



Precious Metals
Consortium

Precious Metals & Rhenium Consortium

Silver Tox Experts Call

9 August 2018

Ag gut microbiome study: food recovery

- Re-test of spiked sample with **different protocol** using NH_3 which eliminates AgCl precipitates: Ag^+ ions were extracted during 24h in the presence of 2M NH_3 (to form a $[\text{Ag}(\text{NH}_4)_2]^+$ complex from AgCl)
- Compared “bioaccessibility” of Ag^+ from **freshly spiked and aged samples** (40 and 400 μg AgAc/kg food)
 - aged samples: 7 weeks old
 - results (cf. Excel file) allow to exclude the possibility of an alteration of AgAc in food
 - no difference in food samples spiked 7 weeks ago compared to spiked just before analysis (40 and 400 mg/kg)
 - Lison confident that Ag remains completely soluble in food for at least 1 month
- Different recoveries in 40 vs 400 samples → homogeneity of the sample is probably not optimal (this will not be the case with the food prepared by Carfil)
- Lison will order the food from Carfil and the animals from Janvier.
- Start the acclimation the week of 20 or 27 August (exposure will then start 3 or 10 September).
- **TE in agreement with conclusion Lison?**



Meeting PMC and ESTF 5 July 2018

- **Read-across** approach ESTF (see also next slides) questioned by Kemi
 - read-across accepted for SZZ, SZ, SCZ and SSZHP (carriers of Ag)
 - unclear how Kemi will assess Ag salts (AgNO_3 , AgCl) and elemental Ag
 - ESTF has bought PMC bio-elution data and would consider generating further TK / bioavailability data (*in vitro* / *in vivo*?)
- **AgNO_3**
 - considered corrosive → read-across from other SCAS
 - ESTF 28d gavage RDT study with AgNO_3 (2016) not accepted by Kemi as incorporating MTD (@ 100 mg/kg bw/d). Need to fill data gaps for carc, genetox, reprotox → options are test and/or read-across (e.g. to AgAc reprotox)
 - Kemi may propose **Repr 1B** classification based on data AgAc (Sprando study), AgCl (Shavlovski study) and SZZ → implications for elemental Ag / other Ag compounds?
 - Kemi intends to contact ECHA for advice
- **Agreement between ESTF and PMC to further share communication Kemi / ECHA and to potentially align efforts / advocacy actions related to Repr classification after internal discussion**



Relationships if ESTF 'bioavailability' (BA) / read-across model is applied to Reprotox

| | | | | | | | | | |
|--------------------------------------------------------------------------|----------------------------------------------------------|---|---------------------------------------------|---|---------------------------------------------------------------------|---|------------------------------------------------------------------------------------|---|---------------------------------------------|
| Rank Ag ⁺ BA (unclear how derived; further info requested) | SZZ | < | Ag (elemental) | < | SSZHP | < | (AgAc) Not a SCAS See footnote | < | AgNO ₃ |
| Key reprotox datapoint (2-gen / OGRTS): | Clear embryo-fetal effects in 2-gen study. Rep Cat. 2 | | Not yet assessed. Read-across proposed ! | | Only limited embryo-fetal effects in 2-gen study. Not classified | | Clear embryo-fetal toxicity (OGRTS). Fertility effect HD. DIT also reported. | | Not yet assessed. Read-across proposed ! |

Notes:

SZZ = Silver Zinc Zeolite Ag elemental is a massive form SSZHP = Silver Sodium Hydrogen Zirconium Phosphate
ESTF bioelution results are from a simple PBS system at pH 4 or 8 (no lower pH data is incorporated).
AgAc not in program / bioelution untested in ESTF model; crude extrapolation is based on comparative solubility to AgNO₃.

Complex picture on bioavailability of Ag

| | Interpretation | Remarks |
|---|-------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 | <i>ESTF bioelution approach flawed: can't model <u>absolute</u> BA Ag⁺ and some doubts over <u>relative</u> BA calls</i> | <ul style="list-style-type: none"> • Due use of PBS systems (plus lower pH for GST) • Systems not proper surrogates for stomach & s. intestine • But PMC Ag bioelution study also affected by limitations <ul style="list-style-type: none"> • Memo: recall the PMC Ag bioelution testing outcomes (see next slide) • Complex equilibria involving AgCl, but also Ag complexes of varying solubility and toxicity. • Plus S/Se sequestration in vivo etc. |
| 2 | <i>ESTF using their model to read-across / gap fill</i> | <ul style="list-style-type: none"> • ESTF use their bioelution ranking ('bioavailability' rank) to read-across (higher to lower) for untested substances. • eCA now questions the dataset and some read-across inferences. • In any event, ESTF have tox data gap for higher BA simple Ag salts (nitrate, chloride); elemental Ag SCAS → see previous slide |
| 3 | <i>Ideally better (new) <u>comparative</u> in vivo TK should be applied to rank BA / justify read-across</i> | <ul style="list-style-type: none"> • May be able to data-mine some existing TK • Possibly support by improved bridging studies in vitro • As discussed by TE, not only clarity on systemic absorption needed but also tissue levels including reproductive system • But would not be sensible for industry to develop such a program without buy-in from stakeholder agencies.... • Catch 22 is that regulatory timelines may not wait ! • Main ESTF strategies seem to be (a) seek deferral on their dossiers; (b) consider collaborative work, e.g. with EPMF |

