European Precious Metals Federation

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Silver Tox Experts Call

23 March 2020

Confidentiality reminder

DO	DON'T
Application of competition law	
Art. 101 and 102 TFEU may be applicable to the conclusion of any preliminary agreement and activities of any preliminary phase.	Don't assume that conflicts with competition law are excluded simply by the fact that the Agreement complies with the provisions of the REACH Regulation.
Consultation in Matters of Competition Law	
Consult an in-house legal expert or the compliance officer of your company or an external lawyer whenever there are uncertainties respecting compliance with competition law. Stop all meetings/discussions which are not in compliance with these Compliance Guidelines until a legal expert has been involved.	Don't assume that these Compliance Guidelines deal with all competition law issues exhaustively. Basically, compliance with Art. 101 and 102 TFEU can be determined only on the basis of market impact in each individual case. These Compliance Guidelines may therefore be regarded only as a means of providing general conduct recommendations.
Activities in any preliminary phase and at any other stage of operation of the Consortium	
Restrict cooperation within the scope of the preliminary phase to the initially defined goals and purposes of the cooperation.	Pursuant to Art. 101 and 102 TFEU, activities which have the object of the effect of preventing, restricting and/or distorting competition are prohibited within the scope of this Agreement, including: - Coming to agreement, including arrangements or collusions, about prices, markets and customers (see Art. 101 paragraph 1 a)-e) TFEU); - Joint boycotting of other companies; - The unjustified unequal treatment of trade partners; - The abusive exploitation of a dominating market position.
Exchange of Confidential Information	
Involve a Trustee for the exchange of Confidential Information.	The exchange of Information concerning market behaviour and having the object or the effect of preventing, restricting and/or distorting competition is inadmissible; in particular, this relates to : - Production capacities; - Productions or sales volumes; - Import volumes; - Market shares; - Price policy; - Distribution and marketing terms; - Marketing strategies; - Information regarding the relationship with suppliers.
Documentation on Cooperation	
Keep minutes of all meetings which detail the subject of the meeting. In case of uncertainty, have the contents of the minutes reviewed by an external legal expert prior to sending them to all parties of the Agreement. Stop all meetings which are not in compliance with these Guidelines until a legal expert has been involved.	

List of participants

- Katrien ARIJS (EPMF)
- Lindsay AVEYARD (Consultant)
- Arno BUTHE (Heraeus)
- Melanie FLACH (BASF)
- Jelle MERTENS (EPMF)
- Mark RAFFRAY (Consultant)
- Nissanka RAJAPAKSE/Eliot DEAG (JM)
- Michael Thiel (BASF)
- Steven VERBERCKMOES (Umicore)

Agenda

1. Welcome and introduction
2. Minutes and actions from 25 Feb TE meeting
3. Feedback meeting Covance/Arcinova 16 Mar
4. Ag TK testing
   - 4.1. Supporting AgNP/AgMP characterisation program
   - 4.2. TK status update
5. Ag EOGRTS + prelim testing
   - 5.1. Status update
   - 5.2. Analyses to be performed in the tests (wish-list)
6. AOB

FOR APPROVAL

Approval of the minutes of the last call (20 Sep 2019) and status of action points (1)

	What?	Who?	Status
AgAc gut biome study			
1	Distribute Danscher & Stoltenberg 2006 publication to TE	EPMF Sec	**DONE**
2	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**
3	Check ESTF study and Boudreau study for info on blood parameters	EPMF Sec	**DONE**
4	Inform EPMF Sec by when a desktop study could be performed on Ag and Cu transport	M Raffray	**DONE (study ongoing)**
5	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	**TO DO**
6	Share PfA neurotox defence doc with Prof. Lison	EPMF Sec	**DONE**
7	Share AMG slides AgAc gut biome study (incl. high magnifications) and neurotox references / PfA neurotox defence doc with Ag TE + send doodle to discuss follow up actions in EOGRTS DRF	EPMF Sec	**DONE**
8	Go through the AMG slides (incl. zoomed in slides) and the DRF study design to suggest modifications to the study design.	Ag TE	**DONE**

Approval of the minutes of the last call (20 Sep 2019) and status of action points (2)

