Message

From: Blackwell, Brett [Blackwell.Brett@epa.gov]
Sent: 2/8/2022 8:15:03 PM
To: Brennan, Amanda [brennan.amanda@epa.gov]
Subject: RE: AltEn soils and effects
Attachments: Transformation Prediction Tools NTA_Product Template_BrennanAA_20220207_3SP_BRB.docx

Sorry about that! Always getting ahead of myself. The proposal looks good, no additional stress needed 😊

Brett

From: Brennan, Amanda <brennan.amanda@epa.gov>
Sent: Tuesday, February 08, 2022 2:13 PM
To: Blackwell, Brett <Blackwell.Brett@epa.gov>
Subject: RE: AltEn soils and effects

Hi Brett,

Thanks for your quick reply, and comments. Could you resend the attachment?

This is my first time submitting a StRAP proposal and I keep forgetting that everything doesn't have to be finalized now. This email has really helped my stress levels a bit, so thanks for that!

Amanda

From: Blackwell, Brett <Blackwell.Brett@epa.gov>
Sent: Tuesday, February 8, 2022 3:00 PM
To: Brennan, Amanda <brennan.amanda@epa.gov>
Subject: RE: AltEn soils and effects

Hi Amanda,

I provided a small edit, feel free to take or leave it. I did add a comment about fractionation and factoring in how that impacts the number of samples you want to characterize. It depends on the objectives you are focused on.

Back to the bioassays, you certainly can use Attagene for this, but I would be concerned you are missing a large fraction of relevant biological space. Cell-based bioassays often need soil or sediment extracts to be diluted out heavily so the natural humic acids and other organic carbon do not cause cytotoxicity. The primary benefit of the eco-htt approach is that is uses whole animals which are not as susceptible to those same cytotoxicity issues, and it covers multiple species across different taxa. This improves the ability to identify true compounds of interest that may be driving toxicity across various species.

The Attagene cross-species assays do cover multiple species, but they are limited to a few receptors. One includes, ER, AR, PPARg (2 species only), and TR (thyroid hormone receptor). Another has PPARg, PPARa, RXRb, and GR. For the pesticides, I don't think any of these targets are especially relevant though.

Right now, all that level of detail does not have to be worked out. The finer details of assays and testing design can be worked out as the project progresses.

Hope that helps,
Brett

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From: Brennan, Amanda <brennan.amanda@epa.gov>
Sent: Tuesday, February 08, 2022 10:38 AM
To: Blackwell, Brett <Blackwell.Brett@epa.gov>
Subject: RE: AltEn soils and effects

I wanted to follow-up on the Attagene. Interestingly, all endpoints for the select neonics were negative (as you said), but not the case for the fungicides. I also saw that there is now an eco-factorial attagene which tests multiple wildlife and human receptors. Thought this might have more applicability when paired with eco-HTTK, or help prioritize samples or portions of the sample extract for eco-HTTK.

Thanks,
Amanda

From: Brennan, Amanda
Sent: Tuesday, February 8, 2022 11:18 AM
To: Blackwell, Brett <Blackwell.Brett@epa.gov>
Subject: RE: AltEn soils and effects

Hi Brett,

Ex. 5 Deliberative Process (DP)

I would greatly appreciate your thoughts and edits (especially if you think more language on the bioactivity portion is needed).

Thanks,
Amanda

From: Blackwell, Brett <Blackwell.Brett@epa.gov>
Sent: Wednesday, February 2, 2022 1:39 PM
To: Brennan, Amanda <brennan.amanda@epa.gov>
Subject: RE: AltEn soils and effects

We have really only done these approaches with targeted data. The NTA work on the S. Platte is not published because I don't have time to figure out all the data analysis! It takes so much time to get that type of workflow running, so it makes sense on projects like this for us to provide support from the bioeffects side (from sample selection through data interpretation) and your team support the NTA work. Myself, Dan, and/or Kevin can all contribute here as needed.

