A Study of Nonylphenol Isomers Using ESR Spectroscopy

Nathan E. Houser '21 and Herbert J. Sipe

Department of Chemistry, Hampden-Sydney College, Hampden-Sydney, VA 23943

INTORODUCTION

A study by John A. McLachlan titled "Synergistic Activation of Estrogen Receptor with Combinations of Environmental Chemicals" scared scientists and casual readers alike. The study proposed that natural estrogenic compounds alone "have potencies 1/50th to 1/10,000th those of DES (synthetic estrogen Diethylstilbestrol)" but that combined these potencies change [1]. McLachlan claimed that a combination of any two of his environmental chemicals lead to synergistic increases of their activation potency; even going so far as to state that a mixture of endosulfan and dieldrin were "160 to 1600 times more potent than the individual chemicals" [1]. His findings also showed that these combinations lead to sex reversal of male determined turtle eggs [1]. These results would show an urgent need to end the use of estrogen disrupting chemicals and clean as many synthetic forms from the environment. Now, while there is certainly no reason to not do the later of these, McLachlan's work was not reproducible by multiple lab studies and he had to release a statement condemning his original paper [2].

McLachlan's paper spawned multiple works of research due to the common fear amongst people that we were exposed to an unhealthy amount of synthetic chemicals. In 2003 a report titled "Critical Evaluation of Observed Adverse Effects of Endocrine Active Substances on Reproduction and Development, the Immune System, and the Nervous System" focused on addressing what effects these Endocrine active compounds (EACs) had on only two systems of the body [4]. It was hard pressed, even with this limited scope of study, to conduct the full length of research it intended to, but nonetheless established data. John O'Connor summarized that how sensitive a bodily system was to chemical presence depended on the compound in question [4]. While the scope of the study was limited, O'Connor collected enough data to conclude that a bodily system's sensitivity to a chemical presence is dependent upon the compound in question [4]. It was noted that the data acquired during this study offered little conclusions as to EAC's impact on the body, a question that remains unanswered.

Previously in 2002 the report "Metabolism and Kinetics of Bisphenol A in Humans at Low Doses Following Oral Administration" found itself seeking the public's answers. It addresses the communal attention and is focused on the effect of one specific compound on the human system [5]. This study found that even after ingesting above average amounts of Bisphenol A the compound was eliminaed within 24 hours [5]. This study noted that the only thing that made Bisphenol A dangerous was a large dosage and that through our metabolism Bisphenol A becomes unreactive with endocrine hormones [5].

Bisphenol-A, more easily referred to as BPA, is a chemical found in a wide range of residential, commercial, and industrial applications that is a part of this fear [3]. As an estrogen disruptor, many people fear that through medical devices and food containers they are indirectly exposed and will eventually be affected by the toxicity [3]. Research conducted by the National Institute of Environmental Health Sciences (NIEHS) and National Center for Toxicological Research (NCTR) found that this fear may be a reality through their study of rats [3]. They found statistical increases of developmental deformities in Sprague-Dawley rats correlating to the females increased BPA exposure, both in dosage and duration, during breeding [3]. This proposes prolonged exposures may be detrimental to human development, and, with more research, BPA plastics may need to be thrown out and replaced entirely.

With EACs and, more specifically BPA, being such a concern and interest, it suits the situation to further study their interaction regarding their point of interference. To carry out such a task we not only look at the compound in question, Bisphenol A, but also its family molecules sharing traits similar and comparable. The molecule I chose to focus on in this experiment was 4-para Nonylphenol due to its similar phenol ring and link in research to estrogenic activity [7]. Due to certain complications with this compound it was deemed worthy to study certain family molecules as well. I studied these compounds using an Electron Spin Resonance (ESR) spectrometer developed to detect the absorption of energy by a radical electron aligned with a magnetic field [6]. The radicals formed by the oxidation of these compounds are short lived, so the instrument must be well calibrated to receive a signal and constantly monitored throughout a trial to receive optimal data [6].

