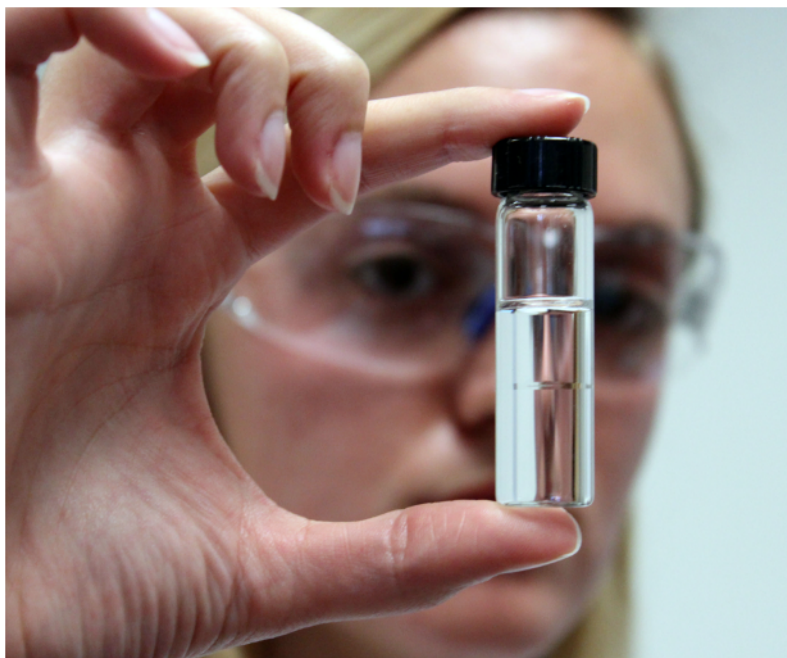


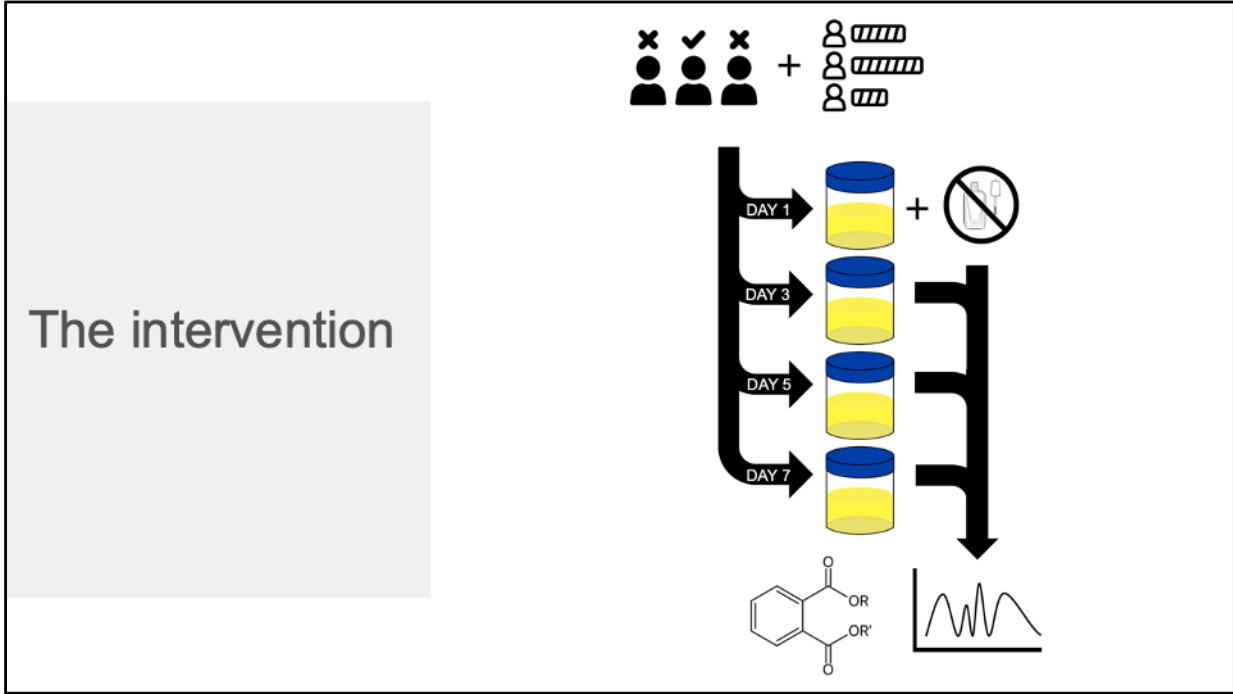
CUREing exposure
to environmental
chemicals from
personal care
products

