

The effect of common food preservatives on the heart rate of *Daphnia magna*

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SUMMARY

Monosodium glutamate (MSG), sodium nitrite, and sodium benzoate are used frequently in the food industry to enhance product flavors and preserve foods, as well as beverages, by preventing bacterial growth. As food additives are being consumed every day worldwide, any side effects of their consumption could have serious implications for human health and should be investigated. The purpose of our study was to examine the effects of MSG, sodium nitrite, and sodium benzoate on the heart rate of *Daphnia magna*, small freshwater crustaceans with hearts comparable to humans. MSG, sodium nitrite, and sodium benzoate have been linked to several health effects including alteration in heart rate. We hypothesized that MSG and sodium nitrite would increase the heart rate and sodium benzoate would decrease the heart rate of *D. magna* based on data from previous studies. Our results show that all three of the compounds had some measurable effect on the heart rate of *D. magna*, with benzoate showing the clearest effect. Sodium nitrite showed slightly increased heart rates as the concentration increased, while in the high concentration of sodium benzoate heart rates were significantly decreased. For MSG the effect was not as clear, and there appeared to be a drop in heart rate at a low concentration. The food industries may use these findings to guide the food additive concentrations in their products as well as to make consumers aware of the possible effects after consumption.

INTRODUCTION

Food additives are natural or synthetic substances added intentionally to food in small amounts to extend the durability of the product and modify its color, flavor, or structure (1). Food preservatives are used to preserve the quality of food and extend its shelf life. Previous studies have reported that chemicals used as preservatives have negative effects in human and animal models, with side effects ranging from very mild to extremely dangerous or life-threatening (2). There has been an increase in concern over food additives due to studies that have shown an increasing number of negative health effects (3). Even though there have been reported side effects, food preservatives and food additives are still used because they are considered to be safe at current usage levels. These reported effects mostly occur at levels much higher than typical human consumption, and their role as preservatives also prevent potentially harmful consequences

from microorganism growth (4,5). In our study, three of the most commonly used food additives - monosodium glutamate (MSG), sodium nitrite and sodium benzoate - were tested on *Daphnia magna* to see if its consumption leads to any alterations in heart rate.

D. magna are freshwater crustaceans with short lifespans and transparent bodies, allowing easy observation of different organs including the heart, and making it an ideal organism for this study (6, 7). The hearts of *D. magna* are similar to those of humans (8). The presence of hemoglobin in the blood of daphnia indicates a similar respiratory function. Like humans, hemoglobin increases when there is a lack of oxygen (9). Ultrastructural studies show that a daphnia's heart wall cells are characterized by long striated myofibrils similar to that found in human cardiac muscle (10). Studies show that caffeine increases heart rate in *D. magna*, which is a similar response to that seen in humans (8, 11). Various substances have been tested on *D. magna* to see if they have an impact on their heart rate that is comparable to what would occur in a human heart (8, 12). Since the *D. magna* heart is similar to a human heart, we chose to test these substances on *Daphnia* because the results would have some relevance to human consumption.

Sodium nitrite (NaNO_2), a water-soluble compound, appears as a slightly yellowish crystalline powder and is used as a food preservative, antimicrobial, and coloring agent (5, 13). Sodium nitrite increases the amount of methemoglobin (14). Methemoglobinemia is a condition which occurs when more than 1% of the hemoglobin in red blood cells is transformed into methemoglobin. The methemoglobin severely affects the oxygen carrying capacity of the red blood cells and an increase of more than 50% of methemoglobin leads to seizures, acidosis, arrhythmias, comas, and even death (15). Sodium benzoate ($\text{C}_7\text{H}_5\text{O}_2\text{Na}$), an odorless sodium salt soluble in water and ethanol, is used as a preservative to prevent the growth of molds, yeasts, and bacteria (4). Sodium benzoate is reported to be associated with hyperactivity and careless behavior in children (16). Other effects attributed to sodium benzoate are an effect on sperm motility, mutagenicity, hormone disruption, and oxidative stress, however these effects are debatable, depending on the study used and the amount of sodium benzoate used is often higher than the recommended daily allowance (17). MSG is one of the most widely used food additives. It acts as a flavor enhancer and creates a unique flavor, known under the umbrella term "umami" which originates from the Japanese word for "savory" (18). According to some studies, there appears to be a link between MSG and obesity, a condition which may lead to diabetes (19). MSG is also associated with changes in heart rhythm, hepatotoxic effects and neurotoxicity (20). Given the details of the previous work, we hypothesized that

the compounds under test would each alter the heart rate of *D. magna*.

