The Effect of Vinegar and Tap Water on the Release of Nickel in Grade 304 Stainless Steel Cups

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Consumption of nickel rich liquids and foods pose a risk to human health, possibly causing reactions such as dermatitis. This study examines the amount of nickel that leaches out of grade 304 stainless steel cups with respect to time. The hypothesis of this experiment is that if given enough time, the liquids will react to the nickel in the stainless steel cups, and then nickel will leach out into the cup's contents. The null hypothesis being that if the cups, since they are not given enough time and/or do not react to the nickel in the stainless steel cups, will not leach nickel into the cup's contents. 30 grade 304 stainless steel cups were tested in this experiment: 15 containing 300 mL of tap water and 15 containing 300 mL of white distilled vinegar. Using the equation of a calibration curve of Absorbance vs. Known Nickel Amounts to calculate the amount of nickel in the liquids, samples were taken from each of the 30 cups at 8, 24, and 32 hours. At 8 hours, on average the cups containing tap water leached 5.16 μg/mL of nickel, and the cups containing vinegar leached 0 μg/mL of nickel. At 24 hours, on average the cups containing tap water leached 24.5 μg/mL of nickel, and the cups containing vinegar leached 110. μg/mL of nickel. The results of 32 hours were not included in the final analysis because of uncertainties. Although the final analysis of this data proved significant, there were statistical uncertainties.

Introduction

The appearance of nickel dermatitis has increased in recent years. Being the fourth most used metal in the world, nickel is commonly found in foods and stainless steel. Grade 304 stainless steel, which is comprised 8% of nickel, is a food grade metal, meaning it is of a sufficient enough quality to be used in food storage, preparation, and production. Ni most commonly causes dermatitis, often on the hands, as a result of an allergy present in "approximately 10% of the population" (Kamerud, 2013). Because of nickel's abundant presence in everyday utensils and consumable items, the average intake of nickel is close to 300 μg a day; "In 2001, the Tolerable Upper Intake Level (UL) of Ni was decreased to 1,000 μg per day. Adults in the U.S. are estimated to ingest an average of 69 to 162 μg of Ni per day" (Kamerud, 2013). Compared to men, women are 10% more likely to have dermatitis caused by nickel because of jewellery containing nickel (Purello, 1998). Used in stainless steel pipes, dental appliances, and kitchen utensils and appliances, nickel and other metals can easily be released into their surroundings because of corrosion and heat. A 2013 study showed the amount of nickel released from stainless steel pans was "dependent on the grade of stainless steel, cooking time, and previous usage or seasoning of the stainless steel" (Kamerud, 2013).

In this study the independent variable is liquid's time spent in the cups. The dependent variable is the amount of nickel released. Distilled white vinegar was used because it has a pH similar to common drinks like Coca-Cola. Tap water will also be used to study what effect a non-corrosive liquid essential to life has on the amount of nickel that leaches out, acting as the control group. The cups containing the liquid will be placed at room temperature, about 70°F. The liquids will be tested for nickel content at 8 hours, 24 hours, and 32 hours. The exploration of this topic was chosen because stainless steel cups, such as Yeti brand tumblers, are sold to insulate both cool and warm liquid. Stainless steel cups and water bottles are becoming more and more popular due to companies like Yeti and S'well, which produce stainless steel water bottles, tumblers, etc. With the market and production of stainless steel water bottles and cups on the rise, it is crucial to test their ability to withstand corrosive liquids and heat, which, if heavy metal does leach out, can be detrimental to the human health.

A 2013 study was similarly conducted to determine the amount of nickel released in stainless steel pans. This study shows as little as "67 μg can cause recurrence of ACD (allergic contact dermatitis), flare up eczema, or lead to systemic dermatitis in individuals sensitive to nickel" (Kamerud, 2013). In Kamerud's study, tomato sauce was cooked in new grade 304 stainless steel pans. The results of the study were that "Nickel. concentrations were highest in the first cooking cycle... Although the amount of metal leaching initially decreased with cooking cycles, significant amounts of both Ni and chromium continue to leach after multiple cooking cycles and appear to reach a constant condition" (Kamerud, 2013).

