



Silver Tox Experts (TE) call

Ag reprotox and study Ag effects on the biome

Draft minutes, call 12 March 2018 (10:00 - 11:00 CET)

Participants

- Katrien ARIJS, Consultant for EPMF (Arche, Belgium)
- Arno BUTHE (Heraeus, Germany)
- Marie-Laure LEDRICH (Traxys, Luxembourg) - *for part of the call*
- Mark RAFFRAY, Consultant for EPMF (Raffray Biosciences Ltd, United-Kingdom)
- Nissanka RAJAPAKSE (Johnson Matthey, United-Kingdom)
- Steven VERBERCKMOES (Umicore, Belgium)

1 Approval of the minutes of the last call (26 Feb 2018)

This call is a follow-up of the 26 February TE call. The minutes of that call were approved.

2 Feedback Prof. Lison on additional questions re gut biome study proposal

The feedback from Louvain University on the additional questions raised during the last TE call is available in Annex 1. Comments / additions from the TE:

- The **mid-point (after 14d) collection of the faeces** will not be done per rat but will be done **per cage** (the lab does not have the possibility to keep 80 rats in individual cages):
 - According to the proposal, there will be a total of 32 cages (with 2 or 3 rats per cage), so there will be 32 mid-point faeces samples. This means that the total number of samples mentioned in the proposal is probably incorrect (total of 640 seems to assume 80 mid-point faeces samples): to be checked (**AP1**).
 - Not having the rats in individual metabolism cages is not considered ideal but given the fact that this is an academic and not a guideline study, the TE do not consider this a major issue. It is noted that this will only influence the mid-point faeces sampling and that we will have individual gut content samples at the end of the study and individual blood samples (both mid-point and at the end of the study).
- Prof. Lison mentioned again that the **ICP-MS analysis of Ag** is the most critical part of the study (cf. agenda point 3).
- Regarding measurement of **luminal Ag concentrations in the GI tract** (possible additional measurement discussed at the 26 Feb TE call), Prof. Lison is not convinced this would add much to the present protocol (except additional efforts and costs). The TE agree measurement of faecal Ag levels will be adequate.

3 Feedback AML on sample analysis

S Verberckmoes contacted AML to check the possibility to outsource the Ag, Cu and Se analyses to them: see Annex 2. It seems they will also need a significant **lead time** and guidance on **methodology for sample**



preparation. Delivery time might even be longer than at Louvain University. They have not provided a cost estimate yet as they need more details on methodology to define pricing. M Raffray thinks there are relatively standardised blood Ag measurement protocols. With due regard for Ag sequestration by macromolecules, and as an adjustment over the initial design for the microbiome experiment, it may be preferable to measure Ag in whole blood rather than serum. In respect of Cu and Se, analysis in serum still makes sense. For Ag tissue distribution / Ag faecal studies: microwave-acid digestion is a common choice (see Annex 3). S Verberckmoes will follow up with AML and ask for pricing (AP2).

There is some concern about Prof. Lison's keenness to perform the Ag biome study but this is probably due to the fact that the study would be an add-on to the lab's main research and that thus far, he does not have any formal commitment from PMC yet. The TE agree that a compromise may be needed for the TK work (like outsourcing to AML), but that for the remainder of the biome study, Prof. Lison's lab is the preferred option and there is no need to approach another lab at this point. It is agreed to contact Prof. Lison again only once we have further information on the timing of the DD and we can commit to the study. Also, the TE agree it would be good to organize a meeting with Prof. Lison to discuss the study design once we commit to the study, in order to ensure confidence in the study outcome (AP3).

4 Further info from ECHA on timing DD

At the 26 Feb TE call, it was agreed to wait for clarity on the timeline of the DD before deciding on the biome study but no reply has been received yet from ECHA on our request for clarity on the timing of the DD (AP4).

5 Discussion Gao et al. 2017 paper

The Gao et al. 2017 study (cf. Annex 4) was identified during the last literature search and suggests potential for **embryotoxicity**. As mentioned in M Raffray's commentary (cf. Annex 4.2), the study is likely to be cited by MS as supporting the reprotox findings on AgAc and could worsen our position. The TE agree this looks like a well performed study and share M Raffray's concern. It is of additional concern that RIVM is apparently keen on the embryonic stem cell test (EST) to be taken into account as standard test in the future. The TE agree it is important to prepare for rebuttal points and to get another expert opinion (AP5-6).

6 Follow-up on Cu homeostasis issue

When discussing the possible MoA of silver during the 9 Nov Ag WG call, it was suggested to re-check the argumentation PMC previously used when commenting on the SZZ CLH that the disruption of the Cu homeostasis caused by Ag should be considered an indirect effect, as M Raffray identified some weaknesses in our position while further looking into studies on this possible MoA. It was discussed that further expert advice may be needed from an expert on micronutrients in pregnancy and Prof. H McArdle was suggested. He has not been contacted yet (although Violaine Verougstraete was contacted, who has already worked with Prof. McArdle and she is willing to help PMC initiate the contact with him), as the biome MoA was considered a priority but the TE agree we should also move forward on other MoAs that could also be relevant. AP7

Annex

1. Feedback Prof. Lison on additional questions re gut biome study proposal
2. Feedback AML on Ag, Cu and Se analyses
3. Examples microwave-acid digestion for Ag tissue distribution / Ag faecal studies:



- 3.1. Poitras et al. 2015 (method development)
- 3.2. van der Zande et al. 2012 (recent TK work)
- 4. Gao et al. 2017:
 - 4.1. 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
 - 4.2. Study review by M Raffray

Actions

Table 1. Actions agreed at the 12 Mar TE call

	What?	Who?	When?
1.	Check with Louvain University if total nr of samples for ICP-MS analysis are correct	PMC Sec	After having further info on DD
2.	Follow-up with AML on methodology and pricing for Ag, Cu and Se analyses	S Verberckmoes	March 2018
3.	Organise meeting with Louvain University to discuss study design of the Ag biome study	PMC Sec + Ag TE + Prof. Lison	After approval to perform study
4.	Follow up request for clarity on timing DD with ECHA	PMC Sec	ASAP
5.	Check with BASF (O Lemke) what their views are on the Gao et al. 2017 paper	PMC Sec	March 2018
6.	Check with Robert Chapin what his views are on the Gao et al. 2017 paper	N Rajapakse	After AP5
7.	Contact Prof. McArdle in 2 steps: 1) Send him the key references and ask for his 'quick' opinion; 2) If he thinks direct effects are involved: ask for a more detailed opinion.	PMC Sec + V Verougstraete	March 2018