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Analyzing the Impact of Residential Chemicals upon the Heartbeat of *Daphnia magna*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors Jihoon Kang, HJ designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SL, RP and VH managed the analyses of the study. Authors JL and Jiwhan Kim managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The controversial consensus among toxicology researchers is that the country's regulation of chemicals has always been insufficient. One critical aspect that has been debated is the government's ability to determine the criteria of what makes a commercially available chemical dangerous, especially considering that these chemicals haven't undergone rigorous testing. In this experiment, the *Daphnia magna*'s heart rate change was evaluated through the serial dilution of residential chemicals, particularly for insecticides and herbicides. We utilized the change in the bpm of the *Daphnia magna* as an indicator of stress, which was used to analyze the environmental

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stress associated with the tested chemicals. An important parameter, the Cardiac Disturbance Indicator (CDI), was defined as the sum of the average heartbeat changes at the set time points we measured. Results showed a broad spectrum of CDI from 0.0 to 90%, while the magnitude of CDI could be predicted by considering other published toxicological data. The CDI was found to be a somewhat useful surrogate for scrutinizing any harmful effects in future studies.

Keywords: Cardiac disturbance indicator; environmental hormones; residential chemicals; toxicological effects.

1. INTRODUCTION

Since 1950, chemical production has dramatically increased fiftyfold and is projected to triple from 2010 to 2050. Even more concerning is that only a tiny fraction of the 350,000 [1] chemicals in use have been thoroughly assessed for safety. The lack of thorough chemical testing is partly to blame for increased chemical pollution and associated risks [2].

Chemical pollution now has the potential to pose one of the most significant environmental threats to humanity, as it serves as a risk in fields such as male fertility, cognitive health, and food security, among others [3]. As of 2015, less than 10 percent of the 80,000 chemicals in general commerce have been tested adequately to determine their health risks. This was considered a significant issue since the United States annually produced over 500 million tons of synthetic chemicals [4]. Commercial chemicals were found at the center of this issue, as unlike pharmaceuticals or pesticides, commercial chemicals were not required to be tested before they were put on the market [5], while only information regarding the production of the chemicals was needed before the chemical could be released [5].

The burden of labeling a chemical as hazardous currently falls under the jurisdiction of the Environment Protection Agency (EPA). Until the passage of the Frank R. Lautenberg Chemical Safety for the 21st Century Act, the EPA was only allowed to require chemical testing once plausible evidence suggested that the chemical posed a risk [6]. However, the EPA could only determine if a chemical posed a risk if it was released to the public, and the soundness of the chemical was then called into question [7]. This created a situation in which the EPA could designate a chemical for testing and risk only after it had already been exposed to the public. Additionally, if a chemical has not been assessed for necessary risk within 90 days of its implementation, it could be used freely with no restrictions [8]. Due to the passage of the Frank R. Lautenberg Safety Act, chemical regulations have become more strict- and with it, more space for newer forms of testing has emerged [9].

Toxicological tests include a variety of models, ranging from bacterial mutation studies, cell models, reproductive and physiological studies with Protista, and multiple invertebrate and vertebrate models. Every model has its own advantages and disadvantages with interconnecting mutual support. Current models of toxicology research offer benefits as well as potential drawbacks. A standard method of testing chemical substances is the *Daphnia magna* [10]. *Daphnia magna* is one of the most favored chemical testing methods due to various factors. Daphnia, as test subjects, satisfy any ethical requirements. This is because Daphnia have a reduced sense of pain, therefore making their use in toxicology research ethical [11]. In addition, Daphnia are classified as non-sentient, meaning they are acceptable for use in toxicology research without violating ethical codes [12]. The Daphnia are also favored for testing due to their biological makeup. Daphnia have transparent bodies, which can be analyzed to determine the heartbeat of Daphnia [13]. This trait has been used in previous experiments to estimate the influence of foreign factors, such as heat or chemicals, upon the Daphnia. The heartbeats would be measured before and after the experiment, which was made possible due to their transparent bodies. [14]. The Daphnia are also valued for the ability to be able to be examined for traits such as swimming speed, distance traveled, heart rate, ingestion rate, feeding rate, oxygen consumption, and thoracic limb activity, all of which are traits that can provide insight to determine toxic influences [15].

In this research paper, we aim to determine the impact of commercially available chemicals on the environment by measuring "stress" in the Daphnia through their change in heartbeats per

minute following exposure to the substances above in varying dilutions of base 10. , a serial dilution method of utilizing Daphnia Magna in chemical testing.

2. EXPERIMENTAL METHODS

2.1 Detailed Material Descriptions

The following tools were used: AmScope model M148B Microscope, plastic cups and slide glasses, test tube rack, six glass test tubes, ZSD-808 stopwatches, DragonLab 100-1000 ul model YE3K126638 micropipette, three pipettes, one of which was cut at the tip for easier handling of Daphnia, and one red Sharpie to make note of the difference in test tubes, for marking down numbers such as "0", "5", "20", etc. All tools, except for any disposable materials, were thoroughly cleaned and replaced after use.

