

Can Turmeric Reverse Effects of Perchlorate on *Daphnia* Heart Rate?



Acknowledgements

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Introduction

I chose this project because perchlorate contamination in the Colorado River is a pollution problem that has been recently identified and may have far reaching environmental impacts. I first chose to use lettuce plants in my experiments with the contaminant perchlorate, but had repeated problems using lettuce in testing. I then selected instead to test *Daphnia magna* (a relatively simple animal form) to study the impacts of both perchlorate and turmeric on heart rate.

Statement of the Problem

Will perchlorate increase the heart rate of *Daphnia magna* and if so, can turmeric (an antioxidant) reverse the effect of perchlorate on the heart rate of *Daphnia magna*?

Hypothesis

Perchlorate will increase the heart rate of *Daphnia magna*. Perchlorate is an oxidizing agent, and just like other oxidizing agents should raise the heart rate of *Daphnia magna*. Turmeric will reverse the effect of perchlorate and reduce its heart rate. Turmeric will reverse the effect of perchlorate on the heart rate of *Daphnia magna* because turmeric is a nontoxic antioxidant.

Abstract

The objective of this project was to evaluate whether perchlorate salts will increase the heart rate of *Daphnia magna* and whether turmeric could reverse this effect.

A dose-dependent increase in heart rate was demonstrated upon exposure of *Daphnia* to perchlorate solutions. In 0.001M perchlorate, the average heart rate (AHR) increased from 215 beats per minute (bpm) in water to 221 at 1 minute; down to 217 after 15 minutes and 215 at 30 minutes. In 0.01M perchlorate the AHR increased from 247 to 274 bpm at 1 minute; 297 at 15 minutes and 289 at 30 minutes. In 0.1M perchlorate the AHR increased from 309 to 383 bpm at 1 minute and 403 at 15 minutes.

Addition of turmeric to *Daphnia* that have already been exposed for 15 minutes to 0.01M perchlorate lowered the AHR from 369 to 331 bpm within 1 minute and continued to decrease it to 114 bpm after 15 minutes.

When *Daphnia* were exposed to solution containing both 0.01M perchlorate and turmeric, the AHR was lowered from 381 bpm in 0.01 perchlorate to 269 in the combined solution within 1 minute of exposure. This drop continued to an AHR of 181 bpm after 15 minutes.

The findings of this study fully supported the hypothesis. This study definitively demonstrates the dose-dependent increase in heart rate in *Daphnia magna* upon short-term exposure to perchlorate in water. The results also showed a dramatic reversal of this phenomenon upon addition of turmeric. In the event of an acute exposure to high perchlorate levels due to industrial accidents, turmeric represents a natural, potent, economic, and readily available antidote that can immediately reverse the effect of perchlorate.

Review of the Literature

Perchlorate salts are found in the Colorado River. Perchlorate salts of ammonium (NH_4ClO_4), sodium (NaClO_4), potassium (KClO_4), and magnesium [$\text{Mg}(\text{ClO}_4)_2$] are toxic and are used as oxidizing agents in rocket fuel (www.ewg.org/reports/suspectsalads/part4.php, 2003).

Ammonium perchlorate is a strong oxidizer and a key ingredient in rocket fuel. It is manufactured in several sites in the United States. The perchlorate found in the Colorado River has been traced to effluents from factories that utilize perchlorate salts. Perchlorate salts are believed to have washed down a stream into Lake Mead, which passes the contaminated water into the Hoover Dam reservoir. The perchlorates then drift down the lower Colorado River and eventually get into the Colorado River's main course. "It's hard to imagine more people exposed from any one release anywhere in the country," said Kevin Mayer, the perchlorate coordinator for EPA's San Francisco-based Region 9 (Hogue, 2003).

The Colorado River supplies water not only to Colorado, California and Nevada, but also many other locations. Sampling by officials has confirmed that there is perchlorate in "more than 500 drinking water sources in 20 states, serving well over 20 million people in California, Arizona, and Nevada, and unknown millions elsewhere" (www.ewg.org/reports/suspectsalads/part4.php, 2003). Many efforts have been made to cut contamination in the Colorado River, but the process is slow. Perchlorate still contaminates the water, and purification processes are far from efficient. "We're facing a situation we didn't create," said Alberto Ramirez, an environmental director for the Torrez Martinez tribe in the Desert Cahuilla Indian reservation in California (Hogue, 2003).

