Review of Sprando et al. (2017)[a] / commentary on study significance

Sprando and co-workers at the US FDA Center for Food Safety and Applied Nutrition have recently published the outcomes of a one-generation reproductive toxicity study (OGRTS) relevant to ionic silver. Its basic design and conduct aligned with US requirements for food additives (FDA CFSAN Redbook, 2000). In brief, Sprague–Dawley rats (n=20/sex per group) received silver acetate (AgAc) administered in drinking water at dose levels of 0, 0.4, 4 or 40 mg AgAc/kg bw/day (equivalent to 0, 0.26, 2.6 or 26 mg ionic Ag/kg bw/day). Parental male animals were exposed 10 weeks prior to mating and parental female animals for 2 weeks prior to mating. The F1-pups were sacrificed on postnatal day (PND) 26. Further remarks on study methodology are provided in Table 1 of this report: *Sprando et al. (2017) rat one-generation study in rats – deviations in comparison to OECD Test Guideline 443 (Extended One Generation Reproductive Toxicity Study)*.

Comments and conclusions hereafter focus on areas identified by PMC Secretariat as being of main interpretative interest. Any referenced dose levels in dietary and drinking water route of administration studies are stated as approximate nominally achieved values. For key / background references, please also refer to the section: *Key References reviewed or re-reviewed during project*.

1 - Significance of Sprando et al. (2017): The study is important in the broader consideration of the reproductive toxicity of silver for the following main reasons:-

(a) Developmental toxicity was observed based on endpoints of decreased implant numbers; increased pup mortality; an increased incidence of runts and F1 generation weight retardation/delayed growth. In qualitative terms, this could be argued just to be congruent with the effects seen in some other studies (e.g. the embryotoxicity observed with high doses of silver chloride, and the outcomes of the 2-generation study conducted with SZZ[b] where effects such as increases in stillbirths and pup development retardation were observed). However, in Sprando et al. the apparent developmental toxicity LOAEL was 4 mg AgAc/kg bw/day with an assigned NOAEL of 0.4 mg AgAc/kg bw/day (equivalent to 2.6 and 0.26 mg Ag+/kg bw/day, respectively). So as well as being broadly confirmatory in qualitative terms, the LOAEL was lower than the mid-dose of the 2-generation study with SZZ (~10 mg Ag+/kg bw/day) where effects were detected for F1 generation pups.

(b) As a more novel finding, Sprando et al. also reported adverse effects on fertility as evident for high-dose group animals receiving 40 mg AgAc/kg bw/day (26 mg Ag+/kg bw/day equivalent), with a NOAEL for this effect of 4 mg AgAc/kg bw/day (2.6 mg Ag+/kg bw/day equivalent). The main parameters impacted were reduced fertility and the number of litters in the high-dose group. Previous evidence from reliable studies for antifertility effects due to treatment with ionic silver has been scant. For instance, in the 2-generation studies with SZZ and SSZHP[c], effects on fertility and associated reproductive parameters were not evident for parental generation at levels up to circa 40 and 23 mg Ag+/kg bw/day equivalents, respectively. An explanation for this disparity between Sprando et al. and the 2-generation studies with SCAS[d] is currently lacking. On a point of correct interpretative balance, Kemi have characterised the antifertility effect reported by Sprando et al as being "severe" in degree (e.g. refer to their comments on the study in the SZ[e] CLH dossier; p. 59). This reviewer considers such a statement to be an exaggeration.

[a] Sprando RL, Black T, Keltner Z, Olejnik N, Ferguson M (2017) Silver acetate exposure: Effects on reproduction and post natal development. Food Chem Toxicol. 106: 547-557.
[b] SZZ = Silver Zinc Zeolite
[c] SSZHP = Silver Sodium Zirconium Hydrogen Phosphate
[d] SCAS = Silver-containing active substance
[e] SZ = Silver Zeolite

(c) In terms of mitigating factors, evidence of appreciable maternal or paternal toxicity in the Sprando et al. study was absent, and therefore would be excluded by regulatory agencies as a reason for confounding influences on reproductive toxicity. However, it should be noted that some other potential secondary effect influences were not examined during the study – such as gut microbiota changes.

