The Effect of Bisphenol A on Prostate-Specific Antigen Level and Motor Activity of C57/BL-6 Mice[1]



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Allegheny College Department of Biology

Senior Comprehensive Project

April 1, 2013

[1] *This research involves vertebrate animals and procedures were approved in accordance with guidelines and policies of the Allegheny College Animal Review Committee*

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Senior Comprehensive Research Project

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April 1, 2013

Submitted in fulfillment of the senior thesis requirements for a Bachelor of Science Degree in the Department of Biology at Allegheny College and approved by the senior thesis committee.

_____	_____
Dr. Jim Palmer, Ph.D	Dr. Ron Mumme, Ph.D
_____	_____
Date	Date

I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College community as a whole.

_____
Pledge

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ABSTRACT[2]

There are many chemicals in the environment that pose a threat to the endocrine system by causing estrogenic effects. Bisphenol A (BPA), one identified endocrine disruptor, is found in polycarbonate plastics and epoxy resins that are used to manufacture everyday items, such as plastic bottles, dental fillings, and the lining of tin food cans. Epoxy resins that line food cans leach into the food, causing consumers to ingest BPA. BPA, and testosterone have been found to affect prostate-specific antigen (PSA) levels; higher PSA levels indicate an abnormality with the prostate, which can be cancerous. BPA has also been observed to induce a behavioral effect on organisms that leads to increased motor activity at a low-dose exposure. Although a correlation between BPA exposure and increased levels of PSA has been found in previous studies, few studies have been completed to determine if the leachate from autoclaved tin food cans raises PSA levels or leads to prostate cancer. Moreover, the determination of effects from BPA on motor activity has also not been examined in depth. In this study, C57/BL-6 male mice were exposed to low and high doses of BPA, as well as an unknown concentration of BPA in epoxy resin leachate from autoclaved corn cans, in order to determine their effect on PSA level. A behavioral assay was used to assess the dose-dependent relationship between BPA and motor activity. I predicted that a low-dose of BPA and leachate from autoclaved cans would show an increase in PSA levels as well as motor activity. Neither hypothesis was supported, as there were no significant differences between treatment groups for PSA levels or motor activity. The only result of significance was that both low-dose BPA and the autoclaved canned corn water treatment groups had greater body mass gain than the ethanol negative control and testosterone positive control. HPLC-Mass Spectrometry test determined that only small amounts of BPA (< 10 μg/mL) were present in the corn can water.

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INTRODUCTION

Prostate cancer is the second leading cause of cancer deaths in North American men (Hu *et al*. 2012). The highest instances of prostate cancer are found in the United States and Canada, while the lowest have been found in China and other parts of Asia. The reasoning behind this is the Western lifestyle's increased intake of fats, meats, and dairy products (Crawford 2003). Steroid hormones, such as testosterone, have also been known to play a role in the formation and progression of prostate cancer, which has led to hormonal treatment options including androgen receptor blockade (Hu *et al*. 2012). One means of prostate cancer testing is with a prostate-specific antigen (PSA) level test. PSA is a 33-kd glycoprotein that contains 237 amino acid residues, multiple disulfide bonds, and 4 carbohydrate side chains. PSA is encoded by the KLK3 gene, which is regulated by an androgen receptor and progestin (Altuwaijri 2012). Cells of the prostate gland generate PSA, and the common PSA test assesses the PSA level in the blood. The PSA test is relatively non-invasive, and only requires a sample of blood, which is sent to the lab for PSA level analysis. The results are reported in nanograms of PSA per milliliter of blood (ng/mL) (Barry 2001).

The U.S. Food and Drug Administration first approved the PSA test in 1986, not to diagnose, but rather to monitor the development of prostate cancer. The FDA approved the use of the PSA test, in concurrence with a digital rectal examination (DRE), in order to test for prostate cancer beginning in 1994 (National Cancer Institute 2012). Previously, it was recommended that any male age 50 or older undergo a PSA test in conjunction with a DRE in order to help detect prostate cancer in those with no symptoms of the disease. The PSA test is currently under debate as to whether or not it serves as a beneficial screening method

for prostate cancer because of the high number of false positive results it produces (Barry 2001). There are many factors that can influence the level of PSA, including: prostatic diseases, clinical manipulation, and testosterone levels (Bunting, 1995). Thus, PSA levels can easily fluctuate, which may lead to false positive test results (Eastham *et al*., 2003).

The American Cancer Society (2012) currently recommends the pros and cons of a PSA level and DRE screening to be discussed with a physician before undergoing the test for males with no family history of prostate cancer beginning at the age of 50. False positives in testing could lead not only to negative psychological effects, but could also lead to unnecessary medical procedures, especially prostate biopsies, which can lead to side effects of pain, fever, bleeding, and infection (Rosario *et al.* 2012). Due to the high level of potential harm compared to benefits, the U.S. Preventive Services Task Force has issued a recommendation against PSA-based screening for prostate cancer (Chou *et al.* 2011).

Clinicians previously considered a PSA level of 4.0 ng/mL or less to be a "normal" and safe range (free from prostate cancer). However, Thompson *et al.* (2004) found that high or medium-grade prostate cancer (where the cells no longer look like healthy prostate cells) can be present in men with PSA levels lower than 4.0 ng/mL. In fact, as many as 15% of men with a seemingly normal PSA level were found to have prostate cancer. The question thus is: should the test be more sensitized? An increase of sensitivity may increase the detection rate of cancer, but will also create a chance for more false-positive results (Bunting 1995). Previous studies have shown that a single abnormal PSA level should not alone be the basis for prostate cancer treatment. For example, in one study nearly half of the men tested showed a single abnormal PSA level and subsequently had normal baseline levels (Eastham *et al.* 2003). It has been recommended, thus, that a baseline PSA level be

established in asymptomatic males so that it can be used in comparison to future tests (Chou *et al.* 2011).

Bisphenol A, a Xenoestrogen:

Estrogens have been found to play a key role in prostate carcinogenesis and progression, but the mechanisms are not completely understood (Hu *et al*. 2012). Xenoestrogens imitate estrogen – providing estrogenic effects. Bisphenol A (2,2-Bis (4-hydroxyphenyl) propane), is considered a xenoestrogen, and exhibits estrogenic effects both *in vivo* and *in vitro* (Steinmetz *et al.* 1998). Bisphenol A (BPA) has been shown to quicken growth and puberty in experimental animal studies, and such evidence suggests that BPA can act as an endocrine disruptor (Dong *et al.* 2010). In fact, there is evidence from rodent models that BPA can influence carcinogenesis and alter prostate cancer cell proliferation (Hu *et al*. 2012). Short, early-life exposure to low-doses of BPA was found to increase susceptibility to precancerous lesions and hormonal carcinogenesis (Ho *et al*. 2006). Bosland *et al*. (1996) found that in rodents, estrogens combined with androgens induce prostate cancer.