Many states are setting limits of perchlorate concentration in their drinking water, for perchlorate is very harmful to the human body. Maryland, New Mexico, and Massachusetts have set the upper limit at 1 part per billion (ppb). New York and Texas set it at 5 ppb. California limits the level to only 4 ppb, and Arizona limits it to 14 ppb (www.ewg.org/reports/suspectsalads/part4.php, 2003).

By disrupting the intake of iodine, perchlorate can interfere with thyroid function. People with hypothyroidism (not enough thyroid hormone being secreted by their bodies) are particularly vulnerable because their iodine intake is already unbalanced, and further disruptions could be problematic. Newborns and fetuses are at high risk because their nervous systems are not fully developed yet (Hogue, 2003). Young children who ingest perchlorate at toxic levels may be at risk of lowered IQ, mental retardation, loss of hearing and speech, and motor skill deficits (www.ewg.org/issues/perchlorate/20030919, 2003).

Perchlorate is not only contaminating water sources, but also the produce that has been irrigated with the contaminated water. "There can be no question that at least some vascular plants absorb perchlorate from their local environments," states the EPA (www.ewg.org/reports/suspectsalads/part4.php, 2003). The Environmental Working Group (EWG) bought 22 samples of lettuce and tested them for perchlorate. The samples came from different suppliers, were of different ages, and different kinds. 18% had "measurable levels" of perchlorate (www.ewg.org/reports/suspectsalads/part1.php, 2003). The

perchlorate in the lettuce may also be affecting those who consume the contaminated lettuce. Almost 3 million women are consuming contaminated winter lettuce each day, and approximately half of them get a dose of perchlorate greater than the EPA's reference dose from winter lettuce. Over 1 million women are getting a dose of perchlorate at least twice the EPA's reference dose from winter lettuce (www.ewg.org/reports/suspectsalads/figureA.php, 2003).

Perchlorate has also been found in blackberries, chinaberries, mulberries, strawberries, cucumbers, soybeans, field-grown wheat, and lab-grown lettuce (www.ewg.org/issues/perchlorate/20030919/, 2003).

This widespread contamination of water and fresh produce with perchlorate salts could be a significant threat to public health. "If perchlorate is shown to collect in vegetables and affect people who eat them, the findings would have significant consequences." (Bustillo, 2003) Unfortunately, more and more samples of produce have been shown contaminated, and consumers are starting to get perchlorate into their systems also

(www.ewg.org/reports/suspectsalads/figureA.php, 2003).

Cow's milk has been found to be contaminated with perchlorate at levels that are higher than the federal government's recommended safe level for drinking water. Researchers from the Institute of Environmental and Human Health at Texas Tech University reported in *Environmental Science and Technology* that perchlorate was "unambiguously detected" in all seven out of seven cow's milk samples from grocery stores in Texas. "These troubling results are some of the first indications that perchlorate is not only contaminating drinking water and irrigation water, but that livestock can pass it on to humans," said EWG Senior Analyst Renee Sharp

(www.ewg.org/issues/perchlorate/20030919/, 2003).

Cow's milk is not the only milk affected by perchlorate. Studies have shown one sample of human breast milk to contain approximately 4 parts per billion (ppb) of perchlorate. This is a big problem because if perchlorate gets into an infant's system, the consequences could be catastrophic. Newborn babies are especially vulnerable to perchlorate because their nervous systems are still developing at a very rapid rate. If a newborn ingested perchlorate the consequences could be lowered IQ, mental retardation, loss of hearing and speech, and motor skill deficits (www.ewg.org/issues/perchlorate/20030919/, 2003).

Daphnia magna, also known as water fleas, are small fresh water crustaceans found in lakes, ponds, rivers, and streams. *Daphnia magna* make up part of the freshwater and marine ecosystems. They are at the bottom of the food chain (Avila, 1992) (<http://ei.cornell/toxicology/bioassays/daphnia/>, 2004).

