Silver Tox Experts (TE) call

Ag reprotox and study Ag effects on the biome

Draft minutes, call 26 February 2018 (14:00 - 15:00 CET)

Participants

- Katrien ARIJS, Consultant for EPMF (Arche, Belgium)
- Arno BUTHE (Heraeus, Germany)
- Mark RAFFRAY, Consultant for EPMF (Raffray Biosciences Ltd, United-Kingdom)
- Nissanka RAJAPAKSE (Johnson Matthey, United-Kingdom)
- Steven VERBERCKMOES (Umicore, Belgium)

1 Introduction

At the 16 Jan TE call, the design aspects of the gut biome study (enabling study for EOGRTS) were discussed (cf. slides 2-3 in Annex 1). Based on the design recommended by the TE, a proposal for the study was drafted by Prof. Lison and Dr. van den Brule (Louvain University) – cf. Annex 2. **The aims of this call are to 1) check if the TE have any specific comments / questions / additions on the gut biome study proposal; 2) re-discuss the timeline and 3) highlight the importance of a recent study identified during the latest literature review**.

2 Gut biome study design

The proposal from Louvain University is available in Annex 2 and summarised on slides 4-6 in Annex 1. Questions / comments from the TE:

- **Dietary formulation**: the proposal suggests to do the incorporation of AgAc in food pellets at Carfil (cost 1000 €) but PMC is free to select another provider. Judging from the costs, the dietary formulation **analysis** is probably not included but to be checked (**AP1**).
- The graphical outline of the study design mentions measurement of 'serum ceruloplasmin' at week 6; this should read '**serum ceruloplasmin oxidase activity**'.
- The proposed **biome sequencing** is a copy from what was done in the van den Brule et al. 2016 study.
- **Sampling blood for Ag, Cu and Se analysis and sampling faeces for Ag analysis**: M Raffray suggests to do the mid-point sampling at **week 4** (= week 2 of dosing). This aligns with the 2 week pre-mating in the EOGRTS protocol + the food consumption is expected to be steady state by that time.
- **ICP-MS analysis**: the proposal suggests to do these analyses at **St Luc**, the Louvain University hospital:
    - None of the TE have experience with this lab; **accreditation** to be checked (**AP1**).
    - Total **costs** for these analyses are rather high (~20 k€) but cost per analysis (30 €) seems realistic.
    - The cost overview mentions a total of **640 samples** but this doesn't correspond with the number of samplings mentioned in the description (cf. slide 5 in Annex 1); to be checked (**AP1**).
    - It is assumed that (mid-point) **collection of the faeces** will be done per rat in individual metabolism cages but to be cross-checked (**AP1**).

  - Whereas Cu and Se analysis should be standard measurements, the **ICP-MS analysis of Ag** in biological tissues at St Luc is the most critical in terms of timing; this might require significant development. There is no formal guarantee that all Ag measurements will be available at the end of the 5 month study period (but this would not affect main study objective, i.e. impact on the biome). An alternative could be to outsource the ICP-MS measurements but other labs that may have more experience with Ag analysis, would not necessarily have experience with analysis of biological tissues. S Verberckmoes suggests checking with **AML** (https://www.aml-lab.be), an independent lab based in Antwerp that did some of the Au analyses and is experienced in analysing biological samples (mainly human) (**AP2**). Ideally, all analyses (i.e. for Ag, Cu and Se in all tissues of interest) would be performed at the same lab. It is noted that AML is not a standard CRO / not GLP certified and as such, their reporting is rather brief. Since this study is enabling work and not a standard OECD test, the TE do not consider this an issue.
- **Time schedule** (cf. slide 6 in Annex 1):
  - The study would take about 5 months and an additional **2 months lead time** would be needed (for ordering rats, food, development Ag analysis…).
  - The proposed time schedule with week estimates looks completely sequential, whereas it should be possible to do some of the analytics in parallel (e.g. Cp oxidase activity, ICP-MS analysis, mrDNA sequencing). It is suggested to ask for a **Gantt chart** instead (**AP1**).
- Since the test would be done at a University lab, we need to think in advance about possible **publication of test results**. Usually, universities include a clause in their contracts to arrange their right to publish. This is not considered a problem as such but it is suggested to check the possibility for a time delay before publication so we have time to prepare a strategy as needed (**AP1**).