2.2 Materials Setup

2.2.1 Preparation of the incubation solution

The experimental materials were collected and confirmed to be on hand, particularly from the aspect of safety activity, as shown in Fig. 1 below. Personal protective equipment, i.e. safety goggles and gloves were used. The conditions of Daphnia were carefully inspected before initiating the solution preparation procedures.

Similar methods of preparing the incubation have been described in other publications in our laboratory [16, 17]. Describing our methods with these materials, the six glass test tubes were placed in the test tube rack and the tubes were written “0”, “5”, “10”, “15”, “20”, and “25” respectively in red Sharpie. The DragonLab micropipette was then set to 900 uL (microliters), and a fresh pipette tip was fitted. The micropipette then transferred 900 uL of water from the Daphnia’s natural habitat’s culturing water to each glass tube twice for a total of 1800 uL in each test tube. Next, the micropipette was adjusted to 200 uL, and 200 uL of the tested chemical was inserted in the initial test tube labeled "0." This solution was then thoroughly mixed utilizing "active mixing," in which the solution was repeatedly dispensed and withdrawn for 15 seconds. 200 uL of this freshly mixed solution was then transferred to the next test tube labeled "5" and would be repeated until the final test tube "25.", resulting in solutions of base-10 concentrations of the tested chemical.

2.3 Experimental Design and Procedure

2.3.1 *D. Magna* culturing and selection

In the weeks leading up to the experimentation, the Daphnia were fed a strict diet of yeast and algae. 40 Daphnias from four different containers were initially measured for the time of 40 heartbeats, and the average was determined. Daphnias were excluded from the study if their initial time for 40 beats escaped the range of 8.548 ± 3 seconds. Daphnias were not used if eggs were present in their brood chamber or if their length was less than 2.5 mm.

Fig. 1. The experimental materials used for the study (a: compound microscope, b: Eppendorf pipette (100~1000 ul capacity), c: stopwatches, d: tube rack, e: test tubes, f: pipette tips, g: slide glasses, h: safety goggles, i: insecticide, j: disposable gloves, k: herbicide)

	Group 1		Group 2		Group 3		Group 4	
Daphnia#	40 CTT	BPM	40 CTT	BPM	40 CTT	BPM	40 CTT	BPM
1	8.75	274	9.36	256	8.47	283	8.44	284
2	8.16	294	6.19	388	6.56	366	8.61	279
3	9.25	259	8.38	286	7.6	316	8.34	288
4	8.82	272	8.97	268	7.65	314	9.19	261
5	8.84	271	9.34	257	8.72	275	9.56	251
6	8.81	272	12.19	197	8.53	281	8.37	287
7	7.85	306	9.25	259	8.03	299	8.29	290
8	9.53	252	8.78	273	8.1	296	6.69	359
9	9.31	240	8.56	280	7.72	311	8.24	291
10	10.09	238	7.88	305	7.97	301	8.54	281
MEAN	8.94	268	8.89	277	7.94	304	8.43	287
STD	0.65	22	1.50	48	0.62	26	0.74	28

Fig. 2. A summarized data table from 40 daphnia measurements, which was used to obtain the mean heartbeat of our *Daphnia population* (Legends: 40 CTT = 40 heartbeat counting time, BPM = Beat Per Minute)

2.3.2 Measurement of daphnia's heartbeats

Utilizing the cut pipette, a singular Daphnia was collected and promptly placed onto a glass slide under the microscope. Excess water on the Daphnia was then removed using the pipette and paper towels so that the *Daphnia magna* was left with a small amount of water in order to live and "breathe" while not being able to move freely to allow better observation. The *Daphnia magna*'s heartbeat was then synchronized with tapping on the table using a finger for ten counts, then the stopwatch was started. While the stopwatch ran, using the beat of the finger and counting mentally, 40 heartbeats were counted. Once 40 counts were reached, the stopwatch was immediately stopped.

2.3.3 Organization of data collection

After an individual Daphnia's time for 40 heartbeats was measured, the Daphnia was placed in the first tube labeled "0," and a separate stopwatch was started. Once this stopwatch displayed 4 minutes and 30 seconds, the time for 40 heartbeats was measured for a second Daphnia utilizing a separate stopwatch. This Daphnia was then placed into the second test tube, labeled "5," at the 5-minute mark. This process was repeated, with the time of Daphnia's heartbeat being measured at 30 seconds before intervals of 5 minutes. Once the final Daphnia was measured and placed in the container labeled "25," after 5 minutes, the Daphnia in test tube "0" was extracted and measured for the time for 40 heartbeats. Every 5 minutes, another Daphnia was extracted and measured, giving each Daphnia an incubation period of 30 minutes. The Daphnias were then placed into a separate cup labeled "contaminated" filled with culturing water and disposed of later.

2.4 Statistical Analysis

The times taken for 40 heartbeats before and after exposure were recorded on a Google spreadsheet as seen in Fig. 3. If the Daphnia had no visible heartbeat and did not react to prodding, it was considered dead. The time for 40 heartbeats was noted as 1000 seconds for a mathematical approximation of being dead with a slow heartbeat. Points were then plotted on a graph, with the percentage change of the bpm following exposure plotted on the y-axis and the x-axis representing the base-10 dilution factors. The regression that most successfully matched the points was used to formulate an equation and to determine the R^2 value. A strong correlation was defined as an R^2 value greater than or equal to 0.6, while a weak correlation was defined as an R^2 value less than or equal to 0.4. Our study defined a comparative parameter as the "Cardiac Disturbance Indicator (CDI)" by summing up the magnitude of the BPM% of each time we measured. It was reasonably understandable, considering the magnitude of the number should be the least when no heartbeat disturbance was caused, such as being exposed to the culturing water. In contrast, the magnitude of the CDI should be more significant when causing maximal heart rate changes.

