

Lymphoma-Causing Aspartame in Dole Whip Soft Serve Sold at Disneyland

Jashryl Tutor¹, Leeroy Jenkins¹, Rick Astley¹, and Tagert Ellis²

¹Orange County Department of Health

²The Best Writing 39B Professor

June 4, 2018

Abstract

Dole Whip® samples from both the Tiki Bar Lounge in Disneyland and the original manufacturer, Dole®, were tested in a 90-day long toxicological study to test the effects each sample would have on *Mickeylius myceus*, the model organism for this experiment. Those within the Disneyland group that were treated with Dole Whip were diagnosed with lymphoma, which was caused by aspartame, an artificial sweetener.

Introduction

It was brought to the attention of the Orange County (OC) Department of Health that the Dole Whip® being sold to park visitors at Disneyland may have been altered in a way that was potentially life threatening to consumers via an anonymous tip. Knowing the popularity of the park itself and the amount of people that visit everyday, the OC Department of Health decided to investigate into this matter.

Dole Whip® is a soft serve dessert that is sold at Disneyland at the Tiki Juice Bar in Adventureland ([bar](#)). Although there is a menu for the Tiki Juice Bar showing that Dole Whip soft serve is being sold there ([res](#)), there is no official ingredient list or table of nutritional facts for this specific food item. However, the company that is the official producer of this dessert does have this information available ([inf](#)). Despite this, it cannot be guaranteed that Disneyland uses this exact same recipe in the soft serve they give to guests at the park. Correspondence with Disneyland Director of Dining was attempted. However, when asked about the ingredient list for the Pineapple Dole Whip(r), they declined to comment and replies to e-mails and phone calls stopped. As a result, this caused suspicion on what Disneyland put in their Dole Whip(r). Consequently, this study is meant to explore the possible differences in content between the Dole Whip produced by the original company and the Dole Whip being sold to park goers at Disneyland.

Aspartame, an artificial sweetener, was first approved for use in food in 1981 by the Food and Drug Administration (FDA) and has become one of the most widely used sweeteners worldwide ([Soffritti et al., 2007](#)) with the allowed daily intake for aspartame in the US being 50 mg/kg ([Soffritti et al., 2005](#)). When ingested, aspartame is broken down and metabolized into aspartic acid, phenylalanine, and methanol ([Soffritti et al., 2007](#)). In addition, several studies were done on the carcinogenic effects of aspartame in which the findings showed that aspartame caused an increased incidence of lymphoma within their test subjects. For example, a study in which 20% of the mice were treated with amounts of aspartame well below the daily limit were found to have developed some form of lymphocytic tumor ([Soffritti et al., 2005](#)).

What exactly causes these tumors to develop and grow larger in size are antigens and growth factors that are specific to the specific cancer cell type. For lymphocytic cells found within the lymph nodes and spleen, a differentiated B-cell antigen known as CD45R (Ward, 2006) as well as basic fibroblast growth factors (bFGFs) (Lodish H, 2000) are responsible. These bFGFs are what promote cell growth via angiogenesis, a process that causes new blood vessels to form around the tumor cell which enables it to utilize the increased amount nutrients to become larger in size (Lodish H, 2000).

Material and Methods

2.1 - Sample Collection

One unit of the Dole Whip soft serve was obtained from the Tiki Juice Bar in Adventureland in Disneyland. In addition, another unit of the Dole Whip soft serve was obtained straight from the manufacturer.

2.2 - Centrifugation of Samples

To make analysis of what is inside the soft serve easier, small samples of each Dole Whip were be placed within a centrifuge to separate the compounds within the actual soft serve itself. An Eppendorf Centrifuge 5920R was used to perform this task. The different layers were then extracted out of the centrifugal tube and into separate vials for spectroscopy analysis.

