Chat with Kathleen 10/13/2020

**Vehicle Solvent Q’s**

Confirm 0.1% DMSO for all. I did not check for every culture, but most every culture.

* NTP91 - I have Dosing plate info for 2 cultures
  + 20160720 -> Looks like 5% DMSO in treated wells, 100% DMSO in control wells?? Then 1:50 dilution?
  + 20160907 -> Looks like 5% DMSO by volume in all wells in Dosing plate. Then 1:50 dilution? (just want to confirm, don't see that written anywhere)
  + What should I assume about the conc of DMSO in the rest of the cultures?
* ToxCast, Frank2017, PFAS2018
  + All 5% DMSO in Dosing plates! Can I assume always 50X dilution to MEA plate, for a 0.1% DMSO final volume? Regardless, the conc of DMSO in dosing plate is consistent for all columns and cultures!!
* Brown 2014
  + All appear to be 0.1%. Confirm that control wells are DMSO/Water according to what was tested in each row (perhaps pull up lab notebook from first culture 20140402, and solvent controls list in paper)
* OPP2015 -> I don’t have the lab notebook, so what to confirm this should be the same.

Kathleen: Yes, should be 0.1% DMSO for all, except occasionally Water or 1:1 DMSO:Ethanol. Do be on the look out for that.

Also, I just checked some Dilution sheets for OPP2015, and it is the same.

Except, I’m confused about DNT/GF:

DNT/GF Control well concentrations:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| DMSO | 20190807, 20190904,20190918, 20191016, 20191030, 20191113 A2 - D2 | Add 5 uL DMSO to Dilution/Dosing Plate | Add 95uL of Media | 5% DMSO by volume | 1:50 Dilution | 0.098% DMSO |
| DMSO | 20190807, 20190904 (G3), 20190918, 20191016, 20191030, 20191113 E2 - F2 | Add 7.5 uL DMSO to Dilution/Dosing Plate | Add 95uL of Media | 7.3% DMSO by volume | 1:50 Dilution | 0.14% DMSO |
| Water | 20190904 (G4) wells E2 - F2 | Add 5 uL Water to Dilution/Dosing Plate | Add 95uL of Media | 5% Water by volume | 1:50 Dilution | 0.098% Water |
| Water | 20190710, 20190724 all control wells | Add 10 uL Water to Dilution/Dosing Plate | Add 190uL of Media | 5% Water by volume | 1:50 Dilution | 0.098% Water |

Questions: (see DNT Group 1 case example)

* For DNT and GF, it lists 5uL for A2-D2, then 7uL for E2-F2. Is that difference real, for every culture? (I am suspicious that the format was just copied, and that this is not right). Is it right?
  + Yes, wells E2 and F2 are a higher % of DMSO. Kathleen did this because she had to have a higher % DMSO for the highest conc tested, so she wanted to have some controls with same DMSO conc.
    - In fact, the row of the higher-conc controls rotated with the treatment row rotation (so e.g. culture 1, 0.15% DMSO wells are always in same row as treatments 11 & 12). – check, implemented.
    - Look and see if the DMSO conc makes a difference, then decide if should normalize to separate controls (as Katie talked about, have subsets of apid!)
* Is it correct that the highest dose tested has 7.5uL test compound+92.5 Media -> 7.5%DMSO -> 0.15% DMSO in the final? - yes
* Loperamide – Looks like dissolved in Water in GF (2019), dissolved in DMSO in DNT 20191113 Group 12. (is that correct)? If so, what are your thoughts on whether we can combine that data in 1 dose-response curve, or should these be kept separate? (there might be other compounds in this category)
  + Loperamide is being used as an assay control, so it could be treated differently (e.g. don’t even have a dose-response plot?) – Let’s do the analysis of vehicle controls, see how all the replicates look, then will probs chat with Tim to see which culture to include for Lop, if any
  + For the DNT culture: she thinks she most likely diluted Loperamide in water, the same as in GF. But, the control in the corresponding well is most likely DMSO for this culture.

Want to confirm: Other than explicit control well info for DNT/GF above, If a compound is dissolved in something other than DMSO (e.g. H2O), does that usually mean that the control well in the individual corresponding well is also H2O?

* + See e.g. 20141203, Sodium Orthovandate is dissolved in H2O. Is well E1 H2O, while e.g. all others are DMSO?
  + K: yes, the content of control well should by default match the solvent for the compound in same row (unless otherwise noted, as in DNTGF2019)

Summary of what I know re solvent controls:

* + OPP2015 – sufficiently confident all are DMSO
  + Brown2014 – solvent control is stated for every compound. Already implement
  + DNTGF2019 – content of control wells is clearly stated, already done
  + PFAS2018 – DMSO is definitely the control for all
  + Frank2017 – I have the table… but also I lack confidence in the table!
  + ToxCast – confident DMSO is solvent for all.
  + NTP91 – confident DMSO for all

So I really only need to think about this for Frank2017

I found 1 mistake in Table 1 already – Acteaminophen dissolved in Water in 20141203, but table says dissolved in DMSO!

But, I don’t think it is worth it for me to read through every notebook.

