewise, several case studies have linked azo pigments with Basal Cell Carcinoma.[9] In the second investigation of my experiment the Azo-Dye will be

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[7] A thesis SUBMITTED BY Kamayani Sharma to the UNIVERSITY OF RAJASTHAN :- Environment impact assessment of textile industry wastewaters in sanganer environment

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[8] "Health & Safety in the Arts, A Searchable Database of Health & Safety Information for Artists". *Tucson University Studies*.

[9] http://en.wikipedia.org/wiki/Azo_compound#As_dyes_and_pigments

added to the fish's environment and there direct response would be shown by the difference in their opercular movement.

HYPOTHESIS

As both our independent variables are toxic pollutants, the opercular response of the fish should show a similar effect, when placed under the tested concentrations of DDT and the Azo-Dye. The effect should preferably show a gradual decline as with prior knowledge I have understood that the decrease in the opercular movement can be regarded as a protective mechanism adapted by the fish to prevent the entry of toxic molecules present in the medium to minimi ze damage to the fish internally.

SELECTING VARIABLES:

- **INDEPENDENT VARIABLES**:

I. **The amount of the DDT solution added** to the beakers will remain independent as we change the amount added in order to change the concentration. (1ppm, 2pp m, 4ppm, 6ppm)

II. **The amount of the Azo-Dye solution added** to the beakers will also remain independent as we change the amount added in order to change the concentration. (0.03ppm, 0.6ppm, 0.09ppm and 0.12ppm)

III. **The amount of the mixture of these two toxins added to the beakers** will again remain independent as we change the amount added in order to change the concentration. (1ppm DDT + 0.03 ppm Azo-Dye)

CONTROLLED VARIABLES:

- **DEPENDENT VARIABLES**:

I. **The opercular rate of the fish** in the first investigation will remain directly dependent on the different concentrations of the DDT solution added to the beaker.

II. **The opercular rate of the fish** in the second investigation will remain directly dependant on the different concentrations of the Azo-Dye solution added to the beaker.

III. **The opercular rate of the fish** in the third investigation will remain directly dependent on the mixture of DDT and Azo-Dye added to the beaker.

- **FIXED VARIABLES**:

I. **The variety of the fish** should be the same in order to provide similar opercular responses. *(Cirrhinus cirrhosus)*

II. **The time the fish are left in the beaker**. The fishes would be added to a new environment and should be kept there for a fixed amount of time.

III. **The source of water**, in which the toxins are dissolved should be the same so that, the created environment is the same for all fishes.

IV. **The temperature** needs to remain constant to provide accurate results .

V. **The same amount and type of Fish food** is provided to all the fish.

VI. **The time of performing the experiment** needs to remain the same.

CONTROL OF VARIABLES:

- **INDEPENDENT VARIABLES:** I individually weighed the concentration of DDT (1g, 2g, 4g and 6g), the Azo Dye (0.03g, 0.06g, 0.09g and 0.12g) and the Mixture of the two (1g DDT +0.03g Azo Dye) with the help of an electronic weighing balance. Then thes e concentrations were added using china dishes into measuring cylinders of 1000ml, each. Thus creating 1ppm, 2ppm, 4ppm and 6ppm for the first investigation. 0.03ppm, 0.06ppm, 0.09ppm and 0.12ppm for the second investigation. And a mixture of 1ppm of DDT and 0.03ppm of Azo Dye for the third investigation. Next, 200ml from each measuring cylinder (DDT and the Azo-Dye) was poured into their respective beakers: (B, C, D and E). For the third investigation however 100ml from its measuring cylinder will be poured into its respective beaker: (B).The concentrations of DDT were selected as referred in our IB Biology Text book in the ecology lesson. The concentration of the Azo-Dye had to be kept in mind as they turned immensely opaque as I increase its concentration; this is why I used the minimal concentrations of the Azo-Dye.

- **FIXED VARIABLES:**

I. **The variety of the fish** *(Cirrhinus cirrhosus)* has been bought from a local fishery shop. Thus ensuring that they are from the same source. As studied in my introduction the Mrigal would be able to show me an accurate impact of these toxins in nearby areas as it is widely cultured in India.

II. **The time the fish are left in the beaker** is monitored with the help of a stop watch. Ensuring that the fish don't remain in the test -beakers for more than 6 minutes.

III. **The source of water** used was fixed as, filtered water was provided to me by the lab assistant for the experiments conducted.

IV. **The temperature** was kept constant during the process of the experiment as no external factors affecting the temperature were turned on. (Fans, Coolers or Windows)

V. **The same amount and type of Fish food** is provided to the Fish. The fish food was bought by from the same local fishery thus providing equal nutrients to all the fishes. (Crude Protein: 32%, Crude F at: 45, Crude Fiber: 55, moisture: 10%) - As per the proximate analysis provided behind the cover of the food.

VI. **The time of performing the experiment** was kept controlled. All experiments and observations carried out started at 12 noon in order to attain an equal response from the fish.

APPARATUS REQUIRED:

- Glass beakers 12 × 200ml
- Mrigal fishes × 60
- Fish tanks × 2
- Measuring cylinders 12 × 100ml
- Electronic weighing balance (± 0.05 mg)
- Stop-watch (± 0.5 seconds)
- DDT powder × 66 g
- Azo Dye × 1g
- China dish × 12
- Watch glass × 1
- Filtered Water × 13 liters
- Spatula × 1
- Spoon × 1
- Gloves × 1 pair
- Goggles × 1pair

PROCEDURE:

I have divided my analysis into three separate primary investigations. The first investigation consists of the different concentrations of DDT; the second consists of the different concentration of the Azo Dye and the third which consists of a mixture of these two toxins. Through an analysis of these three investigations I will determine the change in the opercular movement of *Cirrhinus Cirrhosus*.