2.3 - Spectroscopy of Each Sample

Before any toxicological tests were conducted with the samples, infrared (IR) and nuclear magnetic resonance (NMR) spectra were taken for each of the layers previously extracted. IR spectroscopy was performed using a Nicolet iS5 FTIR Spectrometer and NMR spectra were taken using an NMReady-60PRO compact spectrometer.

2.4 - Toxicological Testing

A 90-day sub-chronic oral toxicity study was conducted using 18 mice (*Mickeylius myceus*). One control group was established in which 2 mice (one male, one female) did not receive any dosage of Dole Whip and were instead given deionized water and mouse pet food twice a day. A group of 6 mice (3 male, 3 female) were set up to receive high doses of Dole Whip in which their diet consisted only of deionized water and Dole Whip for the duration of the study. The third and final experimental group had 10 mice (5 male, 5 female) that were fed deionized water and mouse pet food twice a day but every other day, one of their meals would be replaced with Dole Whip instead. Another sub-chronic oral toxicity study was conducted under the same conditions but instead of the Dole Whip from Disneyland being administered, the Dole Whip obtained straight from the manufacturer was given to the subjects. So in total, 36 mice were involved in this experiment.

During the course of this study, the physical condition of the mice were regularly observed and recorded. At the end of each week, blood samples were taken and physical examinations that involved weighing the mice and assessing any changes in physical appearance were also performed. Any mice that died prior to

the completion of the study underwent histopathological analysis. The mice that were alive by the end of the study were humanely sacrificed under anesthesia and were also subject to histopathological analysis.

2.5 - Immunohistochemical (IHC) Analysis of Affected Tissues

This analysis involved the use of anti-CD45R antibodies [RA3-6B2] (FITC) from AbCam in order to visualize the antigen in question, CD45R. The steps for this analysis were done as outlined in the manufacturer's IHC-Paraffin Protocol (IHC-P)(6hg) in which the tissues of the retrieved organs were prepped for staining via tissue fixation (in 10% neutral buffered formalin), cut with a microtome to be placed into slides, dried, and then deparaffinated using various washes of Xylene and varying concentrations of Ethanol (100%, 95%, 70%, and 50%). The antigen retrieval was performed using a pressure cooker with Tris/EDTA pH 9.0 as the buffer solution. For the staining process, the tissue from the livers obtained from the necropsy of the mice were used as a negative control since the antigen in question is not known to be synthesized within the liver.

Results & Discussion

3.1 Spectroscopic Analysis of the Compounds Within Disneyland Dole Whip(r)

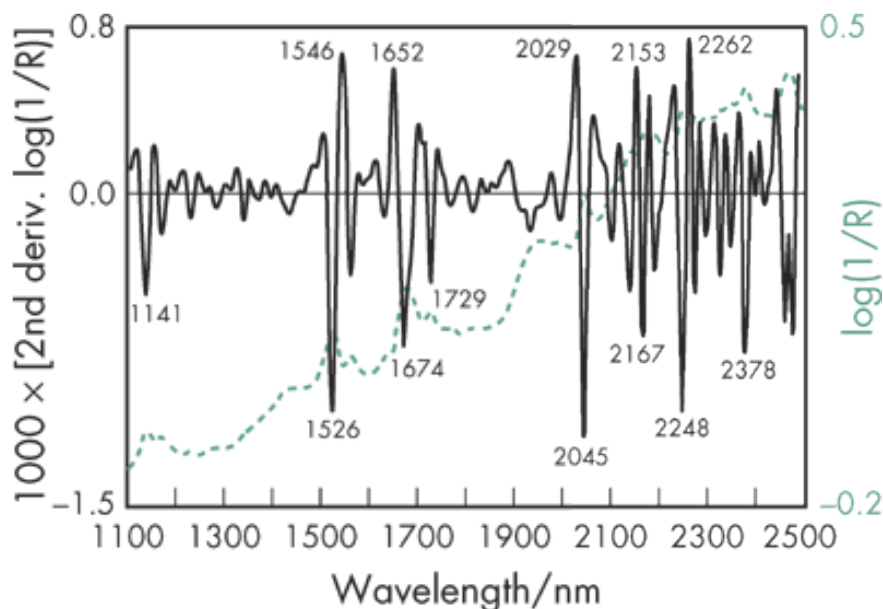


Figure 1: Infrared spectrum of Aspartame Obtained (asp, a)