Erika Doctor, PhD

Cassandra Korte, PhD

LYNN
UNIVERSITY





Our CURE has two parts:

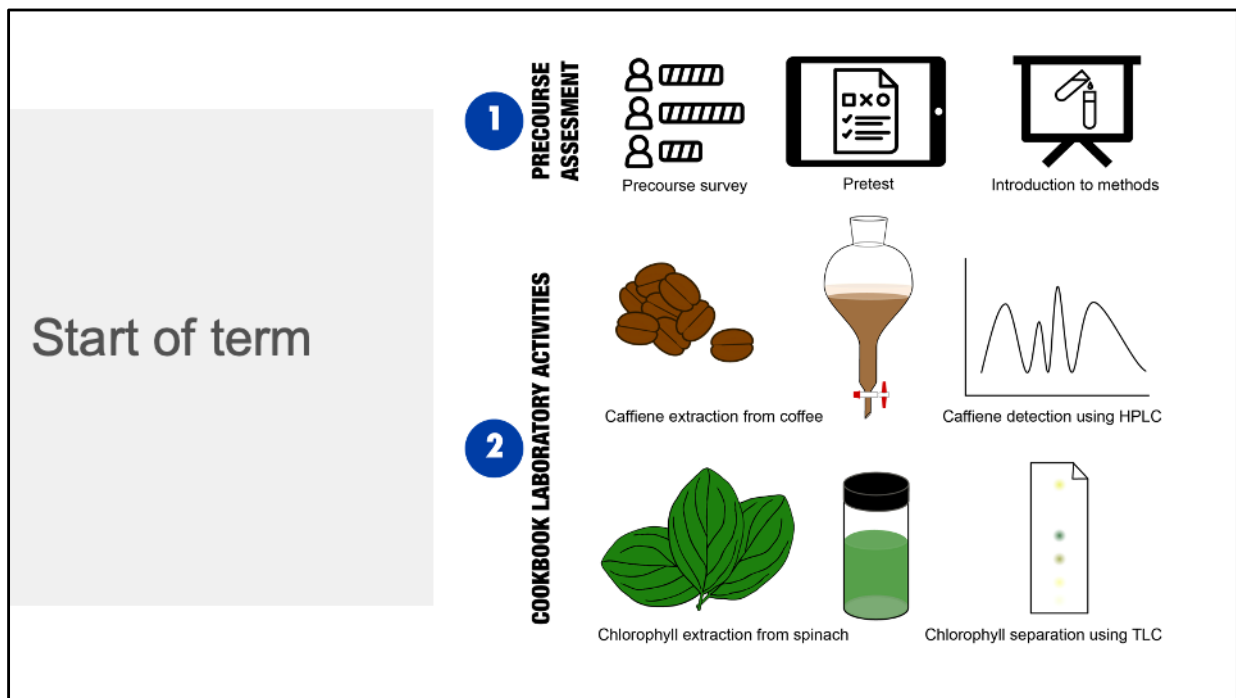
The first is an intervention study in which nail polish-wearing participants are recruited, give consent, and answer a questionnaire regarding their personal care product use.

Then, on the first day of the study, they collect their first urine void and then remove their nail polish. This gives us the participant’s baseline exposure.

On days 3, 5, and 7 participants collect their first voids again and submit them for analysis.

The analysis takes place in the CURE.

