Tasty or Toxic: Evaluating the Effects of Common Food Additives on *Daphnia Magna*

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ABSTRACT: The adverse effects of three of the most used food additives, MSG, food color, and sodium nitrite, were investigated in this research. The hypothesis was that these food additives would have harmful effects on *Daphnia magna*. Treatment groups called control, MSG, sodium nitrite, and food color each were tested in four different concentrations. Five *Daphnia magna* that were less than 24-hours-old were tested per group and were observed after thirty minutes and after overnight incubation with each of the additives. Heart rate and mobility were measured to determine the acute toxicity of the test compounds. In MSG, the heart rate decreased significantly in all concentrations after overnight incubation. All *Daphnia* numbers were deceased within thirty minutes in sodium nitrite in both 1% and 2% concentrations, showing that sodium nitrite is toxic. However, cardiac effects did not cause this because heart rate in lower concentrations of 0.1% and 0.01% did not change significantly from control. With food dye, the heart rate decreased significantly in concentrations as low as 0.1% after overnight incubation. Finally, we can conclude that these food additives have a toxic effect on *Daphnia magna* and the severity of these effects is based on the time of exposure and quantity of food additives consumed.

KEYWORDS: Toxicology; Food Additives; *Daphnia magna*; Monosodium Glutamate; Food Color; Sodium Nitrite.

Introduction

Foods have evolved and changed tremendously in the past decade. Food additives are now applied to many different food types. Food additives are substances used to preserve the food or enhance its flavor or appearance. Recent studies have shown that these substances may have negative effects on the human body.[1,2] To test this, we decided to do experiments with the three most common food additives: MSG, food color, and sodium nitrite. MSG, or monosodium glutamate, is the sodium salt of glutamic acid, one of the most abundant naturally occurring, non-essential amino acids. It is used to intensify and enhance the flavor of food.[3] Studies have shown that it can cause headaches, asthma, and even brain damage.[4-6] Food coloring is made in a lab with chemicals derived from petroleum, a crude oil product, which is also used in gasoline, diesel fuel, asphalt, and tar.[7] Food color is used to improve the appearance of foods and is often used in advertisements and fast-food restaurants. The excessive use of food color has been shown to reduce the attention span and cause ADHD and other behavioral problems in children.[8,9] Sodium nitrite is often found in processed meats as a preservative to prevent bacteria growth.[10] Studies have shown that over-consumption of sodium nitrite can cause kidney damage and low blood-pressure.[11,12] We tested each food additive on *Daphnia magna*. *D. magna* is a tiny, semi-transparent freshwater crustacean with long antennae and prominent eyes. *D. magna* was used because they are a perfect model system, easy to handle, and are an ideal system for studying multiple stressors. They are good test subjects because of their transparent bodies, early reproduction rates, short lifespans, and gender change response system. We hypothesized that these food additives would have harmful effects on the *D. magna*.

Results and Discussion

To observe the short-term effects of food additives, *D. magna* were incubated with each of the test compounds for 30 minutes at four different concentrations: 2%, 1%, 0.1%, and 0.01%. These concentrations represent the range of relevant concentrations of food additives that can be found in the human body system. Five *D. magna* were included in each group. After 30 minutes the heart rates were measured. The average heart rate for the control group was 193 beats per minutes (bpm). No significant effect was observed in any groups for any concentration tested after 30 minutes of incubation. However, all of *D. magna* died within 30 minutes of incubation in 2% and 1% sodium nitrite and all *D. magna* died in the 2% food color group (Table 1 and Figure 1). At least one *D. magna* died in each of the concentrations tested in the food additive groups. No *D. magna* died in the control group (Table 3 and Figure 2). The deceased *D. magna* were not included in heart rate analysis.