(d) As an overall position for silver, the key extended dosing reproductive toxicity studies previously comprised the 2-generation investigations (mentioned previously) on SZZ and SSZHP, where the former had more adverse outcomes in its developmental toxicity endpoints. Integration of the outcome of the SCuZ[f] 2-generation study for read-across to silver is problematic because of the presence of copper (with its potential impact on Cu supplementation). A counterbalanced position also existed between the clear embryotoxicity reported with high doses of silver chloride (Shavlovski et al.; 1995) versus the more favourable outcomes of the prenatal developmental study conducted by NTP (2002)[g] on AgAc. Hence in weight-of evidence terms, Sprando et al. does tip the balance in conclusions that ionic silver can cause developmental toxicity (LOAEL 2.6 mg Ag+/kg bw/day). However, it is important to state that the study does not shed light on mechanism(s) of action, and on whether indirect effects of lesser concern could be involved (further discussed later). Exactly the same limitation applies to all the 2-generation studies on SCAS.

(e) If equivalent findings as those described in Sprando et al. were replicated in an EOGRTS then these would fulfil the internal trigger requirements for Cohort 1B extension (F2 generation). This conclusion is based on current ECHA guidance and also rationales laid down in reviews of EOGRTS conduct, such as by Moore et al. (2016).

(f) The FDA sponsored OGRTS on silver acetate is an important investigation for one other key reason. Developmental immunotoxicity endpoints were also integrated and then reported separately[h] from the main reproductive toxicity outcomes. These findings are not reviewed in depth in this document, but clear effects on the immune system of F1 offspring were described by the investigators (Babu et al., 2016) following P generation exposure to AgAc (including exposures during the gestation and lactation periods). In brief, the effects comprised of downward shifts in several key splenic lymphocyte subsets, and depressed mitogen-induced lymphocyte proliferation in pups. For the latter, which is a marker of normal immune system function, a NOAEL could not be established since differences were noted at the lowest dose level of 0.4 mg AgAc/kg bw/day (0.26 mg Ag+/kg bw/day equivalent). Subject to confirmation/replication, developmental immunotoxicity (DIT) following ionic silver exposure is an important new finding as it has not previously been reliably demonstrated[i]. Triggers related to adult animal immunotoxicity in previous repeat dose studies conducted by the oral route with ionic silver or silver nanoparticles have also been limited. Other than splenic disturbances, Babu et al. report that no comparable detrimental effects on thymic development or related thymocyte phenotypic markers were evident. However, a risk exists that regulators could attempt to conflate the thymus weight depression in offspring previously reported in 2-generation studies on SCAS (e.g. refer to the study with SZZ) to suggest that more generalised DIT occurs after silver exposure. As a final remark, the low-end ionic silver exposures achieved during the FDA OGRTS were only modest and selected deliberately to be relevant to (adult) human exposures, albeit worst-case situations.

[f] SCuZ (SCZ) = Silver Copper Zeolite.
[g] Sometimes also cited as the 'Price and George' study.
[h] Babu US, Balan KV, Bigley E, Pereira M, Black T, Olejnik N, Keltner Z, Sprando RL (2016) Effects of maternal silver acetate exposure on immune biomarkers in a rodent model. Food Chem Toxicol. 98: 195-200.
[i] See also the dataset review assembled for the PMC Testing proposal: EOGRTS (TG 443) – Ag soluble compounds (2015)

2 - Mechanistic considerations: As previously stated, in common with the conventional 2-generation studies conducted on SCAS, the Sprando et al. study does not inform on mechanism of action (MoA) in respect of reproductive toxicity markers. The MoA and other methodological issues applying to the Shavlovski et al. study have previously been well covered in the PMC comments[i] submitted on the SZZ CLH dossier. Given it is a conventional OGRTS, Sprando et al. provides minimal additional insights into the potential reproductive toxicity MoA of ionic silver. This remains an important data gap relevant to both hazard assessment and risk assessment.