To determine whether MSG, sodium nitrite, and sodium benzoate consumption would have an impact on the heart rate of *D. magna*, we exposed *D. magna* to different concentrations of the compounds and recorded both pre-exposure and post-exposure heart rates. We found that after exposure to sodium nitrite, the heart rate increased slightly as the concentration increased and heart rate decreased significantly after exposure to a high concentration of sodium benzoate. The effects of the MSG was not clear; there was a drop in heart rate only at a low concentration. In addition, our findings reveal that certain concentrations of these food additives cause an alteration in heart rate which consumers should be aware of. Given these side effects, food industries that use these food additives in their products as well as consumers should find better alternatives to avoid these possible consequences.

RESULTS

To determine the effects of MSG, sodium nitrite, and sodium benzoate on the heart rate of *D. magna*, we first recorded their pre-exposure heart rate. We then placed them in in 0.5%, 1%, 2% and 5% solutions of MSG, sodium benzoate, and sodium nitrite for 15 minutes before recording the heart rate again under the microscope. The 15-minute incubation allowed time for them to adjust to the new environment and reduced the possibility that the changes in heart rate may be caused by handling or exposure to a new environment instead of the substances. The concentrations used in our study were chosen based on similar concentrations used in other studies (8, 12, 21-22).

We found that sodium nitrite increased the heart rate (beats per minute, bpm) while MSG and sodium benzoate decreased the heart rate (Figure 1). We observed some variation between the heartbeat differences due to the different concentrations of the compounds; however, the spread of the data of each of the groups was not the same and this could have potentially affected the results of an ordinary ANOVA test. We found that the null hypothesis of equal variances was rejected for the data for the concentration groups in sodium benzoate and MSG, with p -values of 5.74×10^{-6} (extremely significant) and 0.002 (very significant) respectively (Table 1). However, the concentration groups for nitrite had a p -value of 0.750, therefore equal variances could be assumed for this compound.

The results of Welch's ANOVA showed that there were some differences between the group mean of each concentration for all compounds (p -value range from 0.021-0.039). We then performed the Games-Howell post-hoc test on all of the compounds (Figure 2). There was a difference between the highest concentration of 5% and the concentrations of 0.5% and 2% of sodium benzoate (adjusted p -values of 0.046 and 0.039, respectively), and the highest concentration produced a larger negative difference in heart rate. This suggests that the more sodium benzoate consumed, the lower the heart rate will drop. Only one of the comparisons showed significant differences in both sodium nitrite and MSG. There was a significant difference between the two lowest concentrations of MSG; 0.5% and 1% (adjusted p -value of 0.029), which showed a decrease in heart rate from 0.5% to 1%, while sodium nitrite showed an increase in the heart rate after dosing with 5% compared to 1% (adjusted