Daphnia are of the Phylum Arthropoda, SubPhylum Crustacea, Class Branchiopoda, Superorder Diplostraca, and the Order Cladocera (www.daphnia.com/daphnia.html). *Daphnia* are herbivores, and live mainly on yeast (www.cladocera.uoguelph.ca/about/about.htm).

Daphnia magna are commonly used as bioassays because they are relatively easy to care for and give accurate test results for water quality and toxicity. A bioassay is the use of a biological organism to test for chemical effect

on living organisms. Bioassays are an accurate way of testing for toxicity, and have been used for a long time. One of the earliest bioassays was the “canary in the coal mine”. Miners would bring a canary down into the mine with them because canaries are more sensitive to methane concentrations than are humans. If the canary died, the miners knew to get out of the mine as soon as possible (<http://ei.cornell.edu/toxicology/bioassays/Uses.asp>, 2003).

Daphnia magna have been shown to be a good model for *in vivo* assessment of cardiac toxicity. Experiments were conducted that study the effects of the glycosides digoxin, oleandrin, and uzarin on *Daphnia magna* and human heart rates. This study showed that *Daphnia magna* showed similar pharmacological response as humans with cardiac glycoside. *Daphnia magna* is thus a good model in testing cardiac toxicity as both *Daphnia magna* and human exhibited similar effects when exposed to the different compounds (Grant, 2000).

The effects of nicotine and antioxidants such as Vitamin E on the heart rate of *Daphnia magna* has been reported. It was concluded that nicotine raises the heart rate of daphnia while antioxidants such as Vitamin E can reduce the heart rate (http://school.discovery.com/sciencefaircentral/dys/hines_a.html).

Another study tested the effect of nicotine, caffeine, and Ritalin on the heart rate of *Daphnia magna*. Nicotine and caffeine had the most long-term stimulating effect on *Daphnia*. Ritalin was shown to have the strongest short-term effect, but the effect soon wore off. The nicotine and caffeine however, increased the heart rate, which consistently stayed high even after 24 hours (Applebaum, 2003).

Turmeric is a common ingredient in Indian and other south Asian food, and is also used in Indian medicine. Turmeric is an antioxidant and is made from the roots of the herb *Curcuma longa* L., Zingiberaceae. People from South Asia statistically have the lowest rates of Alzheimer’s disease and other neurological disorders. A study performed to see if there was a correlation between the consumption of turmeric and memory loss concluded that there was a dose related, direct improvement of memory when turmeric was added to drinking water (Suri, 2003). Curcumin, the main ingredient in turmeric, has also been shown to delay or prevent memory loss in Planaria. The bright yellow color of turmeric comes from the pigment Curcumin (C₂₁H₂₀O₆). Curcumin is used as an anti-inflammatory and is sparingly soluble in water (Merck Index, 2001; www.foodproductdesign.com/archive/1994/0394DE.html, 1994).

A literature search performed in Medline found no reports of a study of the ability of turmeric to reverse the effect of perchlorate salts in *Daphnia*.

Materials

- A culture of *Daphnia magna* from Carolina Biological Supplies
- Critter Keeper (to culture *Daphnia magna*)
- Yeast (to feed *Daphnia magna*)
- Spring water (to culture *Daphnia magna*)
- Distilled water (for preparing solutions)
- Cavity slides
- Microscope with Video Flex video camera
- Stopwatch
- Sodium perchlorate hydrate ($\text{NaClO}_4 \cdot \text{H}_2\text{O}$)
- Turmeric
- Graduated pipette
- Disposable pipettes
- Disposable glass vials
- Kleenex tissues
- Balance with accuracy of 1 mg

Procedures

Preparation of sodium perchlorate solutions:

0.1 M Solution:

A 0.1 molar (M) solution of sodium perchlorate was prepared by dissolving 141 mg of sodium perchlorate hydrate (molecular weight 141) in 10 mL distilled water.

0.01 M Solution:

A 0.01 M solution of sodium perchlorate was prepared by diluting 1 mL of 0.1 M solution with 9 mL distilled water.

