Pharmaceuticals and Personal Care Products: The effects of Boron Exposure on crayfish (*Faxonius virilis)* Motor Function & Behavior

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Abstract

Water contamination has become a great concern for scientists. Pharmaceuticals and personal care products (PPCPs) are threatening environmental health through water contamination. PPCPs are common products, such as mascara, sunscreen, etc. A potentially toxic chemical, boron, is a component to many of these products, which can be harmful at elevated concentrations in nature (Anasaloni & Sousa, 2013). In this study, the effects of low to high boron concentrations are examined based on the variable exposure. The motor function of *Faxonius virilism*, native crayfish species, were tested. Artificial flowing-water and still-water streams were built at the University of Michigan Stream Lab Facility in Pellston, Michigan. Crayfish were placed in streams of different boron concentrations (control = 0 mg/L, low = 1.75 mg/L, medium = 8.75 mg/L, high = 43.75 mg/L).Their performance was recorded by a series of righting tests before and after exposure to a certain concentration. The results indicated no statistical difference before or after chemical treatment, indicated by no distinct change in average righting time or 30 second flip count. Additionally, before and after treatment periods, varying stream water samples around Pellston were tested for boron concentration using ICP-MS. The results do not contribute any physically damaging affects due to boron exposure, there still is a potential danger of boron pollution. The toxicity of this chemical depends on other characteristics of the water, in this case not controlled in this study but could have influence the toxicity in similar amounts in the natural environment.

Introduction

Pharmaceuticals and personal care products are used in various ways such as vetinary medicine, agricultural practices, human health, and cosmetic products (Barceló & Petrovic, 2007). Many PPCPs are harmful to organisms at concentrations above the natural standards in the environment (Ebele et al., 2017). Specifically the category of focus is on cosmetic products, which are products made by humans to modify appearance in any way. These products can be rinsed down the drain through bathrooms, showers, swimming, etc., which can infiltrate nearby watershed systems. However, since there is some dilution, it had not previously caused much panic, but the mass accumulation over time is a concern for the future. These cosmetics are also in a largely unregulated, discredited market (Kumar, 2005). Many of these products have even been linked to serious illness including cancer, ovarian problems, nerve damage, obesity, Alzheimer's, endocrine disruption, and others (Maqbool, et al., 2016).

Many PPCPs are synthetic, some contain material found in nature that are harmful with increased dosages, such as the naturally occurring element Boron (Farer, 2017). The use of boron in makeup products has been patented for reducing wrinkles (Dreher, 2003). As well as several other uses including: make-up foundation to cover blemishes, antibiotics, hygiene products, sunscreen, estrogen supplements, and many others (Richold, 1998) (Hunt et al., 1991). Studies have demonstrated the verge of observable effects of boron exposure on aquatic organisms and evidence demonstrating the toxicity of boron to aquatic and terrestrial organisms above certain concentrations (Butterwick, de Oude, & Raymond, 1989). Many of these studies used boron in reconstituted water and found a variety of results for the lowest observable effect concentration (LOEC) (Butterwick et al., 1989). The LOEC of boron has been evaluated for invertebrates, amphibians, algae, and other aquatic organisms, however none have examined crayfish. LOEC for freshwater fish was found to be .1 mg/L in reconstituted water (Birge & Black, 1977). Concentrations of boron in naturally occurring surface freshwaters are typically < 0.1 – 0.5 mg/L (Howe, 1998). The LOEC differs significantly throughout studies, prompting the uncertainty regarding the most accurate way to perform these analyses (Butterwick et al., 1989).

This paper will examine the effect of elemental amounts of boron concentration exposure on crayfish. Crayfish were chosen because they are a common biological indicator of species used when determining pollutions in aquatic systems (Schilderman et al., 1999). The experimental testing will occur to analyze any physical or behavioral effects on crayfish based on carefully selected concentration ranges, noting the reported concentrations from collected water samples in northern Michigan. Anticipated higher concentrations of toxicity, will allow us to predict the crayfish exposed to higher concentrations of boron will have reduced capability to flip themselves in the series of righting time tests, due to expected damage to motor function.

Methods

Materials

This study was completed using the University of Michigan Stream Research Facility, where water pumped from the East Branch of the Maple River. The groundwater was then distributed throughout the site using PVC pipes and valves. Then, four mock streams were built using bricks, cinder block, plastic material, mesh netting, and rocks. For Trial 1, mesh was surrounding the interior cinder blocks for each stream to impede escape (Fig. 1).

For Trial 2, multiple mesh layering's, cinder blocks, and rocks were placed over the top of each stream to assure mnimal escape (Fig. 2).