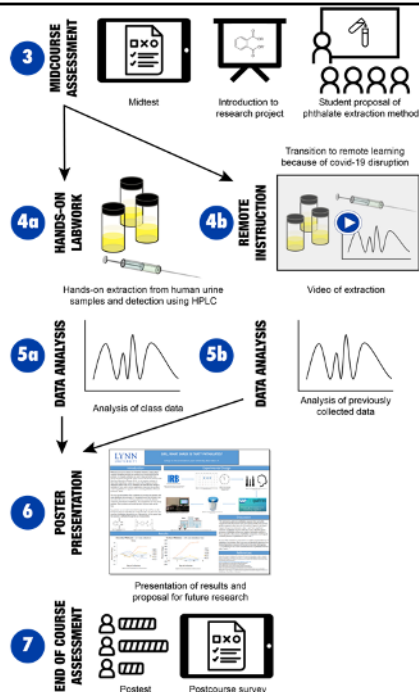
Just a note, because our study involves human participants and IRB approval, these samples are collected ahead of time. Research assistants help with recruitment and collection. Therefore our question is not student driven in the first iteration



We have run this course in Run in three ways: in-person, virtual, and hybrid
 Virtual/hybrid students were giving video demonstrations of any laboratory work –
 this is due to the constraints of the lab (safety, equipment etc)

In order to get data on the success of our CURE student were given precourse and
 post course survey and a content exam at the beginning, middle and end of the
 course. The course begins with a lecture on the methods use in the entire laboratory
 (extraction, chromatography separation and UV detection) This is followed by
 cookbook style experiments applying different methods of liquid liquid extraction and
 separation (HPLC and TLC)

Making our CURE viable



Once students have experience with the methods through the cookbook experiments we then delved into our research project for the rest of the course. Students were first introduced to the Phthalate project through access to the IRB and a lecture which we will be giving next to introduce all of you to the project.

After this background information on the project, the CURE begins with students using their previous knowledge on extraction to propose their own method for extraction phthalates from urine. We will talk more about this later. Students who were in the classroom then performed the methods in the laboratory while remote students were provided with video demonstrations. The culmination of the CURE is students using their data to propose a new intervention project through a poster presentation.

Today we will be walking you through this CURE from the introductory lecture. The amount of content and what content you want to provide will depend on the course that you want to have this project embedded into and the previous knowledge of your students. As this is in an Organic Chemistry laboratory, some of the concepts have been introduced earlier in the course, this lecture focuses on the intervention and how we perform these types of studies

Isn't this for organic chemistry class?

What does toxicology have to do with it?

Toxicology

- Study of and detection of compounds that cause adverse effects on the body
 - Most are organic compounds
 - Need a background in organic chemistry and biochemistry

Organic Chemistry

- Study of carbon-based compounds

Toxicity

- ALL compounds are toxic
- The dose makes the poison

10 g Caffeine – 80 – 100 cups
of coffee all at once!

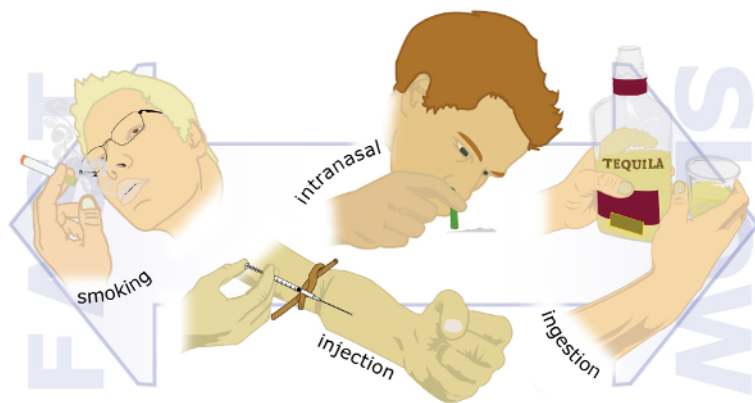


In humans a blood concentration greater than 100 µg/ml
is considered lethal [10,11].

Since all Compounds are toxic, even the caffeine extracted earlier in the semester, what makes something toxic is the amount you are exposed to also called the dose. Since many of our students want a career in the health professions, this type of project peaks their interests.

Route of exposure

- Swallowing
- Inhalation
- Absorption through skin
- Injection



Genetic Science Learning Center, 2013

What are the routes in which an individual can be exposed to a chemical?