Figure 2: H NMR Spectrum of Aspartame (asp, b)

After spectroscopic analysis of one of the layers from the Disneyland Dole Whip, aspartame was found to be one of the ingredients. The way this was identified was through peaks that were characteristic of the functional groups within aspartame. For example, in the H NMR spectrum (Figure 2), at around 9ppm, there is a peak associated with . Aspartame has multiple carbonyl functional groups, namely In addition, the

large peak at around 7ppm is characteristic to that of an aromatic ring, which is also a part of aspartame's chemical structure.

3.2 - Blood Test Results

Knowing that aspartame was present within the Disneyland Dole Whip®, data regarding the compounds it becomes once metabolized were paid close attention to (methanol, phenylalanine, and aspartic acid). Data regarding glucose levels were used to gauge whether or not the mice were losing weight as a result of having more or less aspartame in their diet. The results from the weekly blood tests were averaged out

		No Dole Whip (Control)	Dole Whip + Regular Diet	Dole Whip Only
Glucose (mg/dL)	Male	73.4	63.9	49.2
	Fe- male	70.8	61.2	48.8
Methanol (µg/dL)	Male	50.8	71.3	124.7
	Fe- male	51.2	71.9	125.3
Phenylalanine (µg/dL)	Male	33.8	58.5	99.3
	Fe- male	31.9	54.6	98.7
Aspartic Acid (µg/dL)	Male	45.8	66.6	84.9
	Fe- male	47.1	67.8	85.6

Table 1: Averaged Blood Test Results for Disneyland Dole Whip Group

to provide an overall summary of the difference between each group's blood content levels. As expected, the control group that did not receive any Disneyland Dole Whip® exhibited normal levels of the above each compound. For the mice that had both Dole Whip and a regular diet, they exhibited increased levels of every compound except glucose. The same effect can be seen in the Dole Whip(r) Only group but on larger scale. This can be explained by the lack of a nutrient-rich food source due to a lack of regular feed which lead to an increase in glucose uptake within the mice's cells to make up for a loss of proper nutrients and maintain homeostasis. Because of this drop in glucose for both groups that consumed Dole Whip(r), a loss in weight is to be expected. However, findings which will be discussed in the next section go against what was just predicted. These results were also averaged out for the same reason they were averaged out for the Disneyland group. For the most part, all three of the groups are exhibiting similar blood content levels except for glucose levels. The levels for methanol, phenylalanine, and aspartic acid are all within the normal range. As for glucose levels, a possible explanation for this is, again, the difference in nutrient sources for each group. Since the control group is being a diet typical for mice, it is no wonder that the glucose concentration within the bloodstream would be normal. As for the Dole Whip and Regular Diet group, a spike in glucose levels is most likely due to having access to both a steady source of nutrients as well as a constant source of sugars via the Dole Whip. The low glucose levels in the group receiving only Dole Whip as their food source can be explained using the same reason that was used to explain the low glucose levels for the Disneyland rats in the Dole Whip only group. Since the nutritional value for Dole Whip is quite low

		No Dole Whip (Control)	Dole Whip + Regular Diet	Dole Whip Only
Glucose (mg/dL)	Male	72.4	102.3	54.2
	Fe- male	71.8	101.1	52.4
Methanol ($\mu\text{g}/\text{dL}$)	Male	51.8	52.1	54.7
	Fe- male	50.2	49.8	55.3
Phenylalanine ($\mu\text{g}/\text{dL}$)	Male	31.8	32.8	33.3
	Fe- male	33.9	33.2	32.7
Aspartic Acid ($\mu\text{g}/\text{dL}$)	Male	47.8	44.9	45.3
	Fe- male	45.1	46.5	47.2

Table 2: Averaged Blood Test Results for Original Dole Whip Group

and doesn't contribute to maintaining the overall health of whoever consumes it, the cells within the mice's bodies have to compensate by using up more glucose to maintain homeostasis.

