



Silver Task Force  
North America

**Silver Task Force North America**

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European Chemicals Agency  
Annankatu 18, P.O. Box 400,  
FI-00121 Helsinki, Finland

July 29, 2015

**Re: Evaluation of Silver Substances under Regulation 528/2012**

Dear Sir/Madam,

The Silver Task Force North America (STF NA) is submitting the attached comments relating to the evaluation of silver substances under Regulation 528/2012. These comments on the classification recommendations are based upon the evaluation by the Swedish Chemical Agency (KemI) as the Competent Authority of the rapporteur state. The STF NA is concerned by the KemI recommendation as it is not supported by the available scientific evidence.

If you have any questions or require additional information or clarification, please do not hesitate to contact me by phone at (202) 828-8966 or by email at [etesch@tsgusa.com](mailto:etesch@tsgusa.com).

Regards,

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Silver Task Force North America

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**Evaluation of silver substances under Regulation 528/2012 and issues of potential concern to the evaluation of silver substances by the US EPA.**

The following are comments on classification recommendations, based upon the evaluation by the Swedish Chemical Agency (KemI) as the Competent Authority of the rapporteur state, that the STF North America considers to be of concern and not supported by the available scientific evidence:

**Argyria**

The main consequence of long term high level exposure to silver is argyria (pigmentation of tissues and organs). This appears to be due to the formation of insoluble silver precipitates of silver selenide which are not associated with reactive changes in tissue biopsies of humans (Landsdown, 2010). This formation of silver precipitates is regarded as a toxic effect by KemI in contrast to the general opinion of the scientific community and the United States Environmental Protection Agency (US EPA) that the effect is cosmetic and an indicator of exposure rather than toxicity. KemI concludes *“although the toxicological significance of tissue pigmentation is somewhat unclear, it is considered appropriate to be precautionous and deposition of silver in tissues is therefore regarded as an undesired effect that may lead to adverse effects in humans”* (see page 56, Section 4.7.1.5). The deposition of silver in tissues after repeat dosing is now known to be due to the formation of nano-size silver granules which also contain sulphur and selenium (Loeschner et al., 2011). The formation of insoluble silver sulphide and selenide appears to be protective because the bioavailability of silver ions is reduced in this stable and insoluble complex.

Tissue pigmentation is used to conclude a classification of silver zinc zeolite for Specific Target Organ Toxicity (STOT) according to the CLP Regulation (1272/2008) and is also used to derive the long term Allowable Exposure Level (AEL) for silver zinc zeolite and by extrapolation, based on availability of the silver ion ( $\text{Ag}^+$ ), also for silver. The proposed AEL value for silver is  $0.045 \mu\text{g}/\text{kg bw}/\text{day}$  and this is based on internal organ pigmentation and the application of a one hundred thousand-fold uncertainty factor to the lowest dose level resulting in tissue pigmentation. This value is applied to all the silver substances included in the EU biocide active substance review as a common end-point for risk assessment. The long term AEL is approximately 200 times lower than background dietary exposure to silver (ATSDR, 1990). KemI does not address significance of background exposures to this element in their recommendations.

Although humans are susceptible to argyria and the US EPA Reference Dose of  $5 \mu\text{g}/\text{kg bw}/\text{day}$  is, in fact, based on argyria, this endpoint is not associated with a toxicological response and, given our knowledge of the mechanism of argyria causation in humans and laboratory animals, does not warrant the application of conservative uncertainty factors. The US EPA has applied a total uncertainty factor of three in their derivation of an acceptable amount of human intake of silver. It is recommended that the need for classification for STOT and the proposed AEL be revisited based

on the lack of adverse consequences of this endpoint in humans and laboratory animals. As is noted in the RAC, argyria has been associated with toxicity after very high exposures but is more appropriately considered to be a biomarker of exposure rather than an indicator of toxicity.

### Dermal Absorption

Dermal absorption may be used to convert external exposure to internal exposure in the risk calculation. The RMS states that "A dermal absorption of 4% is however expected to be conservative since it is based on the assumption that all radioactivity that disappeared from the test area entered the systemic circulation through the skin" (see Section 4.1.1, p. 29). In fact, there is no evidence of penetration of silver ions through the intact dermis. The publication of Skog and Wahlberg (1963) is cited as the basis for the dermal absorption value of 4%. This study is not a measure of systemic absorption but rather of disappearance of radiolabelled silver nitrate from the application site on the back of guinea pigs. The estimated disappearance rate may include sequestration in the dermis and, in the case of silver, this material may not be absorbable due to protein binding. In addition, examination of the individual animal data from the study found that only one of the 80 animals administered silver nitrate had a disappearance rate between 3.0 and 3.9% and no animal was reported to have a silver disappearance rate greater than 3.9% percent. Sixty one of the eighty animals were described as having disappearance rates of less than one percent. The mean disappearance rate in this study was 1.26%. This negligible absorption is consistent with absence of dermal penetration of <sup>110m</sup>Ag-labeled silver zinc zeolite applied for 24 hours to rats in the form of a cream (Hajima and Mukai, 1992). In that study only 0.12, 1.10 and 0.17% of the radiolabeled silver was found in the urine, feces and other sites, respectively. An additional 1.0% was found sequestered in the dermis.