0.001 M Solution:

A 0.001 M solution of sodium perchlorate was prepared by diluting a 1 mL of 0.01 M solution with 9 mL distilled water.

Preparation of turmeric solution:

Powdered turmeric (50 mg) was suspended in 3 mL of distilled water and vigorously shaken for approximately 1 minute. The yellow suspension was allowed to settle for 24 hours. The slightly turbid supernatant was transferred to another vial using a disposable pipette. This solution was drawn into a clean disposable pipette through eight layers of Kleenex by suction. The clear yellow solution was used for the experiment.

Determination of the heart rate of *Daphnia magna*:

1. A *Daphnia* was removed from the culture with a wide mouth pipette (plastic disposable pipette with the tip cut off).
2. The *Daphnia* was placed in the cavity of the slide.
3. Excess water was soaked up from the slide with a Kleenex so that the *Daphnia* could no longer swim around.
4. The slide was examined under 40× magnification using a microscope. The heart appeared as a contracting transparent sac on the backside just underneath the head.
5. The time for 100 contractions was recorded using a stopwatch. This count was repeated twice more.
6. The heart rate for three different *Daphnia* was determined.

Determination of the heart rate of *Daphnia magna* in test solutions:

1. A *Daphnia* was removed as described above and placed on the cavity of the slide.
2. Excess water was removed from the slide using a Kleenex.
3. A drop of the test solution was added to the *Daphnia* and immediately removed using Kleenex. This would rinse the *Daphnia* of cultured water.
4. Two drops of the test solution was then added and the *Daphnia* was allowed to remain in the solution for the time specified.
5. The heart rate was then counted three times as described before.
6. After the counting, the *Daphnia* was kept in two drops of the test solution until the next test.
7. The experiment was performed on two more *Daphnia*.
8. The test solution was then rinsed off the slide and the *Daphnia* were placed back in cultured water.

Results

When *Daphnia magna* were exposed to perchlorate, their heart rates were raised. Their response to perchlorate was directly dose related. See Table 1 and Figure 1.

In 0.001 M (0.014%) perchlorate solution, the average heart rate (AHR) of *Daphnia magna* did not show any significant difference from that without perchlorate. This was most likely because the dose was not strong enough to elicit a short-term effect. See Table 2 and Figure 2. After 24 hours back in cultured water, one *Daphnia* was dead, but two were still alive.

After one minute in 0.01 M (0.14%) perchlorate solution, the AHR was 274 bpm as opposed to 247 bpm in water. After fifteen minutes, the AHR was 297 bpm. However, after thirty minutes, the AHR did not increase any further, and was 289 bpm. This could be because the *Daphnia magna* were getting accustomed to their surroundings. See Table 3 and Figure 2. After 24 hours back in cultured water, all three *Daphnia* were dead.

In 0.1 M (1.4%) perchlorate solution, the results were dramatic. After one minute in perchlorate, the AHR went up from 309 to 383 bpm. After fifteen minutes, the AHR went up even more, to 403 bpm. A thirty-minute experiment was not conducted, because the *Daphnia magna*'s AHR was dangerously high. See Table 4 and Figure 2. After 24 hours back in cultured water, all three *Daphnia* were dead.

It was concluded from the dose-ranging experiment that 0.01 M perchlorate solution was the best concentration to use because 0.1 M had too strong an effect on *Daphnia* heart rate and 0.001 M had no significant effect. When exposed to 0.01 M perchlorate, the heart rate of *Daphnia* did change significantly after 15 minutes. See Figure 2. The rest of the experiments were thus conducted by exposing 0.01 M perchlorate solution for 15 minutes.

When another group of *Daphnia magna* was placed in the 0.01 M perchlorate solution for one minute, the AHR was raised from 282 to 362 bpm as observed before in the dose-ranging study. When left in perchlorate for 15 minutes, the AHR escalated even more, to 369 bpm. See Table 5 and Figure 3.

