THE TRUTH BEHIND ASPARTAME: AN EXAMINATION OF THE ADVERSE HEALTH EFFECTS ASSOCIATED WITH ITS CONSUMPTION

by

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B.S., University of California, Los Angeles, 2011

Submitted in partial fulfillment of the requirements for the degree of

Master of Science

2015
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Aspartame (APM) is one of the most widely used artificial sweeteners in the world today. It is present in over 6,000 different products and is consumed by millions of people on a daily basis. Since its approval by the United States Food and Drug Administration (FDA) in 1981, APM has made its way into the normal diets of many people. The negligible caloric content of APM has led to increases in its use by not only diabetics but also a large number of health conscious people. Since the introduction of APM into the food industry, there has been great debate and controversy over the safety of its use. Numerous animal and human studies have shown that APM use is fully harmless and not linked to any negative health effects in humans. On the other hand, other studies have found APM consumption to be associated with a variety of alarming health consequences. These include various types of cancers, genotoxicity, diabetes, metabolic syndrome, oxidative stress, neurochemical imbalances, and shifts in gut microbial balance. Many APM users believe that through consuming products that contain APM, they are reducing their risk of some of these conditions while in reality it is possible that quite the opposite is happening. APM use may not be as safe as the public generally assumes. The circumstances that surrounded the FDA’s approval of APM and the body of evidence that exists pertaining to APM toxicity raise many questions. An extensive look into the literature has revealed that there are enough reasons to doubt the
complete safety of APM. It is important for the public to be made aware of these findings in order to make well-informed decisions and more importantly, to best protect their health.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE</td>
<td>i</td>
</tr>
<tr>
<td>COPYRIGHT PAGE</td>
<td>ii</td>
</tr>
<tr>
<td>READER APPROVAL PAGE</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iv</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>x</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Aspartame’s Path to FDA Approval</td>
<td>4</td>
</tr>
<tr>
<td>Goals</td>
<td>6</td>
</tr>
<tr>
<td>PUBLISHED STUDIES</td>
<td>8</td>
</tr>
<tr>
<td>The Carcinogenic Risk of Aspartame</td>
<td>8</td>
</tr>
<tr>
<td>Aspartame’s Association with Metabolic Disturbances and Diabetes</td>
<td>20</td>
</tr>
<tr>
<td>The Role of Aspartame in Oxidative Stress</td>
<td>25</td>
</tr>
<tr>
<td>Changes in Neurological Behavior, Function, and Chemistry due to Aspartame</td>
<td>30</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>38</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>50</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The occurrence of precancerous and cancerous lesions in male Sprague-Dawley rats treated with APM throughout entire life</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>The occurrence of precancerous and cancerous lesions in female Sprague-Dawley rats treated with APM throughout entire life</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Comparing the occurrence of lymphomas and leukemias in female Sprague-Dawley rats when fed APM postnatally and prenatally</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>The association between diet soda intake and risk of developing metabolic syndrome or type 2 diabetes in people from the MESA population</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>Serum concentrations of glucose, cholesterol, and triglycerides in the control and APM-treated rats</td>
<td>29</td>
</tr>
<tr>
<td>6</td>
<td>Serotonin, Dopamine, and Noradrenaline levels in the brains of mice treated with APM, Lipopolysaccharide, and APM with Lipopolysaccharide</td>
<td>35</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The metabolism of aspartame</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Prevalence of death in APM-treated female Sprague-Dawley rats who had leukemias and lymphomas</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>Changes in the expression of <em>c-myc</em>, <em>Ha-ras</em>, and <em>p53</em> genes in various organs of mice treated with three different doses of APM</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>Average Blood Glucose in Sprague-Dawley Rats after Insulin Tolerance Test</td>
<td>23</td>
</tr>
<tr>
<td>5</td>
<td>Average time required for APM-treated mice to find a submerged platform in the first trial of the Morris water maze</td>
<td>33</td>
</tr>
<tr>
<td>6</td>
<td>The effects of lowered serotonin levels on the blood-brain barrier</td>
<td>36</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

AARP ................................................................. American Association of Retired Persons
ADI ................................................................. Acceptable daily intake
ADH ................................................................. Alcohol dehydrogenase
APM ................................................................. Aspartame
BMI ................................................................. Body mass index
BBB ................................................................. Blood-brain barrier
cAMP ............................................................. Cyclic adenosine monophosphate
EFSA ............................................................. European Food Safety Authority
FDA ................................................................. Food and Drug Administration
GABA ............................................................. gamma-Aminobutyric acid
HPFS .............................................................. Health Professionals Follow-up Study
HR ................................................................. Hazard ratio
MESA ............................................................. Multi-Ethnic Study of Atherosclerosis
MTX ............................................................... Methotrexate
NAAT ............................................................. Neutral amino acid transporter
NHL ............................................................... Non-Hodgkin lymphoma
NHS ............................................................... Nurses’ Health Study
NIH ............................................................... National Institutes of Health
RBC ............................................................... Red blood cell
ROS ............................................................... Reactive oxygen species
RR ................................................................. Relative risk
SCFA..................................................................................Short-chain fatty acid
SOD.......................................................................................Superoxide dismutase
INTRODUCTION

Obesity rates in the United States have been increasing significantly every year over the past several decades and this trend is not expected to slow down anytime soon. Currently over one-third of Americans are obese and over two-thirds are either overweight or obese. By the year 2030, the prevalence of obesity in the United States is projected to reach approximately 50% (Levi et al., 2012). In efforts to lose weight and reduce their risk of conditions such as diabetes, cancer, heart disease, and hypertension, millions of Americans and people around the world have incorporated low-calorie foods and beverages into their normal lifestyle. This has resulted in a huge expansion of this sector of the food industry and the production of a large number of low-calorie products. With sugar being a main source of calories, artificial sweeteners have made their way into these products and gained a lot of popularity in the public due to their negligible caloric content. The first artificial sweetener approved for human consumption was saccharin. It was discovered in 1879 and is still used in a variety of products (Saccharin, 2015). Since then many other artificial sweeteners have found their way into the market, such as neotame, sucralose, and acesulfame potassium. One of the most widely used artificial sweeteners today is aspartame (APM), which has been produced under the brand names of NutraSweet and Equal.

Aspartame (methyl L-α-aspartyl-L-phenylalanine) is an artificial sweetener that is currently being used in over 6,000 products (Aspartame Information Center, 2015). Some of these products include diet beverages, yogurts, cereals, candies, desserts, and tabletop
sweeteners. It is even found in a variety of pharmaceutical products such as Zofran, children’s Benadryl, and sugar-free cough drops. APM has a caloric value of 4 kilocalories/gram; however, since it is 200 times the sweetness of sucrose (table sugar), small amounts are used in products and it is considered a calorie-free ingredient.

APM is a methyl ester of two amino acids, L-phenylalanine and L-aspartic acid. It was originally discovered by James Schlatter in 1965. After APM is ingested, it is broken down in the intestinal tract into 50% phenylalanine, 40% aspartic acid, and 10% methanol (Ashok & Sheeladevi, 2014). After absorption into the circulation, phenylalanine can be further metabolized to tyrosine. Because phenylalanine is a metabolite of APM, individuals who suffer from the genetic condition of phenylketonuria should avoid the consumption of APM. Aspartic acid is converted into oxaloacetate and alanine. Methanol is further metabolized into formaldehyde by the enzyme alcohol dehydrogenase (ADH) and then ultimately into formic acid. The International Agency for Research on Cancer has identified formaldehyde as a known human carcinogen (“Formaldehyde and Cancer Risk,” 2015). The metabolic fate of APM can be seen below in Figure 1.
Today, APM consumption is deemed to be safe by food regulatory agencies in over 100 countries. The acceptable daily intake (ADI) recommended by the United States Food and Drug Administration (FDA) is 50 mg/kg body weight/day (“Aspartame,” 2015). The European Food Safety Authority (EFSA) has set its ADI at 40 mg/kg body weight/day. One can of diet soda normally has around 200 mg of APM. Small packets of the sweetener typically have around 35 mg and one two-liter bottle of diet soda contains around 1100 mg of APM. Therefore, the upper daily limit for an adult weighing 170 pounds would be three and a half two-liter bottles or 19 cans of diet soda, according to the current FDA recommendation. As will be discussed later, several studies have shown APM to be associated with negative health effects when consumed at doses much lower than what both the FDA and EFSA currently recommend.