Comparative in-vitro bioaccessibility of Ag, Ag₂O and AgNO₃ (KTH; PMC Study, 2009)

Table IV. Total concentration of released silver [$\mu\text{g/L}$] in the different test media

| Test Material | GST Ag conc. $\mu\text{g/L}$ | ALF Ag conc. $\mu\text{g/L}$ | ASW Ag conc. $\mu\text{g/L}$ | GMB Ag conc. $\mu\text{g/L}$ | PBS Ag conc. $\mu\text{g/L}$ |
|-----------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| Ag-1 2h | 36.7 \pm 6.8 | 131.3 \pm 1.2 | 215.0 \pm 1.0 | 282.7 \pm 6.7 | 349.0 \pm 7.5 |
| Ag-1 24h | 35.3 \pm 1.2 | 123.0 \pm 1.0 | 224.0 \pm 59.8 | 270.0 \pm 3.5 | 352.3 \pm 6.5 |
| Ag ₂ O 2h | 36.0 \pm 1.0 | 129.3 \pm 1.2 | 190.0 \pm 7.8 | 237.3 \pm 41.3 | 281.7 \pm 6.4 |
| Ag ₂ O 24h | 36.0 \pm 1.0 | 123.0 \pm 0.0 | 184.7 \pm 2.1 | 264.3 \pm 1.5 | 338.0 \pm 2.6 |
| Ag-2 2h | 42.0 \pm 2.6 | 127.7 \pm 1.2 | 209.3 \pm 14.6 | 278.3 \pm 8.1 | 280.7 \pm 92.2 |
| Ag-2 24h | 42.0 \pm 1.0 | 120.3 \pm 0.6 | 184.0 \pm 1.0 | 260.3 \pm 1.5 | 340.0 \pm 1.0 |
| AgNO ₃ 2h | 36.0 \pm 1.0 | 127.0 \pm 1.0 | 190.0 \pm 1.0 | 272.0 \pm 5.2 | 355.7 \pm 11.6 |
| AgNO ₃ 24h | 34.0 \pm 1.0 | 120.0 \pm 1.0 | 186.0 \pm 2.6 | 259.3 \pm 1.5 | 347.3 \pm 2.1 |

In these models:

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

Possibilities for closer alignment / cooperation PMC and ESTF

- Focus on science argumentation → better **TK data** needed before comparative bioavailability can be read-across properly
 - further *in vitro* / *in vivo* testing?
 - cf. EM work on bio-elution with ECVAM (workshop 10 Aug); possibility to use other media?
 - important to have regulatory acceptance of study design → MISA workshop 2 Oct read-across human health (agreement under MISA could help slow down BPR process?)
 - further technical discussion with PMC TE / ESTF (TE?)
- Continue ongoing work on **reprotox MoA**
- Joint meeting **ESTF / PMC / ECHA (REACH + BPR people) / Kemi (idem)**
 - explain importance of interaction different legislations applying to Ag substances (REACH/CLH/BPR)
 - after we have DD on EOGRTS TP
 - use informal call with ECHA after receiving DD on EOGRTS TP as opportunity



DTPA case study

- Info from Stuart Hindle (Dow); cf. Arts et al. 2018
- Diethylene Triamine Penta Acetic acid (**DTPA**), chelating agent
- RAC classified as **Repr 1B** despite significant evidence that dev effects cannot be considered an intrinsic property of the substance itself and are **secondary to Zn depletion**
 - animals fed a Zn deficient diet during gestation (in the absence of DTPA) exhibit dev toxicity of a similar nature and severity to that observed in studies involving DTPA
 - sufficient Zn supplementation in the diet, or administration of Zn bound chelates, completely negates the dev effects
 - bioavailability of DTPA is very low
 - relevance of classification is highly questionable since worker or consumer exposure could not lead to a scenario whereby sufficient Zn deficiency would manifest itself
- RAC concluded that DTPA should be considered a Cat 1B developmental toxicant since the **MoA was relevant for humans** and the secondary effect was not non-specific
- RAC very conservative, '**secondary**' effect must be unequivocally demonstrated to avoid cat 1B classification
- **Importance of providing evidence on overall MoA**





THANK YOU

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