European Precious Metals Federation

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Silver Tox Experts Meeting EPMF / ESTF

19 December 2018 | Brussels

European Precious Metals Federation

1. Welcome and introduction

1.1 Confidentiality reminder

Terms of the non-disclosure agreement signed in December 2013 by PMC and STF apply.

1.2 Tour de table

EPMF:
- Katrien ARIJS
- Arno BUTHE
- Jelle MERTENS
- Mark RAFFRAY
- Nissanka RAJAPAKSE
- Steven VERBERCKMOES

Via conference call:
- *Marie-Laure LEDRICH*
- *Olga LEMKE*

ESTF:
- David ANDREW
- Rob COLLINS
- Andrew GOODYEAR
- Germaine TRUISI
- Ian WATT

Via conference call:
- *Sage BEGOLLY*
- *Carol MACKIE*

1.3 Agenda

1. Welcome and Introduction
2. Background and brief update on respective regulatory processes
   - 2.1. Scope EPMF / ESTF
   - 2.2. Recap situation silver reprotox, challenges and defence
   - 2.3. Draft decision (DD) EOGRTS TP *(placeholder item)*
3. Ag read-across
   - 3.1. Brief summary current read-across approaches EPMF / ESTF
   - 3.2. Learnings from first MISA workshop
   - 3.3. Science strategy: tiered approach to strengthen read-across
     - Tier 1: data-mining of existing TK data
       Preliminary results; Need for further work (e.g. peer review, modelling)?
     - Tier 2: support of existing TK with in vitro testing
       Study design; Test substances
     - Tier 3: validation of in vitro models by in vivo TK testing
       Preliminary study design (cf. background doc 4); Selection test substances depending on results Tier 2 testing
   - 3.4. Recap next steps / consensus plus timeline projection
4. AOB, next meetings/calls and closing remarks

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2. Background and brief update on respective regulatory processes

2.1 Scope EPMF / ESTF

	Ag REACH	Ag BPR
Scope	**EPMF** Ag project includes 8 substances/Dossiers: **1. Silver** (incl. nano) 2. Disilver oxide **3. Silver nitrate** 4. Disilver sulphate 5. Disilver carbonate **6. Silver chloride** 7. Silver bromide 8. Silver iodide	**ESTF** single core active substance dossier supporting 10 substances ('SCAS'): **1. Silver** 2. Silver (reaction mass with SiO2) (nano) **3. Silver chloride** 4. Silver chloride (reaction mass with TiO2) **5. Silver nitrate** 6. Silver sodium hydrogen zirconium phosphate 7. Silver phosphate glass 8. Silver zeolite 9. Silver zinc zeolite 10.Silver copper zeolite
Under review by	ECHA (Dossier Evaluation)	KemI, Swedish CA
CLH	Not a requirement	Requirement

Silver REACH dossier covers 3 forms:
- Massive (> 1 mm)
- Powder (100 nm – 1 mm)
- Nano (< 100 nm)

Obvious that regulatory decisions have shared relevance for both sectors

2.2 Recap situation silver reprotox

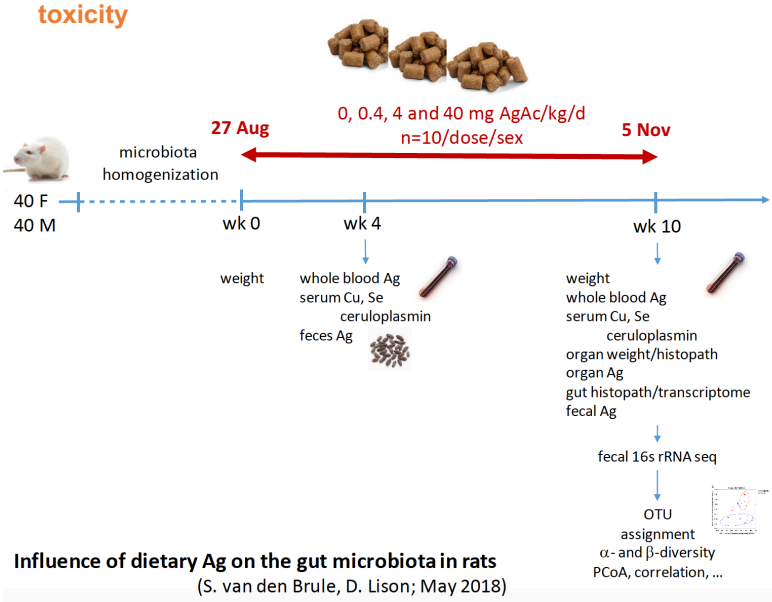
- Ongoing discussions under BPR on **SCAS classification**
  - SZZ: Repr cat. 1B proposed, cat. 2 agreed; RAC attribute effects to Ag^+
  - SZ and SCZ: Repr cat. 2 proposed (no RAC decision yet, June 2019?)
  - Elemental Ag and $AgNO_3$: It is suspected that Kemi will propose Repr cat.1B
  - **Repr cat. 1B problematic for biocidal & general Ag sectors**
- **Data gap** on reprotox → EPMF submitted original **TP for EOGRTS** in 2015 (test article AgAc)
- Since 2015: **further reprotox data** published on ionic Ag (Sprando, Babu)
  - apparently confirm dev tox, fertility, dev immune effects → **Repr cat. 1B?**
  - several weaknesses in studies / uncertainties → data gap still remains, but **update of TP** necessary