1. Taking 5 glass beakers arrange them in a line and marked them as: A, B, C, D and E.
2. Fill each beaker with 200 ml of filtered water.
3. With the help of a fish net, add one of the five fishes to beaker A for the controlled experiment.
4. As soon as the fish is added into the beaker a Watch glass should be placed on the lid of the beaker in order to avoid any loss of water due to the splashing of the fish.
5. The beaker should be left undisturbed for 60 seconds. To allow the fish to adjust to the change in environment.
6. With the help of a stopwatch, at the end of 60 seconds begin counting the opercular movement of the fish in the beaker for 5 minutes .
7. The results should be recorded after each minute.
8. Next, repeat the above procedure for beakers B, C, D and E.
9. The entire set-up should be washed and the observed fishes need to be added into a separate fish tank.
10. The set-up next, should be placed in the identical manner as earlier however, this time I begin adding the DDT solution into the beakers. (ensuring that gloves and goggles are worn while in contact with these toxins)
11. Add 1ppm of DDT into the beakers marked A, B, C, D and E respectively.
12. Give the beakers enough time to let the DDT dissolve. Stir if necessary.
13. With the help of a fish net, add one of the five fishes to beaker A.
14. As soon as the fish is added into the beaker a Watch glass should be placed on the lid of the beaker.
15. Leave the beaker undisturbed for 60 seconds.

16. With the help of a stopwatch , at the end of 60 seconds begin counting and recording the opercular movement of the fish in the beaker for 5 minutes
17. Repeat, the same for beakers B, C, D and E
18. Continue the above procedure with different concentrations of DDT as 2ppm, 4ppm and 6ppm respectively.
19. The same experiment now has to be laid out to carry out the second and third investigation regarding the Azo Dye and the mixture between DDT and the Azo Dye.
20. The results should be recorded in a manner like the result table s shown below.

INVESTIGATION I: TO STUDY THE OPERCULAR RESPONSE OF *CIRRHINUS CIRRHOSUS* UNDER DIFFERENT DOSES OF DDT IN LABORATORY CONDITIONS.

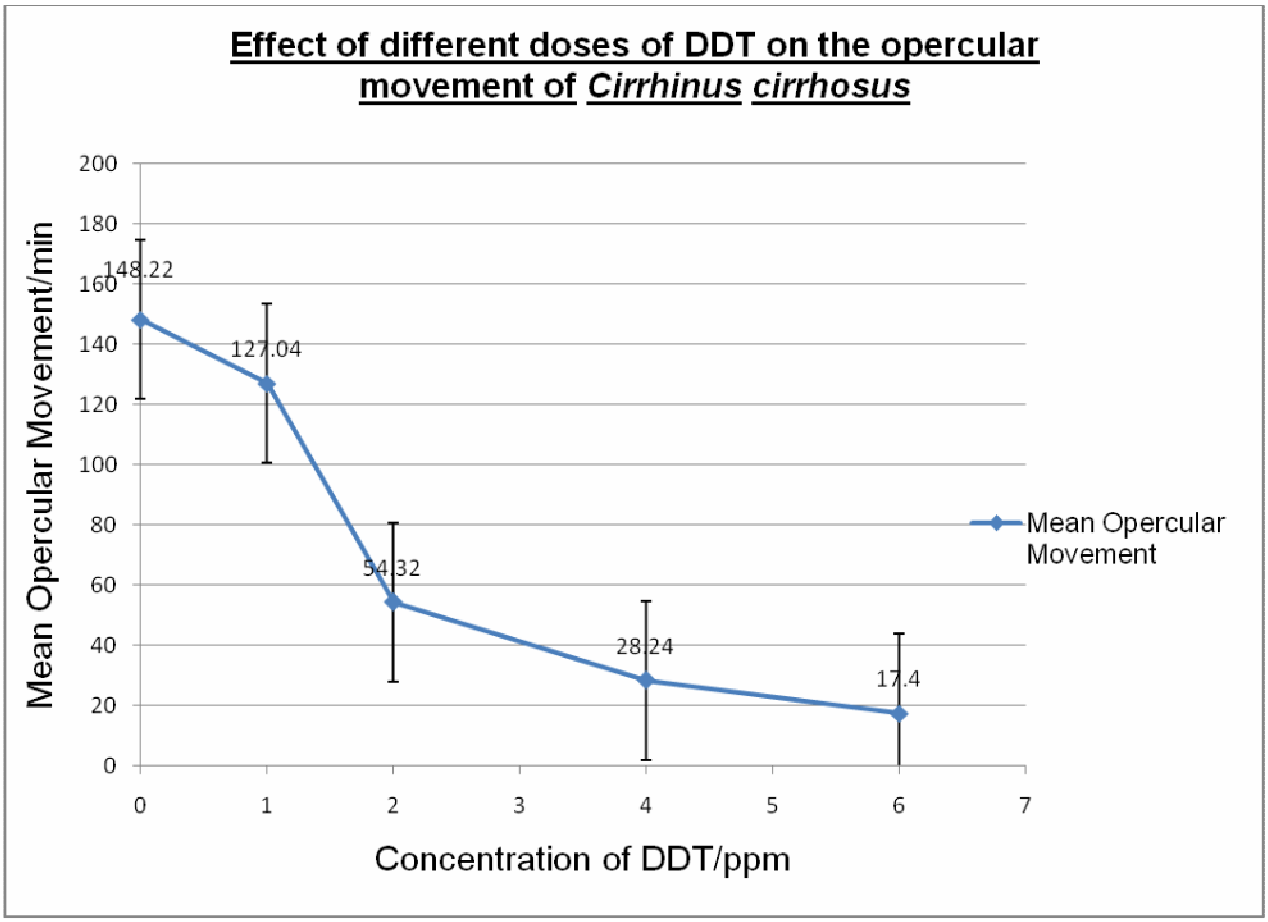
After carrying out the procedure suggested for Investigation 1. I arrive at the following results.

RESULT TABLE:

