Silver Tox Experts (TE) call

Minutes, Call 23 March 2020 (14:00-15:30 CET)

1 Welcome and Introduction

1.1 Reminder on Confidentiality and Competition Law

Participants were reminded on their obligation to comply with Confidentiality and Competition Law.

1.2 Tour de table and apologies

The list of participants is available in Annex 1.

1.3 Approval of the agenda

The agenda is available on slide 3 in Annex 2. Agenda approved.

2 Minutes and actions from 25 February TE meeting

Cf. slides 4-5 in Annex 2. Some actions related to the AgAc biome study are still ongoing (**AP1-3**).
Draft minutes from 25 Feb meeting approved.

3 Feedback meeting Covance / Arcinova 16 March

Cf. slides 6-8 in Annex 2. Additions / comments:

- **Methodology for determination of Ag in blood / tissues / dose formulations:**
  - EPMF Sec is seeking further expertise in Ag chemistry through its members and already received some input confirming the importance of HCl addition and sufficient heating for proper digestion and solubilisation of Ag in organic matrices (like blood, tissues and dosing formulation).
  - The TE have serious concerns around the impact of the way Covance is currently operating on the projects we have currently contracted with them and their management of subcontractors. EPMF Sec and its study monitors have since the beginning of the project been pushing for updates from Covance but have been misled on several occasions. Last week it became clear that Covance has only just placed contracts with Arcinova for the analytics and Arcinova has only recently been given access to the appropriate references for method development which have been provided to Covance in August 2019 by the EPMF secretariat. As a consequence, Arcinova started their own method development from scratch and they seem unwilling to adapt to our methodological requests (likely because this is logistically not feasible).
  - The TE agree that there is no excuse for not using this most robust method. It is noted that our TK study will be compared with what others have done (like US NTP, RIVM, LTAP) and if we diverge, this will not be favourable for our position and our study may be invalidated.
  - Cf. also agenda point 4.2.
- **EOGRTS and prelim reprotox testing**: EPMF was only given 1 day notice on the change of study director (SD - but now changed back to the original SD) and change of location (from Eye to Huntingdon) for the palatability study. The latter makes sense since diet formulation will also be done

at Huntingdon. The revised protocol for the palatability study has been received today from the SD. We are still waiting for a date from the Huntingdon site for the diet formulation.

4 Ag TK testing

4.1 Update on supporting AgNP / AgMP characterisation program (Vito / ECTX)

Cf. slide 9 in Annex 2. The characterisation program showed its value, was very well executed and confirmed the suitability of both the selected test substances (AgNP and AgMP) and the vehicles (1% methylcellulose for oral admin and 5% glucose for i.v. admin) for the *in vivo* TK study. The sedimentation of AgMP in methylcellulose stressed the importance of using proper resuspension techniques (this information has been passed on to Covance already). However, when properly homogenised, the experiments confirmed acceptable stability and dissolution of the formulations over time. The appreciable amount of ionic Ag in the AgNP suspension is in line with earlier studies.

4.2 TK status update

Cf. slides 10-14 in Annex 2. Because of the very insoluble nature of Ag depots in tissues (mainly as Ag_2S and Ag_2Se), **severe digestion techniques** are usually applied before analysis (**AP4**). From the identified previous robust TK studies, conclusion is that most used microwave augmented digestion techniques ('MARS'), including the LTAP Microbiome study performed for EPMF. The only study not using MARS only analysed Ag in plasma (no whole blood or tissue analysis included). Additions / comments:

- It is noted that Covance was already asked to apply MARS in August 2019.
- **The TE agree that we should ask Covance / Arcinova to adapt their Ag analysis methodology to include HCl and microwave digestion** in order to be in line with available robust studies and this way anticipate regulatory and scientific scrutiny of our data. It is noted that there is resistance from Arcinova to change methodology mainly because they do not have the proper equipment available.
- **If Arcinova does not manage / is unwilling to change methodology and meet our request (i.e. include HCl and microwave digestion), the TE recommend to change CRO.**
- EPMF Secretariat has a call planned with a lawyer tomorrow to discuss the Covance contract and the options we have to change CRO.
- Logistics issue: if only the analytics should be placed at an alternative CRO to Arcinova, this should ideally be located in the UK to avoid shipping issues.
- Suggestions for alternatives: Charles River in Edinburgh (quite well equipped and they had capacity when Lindsay approached them), Triskelion in the Netherlands, Fraunhofer in Germany, Bioservice, TNO, BASF.
- Suggestion to talk to Prof Vasseur who knows the metals toxicology very well; ML Ledrich will send contact details.