Pre-Incu Time	40 CTT	Pre-BPM	Post-Incu Time	40 CTT	Post-BPM	Diff BPM	Dilution F	%Change
0	11.22	214	30	100.00	24	-190	1	-88.78
5	9.00	267	35	9.88	243	-24	2	-8.91
10	8.41	285	40	8.30	289	4	3	1.33
15	10.20	235	45	7.81	307	72	4	30.60
20	9.11	263	50	9.30	258	-5	5	-2.04
25	7.78	308	55	8.21	292	-16	6	-5.24
MEAN	9.50	239		54.65	141	-98		-45.41
STD	2.43	67		64.91	190	123		59.07

Fig. 3. A Google spreadsheet sample with data from our single trial (Legends: Incu=Incubation, Diff= Difference, Dilution F=Dilution Factor)

3. RESULTS AND DISCUSSION

3.1 Positive Control Study with 70% Ethyl Alcohol

Measuring the BPM% change when exposed to positive control should be very informative. The positive control was defined as a representative chemical compound that could be expected to cause some heartbeat disturbances, allowing for easy data comparison to that of other peer scientists. The data in Fig. 4 below showed that the heart rate slowed significantly at ten times dilution (x=1) up to 93 BPM% change, but the heart rate didn't change much at the 100 times dilution and forward. The CDI was estimated to be a change of 140 BPM%. The data seemed to show similar results to the study by other researchers [18]*.

3.2 Negative Control Study with Culturing Water

A negative control was chosen as a chemical compound that should not disturb heartbeat change within our observation period. As seen in Fig. 5, the heartbeat changed minimally during the 30-minute incubation. The CDI was measured as a 32 BPM% change, which was significantly smaller than that from the positive control. And, the BPM change was fallen within 10% of the normal heartbeat rate.

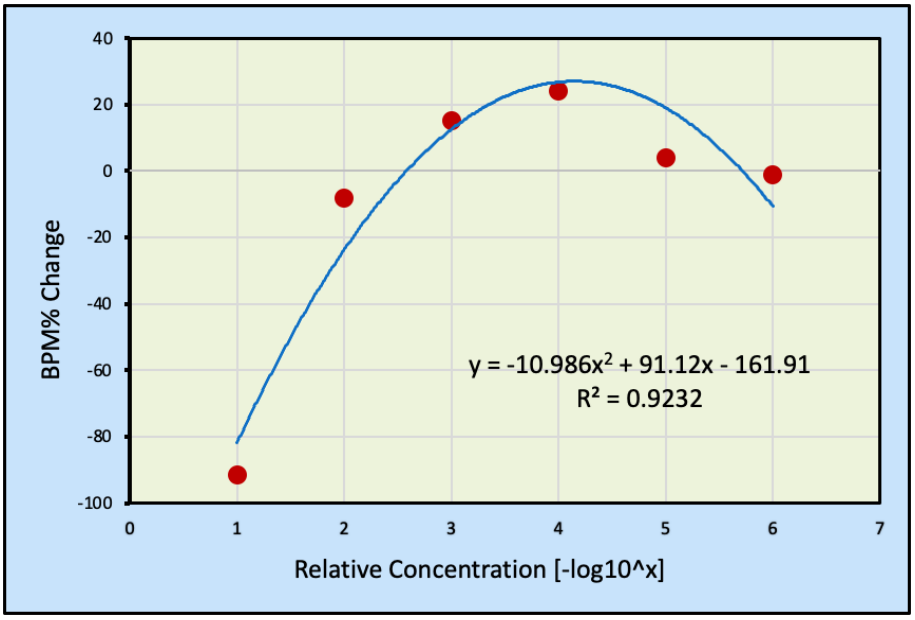

Fig. 4. The BPM% Change under exposure to 70% ethyl alcohol as a positive control

