INFORMATION RELEASE – 15/06/2021
New evidence on the comparative toxicokinetics of different forms of silver (Ag) and its implications for read-across

In this communication, the European Precious Metals Federation (EPMF) relays important new scientific information relating to the toxicokinetic (TK) behaviour[1] of silver substances. Commentary is provided on how this informs on read-across and grouping between various forms of silver – in terms of assessment of potential hazard to human health and harmonised classification and labelling (CLH).

Prior state of scientific knowledge

The main industrial forms of silver are categorised as: **ionic** compounds, e.g. silver[I] acetate; elemental Ag in **nanoform** (typically Ag nanoparticles); and **bulk** elemental Ag in several physical formats, ranging in size from micron-diameter powders to massive forms of silver metal. It has been recognised that the bioavailability[2] of these forms differs; but to date, robust comparative toxicokinetic (TK) data has been lacking. Furthermore, **no quantitative TK information** has been available on the relative absorption characteristics of bulk elemental Ag (i.e. from a reliable OECD guideline study).

Surrogate physiological fluid models which examine the dissolution behaviour of metals or their compounds can be a valid way to predict the relative bioavailability of different forms of a metal (and estimate their potential for uptake into the body). However, in the case of silver and silver ion (Ag+), some of these simplified *in vitro* test systems have limited applicability (Figure 1). Instead, it is more scientifically appropriate to focus on more definitive data from *in vivo* TK assessments.

This TK information gap affecting bulk silver has necessitated assumptions on how it should be grouped in terms of read-across of data covering human health endpoints. For example, whether health effects data relating to ionic Ag compounds, or to nanosilver, are also applicable to bulk silver. As a case in point, a current proposal for harmonised classification and labelling which covers all forms of elemental silver[3] applies a conservative read-across approach.

A comparative TK study commissioned by EPMF – further described below – addresses this key data gap and provides important insights on the relative bioavailability of different elemental silver forms.

New comparative toxicokinetic (TK) information on various Ag forms

The new study[4] was an *in vivo* oral route investigation using a rodent model. The design conformed to OECD and EU norms[5], and was conducted according to full Good Laboratory Practice (GLP). The test items comprised two **ionic Ag salts** commonly selected as investigational references (i.e. silver acetate and silver nitrate); a well-characterised **Ag nanoparticle** reference material (15 nm AgNP); and also a powder-form (~0.3 µm) of **bulk elemental silver**[6]. The

[1] **Toxicokinetics (TK)** is the study of the absorption, transformation/metabolism, distribution, and elimination of chemical substances. EU guidance points out the value of establishing the TK profile of a substance when considering human health hazard assessment since toxic responses to chemical substances – including metals – are based on the nature and quantity of the ultimate toxicant (e.g. a metal ion) presented to a sensitive tissue within the body.

[2] **Bioavailability** is a measure of the proportion of a substance entering the circulation when introduced into the body via a particular route (e.g. oral intake) and its availability for metabolism and interaction at target organs/sites. This parameter bears directly on the toxicity potential of a particular form of a substance.

[3] Proposal for Harmonised Classification and Labelling under Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2. Substance Name: Silver (CAS Number: 7440-22-4). Prepared by Swedish Chemicals Agency (KEMI), Version number: 3. Dated: September 2020. Released by ECHA for public consultation on 19 October 2020.

[4] Silver acetate, Silver nitrate, Micron-sized Silver and Nanoparticulate Silver: A comparative toxicokinetic study in rats by single and repeat administration. Sponsor: European Precious Metals Federation (EPMF). Study identifier: 8430567. Originating institution: Covance, UK.

[5] OECD Guidelines for the Testing of Chemicals Section 4: OECD 417 (Toxicokinetics); and also relevant ECHA guidelines, e.g. those relating to Regulation (EC) No. 1907/2006 [REACH] and the Biocidal Products Regulation [BPR, (EU) No. 528/2012].

[6] Registered under the REACH Regulation and currently placed on the market.