BPA is used widely throughout the world in the production of polycarbonate plastics and epoxy resins (Dong *et al.* 2010). BPA is utilized in the manufacturing of common household items such as water and baby bottles, dental fillings, and metal food can linings (LaPensee *et al.* 2010). Although BPA degrades rapidly in the air, it persists in soil and water. When people consume food and water out of plastic containers made with BPA, they are exposed to the chemical. For example, epoxy resins are utilized as plastic coatings in cans. A significant proportion of unreacted epoxy resins remain in the cans after they are manufactured, which begin to alkylate and become toxic over time, while sitting on the shelf.

The alkylated epoxy resins then leach into the water that the food is preserved in. As canned food is a popular component of modern diets, it is important to study the effects of the BPA present in the lining of the food cans. One study extracted a range of 0-33 µg of parent chemical BPA breakdown products per food can. Canned peas were found to have 33 µg of BPA per can, while canned corn was found to have about 4.5 µg/can (Brotons *et al.* 1995). The amount of BPA that leaches into the food may depend on what type of food is contained in the can. I sought preliminary evidence for the possibility that BPA was present in corn can leachate at levels sufficient to alter either PSA level or behavioral activity. Thus, my research will address the possible xenoestrogenic effects of the BPA lining in the food cans on PSA levels, as well as motor activity.

BPA and Motor Activity:

Additionally, the possible effect of BPA on motor activity has been thoroughly studied in Wistar rat pups treated with a 20 µg/kg intracisternal injection of BPA. The pups were found to be hyperactive at the age of 4 weeks (Masuo *et al.* 2004). Prenatal and lactational exposure to BPA also resulted in an increase in motor activity in rats (Richter *et al*. 2007). Palmer Lab (2011, Unpublished) found BPA to have a significant increase on motor activity when tested in the Opto Varimex Micro Animal Activity System. However, the effect of leachate from autoclaved cans on motor activity has not been as thoroughly studied.

Aims of the Study:

As there has been much debate over the use of PSA tests for the detection of prostate cancer, I attempted to quantify the effect of dose-dependent BPA exposure on the serum PSA level in male mice. I also aimed to determine whether BPA or leachate from autoclaved food

cans could induce elevated PSA levels. A positive control of testosterone was used to compare possible PSA level effects. The negative control was ethanol, which was the vehicle used to dissolve the testosterone and BPA. As most research conducted on BPA and motor activity has been on prenatal or neonatal mice, I expanded on the effects of BPA and leachate from autoclaved food cans on motor activity of weaned mice. Based on previous studies, I hypothesized that a low-dose of BPA and leachate from autoclaved food cans would coincide with elevated prostate-specific antigen level in a negative dose-dependent response in male mice, as well as an increase in motor activity.

METHODS

Chemicals:

BPA, ethanol, and testosterone were obtained from Allegheny College. BPA and testosterone were first dissolved in 100% ethanol and then added to tap water to achieve final concentrations of 0.3 µg BPA/50 mL water per day, 3.0 µg BPA/50 mL water per day, and 3.0 µg testosterone/50 mL water per day. The desired dose per mouse was 10 µg/kg/day for the low-dose BPA and 100 µg/kg/day for the high-dose BPA and testosterone. The concentrations were adjusted based on the daily water consumption of roughly 5 mL water per day so that the mice would consume the desired dose. The concentrations were thus increased tenfold as follows: 0.3 µg BPA/5 mL water per day, 3.0 µg BPA/5 mL water per day, and 3.0 µg testosterone/5mL water per day. These are widely used concentrations in previous studies that result in a low dose dependent relationship. A PSA ELISA kit was purchased from Novatein Biosciences (Cambridge, MA). Carbon dioxide, obtained from Allegheny College, was used to euthanize the mice.

Animals:

A standardized strain of C57/BL-6 Jackson Lab male mice was used. They were bred at Allegheny College and fed *Purina Mouse Chow* and water. The treatment period began on postnatal day 30, when they were fully weaned and it was possible to determine the sex. Four male mice were used for each treatment group (there were five groups), equaling 20 total male mice used. The 20 male mice were housed 2 per cage (when possible based on litters) and provided food *ad libitum*. They were provided water with a concentration of a chemical pertaining to the treatment group. The cages were cleaned twice weekly and the animals were kept at a controlled temperature of 22ºC. Due to an unexpected mortality in

the testosterone treatment group, two mice were replaced, and treatment began at approximately the same age.

Treatment Groups:

Mice were dosed through water intake from controlled water bottles. Four mice received the BPA dissolved in ethanol at a low dose of 10 µg/kg/day, while four mice received the high dose of 100 µg/kg/day. Four mice received the leachate from autoclaved cans (into standard tap water used for mouse water bottles). Four mice received the positive control (testosterone) dissolved in ethanol at a dose of 100 µg/kg/day, and four mice received the negative control of ethanol (Table 1). Exposure began on postnatal day 30 and lasted 21 days until the concentrations were increased tenfold and exposure continued for another 14 days, totaling an exposure period of 35 days (5 weeks). The water loss from each water bottle was measured each day to estimate the amount of treatment consumed. The mice were weighed on postnatal day 30 (when treatment began) and at the end of the exposure time.

Table 1. Dosage of each treatment group. Each chemical was dissolved in ethanol and testosterone served as the positive control whereas ethanol functioned as the negative control.

(All Dissolved in Ethanol)	
Bisphenol A	Low concentration (10 µg/kg day) High concentration (100 µg/kg day)
Leaching from Autoclaved Cans	Unknown concentration
Testosterone	Positive Control (100 µg/kg day)
Ethanol	Negative Control

Autoclaved Cans:

Corn food cans were purchased from Wal-Mart, emptied of corn contents, and filled with distilled water. Corn was chosen because it is a relatively non-acidic food. Acidic food residue may interfere with the BPA leached into the can. The cans were autoclaved for 30 minutes at 120ºC. That water was poured into a jug and more distilled water was added to each can, which was then autoclaved twice additionally (each time for 30 minutes at 120ºC). The resulting water was added to the jugs so that each jug contained water that had been autoclaved a total of three times per can. The water was poured into a drinking bottle for each cage and served as the drinking water for the mice.

Behavioral Assay:

At the end of exposure at postnatal day 65, a behavioral assay was performed on each mouse for 20 minutes using the Opto Varimex Micro Animal Activity System (Fig. 1), which consisted of 16 infrared beams in the x, y, and z positions. Motion was measured, just as in the study by Palmer Lab (2011, Unpublished). Every time the mouse crossed a beam, the activity level was recorded. The number of x-axis beam crosses was combined with the number of y-axis beam crosses for each 5-minute segment. The data collected from the Animal Activity System was compared between treatment groups.