Figure 1: The metabolism of aspartame. ASP=aspartame. PHE=phenylalanine. Figure taken from Stegink, 1987.
Aspartame’s Path to FDA Approval

APM was first approved by the FDA in 1974 for dry foods only, but it was not until 1981 that the FDA allowed APM to be distributed to the public (“Food and Drug Administration,” 1987). Obtaining the approval of APM from the FDA was no easy task for G.D. Searle & Company, the producer of APM at the time. From 1973, when Searle first petitioned for FDA approval of APM, until 1981, when the FDA gave the green light for APM to be consumed by the public, many questionable events took place. In 1973, Searle provided the FDA with over 100 studies that attested to the safety of APM. It should be noted that all of these studies were fully funded by Searle and that at the time, the FDA had no guidelines in place in regards to what sorts of studies are required in order to deem a food additive as safe. Dr. Martha Freeman was a physician working in the FDA Division of Metabolic and Endocrine Drug Products and a reviewer of the APM studies submitted by Searle. She concluded that the studies submitted to the FDA by Searle were deficient in regards to exhibiting the safety of APM. In her analyses, she cited the lack of vital information on APM such as its chemistry and pharmacokinetics, along with many flaws such as how the studies followed no protocols in their design and how results were reported very vaguely (Gold, 2002). Dr. J. Richard Crout, who was the director of the FDA Bureau of Drugs at the time, also recognized these limitations. Despite these findings, APM was given approval by the FDA for use in dry foods in July 1974. Shortly following that in August 1974, legitimate complaints were filed with the FDA asking them to reevaluate their stance on APM and its safety. In July 1975, a special
task force was arranged by the FDA commissioner to further investigate the studies done on APM by Searle. Preliminary investigations led the FDA to suspend its approval of APM in December 1975, before APM distribution to the public had begun. After an in-depth analysis, the task force found that Searle had omitted a lot of information and facts from their studies that would have been detrimental to their case. Examples included not reporting the presence of tumors and seizures in animal subjects, editing pathology reports, and documenting animals as alive when in fact they had died. In January 1977, the FDA launched a criminal investigation into Searle for their falsifications and misrepresentations of information related to APM and assigned United States attorney Sam Skinner to the case. In the months following, Donald Rumsfeld, a distinguished leader in Washington, became the President of Searle, and Sam Skinner ended up moving over to Searle’s litigation team. Charges against Searle were eventually dropped. A Public Board of Inquiry was formed by the FDA, which firmly came to the conclusion that there was still not enough evidence to show that APM is safe for human consumption. In July 1981, FDA commissioner Arthur Hull Hayes, who had been appointed by Donald Rumsfeld, disregarded the recommendation of the public board and officially approved APM for use in dry foods (Gold, 2002). FDA approval of APM was further extended for use in carbonated beverages in 1983. In 1996, the FDA ultimately approved APM to be used in all foods and beverages.
Goals

Despite numerous studies that have linked APM consumption to a large number of adverse health effects, the FDA has yet to conduct a formal reevaluation of its stance on the safety of APM use. As will be discussed in detail, the intake of APM has been associated with many diseases and conditions such as cancers, metabolic syndrome, compromised insulin function, changes in gut microbial balance, increases in oxidative stress, depression, memory impairments, and increases in blood glucose. At the same time, there are a large number of studies that show APM use by humans to be completely safe. In the most recent comprehensive safety review on APM, many of these studies were explored (Magnuson et al., 2007). Examination of studies over the past 20+ years showed APM consumption to not be associated with carcinogenicity, genotoxicity, neurotoxicity, changes in blood glucose, insulin response, behavior, or cognition. Of significance, however, is that the sponsor of this APM evaluation was the largest manufacturer of APM in the world, Ajinomoto. In an examination of medical literature on APM over a period of approximately 20 years, one physician revealed that in 74 published studies that were funded by APM or APM-related industries, all 74 of them had determined APM to be safe (Walton, n.d.). In 91 published studies that were independently funded, 84 of these studies had associated APM with harmful health effects. The other seven studies that had shown APM to be safe were funded by the FDA, which could be argued to be an APM-related industry after seeing how it handled the approval of APM. The large discrepancy observed in these findings raises a lot of
skepticism about the validity of certain information that is out there in the public regarding APM safety. Once a product only aimed at helping diabetics with blood sugar maintenance, APM has found its way into the regular diets of millions of people and its consumption is predicted to only increase, which is very concerning. The goals of this literature review are to inform readers of the multitude of health consequences that can result from APM intake, to raise awareness of misleading information that exists on APM safety, and hopefully to encourage people to think twice before consuming APM.
PUBLISHED STUDIES

The Carcinogenic Risk of Aspartame

The Cesare Maltoni Cancer Research Center in Italy was the first laboratory to directly link the consumption of APM to a variety of cancers. Using six different dosages of APM, two of which were below the recommended ADI by both the FDA and EFSA, researchers incorporated APM into the normal daily diet of 8-week-old Sprague-Dawley rats. The rats were fed this diet until they naturally died. There were either 100 or 150 rats of each sex in each group, including the controls. After their deaths, the rats had complete autopsies performed on them as tissues from numerous organs were examined (Soffritti et al., 2006).

In both the male and female groups, increasing APM consumption showed an upward trend in the occurrence of all malignant tumors. The only group that exhibited a statistically significant increase in all tumor types was the females who were fed 2,500 mg/kg body weight/day of APM (p<0.01), a dose much higher than what the FDA currently recommends (40 mg/kg body weight/day). An upward trend in both males and females was also observed in the occurrence of lymphomas and leukemias. In regards to these two specific cancers, statistically significant increases in their prevalence were seen again only in females. However in this analysis, five out of the six female groups treated with APM had this notable increase (p<0.05 or p<0.01, see Table 2 below). These were the females who were fed 5,000, 2,500, 500, 100, and 20 mg/kg body weight/day of APM. In the analysis of renal pelvis and ureter tissues, researchers found dose-dependent
increases in dysplastic hyperplasias and papillomas in females. Increases were seen in the males as well; however, they were not in a dose-dependent manner. Carcinomas of these tissues were also observed in both the male and female groups. Only the males who were fed 5,000, 2,500, 500 and 100 mg/kg body weight/day of APM exhibited carcinomas, whereas carcinomas were present with an upward trend in all the female APM treated groups. Among the females, all groups but the 4 mg/kg body weight/day APM treated one had statistically significant increases when the total number of carcinomas and dysplastic hyperplasias/papillomas were looked at (p<0.05 or p<0.01, see Table 2 below). In both the male and female control groups, no carcinomas were found. A total of 21 carcinomas were found in all the APM-treated groups. Furthermore, the researchers also analyzed nervous tissue to look for schwannomas. Malignant schwannomas were observed with an upward trend in males. Although the females did not exhibit a similar upward trend, a total of 9 malignant schwannomas were seen in all the APM-treated females; no schwannomas were seen in the female controls. A summary of all the findings discussed above can be seen in Tables 1 and 2 below.
Table 1: The occurrence of precancerous and cancerous lesions in male Sprague-Dawley rats treated with APM throughout entire life. Table taken from Soffritti et al., 2006.

<table>
<thead>
<tr>
<th>Dose, ppm (mg/kg bw)</th>
<th>Animals at start</th>
<th>Malignant tumors&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total animals bearing lymphomas/leukemias&lt;sup&gt;ab&lt;/sup&gt;</th>
<th>Animals bearing dysplastic lesions and carcinomas of the renal pelvis and ureter&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Animals bearing peripheral nerve malignant schwannomas&lt;sup&gt;d&lt;/sup&gt;</th>
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<td>No.</td>
<td>%</td>
<td>No.</td>
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</tr>
<tr>
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<td>35.3&lt;sup&gt;*&lt;/sup&gt;</td>
<td>59</td>
<td>39.3</td>
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<sup>a</sup>The tumor rates are based on the number of animals examined (incorporated).<br><sup>b</sup>Values associated with the trend test are near the control incidence.<br><sup>c</sup>Tissues from 148 animals were analyzed. *Statistically significant (p < 0.05) using Cochran-Armitage test. #Statistically significant (p < 0.05) using poly-k test (k = 3).

Table 2: The occurrence of precancerous and cancerous lesions in female Sprague-Dawley rats treated with APM throughout entire life. Table taken from Soffritti et al., 2006.

<table>
<thead>
<tr>
<th>Dose, ppm (mg/kg bw)</th>
<th>Animals at start</th>
<th>Malignant tumors&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total animals bearing lymphomas/leukemias&lt;sup&gt;ab&lt;/sup&gt;</th>
<th>Animals bearing dysplastic lesions and carcinomas of the renal pelvis and ureter&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Animals bearing peripheral nerve malignant schwannomas&lt;sup&gt;d&lt;/sup&gt;</th>
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<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
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<td>36.7&lt;sup&gt;**&lt;/sup&gt;</td>
<td>69</td>
<td>46.0</td>
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</table>

<sup>a</sup>The tumor rates are based on the number of animals examined (incorporated). <sup>b</sup>Values corresponding to pairwise comparisons between the controls and the dosed group are near the dosed group incidence. <sup>c</sup>Values associated with the trend test are near the control incidence. <sup>d</sup>Values in parentheses indicate the number of tumors (one animal can bear bilateral tumors). *Tissues from 89 animals were analyzed. *Statistically significant (p < 0.05) using Cochran-Armitage test. **Statistically significant (p < 0.01) using Cochran-Armitage test. #Statistically significant (p < 0.05) using poly-k test (k = 3). ##Statistically significant (p < 0.01) using poly-k test (k = 3).
The same laboratory carried out a very similar study in which they evaluated the carcinogenic effects of APM when it was given to Sprague-Dawley rats starting in fetal life (Soffritti et al., 2007). APM doses of 100 mg/kg body weight/day and 20 mg/kg body weight/day were added to the normal diets of female Sprague-Dawley rats and given to them starting on day 12 of pregnancy. After their births, the newborn rats continued on the APM diets until they naturally died. There were 70 rats of each sex in the control groups and 95 rats of each sex in the APM treated groups. Exactly like in the previous study, all rats had complete autopsies performed on them after their deaths and tissues from many different organs were analyzed (Soffritti et al., 2007).