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latter sub-micron sized powder was selected to represent a reasonable worst-case type of bulk silver, i.e. other larger-size bulk Ag forms are expected to be less bioavailable due to their physico-chemical characteristics. **Comparative toxicokinetics** data for the various test substances were obtained after both single and repeated dose administration for 28-days, including the measurement of Ag levels in blood and in tissues. This approach facilitated a direct quantitative comparison of parameters such as bioavailability, and the delivered dose to tissues occurring after administration of the different Ag forms.

Key findings from the new TK study

(1) Simple soluble salts of Ag (both Ag nitrate and acetate) do undergo some absorption when orally administered. Bioavailability via this route appears consistent with other estimates presented in the scientific literature – in the order of 3-5%.

(2) In comparative terms, bulk elemental Ag was **substantially less well absorbed** (Figure 2). Based on matched dose[7] assessments, the extent of systemic exposure was about **10 to 30-fold lower in the case of bulk Ag versus a reference ionic Ag salt**. Unlike the situation with ionic Ag, the degree of uptake was not linear as the amount of administered bulk Ag was increased up to a limit dose, but instead there was evidence of absorption plateauing (Figure 2).

(3) In line with the above, **the dose of silver distributed to tissues and organs was much smaller in the case of bulk Ag** than for ionic Ag forms (Figure 3). This links to predictions that bulk silver represents a correspondingly lower health hazard, i.e., is less likely to cause toxic effects.

(4) A smaller-sized nanosilver form (15 nm AgNP) exhibited an intermediate TK profile. Absorption characteristics and achieved tissue levels more closely resembled those of the ionic Ag test items rather than bulk Ag.

Conclusions

It is commonly considered that the systemic toxicity of inorganic silver substances is driven by the silver ion (Ag^+) as the primary species relevant to tissue exposure, and hence hazard assessment. The EPMF TK study is a high quality GLP investigation which permits a direct comparison of ionic Ag, nanosilver and bulk silver – in terms of relative oral uptake and then tissue distribution of Ag^+.

- The study outcomes confirm that oral intake of **bulk elemental silver results in markedly lower systemic exposure than seen for more bioavailable forms**.
- This conclusion from the experimental model is expected to be **relevant to humans**.
- The study findings strongly suggest that the direct read-across of mammalian toxicity datasets for simple ionic silver salts and nanosilver to bulk silver is **not supported** (from a scientifically valid toxicokinetic perspective).
- Hence, we ask that certain read-across assumptions within the previously referenced CLH proposal[3] are **reconsidered, particularly in respect of the assessment of bulk silver forms**.
- With due regard for this newly available scientific information, the European Chemicals Agency (ECHA) Read-Across Assessment Framework (RAAF)[8] should be appropriately applied to better structure the scientific evaluation of grouping and read-across for elemental silver forms. Metals are within scope of this schema, and RAAF acknowledges that differences in relative bioavailability of metal species represent a basis for differentiation in read-across/grouping.

[7] Comparisons made via normalised administered doses (i.e. Ag equivalent dose) between Ag acetate and bulk Ag. Some variation was seen dependent on the gender of the test animals, with slightly higher systemic exposure evident in the case of females.

[8] The European Chemicals Agency (ECHA) has codified a systematic and consistent approach to assessing such read-across situations, viz. their Read-Across Assessment Framework (RAAF). ECHA-17-R-01-EN. ISBN: 978-92-9495-758-0. March, 2017.

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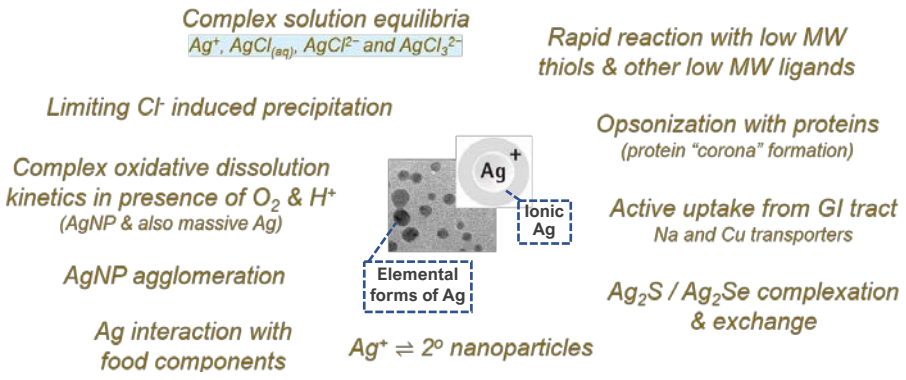

Figure 1. The complex pattern of transformations and interactions of chemical species of silver (under physiological conditions) influencing the bioavailability of Ag for uptake into the body.