Figure 1. The Opto Varimex Micro Animal Activity System utilized for the motor activity behavioral assay. Image from: http://olivier.montagne.free.fr/image_220.jpg

Serum Extraction:

At exposure period completion, the mice were euthanized with carbon dioxide and the heads were removed in order to collect the trunk blood serum. The whole blood was centrifuged for 10 minutes at 14,800 rpm and the serum (supernatant) was removed and saved. The serum was frozen at -40ºC for subsequent analysis. Serum PSA levels were measured using a PSA ELISA kit (Novatein Biosciences), which contained standards, buffer, Biotin-antibody, HRP-avidin, and TMB substrate. The ELISA assay utilized the quantitative sandwich immunoassay. Samples were added to the plate along with the conjugate (HRP), which was then incubated at 37°C for 1 hour. The plate was then washed 5 times with diluted wash solution. Chromogen Substrate A and Chromogen Substrate B were added sequentially and the plate was incubated at 25°C for 10 minutes, covered to avoid light exposure. Stop solution was added and the absorbance of the PSA ELISA was measured with a microplate reader set to 450 nm.

Autoclaved Canned Corn Water Analysis:

HPLC/Mass Spectrometry was used with a Phenomenex hyperclone 250 x 4.6 mm 5µ C18 reverse phase column in order to determine the BPA concentration in the canned corn water. The column was equilibrated with 80% acetonitrile solution and 20% HPLC water. An HPLC-grade BPA standard was made up to a concentration of 10 µg/mL in methanol and run through the column for 20 minutes with an absorbance monitoring at 280 nm. The autoclaved canned corn water sample was then run through the column in triplicate, and the resulting peaks were compared to that of the standard in order to determine the BPA concentration.

Data Analysis:

A standard curve was established and used to calculate the PSA levels. The known concentration of each standard from the PSA ELISA kit was plotted against absorbance at 450 nm and the equation from the trend line of best fit was utilized to calculate the unknown PSA concentration for each mouse (Appendix Fig. B). Two outliers were thrown out in formation of the standard curve.[3] The motor activity, PSA levels, and mass gain were analyzed for statistical significance using ANOVA tests in StatView (SAS Institute, Cary NC). The treatment difference was isolated with post-hoc multiple comparisons test, and statistical significance was accepted at P=0.05 and below.

[3] The ELISA kit was wrongly put into the freezer instead of the refrigerator for one week, which could have affected the formation of the standard curve.

RESULTS

Body Mass:

When the mice were weighed initially, there was no significant difference between average body masses of each treatment group. The one-way ANVOA test showed that P=0.89 and F=0.27, indicating that this was a random assignment of mice per treatment group with regards to body mass with no statistical significant difference between treatment groups (Appendix Fig. A).

However, there were significant differences between treatment groups in relation to change in body mass during the treatment period (Table 2). Specifically, the Student-Newman-Keuls test showed significant differences between the treatments of ethanol and low-dose BPA, ethanol and autoclaved canned corn water, testosterone and low-dose BPA, and testosterone and autoclaved canned corn water. Both low-dose BPA and autoclaved canned corn water showed significantly greater increases in body mass than ethanol and testosterone (Fig. 2 & Table 2).

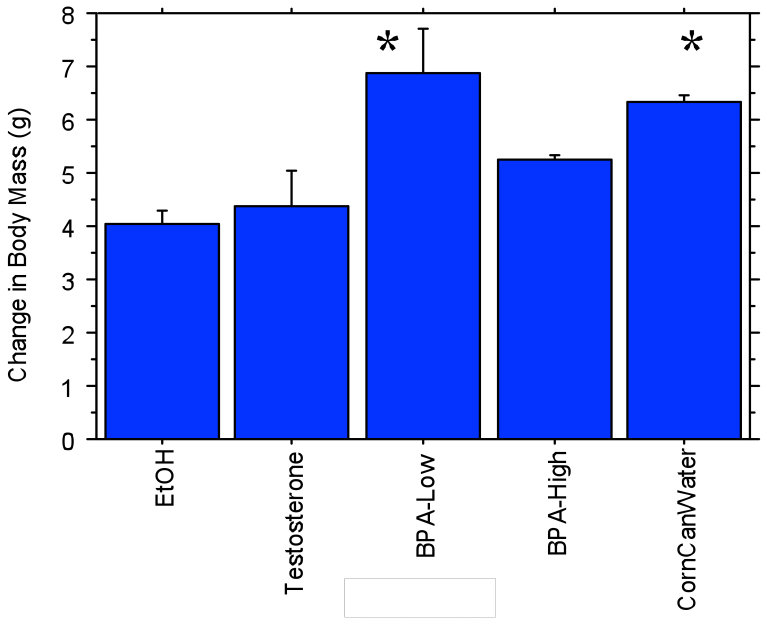

Figure 2. The average change in body mass of each treatment group over the 5-week period in which mice were exposed to different treatments through the consumption of water. Starred error bars indicate significantly greater increase in body mass relative to ethanol controls.

Table 2. (A) ANOVA table of change in body mass (g) of each treatment group. Significant differences between treatment groups (P=0.0036). (B) Student-Newman-Keuls test with S marking the significant differences between treatment groups.

A

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Treatment	4	23.924	5.981	6.279	.0036	25.117	.951
Residual	15	14.288	.953				

B

	Mean Diff.	Crit. Diff.	
EtOH, Testosterone	-.357	1.469	
EtOH, BPA-Low	-2.857	2.133	S
EtOH, BPA-High	-1.200	1.791	
EtOH, CornCanWater	-2.287	1.991	S
Testosterone, BPA-Low	-2.500	1.991	S
Testosterone, BPA-High	-.843	1.469	
Testosterone, CornCanWater	-1.930	1.791	S
BPA-Low, BPA-High	1.657	1.791	
BPA-Low, CornCanWater	.570	1.469	
BPA-High, CornCanWater	-1.087	1.469	

Motor Activity:

A one-way analysis with repeated measures showed that when the behavior analysis was performed on the mice, it was found that activity generally decreased for all treatment groups during the last 15-20 minute segment (Fig. 3). A Student-Newman-Keuls test showed there was a significant decrease in activity between the 0-5 minute segment and the 15-20 minute segment (Table 3). Due to the depressed activity, the last 5-minute segment was omitted when calculating the activity score (Fig. 4).