3 - Tissue pigmentation (argyria): As would be expected, histopathology of tissues from parental animals in the FDA OGRTS identified pigmentation (argyria), which occurred in a dose-dependent manner at 40 and 4 mg AgAc/kg bw/day, though apparently not at the low dose. This was evident particularly for the kidneys, but also in other tissues and organs. However, it is also clear that no adverse histopathological changes coexisting with this phenomenon were detected in P generation animals. There was some evidence of argyria in F1 pups, though this was not intensively studied – but importantly, no obvious histopathological changes were noted in the F1 pups (exposed in utero and during lactation) and then sacrificed at PND 26. Neither was it reported that argyria in pups correlated with the immune system findings reported in Babu et al. (2016). A finding of argyria is in line with many other previous reports involving administration of silver to experimental animals and humans. The generally benign nature of argyria was extensively commented upon by PMC in submissions to ECHA[j] (q.v.). In terms of regulatory precedent, it is notable that, in 2015, ECHA RAC did not consider the argyria reported for a silver-containing active substance (SZZ) to be of toxicological significance (and hence did not accord STOT-RE classification for this effect). However, Kemi's viewpoint on this particular finding from Sprando et al. is notably more adverse, together with equivalent comments on other SCAS in the latest draft CLH dossiers. For example, in the SZ CLH dossier, on p. 59 Kemi state that a parental No Observed **Adverse** Effect Level cannot be set in the Sprando et al. study due to tissue pigmentation. Furthermore, it is clear from their remarks elsewhere (e.g. dossier pp. 77-79) that they regard argyria to be of toxicological significance in respect of ionic silver. Independent of any reproductive toxicity considerations for ionic silver, a change of regulatory mindset about the structural and functional significance of argyria (i.e. regarding its adverse) would have broad impact. It is therefore recommended that PMC at least reiterate the previous rebuttal position on argyria's significance in any comments submitted on the latest SCAS CLH dossiers (also correcting the misperceptions regarding terminology when referring to NOAEL instead of NOEL for Ag-induced tissue pigmentation).

4 - Protocol comparison of Sprando et al. OGRTS vs. EOGRTS TP 443: The question arises as to whether – purely due to methodological overlaps – the availability of a new one generation reproductive toxicity study on ionic silver negates the need for the PMC TP for an EOGRTS. To assist in decision-making by PMC, a comparative assessment was performed of the methodology of the OGRTS performed by Sprando et al. to assess its protocol differences relative to the requirements of a standard OECD Test Guideline 443 Extended One Generation Reproductive Toxicity Study. These differences are summarised in Table 1, together with some remarks on the relative importance of each.

[j] Comments on the proposal for harmonised classification and labelling for silver zinc zeolite by the Precious Metals and Rhenium Consortium (PMC), 16 July 2015.

TABLE 1: Sprando et al. (2017) rat one-generation study in rats – deviations in comparison to OECD Test Guideline 443 (Extended One Generation Reproductive Toxicity Study)

Sprando et al. (2017)	**OECD TG 443 EOGRTS**
In line with the protocol requirements of US FDA Redbook (CFSAN, 2000).	Protocol meeting requirements of current OECD TG[k].
By design, limited to a single parental cohort and F_1 generation.	Potential triggers exist for extension of basal study to include an F_2 generation. Per previous comments, had Sprando et al. (2017) been initiated as an EOGRTS protocol the outcomes apparently fulfil at least one internal trigger[l] condition for extension of Cohort 1 to provide an F_2 generation.
Not Good Laboratory Practice (GLP) compliant. However, from the published details the study appears to be robust and would be expected to be ranked as KL1/2.	GLP norm expected.
Individual animal observations are unavailable, which is not ideal. In respect of one important aspect, no obvious clinical sign evidence of maternal toxicity was reported.	Individual animal observations would be transparent, e.g. clinical observations; bodyweight, food and water consumption data.
Test article (silver acetate) administered via the oral route in the drinking water rather than in the diet. However, the approach taken in Sprando et al. (2017) does not represent a major point of deviation to TG 443.	Drinking water administration of test article is permissible within the TG. However, it can be less preferred than dietary route as first choice, e.g. if potential depression of fluid intake occurs (which can be an effect caused by ionic Ag). Deposition of Ag on container surfaces, particularly affecting the integrity of low concentration solutions, is a further potential complication of inclusion in water. Dietary intake rather than gavage is stated in TG 443 as the preferred administration mode (for exposure of offspring), but incorporation in drinking water could be justified as an alternative with similar advantages in that respect.
Haematological parameters were not assessed (although clinical biochemistry was evaluated).	Included standard parameter.
Thyroid hormone assessment (TSH, T4) was not conducted[m].	Included standard parameters.
Urinalysis was not reported as conducted. This would have been appropriate for a mode of administration/test article where fluid homeostasis may be affected.	Included standard parameter.