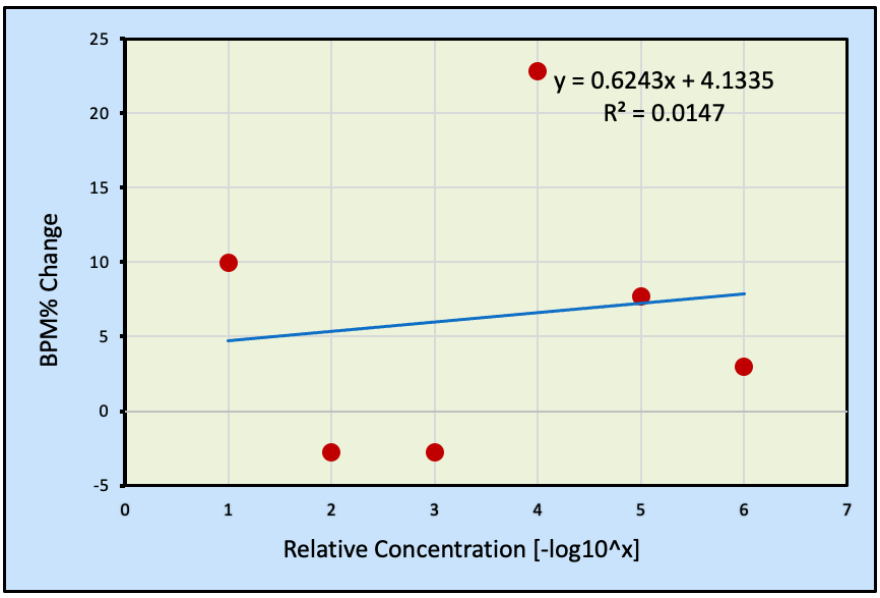

Fig. 5. BPM% change in exposure to culture water as a negative control

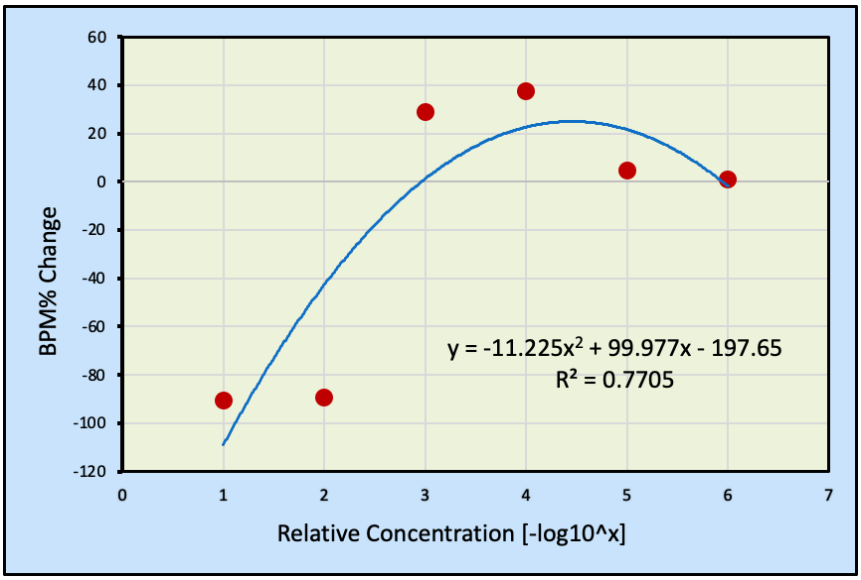

Fig. 6. The BPM% change in exposure to Clorox

3.3 Exposure Study to Clorox

Clorox can sanitize and disinfect washrooms, shower stalls, toilets, countertops, and food prep areas. It is used to brighten whites and sanitize the surfaces for less. It is EPA-registered and known to be effective against *E. coli*, HIV-1, Norovirus, *Salmonella*, Staph, and Hepatitis [19]. So, Clorox is the most ubiquitous cleaner found in our residential areas. Fig. 6 above presents the change in heartbeat after the 30-minute incubation. It demonstrated that Clorox induced severe heartbeat depression of nearly 90%, even at 100 times dilution. Its CDI was 250 BPM% Change.

3.4 Heartbeat Change in Exposure to Gasoline

Gasoline is one of the most sold chemicals. When gasoline is exposed to the environment, such as through runoff or seeping into the soil, it can contaminate groundwater used for drinking. Exposure to contaminated water can lead to nose and throat irritation, headaches, dizziness, nausea, vomiting, confusion, and breathing

difficulties [20]. When gasoline was tested in our experiment, as shown in Fig. 7, it was determined that the CDI was about 330%. Notably, the Daphnia in the first three dilutions died, highlighting the potency of gasoline when exposed to the environment - even at a 1/1000 dilution, gasoline was found to pose a fatal level of environmental stress.

3.5 Heartbeat Change in Exposure to Dimethylacetamide

Dimethylacetamide is commonly used as the primary active reagent in pesticides. Other than its use in pesticides, Dimethylacetamide (DMA) is also used in water treatment, rubber processing, corrosion inhibitors, and dyes [21]. In our experiment, it was determined that DMA had an R^2 value of 0.849, which exceeds the threshold we had previously set for a strong correlation in Fig. 8. The CDI of DMA was determined to be about 45%. Although the data points used to create a line for a polynomial regression had a high correlation, the BPM% change was minimal, at most a difference of 15%. No Daphnia were killed in this exposure, indicating that the level of stress imposed by DMA was minimal. Therefore, while DMA had a strong correlation to the change in the heartbeat of Daphnia, the actual stress imposed by DMA was minimal.