The *Daphnia magna* were then placed in a turmeric solution. One minute later, their AHRs dropped from 369 to 331 bpm. After 15 minutes, the AHR dropped drastically from 331 to 114 bpm, much lower than the AHR in cultured water (control). The heart of one *Daphnia* even stopped beating altogether, so the turmeric solution was rinsed from the slide and the *Daphnia* was put back in cultured water in a separate container. This shows that a small amount of turmeric is beneficial to the *Daphnia*, but in large amounts can be harmful to them. See Table 5 and Figure 3.

The AHRs of another set of *Daphnia magna* were measured, and the average was 305 bpm in cultured water. After being in a 0.01 M perchlorate solution for 1 minute the AHR went up to 362 bpm. After 15 minutes, the AHR increased even more to be at 381 bpm. The *Daphnia magna* were then put in a mixture of both 0.01 M perchlorate and turmeric solution. After one minute in this solution, the AHR was down to 269 bpm. After 15 minutes, the AHR dropped

dramatically to 181 bpm. See Table 6 and Figure 4. The *Daphnia magna* were then restored to cultured water.

Conclusions

This project was designed to study the short-term effects of exposure to high levels of perchlorate and the counter-effect, if any, of turmeric. The results supported the hypothesis: perchlorate increases the AHR of *Daphnia magna* and turmeric reversed this effect.

An initial dose-ranging experiment was conducted to determine the optimal concentration of perchlorate to use for the study. The 0.01 M perchlorate solution was chosen, and used for all other experiments in this project.

When *Daphnia magna* were placed in 0.01 M perchlorate there was an immediate increase in AHR. This increase continued for fifteen minutes, increasing by 20% from the control. The addition of turmeric resulted in an immediate and significant decrease in AHR within one minute. Over a period of fifteen minutes, the AHR continued a dramatic drop to as much as 50% below normal levels. It was concluded that perchlorate acted as a stimulant and increased the AHR of *Daphnia magna* and turmeric immediately reversed this effect.

When *Daphnia magna* were placed in a solution containing both turmeric and 0.01 M perchlorate, the AHR decreased significantly, but not quite as much as in the solution containing only turmeric. As before, the AHR continued to drop over the next fifteen minutes to 40% below normal levels. This shows that it is not necessary to remove the *Daphnia magna* from the perchlorate for turmeric to have an effect.

This study demonstrates the dose-dependent increase in average heart rate in *Daphnia magna* upon short-term exposure to perchlorate salts in water. Therefore rapidly increasing average heart rate may be used as an indicator of potential perchlorate poisoning.

The results also showed a dramatic reversal of this phenomenon upon addition of turmeric. In the event of an acute exposure to high perchlorate levels due to spills or industrial accidents, turmeric represents a natural, potent, economic, and readily available antidote that can immediately reverse the effect of perchlorate.

Recommendations

If I were to repeat this project, I would study not only the short-term effects of perchlorate and turmeric, but also the long-term effects. It would also be beneficial to study the effect of lower concentrations of perchlorate for longer periods of time. I would measure *Daphnia* heart rates after 30 minutes, 1 hour, 3 hours, 24 hours, and maybe even 48 hours of perchlorate and/or turmeric exposure. I would also experiment with other antioxidants such as ascorbic acid and Vitamin E to see if they might have the same positive effects as turmeric.

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Appendix A: Tables

Table 1: Range-finding Experiment: Effect of Different Concentrations of NaClO₄ on <i>Daphnia</i> Heart Rate					
NaClO₄ Conc.	Heart Rate	0 min	1 min	15 min	30 min
0.001 M	bpm*	261.69	220.9	217.5	216.2
	% Control	100%	104%	105%	103%
0.01 M	bpm*	247.1	274.3	296.8	289.2
	% Control	100%	111%	120%	117%
0.1 M	bpm*	309.2	382.9	403.0	-
	% Control	100%	124%	130%	-

* bpm = beats per minute

Table 2: <i>Daphnia</i> Heart Rate in 0.001 M NaClO₄					
Time (min)	Heart Rate	<i>Daphnia</i> 1	<i>Daphnia</i> 2	<i>Daphnia</i> 3	Average:
0	bpm*	261.7	248.5	138.4	216.2
	% Control	100%	100%	100%	100%
1	bpm*	250.5	258.5	153.9	220.9
	% Control	96%	104%	111%	104%
15	bpm*	225.1	247.7	179.6	217.5
	% Control	86%	100%	130%	105%
30	bpm*	215.9	267.8	164.9	216.2
	% Control	83%	108%	119%	103%