AP5-7

5 Ag EOGRTS and prelim reprotox testing

5.1 Status update

Cf. slide 15 in Annex 2 :

- The draft study plans for the palatability study and DRF are currently under discussion. The study plan for the DRF will not be finalised before we have results of the palatability study.
- As soon as we have a date for the dietary formulations, we can proceed. Stability of the dose formulations need to be demonstrated before animals can be ordered.
- Suggested dosing for the palatability study: 0, 4, 40, 120, 160 mg AgAc/kg bw/d. The intermediate and low dose levels have been selected based on replication of equivalent dose levels in the Sprando drinking water study. As a high dose level with mild to moderate toxicity is required, dose levels of 120 and 160 mg AgAc/kg bw/d have been selected as high dose level for the study. **The TE agree on the dose selection for the palatability study.**

5.2 Analyses to be performed in the tests (wishlist)

Wishlist: cf. Excel file in Annex 3. Comments:

- Main objective of the DRF is of course dose selection for the main EOGRTS, further analytical results are a “nice to have”.
- It is suggested to focus on the most important analyses given the issues we are facing with Arcinova.
- Sampling on DD2 will not give us a lot of information – suggestion to remove. GD6 will indicate potential issues during implantation, GD17 is the latest feasible day during gestation to not interfere with littering and in line with the OECD 443 guidance. Pending question: do we need these 2 occasions during gestation?
- It is noted that Ag/Cu/Se analyses are done on different aliquots from the same sample. If only 1 element need analysis, the suggestion is to still take the same amount of sample and not reduce.
- Pups: mean litter size is 15, of which 10 are selected, but amount may vary from 1 to 20 pups per litter. We may not have sufficient culled pups to do all analyses so we need to prioritise. Ag/Cu/Se and Ceruloplasmin are priorities for the culled pups. For the other (potential) analyses it is suggested to take samples and store them for possible later analyses, depending on the outcome of the DRF (like neurotoxic endpoints).
- It is important to agree on the preservation technique for the tissues in advance, e.g. for AMG the tissues need to be preserved differently than or Ag analysis.
- Since we still have some time to decide on the analytic priorities It is suggested to modify the wish-list table and add rationale for each analysis. **AP8-10**

6 AOB

No AOB.

Annexes

1. List of participants
2. Slides presented at the meeting
3. Wish-list analyses to be performed in the DRF

Actions

Table 1. Actions agreed at the 23 March Ag Tox Experts meeting

	What?	Who?	When?
Follow-up AgAc gut biome study – AMG results			
1.	Discuss adjacent brain and testes slides in further detail with a neuropathologist (blind read – start with control and HD, include MD if differences observed at HD slides)	Prof. Lison	Ongoing
2.	Perform desktop study on Ag and Cu transport	M Raffray	Ongoing
3.	Inform prof. Lison: (1) if AgAc gut biome results can be published (shortly) and (2) when the systemic results/AMG can be published	EPMF Sec	As deemed appropriate by EPMF
Ag TK testing			
4.	Send further feedback from company chemists on optimal methodology for determination of Ag in blood / tissues / dose formulations to EPMF Sec	Ag TE	asap
5.	Investigate Covance contract with lawyer and options to change CRO for TK study and/or TK analytics	EPMF Sec	ongoing
6.	Discuss change of analytical method with Covance/Arcinova during 24 March TC and if unwilling to change, discuss change of CRO	EPMF Sec	scheduled 24/3
7.	Send contact details Prof. Vasseur	ML Ledrich	asap
Ag EOGRTS			
8.	Send input on wishlist of samplings / analyses to include in the EOGRTS DRF study to L Aveyard	Ag TE	By 3 April
9.	Modify the wishlist based on TE input and add rationale for each analysis	L Aveyard + M Raffray	By 15 April
10.	Discuss and agree on wishlist of samplings / analyses to include in the EOGRTS DRF study	L Aveyard and EPMF Sec with Covance	By mid-May

Annex 1: Participants

Wasma ALHUSAINY, Shell (The Netherlands)

Katrien ARIJS, consultant for EPMF (ARCHE, Belgium)

Lindsay AVEYARD, Consultant, GPC Consulting Ltd (United Kingdom) - reprotox study monitor

Arno BUTHE, Heraeus (Germany)

Eliot DEAG, Johnson Matthey (United Kingdom)

Melanie FLACH, BASF (Germany)

Marie-Laure LEDRICH, Consultant for Traxys (Luxemburg)

Jelle MERTENS, EPMF (Belgium)

Mark RAFFRAY, Consultant, Raffray Biosciences Ltd (United Kingdom) - TK study monitor

Nissanka RAJAPAKSE, Johnson Matthey (United Kingdom)

Michael THIEL, BASF (Germany)

Steven VERBERCKMOES, Umicore (Belgium)