K10 Study of the Effects of pH, Temperature, and Time on the Migration of Endocrine Disrupting Compounds From Polyethylene Terephthalate (PET) Bottles

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After attending this presentation, attendees will be informed of an important toxicological topic that has an effect on everyone. The attendees will also be exposed to new optimized extraction and analysis methods of several endocrine disrupting compounds which they can apply to further this aspect of work or use in their own current research projects.

This presentation will impact the forensic community by informing the audience of the constant presence of endocrine disrupting compounds (EDCs) and their possible effects on our health and environment. Although this work is only a start, it may lead to new safety measures to ensure that all levels of EDCs are below the oral reference dose. This work could have an effect on humanity because if these new measures were established, it could affect our everyday lives. Also, several extraction and analysis methods that are commonly used in forensic laboratories, have been used in this study. These methods include solid phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS). This presentation may allow the audience to become more knowledgeable about these different techniques which they could possibly apply to their own work. Finally, a major challenge in this work was preventing external contamination. The clean techniques that were utilized in this study could also be useful in a forensic laboratory.

Several toxicological studies have shown that many common endocrine disrupting compounds (EDC), specifically those that display estrogenic properties, could cause toxicity from chronic exposure to levels as low as 20 µg/kg/day. Any compound that has the ability to alter hormonal homeostasis is considered to be an endocrine disrupting compound. Effects of exposure include abnormal cell growth, teratogenicity, liver injury, abnormal thyroid function, and reproductive toxicity. Two types of EDCs, alkylphenols and phthalates, have been found to migrate from plastic containers into the food supply. Recent studies have suggested that phthalates may have a cumulative effect, which has lead to a great interest in studying their presence in the environment. Two different extraction methods, solid phase extraction (SPE) and liquid liquid extraction (LLE), have been performed and compared. LLE has been found to be the most effective extraction technique for studying trace levels of EDCs in water. Gas chromatography- mass spectrometry (GC-MS) was chosen as the analysis method because of the reduced risk of contamination when compared with liquid chromatography- mass spectrometry (LC-MS). Many studies have suggested that LC-MS is not an efficient analysis method when studying EDCs, such as phthalates, because of the use of organic solvents and plastic tubing which can increase the risk of sample contamination. The specific compounds that are being studied include dimethyl phthalate (DMP), dimethyl terephthalate (DMT), diethyl phthalate (DEP), dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), bis-(2-ethylhexyl) phthalate (DEHP), nonylphenol, and octylphenol. Several conditions were established in order to study the effects of temperature, plastic thickness, and pH on migration. When studying the effects of temperature, two plastic bottles were filled with drinking fountain water and were heated to 60°C for six hours. Samples were collected after every two hours. The second bottle had a thickness that was 50% less than the first bottle. Another bottle was filled with fountain water and stored at room temperature. This was used as a control for the temperature experiment and was sampled after three days. The effect of two different pH values was also studied. The two values used were 3.75 and 6.6. These values were chosen because they are comparable to different beverages that are commonly stored in plastic containers. The pH of each sample was adjusted by adding glacial acetic acid until the desired pH value was obtained. A third bottle was filled with fountain water and was not adjusted. This bottle was used as a control for the pH experiment and had a pH value of approximately 7.2. The three pH samples were collected after one day. When heated to 60°C for two hours, five of the eight compounds were detected. These compounds include DMP, DMT, DEP, OP, and DEHP, at concentrations of 2.144, 1.78, 1.258, 0.783, and 1.539 µg/L, respectively. These compounds were found in the control sample at concentrations of 0.619, 0.875, 0.18, 0.475, and 0.483 µg/L, respectively. Therefore, heating plastic to 60°C increased the amount of migration occurring. When the thickness of the plastic bottle was reduced by 50% these compounds were found at concentrations of 1.627, 0.979, 0.779, 0.601, and 1.562 µg/L, when heated at 60°C for two hours. Although lower concentrations were found to migrate from thinner plastics when heated, the migration occurred over a longer period of time when compared to the 50% thicker plastic. Migration was also found to increase as the pH of the water decreased, when stored at room temperature. All reported concentrations were determined using calibration curves prepared from standard solutions. Standard solutions containing all eight compounds were prepared at concentrations of 5×10^5, 5×10^4, 5×10^3, 500, 50, 25, 12.5,

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and 6.25 µg/L. These standard solutions were also used as controls in order to determine the retention times of each of the studied compounds. A selected ion monitoring (SIM) method was also

developed and used to analyze trace levels of these compounds. When compared to a NIST library the highest matches obtained were 80% (DMP), 81% (DMT), 81% (DEP), 73% (OP), and 72% (DEHP). All identified compounds had approximately the same retention times as the standard solutions. The average concentration found from all experiments was 1.046 µg/L. When comparing to the previously reported value of 20 µg/kg/day, a 70 kg person would have to drink approximately 1339 L/day in order to be exposed to this toxic level. Overall, temperature, pH, and plastic thickness have been found to have an effect on EDC migration from PET bottles; however, all concentrations have been below any known toxic level. Further work will be performed in order to study the effect time may have on migration and to test the reproducibility of this method. It is important to understand the effects any of these factors may have on EDC migration because it may or may not suggest that certain safety measures need to be established in order to ensure that all levels are below the oral reference dose (RfD) values set by the United States Environmental Protection Agency.

Toxicology, Endocrine Disrupting Compounds, Gas Chromatography-Mass Spectrometry

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