[k] OECD (2012) OECD Guideline for the testing of chemicals. Test Guideline 443: Extended One-Generation Reproductive Toxicity Study. Organisation for Economic Cooperation and Development, Paris.
[l] Internal trigger: An adverse finding occurring during the conduct of the standard EOGRTS which then requires extension of the study.
[m] Thyroid hormones play a crucial role in normal development, especially maturation and function of the central nervous system, and are included in various reproductive toxicity study protocols for that reason.

TABLE 1 (continued)

Sprando et al. (2017)	**OECD TG 443 EOGRTS**
The tissue set taken for histopathology was more limited than in a standard TG 443 protocol, though it was examined for all treated groups (notably excepting testicular histopathology evaluated for control and high-dose only). There were several important omissions in key primary and secondary reproductive tissues versus the TG 443 protocol (such as uterus, ovaries, epididymides, prostate, and seminal vesicles).	Standard tissues for histopathology are more extensive – in particular for the study of primary and secondary sex organs.
Oestrus cycle evaluation, including oestrous cyclicity assessment, was not evaluated.	Included standard parameter.
Sperm parameters (including motility and morphology by a standardised method) were not evaluated. Although the investigators concluded that silver acetate exposure did not adversely affect spermatogenesis, this was based on limited histopathology and testis weight alone. Detailed investigations comparable to those in an EOGRTS were not conducted, and it is notable that the investigators were unable to discriminate whether the antifertility effects apparently evident at the high-dose were due wholly or in part to effects on parental males.	Included standard parameters.
Anogenital distance measurement (sexual differentiation marker) in pups not evaluated.	Included standard parameter.
Nipple/areola retention in males (maturation/ endocrine disruption marker) in pups not evaluated.	Included standard parameter.
Culled F_1 generation pups were subjected to developmental immunotoxicity (DIT) evaluation and toxicogenomic investigations. For the methods applied to DIT refer to Babu et al. (2016) – see References section. DIT outcomes were reported as significant. Developmental neurotoxicity investigation was not included.	Developmental immunotoxicity (Cohort 3) is an externally triggered[n] extension of the standard EOGRTS. The EOGRTS design foresees inclusion of Cohort 2 (DNT) and/or Cohort 3 (DIT) if trigger justification exists. Note: The current PMC Testing Proposal is for the basic design lacking these cohorts. Toxicogenomic investigations are not part of the TG 443 protocol.
No concurrent toxicokinetic studies (TK) were performed. Pre-existing TK datasets for ionic silver relevant to selected dose levels in the study are acknowledged to be limited – especially regarding achieved fetal/pup exposures.	Toxicokinetic evaluations are stated to be "extremely useful" in the description of the TG, and may be conducted separately as enabling studies or integrated into the main EOGRTS (see TG Section 17).

[n] External trigger: Typically, an adverse finding from previous investigations giving cause for concern that developmental immunotoxicology/neurotoxicology effects are possible.

TABLE 1 (continued)

Further remarks Whilst not a standard parameter in either one- or two-generation reproductive toxicity studies, the Sprando et al. (2017) study includes no assessments of certain parameters which take into account pre-existing knowledge about the substance-specific effects of ionic silver, and which could be relevant to discrimination of MoA and indirect influences impacting on reproductive parameters. For example, evaluation of G.I. tract microbiota populations, determinations of ceruloplasmin levels, tissue selenium levels, or stress hormones.