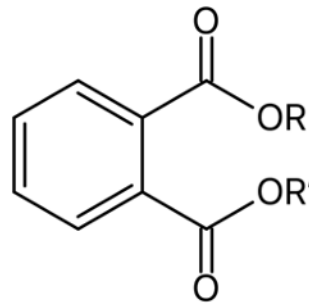
Routes: Ingestion, inhalation, dermal

Routes can determine the level of toxicity and how quickly the compound enters the bloodstream (similarly, this is why some drugs are given intravenously instead of as a pill). In a study looking at exposure to a potentially toxic chemical we need to think about how the chemical enters the body.

<https://learn.genetics.utah.edu/content/addiction/delivery/>

Phthalate exposure assessment

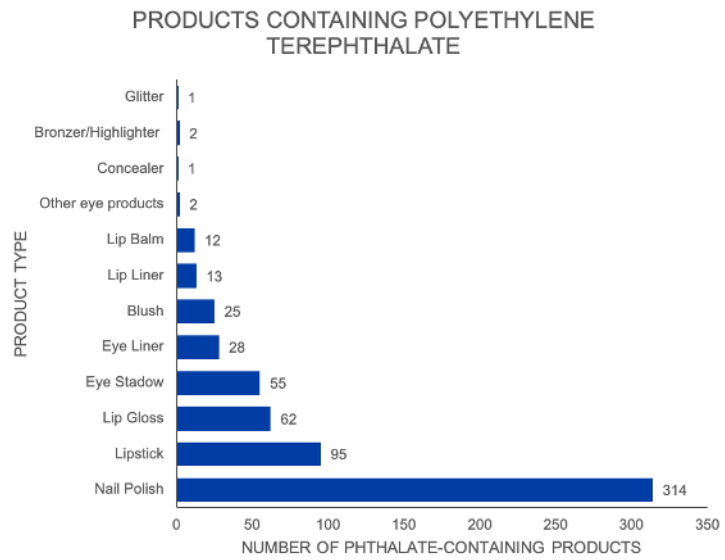
- What's a phthalate?
- What are they used for?



- Why do we care?

- Our study is interested in looking at exposure to phthalates.
- phthalate esters, are esters of phthalic acid. They all contain this same base structure; the differences lie in different R groups which change their chemical properties.
- They are mainly used as plasticizers, i.e., substances added to plastics to increase their flexibility, transparency, durability, and longevity. And are found in personal care products – our study is looking at exposure from nail polish and they provide flexibility of the coating.
- We care because many studies indicate the likelihood that phthalates act as endocrine disruptors. These types of compounds interfere with the normal hormonal mechanisms that allow a biological organism to interact with its environment. This is important because the susceptible population of people who can bear children, are known to use personal care products like nail polish at higher rates than the general population. And their resultant exposures is thus fairly high.

Why nail
polish?



Lunsford et al., 2018

A recent examination of the Environmental Working Group's Skin Deep database found nail polishes to be amongst the products that most commonly contain phthalates.

Why nail polish?

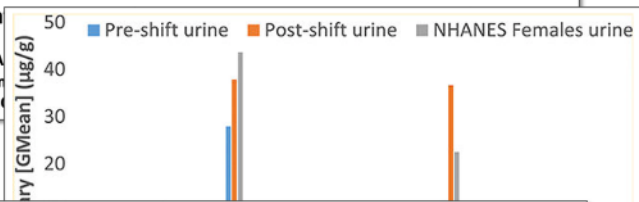
Published in final edited form as:

Environ Sci Technol. 2019 December 17; 53(24): 14630–14637. doi:10.1021/acs.est.9b02474.

Exposure of Nail Salon Workers to Phthalates, Di(2-ethylhexyl)

Tereph

Jessica A
Allen[†], An
Rebecca



Published in final edited form as:

Environ Int. 2016 January ; 86: 45–51. doi:10.1016/j.envint.2015.10.005.

