



Supplemental Materials
for
Implementing CUREs with Cookbook-Style Laboratory Exercises in In-Person, Online, and Hybrid Formats

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Received: 17 December 2020, Accepted: 12 February 2021,
Published: 31 March 2021

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Appendix 1: Laboratory format and transitional changes, including example data

The first seven weeks of the course remained the same for the Spring 2019 and Spring 2020 groups as this was before the transition to online instruction. The experiments during this time were mainly cookbook style experiments and were all able to be completed in-person. A typical week was structured as follows: students were expected to read the material prior to class and write procedures in their notebooks which were checked upon entering the classroom. Pre-lab lectures were given by the instructor in real time along with any safety protocols that needed to be followed. Students performed the experiments, and their results were checked by the instructor. The completion of the pre-lab and end results composed a grade for Notebook Check assignments. Post-lab questions were completed outside of class time. Notebooks were collected once for all grading prior to the transition to online instruction where individual activities were graded separately. Exams 1 and 2 were both given through our learning management software with the addition of LockDown Browser. Students were in the classroom while taking the exam.

The CURE was broken down into a number of distinct parts to allow students more guidance through the different stages of the project: extraction, separation and detection, and data analysis. The different parts build on one another to culminate into the final project. How each part was presented and conducted in Spring 2019 vs. Spring 2020 are described below.

Proposal of Extraction Methods of Phthalates from Urine occurred before the online transition was able to be completed in the same manner for the Spring 2019 and Spring 2020 groups. In the classroom, students were provided with background information about the exposure assessment and used their knowledge about the chemical structure of phthalates and extraction techniques used in the cookbook style experiments to work in small groups (3-4 students) to research and propose their own extraction plan. Individual students were graded separately on this project based on participation in the presentation. Assessment looked at the scientific validity of the method but also the feasibility of the extraction in our laboratory. These were assessed by the faculty, and the most feasible method across sections was to be used in the actual extraction. In Spring 2019, the most viable method proposed was used by the class. In Spring 2020, the previous year's method was used as described below. This step can easily be transitioned to an entirely online component through the use of Zoom breakout rooms in future iterations of the course.

At this point in the 2020 term, the instruction transitioned to entirely online instruction. Due to this disruption, the overall format of completion for each week changed. This was necessary to provide flexibility to students and faculty who could no longer attend the course synchronously.

With the transition to online, rather than a real-time lecture, students were provided with a recorded pre-lab lecture as well as a video demonstration of the process for experiments. Our LMS, Canvas, allows instructors to view whether a student has watched a video, and this was used in replacement of the Notebook Checks. Students were given one-week window to watch the content provided which ended at their scheduled class time (this was necessary due to attendance records). Due dates of the notebooks changed from a bulk submission (Spring 2019) to weekly submissions to keep students engaged in the online format. Students submitted their results and answers to post lab questions to the LMS as images of their notebooks. The post-lab questions were not changed from the in-person Spring 2019 term to the COVID affected term. Additionally, Exam 3 was still taken on the LMS with LockDown Browser but with the addition of video monitoring while the students took the exam. The differences between the terms are shown in Supplemental Table 1. Additionally, the faculty member was available on Zoom for the entire class period. Specific transitions in presentation and assessment for the other portions of the CURE are described below.

In Spring 2019 the Extraction of Phthalates from Urine procedure was informed by the most successful students' extraction proposals. However, during Spring 2020 the faculty did not have access to campus and thus had to rely on the extraction method used in Spring 2019. Students were provided with a video demonstration of the entire process.

In Spring 2019 the Separation of Phthalates Extracted from Urine was spread over three class periods (weeks) due to the length of an HPLC run and our limitations on instrumentation. In Spring 2020, students had used the HPLC previously in a cookbook style experiment and only answered the post-lab questions in their notebook for this part of the experiment as their notebook grade.

In order to have sufficient data to provide a valuable analysis, all class data (peak areas for phthalates of interest) was combined and distributed to all students in an Excel spreadsheet. Supplemental Figure 1 shows a screenshot of what students were provided in the LMS. In Spring 2019, students worked on manipulating the data with their group during class time in order to draw conclusions about what the data means for exposure. In Spring 2020, students

were provided with the same Excel spreadsheet from the previous year (this is our banked data) and performed the analysis independently. Additional resources were provided to these students including a video screen capture of how to use Excel to perform the analysis of the data. Their graphs and post-lab questions were submitted to the LMS as photos from their notebook.