I will just start by nailing down the situation with Acetaminophen

**Other data set Well quality/inclusion questions**

* PFAS question:
  + Last time, you explained the note in lab notebook "Misdosed with both 1 + 2" means that column 1 and 2 doses were switched (lab notebook says col 1=0.03, col2 = controls). Doses for columns 1 & 2 are switched in the Calc file and master chem list for MW1208-3 20181017. However, columns 1&2 are also switched for every other plate in this culture! (both groups!) I am assuming that was a mistake, and that only the doses for 1208-3 should be switched?
    - Yes, should just be the one plate. Correct this (in both calc and master chem lists) – check, done
* Specific Aim 1:
  + Domoic Acid, published data doesn't include conc's at 0.0003 or 0.001 (lowest conc included is 0.003). Should I remove those points, or keep for the data to pipeline? (try to have pic's, or a least a table to reference as we look at it)
    - Yes, remove the data for those conc’s, since would be only an N of 1. – check, implemented in wells\_with\_wllq\_zero.csv
  + Sodium Orthovanadate - published data includes 0.01, which was tested in 20140716. We don't have that spike list files for that culture date right now. Okay to just not include that? (again, have pics/tables ready). Current available cultures only go down to 0.03. Glanding at paper, looks like it was active at 10+uM, so maybe not a prob
    - Kathleen will look for the spike list files for those 2 cultures (20140716,30)

**Specific Aim 2 – DNT 2 well Quality questions:**

(Preface: Just reading through the lab notebook, not sure which notes are noteworthy, what's not a big deal)

* 20140827: Looks like there was a mistake made for dosing on DIV 9 all plates. But, she caught it and removed the media, added new media, then added correct dose.
  + The incorrect dose was on the plate for about 5 hours before redosing.
  + The mistake: "Column 1 went into Row A, col 2 into Row B, etc." - so, not just wrong dose, but temporarily wrong compounds!
  + I’m not even sure what kind of image I would need to assess this…
  + Compounds: "Acetaminophen" "Bisphenol A" "Cocaine" "Cytosine Arabinoside" "DEHP" "Deltamethrin"
  + All of these were re-tested on 20141112
  + We decide these are okay!!! - K
* 20141015 – “at some point during dosing, pipette switched from 10 microliters to 9.8 microliters on plate” for MW1008-39 and MW1007-108. I really don’t know, and I am just curious what Tim/Kathleen think.
  + See plots of compounds at highest conc’s for a few endpoints, there does seem to be a difference
  + It’s not total evidence… there are just enough points where those 2 plates are a lot higher than the other plate that make me concerned.
  + Don’t worry! Within the error of the person using the pipette. E.g. can push past the stop
* 20141029 – [stance: hey, I just found this to be a curiosity, wondering what you think] – pipette tip was loose. All 3 plates affected
  + “When preparing dose plate I noticed (at nicotine) that the pipette was loose/unscrewed which affected the accuracy of the amount of chemical being dispensed. The pipette may have been like this each time I prepared my dosing plate, resulting in inaccurate dilutions. I fixed the pipette at nicotine. All chemicals before were affected.” (for DIV 9).
    - Plates involved: 1008-41, 1008-42, 1036-8
    - I can't find this note in the scanned notebook… maybe it was on a different page. I feel confident that I did not copy this down mistakenly.
    - Compounds:
      * 1008-41, 1008-42: Nicotine, lead acetate, sorbitol, Dieldrin (3 rows for ea across the 2 plates)
      * 1036-8: Methylmercury, Cadmium
    - After chat with Seline, a loose pipette might slightly decrease the amount of the dose. However, JB would have known that she could redo the dosing plate. Since she did not, she must have thought it was okay.
    - All only tested in 20141029, except Sorbitol re-tested in 20151014
    - All wells currently included in published AUC data
  + K: we didin’t worry about it here either (because only during last dose on DIV 9)
* 20150805
  + N=1 for LDH for 5 of the compounds. Jasmine mentioned perhaps we should exclude all LDH for these chem. What do you think? Show the tcpl plots from previously pipelined data. "Additional Well Quality Notes" page
  + Compounds: "Acetaminophen" "Hexachlorophene" "Hydroxyurea" "Methotrexate" "Tebuconazole" "Thalidomide"
    - Only Acetaminophen was retested elsewhere.
    - Published LDH table did not include any LDH data for Acetaminophen from this culture, but did include all 3 Plates for the other 5 compounds in this culture! (Those 5 compounds were not tested anywhere else).
    - This is Tim question (put this in pre-final report to Tim). K says she thinks it’s okay, as long as you say it’s just 1. – in my notes to run by him.
* 20151125
  + MW1088-3: Note on LDH plate spill. Won't use that data. But also, no AUC data from this plate is included in published data. I am concerned that was just a misunderstanding, and should actually be included. I don't see a specific note saying that MEA data be excluded. Other 2 plates from this culture are present.
  + 3/6 control wells have less than 50 MFR at DIV 12, so that could be why. But I don't plan to universally apply to rule until later.
  + From CF's notebook: This culture not included for his abstract for 2 select compounds.
  + So, should I include this plate's mea data or no??
  + (will add to this as I get more info from other sections \*\* Note next page, info from Kathleen on the 3 re-tested compounds!!)
  + Tim question whether to include or not
  + Remember: include the lower conc’s especially for Emmamectin benzoate!
* 20160601
  + (I think this is fine, just want to get Kathleen's opinion)
  + I accidentally skipped this one when chat with Kathleen. Based on the fact that they decided that most other notes above are okay, and my results with the graphs, I think this is okay too.
* There is a culture that I am not sure why these aren’t included in the published data file. Is it worth investigating? (all of these compounds were retested)
  + 20150422 - DNT Reference Compounds.xlsx says "Issues with cytotox assay". No AUC data included from this culture in published file. Do we really need to remove MEA data as well? Some controls seem a little low, but not that bad.
    - Controls did not develop. That’s why not included. So don’t include it