Nail Polish as a Source of Exposure to Triphenyl Phosphate

Emma Mendelsohn¹, Audrey Hagopian¹, Kate Hoffman¹, Craig M. Butt¹, Amelia Lorenzo¹, Johanna Congleton², Thomas F. Webster³, and Heather M. Stapleton^{1*}

¹Nicholas School of the Environment, Duke University, Durham, North Carolina USA

²Environmental Working Group, Washington DC, USA

³Boston University School of Public Health, Boston, Massachusetts, USA

Further, occupational exposure to nail polish is linked to higher phthalate exposure.

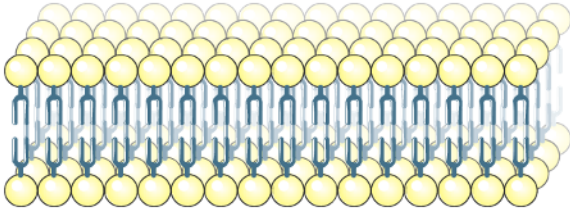
And plasticizing compounds in nail polish can absorb through the nail bed.

Toxicity
depends upon
ADME

- Absorption
- Distribution
- Metabolism
- Excretion

Whether a toxicant will be toxic also depends on these four variables as well

Absorption



[Cell membrane](#) diagram by [Smart Servier Medical Art](#) under [CC 3.0](#)



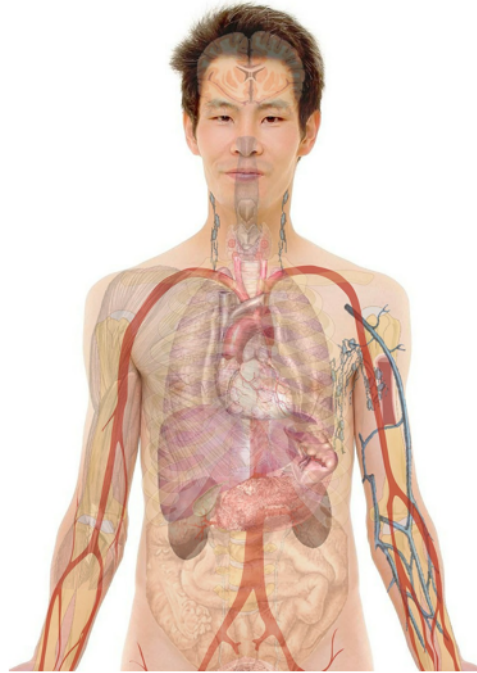
Photo by [Karolina Grabowska](#) from [Pexels](#)

After exposure, the toxicant enters the body (absorption) Dermal, inhalation, ingestion Fat soluble vs water soluble compounds

Phthalates have been found to absorb through the skin into the blood stream – the more polar the more it is absorbed into the blood stream

Distribution

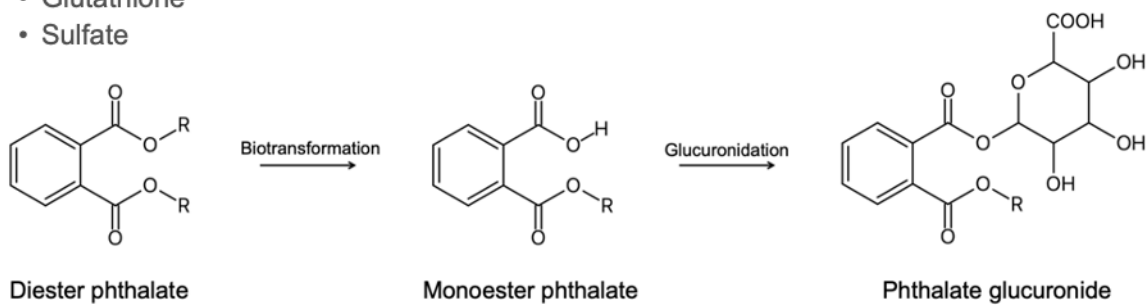
- Movement of the toxicant through the body via the bloodstream
- Distribution depends on solubility



- Move through the bloodstream to all parts of the body – often by diffusion
 - High concentration to low concentration
 - Often distribute by adhering to blood proteins
 - Can also distribute based on solubility – fat soluble vs. water soluble
- If they are removed by the kidneys or liver – when blood passes through these organs, the toxicant can be distributed out of blood and excreted without entering other organs – depends on where it was adsorbed.
- https://commons.wikimedia.org/wiki/File:Male_with_organs.png

Metabolism

- Many toxicants are metabolically altered
- Conjugation with charged species increases solubility
 - Glucuronide
 - Glutathione
 - Sulfate



Frederiksen et al., 2007

Toxicants can be metabolically altered. Mostly occurs in the liver through enzymatic reactions. Function is to increase reactivity then add polar/charged groups to increase water solubility.