As discussed in the comprehensive review article by Hostynek (2003), the electrophilic nature of many metals such as silver result in reaction with proteins in the skin and this inhibits further diffusion. Silver has a strong affinity for sulfhydryl groups on proteins and this limits diffusion through the strata of the skin. Hostynek (2003) notes that "experiments to determine the penetration of human skin by water-soluble salts have not given measurable results". The strong affinity of silver for proteins and the negligible skin penetration rate of 1% indicate that no systemic absorption of silver ions is expected to occur from biocidal uses of SCAS. Kemi extrapolates 5% dermal absorption for Ag<sup>+</sup> to silver zinc zeolite itself which is unrealistic considering the substance is a high molecular weight inorganic crystalline solid.

Adopting an unrealistic value for the dermal absorption of silver results in an exaggerated risk estimate for the antimicrobial uses of silver. The US EPA concluded that silver ions will not be absorbed through intact skin and a quantitative risk assessment for dermal exposure is not necessary. We recommend that Kemi take the approach of the US EPA with respect to dermal absorption.

### Carcinogenicity

Kemi propose GHS classification as Category 2 for carcinogenicity. This proposal is not supported by the available data and weight of evidence supporting the lack of carcinogenic effects of silver. The interpretation of the carcinogenicity data relies on questionable statistical interpretations without taking into account biological significance, dose-response relationship or plausibility. In this respect

it is notable that neither zinc nor silver are considered to be carcinogenic and zeolite is toxicologically inert<sup>1</sup>

KEMI concluded that a carcinogenicity study with silver zinc zeolite in the rat (Takizawa, 1992) showed an increase in leukemia in treated animals despite the absence of a statistically significant difference at any dose level. No increase in tumor incidence at any site was found in a corresponding mouse carcinogenicity study conducted at the same laboratory. The US EPA has noted that there is no evidence of silver carcinogenicity in humans despite frequent use of therapeutics involving exposures over many years. US EPA has not concluded that silver is carcinogenic.

Interpretation of the carcinogenicity data by Kemi appears to be influenced by positive *in vitro* clastogenicity data. KEMI concludes that there is evidence for clastogenicity *in vitro*, but is not reassured by the two negative studies of clastogenicity conducted *in vivo*. Studies of genotoxicity are generally considered more relevant for predicting hazard because *in vitro* genotoxicity studies typically employ concentrations that cannot be achieved from human exposures. The relevance of the *in vivo* studies is questioned by Kemi on the basis of inadequate evidence of exposure of the target tissue (bone marrow) (see Section 4.1.1, page 30).

Distribution studies confirm the wide distribution of silver to many tissues including blood and bone marrow (Lansdown, 2010; Hadrup and Lam, 2014). Given that silver is systemically distributed to a wide variety of tissues, it is reasonable to assume that silver reached bone via blood circulating through bone marrow. The assertion that the *in vivo* micronucleus test results, negative for mutagenicity, are invalidated through a lack of target tissue exposure is not supportable by the distribution data. On this basis the demonstrated absence of silver-induced clastogenicity *in vivo* should be recognised as evidence that insufficient silver can be administered *in vivo* to induce a clastogenic effect.

The STFNA requests that the classification be reconsidered because the weight of the evidence does not support the conclusion that silver is genotoxic or carcinogenic.

### Reproductive Toxicity

Reproductive toxicity is identified by KEMI in a study of silver zinc zeolite, a developmental study of silver acetate and a developmental study of silver chloride. The mid- and high dose levels of the study of silver zinc zeolite were characterized by parental toxicity which indicated excessive dosing and zinc, rather than silver, toxicity. Developmental effects in this study were clearly secondary to parental toxicity. The authors of this US National Toxicology Program study, subjected to extensive peer review, concluded that the study showed “the absence of any statistically or biologically significant developmental toxicity”. The third study cited as support for classification, only showed evidence of developmental toxicity at the sole dose level of 250 mg/kg/day. The extent of maternal toxicity is unknown in this study. The Silver Task Force of North America recommends that weight of the evidence for reproductive toxicity be revisited for purposes of classification. While reproductive effects seen in the two-generation study were not associated with marked mortality or severe

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<sup>1</sup> At sufficiently high dose levels zeolite is associated with crystalline deposits in the kidney and bladder following long-term administration (see HERA, 2004).

bodyweight effects in parental animals, data show an association with anaemia in the reproduction study and a subchronic study conducted at the same dose levels. Mechanistic data indicate that the anemia and reproductive effects are due to an induced copper deficiency due to excessive zinc intake in parents and the reproductive effects are therefore secondary to systemic toxicity.

## References

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