[SQUEAKING]

[RUSTLING]

[CLICKING]

JOHN DOLHUN: Good morning, everyone. And welcome, or good afternoon. Welcome to The Ellen Swallow Richards Lecture Series.

This is our beloved Charles River. This is the river you're going to be testing.

Once you've tested this river, you can actually take this testing and apply it to any body of water. Why should we be concerned about this Charles River? Yes?

Because we live next to it.

Because we live-- that's a good reason. Because we live next to it. Anyone else? Aisha?

AUDIENCE: To care about your environment.

JOHN DOLHUN: You care about your environment. And speaking of caring about your environment, we've got some problems with phosphate out there. We've got high phosphorus concentrations. And those high phosphorus concentrations lead to algal blooms.

And those algal blooms produce toxic chemicals and odors. And when all this algae dies, it heads down to the bottom and breaks off and it's acted on by decomposers. These microbes start to chew it apart and they use up the dissolved oxygen so that we get lower-- lower dissolved oxygen in the water. And what that does is it leads to fish kills. And our recreational ability is impaired.

So here are some examples of what I'm talking about. This is Florida, 2016. This is Lake Okeechobee, one of the largest fresh water lakes in Florida. This is a 30 mile long fish kill.

It involves some 50 species of fish. The cause, most probably, an algal bloom, the result of leaking septic systems, fertilizers from lawns going into the water. This is an economic disaster. It's going to take decades to recover from this.

Is Bradenton, Florida, 2018. This is their harbor filled with dead fish. This started from an algal bloom that turned into a red tide. And the red tide stretched about 150 miles off the coast of Florida, and gradually, the wind and the currents brought it into shore. And when that red tide came in to shore, it started releasing toxins into the air and water, and then the nutrients broke off and microbes started digesting them and used up the oxygen, and all these fish died.

This is Australia, 2019. This is the Darling River, but it also happened in the [INAUDIBLE] River. And they had a successive run of these fish kills, one right after the other. Again, it's global hot temperatures and agricultural runoff.

This is the Charles River, August, 2019. This is the largest algal bloom, I think, that we've had out there. It actually stretched from the BU bridge to the Museum of Science. And cyanobacteria was detected. This is a health problem.

So we're going to be measuring phosphorus in the waters out here. That's one of the things we're going to do. And there are two types of phosphorus, there's the inorganic, which is simply salts of phosphoric acid, and then if you create esters of the inorganic form, you have the organic form of phosphate.

So let's take a look for a moment at the inorganic phosphate. I mean, phosphorus is like, the 11th most abundant element on the planet. And it's not found in elemental form. You won't find any pure phosphorous anywhere on the Earth.

It's found in these types of phosphates, in rocks and soils, and that's how it exists. So the main inorganic player here is the orthophosphate. And that's the PO4 to the 3 minus. This is the most stable form of phosphorus. And this is the form that's readily available to plants for uptake by the plants.

I mean, all plants and animals need phosphorus for growth. It's the backbone of our DNA and the Krebs cycle. Plants need it in photosynthesis. They have to extract it from their environment because they're not going to make sugar unless they first make ATP to actually connect those bonds in the sugars that they're making.

So we've got this orthophosphate, the main player here, but in the inorganic category, you can actually connect several of these together and form a polyphosphate, such as our detergents. A lot of detergents are polyphosphates. P3O10 to the 5 minus would be a polyphosphate. The interesting thing about the polyphosphates is as soon as they hit the water, they hydrolyze into the orthophosphate.

And then we've got the organic phosphates, which are esters of the inorganic form. And organic phosphates are found in all living tissues. So all living tissues, both plants and animals, have organic phosphates.

And waste is also another form of the organic phosphates. So when something living dies, it starts to decay. What happens is the phosphates actually convert to the orthophosphate. That's what happens to them.

So this is what we're going to be measuring. And now, I'd like to talk for a few minutes about how it gets into the water, where all this stuff is coming from. So we're going to look at some of the primary sources of the phosphorus.

The first one is storm water runoff. Can anyone give me an example of some phosphoruses, phosphates that can get into the water from storm water runoff? Yes, Aisha.

AUDIENCE: I'm not sure if there's a phosphate in dirt.

JOHN DOLHUN: Absolutely. There's phosphate in the dirt. And that would get washed in. What else? Yes, Jimmy?