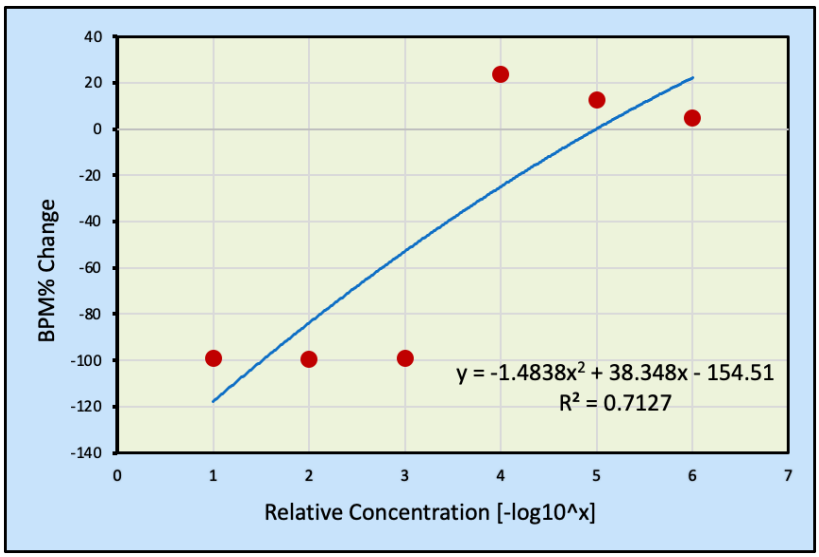

Fig. 7. The BPM% change in exposure to Gasoline

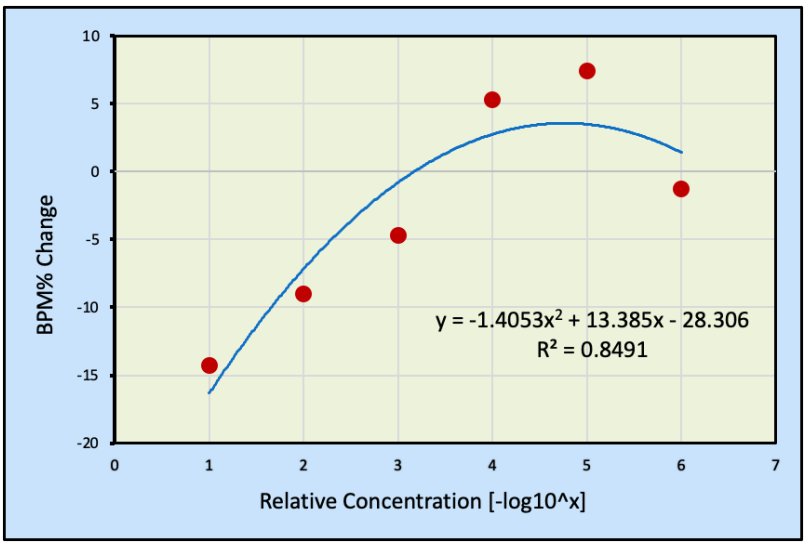

Fig. 8. The BPM% change in exposure to Dimethylacetamide (DMA)

3.6 Heartbeat Change in Exposure to Lambda-cyhalothrin

Lambda-cyhalothrin is a pesticide chemical that was approved by the EPA in 1988 [22]. In the previous testing of this chemical did not used Daphnia as a medium. In our experiment, it was determined that the CDI value was about 100%. No Daphnia were killed in this experiment, and the extent of the change in BPM was about 38%, indicating a moderate value for the maximum heartbeat change as in Fig. 9. In the prior testing of Lambda-cyhalothrin, it was determined that the chemical is hazardous to certain insects such as bees and highly toxic to fish. Our experiment determined that Lambda-cyhalothrin poses a moderate level of environmental stress by testing planktonic crustaceans.

3.7 Heartbeat Change in Exposure to Sodium Lauryl Sulfate

Sodium Lauryl Sulfate (SLS), also known as Sodium Alkylethersulfate, is an anionic detergent and surfactant in many personal and industrial products [23]. Previously tested by the EPA, SLS was determined to be inert, posing no significant hazard to the environment or humans. However, our experiment determined that the CDI% of SLS was about 275%, demonstrating a high level of applied stress as presented in Fig. 10. Additionally, 2 of the tested Daphnia were killed at respective concentrations of 1/10 and 1/100, further signifying the associated risks of this chemical. The R^2 value was determined to be 0.879, which, according to our previously set threshold, is a significant indicator of correlation in a change in heartbeat following exposure to the chemicals.

3.8 Heartbeat Change in Exposure to Geraniol

Geraniol is a naturally occurring terpenoid used as a fragrance and in pesticides, primarily sold as a mosquito and tick repellent for pesticides [24]. In previous tests regarding Geraniol, the maximum dilution used in testing the chemical was 1%, and used rodents as a medium. Although Daphnia Magna was used in testing in prior studies, the maximum dilution was 1/1000, and minimal disturbance was reported, as reflected in our research. However, our experiment increased the concentration up to 10%, resulting in fatalities. The CDI% was about 215%, most directly influenced by the deaths at the highest dilution as in Fig. 11. Apart from these two values, there was minimal change in BPM, implying that Geraniol poses a moderate risk.

3.9 Heartbeat Change in Exposure to Prallethrin

Prallethrin is a commercially used chemical found in most mosquito repellents [25]. The EPA previously tested it and determined it to be of minimal environmental risk. Our research found a CDI% value of about 53%, signifying minimal ecological stress imposed by the chemical, as in Fig. 12.