Figure 1: University of Michigan Stream Research Facility stream lab set up for Trial 1. Drip buckets shown alongside left of the picture and hoses connected to buckets flowing water into artificial stream.

Crayfish were collected at Maple Bay State Forest Campground in Brutus, Michigan located on Burt Lake. The native crayfish species (Faxonius virilis) were collected, both males and females, and stored in moving water tanks before testing began.

Figure 2: University of Michigan Stream Research Facility stream lab set up for Trial 2. Extra cinder blocks, rocks, and mesh placed on stand still water to secure crayfish.

Experiment Trials

The crayfish were tested based on a standard flip test, consisting of three timed righting tests, where each crayfish was placed on its back and the time taken to flip to an upright natural position was recorded. The three consecutive tests were averaged and these values were used in statistical tests after the experiment's completion. A 30 second flip test was also done, where the number of times a crayfish flipped off its back, up to the natural position, in the 30 second period was recorded.

Trial 1

On July 14, 2019 the first group of crayfish was collected and kept overnight in a holding tank. On July 15, ten crayfish were randomly put in each of the 4 flowing streams for over-night acclimation. Each crayfish was given with a number (1-40) on its back using white-out. On July 16, pre-treatment flip tests were performed and noted. Some non-acclimated crayfish took the place of crayfish that had escaped overnight. Three of the four streams were treated with boric acid solution with a drip bucket that streamed water at a constant rate to maintain the goal concentration throughout the stream (Table 1). Each bucket had a narrow tube to deliver the contents of the bucket to the stream water, all done at a rate that diluted the solution by a factor of 35. The boric acid was weighed and dissolved in 1 L of water. Each solution was added to a 5- gallon drip bucket and then filled with 17 liters of stream water, for a total of 18 L per drip bucket. Stream 1 acted as the control was not spiked with boric acid, but 18 L (1 bucket) of stream water were added through the drip bucket system. Stream 2 (1.75 mg/L B) was given a constant flow of a solution of 6.3g boric acid dissolved in 18 L water. Stream 3 (8.75 mg/L B) was given a constant flow of a solution of 31.5g boric acid dissolved in 18 L water. Stream 4 (43.75 mg/L B) was given a constant flow of a solution of 6.3 g boric acid dissolved in 18 L water. After around 20 minutes of flow, one water sample was collected from each stream in 125 mL Nalgene bottles. On the morning of July 17 and July 18, the buckets were refilled and water samples were collected. On the evening of July 18 crayfish were removed from their streams and a post-treatment flip test was performed, including the timed righting tests and 30 second flip test. All water samples were filtered and tested using ICP-MS to determine the boron concentration of each stream.

Stream	Mass of Boric Acid (g)	Volume Water (L)	Drip Bucket Dilution Factor	Concentration Boric Acid (mg/L)	Goal Concentration (mg/L B)
1	0	18	35	0	0
2	6.3	18	35	10	1.75
3	31.5	18	35	50	8.75
4	157.5	18	35	250	43.75

Table 1: Boric acid solution makeup for drip buckets used in four streams and the goal concentrations

Trial 2

The second trial began on July 22 consisting of crayfish of the same origin as Trial 1. The streams used in Trial 1 were emptied, cleaned out of past reminants, and re-filled with approximately 102 L of stream water; however, not the flow from the Maple River was blocked. The crayfish were held in the tank overnight and on July 23, 40 new crayfish were labeled (1-40) with whiteout. Pre-treatment flip tests were completed on each animal in the same approach as Trial 1. Ten crayfish, at random, were set in each of the four streams. A solution of a known mass of boric acid that was dissolved in water was added to each stream to achieve the goal concentration (Table 2). Stream 1 (control) received no acid in the stream. Stream 2 received 1.02 g boric acid (dissolved in water) to reach a goal concentration of 1.75 mg/L boron. Stream 3 received 2.04 g boric acid (dissolved in water) to reach a goal concentration of 8.75 mg/L boron. Stream 4 received 25.5 g boric acid (dissolved in water) to reach a goal concentration of 43.75 mg/L boron. A water sample was collected from each stream, after the boron solutions were added. Crayfish were kept in the streams for 72 hours and after the treatment period, a post-treatment flip test was performed. Subsequently, water samples were collected from each stream. All water samples were filtered and tested using ICP-MS to determine the boron concentration.