3.3 - Body Mass Data for Both Original and Disneyland Dole Whip® Groups

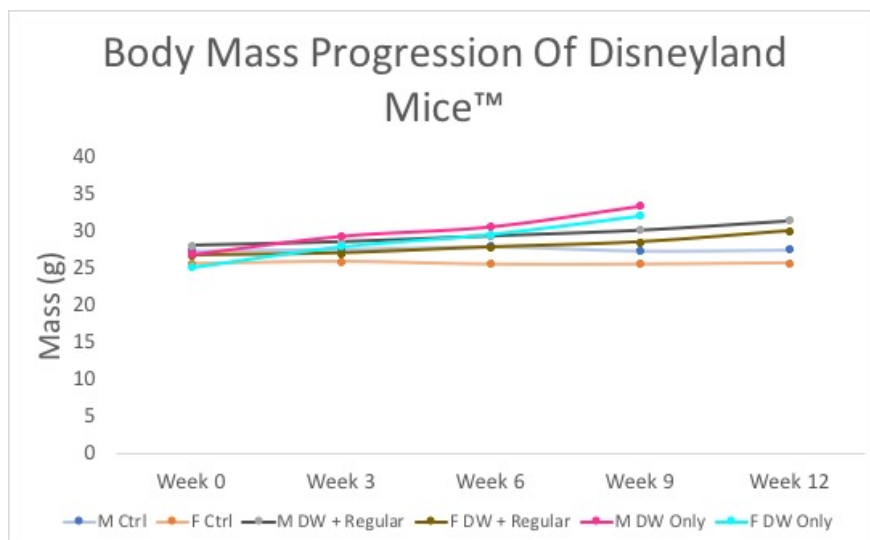


Figure 3: Body Mass of Disneyland Dole Whip Group

The control mice in this group maintained a normal weight throughout the study as expected. However,

both the DW+Regular and DW Only groups experienced an unexpected increase in mass over time, going against the expected loss in weight based on the blood test results (Table 1). These two groups should have undergone a decrease in weight because of the drop in glucose as mentioned in the previous section. Additionally, this graph shows data only until Week 9 for the DW Only group because the mice in this group did not survive to the end of the study. The results from histopathological results of this group as well as the other two groups will be discussed in section 3.4.