In the males, an increase in the occurrence of malignant tumors was observed with increasing APM consumption, with a statistically significant one seen in the group fed 100 mg/kg body weight/day of APM (p<0.01). In the females, although not statistically significant, an increase in the occurrence of malignant tumors was only seen in the 100 mg/kg body weight/day group. Increases in the incidences of lymphomas and leukemias were observed in both the males and females with increasing APM dose. The increases in both sexes were only statistically significant at 100 mg/kg body weight/day (p<0.05 in males, p<0.01 in females). Analysis of mammary gland tissue of both the males and females revealed the presence of carcinomas. The female rats exhibited an increase in mammary carcinomas with increasing APM dose, with a statistically significant increase in the 100 mg/kg body weight/day group (p<0.05). Mammary carcinomas were seen in two of the male rats treated with 100 mg/kg body weight/day APM. As expected, no mammary carcinomas were found in the male controls. The
results of this study further reinforced previous findings on the carcinogenic effects of APM and additionally showed that exposure to APM during the fetal period can potentiate its harmful effects. Compared to the occurrence of lymphomas and leukemias in female rats given APM starting at 8 weeks old (Soffritti et al., 2006), the female rats given the same dose of APM beginning in fetal life had a greater occurrence of these cancers. This can be seen below in Table 3.

**Table 3:** Comparing the occurrence of lymphomas and leukemias in female Sprague-Dawley rats when fed APM postnatally and prenatally. Table taken from Soffritti et al., 2007.

<table>
<thead>
<tr>
<th>APM dose, ppm (mg/kg bw)</th>
<th>Percent of animals bearing lymphomas/leukemias</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Postnatal exposure^a (No. of animals at start)</td>
</tr>
<tr>
<td>2,000 (100)</td>
<td>18.7 (150)</td>
</tr>
<tr>
<td>400 (20)</td>
<td>20.0 (150)</td>
</tr>
<tr>
<td>0 (0)</td>
<td>8.7 (150)</td>
</tr>
</tbody>
</table>

^aData from Soffritti et al. (2006).

Furthermore, when looking at the female rats that exhibited lymphomas and leukemias in both experiments, the researchers observed that those who were fed APM at a dose of 100 mg/kg body weight/day during fetal life had greater death prevalence than those fed the same dose postnatally. This can be seen in Figure 2 below.
Figure 2: Prevalence of death in APM-treated female Sprague-Dawley rats who had leukemias and lymphomas. Arrows show when APM feeding was started. A. Postnatal APM Consumption. B. Prenatal APM Consumption. Figure taken from Soffritti et al., 2007.

It is well known that changes in the expression of various oncogenes and tumor-suppressor genes occur during the initiation and progression of cancers. To investigate whether APM has any effect at this molecular level, researchers looked at the expression of two oncogenes and one tumor-suppressor gene in mice after they were treated with APM. The tumor-suppressor gene examined was p53 and the oncogenes examined were Ha-ras and c-myc. The expressions of these genes were looked at in seven different organs: lymph nodes, bone marrow, thymus, kidney, liver, spleen, and lung (Gombos et al., 2007).

Five-week old female mice were administered APM by mouth for one week. The APM was added to water. Three different doses of APM were used: 40 mg/kg body weight, 200 mg/kg body weight, and 2500 mg/kg body weight. There were six mice in each dosage group, including the control group. After the one week, all the mice
underwent autopsies. The target tissues were examined and the total RNA from each
tissue was extracted. Using probes specific for *Ha-ras*, *c-myc*, and *p53*, the expression of
these three genes was determined. The beta-actin gene in each organ was used as the
control since this gene is constantly expressed.

Increases in the expression of the *p53* tumor-suppressor gene and both the *Ha-ras*
and *c-myc* oncogenes were observed in the lymph nodes, bone marrow, thymus, and
kidneys of all the APM fed mice. Increased activity of *p53* has been observed in a wide
range of cancers (Bártek et al., 1991). In the liver, spleen, and lung tissues of the APM
fed mice, the expression of these three genes was only remarkably increased in the 200
mg/kg body weight APM dose group. The changes in the expression of the *Ha-ras*, *c-
ymyc*, and *p53* genes at the various APM doses can be seen in Figure 3 below. It should be
noted that in the 2500 mg/kg body weight APM dose group, the researchers observed an
osmotic diarrhea in the mice because of the large osmotic concentration of the APM
mixture they were fed. This was believed to prevent the absorption of APM into the
bloodstream and consequently result in a decreased expression of these genes that were
studied in the various organs.
Figure 3: Changes in the expression of c-myc, Ha-ras, and p53 genes in various organs of mice treated with three different doses of APM. Gene expression is shown as percentage of beta-actin gene activity. Figure taken from Gombos et al., 2007.
In an effort to investigate any link between APM consumption and cancers in humans, a study was carried out that looked at the prevalence of hematopoietic and malignant brain cancers over the course of five years in people who participated in the National Institutes of Health-American Association of Retired Persons (NIH-AARP) Diet and Health Study (Lim et al., 2006). In 1995 and 1996, comprehensive food questionnaires were sent out to AARP members between the ages of 50 and 71 in eight different states. Along with basic information such as age, sex, weight, ethnicity, and family and medical history, these questionnaires assessed the frequency at which participants drank four different types of APM-containing drinks over the course of a year. The four drinks asked about were diet sodas, APM-containing fruit juices and iced teas, and APM that was added to coffee and tea. The questionnaire also asked about the consumption frequency of 124 different foods over the course of a year. However, the APM content of these foods, if any, was not taken into account in the determination of APM intake of these individuals. The participants had to answer these questions by estimating their consumption of these products from “never” to “greater than six times a day.” In addition to inquiring about food and drink habits, the researchers gathered information regarding people’s lifestyles. The factors looked at were activity level, alcohol usage, smoking history, and caffeine intake. After the gathering of this foundational information, daily APM intake for 285,079 men and 188,905 women was estimated. None of the participants had any prior history of cancer.

After looking at the incidence of all hematopoietic cancers and malignant gliomas in these men and women for the next five years, no relationship was found between APM
consumption and the occurrence of these cancers. There were six groups of people examined, ranging from no reported APM intake to an APM intake of \( \geq 600 \) mg/day. In the analysis, age, sex, body mass index (BMI), ethnicity, and history of diabetes were adjusted for using Cox proportional hazards models. With these adjustments, for all hematopoietic cancers in men and women combined, the relative risk (RR) for the largest APM consuming group, \( \geq 600 \) mg/day, was determined to be 0.98, 95% Confidence Interval (CI) [0.76 – 1.27]. For malignant gliomas in men and women combined, the RR for the largest APM consuming group was determined to be 0.73, 95% CI [0.46 – 1.15]. Even in the lowest APM consuming group of men and women, < 100 mg/day, there was no significant relationship found between APM use and the occurrence of these cancers. For all hematopoietic cancers in this group, the RR was 0.91, 95% CI [0.81 - 1.03], and for malignant gliomas, the RR was 0.99, 95% CI [0.75 - 1.29]. In a more detailed analysis, the researchers adjusted additionally for activity level, family history of cancer, smoking history, alcohol usage, and daily caloric and caffeine intake. They found a less than 10% change in their results across both genders and all ages. Although the changes were not specifically presented in the study, it was determined that these variables contributed minimally to the overall incidence of the cancers examined. In conclusion, no association was found between APM intake and the incidence of hematopoietic cancers and malignant gliomas (Lim et al., 2006).

On the contrary, in a different study that also used surveys to assess the APM consumption of humans over a period of time, APM was linked to an increased risk of several cancers (Schernhammer et al., 2012). Participants in two different groups, the
Nurses’ Health Study (NHS) and the Health Professionals Follow-up Study (HPFS), completed questionnaires that assessed how much APM they had consumed in the past year. The weight, height, smoking history, alcohol use, medical history, activity level, multivitamin use, and consumption frequency of approximately 130 foods of the participants was also recorded. Unlike the previous questionnaire study, the frequency of regular soda consumption was also assessed. Three APM-containing beverages were asked about: diet coke with caffeine, diet coke without caffeine, and any other diet soda. Participants were given several answers to choose from ranging from “never” to “greater than or equal to six times a day.” People in the NHS were female nurses between the ages of 30 and 55. Data collection from the NHS began in 1984. People in the HPFS were male health professionals between the ages of 40 and 75. Data collection from the HPFS began in 1986. The APM and food consumption of the participating individuals was reassessed every four years to account for changes in peoples’ drinking and eating habits. Every two years, the participants updated their medical histories and reported any new diagnoses. The gathering of all this information from both groups continued until 2006, or until an individual was either first diagnosed with cancer or passed away. The data from men and women was kept separate in order to account for any potential gender differences in cancer onset.

