Silver Tox Experts (TE) call

Ag reprotox and study Ag effects on the biome

Draft minutes, call 24 May 2018 (15:00 – 15:30 CET)

Participants

- Katrien ARIJS, Consultant for EPMF (Arche, Belgium)
- Arno BUTHE (Heraeus, Germany)
- Marie-Laure LEDRICH (Traxys, Luxembourg)
- Mark RAFFRAY, Consultant for EPMF (Raffray Biosciences Ltd, United-Kingdom)
- Nissanka RAJAPAKSE (Johnson Matthey, United-Kingdom)

Updated proposal Prof. Lison gut biome study

The updated proposal from Louvain University is available in Annex 1. There are some remaining points for discussion:

- **Cu, Se, ceruloplasmin analyses: in plasma or serum?** Prof. Lison suggested plasma because this would limit the number of tubes (tubes to be doubled for serum). It is perfectably acceptable to do the analysis in plasma but this would not allow us to directly compare the results to e.g. the Shavlovski work with AgCl. The TE confirm that for comparability, these analyses should be done in **serum**. It is noted that in this case, we may have some more sample loss -> **AP: PMC Sec to check if there are any issues with blood volume.**
- **RNA sequencing**: the TE agree that a **storage duration of 1 year** should be sufficient for PMC to decide if any further analysis is needed.
- **Stability in food**: which analyses needed?
    - Prof. Lison suggests only checking the stability of AgAc in food in the lowest dose. At the previous TE call, it was suggested to check how test substance stability in the diet was analysed for PGM studies: see Annex 2 for platinum dinitrate example. Groups refer to dose groups (Group 1 = control; Groups 2, 3, 4 = concentrations of 300 ppm, 1000 ppm and 3000 ppm respectively). Concentration, homogeneity and stability were checked in all doses. However, this study was performed at a CRO instead of at a University lab.
    - Prof. Lison did not mention the additional costs for additional analyses but expects that if we can exclude the possibility that AgAc is transformed into a non-soluble form at the lowest dose in 1 batch, there is no reason to suspect that it will occur in other batches or at a higher dose.
    - The TE agree that Prof. Lison's explanation is acceptable and agree to **only measure the stability in the lowest dose** but suggest **to hold archive samples of the actual experimental diets so we can analyse these later if we are ever challenged** -> **AP: PMC Sec to check if Carfil can retain samples of all concentrations and all batches.**
- **ICP-MS analyses**:
    - Prof. Lison believes that sample preparation is the critical aspect of the procedure, that the ICP-MS part is not as delicate and that it is not a good idea to separate sample preparation from the measurement in itself because the lab who performs the mineralization needs to control the procedure. The complete mineralization and recovery need to be assessed and this

includes ICP-MS measurements. Van der Zande et al. mention an average recovery of 70% for blood and organs.
    - No further information from AML at this point -> **AP: PMC Sec to check with S. Verberckmoes regarding actions last call.**
    - The TE would prefer to check with their analytical department regarding the separation of the sample preparation from the measurement -> **AP: TE to check internally with their analytics department whether they agree it is not a good idea to uncouple the sample preparation from the ICP-MS measurement.**
- Comments on contract for the study: see Annex 1.4. The TE agree with the suggested additions, and agree to ask for full PMC ownership of the study results.

Annexes

1. Proposal AgAc gut biome study, Louvain University, 15 May 2018
2. Example diet analysis Pt dinitrate