To observe the effects of the food additives after longer incubation, *D. magna* were left overnight (12 hours) with food additives. The heart rates were measured the following day. After overnight incubation, the average heart rate for the control group was 173 bpm. The MSG and sodium nitrite groups displayed a dose-dependent increase in death of *D. magna*, indicating increased toxicity of these food additives with increased dosage (Table 3 and Figure 2). There was a significant reduction in heart rate in *D. magna* in the presence of MSG and food dye (Table 2 and Figure 1). Interestingly, 0.1% and 0.01% sodium nitrite did not display a significant

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change in heart rate compared to the control. However, all *D. magna* in 2% and 1% sodium nitrite died within 30 minutes. This indicates that sodium nitrite is highly toxic at higher concentrations but not due to cardiac toxicity.

Table 1: Heart rate in *D. magna* after 30-minute incubation with various concentrations of food additives. The black filled cells indicate the *D. magna* that died in the presence of food additives.

	Heart Rate (30 min)								
		D. magna individuals							
	Concentration	D1	D2	D3	D4	D5	Average	STDEV	P Value (Student's t-Test)
Control	N/A	200	196	192	194	184	193.2	5.9	N/A
MSG	2%	184	196		200	164	186	16.2	0.32
	1%	200	192	204		200	199	5.0	0.30
	0.1%	204	188	208		208	202	9.5	0.29
	0.01%	168	168	172	168		169	2.0	0.00
Sodium Nitrite	2%							N/A	N/A
	1%							N/A	N/A
	0.1%	188	196		168	184	184	11.8	0.22
	0.01%	184	188		180	188	185	3.8	0.16
Food Dye	2%							N/A	N/A
	1%	196		200	176	192	191	10.5	0.82
	0.1%	196	180	172	196		186	12.0	0.16
	0.01%	200		184	212	224	205	17.1	0.33

Table 2: *Daphnia magna* heart rate after 12-hour incubation with various concentration of food additives. The black filled cells indicate the *D. magna* died in the presence of food additives.

	Heart Rate (Overnight)								
Name	Concentration	D1	D2	D3	D4	D5	Average	Standard Dev	P Value (Student's t-Test)
Control	N/A	168	184	176	188	152	174	14.3	N/A
MSG	2%		128	136			132	5.7	N/A
	1%	120	128			116	121	6.1	0.015
	0.1%	116	128			124	123	6.1	0.035
	0.01%	136	132	128		156	138	12.4	0.087
Sodium Nitrite	2%						N/A	N/A	N/A
	1%						N/A	N/A	N/A
	0.1%	176			160		168	11.3	0.677
	0.01%	160	156			152	156	4.0	0.286
Food Dye	2%						N/A	N/A	N/A
	1%	92	112	96	76	112	98	15.1	0.003
	0.1%	116	140	144	96		124	22.4	0.024
	0.01%	180	164	156	120	144	153	22.5	0.189

Table 2: *Daphnia magna* heart rate after 12-hour incubation with various concentration of food additives. The black filled cells indicate the *D. magna* died in the presence of food additives.

Name	Concentration	# of Mobile *D. magna*	
		30 min	Overnight
Control	N/A	5	5
MSG	2%	4	2
	1%	4	3
	0.1%	5	3
	0.01%	5	4
Sodium Nitrite	2%	0	0
	1%	0	0
	0.1%	5	2
	0.01%	5	3
Food Dye	2%	0	0
	1%	3	3
	0.1%	5	4
	0.01%	5	5

Discussion

These results show that food additives have harmful effects on the health of *Daphnia magna*. Food dye had significant cardiovascular effects corresponding to its concentration. MSG slowed the heart rate of *D. magna* while sodium nitrite did not display any significant cardiovascular effects. However, sodium nitrite was shown to be extremely harmful in high concentrations as none of the *D. magna* were alive in trations above 0.1% after 30 minutes of incubation. MSG and food dye impacted mobility of *Daphnia magna* in a dose dependent manner. The dose dependent effects were highly evident after overnight incubation. This also shows that the