Updated literature search on Ag (conducted 24 August 2017)

Scope of search:

References of possible relevance to human health endpoints with the emphasis on reproductive and repeat dose toxicity of ionic silver and related forms of silver. Secondary search themes were also included covering significant toxicokinetic developments, and findings potentially relevant to developmental neurotoxicity and developmental immunotoxicity.

The search boundaries set for publication date were limited to 2016 and 2017 (to date).

Sources researched include:

- **(a)** Standard biomedical and toxicology online bibliographic databases (Scholar, Pubmed, Toxnet, DART etc.)
- **(b)** Secondary references in recently identified papers on silver.
- **(c)** Grey literature, including publicly available databases from EU ECHA, US FDA, US EPA, US NTP, EU EFSA, Swedish Kemi, and OECD (including WPMN).

Search findings:

Note: to keep the listing to a manageable size, some less relevant references within this publication period have been gated out. This particularly excludes in vitro studies ranked as less than high relevance. Hence it should be noted that the listing in Table 2a is selective and not exhaustive.

TABLE 2a: Updated literature search on silver – Research publications

	Reference	Remarks
1	Ansar S, Abudawood M, Hamed SS, Aleem MM. Sodium Selenite Protects Against Silver Nanoparticle-Induced Testicular Toxicity and Inflammation. Biol Trace Elem Res. 2017 Jan;175(1):161-168.	In vivo model (rat) evaluating testicular effects of Ag NP and protective influence of selenium. ***Recommend review of paper.***
2	Ansari MA, Shukla AK, Oves M, Khan HM. Electron microscopic ultrastructural study on the toxicological effects of AgNPs on the liver, kidney and spleen tissues of albino mice. Environ Toxicol Pharmacol. 2016 Jun;44:30-43.	In vitro ultrastructural studies on Ag NP. May be relevant to argyria theme. ***Recommend review of paper.***
3	Ansari MA, Khan HM, Khan AA, Alzohairy MA, Waseem M, Ahmad MK, Mahdi AA. Biochemical, histopathological, and transmission electron microscopic ultrastructural changes in mice after exposure to silver nanoparticles. Environ Toxicol. 2016 Aug;31(8):945-56.	Sub-acute study on Ag NP via the i.p. route. Dubious relevance.
4	Austin CA, Hinkley GK, Mishra AR, Zhang Q, Umbreit TH, Betz MW, E Wildt B, Casey BJ, Francke-Carroll S, Hussain SM, Roberts SM, Brown KM, Goering PL. Distribution and accumulation of 10 nm silver nanoparticles in maternal tissues and visceral yolk sac of pregnant mice, and a potential effect n embryo growth. Nanotoxicology. 2016 Aug;10(6):654-61.	In vivo study (prenatal exposure) comparing Ag NP and Ag+ (but i.v. route). Probably dubious quality/relevance but abstract insufficient to assess. ***Recommend review of paper.***