This research is meant to garner a better understanding of the base reactions these molecules have. I studied BPA, Nonylphenol and family phenols to test their reactions against each other for further study. Hopefully, a better knowledge of these

chemicals can lead to a better understanding of their effects on the body.

MATERIALS & METHODS

The ESR Spectrometer is an advanced instrument used in experimentation. Before running with a flat cell and actual compound, we studied stagnant radical compounds. The list of these compounds includes Fluoranthene, Anthracene and Pyrene. These compounds required a simpler method, as I didn't need to focus my attention on so many dynamic functions such as flow rate and clogging of the cell. To run these compounds all that was necessary was to place the appropriate vial into the cell, turn on and tune the instrument, then adjust the instruments gain, modulation etc. to obtain the optimal spectra.

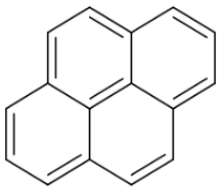


Fig. 1: Pyrene (C16H10) [8]

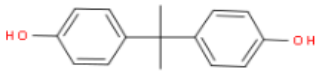


Fig. 2: Bisphenol A (C15H16O2) [8]

The compounds BPA and Nonylphenol were the first chemicals worked with by using fast flow spectroscopy during this experiment. Normal experiments were made to 2milliMolar (mM) with four liters, which included ethanol to suspend the compound into solution and di H2O. To oxidize the Nonylphenol and BPA 1.8mM solutions of cerium sulfate (Ce(SO4)2) were made with 100ml of sulfuric acid and di H2O. Hemoglobin was also tested as a possible biological oxidizing agent to Nonylphenol, replacing the costlier horseradish peroxidase; this solution contained 2 L with 42.14mg DTPA 1.7g hemoglobin and purified water. All solutions were bubbled before experimentation with N2 gas; the hemoglobin solution was bubbled before adding the Hemoglobin. These solutions were adjusted accordingly during some experiments doubling the molarity and increasing the liters of solution from four to five to increase the intensity of the spectra. Other family molecules used for research were 4-Isopropylphenol, p-tert-butylphenol, 4-Ethylphenol and p-cresol were all in solution the same way and kept in ideal conditions.

To identify impurities and flaws in our study, the BPA and Nonylphenol compounds were run through a gas chromatography mass spectroscopy column. This displayed their relative purity and molecular weight of any deviant molecules in solution. Understanding these compounds' purity helped to shed light on more interesting aspects of their spectra. Optimizing flow rate (mL/Min) and power level (milliwatts) to a desirable intensity also allowed spectra with flushed out hyperfine interaction. This made obtained spectra more recognizable in both their detailed hyperfine structure and to the Winsim database.

Fast flow ESR spectroscopy was accomplished by using a flat cell attachment to the JEOL RE-1X Electron Spin Resonance spectrometer and to interpret data Winsim 2008 was installed. The flat cell attachment allowed for speedy oxidation of these phenol compounds enough to recognize they unique absorption by the instrument. WinSim interpreted datapoints set out by the computer within a scanned frequency. It was then possible to identify different hyperfine interactions and optimize the identification of these interactions using parameters.

RESULTS

The goal of this experiment was to compare the radical of Nonylphenol with BPA. Although we can compare the spectra of both, impurities in the Nonylphenol compound obscure detailed hyperfine interactions which we expect in its spectra.

To further understand why this happened we ran both compounds through GCMS. This yielded the results we expected; the BPA sample showed as a single spike on the chromatograph with its highest mass peak around the lower 200. Meanwhile, the Nonylphenol isomers we worked with showed multiple peaks between 12 and 13 minutes on the chromatograph and mass peaks lower than anticipated suggesting more isomers that were not as we anticipated shown in figure 3. These different isomers may cause overlap in their hyperfine interaction obscuring the spectrum shown in figure 4.