Stream	Mass of Boric Acid (g)	Volume Water (L)	Concentration Boric Acid (mg/L)	Goal Concentration (mg/L B)
1	0	102	0	0
2	1.02	102	10	1.75
3	2.04	102	50	8.75
4	25.5	102	250	43.75

Table 2: Boric acid solution makeup for the addition to four streams and the goal concentrations

Environmental Water Samples

Water samples were collected on July 31, in 250 mL Nalgene bottles from the surface of Douglas Lake, Burt Lake, and Mullett Lake. These samples were filtered and tested using ICP-MS for boron concentration.

Statistical Testing

All statistical analyses were completed using the 3.6.1 version of R. A Shapiro-Wilks test was used to assess the normality of the population from a sample of data, as well as a prerequisite for the use of an ANOVA test. An ANOVA test was used to indicate whether the difference in average righting time for crayfish before versus after treatment contrasted significantly between the four streams. An ANOVA was used to assess the statistical significance of the difference in number of flips in the 30 second test for all four streams.

Results

Trial 1

Many of the crayfish in our streams had escaped just after the time allotted for acclimation, including complete loss of crayfish or transfer to a different stream. Thus, prior to the pre-treatment flip test, many crayfish were replaced with non-acclimated crayfish in order to begin the experiment. Data for boron concentrations in Trial 1 stream water samples express the intended concentrations for our streams were not achieved (Table 3).

Stream	Goal Concentration (mg/L B)	Pre-Treatment Concentration (mg/L B)	Post-Treatment Concentration (mg/L B)
1	0	0.274326	0.268784
2	1.75	13.01327	46.88479
3	8.75	39.76452	77.07168
4	43.75	164.6557	4.594866

Table 3: Boron concentrations found in Trial 1 streams for before and after treatments

Several crayfish were not seen in their respective tanks when checked on day 2 and on the final . treatment day, when collected for post-treatment flip tests, 20 animals were missing. This included recoating to a different tank or new area completely. The raw data for the flip tests for the remaining crayfish were recorded (Appendix A). Statistical testing was not completed due to lack of sufficient data.

Trial 2

All crayfish remained in their tanks for the complete duration of Trial 2 and survived the treatment period. Pre-treatment and post-treatment flip test data was collected for all 40 crayfish. A Shapiro-Wilks Test for Normality implies that the population of data for average righting time is not normally distributed (p = .0005). A boxplot was created to model the righting time data, determining that the population is normal enough to proceed with an ANOVA test (Figure 3). The average difference in righting time, before treatment-after treatment, according to the ANOVA was not statistically significantly different for any of the streams (p = .773) (Figure 4).

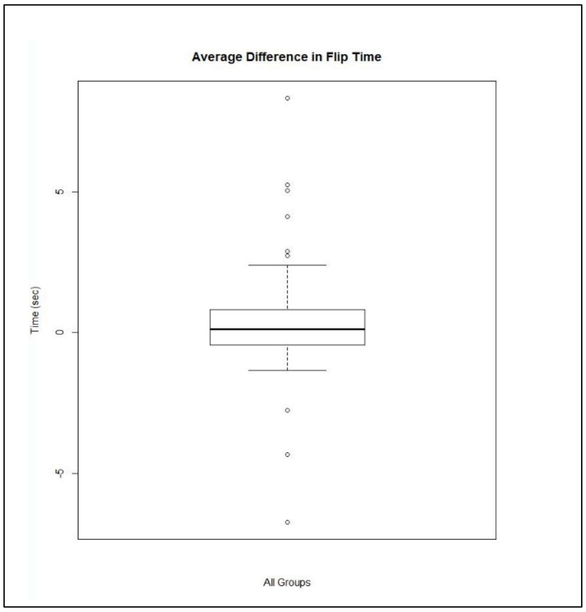

Figure 3: Boxplot of average difference distribution in flip time before minus after treatment (showing normality)