Information gathered from a total of 47,810 men and 77,218 women was used to look at the relationship between APM consumption and various cancers. Using Cox proportional hazards models, the following variables were adjusted for: age, race, BMI, activity level, smoking habits, alcohol intake, daily caloric intake, multivitamin usage,
menopause and hormone replacement therapy (in women), and fruit, vegetable, sugar, animal protein, and saturated fat intakes. In men, APM intake was observed to result in an increased risk of developing Non-Hodgkin lymphoma (NHL) and multiple myeloma. In the group of men who drank $\geq 1$ serving of diet soda/day, the RR for NHL was 1.31, 95% CI $[1.01 – 1.72]$. For multiple myeloma, the RR was 2.02, 95% CI $[1.20 – 3.40]$. For men who consumed $\geq 2$ servings of diet soda/day, the RR for NHL was even higher at 1.69, 95% CI $[1.17 – 2.45]$. In the women, there was no indication that APM consumption resulted in an increased risk of developing NHL and multiple myeloma. However, when the relationship between APM consumption and leukemia was analyzed, it was seen that both men and women who ingest APM are at an increased risk. When the data from both sexes was combined, the RR for those who drank $\geq 1$ serving of diet soda/day was determined to be 1.42, 95% CI $[1.00 – 2.02]$. Similar findings in the occurrence of NHL, multiple myeloma, and leukemia in both men and women were observed when those who drank regular soda were compared to those who did not drink any kind of soda. In summary, greater intakes of APM were linked to increased prevalence of NHL and multiple myeloma in men and leukemia in both men and women (Schernhammer et al., 2012).
Aspartame’s Association with Metabolic Disturbances and Diabetes

Several studies have shown that APM consumption can contribute to metabolic disturbances and the onset of type 2 diabetes. To look at this relationship, information on the diet and lifestyle habits of men and women in the Multi-Ethnic Study of Atherosclerosis (MESA) population was gathered (Nettleton et al., 2009). The goal of the study was to determine if diet soda intake puts people at a higher risk for developing type 2 diabetes, metabolic syndrome, or any of the conditions of metabolic syndrome. Individuals with a fasting glucose of >126 mg/dl and those on diabetic medications were classified as type 2 diabetics. Metabolic syndrome was identified in participants as the occurrence of three or more of the following measurements: waist circumference ≥ 102 cm in men or ≥ 88 cm in women, HDL cholesterol ≤ 40 mg/dl in men or ≤ 50 mg/dl in women, blood triglycerides ≥ 150 mg/dl, fasting glucose ≥ 100 mg/dl (or diabetic medication treatment), and blood pressure ≥ 130/85 mmHg (or blood pressure medication treatment) (Grundy et al., 2005).

The MESA population consisted of men and women in six different states between the ages of 45 and 84 who were Caucasian, African American, Hispanic, or Chinese. Baseline information was gathered from 2000-2002. Data was reevaluated three times following the initial collection: in 2002, 2004, and 2005. Participants were asked to estimate their diet soda consumption ranging from “rarely/never” to “greater than 6 times a day”. A detailed breakdown of their food intake, many lifestyle routines, and complete medical histories were also gathered. Furthermore, at each of the four data collection
times, fasting glucose, fasting insulin, waist circumference, blood pressure, triglycerides, and HDL cholesterol were measured. There were 5,011 participants in this study, none of which were diabetic or prediabetic. However, it should be noted that at the start of the study some participants had certain aspects of metabolic syndrome.

In the data analysis, all adjustments were made using Cox proportional hazards models. After adjusting for demographics, caloric intake, and lifestyle factors, consumption of ≥ 1 serving of diet soda daily was shown to result in a 36% increased risk of developing metabolic syndrome and a 67% increased risk of developing type 2 diabetes. When further adjustments were made for BMI and waist circumference, a statistically significant increased risk of 38% remained for type 2 diabetes. However, no significant relationship between diet soda intake and metabolic syndrome was observed after these additional adjustments. This can be seen in Table 4 below in models 2, 3, and 4. Looking at the individual components of metabolic syndrome, diet soda intake was linked only to increases in waist circumference and blood glucose. For those drinking ≥ 1 serving/day, the hazard ratio (HR) was 1.59, 95% CI [1.23 – 2.07] for waist circumference. The HR for fasting blood glucose was 1.28, 95% CI [1.08 – 1.52]. These results were determined after making the Model 2 adjustments, which are shown in Table 4 below.
Table 4: The association between diet soda intake and risk of developing metabolic syndrome or type 2 diabetes in people from the MESA population. Table taken from Nettleton et al., 2009.

<table>
<thead>
<tr>
<th>Metabolic syndrome</th>
<th>Rare or never</th>
<th>&gt; rare/never but &lt;1 serving/week</th>
<th>≥1 serving/week to &lt;1 serving/day</th>
<th>≥1 serving/day</th>
<th>( P_{\text{model}} ) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2,288</td>
<td>367</td>
<td>722</td>
<td>501</td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>478</td>
<td>95</td>
<td>169</td>
<td>129</td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.00†</td>
<td>1.34 (1.07–1.67)</td>
<td>1.20 (1.00–1.43)</td>
<td>1.31 (1.07–1.60)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>1.00†</td>
<td>1.42 (1.14–1.78)</td>
<td>1.28 (1.06–1.53)</td>
<td>1.36 (1.11–1.66)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>1.00§</td>
<td>1.31 (1.05–1.64)</td>
<td>1.13 (0.94–1.37)</td>
<td>1.18 (0.96–1.44)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>1.00‖</td>
<td>1.30 (1.04–1.62)</td>
<td>1.15 (0.95–1.38)</td>
<td>1.17 (0.96–1.44)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type 2 diabetes</th>
<th>n = 5,011</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>2,961</td>
<td>455</td>
<td>914</td>
<td>681</td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.00†</td>
<td>1.06 (0.73–1.52)</td>
<td>1.39 (1.07–1.80)</td>
<td>1.63 (1.24–2.13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>1.00†</td>
<td>1.10 (0.76–1.59)</td>
<td>1.46 (1.12–1.89)</td>
<td>1.67 (1.27–2.20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>1.00§</td>
<td>1.00 (0.69–1.45)</td>
<td>1.23 (0.94–1.60)</td>
<td>1.40 (1.06–1.84)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>1.00‖</td>
<td>0.98 (0.68–1.42)</td>
<td>1.23 (0.96–1.62)</td>
<td>1.38 (1.04–1.82)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\( n = 5,011. \) *\( P_{\text{model}} \) with categorical variable modeled continuously. †Model 1 adjusted for study site, age, sex, race/ethnicity, and energy intake. §Model 2 adjusted for the variables in model 1 above plus education, physical activity, smoking status, pack-years, and weekly or more supplement use. ‖Adjusted for the variables in model 2 above + waist circumference (centimeters) and BMI (weight in kilograms divided by the square of height in meters).

Whenever humans are used as subjects, there can be a large number of variables that unknowingly contribute to observed results. To more directly look at the effects of APM on metabolic parameters, researchers in one particular study used animals to investigate this topic (Palmnäs et al., 2014). Chronic low-dose APM treatment in Sprague-Dawley rats was found to be associated with increased blood glucose, compromised insulin function, and a shift in gut microbial balance (Palmnäs et al., 2014). APM was administered to the rats in water at a dose of 5-7 mg/kg body weight/day, a dose much less than the ADI recommended by both the FDA and EFSA. This treatment was done for eight weeks. For the two weeks prior to the start of APM treatment, the rats were put on either a normal chow diet or a high fat diet. Each diet type had a control group with no APM consumption; therefore, there were a total of four rat groups.
examined, with 10-12 rats in each group. At the end of the ten weeks, all the rats were sacrificed.

During the eighth week of the experiment, fasting blood glucose levels of all rats was obtained. In both the chow diet and high fat diet APM-treated groups, blood glucose levels were higher compared to the controls. Average blood glucose was $5.4 \pm 0.2$ mM in the chow diet control group, whereas in the APM-treated chow diet group it was $6.9 \pm 0.4$ mM, a statistically significant increase ($p<0.05$). In the high fat diet control group, average blood glucose was $5.6 \pm 0.2$ mM. The average in the APM-treated high fat diet group was $7.4 \pm 0.6$ mM, also a statistically significant increase ($p<0.05$). During the ninth week, insulin tolerance tests were performed. The average blood glucose levels of the APM-treated rats remained notably higher than those of the control rats throughout these tests. This disparity was determined to be statistically significant ($p<0.05$) and can be seen in Figure 4 below.