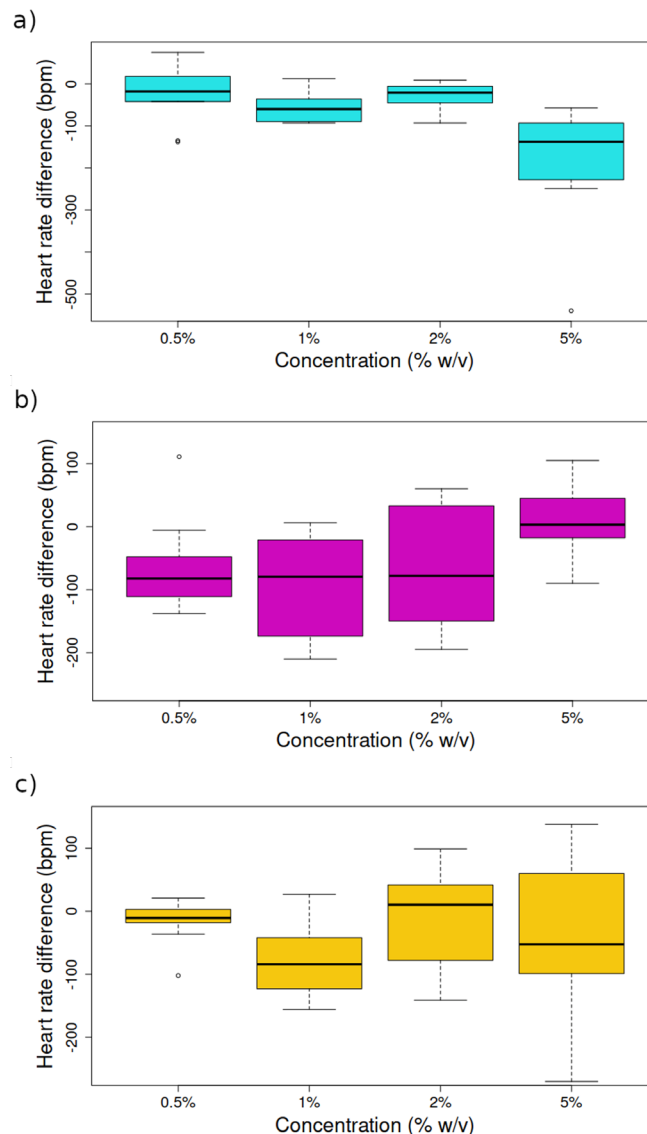


Figure 1: Effect of MSG, sodium nitrite, sodium benzoate on the heart rate of *D. magna*. The boxplots show the heart rate differences (heart rate after exposure - heart rate before exposure) for the *Daphnia* exposed to 0.5%, 1%, 2% or 5% w/v of (a) sodium benzoate, (b) MSG, or (c) sodium nitrite (10 *Daphnia* were used for each concentration).

p -value of 0.031). This would imply that consumption of higher doses of sodium nitrite leads to an increase in the heart rate.

DISCUSSION

Our study was conducted to find the effects of sodium nitrite, sodium benzoate and MSG on the heart rate of *D. magna*. We hypothesized that MSG and sodium nitrite will increase *D. magna*'s heart rate and sodium benzoate will decrease the heart rate, based on previous research (14, 21, 23). We showed that all three compounds significantly affected heart rate of the *D. magna* (Figure 2). Sodium benzoate led to a significant decrease in the heart rate at the highest concentration when compared to most of the lower concentrations. This result is supported by work done by Tiruvannamalai V. et al., which shows similar results in the

	Sodium Benzoate	Sodium Nitrite	MSG
Bartlett's K ²	27.051	1.213	14.625
p-value	5.74 x 10 ⁻⁶ ***	0.750	0.002 **

Table 1: Homogeneity of variances for the heart rate differences.

The distribution of heart rate differences for the nitrite test compound shows a good degree of similarity between the concentration groups. Variances of the group for MSG and sodium benzoate are not homogeneous. * denotes a significant result (p < 0.05), ** very significant (p < 0.01), and *** extremely significant (p < 0.001; n=40 for each compound).

D. magna that were tested (21). It should be noted that the presence of an outlier in the sodium benzoate data, with a heart rate difference of -540 (*D. magna* number 39 in the sodium benzoate group) impacted the data analysis and the significance of the results. Unfortunately it was hard to justify the removal of the outlier. Although the individual in question did display slower movement and it is possible it may have been dying during the test procedure, it is difficult to be certain, and therefore we are unable to remove this data point simply because it does not fit.

Sodium nitrite appears to show an increase in heart rate with increasing concentration (Figure 1), but the effect is only statistically significant for the comparison between the 1% vs 5% concentrations. This is in contrast to results from some previous work where sodium nitrite decreased the heart rate of the *D. magna* (21). This discrepancy could be due to the dose and exposure time used in the study; a dose of 0.015% w/v sodium nitrite, which is 10 times lower than the lowest concentration we tested, and an exposure time of up to 30 minutes. However, others describe how sodium nitrite causes increased heart rate in humans when exposed to high concentrations, similar to our results (14, 23). Sodium nitrite is a toxic compound which can cause severe methemoglobinemia. Methemoglobin is produced when the sodium nitrite enters the red blood cells and reduces the hemoglobin. The ability to carry oxygen to where it is needed decreases with the rise in methemoglobin, causing the body to compensate for the low oxygen level by increasing the heart rate. A person with methemoglobinemia may experience an accelerated resting heart rate, weakness, nausea, and death in severe cases (23). As sodium nitrite is linked with numerous side effects, alternatives for sodium nitrite could be considered. Various plant extracts and organic acids such as lactate and sorbate can be used effectively as substitutes for sodium nitrites in processed meats; however, there have been no substitutions that can fulfill all of sodium nitrite's functions (5).