Sometimes biotransformation can make a toxicant more toxic!

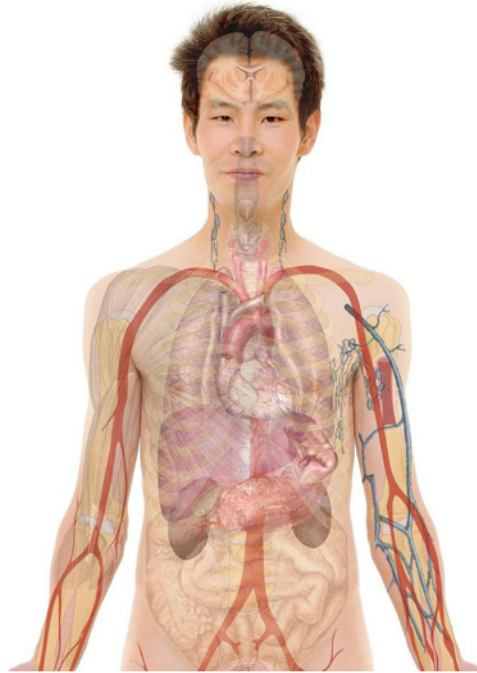
Phase I—reveals functional groups. Revealing functional groups primes the metabolite to be more reactive with Phase II conjugates.

Phase II—conjugation, can increase polarity by addition of glutathione, glucuronide, or sulfate to the phase I functional groups.

<https://onlinelibrary.wiley.com/doi/abs/10.1002/mnfr.200600243>

Excretion

- Kidney (from blood)
- Liver (blood/biliary system)
- Lung (volatile organics)
- Others:
 - Lactation
 - Sweat
 - Saliva
 - Tears
 - Deposition in hair and nails



Exposure assessment methods

Direct

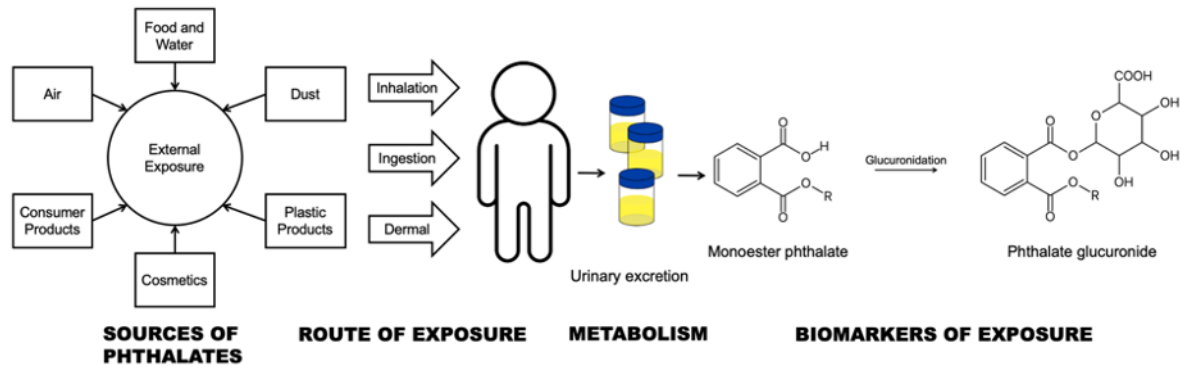
- Measures of absorption in biological media
 - Blood, urine, stool, hair, nails, etc.
 - Assess metabolites and parent compounds