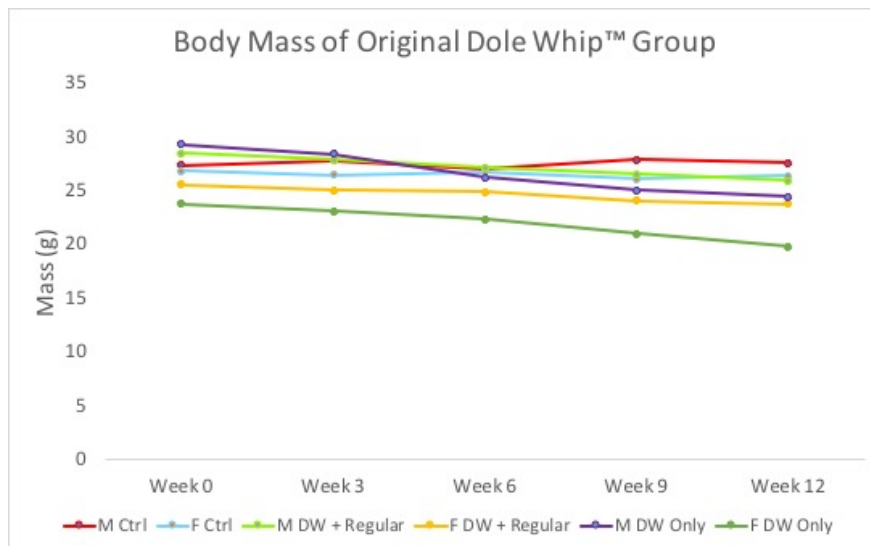


Figure 4: Body Mass of Original Dole Whip Group

Overall, the subjects in this group remained generally healthy throughout the entirety of the study. Only minor shifts in weight were seen within the DW+ Regular and DW Only groups. All mice managed to survive to the end of study, suggesting that no harmful chemicals were in this sample of Dole Whip®. In addition, the spectroscopic data taken for this sample backs up this statement in which the peaks shown in the IR and H NMR spectra matched up with the ingredients listed in the manufacturer's website ([inf](#)).

3.4 - Physical Condition of Test Subjects After Toxicological Study

Upon the end of the toxicological study, the condition of the different testing groups were photographed. Figure 5 shows the condition of the mice that were treated with the Original Dole Whip® directly from the manufacturer in which the test subjects show no visible signs of negative effects from consuming the manufacturer's soft serve. In addition, Figure 4 reflects that the mice in this test group remained healthy throughout the study. A histopathological analysis was performed on these mice as part of standard operating procedure in examination of the mice's anatomy found all internal organs unaffected by the study. Slight muscle atrophy was observed in the Dole Whip® Only trials. However, this was to be expected considering the recorded decrease in weight as the study progressed considering the low nutritional value that the soft serve had. Since there were no negative impacts as a result of consuming the Dole Whip® sample that came directly from the manufacturer, this sample of soft serve is safe for consumers to eat.

Aside from the control group, the physical condition of the Disneyland Dole Whip® mice were drastically different than that of the Original Dole Whip® mice. It should also be reiterated that all the mice in the Dole Whip® Only trial died during Week 9 of the Toxicological Study. Figure 6 shows that there were



Figure 5: Physical Condition of Original Dole Whip® Mice at the End of Toxicological Study



Figure 6: Physical Condition of Disneyland Dole Whip® Mice at the End of Toxicological Study

noticeable lumps in the necks of the two groups tested with Dole Whip® from Disneyland. It was because of these growths that these two trials experienced an unexpected gain in weight instead of losing weight as the study progressed. Shown below, Figure 7 compares the sizes of these two trials in relation to the control mice for this group:

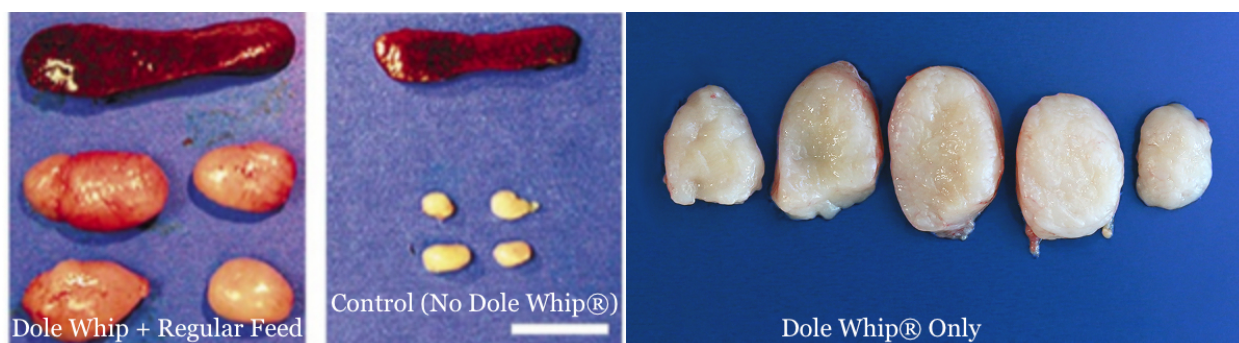


Figure 7: Lymph Nodes and Spleens of Disneyland Dole Whip® Mice

The histopathological study revealed that the cause of death for the Dole Whip® Only was the rupturing of the spleen; hence no spleens were shown in the above figure. In addition, the mice in the DW Only group suffered from acidification of the blood due to high levels of formic acid within the bloodstream. The