AUDIENCE: Probably less here, but in agriculture, I'm pretty sure they're used in fertilizer.

JOHN DOLHUN: Oh, gosh, yes. Fertilizers are a big thing because of lawns. So we've got soil here and fertilizers. Anything else? Jimmy?

AUDIENCE: I guess animal droppings?

JOHN DOLHUN: Absolutely. Animal waste. I mean, think about where do they put pig farms? They put them all by a river, right?

And all those geese, the guano, droppings, everything, they're all out there by the river. And all that stuff is being washed in. So animal waste is a big, big source here.

Anything else? You ever got your car washed in a car wash? Do you ever sit in it while you're going through, see all that soap coming down? Tons of soap. And all that soap's going into the sewer systems, and when it starts to rain, heavy rains, it bubbles over. So car washes would be a big source, and there's your polyphosphates.

Another one that you might not be as familiar with is car exhausts. Car exhausts have a catalyst tube in there. When that tube gets hot, like 500, 600, 700 degrees, it can form cerium phosphate, which is released in the exhaust effluent. So cerium phosphate would be a culprit here.

And then probably the biggest source is discharge from wastewater treatment plants. Did any of you ever go to tour a wastewater treatment plant? Anyone? Oh, you did? Kelly, right? Could you tell us-- tell us about it, what your experience was?

AUDIENCE: It was a school fieldtrip a long time ago, I think. It was interesting. So they purified or treat the water with several different methods. On of them was just like, bacterial, and then they introduce it [INAUDIBLE].

JOHN DOLHUN: Yeah, they aerate it with bacteria, I think, in the secondary treatment. And they do that to get rid of organic matter or to digest it down to sludge.

AUDIENCE: Yeah. And then there's a UV method.

JOHN DOLHUN: Very good. I mean, the most modern plants have this ultraviolet rays at the very end. You can walk along and you can see a glass floor. You can see the water going through with the ultraviolet rays hitting it.

And then, at the end, they have a faucet and they offer you a drink. I mean, there was no one in my group that availed themself to take a sip of that water after going through that. But let's put this in perspective.

So wastewater treatment plants, they're all built along rivers or oceans. And when a heavy rain, they start to discharge. They have to discharge. They can't take the flow.

First thing, they may discharge some of the secondary treated water. Sometimes, they have to discharge the raw sewage. But to put it in perspective, all of us produce about 2.2 grams of pee per day.

I'm talking about phosphorus here. That's about 1.8 pounds per year. Now, when that phosphorus hits the sewage treatment plant, it's in the effluent, it gets diluted down. You're talking somewhere between 2 and probably 20 milligrams per liter PPM of phosphorous in the water.

After the secondary treatment, they only take out between 1 and 2 milligrams per liter. So you end up with a large excess of phosphorus in the treated water. So when they release, it's a ton of phosphorus hitting the water.

But the good news is-- I'm going to draw a smiley face here, because the EPA just filed a regulation forcing-- they're going to force all these wastewater treatment plants to cut back, get rid of the phosphorus in the water, and they're going to have to-- they're going to have to take it down to less than 1.0 milligrams per liter. So all these plants are running around now trying to figure out how they're going to do that. This, I believe, just went into effect this year, and may go-- may be starting in January of 2020, but there are EPA regulations that are just coming out.

The other major source is sewer overflows. And in New England, everyone-- it's notorious that you have a sump pump in your basement. So when your basement floods, the pump starts up and it pumps everything.

They're connected to the sewer systems. So everything in the kitchen sink is going into the sewer system and it's bubbling over. So those are the primary sources.

Now, let's look at the ecological effect of all this phosphorus. This is the Charles River, the famous Charles River. This is up by Newton, Mass. There's a guy in a canoe trying to pull out the vegetation out of the water.

What this vegetation does is two things. First, it's blocking the sunlight to organisms and other plants down below the surface that need that sun. And the second thing is it's going to die and produce these swamp-like odors, and then it's going to get decomposed by microorganisms that are going to use up the oxygen.

This is the result. This is blue-green algae. This is really-- another name for this is cyanobacteria. It's the cyanobacteria that give it that green color on the surface of the water.

Here are some other forms of it. You can get this pea soup kind of look, or you can get this mossy look, or you might get a painted look. The bottom line is it's all bacteria.