The purpose of this study is to determine the amount of nickel released from grade 304 stainless steel cups with respect to the time the liquid contents spend in the cups. The hypothesis is that if given enough time, the liquids react to the nickel in the stainless steel cups, and then nickel will leach out into the cup's contents. The null hypothesis being that if the cups, since they are not given enough time and/or do not react to the nickel in the stainless steel cups, will not leach nickel into the cup's contents.

Methods

A serial dilution performed by adding 0.619g of solid nickel nitrate, which contains 500 mg of Ni, into 250mL of distilled water. 3mL of the solution was pipetted into a cuvette and labeled "1." 125mL (half) of the original solution was then added to 125 mL of distilled water. The process of pipetting the solution into a new cuvette, labeling it with a new number, and halving the solution was repeated until 6 cuvettes were filled. Logger Pro was then used to calibrate the SpectroVis Plus Spectrophotometer using a cuvette filled with distilled water. Using the spectrophotometer, each cuvette of the serial dilution was then tested to see how much purple-blue light was absorbed. A new graph was made for each cuvette. The absorbance where each of the solutions peaked in the purple-blue region was plotted on a single graph, which was labeled "Absorbance vs Nickel μg/mL." By plotting a graph in which Ni amounts were known, a equation was produced which was then used to calculate the amount of Ni in the vinegar and water after completing the experiment. 30, 16oz grade 304 stainless steel cups were washed with dish soap and a sponge. The cups were dried with a soft rag to ensure they are not scratched, then 15 cups were labeled "V1" through "V15," and 15 cups were labeled "W1" through "W15," "V" standing for vinegar and "W" standing for water. Starting at a set time each cup labeled with a "V" was filled with 300 mL of vinegar and each cup labeled with a "W" was filled with with 300 mL of tap water; a graduated cylinder was used to measure out exactly 300 mL per cup. A lid was put on each cup to ensure that the least amount of liquid was evaporated, and the least amount of contaminants can contact the liquids. 8 hours later, starting with the cup labeled "V1", 3 mL was pipetted into a cuvette and labeled "V1." A 3mL sample was taken from each of the 30 cups, using a new pippett each cup to ensure that there is as little contamination as possible, and the lids were labeled according to the label on its corresponding cup. Each cuvette was then placed in the spectrophotometer and graphed on their own Absorbance vs. Wavelength (nm) graph. At 24 hours and 32 after starting the experiment, 3 mL samples were once again taken from each cup, placed in the spectrophotometer, and graphed. After completing the experiment, the contents of the cups were emptied down the drain, and washed with soap and water. A wavelength in the purple-blue region, 382.3, was used so that all the absorbances of each data point were on the same wavelength. The equation for absorbance from the calibration curve was then used to calculate the amount of Ni in each cup. The original equation *Absorbance = 0.5536 (Ni) + 0.01900* (or "*Absorbance = slope * Ni + b*") was converted so that Ni could be found.

The new equation was *Ni = (A - 0.01900) ÷ 0.05536* (or "*Ni = (Absorbance - b) ÷ slope*". The absorbances at the wavelength 382.3 recorded from each cup at 8, 24, and 32, hours was then plugged into the equation to find the amount of Ni μg/mL.

Safety and Precautions:

Since the amount of nickel in the serial dilutions was so concentrated, precautions were taken so that it was not consumed by anyone.

Results

The calibration curve shown in Appendix B, Table 1 was used to find the amount of Ni that leached into the stainless steel cups. The equation to find the absorbance, A= mx + b, was transformed into an equation to find the amount of Ni, *(A - b)/m = Nickel* . The slope of the points had a correlation of 0.9541, or 95%, meaning it has a 95% positive correlation. Since the b-value was more than the greatest y-value, .121, it was added to the equation. As shown in Appendix B, Table 2, the 6th data point of vinegar at 8 hours reveals a sharp spike in Ni, which 632.2 μg/mL. At 24 hours, the 6th data point of vinegar at 8 hours shows 18.06 μg/mL of Ni in the water. Since the sixth datapoint was so greatly skewed rom the data, it was considered an outlier. The 6th data point of each group of cups was not considered in the final analysis of data