The results for MSG were more ambiguous (Figure 1). The box plot of the difference in heart rate versus concentration of MSG does not show a clear upward or downward trend. Instead the medians hover around zero difference for each concentration group and there is an obvious increase in variability as the concentration increases. According to the Welch's ANOVA post-hoc analysis, the only concentration comparisons that showed a significant difference was between the lowest concentrations (0.5% and 1.0%), with the 1.0% showing a dip in the heart rate difference. Although our results for MSG show a little effect on the *D. magna* heart rate, the effect is not as pronounced as previous studies (24). This may be due to the fact that our exposure times to MSG

for some *D. magna* were inconsistent; MSG was the first compound tested, starting with the highest concentrations of MSG and the inconsistencies in exposure times might explain the increasing variability seen in these higher concentrations. Previous studies used different acclimation times; some of the studies used five or seven minutes to acclimate the daphnia in the solution, while some used much longer at 30 or 45 minutes (7-8, 12, 21-22). After some preliminary investigation it was felt that 5 minutes was too short, and we did not have sufficient time available to use 30 minutes or more, therefore 15 minutes was chosen as the acclimation time in our experiment. Mahna et al. tested 1% and 2% solutions with

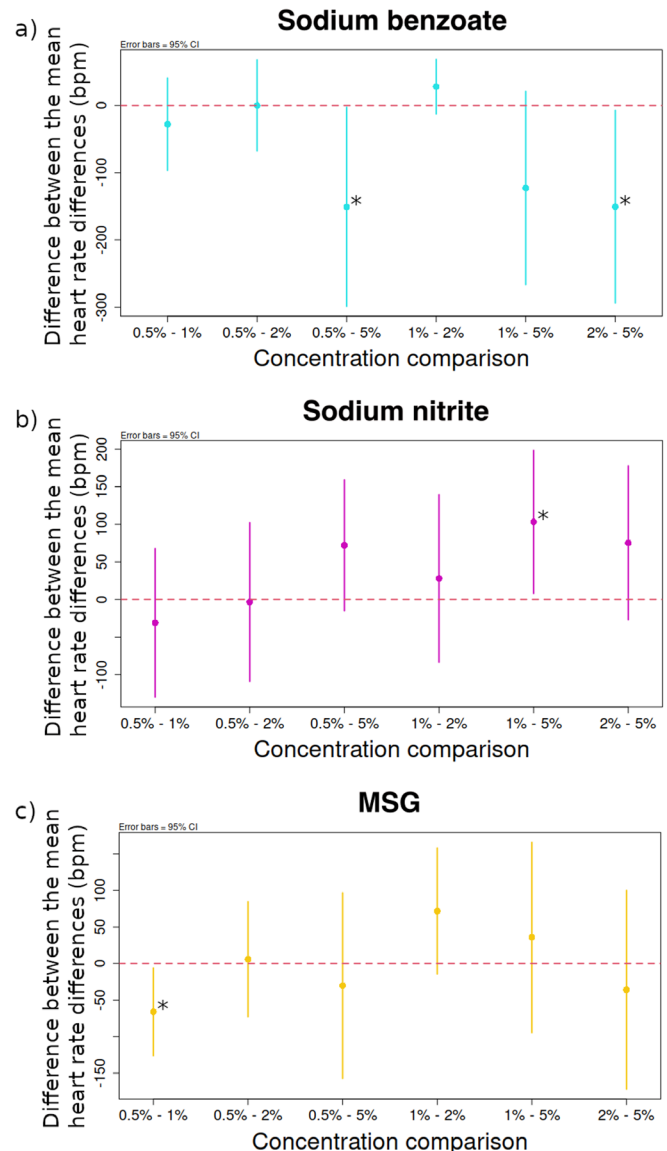


Figure 2: Games-Howell post-hoc comparisons to find which of the concentrations show a significant difference in the heart rate. The plots are for a) sodium benzoate, b) MSG, and c) sodium nitrite, and show the mean and confidence intervals for the concentrations being compared. The red dotted line denotes no difference between comparison groups, the colored dot indicates the differences in the means of the two groups, the lines represent the 95% confidence intervals for the differences in the groups. Any comparisons that show a significant difference are indicated. * p<0.05