2.2 Recap situation silver reprotox (cont.)

- **TP updated** April 2018:
  - Addition of recent studies to the TP / registration dossier + highlight data gaps
  - Addition of developmental immunotoxicity (DIT) cohort to the EOGRTS design
  - Need for enabling studies on mode of action (MoA) prior to EOGRTS final dose-setting / study start (indirect tox?) → biome study *(cf. next slide)*
  - Revision of dose-setting (consideration indirect tox / factor in TK considerations)
  - TP still awaiting response… *(cf. 2.3)*
- EPMF would prefer that EOGRTS can proceed before CLH decision is made by RAC → request for joint meeting EPMF / ESTF / ECHA / KemI
- Even if TP accepted: outcome of EOGRTS is highly unpredictable and **risk of classification as Repr cat. 1B** still exists
- Provided TP accepted and assuming study commissioned in late 2019 then full outcome likely to be reported by **2021**
- May have to accept most complex EOGRTS design (> 1 M€ cost)
- Outcome EOGRTS **relevant for EPMF and ESTF**

"Biome study"

EPMF sponsored support study: Effects of Ag on gut microbial populations / influence re indirect toxicity

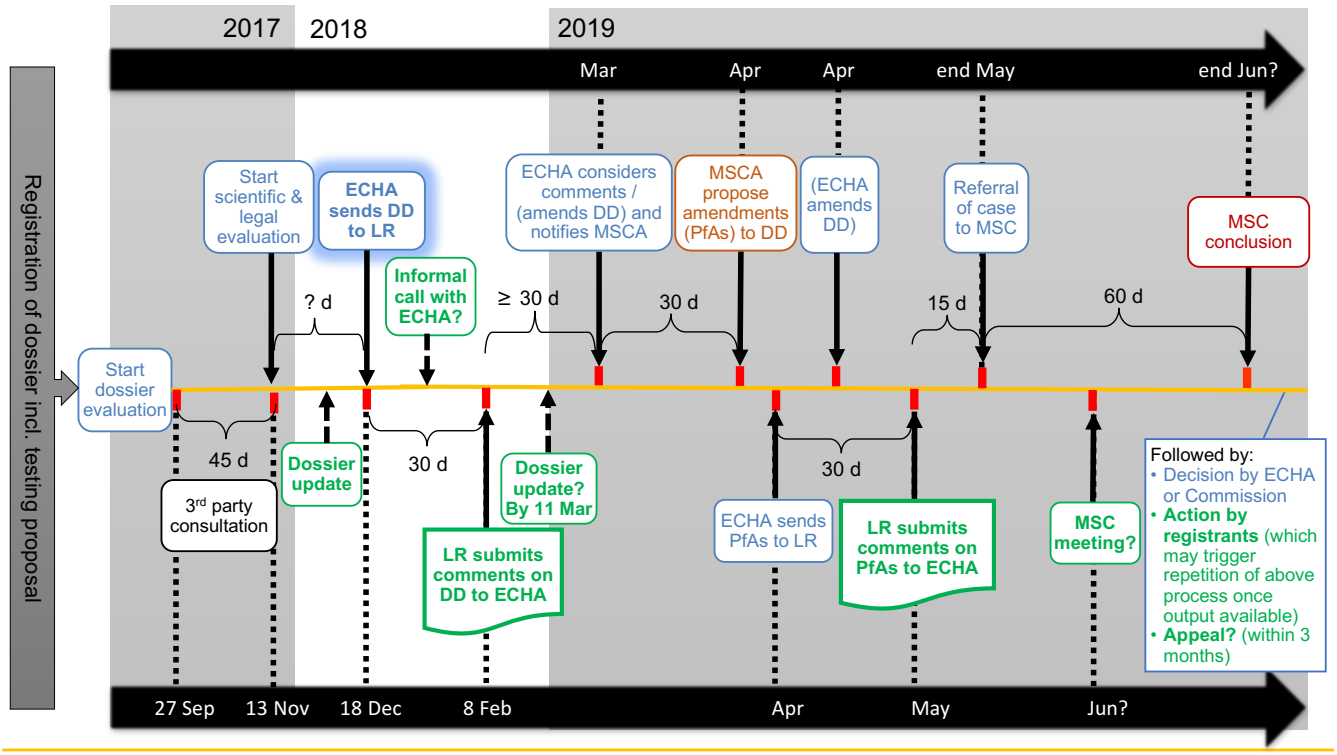


Influence of dietary Ag on the gut microbiota in rats
(S. van den Brule, D. Lison; May 2018)