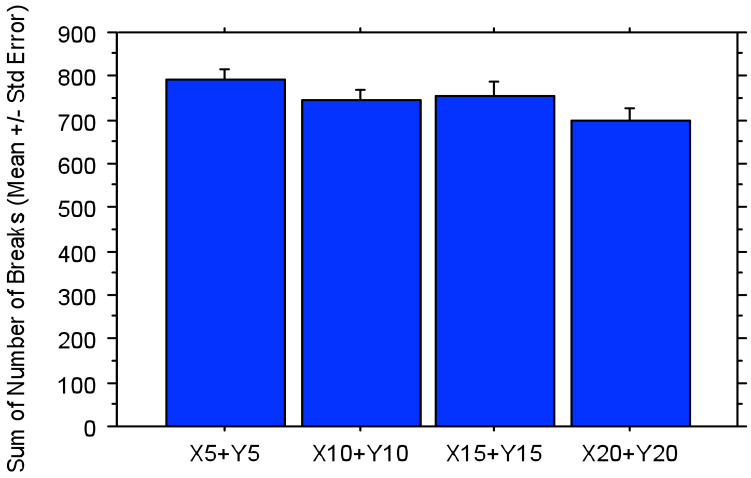

Figure 3. Total 5-minute interval activity for all treatment groups in the form of the sum of x and y-axes breaks in each of the 5-minute intervals.

Table 3 . (A) ANOVA table for 5-minute interval activity of all treatment groups. Significant difference (P=0.0168) between interval activity periods. (B) Student-Newman-Keuls test with S marking significant difference between time periods of X5+Y5 and X20+Y20.

A

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Subject	19	642327.200	33806.695				
Category for Interval Activity	3	89786.700	29928.900	3.694	.0168	11.082	.780
Category for Interval Activity * Subject	57	461804.300	8101.830				

B

	Mean Diff.	Crit. Diff.	
X5+Y5, X10+Y10	42.900	68.551	
X5+Y5, X15+Y15	34.950	57.049	
X5+Y5, X20+Y20	93.750	75.424	S
X10+Y10, X15+Y15	-7.950	57.049	
X10+Y10, X20+Y20	50.850	57.049	
X15+Y15, X20+Y20	58.800	68.551	

There were no significant differences in motor activity levels between the four treatment groups (Fig. 4 & Table 4).

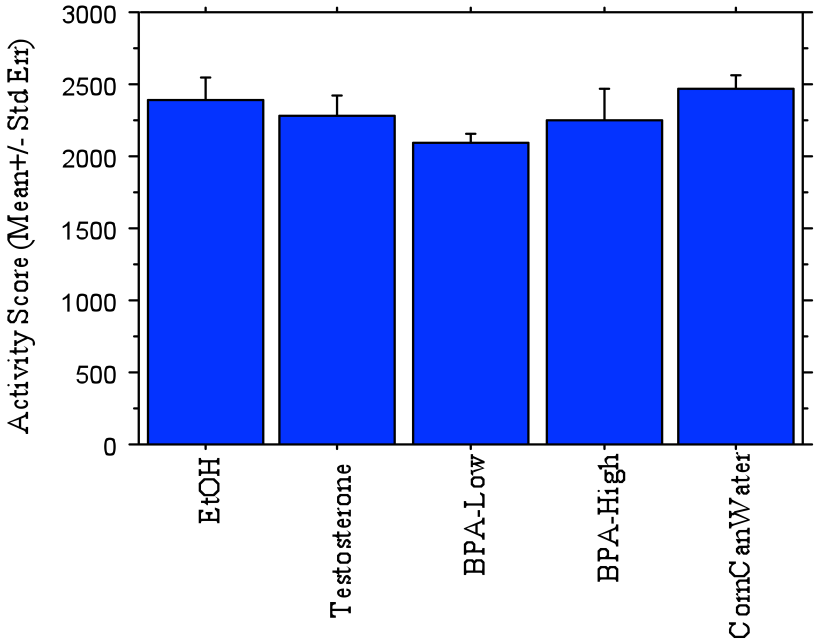

Figure 4. The average motor activity score for each treatment group during the 0-15 minute segments. The number of beam breaks in the x and y-axes were combined.

Table 4. ANOVA table for the average motor activity score of each treatment group. There were no significant differences between treatments (P=0.4820).

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Treatment	4	331094.300	82773.575	.912	.4820	3.649	.220
Residual	15	1361117.500	90741.167				

PSA Levels:

Although there were no significant differences in PSA concentrations between treatment groups (Table 5), there was a slight trend showing the ethanol group to have the lowest PSA levels, whereas the high-dose BPA and autoclaved canned corn water groups had higher PSA levels (Fig. 5).

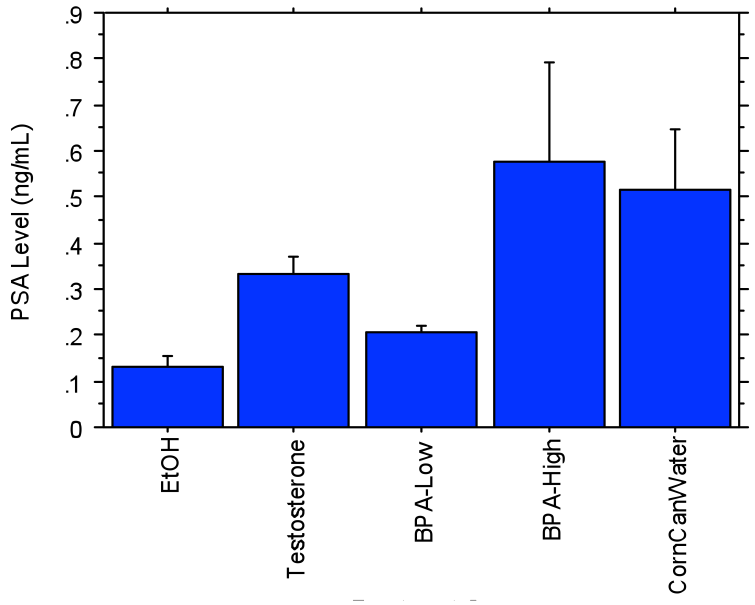

Figure 5. PSA serum concentration of each treatment group (mean +/- standard error).

Table 5. ANOVA table of PSA levels of each treatment group. There were no significant differences between treatment groups (P=0.0661).

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Treatment	4	.583	.146	2.769	.0661	11.078	.612
Residual	15	.790	.053				

Canned Corn Water Analysis:

HPLC/Mass Spectrometry analysis was performed on the autoclaved canned corn water, in order to determine the concentration of BPA that may have leached into the water. A BPA standard diluted to 10 µg/mL in methanol was used as a basis of comparison for the canned corn water. Figure 6-A shows a resulting peak of 227.3 m/z that came out at 3.08 minutes. Although the molar mass of BPA is 228.29 g/mol, the use of negative ions would produce a peak indicating (the molar mass of the compound – 1). The percent area of this peak was 31.32%.