The final project of the course is a poster presentation of the data collected to use as support for proposing a new iteration of the intervention study. Students are told they can change any part of the study as long as they can justify their changes. In Spring 2019, students were given 1.5 class periods (2 weeks overall) to work in their groups on this final project, though they are asked to start thinking about the proposal as they work through all steps of the research project. Students then presented their posters to the class. In Spring 2020, this project was turned into an individual student project to most flexibly accommodate students. Students were provided with a video description of the assignment along with tips on making their proposal successful, this information mirrored what would have been provided by the instructor in class. Students were given the same amount of time to work on the project and submitted their final poster to the LMS, however, they did not present it. Supplemental Figure 2 shows an example of a final student project.

WEEK	Spring 2019		Spring 2020	
	EXPERIMENT	ASSIGNMENTS DUE	EXPERIMENT	ASSIGNMENTS DUE
8	<i>Extraction of Phthalates from Urine</i>	Week 8 Notebook Check	<i>Extraction of Phthalates from Urine</i>	Week 8 Notebook Check
9	<i>Ethics of Human Studies Separation of Phthalates from Urine</i>	Week 9 Notebook Check	<i>Ethics of Human Studies Separation of Phthalates from Urine</i>	Week 9 Notebook Check Extraction of Phthalates from Urine Notebook
10	<i>Separation of Phthalates from Urine Isolation of Cinnamaldehyde</i>	Week 10 Notebook Check	<i>Isolation of Cinnamaldehyde</i>	Week 10 Notebook Check Separation of Phthalates from Urine Notebook
11	<i>Separation of Phthalates from Urine</i>		<i>Data Analysis</i>	Isolation of Cinnamaldehyde Notebook
12	<i>Separation of Phthalates from Urine Formation of a Useful Ester</i>	Notebook Due Week 12 Notebook Check	<i>Formation of a Useful Ester</i>	Week 12 Notebook Check

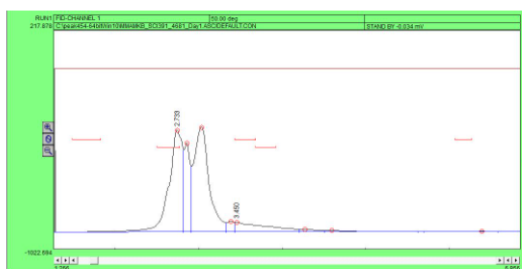
13	Data Analysis Working on Intervention Poster		Working on Intervention Proposal	Data Analysis Formation of a Useful Ester
14	Critiques/Presentations	Notebook Due Intervention Poster Proposal	Working on Intervention Proposal	Intervention Poster Proposal
15	-----	Exam 3	-----	Exam 3

Supplemental Table 1: Weekly changes in content and assignments due between Spring 2019 and Spring 2020

Data Analysis

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In this [Excel spreadsheet](#) you will find all the data collected for the semester between both classes. There are two tables presented, one for dimethyl phthalate metabolite monomethyl phthalate and one for diethyl phthalate metabolite monoethyl phthalate, with peak heights obtained over the 7 days of participant samples. Feel free to use the spreadsheet to perform your calculations. Peak areas were obtained from the chromatograms like the one pictured below.



To analyze these samples you will be calculating the percent change for each participant compared to their Day 1 samples. For example:

$$\frac{(\text{peak area Day 3} - \text{peak area Day 1})}{\text{peak area day 1}} \times 100\% = \text{percent change in phthalate Day on Day 3}$$

If % change is negative, that shows a decrease in the amount of phthalate compared to Day 1, if % change is positive, that shows an increase in the amount of phthalate compared to Day 1. Remember to refer back to the IRB in the [Background Reading On Study](#), for what those samples were.

Supplemental Figure 1: Example data provided to students in the Data Analysis activity of the CURE

Introduction

Phthalates are synthetic compounds used in the production of a variety of consumer products such as water bottles, personal care products, fragrance carriers, detergents, and pharmaceuticals (Serrano et al., 2014, p. 6194). As a plasticizer, phthalates concern the health of the human population because they have shown to disrupt the endocrine system and cause other health risks including pregnancy (Serrano et al., 2014, p. 6195).

Humans buy about 1,000,000 plastic water bottles per minute (Nace, 2017). Bottled water remains in high demand while it is portrayed as a safer alternative to tap water of which quality is uncertain. However, phthalates may be leached into water samples from plastic packaging material or contamination during the bottling process (Al-Saleh, Shinwari & Alsabhaheen, 2011, p. 469). Portable water filters, reusable metal flasks and glass bottles may be used to lower risk of exposure to phthalates.