* bpm = beats per minute

Table 3: <i>Daphnia</i> Heart Rate in 0.01 M NaClO₄					
Time (min)	Heart Rate	<i>Daphnia</i> 1	<i>Daphnia</i> 2	<i>Daphnia</i> 3	Average:
0	bpm*	249.3	240.9	251.2	247.1
	% Control	100%	100%	100%	100%
1	bpm*	275.4	269.0	278.4	274.3
	% Control	110%	112%	111%	111%
15	bpm*	300.0	294.5	295.9	296.8
	% Control	120%	122%	118%	120%
30	bpm*	296.0	374.9	296.8	289.2
	% Control	119%	114%	118%	117%

* bpm = beats per minute

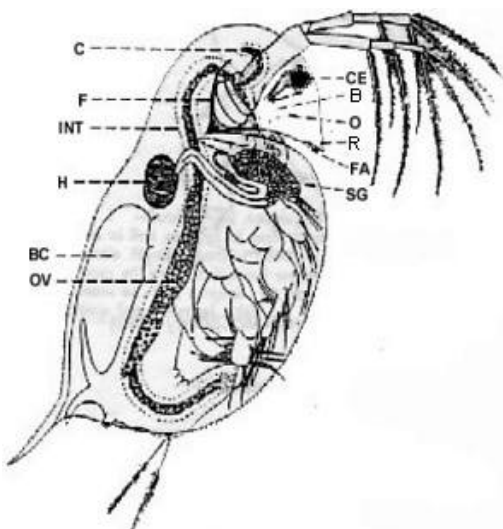
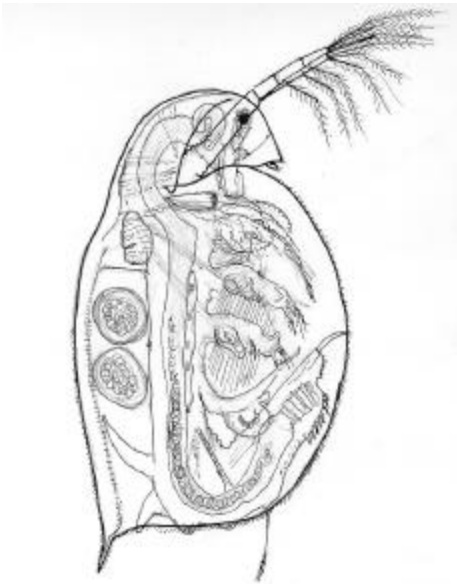
Table 4: <i>Daphnia</i> Heart Rate in 0.1 M NaClO₄ (% control)					
Time (min)	Heart Rate	1	2	3	Average:
0	bpm*	320.63	313.97	292.93	309.18
	% Control	100%	100%	100%	100%
1	bpm	366.41	392.93	389.27	382.87
	% Control	114%	125%	133%	124%
15	bpm	400.20	415.42	393.44	403.02
	% Control	125%	132%	134%	130%
30	bpm	Did not leave <i>Daphnia</i> in NaClO ₄ this long, because the heart rate was too fast.			
	% Control				