The canned corn water sample was run in triplicate, and the resulting mass spectrometry graph of the third trial indicated that a small peak of 227.3 m/z did also come

out (Fig. 6-B). The other trials produced very similar results, however, the second trial did not produce a visible peak at 227.3 m/z (Appendix Figs. C&D).

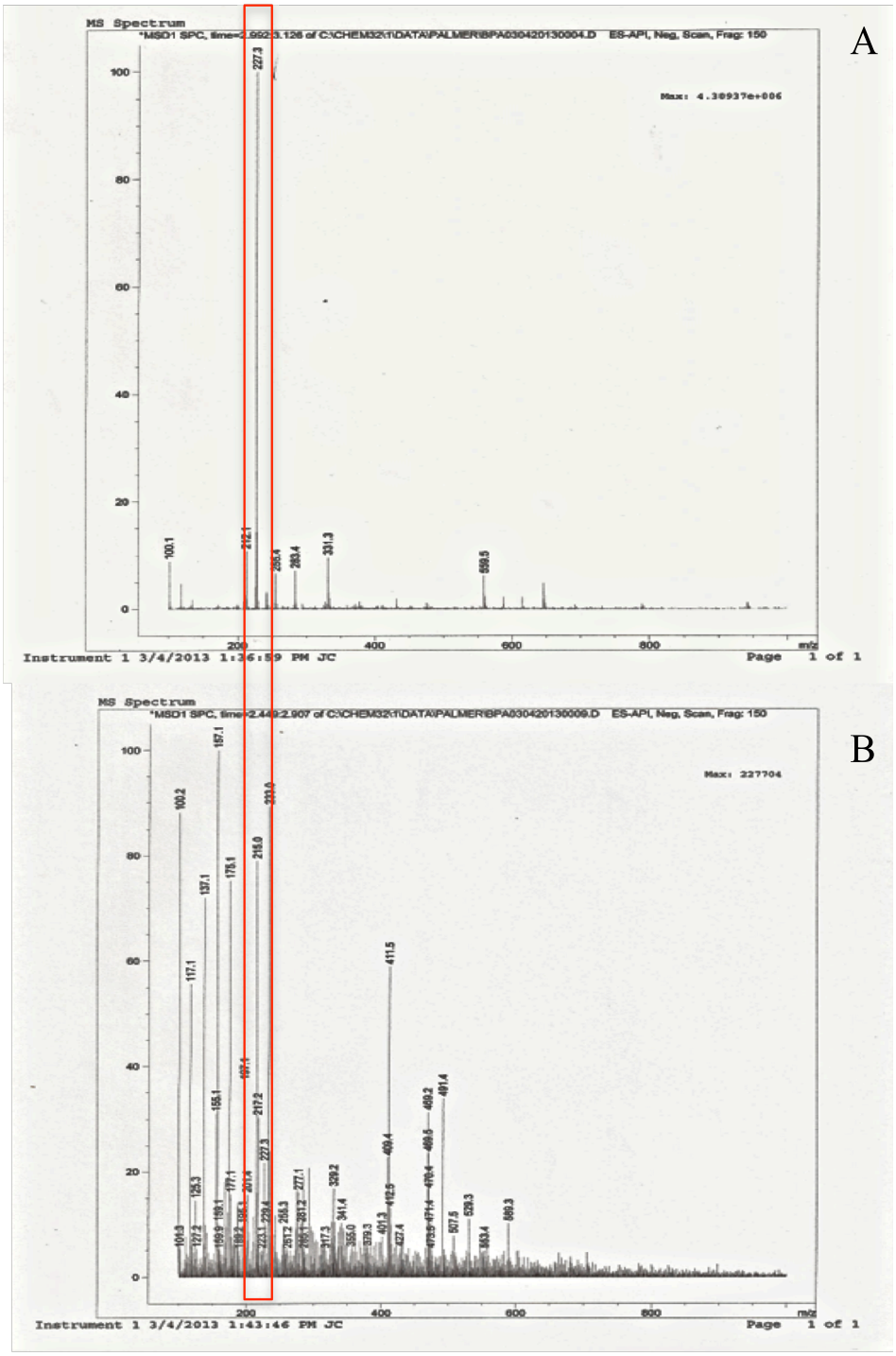

Figure 6. (A) Mass spectrum of the standard BPA (10 µg/mL in methanol). (B) Mass spectrum of sample 3 of the autoclaved canned corn water. The red box indicates the location of the resulting BPA peak (227.3 m/z).

DISCUSSION

The effects of bisphenol A on motor activity and prostate-specific antigen level of mice were tested in this study. In order to ensure a random sample between treatment groups, initial body mass was measured for all mice. There were no significant differences between masses of treatment groups, which indicates that this was indeed a random sample (Appendix Fig. A). The final body masses of the mice in each treatment group were also measured, and significant differences were found between treatment groups. Specifically, the low-dose BPA and autoclaved canned corn water treatment groups showed greater increases in body mass than the ethanol and testosterone treatment groups (Fig. 2). As the low-dose BPA and autoclaved canned corn water showed similar effects of weight gain, so this could be an indication that the canned corn water and low-dose BPA have similar BPA concentrations.

Although weight gain was not one of my original testing endpoints, I found a dose-dependent effect of BPA on weight gain. This is consistent with the results of previous studies, which indicate that exposure to low-doses of BPA (during the perinatal period) can cause an increase in weight gain (vom Saal *et al.* 2012). Akingbemi *et al.* (2004) also found that BPA exposure just prior to puberty also increased body weight gain in mice.

A behavioral assay was performed on the mice, and motor activity was measured by an Opto Varimex Micro Animal Activity System. The number of x and y-axes beam crosses was recorded in 5-minute intervals. I hypothesized that, like the study by Palmer Lab (2011, Unpublished), BPA would also have a potential low-dose effect on motor activity. I expected low-dose BPA and testosterone to increase motor activity in mice. However, I did not find any significant differences in motor activity between treatment groups. Because

activity was significantly depressed across all treatment groups in the last 5-minute testing segment, I omitted the last segment from the composite activity score (Fig. 3). Thus, only the data from the first three 5-minute segments was used. Activity may have been depressed due to the mice becoming tired, or simply losing interest. My results were similar to a previous study, in which BPA was not found to have a significant effect on motor activity in an elevated plus maze (Wolstenholme *et al.* 2012). A previous unpublished study by this lab showed that low-dose BPA significantly increased motor activity in relation to ethanol (negative control) (Palmer Lab 2011, Unpublished). Another study found a dose-dependent effect of BPA on motor activity with a low dose of 0.87 nmol and a high dose of 87 nmol. A high dose (87 nmol) was found to increase spontaneous motor activity in rats, as measured by a Supermex Activity-Monitoring System. This motor hyperactivity occurred at four weeks of age (Masuo *et al.* 2004).