	What?	Who?	Status
Ag EOGRTS			
9	Make wishlist / matrix of samplings / analyses we want to include in the EOGRTS DRF study + analytically confirm Se & Cu content in breeder diet	EPMF Sec	**DONE**
10	Discuss and agree on wishlist of samplings / analyses to include in the EOGRTS DRF study	Ag TE	**AIM OF THIS CALL**
11	Discuss and agree on wishlist of samplings / analyses to include in the EOGRTS DRF study	L Aveyard / EPMF Sec with Covance	**TO DO**
12	Request clear update / timelines from Arcinova for the analytics and decide on that basis if we want to continue working with Arcinova + France to call Covance management (Mark Terry)	EPMF Sec	**DONE**
13	Keep pushing Covance to start palatability study ASAP	L Aveyard	**ONGOING**
Ag TK testing			
14	Check if prof. Lison can do Ag localisation in brain / testes from the Ag TK study & check way of processing the tissues	EPMF Sec	**DONE**

APPROVAL OF DRAFT MINUTES

Feedback meeting Covance/Arcinova 16 Mar (1)

- **Aim of the meeting**:

  discuss the approach and status of the various assays (TK & EOGRTS), clarify on the outstanding points and serve as a re-set meeting to ensure a smooth progress and generation of robust and reliable data

- **Going-in position EPMF**:

  Ag testing will be under high (regulatory and scientific) scrutiny and study quality takes precedence over timing (although regulatory deadlines obviously need to be met)

- **Issues identified**:

  Difficulties and unclarities around:

  - analytics package (subcontracted to Arcinova)
  - inter-business coordination between Covance and Arcinova
  - timelines

Feedback meeting Covance/Arcinova 16 Mar (2)

- **TK study**:
  - info related to e.g. method development / validation and timing of the experimental work received in a fragmentary and incomplete manner
  - analytical development/validation is delayed - work still not finalised
  - EPMF suggested methodology for determination of Ag in blood / tissues / DF was not passed on from Covance to Arcinova when they started method development
    - EPMF suggested method: use of HNO_3 + **HCl** plus **microwave** digestion (MARS) – i.e. in line with what FDA, RIVM, UCL and OECD centres of excellence have used before
    - Arcinova suggested digestion method: HNO_3 plus heating to 40°C for 30 min -> incomplete dissolution and detection of Ag (especially in tissues)?
  - **Follow-up actions:**
    - **Further technical discussion with Arcinova during conf call 24 Mar**
    - **EPMF to investigate the option to cross-verify Arcinova methodology with established method at UCL/Vito**
    - **Covance to pause start of in-life stage until above issues around analytics have been clarified**
    - **EPMF seeking expert in Ag chemistry for further advice**

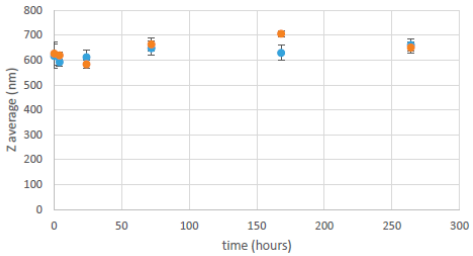
Feedback meeting Covance/Arcinova 16 Mar (3)

- **EOGRT study + prelim reprotox testing**:
  - Various changes since first discussions late/not communicated
  - Inter-business communication questionable (e.g. diet analysis methodology, dietary Ag concentrations…)
  - analytical development/validation is delayed - work still not finalised
  - EPMF suggested methodology for determination of Ag in blood / tissues / DF was not passed on from Covance to Arcinova when they started method development
    - EPMF suggested method: use of HNO_3 + **HCl** plus **microwave** digestion (MARS) – i.e. in line with what FDA, RIVM, UCL and OECD centres of excellence have used before
    - Arcinova suggested digestion method: HNO_3 plus heating to 40°C for 30 min -> incomplete dissolution and detection of Ag (especially in tissues)?
  - **Follow-up actions:**
    - **Palatability study to be initiated ASAP**
    - **EPMF to decide on additional analysis for blood/organs + prioritisation**
    - **EPMF to decide on cross-check LTAP samples**