EFFECT OF THE DIFFERENT CONCENTRATIONS OF DDT ON THE OPERCULAR MOVEMENT OF *CIRRHINUS CIRRHOSUS*

Doses of DDT/ppm	Beaker : (fish)	Opercular movement/min					Mean opercular movement/min	AVG	S.D	Mean of S.D
		First minute	Second minute	Third minute	Fourth minute	Fifth minute				
CONTROL	A	146	155	150	142	148	148.5	148.22	4.82	3.70
	B	147	148	145	152	148	148		2.55	
	C	150	149	142	145	150	147.2		3.56	
	D	149	152	147	142	150	148		3.81	
	E	145	150	147	155	150	149.4		3.78	
1	A	120	138	127	131	124	128	127.04	6.89	5.51
	B	119	128	130	134	120	126.2		6.50	
	C	121	130	127	133	125	127.2		4.60	
	D	130	133	130	125	120	127.6		5.13	
	E	131	120	125	125	130	126.2		4.44	
2	A	66	48	52	48	58	54.4	54.32	7.67	5.63
	B	54	56	60	59	50	55.8		4.02	
	C	50	60	48	55	48	52.2		5.22	
	D	60	62	48	50	50	54		6.48	
	E	54	60	59	55	48	55.2		4.76	
4	A	30	28	26	32	25	28.2	28.24	2.86	3.07
	B	33	31	24	27	24	27.8		4.09	
	C	30	32	24	26	24	27.2		3.63	
	D	30	33	31	27	30	30.2		2.17	
	E	28	27	31	24	29	27.8		2.59	
6	A	21	20	16	18	14	17.8	17.4	2.86	2.68
	B	24	21	21	14	14	18.8		4.55	
	C	16	14	16	18	16	16		1.41	
	D	20	14	18	16	18	17.2		2.28	
	E	14	18	18	16	20	17.2		2.28	

RESULT GRAPH:

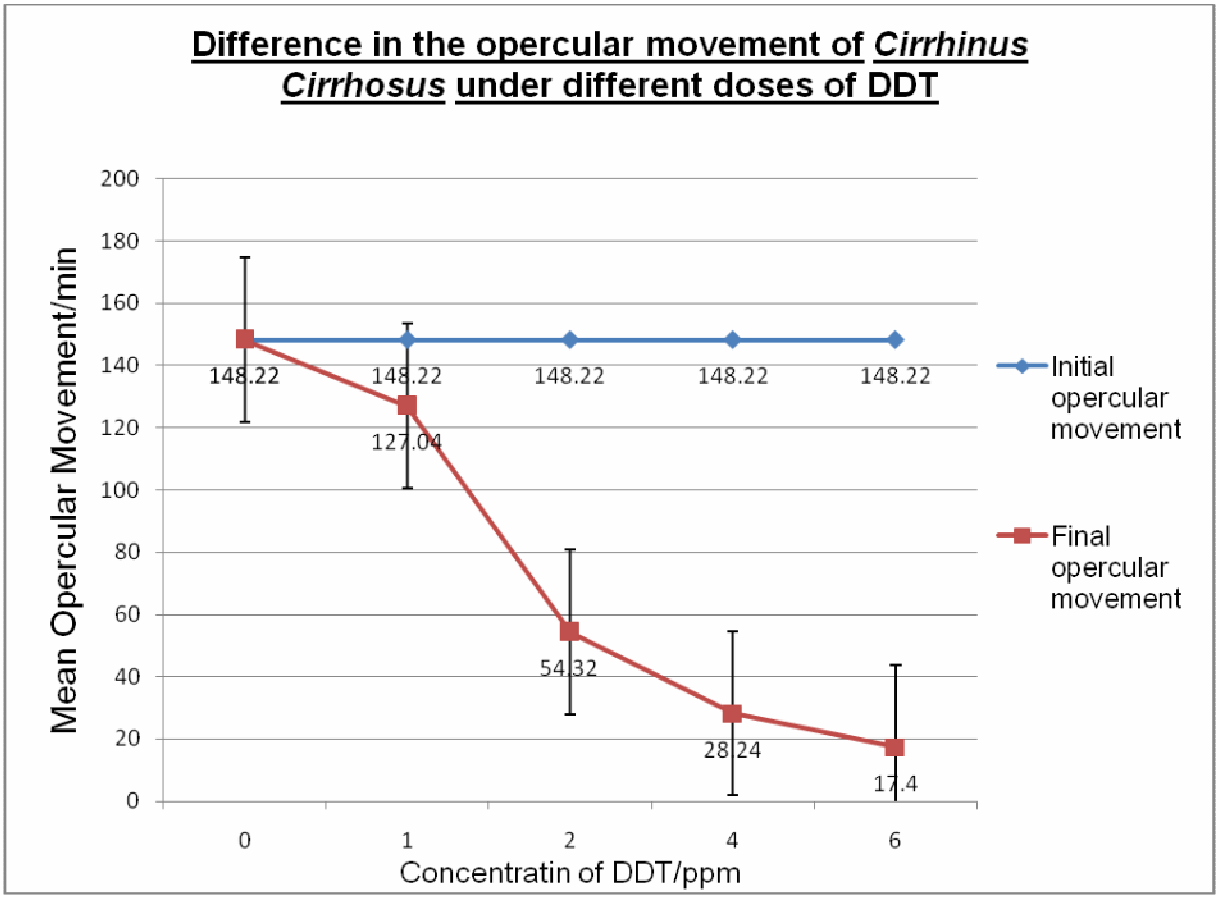


PERCENTAGE DIFFERENCE OF THE OPERCULAR RESPONSE SHOWN BY THE FISH

OBSERVATION TABLE:

Initial Opercular movement in the controlled beaker/min	Doses of DDT/ppm	Final Opercular movement in the different concentration of DDT/min	DIFFERENCE/ppm	PERCENTAGE DIFFERENCE/%
148.22	0	148.22	0	0.00
148.22	1	127.04	21.18	-14.29
148.22	2	54.32	93.9	-63.36
148.22	4	28.24	119.98	-80.95
148.22	6	17.4	130.82	-88.27

OBSERVATION GRAPH:



INVESTIGATION II: TO STUDY THE OPECULAR RESPONSE OF FISH UNDER DIFFERENT DOSES OF AZO-DYE IN LABORATORY CONDITIONS.