One issue that had arisen within my experiment was that I had to increase the treatment concentration in the water for the mice, because the mice were consuming less water than I had previously anticipated. For the first three weeks of the treatment period, the mice were exposed to BPA concentrations of 1 μg/kg/day and 10 μg/kg/day, instead of 10 μg/kg/day and 100 μg/kg/day. This was corrected, and for the remaining two weeks, the BPA concentrations were altered to desired concentrations. This could have impacted my results, and may be a reason why motor activity results differed from those of the previous study in this lab. This also could explain why I did not see significant differences between treatment groups for PSA levels.

I hypothesized that BPA would increase serum PSA levels and the positive control (testosterone) would show the greatest rise in PSA level in comparison to negative control

(EtOH) followed by low-dose BPA and leachate from autoclaved cans. Results did not support this hypothesis; there were no significant differences between treatment groups for the calculated PSA levels (Fig. 5 & Table 5). Although there were no significant effects of BPA on PSA levels, there was a slight trend that showed higher PSA levels for the high-dose BPA treatment group and the autoclaved canned corn water in relation to the ethanol control. Mouse 12 of the high-BPA treatment group had the highest PSA level of 1.12 pg/mL. The lowest measured PSA level (0.082 pg/mL) occurred in mouse 3 of the ethanol treatment group (Appendix Table D). Surprisingly, the testosterone treatment group (positive control) did not show significant differences in comparison to the ethanol (negative control) PSA levels. This is inconsistent with previous studies, which have found that testosterone exposure elevates PSA levels (Bunting 1995).

Although very few studies have investigated the effects of BPA on PSA levels, previous studies have shown that temporary, early-life exposure to a low-dose of BPA (10 μg BPA/kg body weight) amplified susceptibility to adult-onset precancerous prostate lesions (Ho *et al.* 2006). However, a different means of detecting possible prostate cancer, such as histopathology, may be more accurate for detecting prostate cancer. In fact, the U.S. Preventive Services Task Force has recently (as of May 2012) recommended against PSA-based screening for prostate cancer. They argue that in detecting prostate cancer, the PSA test offers a very low amount of potential benefit, and a significant level of potential harm through people undergoing unnecessary procedures (U.S. Preventive Task Force 2012). There is currently much research and experimentation that strives to find a better screening tool for prostate cancer.

One of the more popular biomarkers emerging as a diagnostic tool for prostate cancer is the prostate cancer antigen 3 (PCA3). In greater than 90% of prostate cancer tissues, PCA3 has been shown to be elevated. Unlike PSA, PCA3 has not been shown to be elevated in normal or benign prostate hyperplasia (Presner *et al.* 2012). An interesting follow up to this experiment would be to replicate my experimental portion, but use PCA3 levels as the endpoint. However, more research is needed in order to fully conclude whether PCA3 should be the recommended means of prostate cancer screening.

Because of interesting significant differences between treatment groups for change in body mass, I wanted to quantify the amount of BPA present in the autoclaved corn cans. I performed an HPLC-Mass Spectrometry using a 10 µg/mL BPA standard (dissolved in methanol). The standards peaked at 3.08 minutes, with a mass-to-charge ratio of 227.3 m/z – indicating BPA. The corn water was run in triplicate in comparison to the standard, and no detectable amounts of BPA were found, or the BPA had degraded to a different form. This does not necessarily mean that no BPA was present in the water; it simply means that the mass spectrometer machine used may have been unable to detect such low concentrations. From this data, I can conclude that if there is BPA present in the water, the concentration is lower than 10 µg/mL. This is consistent with a previous study, in which BPA was found to be present in corn cans in a concentration of 4.5 µg/can (0.01 µg/mL) (Brotons *et al.* 1995).

In conclusion, my hypothesis that BPA would have a negative dose-dependent effect of increased motor activity and elevated PSA levels in mice was not supported. There were no significant differences between treatment groups for motor activity or PSA levels. However, there was a significant potential low-dose effect of BPA on an increase in weight gain. The autoclaved canned corn water treatment group also showed a significant increase

in body weight relative to the controls, possibly due to degradation products of BPA or alternatively, increased nutrition or carbohydrates in corn can water (which were not measured). Future experiments could involve using a different prostate cancer detecting endpoint, as PSA levels have recently been found to be a poor means of testing. Further studies could also test different types of canned food in order to see if one type of food has a greater effect on motor activity or weight gain.

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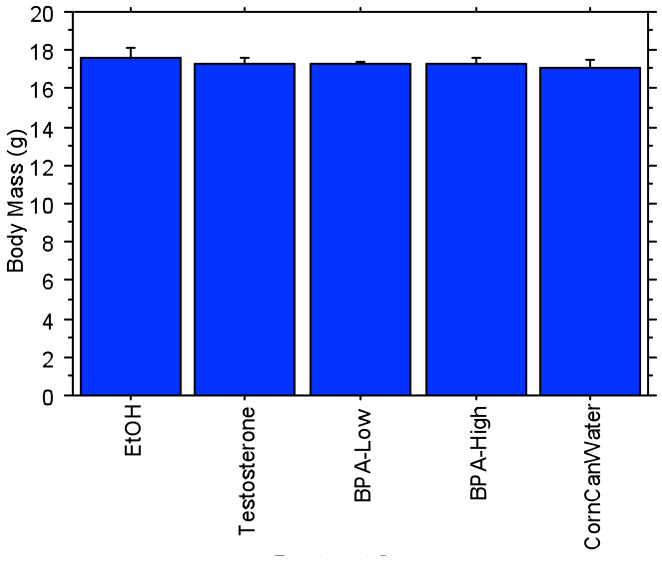
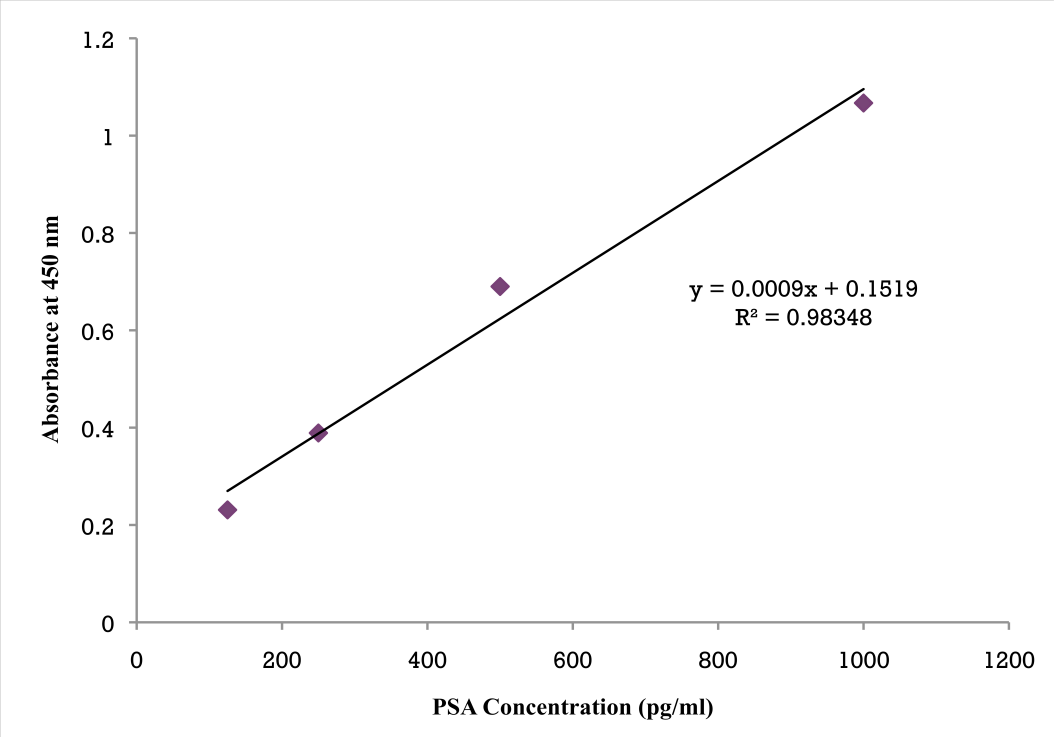
APPENDIX