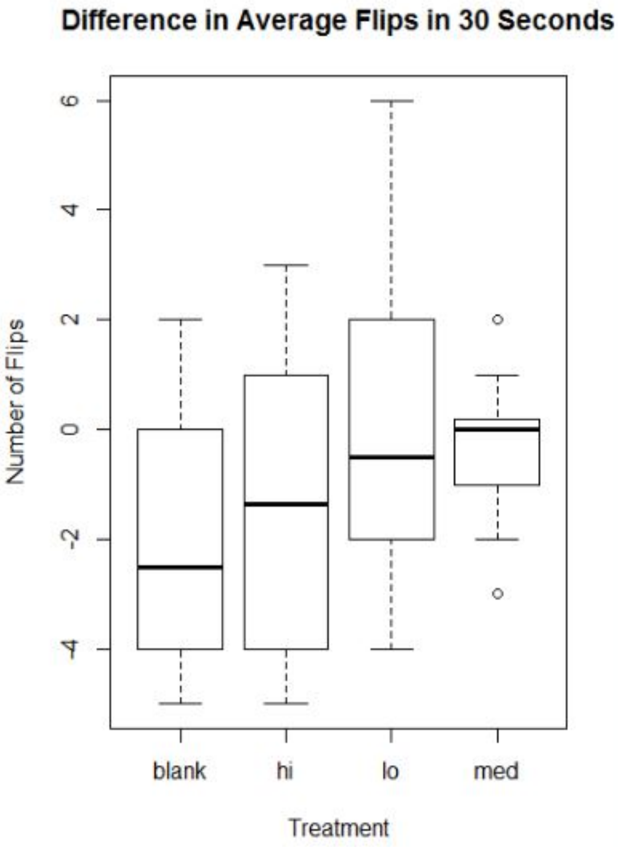

Figure 4: Boxplot of difference in crayfish average righting time for Trial 2

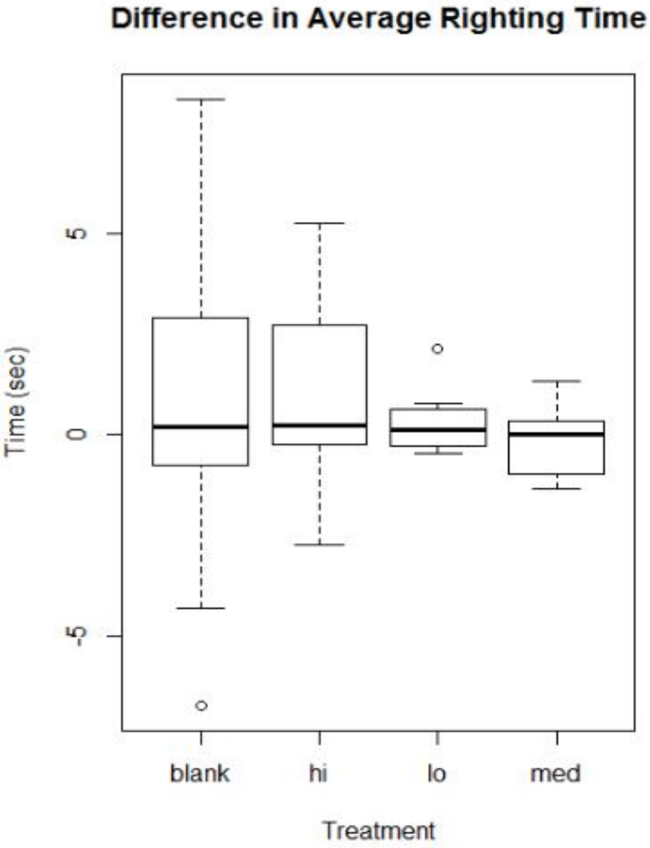

Figure 5: Boxplot of difference in crayfish average number of flips in 30 seconds for trial 2

A Shapiro-Wilks Test for Normality implies that the population of data for number of flips in 30 seconds is normally distributed (p = .2759). The average difference in number of flips in 30 seconds, before treatment-after treatment, was not statistically significantly different for any of the streams (p = .184) (Figure 5), signified by the ANOVA. Each concentration of boron in each of the streams before and after treatment differed from the goal concentrations, yet tested concentrations were similar for each stream before and after the treatment period (Table 4). The measurements of boron concentrations in the environmental samples marginally different between lakes: with concentrations of 4.22 mg/L (Mullett Lake), 7.79 mg/L (Douglas Lake), and 7.01 mg/L (Burt Lake).

Stream	Goal Concentration (mg/L B)	Pre-Treatment Concentration (mg/L B)	Post-Treatment Concentration (mg/L B)
1	0	0.284209	0.329096
2	1.75	7.050183	9.083688
3	8.75	24.19888	20.14323
4	43.75	115.0646	113.9176

Table 4: Boron concentrations detected in four streams before and after treatment

Discussion

Trial 1

As a result of many crayfish escaping their designated streams, data could not be statistically analyzed. Increased aggression (claws facing up and backing away from hands), specifically on post-treatment tests, indicated poor adjustment to their new environments. This could explain the considerable amount of escaping crayfish, however this stress and aggression cannot be associated with boron exposure, but rather some part of experimental setup, handling the crayfish in transition, time without water, etc. which may have led to these behaviors.

