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Recently Discovered Benzo(a)Pyrene Induced Health Effects and Their Threat to the Inland Empire

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Recently Discovered Benzo(a)Pyrene Induced Health Effects and Their Threat to the Inland Empire

Quinn Navarro, B.A. Biology, B.A. Religious Studies, University of Redlands 2018

Abstract

This study investigated recent discoveries of benzo(a)pyrene (BaP) in order to determine the current threat BaP poses to residents of Southern California. Literature reviews comprise the research done on BaP induced health effects. Primary articles reviewed include “Adverse Effect of Sub-Chronic Exposure to Benzo(a)Pyrene and Protective Effect of Butylated Hydroxyanisole on Learning and Memory Ability in Male Sprague-Dawley Rat” by Xiao Liang (2014), and “Bioaccumulation and oxidative damage in juvenile scallop Chlamys farreri exposed to benzo[a]Pyrene, benzo[b]Fluoranthene and chrysene” by Meng Xiu (2014). These studies conclude that BaP potentially impairs memory and learning abilities, and displays bioaccumulative properties, respectively.

Subsequent research conducted in Lake Perris, CA, provided BaP levels from water and sediment samples. Of the water samples, an average of 5.466 µg/mL BaP (standard deviation of 0.30822) was determined at one site, while the other sites failed to yield detectable concentrations. An total average of sediment samples from all three sediment sites was found to be 0.5318 µg/kg (standard deviation of 0.21332). The concentrations used to suggest BaP induced effects on memory and learning were nearly 3000 fold greater than the average level detected in Lake Perris' sediment. In conclusion, while BaP concentrations in Lake Perris may not raise immediate concern, due to its bioaccumulative properties, it should still be rendered as such. This calls for progressive measures to reduce BaP emission into the environment.
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Introduction

A standard range of respiratory rates among humans is 12-20 breaths per minute, according to the Cleveland Clinic (Vital Signs, 2018). The median of this range is 16 breaths per minute. Therefore, it can be estimated that the typical person takes approximately 23,040 breaths per day. This number of breaths per day can be used further to estimate a total of 8,409,600 breaths a year. At the age of 50, the average person would have inhaled a total of 420,480,000 breaths. Considering that breathing is such an innate behavior, is it not fair to question what went through the body’s respiratory system after taking about 420 million breaths over the course of 50 years? The same question can address another innate behavior: drinking water. The average person drinks about 2.5 cups of water a day, in addition to other beverages. This is an annual consumption of 58 gallons of water. Is it not valid to question what floats in the water that so frequently enters the body?

In this day and age of industrial technology, it is evident that pollution is occurring at concerning rates. Among numerous air pollutants, several in particular are classified as polycyclic (or polynuclear) aromatic hydrocarbons (PAHs). The source of these molecules is most often from combustion processes. According to the National Institute of Health, the products that heavily contribute to the release of PAHs into the atmosphere are coal, oil, gasoline, trash, tobacco, and wood. Moreover, PAHs are further labeled as persistent organic pollutants, a term defined by the Environmental Protection Agency to be a group of toxic organic (carbon-based) chemicals that are persistent in the environment due to their tendency to resist degradation and travel via wind and water (“Persistent Organic Pollutants...”). While PAHs tend to be emitted into the atmosphere, they are actually more
likely to settle in water and organic solvents, such as sediment, due to them being highly lipophilic (Abdel-Shafy, 2016).

PAHs have been studied extensively over the past few decades, resulting in illuminating findings concerning their induced health effects. Of many implications, PAHs are known to be probable mutagens and carcinogens in humans. (IARC, 2010; USEPA, 2001) While PAHs as an entity have been classified as dangerous to human health, it is important to know that there are nearly 100 different molecules that are considered to be PAHs. Each molecule is unique in terms of structure and reactivity, thus contributing their own set of risks. Among the vast number of PAHs is the commonly researched benzo(a)pyrene (BaP), which has a number of reactive oxidative derivatives (some shown below).

Benzo(a)Pyrene Structures. BaP on the left, with two reactive oxidative derivatives on the right. Structures were replicated on ChemDraw.