- Ag^+ biocidal activity may cause indirect toxicity/ stress ▶ dysbiosis ▶ repro effects
- Data (rat model) at low dose Ag^+ exposures was unavailable
- Helps interpretation of (adverse) Sprando / Babu studies
- Supports dose-level setting for EOGRTS
- Fallback use: may mitigate re Cat 1B
- Study underway at centre of excellence (Univ. Louvain) – results available by Feb '19

2.3 Status EOGRTS TP

DD = Draft Decision
LR = Lead Registrant
PfAs = Proposal for Amendments



Draft decision - 18 Dec

- **TP accepted**
- EOGRTS (Annex X, Section 8.7.3.; test method: OECD TG 443) in **rats**, **oral route** with the analogue substance **silver acetate** (EC number 209-254-9; CAS number 563-63-3):
  - **Ten weeks premating exposure duration** for the parental (P0) generation;
  - Dose level setting shall aim 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 (**Developmental immunotoxicity**)
- Test design / test article as proposed
- Deadline to comment: 8 Feb
- Deadline for dossier update: 11 Mar
- 24 months after the final decision to submit the EOGRTS test results.

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3. Ag read-across

3.1 Brief summary current Ag read-across approaches

- **ESTF bioelution / read-across**
  - Used to fill SCAS data gaps (e.g. CMR)
  - Validity increasingly challenged (KemI)
  - Unlikely to always predict true bioavailability *(cf. next slides)*
  - ESTF looking to strengthen their existing approach

- **EPMF bioelution / read-across**
  - Attempted bioelution model for various Ag forms (TK data was limited)
  - Also unlikely to always reflect actual bioavailability *in vivo (cf. next slides)*
  - Little option when REACH dossiers prepared but standard bioelution approaches problematic for Ag
  - Need for improvement

EPMF Read-across approach

- Based on 3 parameters which are expected to drive bioavailability and tox:
  1) **Water solubility**
  2) **Valency**: Ag(1+) considered to be the relevant species under physiological conditions which potentially exerts the tox effect
  3) **Bioaccessibility**

- Bioaccessibility data from Midander and Wallinder (2009)

Table IV. Total concentration of released silver [µg/L] in the different test media

Test Material	GST Ag conc. µg/L	ALF Ag conc. µg/L	ASW Ag conc. µg/L	GMB Ag conc. µg/L	PBS Ag conc. µg/L
Ag-1 2h	36.7±6.8	131.3±1.2	215.0±1.0	282.7±6.7	349.0±7.5
Ag-1 24h	35.3±1.2	123.0±1.0	224.0±59.8	270.0±3.5	352.3±6.5
Ag_2O 2h	36.0±1.0	129.3±1.2	190.0±7.8	237.3±41.3	281.7±6.4
Ag_2O 24h	36.0±1.0	123.0±0.0	184.7±2.1	264.3±1.5	338.0±2.6
Ag-2 2h	42.0±2.6	127.7±1.2	209.3±14.6	278.3±8.1	280.7±92.2
Ag-2 24h	42.0±1.0	120.3±0.6	184.0±1.0	260.3±1.5	340.0±1.0
$AgNO_3$ 2h	36.0±1.0	127.0±1.0	190.0±1.0	272.0±5.2	355.7±11.6
$AgNO_3$ 24h	34.0±1.0	120.0±1.0	186.0±2.6	259.3±1.5	347.3±2.1

  - Cl conc. in media drove absolute equilibrium concentration of dissolved Ag (independent of tested Ag substance)
  - Dissolved Ag always <0.5% irrespective of substance; this does not match *in vivo* oral bioavailability data
  - Early dissolution kinetics (stable by 2h)

ESTF Read-across approach

- Ag^+ is the common species released from all SCAS, and is responsible for the biocidal activity
- Read-across (tox effects) based on measured silver release under comparable experimental conditions.
- Availability = Ag^+ release (%) x silver content (%) of material
- Tox effects read across from high availability to low or similar availability, but not vice versa
- Release data from O'Connor and Woolley (2010)

SCAS name	Reaction mass of SiO_2 and silver (nano)	Silver zinc zeolite	Reaction mass of TiO_2 and silver chloride	Silver phosphate glass	Silver phosphate glass	Silver copper zeolite	Silver zinc zeolite	Elemental silver	Silver sodium hydrogen zirconium phosphate	Silver nitrate
Abbreviation	Ag/SiO_2	SZZ B502i	$TiO_2/AgCl$	Ag glass IPL	Ag glass IPM	SCZ	SZZ AK	Ag	$AgZrPO_4$	$AgNO_3$
Silver content	20%	2.1%	15.1%	1.9%	2.4%	3.5%	4.9%	100%	9.9%	64%
24 hour PBS pH4, 37°C	8.6 mg/L[a]	19 mg/L	3.3 mg/L	28 mg/L	27 mg/L	18 mg/L	18 mg/L	1.1 mg/L	14 mg/L	Fully soluble
	0.6%	37%	6.5%	56%	53%	37%	37%	2.2%	27%	100%
Availability	**0.001**	**0.008**	**0.010**	**0.011**	**0.013**	**0.013**	**0.018**	**0.022**	**0.027**	**0.64**