prevalence of formic acid is attributed to the high levels of methanol seen in the blood tests results of this group (Table 1) in which an enzyme known as alcohol dehydrogenase turns methanol (which is typically non-toxic) into formic acid (a toxic compound). The elevated levels of formic acid disrupt the equilibrium of the pH buffers within the bloodstream that keep the pH at a level that allows for cell processes to function properly. With the mice's bodies unable to undergo the necessary processes to survive, they were unable to survive.

As for the organs themselves, typical sizes for a mouse's spleen and lymph nodes were reflected within the Control group, with the other two group's sizes being compared to it. In the DW+Regular group, both the spleen and lymph nodes are visibly swollen yet still maintain its general conformation in relation to the control group's spleen and lymph nodes. In the DW Only group, the lymph nodes are more than four times the size of the control group's lymph nodes and have a different appearance, no longer resembling a typical lymph node. Although it is possible that swelling may have occurred at some point with these lymph nodes as well, the lymph nodes from the DW Only group could not have reached this size without experiencing some type of induced cell growth. Based on prior research on the effects of aspartame consumption, this compound has been known to cause lymphoma [**one of the aspartame studies**].

3.5 - IHC Analysis of Affected Organs

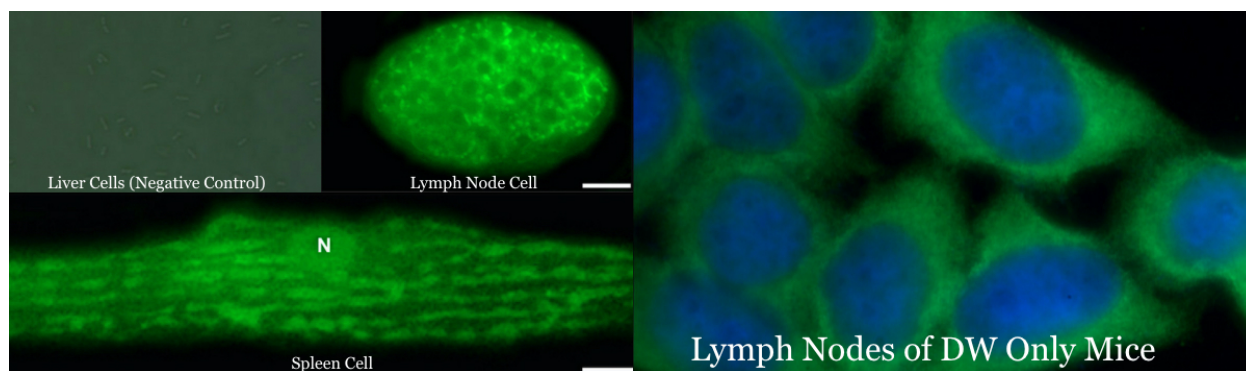


Figure 8: Immunohistochemical Analysis on Dole Whip® + Regular Feed & Dole Whip® Only Mice

The two fluorescently labeled cells on the left are from the Dole Whip® + Regular Feed mice and the labelled cells on the right are from the Dole Whip® Only mice. The green fluorescence is caused by the antibody (tagged with green fluorescent protein, GFP) used to bind to the CD45R antigen. Since liver cells rarely produce the CD45R antigen [**leukemia and lymphoma in mice**], the liver cells from each of the group's mice were used to establish a negative control in order to ensure that the antibody was properly binding to the correct substrate.