**Figure 4:** Average Blood Glucose in Sprague-Dawley Rats after Insulin Tolerance Test. † $p<0.05$. CHW=chow diet with water, CHA=chow diet with APM, HFW=high fat diet with water, HFA=high fat diet with APM. Figure taken from Palmnäs et al., 2014.
At the end of the tenth week, fecal samples were collected from all the rats. DNA was extracted from these samples and microbial analyses were performed. Of importance, a statistically significant increase (p<0.05) in the proportion of Enterobacteriaceae was found in the APM-treated high fat diet group compared to the control high fat diet group. Previous studies have shown that Enterobacteriaceae generate short-chain fatty acids (SCFA) and gases that contribute to insulin resistance and intestinal inflammation (Koren et al., 2012). As part of their symbiotic relationship, gut microorganisms are known to generate many metabolites (Tremaroli & Bäckhed, 2012). Serum samples from all the rats were obtained in order to evaluate a large number of these metabolites. In both the APM-treated diet groups, blood propionate levels were found to be approximately 2.5x greater than those in the controls, a statistically significant increase (p<0.05). Propionate is a SCFA that is produced by bacteria in the gut. Once produced in the colon, it enters the circulation and travels to the liver where it induces gluconeogenesis, which ultimately raises blood glucose levels. Propionate has also been linked to insulin resistance (Kimura et al., 2013). In summary, low-dose APM administration in rats was associated with increased blood glucose levels and decreased insulin function. These changes can possibly be attributed to increases in propionate production as a result of changes in the gut microbial population.
The Role of Aspartame in Oxidative Stress

Oxidative stress is a disruption in the balance of reactive oxygen species (ROS) in the body and the body’s antioxidant defense mechanisms. Increases in ROS and/or decreases in antioxidant defenses have been implicated in a large spectrum of diseases (Aruoma, 1998). Methanol, which is one of the breakdown products of APM, has previously been shown to result in the generation of ROS (Castro et al., 2002). To determine whether long term APM consumption can produce similar effects, one study looked at markers of free radical stress in rats being treated with APM. It was found that APM intake can not only lead to increased oxidative stress in the brain, but also can give rise to apoptosis in the brain (Ashok & Sheeladevi, 2014).

Rodents naturally have high folate levels in their liver. This results in the rapid metabolism of formate and therefore prevents accumulation of this APM-derived compound in the blood. In order to create a folate-deficient environment similar to that in humans, male Wistar strain albino rats were treated with methotrexate (MTX) and fed a diet absent of folate prior to being given APM (Ashok & Sheeladevi, 2014). APM was combined normal saline solution and given to the MTX-treated rats at a dose of 40 mg/kg body weight/day, the ADI recommended by the EFSA and a dose lower than what the FDA recommends. APM treatment lasted for 90 days. Rats were divided into three groups of six. One group was the normal saline control, one was the MTX-treated control, and the other was the MTX-treated rats who were fed APM. After the 90-day treatment period all the rats were sacrificed.
Superoxide dismutase (SOD) is a vital antioxidant enzyme that gets rid of superoxide radicals in cells. Increases in the activity of this enzyme were observed in the APM-treated rats (Ashok & Sheeladevi, 2014). These increases were present in six different regions of the brain: hippocampus, pons-medulla, midbrain, cerebellum, cerebral cortex, and hypothalamus. Increases in the activity of catalase and glutathione peroxidase, two other important antioxidant enzymes, were also observed in all six of these brain regions of the APM-treated rats. Glutathione is an endogenous compound that functions as an antioxidant. In its reduced form it is able to donate an electron to ROS and subsequently alleviate the harmful effects of these species. In the six brain regions analyzed, there were decreased concentrations of reduced glutathione in the APM-treated rats. It has been shown that reduced glutathione is a necessary cofactor in the metabolism of methanol (Pankow & Jagielki, 1993). When plasma methanol concentrations of the rats were measured, significantly higher levels of methanol were present in the APM-treated rats, which could explain the decreased amounts of reduced glutathione observed in the brain. Rather than scavenging ROS, this major antioxidant had to be utilized to breakdown methanol. After reduced glutathione donates an electron, glutathione reductase is responsible for converting it back into its reduced state. In the six brain regions studied in the APM-treated group, decreased activity levels of this enzyme were also observed. These decreases could have also been responsible for the decreased amounts of reduced glutathione in the brain. Glutathione-S-transferase, a detoxification enzyme that conjugates reduced glutathione to other compounds, was found to have increased activity in all six of the brain regions looked at in the APM-treated rats. All the
changes discussed above in the APM-treated rats were found to be statistically significant in their comparison to both control groups (p<0.05) (Ashok & Sheeladevi, 2014).

In the presence of free radicals, polyunsaturated lipids in the membranes of cells can become oxidized (Bergendi et al., 1999). This can compromise the structural and functional integrity of cell membranes and ultimately lead to impaired cell function (Halliwell, 1992). Increased levels of lipid peroxidation were found in all six analyzed brain regions of the APM-treated rats compared to the controls. These increases were determined to be statistically significant (p<0.05). To look at the amount of protein oxidation in tissues that results from interactions with ROS, protein carbonyl levels are assessed (Beal et al., 1993). Oxidative stress in the brain has also been shown to result in lowered levels of protein thiols (Patsoukis et al., 2004). Increased protein carbonyl levels and decreased protein thiol levels were found in all six analyzed brain regions of the APM-treated rats as well. These changes were determined to be statistically significant when compared to the controls (p<0.05) (Ashok & Sheeladevi, 2014).

*Bcl-2, Bax, and caspase-3* are three genes that are involved in the regulation of apoptosis. *Bcl-2* is involved in the prevention of apoptosis whereas *Bax* and *caspase-3* are involved in its promotion. The mRNA expression of these three genes was determined in four different brain regions of the rats: cerebral cortex, cerebellum, hippocampus, and hypothalamus (Ashok & Sheeladevi, 2014). In the APM-treated rats, *Bcl-2* was found to have decreased expression and both *Bax* and *caspase-3* were found to have increased expression in all four of these brain regions. The protein expression of these three genes was also determined. In the APM-treated rats, *Bcl-2* protein expression was decreased in
the cerebral cortex, cerebellum, and hippocampus. Protein expression of \textit{Bax} and \textit{caspase-3} were both increased in these three brain regions of APM-treated rats. In the analysis of both mRNA and protein expression of these genes, the beta-actin gene was used as the control since it is constantly expressed. All the changes in mRNA and protein expression were determined to be statistically significant when compared to the controls (p<0.05). The large number of findings in this study showed that relatively long-term APM consumption at the current recommended daily dose (by the EFSA) can induce a great deal of oxidative stress in the brain and lead to alterations in apoptotic mechanisms at the genetic level which ultimately lead to cell death in the brain (Ashok & Sheeladevi, 2014).

The intake of APM has also recently been shown to induce oxidative stress in the red blood cells (RBC) of rats (Prokic et al., 2014). APM mixed into distilled water was administered to five male Wistar albino rats at a dose of 40 mg/kg body weight/day for six weeks. The control group also contained five rats. At the end of the six weeks all the rats were sacrificed, at which time blood samples were acquired.

In the APM-treated group, increased levels of superoxide anion and hydrogen peroxide were found in the RBCs, both important indicators of oxidative stress. Peroxynitrite is a strong oxidant that is generated by the reaction of superoxide anion and nitric oxide, an endogenous free radical produced by endothelial cells. Considerably higher levels of peroxynitrite were found in the RBCs of the APM-treated rats. Furthermore, greater amounts of lipid peroxidation were observed in the RBCs of the APM-treated rats (Prokic et al., 2014). Reduced glutathione plays an important role in
RBCs, as it is able to reduce free radicals. In the APM-treated rats, increased concentrations of this compound were found in RBCs, indicative of a higher level of oxidative stress. Also observed in the RBCs of the APM-treated rats were increased activity levels of catalase, an antioxidant enzyme that breaks down hydrogen peroxide. All of the changes presented above that occurred in the RBCs of the APM-treated rats were statistically significant when compared to the control group (p<0.05) (Prokic et al., 2014).

In addition to looking at markers of oxidative stress in the RBCs, the researchers measured the serum levels of glucose, triglycerides, and cholesterol in both groups at the end of the six weeks. In the APM-treated rats, average levels of all three of these compounds were elevated (Prokic et al., 2014). This can be seen below in Table 5.

**Table 5**: Serum concentrations of glucose, cholesterol, and triglycerides in the control and APM-treated rats. ASP = aspartame. Numbers presented as averages ± standard error. * p<0.05 compared to control. Table taken from Prokic et al., 2014.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.84 ±0.31</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>1.58 ±0.04</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.56 ±0.02</td>
</tr>
</tbody>
</table>

Previous studies have shown that increased glucose, cholesterol, and triglyceride levels in the blood activate neutrophils (Kummer et al., 2007; Petnehazy et al., 2006; Alipour et al., 2008). The increased affinity of these neutrophils for the endothelium
results in the generation of ROS that can then enter RBCs and give rise to oxidative stress (Lynch & Fridovich, 1978). The pro-oxidative changes seen in this experiment reinforced the likelihood that APM has damaging effects on oxidative stress and metabolic parameters when consumed at the current EFSA recommended daily dose of 40 mg/kg body weight/day (Prokic et al., 2014).