Indirect

- External estimates of exposure
 - Sampling of water, air, food, ambient home environments, personal care products, etc.
 - Measure parent compound and model exposure

Because our study is trying to determine whether or not nail polish is a viable source of phthalate exposure what type of assessment would be more beneficial to answer our question? Direct

Exposure assessment



Adapted from Kumar & Sivaperumal, 2016

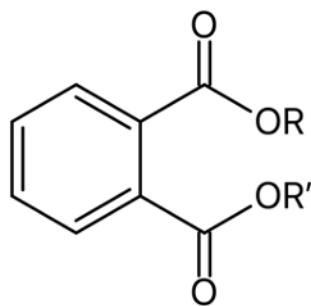
In our prepared exposure assessment the source of the phthalate is the nail polish with the route of exposure being through the nail bed (dermal). In the case of phthalates they are metabolized and excreted into the urine which we are collecting. The main mechanism of metabolism of phthalates produced glucuronide conjugates which will become important in our experiment

https://www.researchgate.net/publication/283558148_Analytical_methods_for_the_determination_of_biomarkers_of_exposure_to_phthalates_in_human_urine_samples

Onto the experiment...

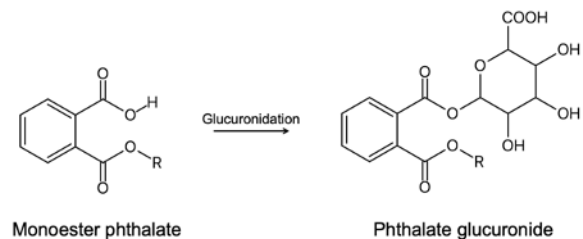
What do we need to consider?

- Extraction (Activity 1)
- Separation (Activity 2)
- Detection & Data analysis (Activity 3)



Extraction

- Goal is to remove the free compounds from urine mixture
- Glucuronide conjugation – removal by enzymatic hydrolysis
- Other considerations
 - Time and feasibility



- Students begin by proposing their own method of extraction. In our first year, where everyone was in person, the best student proposal was used for the whole class to use. However, in our covid affect term and in spring 2021 where many students were still virtual the extraction method was predetermined (see part 1) This is because videos needed to be recorded or data needed to be provided ahead of time.
- However, all students are proposing their own method of extraction using these considerations in their groups. Because we want to detect the phthalates in the same form, the first step of any extraction method you propose must remove the glucuronic acid, leaving the free phthalate – this involves an enzymatic hydrolysis, other conditions require the phthalate to be neutral. You don't just have to consider the science though but also the practicality in your proposal – how long will it take? Is it very expensive? Do we have the equipment
- In-person: students work together in classroom
- Virtual: Zoom breakrooms
- Hybrid: paired via method of instruction
- One class period to complete; best (most feasible) proposal is used to do

extraction

- Reference your binder document on facilitation in the lab

Example proposal method

Original Paper

A liquid–liquid extraction technique for phthalate esters with water-soluble organic solvents by adding inorganic salts

Yaqi Cai^{1,*}, Yu'e Cai^{1,2}, Yali Shi¹, Jiemin Liu², Shifen Mou¹, and Yiqiang Lu²

¹ Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

² Department of Chemistry, University of Science and Technology Beijing, Beijing 100083, China

The first proposal and what the method in Activity 1 of your handout came from this paper. A student found this paper which is not in a biological sample but is chemically sound for removing the phthalate. Additionally an enzymatic hydrolysis method was also proposed to start with.

So lets get into the method. You will be experiencing this as a remote student – for this part of the experiment the students watched the following video and recorded their observations – students in the classroom performed this extraction. This took a whole class period (3 hrs) though depending on your class length you may need to separate the enzymatic hydrolysis (1 hr 45 min) and the extraction (1 hr)

Image References

- iPad by Anna Sophie from the Noun Project
- Presentation by Ikipoh from the Noun Project
- Recruitment by Adrien Coquet from the Noun Project
- Survey by Adrien Coquet from the Noun Project
- Exam by BomSymbols from the Noun Project
- Teaching by Rajive from the Noun Project
- Test tube by Barbara Marsillac from the Noun Project

<https://thenounproject.com>

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