* bpm = beats per minute

Table 5: Effect of NaClO₄ and Turmeric on <i>Daphnia</i> Heart Rate				
Concentration:	<i>Daphnia</i> 1	<i>Daphnia</i> 2	<i>Daphnia</i> 3	Average
	22.14	22.80	19.85	
	21.77	21.99	19.11	
	22.07	22.05	19.84	
	-	-	20.28	
Average H₂O (100 beats/s)	21.99	22.28	19.77	21.35
Average H₂O (beats/min)	272.81	269.30	303.49	281.87
Average H₂O (% control)	100%	100%	100%	100%
	18.31	14.74	16.76	
	16.01	16.27	17.32	
	17.74	15.78	16.42	
	17.35	15.90	16.53	
Average NaClO₄ @ 1 min (100 beats/s)	17.35	15.67	16.76	16.59
Average NaClO₄ @ 1 min (beats/min)	345.77	382.84	358.05	362.22
Average NaClO₄ @ 1 min (% control)	127%	142%	118%	129%
	16.43	14.90	16.39	
	16.64	15.38	17.67	
	16.78	15.14	17.96	
	-	-	16.42	
Average NaClO₄ @ 15 min (100 beats/s)	16.62	15.14	17.11	16.29
Average NaClO₄ @ 15 min (beats/min)	361.08	396.30	350.67	369.35
Average NaClO₄ @ 15 min (% control)	132%	147%	116%	132%
	16.49	17.20	18.74	
	17.21	18.73	18.89	
	18.62	17.48	18.36	
	19.06	17.71	18.85	
Average Turmeric @ 1 min (100 beats/s)	17.85	17.78	18.71	18.11
Average Turmeric @ 1 min (beats/min)	336.23	337.46	320.68	331.46
Average Turmeric @ 1 min (% control)	123%	125%	106%	118%
	48.31	Heart stopped beating during first counting	50.28	
	50.84		58.70	
	53.54			
Average Turmeric @ 15 min (100 beats/s)	50.90	0	54.49	52.69
Average Turmeric @ 15 min (beats/min)	117.89	0	110.11	114.00
Average Turmeric @ 15 min (% control)	43%	0%	36%	26%

Table 6: Effect of NaClO₄ and Turmeric Simultaneously on <i>Daphnia</i> Heart Rate				
Concentration/Time	<i>Daphnia</i> 1	<i>Daphnia</i> 2	<i>Daphnia</i> 3	Average
	21.17	18.74	20.27	
	21.66	19.42	18.71	
	22.17	18.29	18.41	
	21.10	17.83		
Average H₂O (Time for 100 beats, seconds)	21.53	18.57	19.13	19.74
Average H₂O (beats/ min)	278.75	323.10	313.64	305.16
Average H₂O (% control)	100%	100%	100%	100%
	20.03	17.57	17.43	
	17.91	16.55	16.06	
	16.85	15.04	15.56	
	16.64	14.51	15.41	
Average NaClO₄ @ 1 min (Time for 100 beats, s)	17.86	15.92	16.12	16.63
Average NaClO₄ @ 1 min (beats/ min)	335.99	376.94	372.32	361.75
Average NaClO₄ @ 1 min (% control)	121%	117%	119%	119%
	17.62	17.47	19.49	
	15.67	14.47	17.95	
	14.04	13.88	15.20	
	13.64	13.55	16.70	
Average NaClO₄ @ 15 min (Time for 100 beats, s)	15.24	14.84	17.34	15.81
Average NaClO₄ @ 15 min (beats/ min)	393.64	404.24	346.12	381.33
Average NaClO₄ @ 15 min (% control)	141%	125%	110%	126%
	21.28	21.11	23.87	
	21.38	21.62	23.43	
	22.10	21.77	24.07	
	21.63	21.88	24.03	
Average NaClO₄+Turmeric @ 1 min (Time for 100 beats, s)	21.60	21.60	23.85	22.35
Average NaClO₄+Turmeric @ 1 min (beats/min)	277.81	277.84	251.57	269.07
Average NaClO₄+Turmeric @ 1 min(% control)	100%	86%	80%	89%
	24.78	32.68	36.06	
	33.41	33.92	35.35	
	33.95	33.98	34.67	
	32.50	34.09	33.54	
Average NaClO₄+Turmeric @ 15 min (Time for 100 beats, s)	31.16	33.67	34.91	33.24
Average NaClO₄+Turmeric @ 15 min (beats/min)	192.55	178.21	171.90	180.89
Average NaClO₄+Turmeric @ 15 min (% control)	69%	55%	55%	60%

Appendix C: *Daphnia magna*



H	Heart
OV	Ovary
B	Brain
BC	Brood chamber
CE	Compound Eye
F	Fornix
FA	First Antenna
INT	Intestine
O	Ocellus
R	Rostrum (beak)
SG	Shell Gland