It's unicellular bacteria. These are prokaryotic bacteria. They have no nucleus, but they make their own food and they secrete chemicals. These are the same bacteria, believe it or not, that gave us life 3.5 billion years ago.

And now, they're out to get us. Isn't that amazing? Every time I look at this, I can't believe it. I mean, when we had no oxygen, these were the guys that were giving our oxygen atmosphere, and now, look what they're doing.

Now, if these are out there in the water, you don't want to go in the water. If you do, you are dead meat, and I'll tell you why. You can have all kinds of symptoms, you can get covered with a rash, you can have diarrhea, flu-like symptoms, eye and ear problems, respiratory problems.

I gave you an article in the news. This is*The New York Times.* Central Park in New York, all of their ponds, infected with blue-green algae. Here's a good example. This is today, "Ohio strikes a Blow in Algae Fight." This is *The Wall Street Journal* this morning. This article just came out.

Interestingly, Ohio had this algae problem 10 years ago, and they took around one of their lakes and they built up these wetland areas to prevent the runoff from reaching-- the agricultural runoff from reaching the lake, and it's actually working. So there are ideas out there that you can come up with an idea and solve a problem.

So some of these cyanobacteria, some of the fresh water bacteria produce very toxic chemicals. One form of these are the microcystins. There are something like 50 different micocystins that have been identified to date.

It's a cyclic peptide here. And this is a hepatotoxin. So once this gets through your skin into your body, your liver is going to get attacked, and you're pretty much gone. There's not much you can do about it.

The article mentions the dogs-- one woman had three dogs in North Carolina. She let them go for a swim in a pond. All three dogs died within a few hours.

So this is really nasty stuff. And I challenge you to think about coming up with an antidote for this. There's your startup company right here. I'm giving it to you. When this stuff is active, you go out to the Charles River, you'll see these signs posted warning people and pets to stay out of the water.

Here's another example. This is the summer of 2014. This is a satellite picture of Lake Erie, one of the five Great Lakes in this country. And the lake actually borders Canada on one side, separates Canada from the US near Ontario, and then there are several states that border the lake.

Out here to the west is Toledo, Ohio, and Toledo, fourth largest city in Ohio, has a population of about a half million. This was their tap water in 2014. And I had a 5.310 student in class four years ago who remembers this. I mean, this is serious business. And it's happening all over, all over the world.

Here's another picture. This is the largest landlocked body of water on the Earth. Anybody from Europe here recognize this? What? Who said that? Yes, Sean, exactly correct, that's the Caspian Sea.

It's bordered by five countries. But look at the massive, massive algal growth from this satellite picture. Here's the Volga River, Europe's longest river, pouring into it.

It's a 2,000 mile long river. Here's a new word for you, eutrophication. It's from the Greek meaning well nourished.

So how much phosphorus is acceptable? The EPA came out in 2000-- that was almost 20 years ago-- and said 0.0238 milligrams per liter. Well, we know today that if you have anything greater than or equal to 0.016 milligrams per liter, you're going to have really a large algal growth proliferating in your water.

Now, these concentrations of phosphorus, they're low. And it's going to be challenging to measure those. This is what we're going to do.

The other thing that makes it challenging is phosphates are colorless. So how are we going to use UV vis to measure these concentrations? Anybody have an idea? Yes?

Maybe we can react them with something that will make them colorful? Maybe we can react them with something that can make them colorful. Very good. Very good. Yeah, I like that. Keisha, right?

AUDIENCE: Gizelle.

JOHN DOLHUN: Gizelle. I'm sorry, Gizelle. Where's Keisha? There she is. OK. All right, so react them with something that makes it colorful. So here comes chemistry to the rescue, right?

So phosphates are very reactive. So we can actually take river water and this method here, this is the ammonium metavanadate method. When I tried to develop this Charles River water testing, I started with this method because it was written up as the method to detect phosphorus in river water.

So you would take the river water, mix it with molybdate, ammonium metavanadate, and you create this color heteropoly molybdic acid that has a yellow color and it absorbs light at 400 nanometers. What I found is that this method was not sensitive enough to actually measure the phosphorus levels in the Charles River. It's a great method for measuring phosphorus in sewage, but not the rivers.

So I looked around and I found what's called the ascorbic acid method, an EPA-approved method where you actually took river water, mixed it with molybdate, sulfuric acid, and you create this heteropoly molybdic acid, which is colorless. But the good thing is this heteropoly molybdate anion can accept electrons and be reduced down and ascorbic acid can cause that reduction. And you end up getting this molybdenum blue complex, a mixed valence complex that absorbs light at 880 nanometers.