Complex picture on bioavailability of Ag

- Both EPMF & ESTF read-across approach flawed:
  - **EPMF** bioaccessibility testing: complex equilibria involving AgCl, but also Ag complexes of varying solubility and toxicity. Plus S/Se sequestration *in vivo* etc.
  - **ESTF** release testing: PBS systems not proper surrogates for stomach & intestine + lower pH for GST
- Need for improvement → EPMF and ESTF already discussed outline vision for improved modeling / TK:
  - Tier 1: Data-mine existing TK:
  - Tier 2: Improved *in vitro* bridging studies
  - Tier 3: Validation of *in vitro* models by *in vivo* TK testing

3.2 Learnings from first MISA workshop (2 Oct 2018)

- **MISA** (Metals and Inorganics Sectorial Approach):
  - Cooperation framework between ECHA and Eurométaux
  - Aim: improving data in REACH registration dossiers and advancing technical and scientific issues related to metal compounds and inorganic substances
- First workshop 2 Oct on HH Read-Across, EOGRTS / Route of Exposure and Mutagenicity
- Participants: ECHA, Eurométaux, company and consortium representatives
- **Key conclusions/learnings on read-across / grouping**:
  - Clearly define background + boundaries of categories
  - Grouping:
    - Bioelution as tier 1
    - Bridging studies as tier 2
  - Test data need to be available for at least 1 worst-case group/category member
  - Read-across of data/classification to all group/category members (unless you can undoubtedly justify exemption)
  - Bioelution can not be used to fill datagaps (only as '**qualitative**' information)
  - Ensure counter-ion effects on tox and bioavailability are properly considered

3.3 Strategy to strengthen Ag substance read-across

Tiered approach

Tier 1

Data-mine existing TK data

- Literature data covering ionic Ag forms & elemental Ag (incl. AgNP surrogate)
- Evaluate TK studies in detail
- Feed most reliable TK parameters into bigger picture (e.g. systemic exposure/tissue exposure)

Tier 2

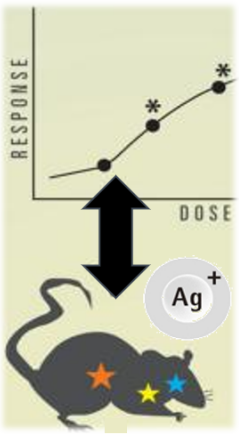
Modelling approaches

- Further adaptation of bioelution models
- Other possibilities ?

Tier 3

Validation

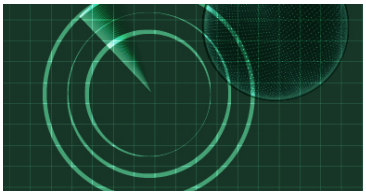
- In vivo TK testing of selected Ag substances (plan was to base selections on Tier 1 & 2)



3.3 Scope of Desktop Study

Publications covering in vivo TK of Ag substances

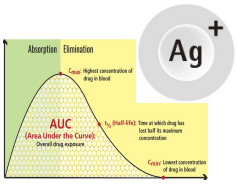
- Emphasis on **systematic exposure parameters** via the **oral route** & **distributional/tissue exposure** parameters
- Data gaps & read-across needs (mamm tox in rat) ▶ **boundaries set accordingly**
- Evaluation of data from other exposure routes (mainly i.v.) only if relevant
- Other papers on modelling, Ag speciation in biological systems



3.3 Aims of Project

Elemental Ag as a problem child

- Is there useable TK info on elemental Ag in massive form?
- If reliance must be on AgNP, which TK studies are key?
- AgNP ▶ primary factors influencing TK profile (esp. BA) e.g. size, coating/stabilisation, co-existent Ag^+
- If further TK work on elemental Ag is to be done, what is optimum choice of TA ?



Integration (especially reprotox)

- Interpretation of reprotox & RDT (which have lacked supporting TK)
- Input into design of new EOGRTS
- Ag read-across/grouping: new program design (viability of bioelution; what validating in vivo TK should be done etc.)