The averages of each test were taken (see Appendix C, Table 1). The results for 32 hours was not included in any of the final analysis of data because of discrepancies between data points. The absorbances for each of the data points was negative, which is extremely unlikely because Ni had already leached into the liquids. At 24 hours, the cups that contained vinegar leached the most Ni, 104. μg/mL, on average. Water at 24 hours leached 22.0 μg/mL, which is significantly less than what was leached at 8 hours. This data was inconsistent, and it was extremely unlikely the amount of Ni leached could be less at 24 hours.

A single factor ANOVA test was run with an alpha value of 0.05 before removing the outlier shown in Appendix C, Table 2. The F value of 2.5057 was less than the F-critical value of 2.7694, therefore the difference in means was statistically insignificant, and the results of the experiment support the null hypothesis.

With the outlier removed, the data became more consistent. At 8 hours, the containing vinegar leached 0 μg/mL of Ni on average, compared to the cups of water which leached 5.16 μg/mL of Ni. At 24 hours, the cups containing vinegar leached 110. μg/mL of Ni on average, whereas at 24 hours the cups containing water leached 24.5 μg/mL of Ni on average (see Appendix C, Table 3).

After removing the outlier, a single factor ANOVA test was run with an alpha value of 0.05 (see Appendix C, Table 4). The F value of 8.0875 was more than the F-critical value of 2.7826, therefore the difference in means was statistically significant, and the results of the experiment support the hypothesis.

Conclusion

The purpose of this study is to determine the amount of nickel released from grade 304 stainless steel cups with respect to the time the liquid contents spent in cups. After using Beer's Law to calculate the equation of the slope from the calibration curve, the absorbances of the 8 and 24 hour tests were used to calculate the amount of Ni μg/mL in each cup. A single factor ANOVA test with an alpha value 0.05 was run on the 8 and 24 hour data, and the results proved to be insignificant because the F value of 2.5057 was less than the F-critical value of 2.7694. Once the outlier was removed from the data tables, the single factor ANOVA test with an alpha value of 0.05 was performed on the 8 and 24 hour data, and the results proved significant, supporting the hypothesis. Although at 8 hours both the water and vinegar leached little to no Ni, at 24 hours vinegar and water leached a significant amount of Ni. Compared to tomato sauce cooked in a new stainless steel pan, which "suggest that TS cooked in a new saucepan would contain 483 μg/kg (0.000483 μg/mL) of Ni per serving of tomato sauce, nearly half the tolerable upper intake level for a day" (Kamerud, 2013), the most Ni, with a mean of 110. μg/mL, leached from the cups that held vinegar for 24 hours. Without applying heat, the cups leached over the tolerable daily upper level intake. The absorbance data for both the water and vinegar for 32 hours were negative numbers. The spectrophotometer had stopped reading accurately, possibly due to the loss of the cuvette used to calibrate the spectrophotometer before each set of data was tested. After careful consideration, the data collected at 32 hours was not considered in the final analyzation of the data because it would be impossible for the Ni content of all the cups to rise well above 0 during the tests at 24 hours, then be less than zero at 32 hours. Although the spectrophotometer, and/or the software did not perform as expected when analyzing the data at 32 hours, it was not considered broken when testing the data for the 8 and 24 hours because of its consisity. Although the final analysis of the data was significantly significant and supported the hypothesis, after comparing the data to others studies it seemed unlikely that the stainless steel cups leached such a large amount of Ni. The Ni that leached out of the stainless steel cups could of been attached to other metals or chemicals, which could possibly skew the wavelength and amount of light absorbed; therefore it is possible that the nickelous nitrate absorbed a different wavelength and amount of light than the metals and chemicals that leached from the stainless steel cups. This study was limited to testing translucent liquids, which the spectrophotometer requires in order to read which wavelengths the liquids absorb. There was also statistical uncertainties concerning the actual amount of Ni that leached from the cups because other metals and chemicals could of leached out from the cups, which may absorb different wavelengths and amount of light compared to the nickelous nitrate. Future studies could test a wider range of liquids, use a greater amount of cups, test the effect of different temperatures, and use qualitative Ni test kits to get more accurate results.