In order to extract maximum value from the study and taking the recent literature into account, M Raffray suggests 2 potential additional measurements in the biome study:

1. Measurement of **testicular Ag levels** (Ag concentrations per g tissue + organ weight + histopath): this is something that was not looked at in the Sprando study while other testicular studies were performed at lower exposure levels. This additional analysis would not require extra animals. The TE agree to check the possibility / cost to measure Ag levels in the testes but not to perform histopath at this point (28d exposure is too short and doesn't cover a complete spermatogenic cycle) and ask to store the tissue for potential histopath later. At the same time, it is suggested to measure Ag levels in ovaries / store ovaries for later histopath as well (**AP1**). When contacting AML, S Verberckmoes will also check costs for Ag analysis in testes and ovaries (**AP2**). Additional costs for these extra measurements are anticipated to be around 5 k€.
2. Measurement of **luminal Ag concentrations in the GI tract** at the end of the study: this was measured in a study on effects of nanoAg in mice (Bergin et al. 2016). However, this measurement may require satellite animals and isolation of the gut lumen is rather complex. The TE think faecal Ag levels may be adequate rather than getting into the complexities and cost of additional animals and tests on gut content. Along with some modelling, it should be possible to estimate microbial exposures. It will be checked with Prof. Lison what his thoughts are (**AP1**).

3 Timeline

At the 16 Jan TE call, it was agreed to wait for the proposal / timeline from Prof. Lison before deciding on the biome study. It was also noted that we might have the Draft Decision (DD) by then. It is still unclear when we

will receive the DD but a mail has been sent to ECHA to ask clarity on the timing (**AP3**). At the 20 Feb Management Committee meeting, the draft Ag strategy was discussed and since it is very unlikely to have results of the biome study before we have the DD, it was felt that there is no rush to start the study. The TE agreed to wait for clarity on the timeline of the DD before deciding on the biome study.

4 AOB

- **Gao et al. 2017** (cf. Annex 3): this study was identified during the last literature search. It reports toxicogenomics work from the US FDA which was done in parallel with the Sprando and Babu studies, and suggests potential for embryotoxicity. M Raffray did not find cause to set aside the findings based on study robustness or other reasons. As mentioned in the commentary (cf. Annex 3.2), the study is likely to be cited by externals as supporting the reprotox findings on AgAc and could worsen our position. Therefore it seems important to discuss its interpretation further within the TE group (**AP4**).
- From recent reading (e.g. recent NAS report https://www.nap.edu/catalog/24960/environmental-chemicals-the-human-microbiome-and-health-risk-a-research) M Raffray believes another possible target is the reproductive system biome as changes in this biome can be very influential; to be kept in mind for future discussions.
- A follow-up call will be held **Monday 12 March at 10:00 (Brussels time)**.

Annex

1. Slides presented at the meeting
2. Study proposal: effects of Ag on the gut microbiome (Prof. Lison and Dr. van den Brule, Louvain University, 5 Feb 2018):
   - 2.1. Description of the study design
   - 2.2. Graphical outline of the experimental design
   - 2.3. Excel file with budget calculation / timeline
3. Gao et al. 2017:
   - 3.1. Gao X, Topping VD, Keltner Z, Sprando RL, Yourick JJ. 2017. Toxicity of nano- and ionic silver to embryonic stem cells: a comparative toxicogenomic study. J Nanobiotechnology, 15(1):31, DOI 10.1186/s12951-017-0265-6
   - 3.2. Study review by M Raffray

Actions

Table 1. Actions agreed at the 26 Feb TE call

	What?	Who?	When?
1.	Check with Louvain University: • Dietary formulation analysis included or to be arranged by PMC? • Accreditation St Luc (lab for ICP-MS analyses)? • Nr of samples for ICP-MS analysis: analysis in faeces after 28d = analysis gut content after 28d? • (Mid-point) collection of faeces done per rat in individual metabolism cages? • Time schedule as Gantt chart?	PMC Sec	ASAP

	• Publication policy and possibility to have time delay between end of study and publication of results? • Possibility / additional cost to measure Ag levels in the testes / ovaries + store tissues for potential later histopath? • Possibility / additional cost to measure Ag levels in GI tract lumen?		
2.	Contact AML to check possibility / pricing / timing to analyse Ag, Cu and Se in blood and Ag in faeces / gut content / testes / ovaries	S Verberckmoes	By 12 March
3.	Follow up request for clarity on timing DD with ECHA	PMC Sec	ASAP
4.	Check Gao et al. 2017 paper + review by M Raffray in order to prepare possible rebuttal points (Annex 3)	Ag TE	By 12 March
5.	Follow-up call TE on gut biome study / timeline / Gao et al. study	PMC Sec + Ag TE	12 March 10:00 (Brussels time)