Benzo(a)Pyrene is studied the most out of the entire group of PAHs. Because of this, BaP is labeled as a human carcinogen, proving to cause numerous health implications to humans (IARC, 2012). Aside from cancer, BaP induced effects on humans include respiratory and cardiovascular problems. Yet, what remains to be determined as potential
threats to the health and well-being of humans is of immense concern. Considering the already evident toxicity of BaP, this study highlights recent discoveries of additional potential health effects induced by exposure. This is done in order to expand the public’s awareness of the dangers anthropogenic organic pollutants pose to humans. Additionally, research was conducted in order to determine the concentration of BaP in Lake Perris, a frequently used recreational site in Southern California. Water and sediment samples used to detect traces of BaP, which would indicate the threat it poses to Inland Empire residents with regards to their memory and learning abilities.

**Literature Review: Memory and Learning Abilities**

After reviewing other studies that suggest Benzo(a)Pyrene induces neurobehavioral toxicity (Saunders et al., 2006), Xiao Liang et al. set out to investigate the BaP induced effects of oxidative stress on hippocampal neurotransmitter systems (2014). Their approach included several points of analysis. They observed butylated hydroxyanisole (BHA), a widely known antioxidant, as a protective agent against harmful chemicals, and tested its ability to do so with BaP. They also recognized that sodium-potassium-ATPase and calcium-magnesium-ATPase are crucial enzymes with regards to maintaining neuronal excitability, stabilizing neuronal cell walls, and contributing to cellular volume control (Erecinska and Silver, 1994). Due to these enzymes’ susceptibility to free radical oxidation, BaP’s influence on their activities was investigated. The overarching hypothesis of this study can be worded as such: Low dose BaP exposure in sub-chronic toxicity could induce changes in oxidative stress and efficiency of ATPase, thus impairing memory and learning
abilities. Also, BHA may play a role in preventing the impairment of such abilities that can be caused by such effects of BaP exposure (Liang et al., 2014).

In order to test the learning and memory ability of rats exposed to BaP, their performance in a Morris water maze (MWM) was observed. The MWM was a circular pool where the rats underwent two different tests. For each test, the rats were divided into 4 different groups: BaP induced, BHA induced, BaP + BHA, and control. Rats exposed to BaP received doses of 2mg/kg of their weight a day, while those exposed to BHA received doses of 50mg/kg of their weight a day. All rats received daily administrations for a period of 90 days prior to undergoing any tests. The hidden platform test consisted of a 4 trial procedure. After undergoing habituation and acclimation processes, the rats were set on top of a small platform that lay 1.5cm beneath the surface of the pool water. The water was mixed with milk powder, which made the liquid translucent so that the rats could not see the platform that was beneath the water. This platform was centered in one of the 4 quadrants that divided the pool. Then, each rat was placed in the water and their goal was to escape the pool by locating the hidden platform. Each trial measured the time for the rat to find the platform. If any rats failed to find the platform in 120 seconds, they were guided to the

![Figure 1. Results of Hidden Platform Tests. Escape latency indicates the measured time for rats to locate the platform. Days chronologically indicate the trial numbers. * Indicates statistical significance from the control. # Indicates statistical significance from BaP+BHA. P-values were less than .05.](image-url)
platform and received a score of 120 seconds. Each rat was tested once a day for 4 days straight. Results of this test can be viewed in Figure 1.

On the fifth day, the rats underwent a 120 second probe test. This test consisted of the rats being placed in the same pool, but the platform was removed. Using an automated tracking system, the rats’ swimming was tracked. The number of times a rat swam through the quadrant that originally contained the platform was tallied. The amount of time spent in the quadrant that originally contained the platform was also measured. The results are displayed in Figure 2.

Proceeding these tests, hippocampus tissue was extracted from rats in order to quantify the amount of malonaldehyde (MDA), as well as measure the activity of the following enzymes: sodium-potassium-ATPase (Na-K-ATPase), calcium-phosphorus-ATPase, (Ca-P-ATPase), and superoxide dismutase (SOD). ATPase enzymes were estimated using the rate of formation of free inorganic phosphate during ATP synthesis. All proteins were quantified via Bradford assays. These results can be seen in Figure 3 and 4.
The presence of MDA in hippocampus tissue indicates oxidative tissue damage. With these levels increasing significantly in the presence of BaP, this suggests BaP exposure can directly induce oxidative tissue damage to hippocampus tissue. BaP’s toxicity is more threatening when considering it was also observed to inhibit the activity of SOD, which actively removes free oxidative species from cell membranes. It was also observed that BaP inhibited both ATPase enzymes. For every enzyme, BHA minimized BaP induced effects,
suggesting that BHA’s antioxidant properties apply to BaP. These observations contribute to the potential for BaP to impair memory and learning abilities.