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References

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Supplemental Information - Appendix A: Experimental Design Diagram Matrix

Experimental Design Diagram Matrix

Title of the Experiment - The Effect of Vinegar and Tap Water on The Release of Nickel in Grade 304 Stainless Steel Cups				
Hypothesis - Given enough time, the liquids will react to the nickel in the stainless steel cups, and then nickel will leach out into the cup's contents.				
Independent Variable - Time, liquids				
Levels of Independent Variable	8 hours	24 hours	32 hours	
Number of Repeated Trials	15 cups of distilled water 15 cups of vinegar (Same for			
Constants/ Controlled Factors (List at least 5) Time liquids spend in cups, type of cup, amount of liquid taken out of cups at each time interval, pH of liquids				
Control Group - 15 of the stainless steel cups contain water, which will be compared to the vinegar.				

Supplemental Information - Appendix B: Calibration Curve and Raw Data

Table 1: Calibration Curve

	Data Set	
	Ni Nickel (μ/ml)	A
1	2000	0.121
2	1000	0.097
3	500	0.036
4	250	0.038
5	125	0.016
6	62.5	0.024
7		
8		
9		
10		
11		
12		
13		

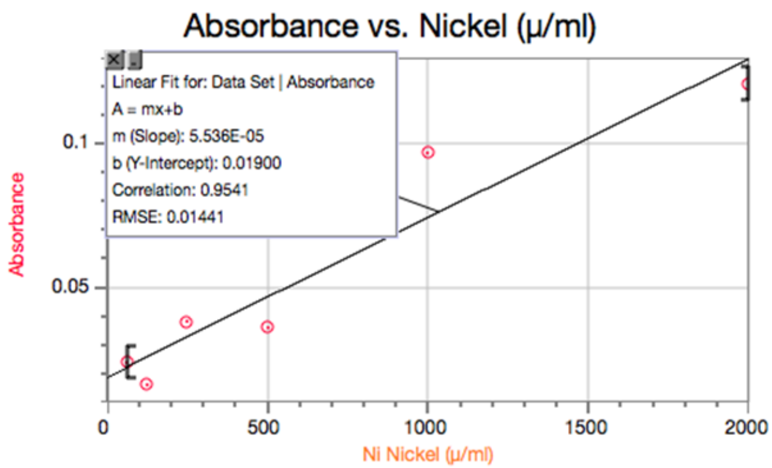

Table 2: Raw Data of Nickel Leached from Cups (μg/mL)

Vinegar cups: Nickel 8 hours (μg/mL)	Water cups: Nickel 8 hours (μg/mL)	Vinegar cups: Nickel 24 hours (μg/mL)	Water cups: Nickel 24 hours (μg/mL)
0	0	307.1	0
0	0	90.32	0
0	0	180.6	0
0	0	0	0
0	0	0	0
632.2	0	18.06	0
0	0	0	18.06
0	0	72.25	126.4
0	0	234.8	0
0	0	343.2	0
0	0	0	0
0	18.06	90.32	0
0	0	180.6	0
0	0	18.06	0
0	54.19	18.06	198.7

Supplemental Information - Appendix C: Nickel Averages (with and without outlier) and ANOVA Tests