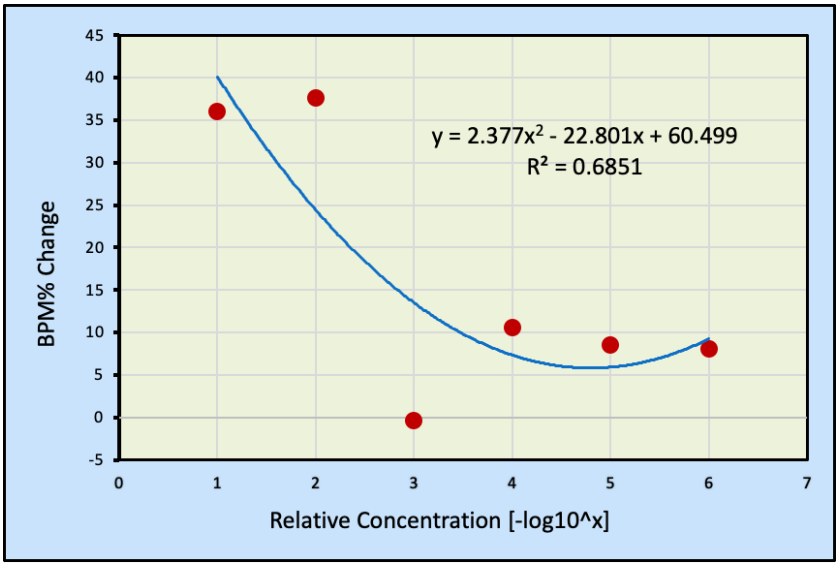

Fig. 9. The BPM% change in exposure to lambda-cyhalothrin

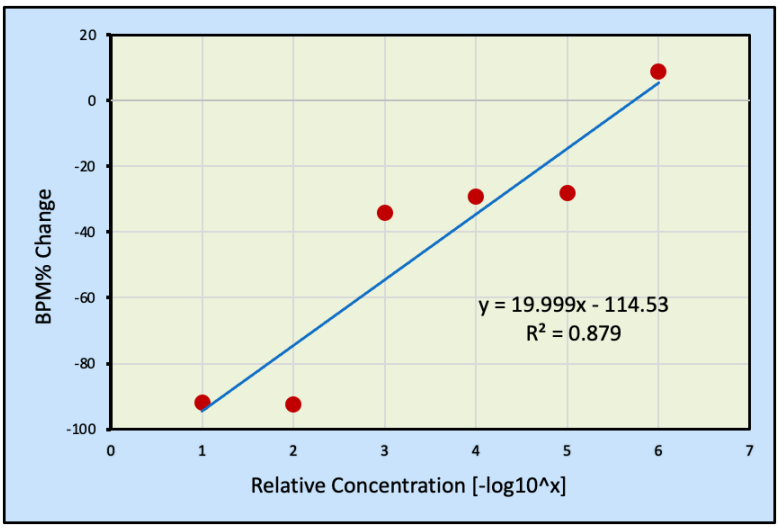

Fig. 10. The BPM% change in exposure to Sodium Lauryl Sulfate

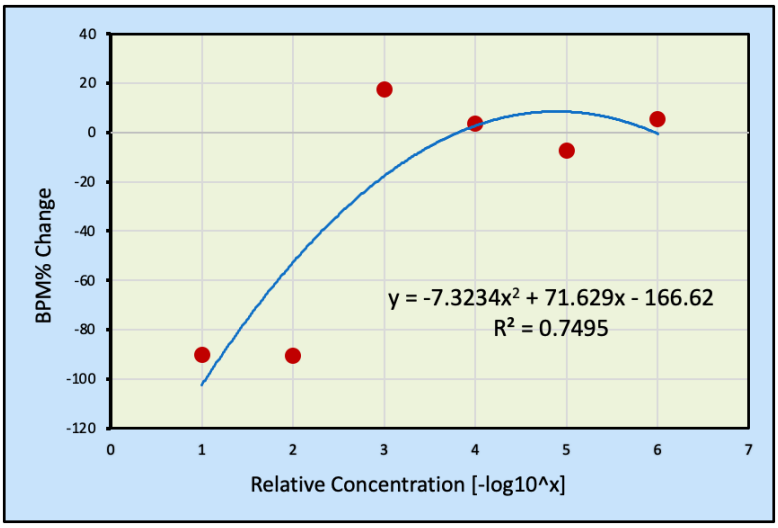

Fig. 11. The BPM% change in exposure to Geraniol

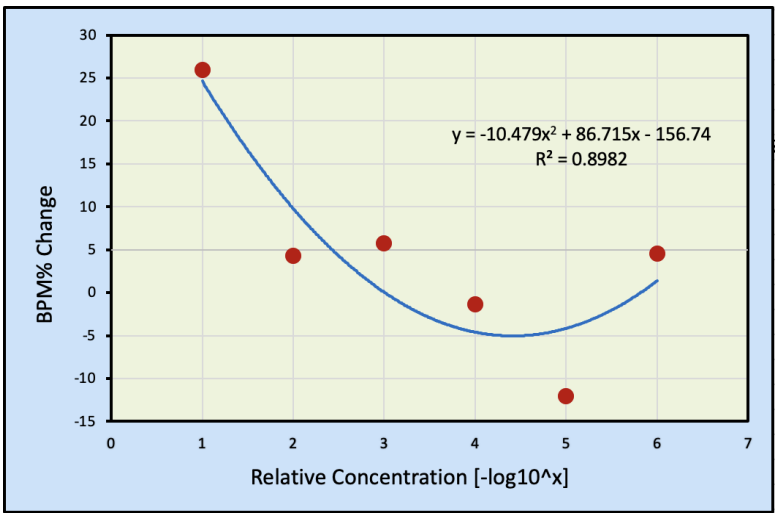

Fig. 12. The BPM% change in exposure to Prallethrin

3.10 Heartbeat Change in Exposure to Dimethylamine Salt

Dimethylamine salt is widely used in pesticides, notably in brands such as ACE [26]. Previous experiments have determined that there is a low acute toxicity associated with this chemical, which means that while there are situations where the chemical could pose a risk it is generally considered safe. In our research, the CDI% was determined to be about 12%, as seen in Fig. 13. A CDI% of 12% implies that there is very minimal environmental stress associated with this chemical. Considering that this chemical has an even lower CDI% than culturing water, we can conclude the minimal stress this chemical exerts on the environment.

3.11 Heartbeat Change in Exposure to Glyphosate

Glyphosate is a chemical widely used to kill certain plants and grasses. Although the EPA classified the chemical minimal risk, it also recognized its potential toxicity in aquatic environments. The EPA noted that Glyphosate is not readily broken down by water or sunlight and raised concerns for the possibility of groundwater contamination. More recent studies have pointed out potential carcinogenic (cancer-inducing) properties