5	Babu US, Balan KV, Bigley E, Pereira M, Black T, Olejnik N, Keltner Z, Sprando RL. Effects of maternal silver acetate exposure on immune biomarkers in a rodent model. Food Chem Toxicol. 2016 Dec;98(Pt B):195-200.	Reproductive toxicity assessment relevant to developmental immunotoxicity. Satellite work during OGRTS. ***Major significance/vital that paper is reviewed.***
6	Bergin IL, Wilding LA, Morishita M, Walacavage K, Ault AP, Axson JL, Stark DI, Hashway SA, Capracotta SS, Leroueil PR, Maynard AD, Philbert MA. 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. 2016;10(3):352-60.	Ag NP and AgAc compared. Possibly relevant to TK considerations.
7	Boudreau MD, Imam MS, Paredes AM, Bryant MS, Cunningham CK, Felton RP, Jones MY, Davis KJ, Olson GR. 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. 2016 Mar;150(1):131-60.	Relevant. Already reviewed by PMC.
8	van den Brule S, Ambroise J, Lecloux H, Levard C, Soulas R, De Temmerman PJ, Palmai-Pallag M, Marbaix E, Lison D. Dietary silver nanoparticles can disturb the gut microbiota in mice. Part Fibre Toxicol. 2016 Jul 8;13(1):38.	Assessment of Ag influence on gut microbiota of mice. Reliable research group. ***Strongly recommend review of paper.***
9	Charehsaz M, Hougaard KS, Sipahi H, Ekici AI, Kaspar Ç, Culha M, Bucurgat ÜÜ, Aydin A. Effects of developmental exposure to silver in ionic and nanoparticle form: A study in rats. Daru. 2016 Oct 6;24(1):24.	Reproductive toxicity assessment relevant to prenatal stage (Ag nitrate and Ag NP). Includes TK. ***Strongly recommend review of paper.***
10	Chen S, Goode AE, Skepper JN, Thorley AJ, Seiffert JM, Chung KF, Tetley TD, Shaffer MS, Ryan MP, Porter AE. Avoiding artefacts during electron microscopy of silver nanomaterials exposed to biological environments. J Microsc. 2016 Feb;261(2):157-66.	Methodological aspects relevant to ultrastructural investigations. ***Recommend review of paper.***
11	Dąbrowska-Bouta B, Zięba M, Orzelska-Górka J, Skalska J, Sulkowski G, Frontczak-Baniewicz M, Talarek S, Listos J, Strużyńska L. Influence of a low dose of silver nanoparticles on cerebral myelin and behavior of adult rats. Toxicology. 2016 Jul 1;363-364:29-36.	Investigational neurotoxicity study on Ag NP and also Ag+ in adult rats. Effects reported. May be relevant to EOGRTS design. ***Recommend review of paper.***
12	Dănilă OO, Berghian AS, Dionisie V, Gheban D, Olteanu D, Tabaran F, Baldea I, Katona G, Moldovan B, Clichici S, David L, Filip GA. The effects of silver nanoparticles on behavior, apoptosis and nitro-oxidative stress in offspring Wistar rats. Nanomedicine (Lond). 2017 Jun 1. doi: 10.2217/nnm-2017-0029. [Epub ahead of print]	Claim of significant prenatal neurobehavioural toxicity in rats exposed to Ag NP. Probably limited relevance but cannot assess from abstract. ***Recommend review of paper.***

13	Dziendzikowska K, Krawczyńska A, Oczkowski M, Królikowski T, Brzóska K, Lankoff A, Dziendzikowski M, Stępkowski T, Kruszewski M, Gromadzka-Ostrowska J. Progressive effects of silver nanoparticles on hormonal regulation of reproduction in male rats. Toxicol Appl Pharmacol. 2016 Dec 15;313:35-46.	Mechanistic studies on Ag NP and reproductive hormones. May be of relevance. ***Recommend review of paper.***
14	Ema M, Gamo M, Honda K. Developmental toxicity of engineered nanomaterials in rodents. Toxicol Appl Pharmacol. 2016 May 15;299: 47-52.	Review. Includes some coverage of Ag NP.
15	Ema M, Okuda H, Gamo M, Honda K. A review of reproductive and developmental toxicity of silver nanoparticles in laboratory animals. Reprod Toxicol. 2017 Jan;67:149-164.	Limited to Ag NP studies but an important updating review and compilation of dataset on reproductive toxicity. ***Strongly recommend review of paper.***
16	Fröhlich EE, Fröhlich E. Cytotoxicity of Nanoparticles Contained in Food on Intestinal Cells and the Gut Microbiota. Int J Mol Sci. 2016 Apr 6;17(4):509.	Review partly covering Ag NP and gut microbiota effects. May be of secondary relevance.
17	Garcia T, Lafuente D, Blanco J, Sánchez DJ, Sirvent JJ, Domingo JL, Gómez M. Oral subchronic exposure to silver nanoparticles in rats. Food Chem Toxicol. 2016 Jun;92:177-87.	Sub-chronic in vivo adult rat study on Ag NP. Influence on tissue levels of other metals investigated, e.g. Cu. ***Recommend review of paper.***
18	Gao X, Topping VD, Keltner Z, Sprando RL, Yourick JJ. Toxicity of nano- and ionic silver to embryonic stem cells: a comparative toxicogenomic study. J Nanobiotechnology. 2017 Apr 11;15(1):31.	Investigational mechanistic study (genomics) related to development toxicity (Ag+ compared to Ag NP). US FDA staff. ***Recommend review of paper.***
19	Gueroui M, Kechrid Z. Evaluation of Some Biochemical Parameters and Brain Oxidative Stress in Experimental Rats Exposed Chronically to Silver Nitrate and the Protective Role of Vitamin E and Selenium. Toxicol Res. 2016 Oct;32(4):301-309. Epub 2016 Oct 30.	Investigational neurotoxicity study on Ag nitrate. Dubious quality. Does assess impact of selenium.
20	Hendrickson OD, Klochkov SG, Novikova OV, Bravova IM, Shevtsova EF, Safenkova IV, Zherdev AV, Bachurin SO, Dzantiev BB. Toxicity of nanosilver in intragastric studies: Biodistribution and metabolic effects. Toxicol Lett. 2016 Jan 22;241:184-92.	Distributional study on Ag NP linked to negative findings on neurotoxicity. Possible relevance to EOGRTS DNT. ***Recommend review of paper.***
21	Jiang X, Wang L, Ji Y, Tang J, Tian X, Cao M, Li J, Bi S, Wu X, Chen C, Yin JJ. Interference of Steroidogenesis by Gold Nanorod Core/Silver Shell Nanostructures: Implications for Reproductive Toxicity of Silver Nanomaterials. Small 2017 Mar;13(10).	In vitro study on Ag NM judged to be of limited relevance to ionic silver.