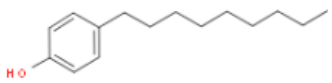


Fig. 3: 4-Nonylphenol (C15H24O) [8]

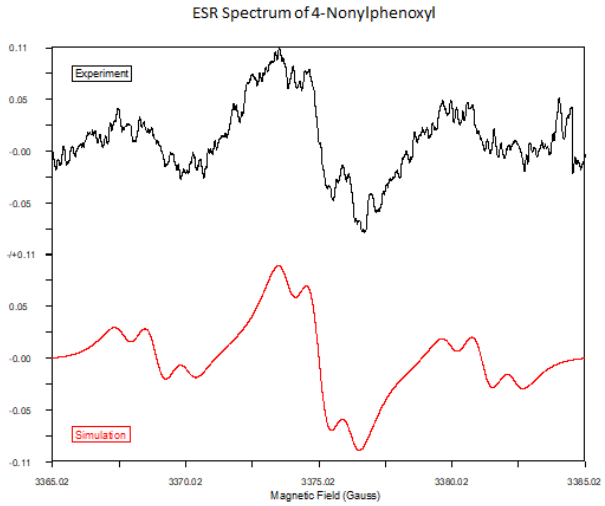


Fig. 4: ESR Spectrum of the Nonylphenoxyl radical oxidized with 1.8mM cerium sulfate

In an attempt to confirm that the interaction we saw were smudged by alternate hyperfine interactions, we tested four other compounds of the same family of molecules. All compounds including 4-Isopropylphenol, p-tert-butylphenol, 4-Ethylphenol, and p-cresol showed distinct hyperfine interactions, characteristic with our simulation for their hydrogen interactions. These results indicated the purity of our Nonylphenol isomer sample was to blame, and with further experimentation, purifying these isomers and testing the new sample for further hyperfine interaction would be the next step.

The radicalization of Nonylphenol using the catalytic process of Hemoglobin, like the catalytic process of horseradish peroxidase shown in figure 5, was our final experiment. This took time to create both solutions properly and, unfortunately, during the experiment the flat cell clogged with polymer and needed cleaning.

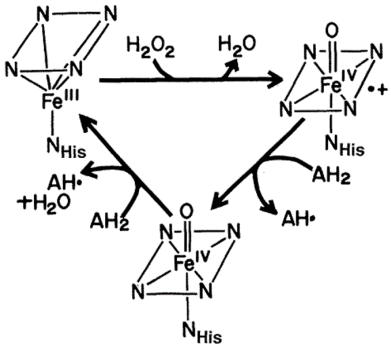


Fig. 5: catalytic cycle of horseradish peroxidase like that of Hemoglobin used to oxidize Nonylphenol