The boron concentrations with the drip bucket did not work properly, the consistent concentration was not maintained throughout the experiment. Although with the intention to model a natural system, the flow of chemicals was not constant throughout the day, instead in flares of chemical. Due to the varied time of samples taken, the measurements do not permit accurate estimation of boron concentration during total drip period, around 20 minutes. Water samples should have been taken every 1-5 minutes over the full drip period in order to provide for a better average estimate of actual concentration of boron.

Trial 2

The crayfish exposed to varying concentrations of boron did not incite motor function impairment or decelerated endurance, represented by the 30 second flip test. Statistical analysis theoretically would have been run with a paired t-test to compare before and after righting time of number of flips for each individual crayfish, however, could not be done due to the confounding variable of relocation to artificial streams. There are possible effects of exposure, since there was increased aggression observed from pre to post-treatment. Again, motor skills or physical impairment cannot directly correlate to boron exposure, however other factors of health may have been affected. This does not diminish the possible harmful effects of chemicals at increased concentration or when comparing differing conditions.

The water samples taken during the experiment (before and after treatment) did not maintain the goal concentrations, however they did have elevated levels of boron that were consistent over the treatment period. The uncertainty, in the boron composition of boric acid to create solutions, may have been reason to the unknown discrepancy. AS well, the highest concentration in Stream 4 was around 115 mg/L. This is a much greater concentration than would be typically found in nature, since the results for boron concentration in three inland lakes in northern Michigan were below 10 mg/L. Therefore, despite the concentrations used in streams 2, 3, and 4 not being high enough to enact change to physical function, These concentrations can represent concentrations higher than crayfish in this region typically are exposed to , regardless of concentrations in 2,3, and 4 not being high enough to show change in physical function or behavior in the crayfish).

The fluctuation of water composition was not properly addressed, as toxicity of boron can influence water, including: hardness, pH, and alkalinity (Nable, et al., 1997). For example, one study to investigate this relationship found that the 24-hour median tolerance limit of sunfish exposed to boron differed significantly (2389 versus 4.6 mg/L). This was depending on the alkalinity and hardness of the water (Turnbull et al., 1954). Other past studies have proven organisms have much longer exposure, as much as up to 60 days. The 72 hours in our experiment had no noticeable effects, but given longer time likely could have proved harmful. This concern remains due to PPCPs likelihood to remain in the natural environment for lengthy periods of time (months to years). This can result in a compilation of larger amounts and longer exposure time to these chemicals, thus leading to harmful effects on the overall health of these aquatic systems.(Ebele et al., 2017). The results do not counter the dangers of boron nor the potential long-term exposure to low concentrations of the chemical. Thus, there is more research to be done in this area to provide more conclusive findings.

This study was meant to assess how subtle increase in boron concentration (that mimic the environment) have on biological indicator species. The results do not grant adequate conclusions of the effects on crayfish. However, this does pivot to the principle that any extreme concentrations of chemicals can bring about death or impairment. As more products containing boron wash off humans or rinsing products down to the water system, chemicals (such as boron) compile. Eventually releasing into the environment and creating higher than natural concentration's. Future studies could explore increased length of exposure time, larger sample size, and a larger area for crayfish. These studies should be pursuing because the long-term ramifications of these products containing copious amounts of chemicals with unknown affects, may incur more damage to overall health than is currently known.

Citations

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Appendix A:

	Pre-Treatment		Post-Treatment	
	Average Righting Time (s)	Number of Flips in 30 s	Average Righting Time (s)	Number of Flips in 30 s
Tank 1				
1	2.23	8	2.76	4
2	3.28	4	1.71	5
3	1.14	11	1.58	5
4	1.21	7	1.82	6
5	0.75	9		
6	0.88	9	0.76	14
7	3.28	8		
8	1.76	8	3.42	6
9	1.49	11	1.08	12
10	1.60	6	1.40	8
Tank 2				
11	1.97	9		
12	2.44	4	5.83	5
13	2.02	7		
14	1.68	7	2.16	10
15	1.12	9	1.08	6
16	1.49	6	0.83	11
17	1.12	12	1.04	12
18	2.24	7		
19	0.94	11		
20	1.42	9		
Tank 3				
21	1.58	5		
22	1.42	8	2.46	6
23	1.20	6		
24	2.91	7		
25	2.56	7		
26	2.10	6	1.39	10
27	0.88	4		
28	3.35	2		
29	0.96	9		
30	2.86	7	1.25	5

Tank 4				
31	2.50	9	1.61	8
32	2.98	7		
33	1.88	8		
34	1.38	12		
35	1.42	8		
36	4.37	7	2.44	6
37	2.71	6		
38	4.33	2	1.95	3
39	3.70	6		
40	3.85	6	1.10	7