Ag TK testing

4.1. Update on supporting AgNP/AgMP characterisation program

- Work at VITO/ECTX
  - characterisation & intrinsic properties (size, ζ-potential, contaminant ionic Ag etc.)
  - suspension characteristics (agglomeration, sedimentation behaviour) & dissolution in vehicles + evolution in time
- Ionic Ag fraction: AgNP ±5,3% ↔ AgMP <<0,01%
- Stability AgNP/AgMP in vehicles confirmed (DLS vs time)
  - 11 days for 1%MC
  - 24 h for 5% gluc
- Dissolution ionic Ag (3 kDa filtration)
  - stable over time
  - 1%MC: Δ = 2500-3500 despite lower Ag(tot)
  - 5%gluc: Δ = 50-300 despite lower Ag(tot)



Good enabling work for in vivo TK!

Ag TK testing

4.2. TK Status update

…delayed…

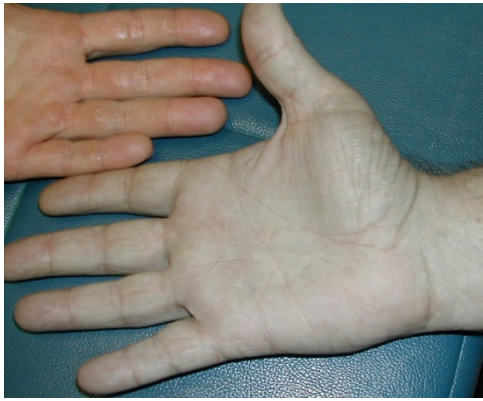
- Main challenge = analytical method (cfr. technical call Tue 24/3)
- Study plan = close to finalised
- Timing ~ analytical progress

Ag in biological tissues

- Relevance: ionic silver (Ag salts) & Ag^0 (since oxid. dissolution yields Ag^+)
- Tissue depots – mainly forms of **Ag sulphide** & **Ag selenide**
- Highly refractory to dissolution – why argyria is so persistent