So this is great because the concentration of the phosphorus in the complexes is proportional to the absorbance of the light in these things. So this is what you're actually making. It's beautiful. This is a Keggin structure. It captures the phosphorus in the center, surrounded by 12 molybdenums and 40 oxygens.

I want you to take a moment and look at this. And I'd like you all to close your eyes for a moment and think about this image. Close your eyes. OK, open your eyes, please.

I want you to carry this image with you into the lab when you do this experiment. You're actually going to be making this in your beakers when you add the color developer to your water samples. You're creating this in about 15 minutes in your beaker. This is inorganic chemistry at its best.

Now, I want to spend the next several slides showing you how we're going to actually measure the concentrations of this. So we're going to be shining electromagnetic energy on a sample. And if you look at the visible light here that I broke out of this electromagnetic spectrum, on the red end of this, we're going to be looking at our samples on that far red end in the near IR. So that's where you're going to be-- where you're going to be taking your readings.

Now, what can happen when you shine radiation on your sample? Anyone? Yes, Kim.

AUDIENCE: Photobleaching

JOHN DOLHUN: Sorry?

AUDIENCE: Some of your sample could get photobleached.

JOHN DOLHUN: Some of your sample could get photobleached, yeah. That's a possibility. What else? Yes?

AUDIENCE: [INAUDIBLE]

JOHN DOLHUN: Exactly. I mean, electrons could get chewed up to a higher energy levels. Little nuclei could see that happen and get nervous and start to rearrange themselves. And the molecule, as Kelly said, could burst into vibration.

And your UV is actually monitoring all of those electronic transmissions that we can't see with our eye. And what the UV does is it draws you a smooth curve through all of those electronic transitions and you end up somewhere with your lambda max. What UV vis is, a good definition is it's just the interaction of light with matter as a function of wavelength.

So here's your UV cuvette. Here's the radiation hitting that cuvette. What happens? What do you see up there happening? Yes, Ryan?

Yeah, the lights focused. And what's happening to the sample? I'm sure it's heating up, yeah. Yes, Autumn?

AUDIENCE: It absorbs sunlight and transmits sunlight and also re-emits.

JOHN DOLHUN: Yeah, good. So some of the light's going through and some's getting absorbed. So let's look at that for a minute. So we've got absorbance minus the log of the transmitted light, which is i over i0.

Now, absorbance is-- we're actually monitoring the absorbance. We're not monitoring the transmittance because absorbance is actually directly proportional to concentration by Beer's law. So we have this relationship. And E, the extinction coefficient, is the molar absorptivity constant, is simply the amount of light absorbed per unit concentration of your sample. Let me just rewrite this. Let me get rid of the logarithm. I want to show you something here. So I'm going to rewrite this.

Let's actually graph the intensity of the incoming radiation here. We're going to graph that as a function of concentration. If you do that, you're going to see from that equation that the transmitted light decreases exponentially as the concentration increases.

I want you to keep that in the back of your mind. This equation fascinated me. I like Beer's law. What this tells you, this extinction coefficient tells you, that it has to be a constant. So if you cut your concentration in half, absorption should be cut in half.

So I wanted to prove this. So I went out and I made this compound, hexacyanoferrate(III). And I made up five solutions of this, and then I measured the absorbance of each solution.

It absorbed light at 420 nanometers. And if you look here, 10 times e to the minus 4, if I cut that in half to 5 times e to the minus 4, indeed, the absorption gets cut in half as it should. So I graph this, got a nice, straight line.

The change in absorption over the change in concentration is my slope. And it's 1,056.7 so that's my extinction coefficient. And this worked really well.

But I want to caution you-- if you try this, and you're graphing absorption versus concentration, and you're doing Beer's law, you've got this nice, straight line, you might end up with something like this, where Beer's law falls apart. And it all comes back to this here-- the intensity of the transmitted light decreases exponentially with concentration. So eventually, if the concentration is too high your sample becomes saturated, and you're not going to be-- this Beer's law falls apart.

I just want you to be aware of that. What I did is I made up all of my concentrations are very low. Beer's law worked fine.

So now we're going to get to some serious business. This is what you have to do for this lab, for it to work. Otherwise, you will be a goner-- gonzo.