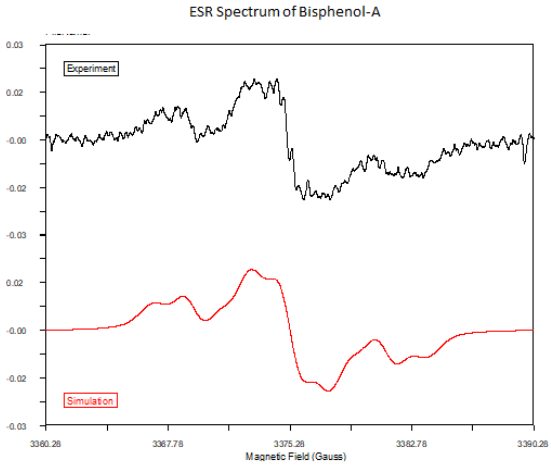


Fig. 6: ESR spectroscopy of BPA with WinSim simulation

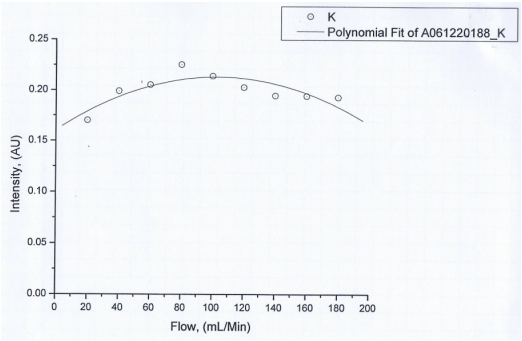
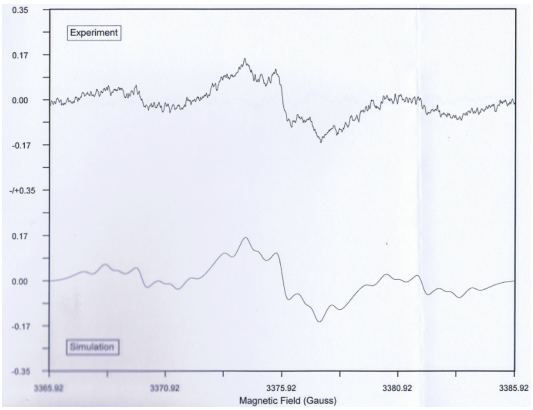


Fig. 7: Flow rate optimization chart for Nonylphenol



{Fig. 8: Nonylphenol Spectra with Winsim simulation of double concentration of Nonylphenol solution}

CONCLUSIONS

Bisphenol A, Nonylphenol and their spectral comparison are the focal point of this experiment. In these tests it may seem simple to say that the spectra are similar just by giving them a cursory glance. This, however, would be a brash judgment. By looking both at the GCMS chromatograph and spectra of family molecules, it is safe to say this structure may be tampered with. Other family molecules show distinct hyperfine splitting. I would hypothesize that purifying these isomers will show a splitting of the largest peak of nonylphenol spectra. I would continue the experiment in this manner.

To further investigate the biological implications of this experiment, I believe it would be advantageous to acquire results of Hemoglobin oxidation. I expect a weaker spectrum than obtained by cerium but still present and able to be simulated with Winsim.

REFERENCES

[1] McLachlan, John A., et al. *Synergistic Activation of Estrogen Receptor with Combinations of Environmental Chemicals*. vol. 272, Science, 1996, pp. 1489–1492, *Synergistic Activation of Estrogen Receptor with Combinations of Environmental Chemicals*

[2] McLachlan, John A. “Synergistic Effect of Environmental Estrogens: Report Withdrawwn.” Received by Science, *Science*, American Association for the Advancement of Science, July 1997, science.sciencemag.org/content/277/5325/459.5.

[3] National Toxicology Program. 2018. *Draft NTP Research Report on the CLARITY-BPA core Study: APerinatal and Chronic Extended-Dose-Range Study of Bisphenol A in Rats*. NTP RR 9. Research Triangle Park, NC. National Toxicology Program. (9): 1-249.

[4] O'connor, John C., and Robert E. Chapin. *Critical Evaluation of Observed Adverse Effects of Endocrine Active Substances on Reproduction and Development, the Immune System, and the Nervous System*. vol. 75, Pure and Applied Chemistry, 2003, pp. 2099–2118, *Critical Evaluation of Observed Adverse Effects of Endocrine Active Substances on Reproduction and Development, the Immune System, and the Nervous System*.

[5] Dekant, Wolfgang, et al. *Metabolism and Kinetics of Bisphenol A in Humans at Low Doses Following Oral Administration*. vol. 15, American Chemical Society, 2002, pp. 1281–1287, *Metabolism and Kinetics of Bisphenol A in Humans at Low Doses Following Oral Administration*.

[6] Chechik, Victor., et al. *Electron Paramagnetic Resonance*. Oxford University Press, 2016.

[7] Preuss, Thomas G., et al. *Nonylphenol Isomers Differ in Estrogenic Activity*. Environment Science Technology, 2006.

[8] *Royal Society of Chemistry*, 2015, www.ChemSpider.com.