Table 1: Average Amount of Nickel Leached Before Removing the Outlier

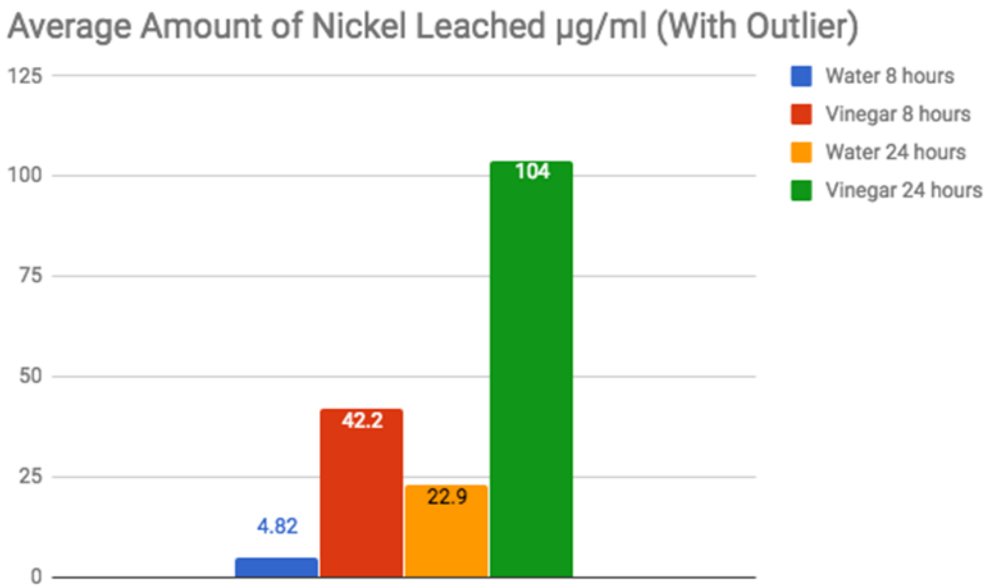

Table 2: Single Factor ANOVA

ANOVA			
Source of Variation	*SS*	*df*	*MS*
Between Groups	82955.98699	3	27651.99566
Within Groups	617991.1384	56	11035.55604

ANOVA			
Source of Variation	*F*	*P-value*	*F crit*
Between Groups	2.505718385	0.06830547253	2.7694309
Within Groups			

Table 3: Average Amount of Nickel Leached (mg) After Removing the Outlier

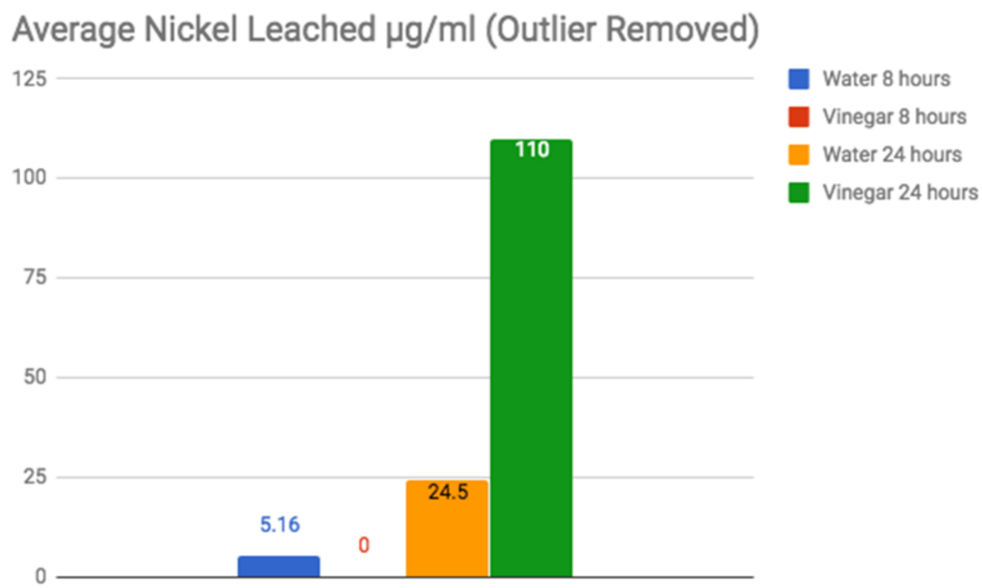

Table 4: Single-factor ANOVA Test

ANOVA			
Source of Variation	*SS*	*df*	*MS*
Between Groups	110291.3192	3	36763.77307
Within Groups	236378.7072	52	4545.74437

ANOVA			
Source of Variation	*F*	*P-value*	*F crit*
Between Groups	8.087514405	0.0001620919031	2.782600438
Within Groups			