The results of the MWM further strengthen this claim. For the hidden platform tests, the escape latencies (time required to find the platform) for the BaP exposed rats were significantly larger than the control. This suggests the rats needed more time to remember where the platform was compared to the control group. The probe test paralleled these results, with BaP exposed rats spending less time on the quadrant that contained the platform compared to the control. These rats also crossed over the original location of the platform a significantly less number of times compared to the control. This contributes to the suggestion that BaP impairs memory and learning abilities. Additionally, BHA proved to reduce the effects of BaP by indicating significantly different results from the BaP group in both the hidden platform and probe tests.

Conclusively, the results show BaP’s ability to inhibit enzyme activities through free radical inactivation. Its free oxidative species also contribute to the accumulation of MDA in hippocampus tissue. Consequently, BaP exposure can lead to impaired memory and learning abilities.

**Literature Review: Bioaccumulation**

Meng Xiu et al. set out to provide further insight to toxic properties of 3 different PAHs. Among these PAHs was BaP, and one of the properties investigated was bioaccumulation. This is defined to be the buildup of a substance, in this case BaP, within an organism’s tissues at a rate that is greater than the organism’s ability to excrete it. The overall hypothesis of this study was that BaP would exhibit bioaccumulative properties,
thus presenting increased concentrations of BaP in scallop tissue after prolonged periods of time (Xiu et al., 2014).

Juvenile scallops were held for a period of 21 days in tanks that contained different concentrations of BaP. There were a total of 4 groups that classified these scallops. There was a control group, and three groups exposed to BaP at different concentrations (0.01, 0.2, and 4 µg/L). Scallop tissue was collected at several points throughout the 21 day period and analyzed for BaP concentrations via high performance liquid chromatography. Results for days 1, 10, and 21 can be viewed in Table 1.

![Table 1. BaP Quantification in Scallop Tissue. Significant differences compared to the control are indicated with asterisks. * uses a p-value of .05, while ** uses a p-value of .01.](image)

The results from this experiment indicate that BaP does in fact accumulate in scallop tissue. Significant results were obtained for the 0.2 and 4 µg/L concentrations (compared to the control). The 0.01 µg/L group failed to produce significant results, but still show how BaP accumulates in tissue compared to the control group. This may be attributed to the higher values of standard deviation among the analyzed scallops. However a trend was still observed for this particular concentration that suggests BaP accumulates in scallop tissue after prolonged periods of time. With these results, BaP may have increased potential for toxicity with its accumulation in organism tissues.
Conducted Research: Introduction

Lake Perris is a commonly used recreational site for residents of the Inland Empire. It provides a place for people to hike, fish, swim, kayak, and other outdoor activities of the sort. BaP poses a threat to humans by its presence in Lake Perris, which is a place where humans would be exposed to this pollutant, as these outdoor activities often immerse humans into solutions containing BaP. Sampling locations were strategically chosen in order to develop a range of values of BaP concentration that represents the ecosystem of the lake as a whole. This meant collecting samples from different geographical locations while considering the amount of human exposure to each site.

Conducted Research: Methods

Methodology for collecting samples from Lake Perris included 2x250mL water samples from 3 different locations. Sediment samples were obtained by collecting soil that was approximately 30 cm below the surface. Upon retrieving the samples, sediment was dried at 80 degrees Fahrenheit for 3 hours. Once dried, the sediment was sonicated in hexane using a Branson 2510 Sonicator (final volumes of 50 mL). Water samples underwent a 3 successive hexane extractions with a net ratio of approximately 1:1. Each hexane solution (both water and sediment samples) was concentrated with a Kuderna-Danish OA-SYS Heating system to a volume ranging from 2-10mL.

BaP standards were created using pure BaP and hexane (50 µg/mL, 100 µg/mL, 200 µg/mL, 400µg/mL). Standards were analyzed with a Jasco FP-750 Spectrofluorometer. The excitation wavelength used was 384 nm (Fioretti et al. 2008). Absorption of BaP emission was determined by peak areas analyzed within the parameters of 448 and 463 nanometers.
Peak areas from samples were compared to those of the generated standard curve which allowed for the determination of BaP concentration. Values for sediment samples were converted to µg/kg of original samples, and water samples to µg/mL of original samples.