Appendix Figure A. The average initial body mass of the mice of each treatment group.

Appendix Table A. ANOVA table for the initial body masses of the treatment groups. There were no significant differences between average initial body masses.

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Treatment	4	.595	.149	.273	.8910	1.091	.094
Residual	15	8.178	.545				



Appendix Figure B. The known concentration of the standards from the PSA ELISA kit plotted against the absorbance measured at 450 nm. A trend line of best fit was created in order to determine the unknown PSA concentrations for each mouse.

Appendix Table B. Raw data for the initial and final masses of each mouse and the change in mass. All masses were measured in grams.

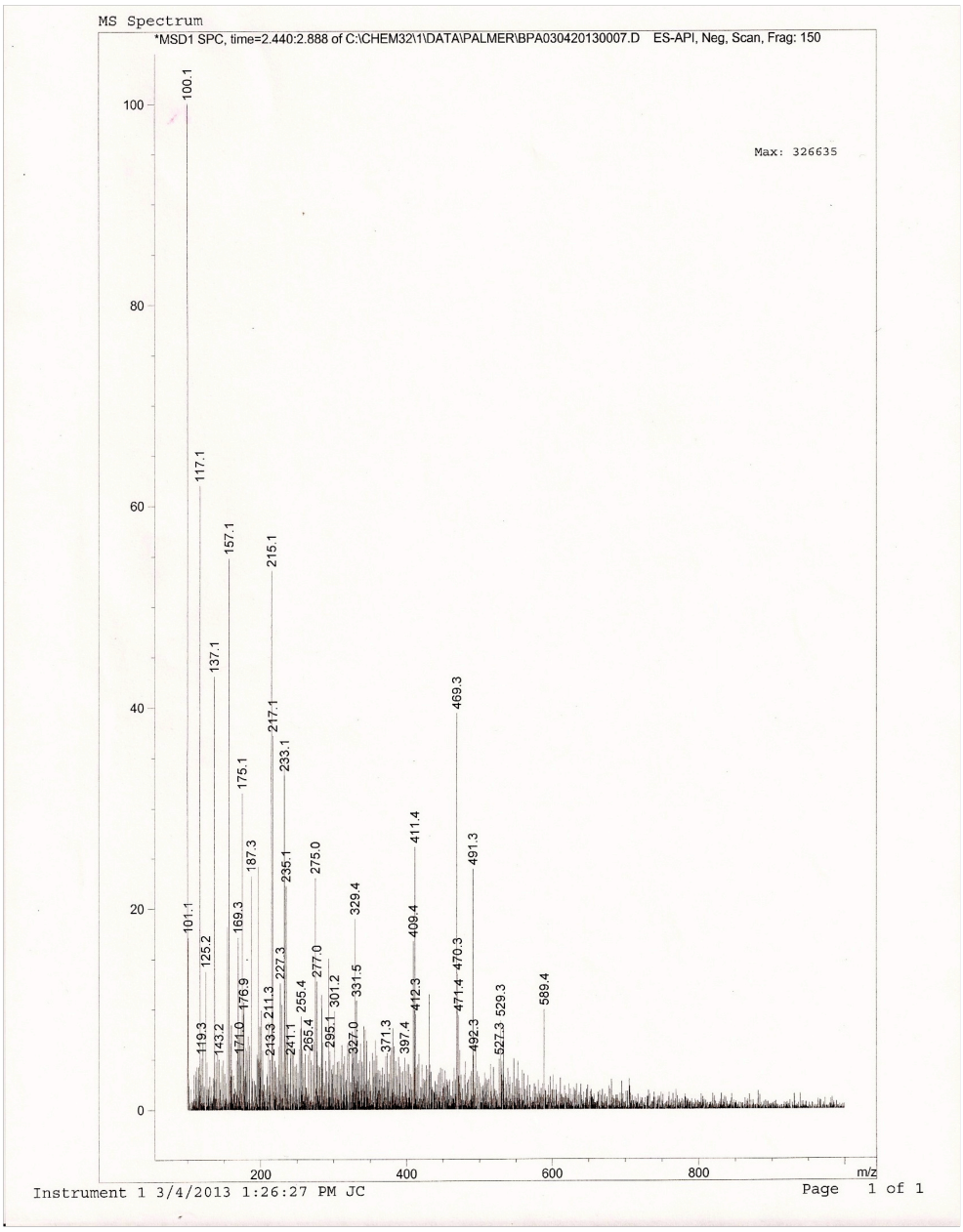
Treatment	Subject Number	Mass 1	Mass 2	Change in Mass
EtOH	1	16.36	20.76	4.40
EtOH	2	17.78	21.03	3.25
EtOH	3	18.78	22.93	4.15
EtOH	4	17.54	21.89	4.35
Testosterone	5	16.55	22.54	5.99
Testosterone	6	16.97	21.37	4.40
Testosterone	7	18.25	20.98	2.73
Testosterone	8	17.32	21.78	4.46
BPA-High	9	17.26	22.65	5.39
BPA-High	10	18.06	23.03	4.97
BPA-High	11	17.15	22.59	5.44
BPA-High	12	16.74	21.89	5.15
BPA-Low	13	17.56	22.69	5.13
BPA-Low	14	16.92	25.88	8.96
BPA-Low	15	17.45	23.78	6.33
BPA-Low	16	17.03	24.19	7.16
CornCanWater	17	16.89	22.87	5.98
CornCanWater	18	15.97	22.53	6.56
CornCanWater	19	17.37	23.83	6.46
CornCanWater	20	18.12	24.42	6.30

Appendix Table C. Raw data for the motor activity of the mice in each treatment group. The number of x and y-axes beam crosses were combined and the activity score combined all beam crosses for each subject.