Main Aim: extract key TK parameters for Ag reference subs

- Coverage ionic Ag ($AgNO_3$; AgAc etc.) + elemental Ag
- Establish **systemic exposure** / bioavailability via oral route
- Focus on AUC. Capture or derive **true oral bioavailability (BA)** if possible
- Evaluate **completeness/security** of total TK dataset for each Ag substance
- Identify **TK data gaps** ▶ targets for new in vivo TK

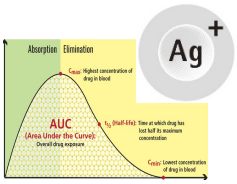
Comparative profiles

- Between various ionic Ag forms
- Ionic vs. elemental Ag
- For individual substance: how congruent is data across various studies? If non-congruent, what might be basis for disparities?

3.3 Aims (cont.)

Secondary aims

- Improved bioelution models ► feasibility of system adaptation re complexities of Ag transformation / absorption?
- Other modelling alternatives which could be leveraged, e.g. valid PBTK models for Ag?
- Some other specifics: e.g. does any useful TK data exist for AgCl? (q.v. problematic reprotox findings)



Secondary aims

- Specific adult animal tissue exposure data of interest: e.g.
  - Tissue distribution and elimination data related to male and female reproductive tract; CNS/brain
  - Influence of BBB, BTB
- Transplacental & embryofetal TK
  - Relevant to EOGRTS design
  - Relevant to interpretation of reprotox studies

3.3 Study Listing

Initial Triage

- >200 potentially relevant studies
- Majority of reports relate to AgNP !
- Initial screen for relevance + study robustness thrifting
- Alignment with Data Mining Project aims ► mainly gated for rat TK, but other species included if study was important (mouse, dog, non-human primate)

Key / Supporting studies

- For those surviving cut ► deep-dive of each study
- KEY / SUPPORTING / EXCLUDED
- Main TK parameters extracted. Individual TK data derivations verified (to extent possible)
- Detailed robustness assessment, e.g. TK design, AgNP characterisation etc.
- Big Picture (inter-study congruency)
- Judgement on relevance to reprotox studies (Sprando et al./Babu et al. OGRTS) and to design of EPMF's proposed new EOGRTS

3.3 Some Headlines

High-level Ag TK picture

1) For a common metal ▶ **Dataset small** < 20 (Key + Supporting) studies id'd

2) Clear **data gap** ▶ elemental Ag in **bulk / massive** form
   - No robust TK in rodents identified for micron+ sized elemental Ag
   - **Forces reliance on AgNP** as surrogate

3) Systemic exposure (via oral route) ▶ **Handful** of robust conventional TK studies yielding **true bioavailability / fractional oral absorption** metrics
   - Challenge re high confidence conclusions
   - Comparisons between ionic Ag vs elemental Ag (AgNP !) can be drawn
   - Individual ionic Ag subs comparisons not particularly secure (datapoints too few)

4) Tissue distribution [individual tissue exposures] ▶ reasonably well established

5) Elimination ▶ securely established that biliary elimination (not renal) predominates

3.3 Some Headlines

Absorption / Systemic exposure (oral route)

1) Ionic Ag (soluble subs) & elemental Ag ▶ True **oral BA low** (certainly < 5%)
   - ~ 4% represents upper boundary (ionic worst-case)

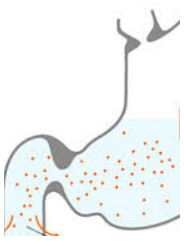
2) Most studies show **ionic Ag form has greater BA** than AgNP*

3) Ionic (NO_3/Ac) / various AgNP types (overall picture) ▶ **Ag^+ ~3.5x higher BA**

4) Two ionic forms where comparison straightforward (NO_3 vs Ac) ▶ bioavailability / systemic exposure **similar after single dose** (Ac somewhat higher), e.g. ▼

	$AUC_{(0-t)}$ / D	
	♂	♀
ESTF, 2017 $AgNO_3$	227	311
Boudreau et al., 2012 AgAc	283	623

Comparison of d1 TK;
Closest dose level matched



* Based on either derived BA (F values) or estimates of orally absorbed fraction of administered dose

3.3 Some Headlines (cont.)

Absorption / Systemic exposure (oral route)

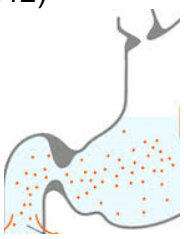
1) Overview ▶ apparent BA of AgNP **higher than might be expected !**

2) Cannot describe as negligible
   - **Significant variation** in reported values
   - But **appreciable oral BA demonstrated in some studies** of AgNP*
   - Values range from 0.9% – 3.4%

3) Data available for **various AgNP** sizes (~5 to 110 nm) & coatings/stabilisation

4) Single-dose studies (e.g. Boudreau et al., 2012; Park et al., 2013) and repeat-dose studies up to 4-wk (e.g. Loeschner et al. 2011; van der Zande et al., 2012)

[Factors which might influence BA of AgNP dealt with in later slide]



* Based on either derived BA (F values) or estimates of absorbed fraction of administered dose

3.3 Integration of this information

Bioelution vs TK data-mining position

	SZZ	Ag (elemental)	SSZrHP	Ag Ac	$AgNO_3$
	Relative bioavailability predicted vs $AgNO_3$ value				
ESTF rank Ag^+ predicted BA (via bioelution) ▶	**~0.03**	**0.03** Not nanoform	**0.04**	**Not in model**	**1**
Rank BA based on in vivo TK data	**No data**	**0.28** AgNP*	**No data**	**1.25 – 2**	**1**

*Note: AgNP rank BA vs $AgNO_3$ is based on value considered to be best supported from overall TK dataset (10 – 60 nm range). Higher rank BA would be possible based on some studies.