  Ag_2S ▶ *K*sp=$5.9 \cdot 10^{-51}$

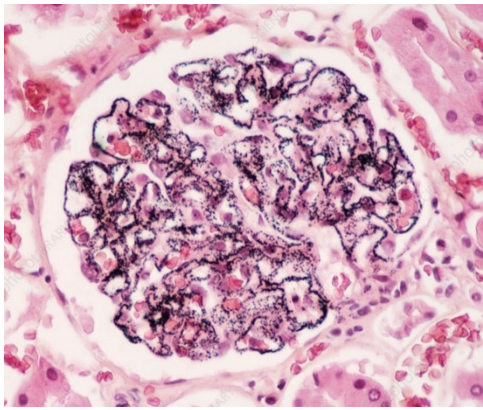
  Ag_2Se ▶ *K*sp=$3.1 \cdot 10^{-65}$



- Robust TK studies have utilised severe digestion techniques

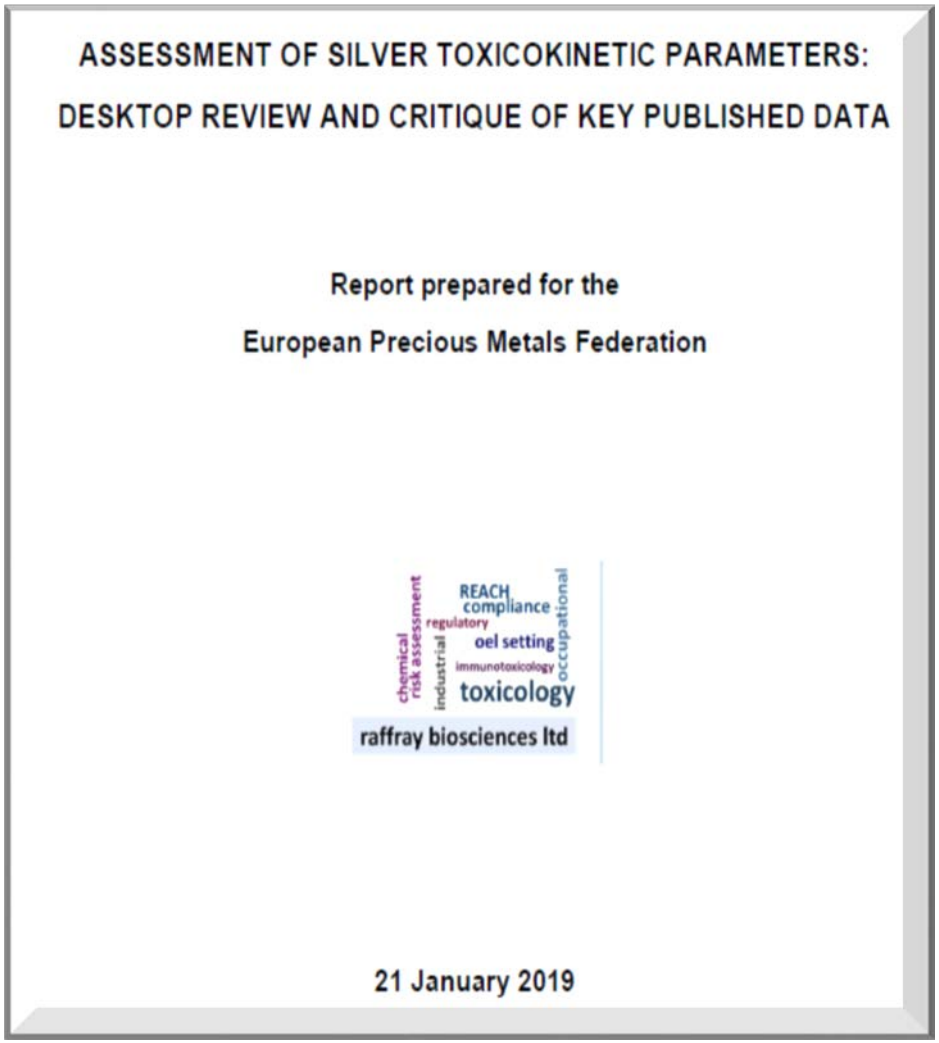
  For example, digestion/mineralisation in conc. HNO_3 augmented by microwave accelerated reaction system

  If Cl^- excess not assured, then HCl also required (included by most investigators even though Cl^- present in tissues)



- EPMF Biome study [adaptation of method ex. Loeschner et al., 2011]; US FDA program; NL RIVM work program etc.

Previous TK Studies (EPMF is facing off to...)



Tissue/blood digestion methodology

Key TK Studies

TABLE KEY

A = absorption D = distribution

E = elimination O = ancillary TK parameter

□ <=10 □□ >10 <100 □□□ >100 mg Ag equiv./kg bw.

Reference	Title	Ag form	Route	Key study	Supporting study	Not relevant / Excluded	Species	Dosing regimen	Dose-level
Barraclough and Cotton, 2017 [Arcinova]	Silver nitrate: 28 day oral (gavage) administration toxicity study in the rat; Final toxicokinetic analysis report. Plasma only - TMAH digest / No tissues	$AgNO_3$	Oral	● A			rat	4-wk	□□
Boudreau et al., 2012	An evaluation of the toxicological effects of discrete sizes of silver nanoscale particles (AgNP) in the Sprague Dawley rat. Presentation / personal communications.	AgAc AgNP (10 nm; 75 nm; 110 nm – citrate capped)	Oral i.v.	● A			rat	Single	□□
van der Zande et al., 2012	Distribution, elimination, and toxicity of silver nanoparticles and silver ions in rats after 28-day oral exposure. ACS Nano. 6: 7427–7442.	AgNP <20 nm; <15 nm (both capped) $AgNO_3$	Oral	● A	● D, E		rat (♂)	4-wk	□ $_{Ag}$ * □□ $_{NP}$
Park et al., 2011	Bioavailability and toxicokinetics of citrate-coated silver nanoparticles in rats. Arch Pharm Res. 34: 153–158.	AgNP (coated)	Oral i.v.	● A		● D	rat (♂)	Single	□
Boudreau et al., 2016	Differential effects of silver nanoparticles and silver ions on tissue accumulation, distribution, and toxicity in the Sprague Dawley rat following daily oral gavage administration for 13 weeks. Toxicol Sci. 150:131-160.	AgAc AgNP (10 nm; 75 nm; 110 nm – citrate capped)	Oral	● D			rat	13-wk	□□□
Klaassen, 1979	Biliary excretion of silver in the rat, rabbit, and dog. Toxicol Appl Pharmacol. 50: 49-55.	$^{110}AgNO_3$	i.v.	● E	● D		multiple	Single	□
Gregus and Klaassen, 1986	Disposition of metals in rats: a comparative study of fecal, urinary, and biliary excretion and tissue distribution of eighteen metals. Toxicol Appl Pharmacol. 85: 24-38.	$^{110m}AgNO_3$	i.v.	● E	● D		multiple	Single	□