You've got to clean this glassware. This is a list of the glassware that you'll need to clean. And you've got to do it with 10% hydrochloric acid, triple rinsed with Milli-Q Water. Why do you think you need to do that? Yes.

AUDIENCE: Any contamination might change the products concentration.

JOHN DOLHUN: Any contamination is going to just destroy the experiment. It's kind of like the Nano Building. You've seen them in there with their bunny suits on. One speck of dust, even a dander from your hair, is like a wrecking ball to the experiment.

Well, one speck of phosphate from any detergent is going to wreck this experiment. So you've got to judiciously sit down. There'll be two of you-- you'll be partnered up for this.

So you clean this up. And you'll do this on day two after the dissolved oxygen testing. This is what you're going to need for day three for the phosphate testing.

So you can leave this glassware in the top drawer above your locker. It's not locked. And it'll be nice and ready for when you come in to do the testing.

So this is what you have to do in this lab. First of all, we need to make up a set of standards that we can interpolate our river samples against to find the concentrations of the phosphate and phosphorus. So you're going to make seven standards up. And you're going to be using a stock solution.

And the formula to use in all these tables is you're going to use this M1V1 equals M2V2. So if you have a 10 to the minus 1/3 molar stock solution, and you're taking 1 milliliter of it out, out of the bottle, how much molarity do you want to make to make up 100 mil solution?

You'll be diluting this 1 mil to 100, and you'll see that your x is 10 to the minus 5. That's your stock solution of phosphate that you're going to create. So you just use that formula for all these. You're taking this much out of your 10 to the minus 5 molar stock solution, and you're diluting it up to 10 mils. What's my concentration? Perfect.

What you'll do is you'll come into the lab, and the TAs will go through this with you. You'll make up your stock solutions. And then you'll go to the river to get your water.

And when you bring the water back to the lab, there'll be two of you. So one of you will go over to the hoods and get the color developer. And these are the four chemicals that we talked about in the color developer-- the ammonium molybdate, sulfuric acid, ascorbic acid, potassium antimonyl-tartrate.

Potassium antimonyl-tartrate is there to actually speed up the reduction that's going on with ascorbic acid. That's important. If I didn't mention that before, that's the purpose of that. The ammonium molybdate, sulfuric acid, you're creating that Keggin structure, that heteropoly, colorless, molybdic acid.

You also have to take this color developer in the order that it's written here. It'll be set up in burettes in the hood. If you, for example, take it in a wrong order, you could have a side reaction, and not get the color developer that you want to put in your sample. So it's important.

So one of you will go to the hood, get the color developer. The other one will go to the river water you brought back, use a clean pipette, 10 mL pipette, and pipette five beakers with river water, five 10-mL beakers. So you'll end up with 12-- seven standards and five samples. And then you add the color developer, and you wait 20 minutes, and you're ready to go.

Just a couple other precautions that you should take-- we're going to be using 4-mL cuvettes. These are big inside. Don't use the 1 and 1/2 mL cuvettes.

The other thing is all these cuvettes have a fill line. It becomes frosted at one point. And you want to stop pouring when you get to that line. You're going to take your beakers, and you're going to swirl them gently. And you're going to pour them by hand until you reach the fill line. Don't go beyond that.

Also, all of these UV cuvettes have an arrow on one side. You want to be sure that the arrow is in the light beam when you put these cuvettes in the UV spectrometer. A lot of people have made mistakes, put them in the wrong way, and then you're not going to get very good readings.

So the arrow's clear to see. What I usually do is arrange all my cuvettes in a box ahead of time. And then I pour my samples and put them in the box, and I know the arrow's in the right place. And when I go to the UV, I just lift them up and put them in.

Also, keep in mind what Dr. Sarah Hewett told you the other day that there's two kinds of pipettes in the lab. This one is a blowout pipette. You've got to blow it all out to get your 10 mLs.

This one is a to deliver pipette. You only go up to that last line. You don't blow the tip in, otherwise you put too much-- going to wreck your experiment. So make sure you look at the pipettes carefully.

So you're going to get your graph here, a nice graph. And what you're going to do is you're going to interpolate now your river absorbances against the curve so you can read off the concentrations. So the concentrations in that graph are in micromolar.

So you're going to want to take those and convert them to milligrams per liter. So you want to convert this first to a molarity, moles per liter. Then you take that, and you want to go down to grams per liter, and then finally milligrams per liter.