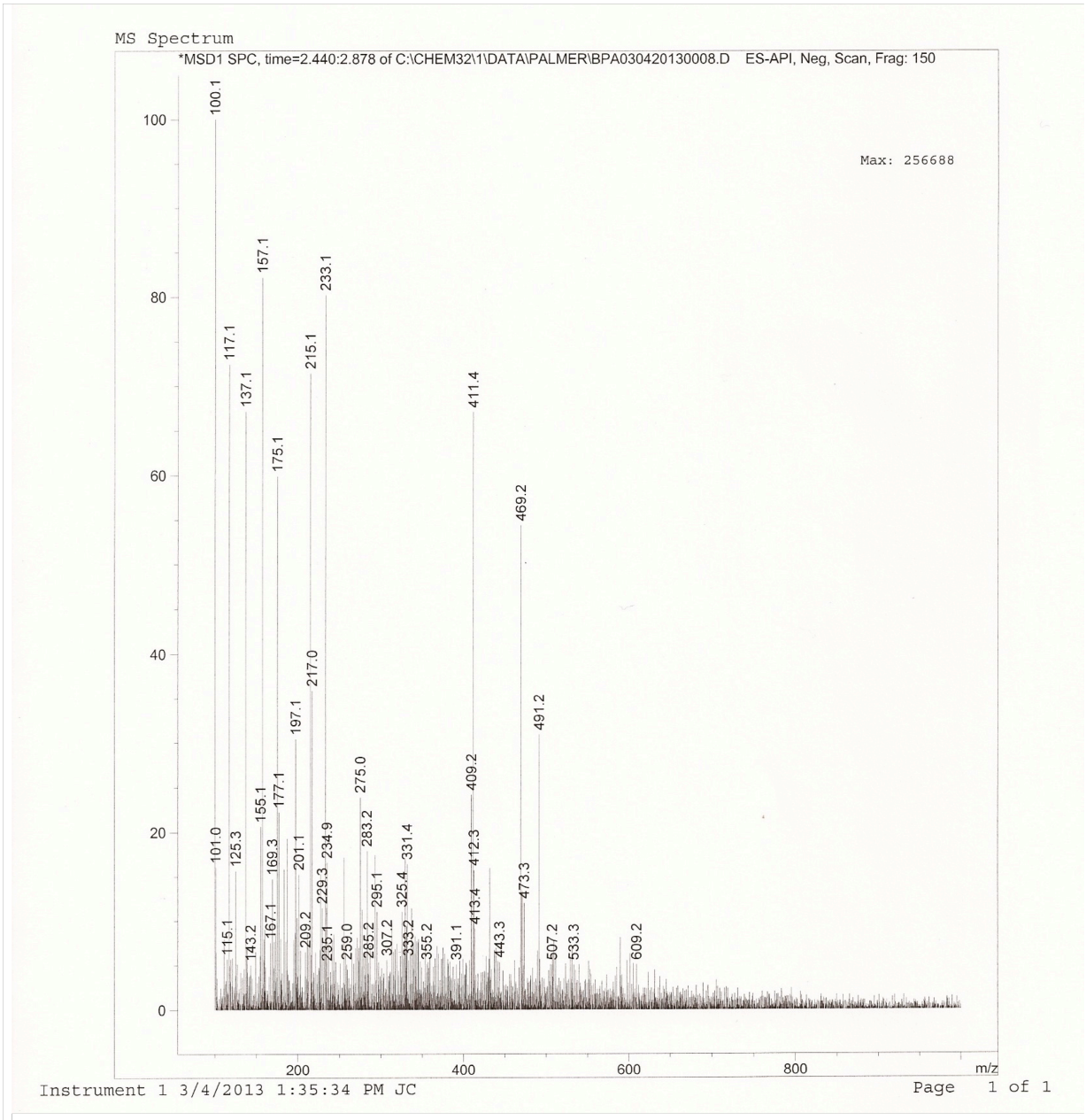
Treatment	Subject Number	X5+Y5	X10+Y10	X15+Y15	X20+Y20	Activity Score
EtOH	1	729	700	791	816	2220
EtOH	2	1064	873	924	478	2861
EtOH	3	806	746	661	546	2213
EtOH	4	792	723	741	843	2256
Testosterone	5	678	592	707	585	1977
Testosterone	6	838	748	705	746	2291
Testosterone	7	738	668	755	784	2161
Testosterone	8	894	798	987	961	2679
BPA-High	9	643	549	665	594	1857
BPA-High	10	624	811	558	578	1993
BPA-High	11	933	885	1057	834	2875
BPA-High	12	777	821	668	644	2266
BPA-Low	13	808	650	639	651	2097
BPA-Low	14	854	654	668	798	2176
BPA-Low	15	809	775	619	561	2203
BPA-Low	16	663	637	575	556	1875
CornCanWater	17	701	755	711	700	2167
CornCanWater	18	857	855	857	876	2569
CornCanWater	19	841	820	904	702	2565
CornCanWater	20	756	887	914	677	2557

Appendix Table D. Raw data for the PSA ELISA plate absorbance at 450 nm and the calculated PSA levels.

Treatment	Subject Number	Absorbance	PSA Level
EtOH	1	0.277	0.139
EtOH	2	0.27	0.131
EtOH	3	0.226	0.082
EtOH	4	0.315	0.181
Testosterone	5	0.408	0.285
Testosterone	6	0.561	0.455
Testosterone	7	0.41	0.287
Testosterone	8	0.417	0.295
BPA-High	9	0.271	0.132
BPA-High	10	0.47	0.353
BPA-High	11	0.777	0.695
BPA-High	12	1.156	1.12
BPA-Low	13	0.323	0.19
BPA-Low	14	0.341	0.21
BPA-Low	15	0.366	0.238
BPA-Low	16	0.323	0.19
CornCanWater	17	0.636	0.538
CornCanWater	18	0.939	0.875
CornCanWater	19	0.478	0.362
CornCanWater	20	0.41	0.287



Appendix Figure C. Mass spectrum of sample 1 of the autoclaved canned corn water. There is a small peak at 227.3 m/z.



Appendix Figure D. Mass spectrum of sample 2 of the autoclaved canned corn water. There is no visibly marked peak at 227.3 m/z.