Silver Tox Experts meeting EPMF

Draft minutes, Brussels, 19 December 2018 (11:00-13:00 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 5 in Annex 2. No remarks / additions; agenda approved.

2 Ag gut biome study: preliminary results

Cf. slides 7-17 in Annex 2. Comments/additions:

- 2 rats died during the exposure but since there are no tissues for further examination available, we are unable to identify exact cause. The death in the MD was classed as incidental since most likely related to the blood sampling procedure. The death in the HD is probably sporadic since there were no other signs of toxicity at this dose but we cannot be sure.
- Uterus tissue samples have been reserved and the **TE agree to proceed to histopath of uterus samples for the control and the high dose if possible** (**AP1**). In the Sprando et al. study, effects of AgAc on the uterus were observed. Hence, results from our study could add to the available database and could provide background info for the EOGRTS design.
- **Ceruloplasmin (Cp) (ferroxidase) activity** was measured in serum (cf. slide 17 in Annex 2) and clear dose-dependent reductions were observed. Notes:
    - Some values had to be eliminated because of hemolysis (especially at week 4). It is unclear if the hemolysis is a consequence of treatment or a sampling handling issue (**AP1**).
    - Rationale for the Cp oxidase activity measurement: to obtain data on Cp oxidase activity depression / serum Cu depletion at exposures comparable to Sprando et al. study and the SZZ 2-gen study (where only fetal homogenate data exists). Loss of Cp oxidase activity was also observed in other papers where rats were exposed to AgCl (e.g. Shavlovski et al.).
    - Although the water solubility of AgNO3 (>2000 g/L), AgAc (10 g/L) and AgCl (2 mg/L) differ significantly, each of these compounds produces exactly the same effect when it comes to Cp oxidase activity. This is an indication that the bioavailability of these compounds is probably very similar (which might explain the EPMF bioelution results, where little difference between Ag substances was shown). Furthermore, it shows that water solubility is probably not a good indicator of bioavailability for Ag substances. It is noted that there are no TK data in AgCl but there are only indirect indicators of bioavailability.
    - During pregnancy, Cp oxidase levels normally increase (approximately doubled).

- Full results are expected early 2019. In light of the 8 Feb deadline for commenting on the EOGRTS TP DD (see also next point), and the fact that the results of the gut biome study may have an impact on the dose-setting of the EOGRTS, it is suggested to check when we would have the final results from the study (**AP1**).

3 Ag EOGRTS TP Draft Decision (DD)

3.1 Process and timeline

Cf. slide 19 in Annex 2. The LR received the DD on 18 Dec. The **deadline for us to comment on the DD is 8 Feb 2019**. The deadline for a dossier update related to the TP is 11 Mar 2019. ECHA will then consider our comments and may amend the DD accordingly. Afterwards, ECHA will notify the MSCA of the (amended) draft decision without undue delay and they will have the chance to submit comments as well. The DD will then be referred to MSC, who may seek agreement in written procedure or discuss and seek agreement at its MSC meeting (the decision on which agreement seeking procedure to initiate will be made by the MSC secretariat based on the proposed amendments and on the likelihood of finding an agreement through a written procedure). In any case, based on the decision-making process, our **current estimate for receiving the final decision is June 2019 at the earliest.**

3.2 Content of the DD

Cf. slides 20-25 in Annex 2. The **TP is accepted with the test design and test article as proposed by EPMF** (EOGRTS in rats via the oral route with the analogue substance silver acetate; 10 wks premating exposure duration for the P0 generation; dose level setting aiming to induce systemic tox at the highest dose level; Cohort 1A (Repr tox); Cohort 1B (Repr tox) without extension to mate the Cohort 1B animals to produce the F2 generation; and Cohort 3 (DIT)). We have **24 months** after receiving the final decision to submit the test results.

Discussion on strategy:

- The TE are pleased with the current outcome. The EOGRTS and its results will be essential in the discussion on a silver reprotox (non-)classification.
- **No technical comments on the DD** will be submitted and we see no need for a dossier update related to the TP at this point. However, given the complexity of the EOGRTS and the preparatory work, and the limited number of labs able to perform the test and their limited availability, we will **comment on the timing** and will ask for more than 24 months (as given in the draft decision). We will request for lab statements to support this request. **AP2-3**
- **Read-across** (see also next point) is still a challenge so we will continue the work on strengthening our grouping and read-across approach, given the importance of the EOGRTS outcome to other silver substances incl. elemental silver (in the DD, ECHA considers our approach to use AgAc as test item plausible for the purpose of the TP evaluation but will reassess the validity of our read-across once we submit the test results). The TE agree that bioelution may not be a good basis to justify our read-across and **additional *in vivo* testing** will be needed to shed further light on the potential to differentiate the effects observed with soluble silver compounds from elemental silver (and further differentiate nanosilver (AgNP) from 'bulk' silver? – see also discussion under next point).
- The DD mentions that **dose-setting** should be based on fertility effect. Currently, this would imply a HD of 40 mg AgAc/kg bw/d (Sprando et al.) unless TK or biome study suggest other possibility / lower

dose levels. Therefore, we may need to rethink the EOGRTS dose-setting based on the outcome of the gut biome study. The TE agree to not raise the issue of dose-setting in our comments but suggest to look into possibilities to **informally raise the importance of the link between reproductive toxicity and gut dysbiosis with ECHA**. AP4