It is hypothesized that people who drink regularly from plastic water bottles have higher concentrations of phthalates in their urine than people who only drink from reusable, nonplastic bottles.



Figure 1. Basic structure of phthalates.

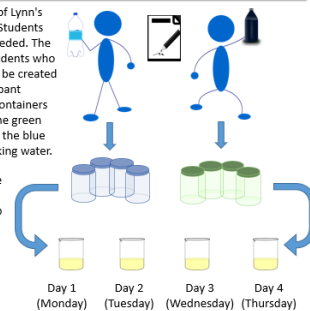


Experimental Design

Two types of Lynn students will be recruited for this study from multiple areas of Lynn's Campus, including the library, athletic events, recreation center, and cafeteria. Students will be recruited from various courses with permission from the instructor if needed. The study requires a mix of students who regularly use plastic water bottles and students who only use nonplastic reusable bottles as sources for drinking water. A survey will be created to determine each participant's preference of water sources. After each participant completes a consent form, a research assistant will hand them four specimen containers that are either blue or green along with paper bags to take home with them. The green containers will be given to students who do not use plastic water bottles, while the blue are given to students who depend heavily on plastic bottles as a source of drinking water.

The assistant will instruct the participants to fill each specimen cup with a urine sample of their first morning void. They will fill the cups every day for four days total as in the image demonstration to the right. They will also be asked to keep a record of the amount of plastics they drink from throughout each day to help eliminate phthalate sources.

The samples from the students who drink from plastic water bottles will be separated from the samples of students who do not drink from plastic water bottles by their cup color differences.



Results

In a previous exposure assessment of nail polish, phthalate levels were tested in urine samples of a handful of Lynn students. The participants were students who regularly use nail polish. They were asked to provide urine samples similar to the design of this proposal, but they removed the nail polish after the first day. The subsequent days were urine samples without nail polish present. The graphs below show the percentage change of phthalates from day one to day seven. On day one, the percentage change was zero because the nail polish was removed after this day. The graphs show levels of monomethyl phthalate and monoethyl phthalate, two common phthalates used in plastic products.

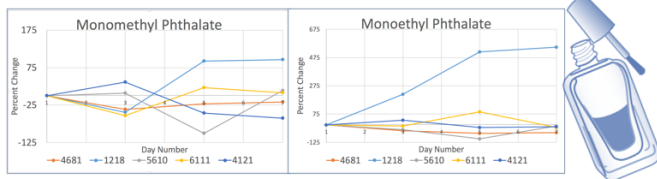
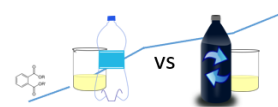


Figure 2. Percent changes of two common Phthalates present in urine of participants who wore nail polish.

The purpose of this experiment was to find if people are being exposed to phthalates via nail polish. The hypothesis was that phthalates would decrease in urine after removing nail polish. An increase in percentage meant that the amount of phthalates in the urine increased compared to the previous day.

Discussion

It is unclear if the intervention succeeded in the previous experiment because the sample size was so small. The results suggest that participants may have been exposed to other sources of phthalates between the collections of urine. If nail polish absence does not pose drastic decreases in phthalate levels, maybe eliminating other plastic sources will. For that reason, this new experiment design will test plastic drinking water bottles as the next possible source of phthalates. The goal of this new design is to see whether drinking from plastic bottles or nonplastic water bottles affects phthalate levels in urine.



References

Al-Saleh, L., Shams, N., & Alsabhaheen, A. (2011). Phthalates residues in plastic bottled water. The Journal of Toxicological Sciences, 86(4), 469-476. doi: 10.1080/10537709.2011.584889

Nace, T. (2017, July 28). 1000+ New 64.4 Million Plastic Bottles Per Minute. 61% Of Which Are Recycled. <http://www.bottles.com/blogs/news/2017/07/28/1000-plus-plastic-bottles-per-minute-61-percent-recycled/>

Serrano, S. E., Kim, C. J., Sobota, N. S., Nguyen, K. H., Norrath, E. S., Jansson, S., Holman, B., Swain, S. H., & Sathyanarayanan, S. (2014). Dietary phthalate exposure in pregnant women and the impact of consumer practices. International journal of environmental research and public health, 11(6), 6159-6215. <https://doi.org/10.3390/ijerph110602019>

Supplemental Figure 2: Example student intervention proposal poster