The attached paper is where we prioritized chemical and bioeffects data from the S. Platte based on known benchmarks of various types, which may be helpful.

Brett

From: Brennan, Amanda <brennan.amanda@epa.gov>
Sent: Wednesday, February 02, 2022 10:55 AM
To: Blackwell, Brett <Blackwell.Brett@epa.gov>
Subject: RE: AltEn soils and effects

Hi Brett,

It's great that contracts are in the place that additional money can be added to!

For the eco-HTTr, would you be able to help with the interpretation of the data, and possibly selection of soil samples that might yield interesting results? We're going to run the results of the NTA work through the hazard comparison dashboard to identify potential hazards so hopefully, that would help with sample selection.

Your group did similar work with some of the samples from the South Platte River, right? Do you have a publication handy that I can look at? My quick search didn't yield what I was looking for, but I remember you presented something at the NTA meeting about it.

Thanks,
Amanda

From: Blackwell, Brett <Blackwell.Brett@epa.gov>
Sent: Tuesday, February 1, 2022 1:45 PM
To: Brennan, Amanda <brennan.amanda@epa.gov>
Subject: RE: AltEn soils and effects

Yes, we have done quite a bit of testing of surface water extracts across multiple assay types. Attagene does work for screening, but it is limited in the endpoints. Most are nuclear receptors, which are not the direct targets of pesticides. Looking at clothianidin for example, it has been tested in Attagene but was a negative for all endpoints. I think ATG makes the most sense for more broad, screening level efforts where diverse sites and a variety of potential contaminants are expected. So overall, I do think the transcriptomics approach using ecologically relevant species (eco-HTTr) would be a much better fit.

For eco-HTTr, the cost is in sequencing the samples, and there will be contracts already in place that could accept additional funding. In the past when we have used ATG, there was an existing contract in place that we loaded money onto. That may have expired, but I would guess there is another one coming online. I don't know that much about that side of things and usually try to avoid it if I can!

Hope this helps
Brett

From: Brennan, Amanda <brennan.amanda@epa.gov>
Sent: Tuesday, February 01, 2022 12:16 PM
To: Blackwell, Brett <Blackwell.Brett@epa.gov>
Subject: RE: AltEn soils and effects

Hi Brett,

No worries at all.

Great news that you might have availability depending on timing, and the cost estimate per sample is extremely helpful.

Attagene was also mentioned as a possibility for looking at bioactivity. I noticed you published a paper on Attagene with some of the Platte River samples. I sat in on a meeting yesterday (for a different project) in which there was a discussion on processing of data from Attagene, and how to possible correlate some of the NTA data with bioactivity. I'm pretty new to the biological side of things, so didn't realize that a bunch of Attagene testing has already been done for a large group of pesticides. I haven't delved into the data to see if there is overlap between the pesticides found in AltEn and that on Toxcast. Given your experience with Attagene in river samples, do you see this being a potentially informative assay if the effects seen from extracts or fractions of extracts can be linked to data already generated from individual pesticides?

As I mentioned earlier, the link to measured effects is tentative depending on the NTA, but one that I'd like to have the possibility of exploring. For the proposal I don't think I need to have it entirely fleshed out, but I'd like to have an idea of which tests (in-vitro or in-vivo based) would yield the best data for comparison to NTA for this sample set (with an already good knowledge of contaminants at the site). Do you have any thoughts on this?

Thanks,
Amanda

From: Blackwell, Brett <Blackwell.Brett@epa.gov>
Sent: Monday, January 31, 2022 2:54 PM
To: Brennan, Amanda <brennan.amanda@epa.gov>
Subject: RE: AltEn soils and effects

Sorry for the slow turn around, the UNL meeting kicked me into action.

Ex. 5 Deliberative Process (DP)

Let me know if you need anything more from our end! Thanks

Brett

From: Brennan, Amanda <brennan.amanda@epa.gov>
Sent: Wednesday, January 26, 2022 8:11 AM
To: Blackwell, Brett <Blackwell.Brett@epa.gov>
Subject: RE: AltEn soils and effects

Hi Brett,

Thanks for the quick reply!