**Changes in Neurological Behavior, Function, and Chemistry due to Aspartame**

The consumption of APM has been linked to several impairments in brain function both at a behavioral level and at a molecular one (Abdel-Salam et al., 2012a, 2012b; Lindseth et al., 2014). In one human study, APM intake was found to be associated with poorer performance on spatial ability tests and worsened moods and depression (Lindseth et al., 2014). In a double-blind setup, 28 young healthy adults were fed a strict low-APM diet of 10 mg/kg body weight/day for one eight day period and a strict high-APM diet of 25 mg/kg body weight/day for another eight day period. After the first APM treatment period and before the next one, participants went back to their normal diets for two weeks. All participants were given specific instructions and informed of the importance of consuming only the foods and drinks assigned during the treatment periods. In order to assess spatial abilities, the Vandenberg Mental Rotation Test was administered. In this test, participants were required to differentiate between various geometrical shapes. Scores were assigned based upon the number of correct answers in a six-minute period. Zung’s Irritability Subscale and Self-Rating Depression
Scale tests were administered to assess mood and depression, respectively. All three of these mental tests had previously been shown to provide reliable results (Vandenberg & Kuse, 1978; Campbell et al., 2012; Sakamoto et al., 2001). All tests were given to the participants within two hours of their last meal in each eight day APM-treatment period.

The scores of the participants on the spatial ability test were notably higher after they consumed the low-APM diet compared to their scores after the high-APM diet. This difference was of statistical significance (p<0.05). Scores obtained from the Zung tests showed that after the participants were fed the high-APM diet they were much more depressed and irritable compared to how they felt after being fed the low-APM diet. These differences were also of statistical significance (p<0.01). Of the 28 participants, three had scores that put them in the clinically depressed category; none of the three had any prior history of depression. It should be noted that the APM dose of 25 mg/kg body weight/day administered in the high-APM diet of this study was half the ADI that the FDA currently recommends. Although the findings in this study do not prove that APM causes these neurobehavioral impairments, the contribution of APM to depression, irritability, and impaired spatial abilities seen in these subjects cannot be overlooked (Lindseth et al., 2014).

One animal study found APM intake to be associated with decreased memory retention and changes in the levels of several important neurotransmitters (Abdel-Salam et al., 2012a). Male Swiss albino mice were given APM subcutaneously once a day at a dose of 0.625, 1.875, or 5.625 mg/kg body weight for two weeks. There were six mice in each dose group and in the control group, which was administered normal saline. In order
to assess the memory of the mice, the Morris water maze was used. This was a maze that required the mice to locate a platform that was submerged underwater in an unknown location. Once the mice were able to locate it, they could leave the water tank. Three trials were conducted each time this test was done. In the first trial, the mice were kept on the platform for 15 seconds after finding it in order to process its location. In the next two trials, the mice were able to leave the tank after successfully locating the platform, which remained in the same spot. Throughout the two weeks of APM administration, this test was done four times a week. At the end of the two weeks all the mice were sacrificed and brain tissues were analyzed.

Dose-dependent increases in the average time required to locate the platform in the first trial were observed in all three of the APM-treated groups over the two weeks, with a statistically significant increase in the 5.625 mg/kg APM dose group (p<0.05). Average time in the control group was 3.58 ± 0.27 sec. In the 5.625 mg/kg APM group it was 5.65 ± 0.66 sec. This can be seen in Figure 5 below. In the second and third trials of each test done, no statistically significant changes in the average time required to locate the platform were observed across the APM-treated groups.
Figure 5: Average time required for APM-treated mice to find a submerged platform in the first trial of the Morris water maze. Data was acquired from eight tests performed over the course of two weeks. * p<0.05 compared to control. Figure taken from Abdel-Salam et al., 2012a.

The amount of the reduced glutathione present in the brain tissue of the APM-treated mice decreased in a dose-dependent manner. A 25.1% decrease was observed in the 1.875 mg/kg group and a 32.7% decrease in the 5.625 mg/kg group, both statistically significant when compared to the control (Abdel-Salam et al., 2012a). Previous studies have linked decreases in the levels of this important brain antioxidant to impairments in spatial memory (Dean et al., 2009). Decreases in the brain levels of serotonin, dopamine, and noradrenaline were also observed in a dose-dependent fashion in the APM-treated rats. In the 1.875 and 5.625 mg/kg APM dose groups, these changes were statistically significant compared to the control (p<0.05) (Abdel-Salam et al., 2012a).

Changes in the levels of these monoamine neurotransmitters were again observed by these researchers when mice were administered APM in a different study design (Abdel-Salam et al., 2012b). Two different controls were used in this study: one with
normal saline and one with bacterial lipopolysaccharide. Lipopolysaccharide was used in order to see the effects of APM when systemic inflammation is present, a condition triggered by this compound. Inflammation is an underlying feature of a wide spectrum of diseases and has been shown to deteriorate neurons (Qin et al., 2007). Both normal saline and lipopolysaccharide were administered intraperitoneally. The lipopolysaccharide treated mice received APM in the following doses: 0.625, 1.875, 5.625, 11.25 and 22.5 mg/kg body weight. The saline treated mice received doses of 11.25, 22.5, and 45 mg/kg body weight. Each treatment group contained six mice. APM was administered as a single dose subcutaneously and after four hours all the mice were sacrificed.

Dose-dependent decreases in the levels of serotonin, dopamine, and noradrenaline were observed in the brains of all the mice treated with APM. Compared to the saline control, the lipopolysaccharide control group had significantly higher levels of these neurotransmitters. This was expected as previous studies had shown this inflammatory substance to be associated with these increases (Borowski et al., 1998; Mori et al., 2003; Ota et al., 2007). APM administration to the lipopolysaccharide reduced the increase of these neurotransmitters. All observed changes were found to be statistically significant (p<0.05) (Abdel-Salam et al., 2012b). Data obtained from all the groups can be seen below in Table 6.
Table 6: Serotonin, Dopamine, and Noradrenaline levels in the brains of mice treated with APM, Lipopolysaccharide, and APM with Lipopolysaccharide. Numbers presented as averages ± standard error. * p<0.05 when compared to saline control. † p<0.05 when compared to lipopolysaccharide control. Table taken from Abdel-Salam et al., 2012b.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serotonin (µg/g tissue)</th>
<th>Dopamine (µg/g tissue)</th>
<th>Noradrenaline (µg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>2.99 ± 0.30</td>
<td>3.29 ± 0.21</td>
<td>1.89 ± 0.08</td>
</tr>
<tr>
<td>+Aspartame 11.25 mg/kg</td>
<td>1.68 ± 0.08*</td>
<td>2.90 ± 0.27</td>
<td>1.52 ± 0.16*</td>
</tr>
<tr>
<td>+Aspartame 22.5 mg/kg</td>
<td>1.19 ± 0.13*</td>
<td>2.03 ± 0.18*</td>
<td>1.02 ± 0.05*</td>
</tr>
<tr>
<td>+Aspartame 45 mg/kg</td>
<td>1.05 ± 0.02*</td>
<td>2.01 ± 0.15*</td>
<td>0.95 ± 0.11*</td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td>8.75 ± 0.69*</td>
<td>6.34 ± 0.38*</td>
<td>3.62 ± 0.21*</td>
</tr>
<tr>
<td>+Aspartame 0.625 mg/kg</td>
<td>3.39 ± 0.12†</td>
<td>3.41 ± 0.32†</td>
<td>1.50 ± 0.06†</td>
</tr>
<tr>
<td>+Aspartame 1.875 mg/kg</td>
<td>3.08 ± 0.26†</td>
<td>3.25 ± 0.18†</td>
<td>1.49 ± 0.07†</td>
</tr>
<tr>
<td>+Aspartame 5.625 mg/kg</td>
<td>2.82 ± 0.22†</td>
<td>3.13 ± 0.36†</td>
<td>1.40 ± 0.09†</td>
</tr>
<tr>
<td>+Aspartame 11.25 mg/kg</td>
<td>2.72 ± 0.19†</td>
<td>2.79 ± 0.13†</td>
<td>1.37 ± 0.20†</td>
</tr>
<tr>
<td>+Aspartame 22.5 mg/kg</td>
<td>2.72 ± 0.31†</td>
<td>1.98 ± 0.09†</td>
<td>0.80 ± 0.03†</td>
</tr>
</tbody>
</table>

The effects of APM metabolites on the brain explain these changes that occur at the neurochemical level (Humphries et al., 2008). Once phenylalanine, one of the metabolites of APM, enters the vasculature, it is either further metabolized into tyrosine in the liver or transported across the blood-brain barrier (BBB) via a neutral amino acid transporter (NAAT). The NAAT is also responsible for the transport of six other amino acids across the BBB. Tyrosine, a precursor for dopamine, and tryptophan, a precursor for serotonin, are two of the amino acids that compete with phenylalanine for entry into the brain using this transporter. If the level of one amino acid is elevated compared to that of another one, it will reduce the entry of the other amino acids into the brain because they all compete for the same site on the NAAT. APM consumption has been shown to increase the concentration of phenylalanine in the blood (Jr & Stegink, 1988). Therefore, if too much phenylalanine is present in the blood as a result of APM intake, it will prevent adequate amounts of tyrosine from crossing the BBB and consequently result in
decreased dopamine synthesis in the brain. Tyrosine transport across the BBB is the only method by which tyrosine can be present in the brain; tyrosine synthesis does not occur in the brain. Since dopamine is a precursor of noradrenaline, decreases in dopamine will consequently lead to decreases in noradrenaline as well. The same mechanism explains the lowered levels of serotonin in the brain that can result from APM intake. With the increased blood levels of phenylalanine, less tryptophan can enter the brain via the NAAT and less serotonin is synthesized. The decreased amounts of serotonin can result in lowered activity of cyclic adenosine monophosphate (cAMP) at the BBB (Humphries et al., 2008). Sufficient cAMP is important in maintaining the structural integrity of the BBB; therefore, decreases in cAMP will weaken the function of this important barrier. A schematic showing the effects of decreased serotonin levels can be seen in Figure 6 below.