22	Lafuente D, Garcia T, Blanco J, Sánchez DJ, Sirvent JJ, Domingo JL, Gómez M. Effects of oral exposure to silver nanoparticles on the sperm of rats. Reprod Toxicol. 2016 Apr;60:133-9.	Ag NP related. May be of secondary interest to interpretation of reproductive toxicity of Ag. ***Recommend review of paper.***
23	Miller DL, Yu IJ, Genter MB. Use of Autometallography in Studies of Nanosilver Distribution and Toxicity. Int J Toxicol. 2016 Jan-Feb;35(1):47-51.	Ultrastructural Ag NP methodology related. May be relevant to argyria theme.
24	Orlov IA, Sankova TP, Babich PS, Sosnin IM, Ilyechova EY, Kirilenko DA, Brunkov PN, Ataev GL, Romanov AE, Puchkova LV. New silver nanoparticles induce apoptosis-like process in E. coli and interfere with mammalian copper metabolism. Int J Nanomedicine. 2016 Dec 15;11: 6561-6574.	Study of dubious quality on Ag NP, but in vivo study component did assess ceruloplasmin as a marker. ***Recommend review of paper.***
25	Pang C, Hristozov D, Zabeo A, Pizzol L, Tsang MP, Sayre P, Marcomini A. Probabilistic approach for assessing infants' health risks due to ingestion of nanoscale silver released from consumer products. Environ Int. 2017 Feb;99:199-207.	Ag NM related risk assessment.
26	Han JW, Jeong JK, Gurunathan S, Choi YJ, Das J, Kwon DN, Cho SG, Park C, Seo HG, Park JK, Kim JH. Male- and female-derived somatic and germ cell-specific toxicity of silver nanoparticles in mouse. Nanotoxicology. 2016;10(3):361-73.	Mechanistic reproductive toxicity investigation on Ag NP. Dubious relevance.
27	Qin G, Tang S, Li S, Lu H, Wang Y, Zhao P, Li B, Zhang J, Peng L. Toxicological evaluation of silver nanoparticles and silver nitrate in rats following 28 days of repeated oral exposure. Environ Toxicol. 2017 Feb;32(2):609-618.	Subacute study in adult rats with TK component comparing AgNP to Ag+.
28	Sun C, Yin N, Wen R, Liu W, Jia Y, Hu L, Zhou Q, Jiang G. Silver nanoparticles induced neurotoxicity through oxidative stress in rat cerebral astrocytes is distinct from the effects of silver ions. Neurotoxicology. 2016 Jan;52: 210-21.	Mechanistic investigation comparing Ag+ and Ag NP. Claim of significant toxicity for the former. Possibly relevant to EOGRTS DNT question. ***Recommend review of paper.***
29	Tiwari R, Singh RD, Khan H, Gangopadhyay S, Mittal S, Singh V, Arjaria N, Shankar J, Roy SK, Singh D, Srivastava V. Oral subchronic exposure to silver nanoparticles causes renal damage through apoptotic impairment and necrotic cell death. Nanotoxicology. 2017 Jun;11(5):671-686.	Oral rat study on Ag NP using doses below established LOAEL region but claiming significant renal toxicity. Probably of dubious quality but abstract is insufficient to fully judge.
30	Wen R, Yang X, Hu L, Sun C, Zhou Q, Jiang G. Brain-targeted distribution and high retention of silver by chronic intranasal instillation of silver nanoparticles and ions in Sprague-Dawley rats. J Appl Toxicol. 2016 Mar;36(3):445-53.	Investigational sub-acute rat study via intranasal route comparing Ag NP to Ag+. Brain distribution claimed. Probably dubious quality/relevance but abstract insufficient to assess. ***Recommend review of paper.***