**Conducted Research: Discussion & Conclusion**

Concentrations of BaP in samples taken from Lake Perris were successfully determined. Values for determined BaP levels in sediment and water samples can be viewed in the appendix. As expected, concentrations were higher in sediment samples. This is attributed to BaP’s lipophilic properties.

Variation in levels of BaP by sampling sites can be explained by observing the sampling site map, which is also in the appendix. Sediment Site 1 was near the primary outflow for the lake. This can be understood to be the location where the solutes would be very dilute, if not the most dilute. As a result, the lowest levels of BaP in sediment were found here. Sediment Site 2 was at a remote location, where the soil was quite marshy. There was a bike path nearby, but for the most part it was surrounded by plants and most likely does not get disturbed by humans. It would make sense that BaP levels would be detected at higher concentrations compared to Site 1, but not to Site 3. Sediment Site 3 was on a beach that serves as a location for human interaction with the lake. It is also

![Image](image.png)

**Figure 6.** BaP Levels in Sediment Samples. Average concentrations of BaP in µg/kg for each sampling site is displayed in the bar graph. Error bars are set by standard deviations.
located near boat docks, which can further serve as a source of BaP emission. Thus, it comes as no surprise that BaP levels in sediment were highest at Site 3 compared to the other sites.

Variation in BaP concentrations at water sampling sites are also within reason. Water site 1 was also at the lake’s outflow location, which is where the water would be very dilute, thus containing untraceable levels of BaP. Water site 2 would also expect to have dilute samples of solute, as this was a deep water sample taken at a depth of 60 m below the surface of the lake. Water site 3 was the only site to yield detectable BaP concentrations. This can be understood from the high levels of human interaction at this site, in addition to the location being near boat docks. Figure 8 provides a map of these sampling sites.

To get an idea of the threat BaP poses to residents of the Inland Empire, the average concentrations of BaP found in Lake Perris can be visually compared to other values. One study conducted on Southern California’s sediment and soil produced average BaP concentrations that are nearly 300 times greater than the concentrations detected in Lake Perris sediment (LaPierre, 2008). Of
importance is that this study had a much larger sample size, as it obtained samples from all over Southern California. Anyhow, the dosage administered to the rats by Liang et al. is well over 3000 times greater than the sediment samples in Lake Perris. Both these values are 100 times larger, in addition to the factors used to compare the lake’s sediment samples, when compared to the water samples. Table 2 provides these values.

Of course, human fallibility should be considered when observing the variation in detected BaP levels. Improper sampling methods can attribute to these variations, such as traces of sediment in the water sample at site 3, contaminated glassware and solvents, and loss of samples due to condensation in storage. Furthermore, the sampling population may not prove representative of the lake as whole due to limitations of sample size. Replicating the study would prove useful in confirming the conclusions drawn from this research.

**Overall Conclusion**

With the detected levels of BaP in Lake Perris displaying no surprising trends, it should be noted that the values are drastically less than those administered to rats by Liang et al.. Rats received doses of BaP at 2 mg/kg of their weight. This is over 3000 fold greater than the concentrations found in Lake Perris. However, the presence of BaP in this lake should not be easily dismissed. As shown by Xiu et al., BaP’s bioaccumulative properties

<table>
<thead>
<tr>
<th>Lake Perris Water</th>
<th>Lake Perris Sediment</th>
<th>So-Cal</th>
<th>Rat Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.466 E⁻⁶ mg/kg</td>
<td>5.318 E⁻⁴ mg/kg</td>
<td>0.16 mg/kg</td>
<td>2 mg/kg</td>
</tr>
</tbody>
</table>

*Table 2. Comparative B(a)P Levels. Average concentrations of both water and sediment samples in Lake Perris are shown alongside both LaPerrys’s average value for sediment and soil samples in Southern California (2008), and Liang et al.’s dosage administered to rats [2014]. All values are given in mg/kg.*

should present BaP as a threat to organisms that interact with Lake Perris (2014). Recalling BaP’s lipophilic properties, this bioaccumulative property implies that BaP would remain in tissue of organisms as opposed to the liquids that would excrete it (blood, urine). Whether it be fish that live there, animals that drink from the lake, or humans that use it recreationally, the threat BaP poses to these organisms should not be undermined. Progressive measurements to reduce BaP concentrations are advised not only for Lake Perris, but for Southern California altogether.

References


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