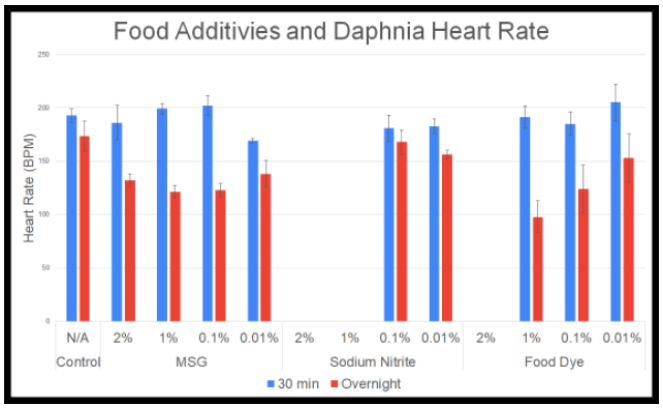

Figure 1: The effects of various food additives on *Daphnia magna* heart rate.

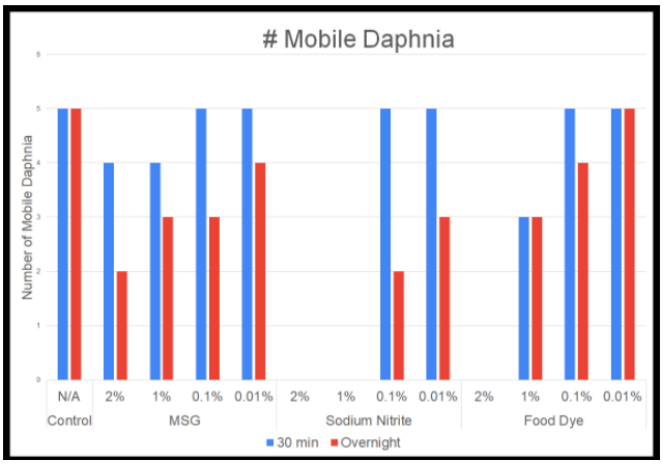

Figure 2: The effect of various food additives on the mobility of *Daphnia magna*.

overnight incubation had a much larger influence on *D. magna* than the 30-minute incubation. Overall, these results indicate that food additives have toxic effects on *Daphnia magna* and these effects are dependent on the concentration and time of exposure.

Conclusion

Based on these results obtained in this study, we can conclude that these food additives have a toxic effect on *Daphnia magna*, and the severity of these effects are based on the time of exposure to these additives and the concentrations at which the additives are used. Using these results, further studies should be performed on humans using the hypothesis derived from this study. Although humans should portray the effects found in this study, they may not because of the variations between the physical and genetic makeup of the two species.

Methods

To determine the health effects of food additives, the *Daphnia magna* were tested in four groups (control, MSG, sodium nitrite, and Quinoline yellow food color). Each food

additive was tested in four different concentrations (0.01%, 0.1%, 1%, and 2%). *D. magna* heart rate and mobility were measured as an indicator of toxicity. Five one-day-old *D. magna* were tested per group. The control group was left in spring water without any additives. The measurements for heart rate and mobility were performed after 30 minutes and overnight incubation with each food additive. The heart rate was measured by looking at *D. magna* through the microscope and counting how many beats occurred in 15 seconds. The results were multiplied by 4 to get the beats per minute (bpm). Lastly, to determine the results of mobility, the test container with *D. magna* was shaken and then observed to see if any *D. magna* moved within fifteen seconds. The results were entered into Excel and graphs were created. The results were analyzed by calculating the average heart bpm and standard deviation. The number of mobile *D. magna* was recorded by counting. The Excel data analysis tool ANOVA was used to determine if there was a significant effect due to any food additive. Once ANOVA determined that there was a significant effect due to food additive, a two-tailed, paired student t-test was performed to determine the significance of effects at each concentration of food additive in comparison to the control group. A p-value less than 0.05 was considered significant.

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