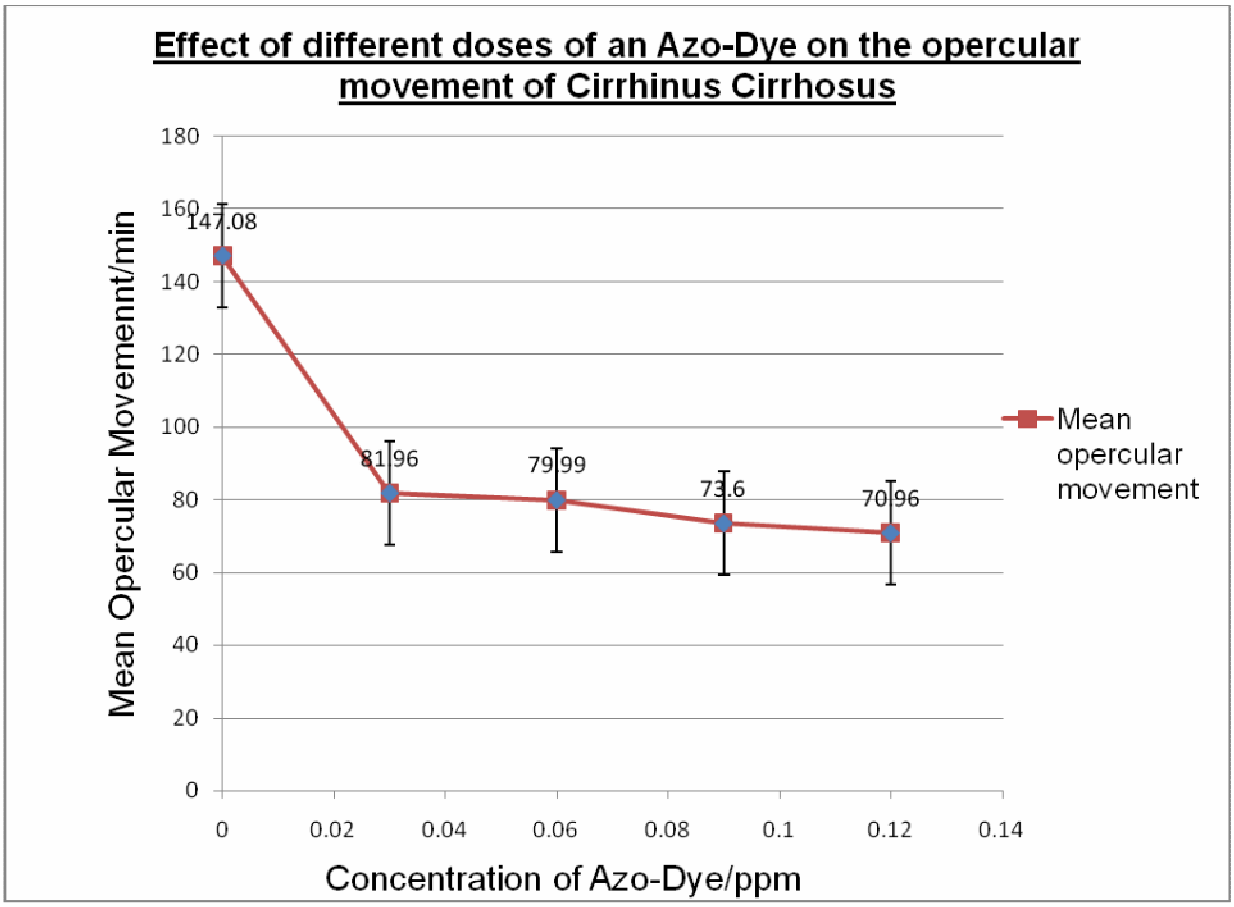
After carrying out the procedure suggested for Investigation II. We arrive at the following results:

RESULT TABLE:

EFFECT OF THE DIFFERENT CONCENTRATIONS OF THE AZO DYE ON THE OPERCULAR MOVEMENT OF *CIRRHINUS CIRRHOSUS*

Doses of DYE/ppm	Beaker: (fishes)	Opercular movement/min					Mean opercular movement/min	Average	SD	Mean of SD
		First minute	Second minute	Third minute	Fourth minute	Fifth minute				
CONTROL	A	150	142	146	148	149	147	147.08	3.16	4.59
	B	149	145	149	146	148	147.4		1.82	
	C	149	149	146	148	146	147.6		1.52	
	D	146	147	144	149	146	146.4		1.82	
	E	148	149	147	146	145	147		1.58	
0.03	A	76	87	84	83	85	83	81.96	4.18	4.4
	B	84	79	74	88	88	82.6		6.07	
	C	78	74	80	88	80	80		5.10	
	D	79	85	88	81	78	82.2		4.21	
	E	83	80	85	83	79	82		2.45	
0.06	A	76	80	84	84	84	81.6	79.88	3.58	5.11
	B	71	78	86	70	88	78.6		8.29	
	C	75	72	79	84	80	78		4.64	
	D	84	76	78	80	74	78.4		3.85	
	E	76	82	88	88	80	82.8		5.22	
0.09	A	72	76	76	72	72	73.6	73.6	2.19	2.92
	B	70	76	75	71	72	72.8		2.59	
	C	76	70	78	70	74	73.6		3.58	
	D	79	71	73	74	78	75		3.39	
	E	72	76	76	71	70	73		2.83	
0.12	A	76	72	72	72	72	72.8	70.96	1.79	1.78
	B	69	72	74	70	70	71		2.00	
	C	70	71	68	69	72	70		1.58	
	D	72	69	70	70	71	70.4		1.14	
	E	74	69	72	68	70	70.6		2.41	

RESULT GRAPH:

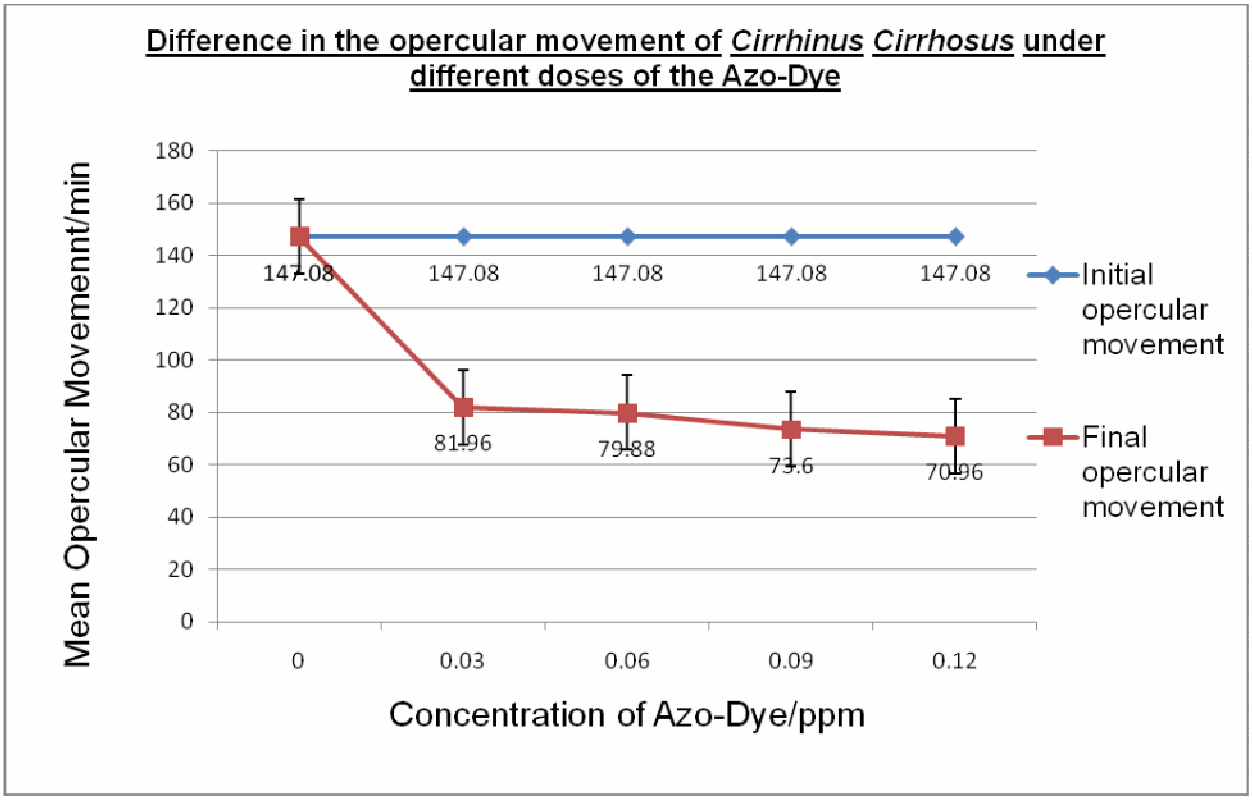


PERCENTAGE DIFFERENCE OF THE OPERCULAR RESPONSE SHOWN BY THE FISH

OBSERVATION TABLE:

Initial Opercular movement in the controlled beaker/min	Doses of DYE/ppm	Final Opercular movement in the different concentration of Azo-Dye/min	DIFFERENCE/ppm	PERCENTAGE DIFFERENCE/%
147.08	0	147.08	0	0.00
147.08	0.03	81.96	65.12	-44.28
147.08	0.06	79.88	67.2	-45.69
147.08	0.09	73.6	73.48	-49.96
147.08	0.12	70.96	76.12	-51.75

OBSERVATION GRAPH:



INVESTIGATION III: TO STUDY THE OPECULAR RESPONSE OF FISH UNDER DIFFERENT DOSES OF A MIXTURE BETWEEN DDT AND THE AZO-DYE IN LABORATORY CONDITIONS.

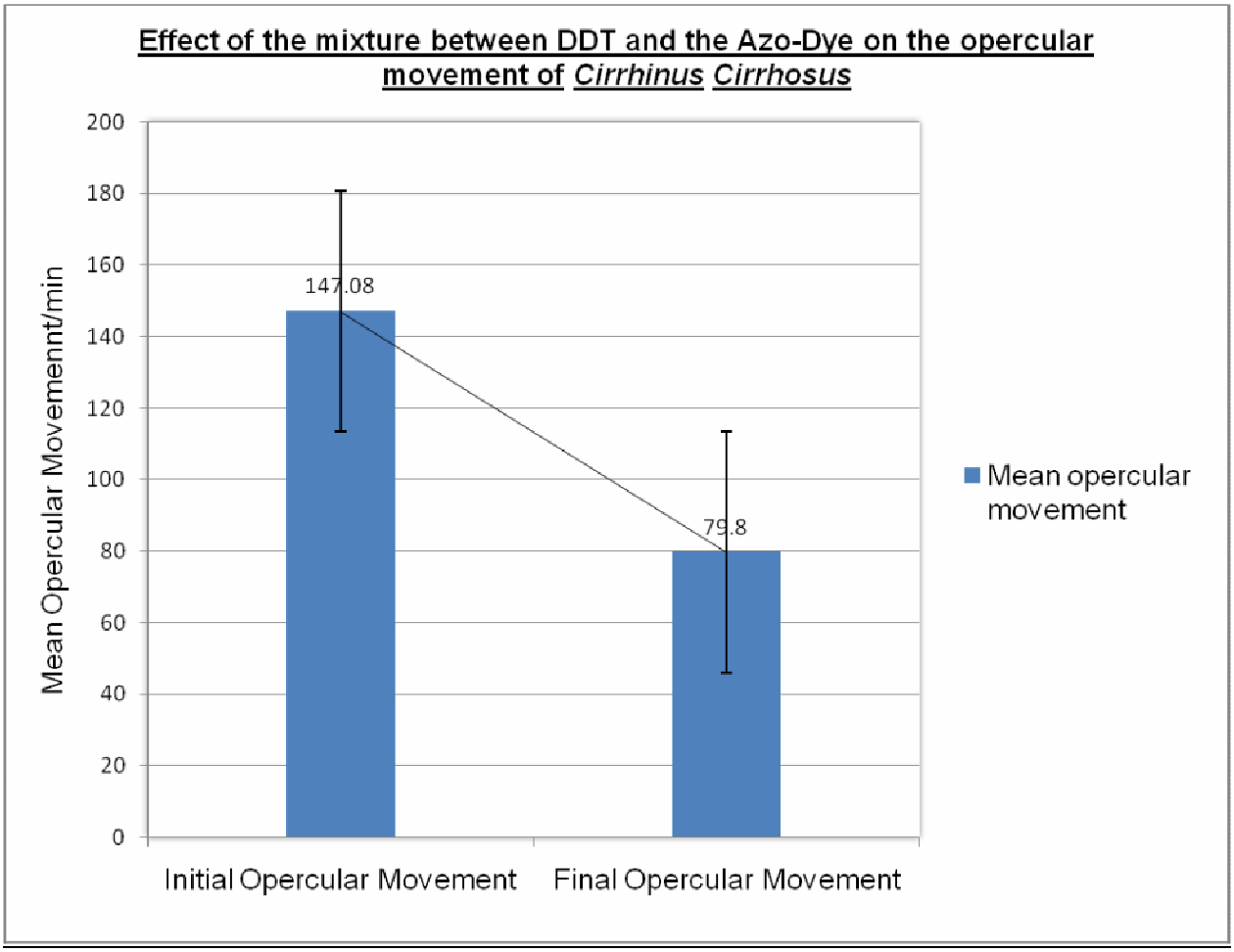
After carrying out the procedure suggest ed for Investigation III. We arrive at the following results:

RESULT TABLE:

EFFECT OF THE MIXTURE BETWEEN DDT AND THE DYE ON THE OPERCULAR MOVEMENT OF *CIRRHINUS CIRRHOSUS*

Doses of DDT + DYE/ppm	Beaker: (fishes)	Opercular movement/min					Mean opercular movement/min	Average	SD	Mean of SD
		First minute	Second minute	Third minute	Fourth minute	Fifth minute				
CONTROL	A	149	148	145	150	146	147.60	147.08	2.07	2.13
	B	150	148	147	146	148	147.80		1.48	
	C	146	145	148	144	147	146.00		1.58	
	D	151	147	148	150	144	148.00		2.74	
	E	150	144	146	149	144	146.60		2.79	
1+0.03	A	80	80	81	75	80	79.20	79.8	2.39	2.09
	B	79	81	84	79	79	80.40		2.19	
	C	78	81	79	82	79	79.80		1.64	
	D	80	79	79	84	77	79.80		2.59	
	E	82	79	81	78	79	79.80		1.64	

RESULT CHART:

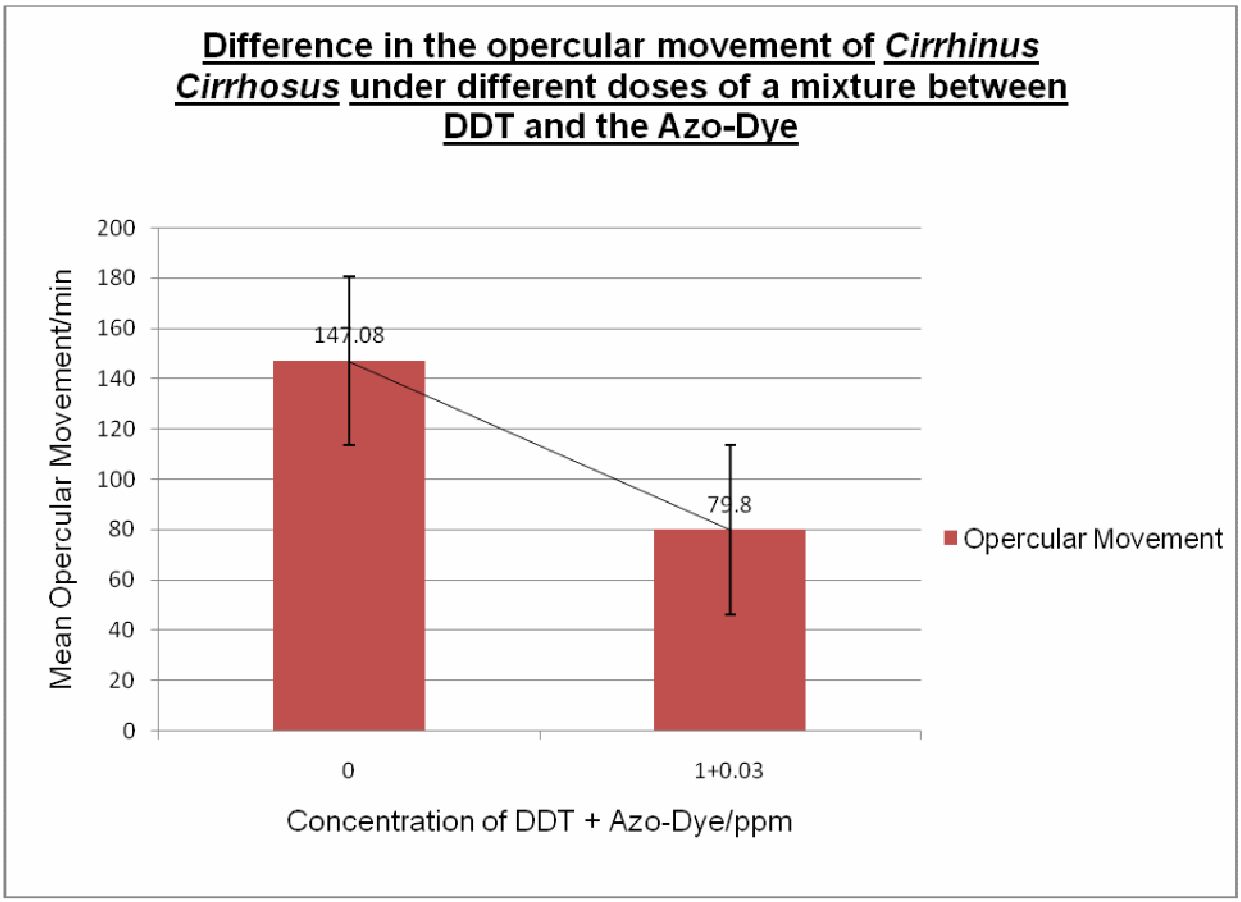


PERCENTAGE DIFFERENCE OF THE OPERCULAR RESPONSE SHOWN BY THE FISH

OBSERVATION TABLE:

Initial Opercular movement in the controlled beaker/min	Doses of DDT + DYE/ppm	Final Opercular movement after adding the mixture/min	DIFFERENCE/ppm	PERCENTAGE DIFFERENCE/%
147.08	0	147.08	0	0.00
147.08	1+0.03	79.8	65.12	-46.22

OBSERVATION CHART:



T-TEST

The following T-test shows us the probability that the means of our results are different simply due to chance.

Doses of DYE/ppm	Beaker: (fishes)	Opercular movement/min					Mean opercular movement/min	Average	SD	Mean of SD	T-Test Value
		First minute	Second minute	Third minute	Fourth minute	Fifth minute					
0.03	A	76	87	84	83	85	83	81.96	4.1833001	4.4	Array 1
	B	84	79	74	88	88	82.6		6.0663004		
	C	78	74	80	88	80	80		5.0990195		
	D	79	85	88	81	78	82.2		4.2071368		
	E	83	80	85	83	79	82		2.4494897		
0.06	A	76	80	84	84	84	81.6	79.88	3.5777088	5.11	Array 2
	B	71	78	86	70	88	78.6		8.2945765		
	C	75	72	79	84	80	78		4.6368093		
	D	84	76	78	80	74	78.4		3.8470768		
	E	76	82	88	88	80	82.8		5.2153619		

T-Test Value:

0.07

Our T-test value tells us that there is a 7% chance that the difference between the 0.03pmm concentration and the 0.06ppm concentration of the Azo-Dye is due to random chance alone.