Microwave augmented

Non-microwave

Study not relevant

Tissue/blood digestion methodology (cont.)

Supporting TK Studies

TABLE KEY

A = absorption D = distribution

E = elimination O = ancillary TK parameter

□ <=10 □□ >10 <100 □□□ >100 mg Ag equiv./kg bw.

Reference	Title	Ag form	Route	Key study	Supporting study	Not relevant / Excluded	Species	Dosing regimen	Dose-level
Park, 2013	Toxicokinetic differences and toxicities of silver nanoparticles and silver ions in rats after single oral administration. Journal of Toxicology and Environmental Health, Part A, 76: 1246-1260.	Ag/$AgNO_3$ AgNP (8 nm; citrate capped)	Oral		● A		rat (♂)	Single	□ / □□
Bachler et al., 2013	A physiologically based pharmacokinetic model for ionic silver and silver nanoparticles. International Journal of Nanomedicine 8: 3365-3382.	$AgNO_3$; AgAc; Ag° AgNP (var.) [Model data]	PBTK		● A, D, E		rat model	Repeat°	□ to □□□
Juling et al., 2016	In vivo distribution of nanosilver in the rat: The role of ions and de novo-formed secondary particles. Food Chem Toxicol. 97: 327-335.	$AgNO_3$ AgNP (~15 nm; POE capped)	Oral I.v.		● A, D		rat (♂)	Single	□ / □□
Liu et al., 2012	Chemical transformations of nanosilver in biological environments. ACS Nano. 6: 9887–9899.	AgNP (5 nm; ~30 nm; citrate cap)	In chemico		● A,		N/A	N/A	N/A
Walczak et al., 2013	Behaviour of silver nanoparticles and silver ions in an in vitro human gastrointestinal digestion model. Nanotoxicology 7: 1198-1210.	$AgNO_3$ AgNP (60 nm; citrate capped)	Bioelution		● A, O		N/A	N/A	N/A
Loeschner et al., 2011	Distribution of silver in rats following 28 days of repeated oral exposure to silver nanoparticles or silver acetate. Part Fibre Toxicol. 8: 18.	AgAc AgNP (14 nm; PVP capped)	Oral		● D		rat (♀)	4-week	□
Lankveld et al., 2010	The kinetics of the tissue distribution of silver nanoparticles of different sizes. Biomaterials 31: 8350-8361. Low dose study / no ionic Ag test article	AgNP (20; 80; 110 nm)	I.v.		● D		rat	5-day	□
Bergin et al., 2016	Effects of particle size and coating on toxicologic parameters, fecal elimination kinetics and tissue distribution of acutely ingested silver nanoparticles in a mouse model. Nanotoxicology 10: 352-360.	AgAc AgNP (20; 110 nm; citrate capped)	Oral		● D, E		mouse (♂)	3-day	□
Furchner et al. 1968	Comparative metabolism of radionuclides in mammals-IV. Retention of silver-110m in the mouse, rat, monkey, and dog. Health Physics 15: 505-514	$^{110m}AgNO_3$	Oral I.v. I.p.		● D, E		multiple	Single	□
Pang et al., 2016.	Demonstrating approaches to chemically modify the surface of Ag nanoparticles in order to influence their cytotoxicity and biodistribution after single dose acute intravenous administration. Nanotoxicology. 10: 129-139.	AgNP (multiple capping types) Ag°	I.v.		● D, O		mouse	Single	□

Microwave augmented

Non-microwave

Study not relevant

Ag EOGRTS + prelim testing

- Dosing formulation analysis pending
- Study plan palatability study ± drafted

- Analysis for prelim443 = wish-list discussion

AOB

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THANK YOU

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