The IHC analysis revealed that all of the organs retrieved from both DW+Regular and DW Only mice contained cells that produced the CD45R antigen responsible for the development of cancerous lymphomas. The main difference between the organs from these two groups is that when exposed to a second antibody stain that was specific to basic fibroblast growth factors (bFGF), only the lymph nodes obtained from the DW Only mice were visualized (shown in blue in Figure 8). These bFGFs are what allowed the lymph nodes from the DW Only mice to increase in size and have no resemblance to a regular lymph node (with new tissue constantly forming, the lymph nodes become deformed).

Conclusion

The findings from this study on Dole Whip® soft serve sold at Disneyland point to the contents of this dessert as harmful to those who consume it. The soft serve has very little nutritional content and most notably contains enough aspartame to cause lymphoma and ultimately death in mice. This effect is specific to Dole Whip® sold at Disneyland because when Dole Whip® produced directly from the original manufacturer was tested, no aspartame was detected and the ingredients list matched up to what was found when a spectroscopic analysis was performed. Extrapolating this data to humans, the Dole Whip® soft serve is carcinogenic, specifically targeting organs that, when affected, lead to lymphoma. This calls for immediate action by health professionals at the OC Department of Health as well as Disneyland. At the discovery of these results, investigative authorities within the OC Department of Health as well as the FDA were notified. Disneyland has also been notified of the contents of their dessert and have been asked to take immediate action in eliminating exposure to the aspartame in their Dole Whip®.

References

IHC-Paraffin Protocol (IHC-P).

IR Spectrum of Aspartame. https://www.medicinescomplete.com/mc/excipients/2012/ExcaspartameF002_default.png,a.URL. Accessed on Sun, May 27, 2018.

H NMR Spectra of Aspartame. https://www.chemicalbook.com/Spectrum/22839-47-0_1HNMR.gif,b.URL. Accessed on Mon, May 28, 2018.

Tiki Juice Bar. <https://disneyland.disney.go.com/dining/disneyland/tiki-juice-bar/>. URL <https://disneyland.disney.go.com/dining/disneyland/tiki-juice-bar/>. Accessed on Mon, May 28, 2018.

DOLE® Soft Serve :: Product Information. <http://www.dolesoftserve.com/prod-info.cfm>. URL <http://www.dolesoftserve.com/prod-info.cfm>. Accessed on Mon, May 28, 2018.

Tiki Juice Bar Menu — Disneyland Resort. <https://disneyland.disney.go.com/dining/disneyland/tiki-juice-bar/menus/>. URL <https://disneyland.disney.go.com/dining/disneyland/tiki-juice-bar/menus/>. Accessed on Mon, May 28, 2018.

Zipursky SL et al. Lodish H, Berk A. *Molecular Cell Biology*. 4th Edition. New York: W.H. Freeman, 2000.

Morando Soffritti, Fiorella Belpoggi, Davide Degli Esposti, Luca Lambertini, Eva Tibaldi, and Anna Rigano. First Experimental Demonstration of the Multipotential Carcinogenic Effects of Aspartame Administered in the Feed to Sprague-Dawley Rats. *Environmental Health Perspectives*, 114(3):379–385, nov 2005. 10.1289/ehp.8711. URL <https://doi.org/10.1289%2Fehp.8711>.

Morando Soffritti, Fiorella Belpoggi, Eva Tibaldi, Davide Degli Esposti, and Michelina Lauriola. Life-Span Exposure to Low Doses of Aspartame Beginning during Prenatal Life Increases Cancer Effects in Rats. *Environmental Health Perspectives*, 115(9):1293–1297, jun 2007. 10.1289/ehp.10271. URL <https://doi.org/10.1289%2Fehp.10271>.

Jerrold M. Ward. Lymphomas and leukemias in mice. *Experimental and Toxicologic Pathology*, 57(5-6): 377–381, jul 2006. 10.1016/j.etp.2006.01.007. URL <https://doi.org/10.1016%2Fj.etp.2006.01.007>.