- The current DD still has to go through a MSCA commenting round, and there is a possibility that they will request changes (like e.g. additional Cohorts) to be added to the study design (mainly **Cohorts 2A and 2B on DNT**). Therefore, EPMF will prepare internally to strengthen our position in this respect. AP5
- **Possible labs for EOGRTS**: Charles River, CitoxLab, LPT, Dow lab (to be checked with ESTF during afternoon meeting). The BASF lab also performs the EOGRTS but they have no capacity for the next few years. AP6
- **Possible study monitors:** RSA, Lindsay Aveyard (UK based consultant). AP6

4 Ag read-across

M. Raffray briefly introduced the findings of the TK data-mining project (cf. slides 27-49 in Annex 2 – to be further discussed during afternoon meeting). Comments/additions:

- There is a small dataset for Ag and Ag substances (< 20 key & supporting studies) and a **clear data gap on elemental Ag**.
- AgAc has somewhat higher bioavailability than AgNO3 and AgNP bioavailability is higher than expected.
- Cf. slides 40-41: the ESTF elemental Ag form tested via bioelution looks like it is nano - to be checked with ESTF during afternoon meeting.
- It looks as if for AgNP, there is contribution from dissolved Ag (which would also partly explain the conflicting data on AgNP) and from particles that may be taken up. A further complication is that ionic Ag in physiological media will form secondary AgNP. This means that uptake is ionic + nano + dynamic. Current hypothesis is that AgNP behave as ionic Ag, so it is **suggested to group AgNP with the Ag salts / ionic Ag and form a separate group for 'bulk' elemental Ag**. This will have to be confirmed by further *in vivo* TK testing.
- Steady state is evident by 14 days exposure so it is suggested to perform **14d *in vivo* tests**.
- As **test substances for *in vivo* testing**, elemental Ag (micron-sized and nano), AgAc, AgNO3 and AgCl are suggested, and Ag2O as poorly soluble compound.
- Cf. slide 45: important argumentation for DNT cohort: there is no passing of Ag through the blood brain barrier. Further data are needed on the localisation of Ag in the brain. It is suggested to check if this can be done by prof. Lison's lab (AP1). If not, S. Verberckmoes could help us find a lab for this.
- Slide 47 summarises why further bioelution testing for Ag substances does not make sense and why bioelution is not a good predictor of bioavailability for Ag substances.
- **The TE agree further *in vivo* testing will be needed for elemental Ag ('bulk' + nano forms representative for EPMF) and some Ag substances,** possibly in parallel with modelling (PBTK or some form of bioelution testing?) for all Ag substances in scope.
- **It is further suggested to try to create a separate group for elemental 'bulk' Ag.** Once we have clear evidence that elemental 'bulk' Ag behaves differently from the nanoform and ionic Ag, it is suggested to flag this to ECHA.

5 AOB

It is suggested to look into the Mo case BoA for learning lessons. This may be relevant to the discussion on human exposure (**AP8**).

Annexes

1. List of participants
2. Slides presented at the meeting

Actions

Table 1. Actions agreed at the 19 December 2018 Ag Tox Experts meeting EPMF in Brussels

	What?	Who?	When?
Ag gut biome study			
1.	Check with prof. Lison: • If possible to proceed to histopath of uterus samples for the control and the HD; • If hemolysis is a consequence of treatment or a sampling handling issue; • If we can do Ag localisation work on the brain; • When we would have the final results from the study.	EPMF Sec	Jan 2019
Ag EOGRTS TP DD			
2.	Prepare comment on the timing and ask for more than 24 months + request lab statements (from Charles River, CitoxLab, LPT) to accompany our comment.	EPMF Sec	Jan 2019
3.	Check if comments / statements on timing used for previous DDs can be shared as an example.	O. Lemke	Jan 2019
4.	Ask advice from Eurométaux on how to informally raise the importance of the link between reproductive toxicity and gut dysbiosis with ECHA.	EPMF Sec	Jan 2019
5.	Prepare offer for DNT-related defence paper (review of distributional TK + link to appraisal of neurotox evidence for Ag).	M. Raffray	By half Jan 2019
6.	Inform EPMF Sec of possible labs to perform the EOGRTS (apart from Charles River, CitoxLab, LPT) and possible study monitors.	EPMF Ag TE	Jan 2019
Ag read-across			
7.	Finalise report on Ag TK data-mining project.	M. Raffray	By half Jan 2019
AOB			
8.	Look into the Mo case BoA for learning lessons.	All	Jan 2019

Annex 1: Participants

Katrien ARIJS, consultant for EPMF (ARCHE, Belgium)

Arno BUTHE, Heraeus (Germany)

Marie-Laure LEDRICH, consultant for Traxys (Luxembourg) – *via conference call*

Olga LEMKE, BASF (Germany) – *via conference call*

Jelle MERTENS, EPMF (Belgium)

Mark RAFFRAY, Consultant, Raffray Biosciences Ltd (United Kingdom)

Nissanka RAJAPAKSE, Johnson Matthey (United Kingdom)

Steven VERBERCKMOES, Umicore (Belgium)