Figure 6: The effects of lowered serotonin levels on the blood-brain barrier. Figure taken from Humphries et al., 2008.
Furthermore, since gamma-aminobutyric acid (GABA) transporters on astrocytes are serotoninergic receptors, decreased serotonin levels can down-regulate these transporters that are responsible for the reabsorption of GABA back into astrocytes. This would result in higher levels of GABA in the synaptic cleft and consequently greater and longer inhibition of the postsynaptic cell (Humphries et al., 2008).
DISCUSSION

The association of APM intake to a variety of adverse health effects raises concern over the consumption of this artificial sweetener that is currently deemed safe for humans by the FDA and a large number of other food regulatory agencies around the world. Although numerous conflicting studies exist regarding the influence of APM on the prevalence of many conditions such as cancer and diabetes, the fact that there are studies showing APM intake playing a role in disease development either directly or indirectly is a red flag for its use.

In 2006 and 2007, two separately conducted studies showed APM consumption to be related to the increased prevalence of several cancers: lymphomas, leukemias, carcinomas of the renal pelvis and ureter, and schwannomas (Soffritti et al., 2006, 2007). Being that it was the first time that this connection was experimentally shown, it caught the attention of many people, including the FDA. The FDA conducted a review of the studies and then released a statement in which they maintained their stance that APM use at an intake of up to 50 mg/kg body weight/day is safe. In the statement, the FDA said that many flaws were present in the study in regards to its design, data collection, and the reporting of its findings (FDA, 2007). However, no specifics were mentioned in regards to what was flawed in the study design. It was stated that many other variables could have contributed to the onset of these cancers, such as infections, and that the cancerous histological changes observed were not analyzed properly and all occurred by chance. The FDA ended the statement by referencing previous studies that had shown no link between APM intake and carcinogenesis. Overall, it was a short and vague statement
lacking any sound scientific backing. In a different article that aimed to invalidate the findings of Soffritti et al., it was mentioned that the APM doses reported in the studies were inaccurate, that certain data from the pregnancy period of the rats was missing, that the cancer occurrences were in the range of historical controls, and that infection was most likely the reason of carcinogenesis in the rats, the same belief held by the FDA (Magnuson & Williams, 2008). Of great importance, the authors of this article received full financial backing for the presentation of this information from the world’s largest producer of APM, Ajinomoto. Therefore, these statements should be interpreted with caution.

In regards to the design of the Soffritti studies, they were the first experiments done that examined the relationship between APM and cancer in which APM was administered throughout the life of the animals and large treatment and control groups were used (Soffritti et al., 2006, 2007). Compared to short-term APM treatment, looking at the effects of APM across the life span of animals gives a clearer insight into the chronic effects of this artificial sweetener. The slower progressing diseases and biological changes that could potentially be related to its use can be better observed. This feature of the study, combined with the large number of rats in each treatment group, improved the sensitivity of the results. In the rats that began exposure to APM starting in fetal life, the prevalence of lymphomas and leukemias in both males and females was almost double the prevalence seen in the controls (Soffritti et al., 2007). Although these differences were observed in only the 100 mg/kg body weight dose group, a dose higher than what is currently recommended in humans, these significant findings cannot be ignored and
should not be compared to historical controls from studies that had different designs and did not assess the impact of APM intake over a life span. Furthermore, a total of 21 renal pelvis and ureter transitional cell carcinomas were observed in the rats that began APM-consumption at 8 weeks old. In the past 20 years at the lab at which this study was conducted, zero cases of these carcinomas had ever been diagnosed in over 4,000 animals that were experimented on (Soffritti et al., 2006).

Pathology working groups consist of highly skilled pathologists who convene in order to conduct an impartial review of existing pathological findings and diagnoses made in previous studies. In 2011, one such group reviewed studies done at the laboratory at which the Soffritti experiments were carried out and determined all their cancer diagnoses to be accurate (Soffritti et al., 2014), contrary to what was believed by the FDA. If infections were the only source of carcinogenesis in the rats, then the control rats would have incidences of cancer similar to those rats in the APM-treated groups, which was not the case as there were notable differences between the two. Additionally, even if the doses were not completely accurate as is believed by Magnuson et al., the increases in total malignant tumors, lymphomas, leukemias, carcinomas, and schwannomas seen in the rats that consumed APM raises a lot of worry about the dangers of APM use. Further studies need to be carried out in order to gain a better understanding between the relationship of APM and carcinogenesis. These studies should assess APM exposure both over life spans and starting in the prenatal period. The findings by Soffritti et al. that prenatal exposure potentiates the harmful effects of APM are very concerning regarding the use of APM by pregnant women.
The large number of products that contain APM as an ingredient makes it very difficult to assess human APM intake. From familiar sources such as sodas, yogurts and desserts, to less familiar ones such as condiments, coffee syrups and pharmaceuticals, APM is present in a wide variety of frequently consumed products. The amount of APM people believe to be consuming vs. the amount they actually are consuming can potentially vary greatly. This adds a gray area in human studies in which people have to recall and estimate APM intake and is one reason why conflicting studies exist when humans are used as subjects.

In the NIH-AARP Diet and Health Study, cancer-free individuals completed a food questionnaire that assessed the frequency of APM intake from beverages over the course of a year. The participants of the study were then monitored over the next five years for occurrences of cancers. After the five year period and data analysis, it was determined that there was no association between APM consumption and increased risk of hematopoietic and brain cancers (Lim et al., 2006). The design of this study had several inherent flaws. Even though self-reported individual and lifestyle factors such as ethnicity, smoking history, activity level, and family history of cancer were all adjusted for in the analyses, to assess participants for APM consumption only once and then extrapolate that information over the next five years is a very inadequate way to determine what effects APM has on people’s health. In the initial questionnaire, it is possible that errors in the reporting of APM intake occurred. Participants had to remember how frequently certain beverages were consumed over a year, a measurement method of APM intake that is prone to recall bias. In the five years that the participants
were observed, it is likely that their drinking, eating, and lifestyle behaviors changed. These changes would have not only impacted estimated APM consumption but also added even more variables that could have contributed to the results. Furthermore, the questionnaire only asked about APM intake through beverages, so any APM that was being consumed through foods was never accounted for. The poor construction of this study and the large number of confounding variables present made it difficult to examine any direct effects APM has on the health of the participants.

A similar study using questionnaires was described in the NHS and HPFS review that determined APM intake to be associated with an increased risk of NHL and multiple myeloma in men and leukemia in both men and women (Schernhammer et al., 2012). The questionnaires in this study were updated every four years over the 20 years that the study was conducted. Although this gave a more precise estimate of APM consumption through diet sodas, the design was still prone to recall bias, omission of other products that contain APM, and transient changes in the drinking and eating behaviors of the individuals during the study period. One strength of the study is that data collection began right after APM was approved for use in beverages in the early 1980s, which allowed investigation of a population who had never been exposed to APM. However, except for in diet coke, all other diet beverages contained a mixture of APM and saccharin up until 1992 when APM became the predominantly used artificial sweetener in diet beverages. This makes it difficult to single out APM as the cause of any perceived negative health effects. The gender differences in the results could have possibly been due to increased activity levels of ADH in men compared to those in women (Chrostek et
al., 2003). Since ADH converts one of the metabolites of APM, methanol, to formaldehyde, men could have been exposed to higher levels of this known human carcinogen; formaldehyde has been linked to leukemias and a variety of other cancers in humans (“Formaldehyde and Cancer Risk,” 2015). Further studies that investigate whether the differences in ADH activity in males and females are enough to be a contributing factor to gender differences in carcinogenesis are warranted. Although this particular study using human subjects showed that there is a possibility that APM use can increase cancer risk, it was inconclusive because of the great number of confounding variables that could not be controlled for and may have influenced the results.