HyGate 4000 [TA in ESTF bioelution – tbc]

Nanosilver Antimicrobials

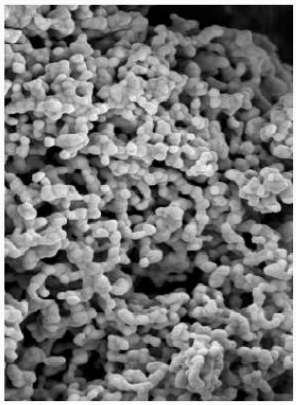
EPA-Registered Antimicrobial Additives: Ciba / Bio-Gate

Ciba

Product: HyGate 4000
Particle size: **50-200 nm**
Agglomerate size: 2-5 µm
FIFRA Reg # 70404-10
Type: 100% Silver
First Registered: 09/05/**2008**[1]

Bio Gate

Product: MicroSilver BG-R
Particle size: **50-200 nm**
Agglomerate size: 2-5 µm
FIFRA Reg # 84146 -1
Type: 100% Silver
First Registered: 03/18/**2008**[1]



Press Release: "Ciba Specialty Chemicals forms marketing cooperation with Bio–Gate for silver antimicrobial technology" 14.12.2005, Basel, Switzerland.

[1] *NPIRS Public* http://ppis.ceris.purdue.edu/npublic.htm

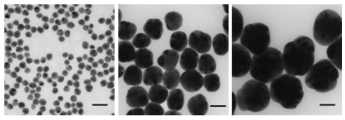
3.3 TK disparities for AgNP

Could NP intrinsic properties be influencing TK outcomes ?

1) NP Size
   - *Data are conflicting & puzzling*
   - *Some studies apparently show systemic exposure inversely correlates with size (monotonic relationship has been reported)*
   - *Others report no or limited influence of NP size (>10 <100 nm)*

2) Coating / stabilisation system
   - *Data here are more congruent*
   - *Most studies indicate limited influence of coating (one exception: study by Pang et al., 2016 via i.v. route showed marked differences)*

3) But it is known that Ag^+ may be formed in AgNP formulations
   - *During synthesis and/or storage. >10% Ag^+ content has been described.*
   - *Not all studies evaluate Ag^+ conc. / control for possible impact*
   - *May provide at least a partial explanation for AgNP TK disparities*
   - *See in particular van der Zande et al., 2012*



Issue of confounding by Ag^+ release

Release values up to 90% (w.r.t. AgNP Ag content) have been reported

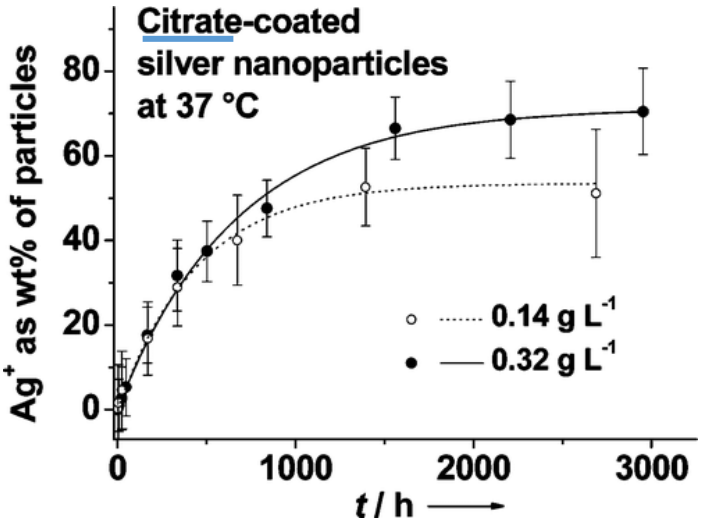


Figure taken from Kittler et al., 2010

Dissolution Citrate-stabilised and PVP-stabilised AgNP in water studied by dialysis for up to 125 days.

In some cases, the nanoparticles released up to 90% of their weight.