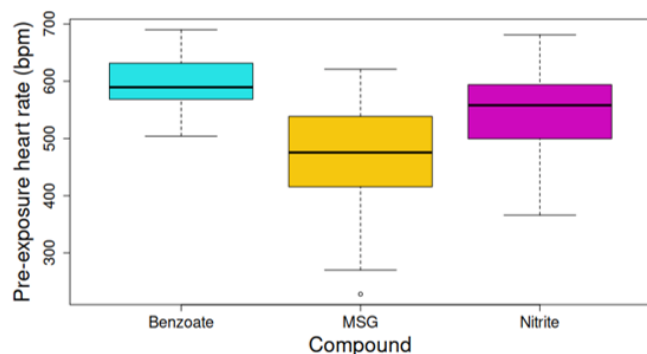


Figure 3: Boxplot comparison of the pre-exposure heartbeats for all *Daphnia* grouped by the compound they would later be exposed to. Note the difference in not only the median heart rate, but also in the variation of the heart rates. (n=40 for each compound).

acclimation times of 30 minutes and overnight, while in this study the acclimation time was altered to 15 minutes to see whether the results show any similar effects (7).

During the data analysis stage of this investigation, we found that the pre-exposure heart rates for *D. magna* for each test substance showed significant variation (Figure 3). We would expect the heart rates to be similar prior to dosing and the difference in variation to be similar, and these should act as a baseline control. The variability in the pre-exposure heart rate decreases from MSG to sodium nitrite to sodium benzoate, which reflects the order in which those substances were tested (Figure 3).

There were other issues which may have affected our results. Some of the *D. magna* used in the experiment, especially for the sodium benzoate and sodium nitrite, were pregnant. It is unknown if this will affect the response, and the magnitude of this potential effect. Another factor that could have introduced unwanted variation is that *D. magna* from different batches were used throughout the experiment and it took some time to create a stable colony. Future research could also consider testing higher or lower concentrations of the compounds as well as other food additives that are being used. The model organism, *D. magna* could also be used to observe effects of the additives on pregnancy as they have clear bodies for easy observation, reproduce both sexually and asexually, and have a short life span.

In summary, our results suggest that sodium benzoate decreases the heart rate of *D. magna* at higher concentrations, and sodium nitrite increases the heart rate, whilst our results for MSG are somewhat inconclusive. Since *D. magna* have hearts comparable to humans, similar effects could be expected in humans. The result of this study could raise the awareness of the side effects of food additives that are widely consumed worldwide on heart rate.

MATERIALS AND METHODS

Organisms and reagents

D. magna were brought from Live Feed Center, Supan Bhuri. The *D. magna* were kept in a container with natural light and darkness. They were fed with a small amount of yeast suspension in water everyday. Food grade sodium benzoate was sourced from Wuhan Youji Industry Company Limited, Wuhan. MSG from Ajinomoto, Bangkok and sodium nitrite from Suksapanpanit, Bangkok.

Heart rate assay

250ml solutions of MSG, sodium nitrite, and sodium benzoate were made by dissolving in filtered water. For each concentration, 10 matured (grown to approximately 2mm in length to ensure that they are easier to monitor under the microscope) *D. magna* were picked using a plastic dropper and taken out of the container to record the pre-exposure heart rates. After recording the pre-exposure heart rates, each *D. magna* was placed in a beaker containing approximately 50ml of either 0.5%, 1.0%, 2.0% or 5.0% w/v concentrations of MSG, sodium nitrite or sodium benzoate. The recorded heart rate pre-exposure to the drug solutions act as the baseline. They were given 15 minutes to adjust to their surroundings before the heart rates were recorded again. After the 15 minutes, each *D. magna* was taken out of the beaker containing the compound solution and placed on a slide and the heart rate measured again. To measure the heart rates, the *D. magna* were placed on to an ordinary microscope slide and a small piece of cottonwool was added to absorb the excess water and reduce the *D. magna* mobility. A video was taken by phone through the microscope lens to record the heart rate for 30 seconds. The video was then cut to 10 seconds and slowed down, allowing us to precisely count the heart rate during the ten seconds. The heart rates were then multiplied by six to get the heart rate in beats per minute (22).

Statistical analysis

Statistical analysis of the data was performed using R version 4.1.3 and RStudio 2022.02.4. Exploratory statistics were performed to check the data for potential issues and boxplots were used for visualization. The ANOVA test can be affected by violations of the assumption of equal variance of the groups therefore a Bartlett's test of homogeneity was performed to check that the groups had equal variance, and a Welch's ANOVA was used to test for difference in the group means (25). Finally, since the ANOVA only checks for differences in the group means and cannot identify which groups are different, a Games-Howell post-hoc analysis was performed on any compound that demonstrated statistically significant differences between group means in order to identify which concentration(s) are different. In each of the statistical tests, a *p*-value of 0.05 was used as the threshold to denote significance, but the *p*-values are also reported in the results.

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