The comparison of the predicted and measured pesticides and transformation products would be the primary goal of this research. The effects testing would likely be dependent from the results of the NTA analysis. I would imagine it would only be a small set of samples, if any. I know that doesn't really help out with the funding needs, but it might from a time management perspective. I think it will also depend on what samples we are able to get from the Univ of Nebraska. It's a bit up in the air at this point, but hopefully, we'll know more about their sampling plans in the next week. I do plan on asking for some funds to do some sort of effects testing in the proposal.

Hope you're staying warm!

Amanda

From: Blackwell, Brett <Blackwell.Brett@epa.gov>
Sent: Monday, January 24, 2022 5:12 PM
To: Brennan, Amanda <brennan.amanda@epa.gov>
Subject: RE: AltEn soils and effects

Hi Amanda,

I saw the extreme winter event hitting NC, hope you made it through safely! 😊 Now that I've adapted to Duluth it would be strange to go back to mostly snowless winters.

The eco-high throughput team (mostly led by Dan V and Kevin Flynn…I'm the research chemist with the project) has been developing the assays and now testing chemicals over the past ~2 years. The 4 species are: green algae (*Raphidocelis subcapitata*), *Daphnia magna,* midge (Chironomus dilutus), and fathead minnow (*Pimephales promelas*). The platform is mainly focused around transcriptomics and generating points-of-departure by running dose-response of chemicals. They have done the most work so far with daphnia and fatheads, and for those species they can also get some behavioral data and apical-level endpoints (mortality, ataxia). I do know that the transcriptomics is not cheap, and we would definitely have to request funding if that is going to be an endpoint of interest. Also, I will need to check with Dan and Kevin about overall workload and when this could fit in the current StRAP. We are facing a lab renovation beginning May 2023, so that is also limiting what we can fully commit to. Let me talk with them both and see about what funding we would need and if they do think this could fit in.

For the extracts themselves, we have not directly tested any soil extracts, only water extracts. For the soils, figuring out a suitable dilution series for testing would be critical, and identifying a clean, reference-type soil of similar composition for testing. These are all aquatic species as well, so the link to soil exposure is less direct. Or perhaps the angle would be runoff from contaminated soils to water bodies? The AltEn project seemed like an ideal project to use some of these approaches, but it has been a mess as far as planning goes, and I haven't delved much deeper since that first meeting.

I'll follow up with Dan and Kevin and get back to you in the next two days. Then I can set up a Teams call with one or all of us to discuss this more!

Thanks
Brett

From: Brennan, Amanda <brennan.amanda@epa.gov>
Sent: Monday, January 24, 2022 7:54 AM
To: Blackwell, Brett <Blackwell.Brett@epa.gov>
Subject: AltEn soils and effects

Hi Brett,

I hope you're staying warm! We had about 3 inches of snow this weekend in Durham, and it made me realize how much I miss winters in Duluth.

We (some of us in Elin's Branch) are proposing an NTA project for StRAP 4 (CSS 402.6- Seth Newton's Output) related to AltEn soils. We would like to compare transformation products predicted by ORD tools (Chemical Transformation Simulator) and others to NTA results. University of Nebraska I believe has a good idea of parent pesticides found near the site from previous and ongoing work. We would also like to predict effects and risk based on the identified pesticides and transformation products in the soils and compare that to measured effects (using a soil extract). Maybe not necessarily in that order.

During the initial AltEn meeting in November, there was discussion of effects directed analysis. You mentioned you have a good workflow in Duluth for testing effects to 4 different species. I was hoping to get a bit more information about the assays you mentioned (such as species, endpoints, applicability to soil extracts, any articles you recommend), if you have any interest in collaborating with this effort, and the feasibility of the effects work that we are thinking about.

I'm more than happy to chat via Teams if that's easier than email.

Thanks,
Amanda