Type of stabilisation was influential on [Ag^+]

[NB: In vitro cytotoxicity studies with aged solutions showed markedly higher toxicity than fresh solutions]

3.3 Other notes on TK

Gender-related differences

- *Majority of studies (rat) covering ionic Ag and AgNP ► higher systemic exposure seen in female animals ► typically 120% - 180% of male values*

Clearance

- *All key/supporting studies indicates absorbed Ag quite rapidly cleared from blood*

Time to achieve steady state (SS) concentrations (blood)

- *4-wk repeat dose TK time-course studies / Extrapolations via elimination half-life*
- *Ionic Ag & AgNP ► via oral route, near SS by 5 to 7-d. Full SS evident by 14-d.*

Tissue distribution

- *Dose-normalised tissue concentrations typically higher for ionic Ag vs AgNP [expected based on relatively higher bioavailability]*
- *General distribution pattern replicated by many studies*
- *Highest tissue levels typically seen in reticuloendothelial system (liver, spleen)*
- *For AgNP re RES ► as might be expected uptake does correlate with increasing NP size and agglomeration*

3.3 Other notes on TK (cont.)

Tissue distribution (critical tissues)

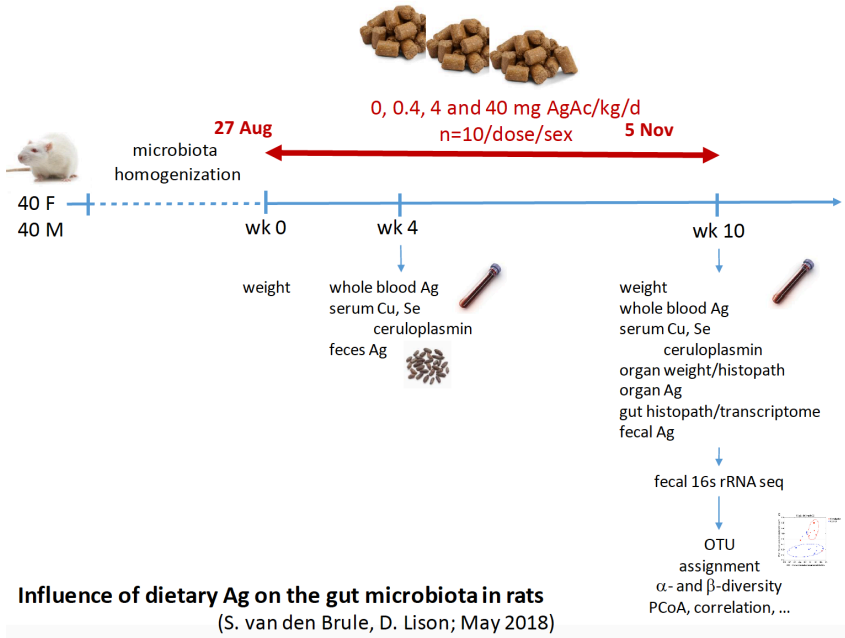
- *Conflicting reports re extent Ag accumulation in the CNS/brain and testis*
- *Publications describing relatively high levels (some were robust studies)*
- *Atypical persistence in these tissues also reported*
- *BBB & BTB may be influential*
- *Most work does not delineate whether Ag is localised within organ or else associated endothelial compartments*
- *Still reviewing weight-of-evidence & possible explanations !*

Transplacental & embryofetal TK

- *Relevant to EOGRTS design and other aspects of reprotox issue*
- *Work ongoing at this time*

3.3 "Biome study" & TK

PMC sponsored support study: Effects of Ag on gut microbial populations / influence re indirect toxicity



Influence of dietary Ag on the gut microbiota in rats
(S. van den Brule, D. Lison; May 2018)

- Rat study (in-life phase recently completed)
- Primary aims involve assessment of Ag impact on gut biome
- Will also provide further TK data on AgAc (limited scope investigations)

3.3 Modelling – Bioelution / bioaccessibility

Inconvenient Truth:
Complex set of transformations & interactions linked to BA of Ag

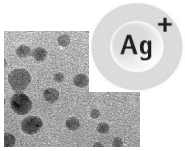
Complex solution equilibria
Ag^+, $AgCl_{(aq)}$, $AgCl^{2-}$ and $AgCl_3^{2-}$

Rapid reaction with low MW thiols & other low MW ligands

Limiting Cl^- induced precipitation

Opsonization with proteins
(protein "corona" formation)

Complex oxidative dissolution kinetics in presence of O_2 & H^+
(AgNP & also massive Ag)



Active uptake from GI tract
Na and Cu transporters

AgNP agglomeration

Ag_2S / Ag_2Se complexation & exchange

Ag interaction with food components

$Ag^+ \rightleftharpoons 2^o$ nanoparticles

Bioelution systems are inherently simplified versus true physiological situation
Difficult to accommodate Ag

Refs:
Liu et al., 2012
Walczak et al., 2012

3.3 Modelling (cont.)

Could there be an intermediate complexity approach?