When the MESA population was examined over a period of seven years, diet soda consumption was found to be associated with increased waist circumference, fasting blood glucose, and risk of developing type 2 diabetes (Nettleton et al., 2009). Similar to the two previous human studies discussed, weaknesses of this study included the use of questionnaires to estimate APM consumption and the fact that a variety of lifestyle variables and substances in diet sodas, including other artificial sweeteners, could have influenced the findings. Simply because consumption of diet sodas was found to be associated with these symptoms does not in any way provide legitimate evidence that they are causing these changes. When people drink calorie-free diet soda, it is possible for them to consume a greater number of calories from other foods and drinks than those calories they saved from drinking the diet soda. An increase in daily caloric intake is one possibility for the larger waist circumferences found in the population that was consuming diet soda. The questionnaire used in this study included a detailed assessment
of the categories of foods that participants typically ate; however, as is always possible, their eating habits could have considerably changed over the course of the study. Given the fact that the types of foods consumed can greatly influence fasting blood glucose and therefore the risk of developing type 2 diabetes, other dietary measures other than diet sodas could have been responsible for the perceived results of this study. With the vast number of products that contain APM these days and other ingredients present in APM products, it is difficult to isolate APM in human epidemiological studies. Combined with the challenge of controlling for confounding lifestyle variables, determining any adverse health effects in humans due to APM use is an uphill task. Future human studies that investigate the impact of APM on health should be designed in a way that participants only consume APM in its isolated form and have all other dietary and lifestyle factors controlled to the highest degree. Studies of this nature will give clearer insights to any health consequences related to APM use.

Animal studies have given a clearer understanding between the relationship of APM use and metabolic disturbances. Rats that were administered very low doses of APM for eight weeks, 5-7 mg/kg body weight/day, were found to have increased blood glucose levels, compromised insulin function, and changes in gut microbiota (Palmnäs et al., 2014). The increase in blood propionate levels discovered in the APM-treated rats was believed to be the underlying cause of these changes in glucose levels and insulin function. Propionate is a SCFA that is produced by bacteria in the gut. Changes in the gut bacterial population were attributed to APM treatment and were what caused this elevation of propionate production. In addition to being a gluconeogenic agent and being
previously linked to insulin resistance, propionate has been associated with a number of other mechanisms and conditions such as changes in gene expression, autism, changes in mitochondrial function, and irritable bowel syndrome (Kimura et al., 2013; Larsson et al., 2012; Frye et al., 2013; Lee & Tack, 2010). It is possible that sustained elevations in blood propionate levels as a result of long-term APM consumption could ultimately lead to the acquisition of any of these conditions or even other negative outcomes yet to be linked to increased propionate production. The APM dose administered to the rats was much lower than what is currently recommended by the FDA, 50 mg/kg body weight/day. The adverse health effects associated with this low dose over a short period of time raises the possibility that even low to moderate APM consumption can contribute to impairments in metabolic homeostatic mechanisms. Further studies looking at APM intake and its contribution to propionate production need to be conducted to confirm these findings.

Research investigating the relationship between APM consumption and oxidative stress has shown that APM plays a role in the increased production of free radicals in the brain and RBCs and induction of apoptotic mechanisms in the brain (Ashok & Sheeladevi, 2014; Prokic et al., 2014). Methanol, one of the metabolites of APM, has been implicated in rises in the production of free radicals (Kadiiska & Mason, 2000). It is possible that increases in the activities of SOD, catalase, and glutathione peroxidase in the brain could have been in response to free radicals generated from the methanol exposure. In the study conducted by Ashok and Sheeladevi, plasma methanol concentrations in the APM-treated rats were found to be significantly higher than those in
the controls (p<0.05). Free radicals also play a role in the initiation of apoptotic processes (Raha & Robinson, 2001). The changes seen in the expressions of the *Bcl-2*, *Bax*, and caspase-3 genes by Ashok and Sheeladevi in the APM-treated rats could have been a secondary effect of APM exposure. When decreases in *Bcl-2* expression and increases in *Bax* expression occur in neurons simultaneously, they give rise to apoptosis (Mbazima et al., 2008). Furthermore, increases in the expression of the pro-apoptotic *Bax* gene leads to mitochondrial release of cytochrome c, which also triggers apoptosis (Thomas et al., 2000). Given the implications of oxidative stress in a wide number of diseases, these findings need to be investigated further in relation to their association with APM use. Further studies using larger sample sizes are required in order to confirm APM as the cause of the changes observed in oxidative stress and apoptotic markers. These studies should also look into whether APM gives rise to oxidative stress in any other organ systems.

From a neurological perspective, the associations of APM to depression, irritability, and compromises in spatial orientation and memory raise even more questions on the safety of this artificial sweetener. In human subjects, these three symptoms were associated with APM when consumed for eight days at a dose of 25 mg/kg body weight/day, half the daily dose that the FDA currently recommends (Lindseth et al., 2014). Even though APM intake was strictly regulated in this particular study and the subjects were both physically and mentally healthy prior, there are so many external variables that could have contributed to the results. However, the effect that APM has on the levels of monoamine neurotransmitters adds greater validity to these findings. Several
investigations of the effects of APM at the neurochemical level have shown APM to be associated with decreased levels of dopamine, serotonin, and noradrenaline in the brain (Abdel-Salam et al., 2012a, 2012b; Humphries et al., 2008). Depression has been linked to decreases in the levels of all three of these neurotransmitters (To et al., 2005). It is possible that the feelings of depression the subjects had after eight days of APM consumption were due to these changes. Decreases in dopamine have also been linked to impairments in working memory and the development of attention deficit hyperactivity disorder (Humphries et al., 2008).

Decreases in serotonin levels can affect sleep cycles, appetite, temperature control, and moods. Serotonin also plays many important roles in brain development. It has been associated with the formation of synaptic circuits, the differentiation and maturation of neurons, and apoptotic mechanisms (Gaspar et al., 2003). Soffritti et al. showed that prenatal exposure to APM potentiated the already existing increased risk of cancer due to APM consumption (Soffritti et al., 2007). The consequences of decreased serotonin production present more cause for concern in pregnant women who consume this artificial sweetener. In regards to noradrenaline, decreased levels of this neurotransmitter have been associated with loss of attention and impulsiveness during child development (Humphries et al., 2008). It is possible for children who frequently consume APM to acquire these deficits over a period of time. Additionally, the down regulation of astrocyte GABA transporters that is a result of decreased serotonin can also have harmful effects during the developmental period. Throughout brain development, GABA plays an excitatory and trophic role and has been associated with the growth of
neurons and shaping of synapses (Owens & Kriegstein, 2002). Shifts in the levels of this neurotransmitter as a secondary consequence of APM consumption could potentially result in impairments in maturation of the brain. In order to confirm the changes APM brings about neurologically at the molecular level, further studies are required that investigate exactly to what extent APM metabolites rise in the blood, exactly what doses are responsible for those rises, and how drastically neurotransmitter levels change.

In conclusion, numerous studies conducted on APM have shown that consumption of this artificial sweetener at both low and high doses contributes to the development of many diseases and conditions. These include lymphomas, leukemias, carcinomas, schwannomas, multiple myeloma, genotoxicity, diabetes, metabolic syndrome, oxidative stress, insulin resistance, premature apoptosis, and neurochemical imbalances. Although there are many other APM studies that say otherwise, there is enough research and evidence out there to show that there is a strong likelihood that APM is not completely safe. There is a vast amount of misleading information out there that can deceive people in regards to the safety of APM. For example, the Aspartame Information Center, an .org website which looks very credible, is ran by an organization that works directly with manufacturers and suppliers of low-calorie foods and beverages (Aspartame Information Center, 2015). People who have conflicts of interest present a great deal of this literature and information on APM that claims its safety. Because of this, future investigations that examine how the funding sources of studies and resources are related to reporting findings of APM safety are warranted, as they would shed more light on this controversial topic. As it stands, consumers of APM, whether it is at high or
low doses, may be at an increased risk of a variety of adverse health effects. The spread of this information to the public and reevaluation of the safety of APM are urgently needed.
REFERENCES


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WORK EXPERIENCE

Ronald Reagan UCLA Medical Center, Los Angeles, CA
Emergency Trauma Technician
October 2011-June 2012
• Supported nurses and physicians in the Emergency Department.
• Performed a wide range of patient care skills, monitored EKGs, and transported patients.

UCLA Emergency Medical Services, Los Angeles, CA
Emergency Medical Technician
June 2009-October 2011
• Responded to 911 calls at UCLA and the surrounding Westwood area.
• Staffed special events on campus and coordinated the campus lost and found.

Field Training Officer
July 2010-October 2011
• Taught trainees medical and equipment guidelines, as well as EMS procedures.
• Directed practice and testing simulation sessions.

Maintenance Coordinator
April 2010-June 2010
• Maintained proper functioning of ambulances and medical equipment.
• Managed and organized all medical supplies.

Team Representative
January 2010-March 2010
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UC Irvine Medical Center Volunteer October 2014-Present
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American Heart Association (AHA) Volunteer November 2012-July 2013
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