So you're going to be calculating two things. You want to calculate the phosphate concentration in ppm, which is milligrams per liter, and the phosphorus concentration. So you'll take your mass from the curve in milligrams per liter, and take it times the ratio for phosphate of PO4 over KH2PO4. And that ratio is 0.70.

And then for phosphorus, do the same thing. The ratio there is 0.23. And this is the most important part of this lab-- this is the whole thing, calculating these concentrations in the end. So it's important to know how to do that.

So once you get your concentrations, you've got a series of things to go through here for your data analysis. There's no error propagation on this part of the lab, which is good for you. You will have to use the LINEST equation that Sarah talked about, which is pretty easy to use. And that'll help you calculate the errors of your slope intercept and y values.

And then you find your average and standard deviation of the five measurements, calculate the 95% confidence interval. Most importantly, report the final concentration of P and PO4. Make sure all these results show up in your abstract.

First thing I do when I get lab reports is I look at the abstract. I want to see a line that shows my results. And they also should appear in your conclusion, and then you can discuss them in your discussion of your lab report.

So any questions about this? I know you're going to have a busy weekend because you're going to be working on your ferrocene lab, your first lab report. And I want to tell you that please, feel free to reach out to me or Sarah if you have any questions.

I'm going to be here tomorrow. I probably will be here Saturday and Sunday. So if there's a last minute question, just send me an email, and I'm happy to invite you in, and we can answer your question. I should be here in the afternoon, probably, on Saturday and Sunday.

Now, I'd like to end the lecture today by doing a demonstration. Actually, did I turn that off? I can show you this here. I'm going to do this Briggs-Rauscher reaction. Actually, we're going to make this iodomalonic acid. We're going to actually attach an iodine to malonic acid. We're going to create this in a beaker.

The reason I'm showing you this reaction is because it has everything in it, including starch. And it has a lot of colors. And these colors are things that we're going to talk about in the next lecture, what starch does and how it works. And this is kind of a preview to that.

This reaction that you're going to see was actually discovered by two high school chemistry teachers. They're Briggs and Rauscher from Galileo High School, my favorite city, San Francisco. So these guys came up with this back in, I think, 1973.

They published a paper in the *Journal of Chemical Education.* And they had every scientist in the country puzzled. And it took about 10 years to work out all the and sundry reactions that are going on in this reaction.

So I'm going to put on some safety glasses. And what I'm going to be doing is going to be mixing three colorless solutions in here. This is my first one. And I'm going to use kitchen chemistry, which means I'm kind of looking at that scale over there and I'm going to pour these in.

So let's put a little more. That's colorless, right? OK, next colorless solution.

That's about right. And the final colorless solution-- keep your eye on it. Oh my goodness, wow. It wasn't supposed to do that. What? Are you kidding me?

What's going on here? Get your clock out. This is a clock reaction. You can keep time with this. So I'll give you a little hint. There iodide in there, I minus. And I minus is colorless.

There is iodine in there, and iodine is amber. When they're both present, it's blue-black. And we're going to see why that is in the next lecture.

So there's a lot of reactions going on in there. When these reactants react, they form hypoiodous acid. And sometimes, the hypoiodous acid actually oxidizes iodide to iodine. So you've got collarless going to amber.

But sometimes, there's so much hypoiodous acid formed, you can't handle everything. And what happens is you have a little bit of I minus and I2 present at the same time. And you get blue-black. This is kind of the color you're going to get when you do your titrations and you add the starch, because there is starch present in this.

So I'm going to leave you with that. And I'll see some of you up in the lab. Yes, you have a question.

AUDIENCE: [INAUDIBLE]

JOHN DOLHUN: She just asked me if this will ever stop. Can someone-- Hannah wants to know if this is going to ever stop. Who can tell me, anyone? Aisha?

AUDIENCE: I feel like it won't, because it's not releasing gas.

JOHN DOLHUN: Aisha says it probably won't because it's not releasing gas. But in a minute, it's going to. Iodine vapor is going to start pouring out of it, and Tristan is going to carry it up to the lab in a bucket quickly.

But in answer to Hannah's question is, will this stop?

AUDIENCE: [INAUDIBLE]

JOHN DOLHUN: Limiting reagent, right? There's always a limiting reagent. OK, see you.