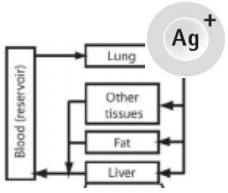
Physiologically-based TK model

Researchers at ETH Zurich

One has been developed for Ag

Institute for Chemical and Bio-engineering

Coverage of Ag^+ and AgNP (sub-models)



May be superior adjunctive approach

Based on WHO IPCS PB-modelling principles

Especially if new in vivo TK data can be added into model

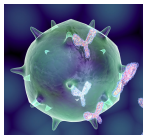
Rodent and human TK application

Seems realistic for elemental Ag

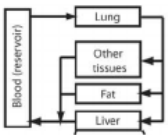
Ref: Bachler et al., 2013

Suggested areas for discussion / follow up

Grouping / Read-across program: Ag TK is a patch-work of studies & overall confidence level not optimal. Should emphasis shift toward proper comparative in vivo TK (at least for key Ag references)? Elemental Ag: care in choice of test article!



Modelling: Given the technical hurdles, is it worth placing reliance on enhanced bioelution approaches? Will doubts (regulator mind-set) still persist?



Other possibilities: Are more sophisticated modelling approaches worth exploring (e.g. PBTK)? Company level experts available? Who else might be approached for advice

3.3 Strategy to strengthen Ag substance read-across

Tiered approach

Tier 1 — Data-mine existing TK data

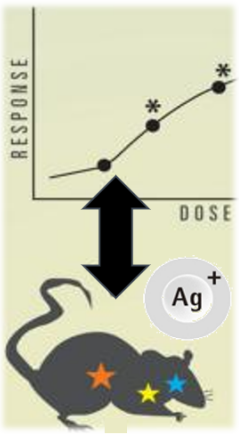
- Literature data covering ionic Ag forms & elemental Ag (incl. AgNP surrogate)
- Evaluate TK studies in detail
- Feed most reliable TK parameters into bigger picture (e.g. systemic exposure/tissue exposure)

Tier 2 — Modelling approaches

- Further adaptation of bioelution models
- Other possibilities ?

Tier 3 — Validation

- In vivo TK testing of selected Ag substances (plan was to base selections on Tier 1 & 2)



Improved *in vitro* testing / modelling

- Ag: greater absorption expected post-gastric (i.e. in the intestine) because lower Cl content → additional bioelution testing in gastric + intestinal fluids?
  - Similar testing performed for other metals (Henderson et al. 2012, Denys et al. 2012, Colombo et al. 2008, Turner et al. 2008)
  - Importance of identifying exactly which Ag substances need to be tested
  - Preliminary cost estimate for combined 'gastric / post-gastric' bioelution (depending on used media / materials): 8-10 k€ for first test item; 6.5-8 k€ for additional test items (versus approx. 30 k€ for *in vivo* TK)
- Cl complexes: gastric bioelution with HNO_3 instead of HCl?
- **Advantages** additional *in vitro* testing:
  - Lower cost / quicker than *in vivo*
  - Allows us to do preliminary grouping of all Ag substances in scope (to then be confirmed by *in vivo* testing for some selected substances)
- **Disadvantages** additional *in vitro* testing:
  - Oversimplification of *in vivo* situation…
- **PBTK modelling instead?**

3.3 Strategy to strengthen Ag substance read-across

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Tier 2

Modelling approaches

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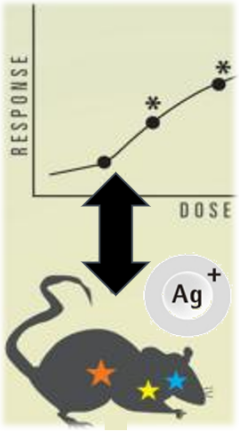
- Other possibilities ?

Tier 3

Validation

- In vivo TK testing of selected Ag substances (plan was to base selections on Tier 1 & 2)



Silver bioavailability: potential study design for *in vivo* testing ESTF

(1) Reference SCAS: soluble salt (e.g. silver nitrate)

- Single oral low dose (comparable to critical study NOAEL)
- Single intravenous low dose (assessment of bioavailability)
- Single high dose (investigate potential saturation of absorption)
- Groups of male and female rats (investigate potential sex differences)
    - Measurement of silver in excreta, tissues, carcass: to provide mass balance
    - Measurement of silver in blood: to give kinetic parameters (Cmax, AUC etc.)
- 7-day study duration

(2) Comparative studies

- Single low dose oral studies with other SCAS (metallic Ag, AgCl, SZZ, SSHZP….)
- Single sex (assuming no sex differences shown in Phase 1)
- Measurement of silver in blood to provide kinetic parameters (Cmax, AUC)
- 7-day study duration (potentially shorter depending on Phase 1)
    - Calculation of oral absorption relative to the reference SCAS, based on comparison of AUC

Study design is based on the following assumptions:

- Excretion of silver is predominantly biliary
- Silver is absorbed in the same form regardless of SCAS
- Absorbed silver behaves in the same way once absorbed

European Precious Metals Federation

4. AOB, next meetings/calls and closing remarks

THANK YOU

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