OR

There is a 73% chance that the differences between the opercular responses of the fish, in concentrations of 0.03ppm and 0.06ppm of the Azo -Dye are due to the variable being investigated which in this case is the Azo-Dye.

CONCLUSION AND EVALUATION

CONCLUSION

The results of the first two investigations carried out go on to support my initial hypothesis, as both our independent variables were toxic pollutants, the opercular response of the fish did show a similar decline when placed under the different test concentrations.

The average opercular rate of the fishes showed a similar decline, when placed in the DDT solution, the AZO-Dye solution and their mixture. We could however, not carry out the investigation under the mixture with any further concentrations. As the test beaker began getting opaque due to the large concentrations. Keeping the animal protocol in mind, I would not have been permitted to perform any further experiments on the expense of life. This is why each fish was tested only once and then placed into a fresh fish tank.

In the first investigation carried out, the 25 fishes were placed under different concentration of the created DDT solution and there opercular response was observed. The fishes observed; recorded an average response of 148.22 opercular movements at the end of five minutes in the controlled experiment. Next I began to add the DDT solution into the beakers and began recording the results. The concentrations of the DDT solution added was 1ppm, 2ppm, 4ppm and 6ppm and the average opercular response to the different doses was 127.04, 54.32, 28.24 and 17.4 respectively. The recorded results show us that there was a gradual decrease in the opercular rate of the fish which is what was suggested in the initial hypothesis.

An XY (scatter) graph too has been added at the end of the result table which shows the effect of different doses of DDT on the opercular movement of *Cirrhinus cirrhosus*. The graph goes on to support the results in the table as it too shows the trend of a gradual decline. To substantiate and understand the difference between the controlled and test experiments I studied the percentage difference between the initial and final opercular movement under the different concentrations of DDT. The percentage difference under the concentrations was -14.29%, -63.36%, -80.95% and -88.27% respectively. Another graph was then added under the percentage

difference table to understand the difference in the in itial and final opercular movements better. This went on to support my result graph as this too showed a gradual decline in the opercular movement.

In the second investigation carried out, another set of 25 fishes were placed under different concentration of the created Azo-Dye solution. The fishes observed; recorded an average response of 147.08 opercular movements at the end of five minutes in the controlled experiment. After adding the Azo-Dye solution into the beakers the results were recorded. The concentrations of the Azo-Dye were 0.03ppm, 0.06ppm, 0.09ppm and 0.12ppm and the average opercular response to the different doses was 82.76, 79.88, 73.6 and 70.96 respectively. The recorded results again showed a gradual decrease in the opercular rate of the fish. The results under the Azo-Dye solution first began to show an immense decline under the initial concentration of the Azo-Dye as compared to DDT which only started with a decline of 20 opercular movements. However, with increasing concentrations, I ob served that DDT had a much greater impact on the opercular movement as compared to the Azo-Dye. Even though we see a significant difference in the opercular response shown under the two toxins we cannot conclude that DDT is more dangerous as compared to the Azo-Dye as the concentrations tested were not the same. The initial attempt to try and have the same concentrations of DDT and the Azo -Dye resulted in the beaker turning completely opaque and turned into a dark purple colour. This is why the Azo-Dye concentration had to be decreased in an attempt to not harm the fishes.

The XY (scatter) graph added at the end of the observation table which shows us the Effect of different doses of the Azo-Dye on the opercular movement of *Cirrhinus cirrhosus* shows us that these results too show the trend of gradual decline. The results of the percentage difference carried out in the second investigation were 44.28%, -45.69%, -49.96 and -51.75 for the concentrations of 0.03ppm, 0.06ppm, 0.09ppm and 0.12ppm of the Azo-Dye respectively. A second graph was then added here as well, under the percentage difference table to understand the difference in the initial and final opercular movements in the Azo -Dye investigation better. This too supports its observation graph.

Moving on to the third investigation carried out. 10 more fish were placed under a mixture between DDT and the Azo-Dye and their opercular response was recorded. The fishes observed; recorded an average response of 147.08 in the controlled experiment. I then began to add the solution of the mixture between DDT and the Azo-Dye into the observation beaker. The concentration s were 1ppm (DDT) + 0.03ppm (Azo-Dye). The mean opercular response of the fish to the mixture was 79.8 opercular movements per minute. The recorded results like the prior investigations too showed a decline in the opercular rate of the fish. The opercular response during the test experiments (79.8) is seen to be lower than the opercular response of the same concentration of DDT (127.04) and the Azo -Dye (81.96). In the first two investigation we saw that yes, these two toxins are dangerous to freshwater fishes. The third investigation now shows us that when added together to a pond or local body they are a lot more impactful as compared to when they are added in isolation.

A bar chart was then added after the observation table which shows the difference in the opercular response shown by the fishes when placed under the mixture of these two toxins. The graph like the prior two investigations goes on to s upport the results as it too shows the trend of decline.

Similar to the prior two investigations, I studied the percentage difference between the initial (controlled) and final (test) opercular movement s. The percentage difference under the concentration o f 1ppm (DDT) + 0.03ppm (Azo-Dye) was -46.22%. This again supports my observation graph and tells us how the percentage difference is lower in the mixture as compared to the first two investigations which had a difference of -44.28% (DDT) and -14.29% (Azo-Dye).

To conclude, as stated above we see that these two toxins do affect the breathing process of the fish. We also find out that when added together these two can have a long lasting impact on the aquatic life.

EVALUATION

- STANDARD DEVIATION:

Despite the fact that the readings of my observations do tend to show a gradual decline, each specific reading however fluctuates around the mean. Thus the calculated standard deviation will help me understand the exact variance of each reading from its respective mean. The standard deviation for each observation was calculated with the help of Microsoft Excel and has been added to the tables containing the observations and the ir mean. I however, was not satisfied with the standard deviation calculated for the con centrations of 1ppm (5.51) and 2ppm (5.63) of DDT, and 0.06ppm (5.11) of the Azo Dye. The results showed me that my readings deviated significantly from the mean. This may have resulted in the accuracy of my investigations.