31	Wilding LA, Bassis CM, Walacavage K, Hashway S, Leroueil PR, Morishita M, Maynard AD, Philbert MA, Bergin IL. Repeated dose (28-day) administration of silver nanoparticles of varied size and coating does not significantly alter the indigenous murine gut microbiome. Nanotoxicology. 2016;10(5): 513-20.	Absence of effect of Ag NP on G.I. tract microbiota.
32	Ziemińska E, Strużyńska L. Zinc Modulates Nanosilver-Induced Toxicity in Primary Neuronal Cultures. Neurotox Res. 2016 Feb;29(2):325-43.	In vitro study.

TABLE 2b: Updated literature search on silver – Grey literature

Reference	Remarks
European Food Safety Authority (EFSA), Scientific opinion on the re-evaluation of silver (E 174) as food additive, EFSA Journal 2016;14(1):4364	Summaries and interpretation of various studies on Ag published up to 2015. Includes reproductive toxicity and repeat dose toxicity findings. ***Recommend review of paper.***
Contact details / updated mini biography of a notable contact at FDA (Dr S Khare). Notice of a new microbiome project. https://www.fda.gov/aboutfda/workingatfda/fellowshipinternshipgraduatefacultyprograms/commissionersfellowshipprogram/ucm544267.htm	Key contact/team leader at FDA/National Center for Toxicological Research (NCTR) – expertise in silver impact on G.I. tract
KEMI (Swedish Chemicals Agency), Uptake and biodistribution of nanoparticles: A review; Report 12/16	Covers all NP. Limited coverage of Ag NP.
KEMI (Swedish Chemicals Agency), Nanomaterials and genotoxicity: a literature review; Report 13/16.	Includes coverage of Ag NP, but limited relevance to current project.

Other references out of scope but noted as potentially of significant interest to PMC:

Hund-Rinke K, Baun A, Cupi D, Fernandes TF, Handy R, Kinross JH, Navas JM, Peijnenburg W, Schlich K, Shaw BJ, Scott-Fordsmand JJ. Regulatory ecotoxicity testing of nanomaterials - proposed modifications of OECD test guidelines based on laboratory experience with silver and titanium dioxide nanoparticles. Nanotoxicology. 2016 Dec;10(10):1442-1447.

Li Y, Qin T, Ingle T, Yan J, He W, Yin JJ, Chen T. Differential genotoxicity mechanisms of silver nanoparticles and silver ions. Arch Toxicol. 2017 Jan;91(1):509-519.

Weldon BA, M Faustman E, Oberdörster G, Workman T, Griffith WC, Kneuer C, Yu IJ. Occupational exposure limit for silver nanoparticles: considerations on the derivation of a general health-based value. Nanotoxicology. 2016 Sep;10(7):945-56. [NB Previously notified to PMC].

During searches of OECD databases it was noted that several new publications exist related to Ag NP testing (human health and environmental endpoints) which advance knowledge beyond the previously released Ag NP dossier from OECD's Working Party on Manufactured Nanomaterials (WPMN) programs.

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