Simple aqueous dissolution or surrogate physiological fluid models alone are not adequate for the prediction of bioavailability of various Ag forms.

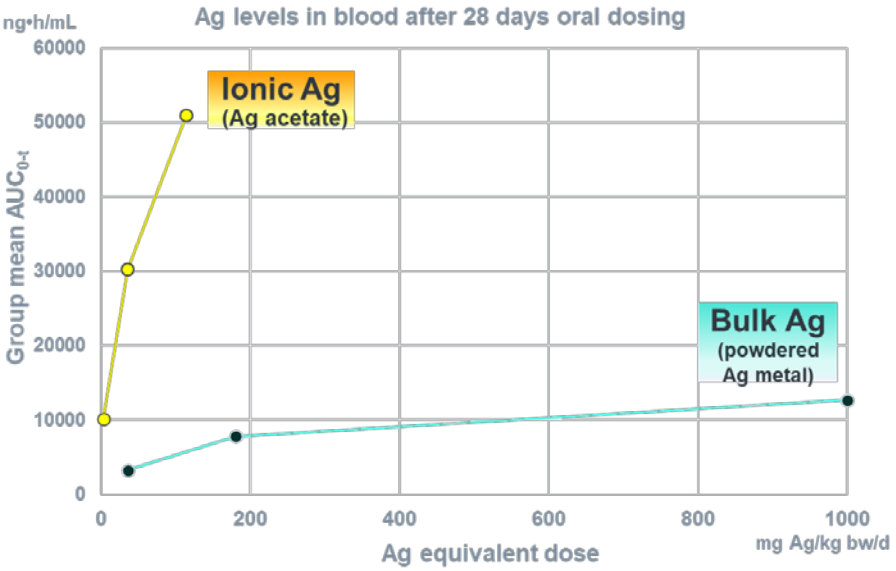

Figure 2. Marked differences exist in the extent of absorption observed for ionic Ag versus that of bulk Ag (sub-micron Ag metal powder).

Results are for female animals receiving the test items (very similar findings were also obtained in the case of males).

A comparable differential absorption picture was evident using Ag nitrate instead as the ionic comparator Ag substance.

Ag concentration (group mean values : ng Ag/g tissue)										
Dose level	**Bulk Ag (silver metal powder)**									
mg Ag/kg bw/d	**Spleen**		**Bone marrow**		**Brain**		**Testis**	**Uterus**	**Ovary**
	♂	♀	♂	♀	♂	♀			
Control	--	--	--	--	--	--	--	--	--
36	<LLOQ	<LLOQ	<LLOQ	<LLOQ	58	56	<LLOQ	<LLOQ	<LLOQ
180	89‡	43‡	47	65	119	128	130	46	525
1000	218	276	130	165	171	223	332	236	3645
Dose level	**Ionic Ag (silver acetate)**									
mg AgAc/kg bw/d [mg Ag/kg bw/d]	**Spleen**		**Bone marrow**		**Brain**		**Testis**	**Uterus**	**Ovary**
	♂	♀	♂	♀	♂	♀			
Control	--	--	--	--	--	--	--	--	--
5 [3.25]	283	990	62	126	142	169	167	188	2197
55 [36]	38656	60783	3500	4501	637	805	1508	8004	24262
175 [114]	96379	141560	21373	46761	1458	1455	1531	11094	39668

Figure 3. Levels of Ag distributed into tissues are considerably lower in the case of bulk elemental Ag than an ionic Ag compound.

Notes:
Results shown for a subset of key tissues.

LLOQ = limit of quantitation.

‡ *only two samples in the group had measurable levels.*

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