- T-TEST:

A T-test was calculated between the concentrations of 0.03 ppm and 0.06ppm of the Azo-Dye. The difference shown in the mean opercular movement of the fish under these concentrations was not conclusive enough as it did not show a great degree of difference which is why I supported my observations with the help of a T-test performed on Microsoft Excel.

The T-Test helped me conclude that there was a 7% chance that the difference between the 0.03pmm concentration and the 0.06ppm concentration of the Azo -Dye is due to random chance alone.

OR

There was a 73% chance that the differences between the opercular responses of the fish, in concentrations of 0.03ppm and 0.06ppm of the Azo -Dye are due to the variable being investigated which in this case is the Azo -Dye.

- STANDARD ERROR:

The standard error has been mentioned for each of the apparatus used which might have had a standard error as this affects the accuracy of my results. The graphs also contain error bars to provide us with an understanding of general errors. This helped me in attaining a more precise understanding of the results.

- ERROR ANALYSIS:

- **Human Error:** The greatest drawback of this experiment is the human error which occurs during the observation of the opercular response of the fish, as this takes place at a rapid rate; the human eye may miss an odd flap or two.
- **Systematic Error:** There wasn't any systematic error as there was no use of technology during the experiment.
- **Random Error:** The fish were selected at random from the 2 fish tanks. It might have been a possibility that the fish suffered from a random physiological defect.

- IMPROVING THE INVESTIGATION:

The experiment could have been improvised, expanded and thus improved in various ways.

- As this experiment is involved with the change of 'environment' of the fishes. The fishes could have been left in their respective beakers for a longer period of time in order for them to get used to the new 'environment'.
- Toxic pollutants are an extremely wide area of toxicology; I could have gathered various other toxic pollutants like detergents, fertilizers, insecticides, herbicides etc, and then observed there results on my test fishes.
- The addition of technology, like time lapse sensors to measure the opercular response of the fishes. Co_2 sensors to measure the breathing rate of the fish could have taken the research process to a much higher level, and provided me with much more substantial data.

EXTENSION OF THE EXPERIMENT

- **Can the toxicants change the behavior pattern of the fishes** :

The behavior pattern of freshwater fishes in terms of their breeding, biological interactions and eating habits can be studied with the help of various experiments, which might be further performed to study the ir response over a long period of time , when affected by such toxicants. This will help us in understanding this topic with a lot more detail.

- **The effects of such toxins on the aspects of the ecosystem like Bio - accumulation and Bio-magnification :**

An abstract from the IB Biology text has shown a study of this principle with an example of DDT:

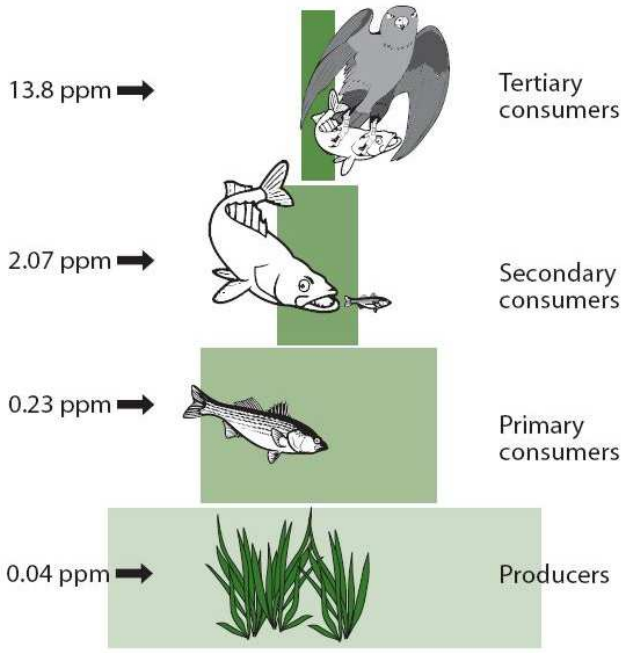


Figure 1835 The accumulation of DDT in a food chain

DDT sprayed on water to eliminat e mosquito larvae will be taken up by algae. As each level of the food chain is eaten, DDT levels increase because it cannot be excreted with urine due to i ts poor solubility. Hence it accumulates within the

organisms and then is magnified as many organisms from one trophic level are eaten by a few from the next trophic level.

- The same experiment or different experiments can be performed on the same lines as this extended essay. Different fresh water fish may be tested upon or different aquatic animals found at contaminated areas. The experiment does not have to stop at aquatic life. Those animals which drink water from nearby areas may also face the effect of these toxins. This is why the response of such toxins can be studied on regular animals as well.

TERMINOLOGY

- **Half-life:** The period of time it takes for a substance undergoing decay to decrease by half. [10]
- **Runoff:** The washing away of nutrients from the soil due to the flow of water.
- **Volatilization:** The process whereby a dissolved sample is vaporized. In atomic spectroscopy this is usually a two step process.[11]
- **Photolysis:** A chemical reaction in which a chemical compound is broken down by photons.[12]
- **Biodegradation:** The chemical breakdown of materials by a physiological environment.[13]
- **Biomagnification:** The increase in concentration of a substance, which occurs in a food chain.[14]
- **Bioaccumilation:** The increase in concentration of a substance in certain tissues of organisms' bodies due to absorption from food and the environment[15]
- **Mutagenic:** A physical or chemical agent that changes the genetic material, usually DNA, of an organism and thus increases the frequency of mutations above the natural background level.[16]

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[10] http://en.wikipedia.org/wiki/Half_life

[11] http://en.wikipedia.org/wiki/Volatilisation

[12] http://en.wikipedia.org/wiki/Photolysis

[13] http://en.wikipedia.org/wiki/Biodegradation

[14] http://en.wikipedia.org/wiki/Biomagnification

[15] http://en.wikipedia.org/wiki/Biomagnification

[16] http://en.wikipedia.org/wiki/Mutagenic

- **Hydrophobic:** The physical property of a molecule that is repelled from a mass of water. [17]
- **Basal cell carcinoma:** the most common type of skin cancer. It rarely metastasizes or kills, but it is still considered malignant because it can cause significant destruction and disfigurement [18]

[17] http://en.wikipedia.org/wiki/Hydrophobic

[18] http://en.wikipedia.org/wiki/Basal_cell_carcinoma

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