present in Glyphosate [27]. As a result, Glyphosate has been banned in a multitude of locations. Our research determined a CDI% of 130%, signifying moderate environmental stress associated with this chemical. Furthermore, the 1/10 dilution proved to be fatal.

3.12 Discussion Summary

The range of CDI% is from 12.0 in DMAS, the lowest, to 330, the highest, in GSL. It was dispersed across all the numbers, as in Fig. 15. The results varied for each chemical. For instance, gasoline showed a high-stress level to the point that every Daphnia up to the 1/1000 dilution died, highlighting the stress gasoline can place when exposed to the environment. On the other hand, other commercial chemicals, such as Dimethylamine Salt, showed very minimal change in BPM—the CDI% was measured to be 0%.

Our experimental methods to determine environmental stress provide a prospective look to further our understanding of chemicals and can be an additional process to determine a chemical's safety before being released to the general public. However, it is true that this method may not capture all aspects of chemicals, as seen in Glyphosate. Despite Glyphosate showing a moderate level of stress on the environment, no differentiating factor revealed its potential properties as a carcinogen. In addition, certain chemicals cause an increase in a heartbeat while others cause a decrease- though this study focused solely on the BPM changes.

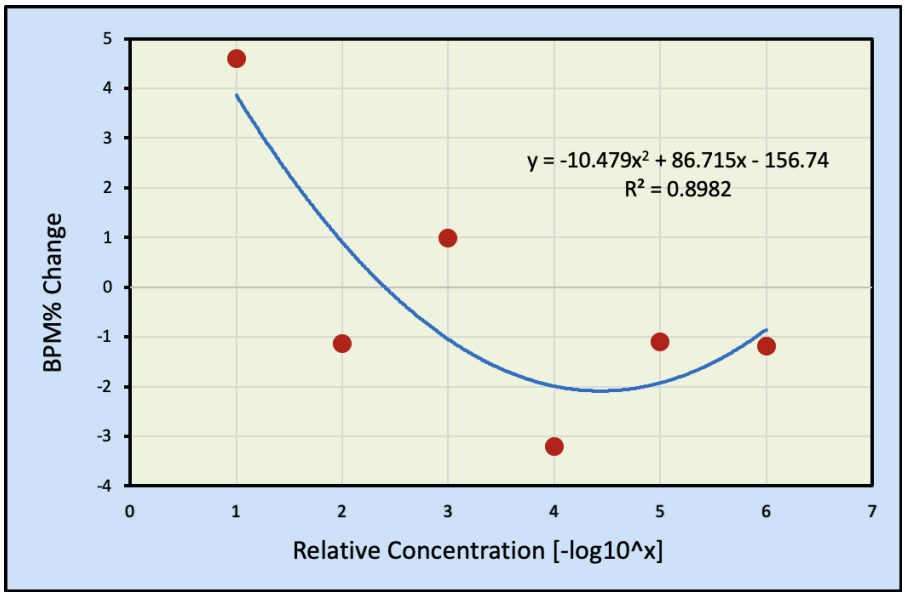

Fig. 13. The BPM% change in exposure to Dimethylamine Salt (ACE Spot Weed Killer)

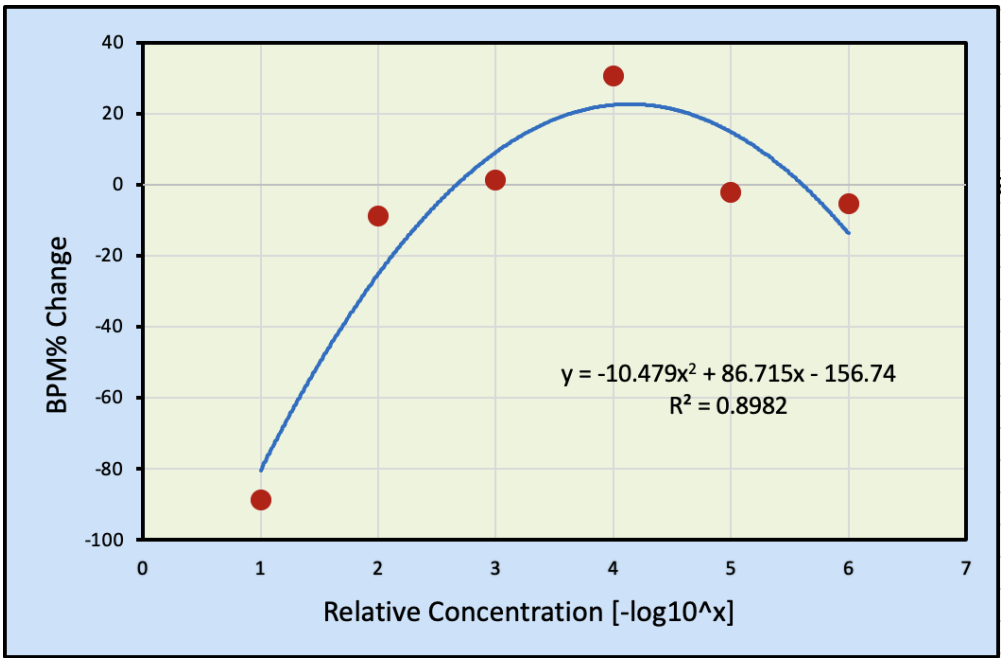

Fig. 14. The BPM% change in exposure to Glyphosate (Roundup)

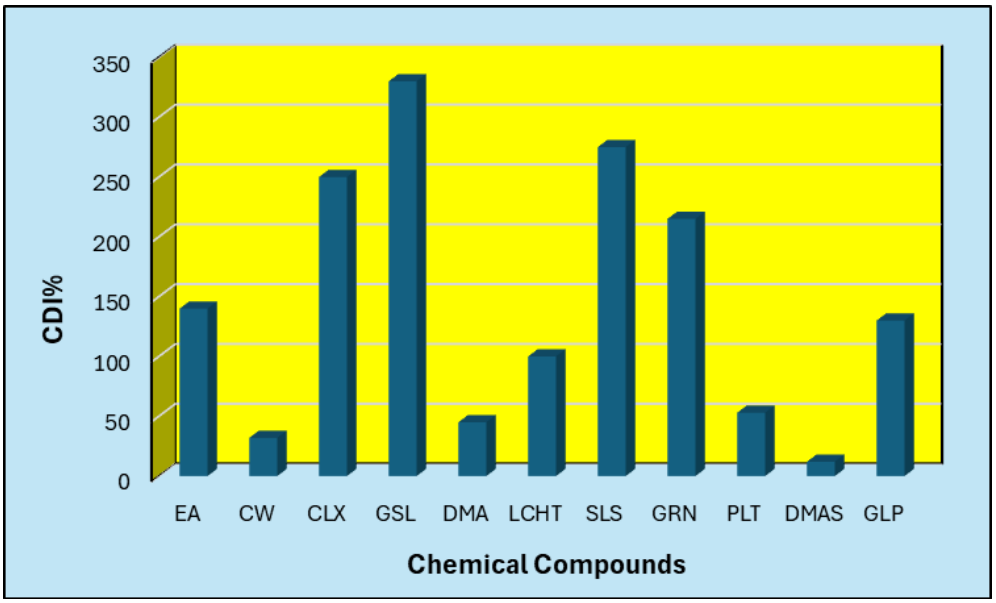

Fig. 15. The magnitudes of CDI% for each chemical compound tested in the study (Legends; EA; ethyl alcohol, CW; culturing water, CLX; clorox, GSL; gasoline, DMA; dimethylamide, LCHT; lambda-cyhalothrin, SLS; sodium lauryl sulfate, GRN; geraniol, PLT; prallethrin, DMAS; dimethylamide salt, GLP; glyphosate)

Future analysis could be done to explore whether the direction of BPM change is another analyzable factor. Nonetheless, it is essential to note that this method of experimentation serves as a single facet of the overall traits of a chemical and does its target goal well, as it can successfully determine environmental stress. Ultimately, our research shows a feasible process for determining the safety of chemicals before they are released to the general public.

4. CONCLUSION

Our experiment measured the environmental stress imposed by various residential chemicals, including herbicides, insecticides, and

detergents. Some of these chemicals have been claimed to act as endocrine disruptors.

This stress was measured with these chemicals by quantifying data, with which we evaluated the change in the BPM of *Daphnia Magna* after exposure to varying dilutions of the tested chemicals. The change in bpm of the *Daphnia magna* was utilized as an indicator of stress, which was used to analyze the environmental stress associated with the tested chemicals. An important parameter, called cardiac disturbance indicator (CDI), was defined as the average sum of heartbeat changes at the set time points we measured. Results showed a broad spectrum of CDI from 12% to 330%. The magnitude of CDI could be predicted, considering other toxic data published. The study could conclude that the CDI should be a useful surrogate for assessing toxic effects in future studies.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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