

**PMC Ag WG project:  
Ag literature follow-up project – Appraisal of key references (Medium priority)**

**TABLE 2: Appraisal of individual publications (cont.)**

|  |   |
|--|---|
| <b>Reference id.</b>                             | <b>18</b>   |
| <b>Title</b>                                     | Toxicity of nano- and ionic silver to embryonic stem cells: a comparative toxicogenomic study.  |
| <b>Bibliographic ref.</b>                        | Gao X, Topping VD, Keltner Z, Sprando RL, Yourick JJ. J Nanobiotechnology. 2017 Apr 11; 15(1) :31. Doi: 10.1186/s12951-017-0265-6. <sup>[20]</sup>  |
| <b>Appraisal keypoints</b>                       | Cytotoxicity was examined 24h after exposure of pluripotent mouse embryonic stem cells (ESC) in vitro to citrate-coated AgNP (20 nm) and compared to ionic Ag <sup>+</sup> (AgAc) over a series of concentrations (0.1–50 µg/ml). In <u>mono-layer</u> culture, both Ag <sup>+</sup> and AgNPs caused significant concentration-dependent cytotoxicity at >1.0 µg/ml, with Ag <sup>+</sup> being relatively much more potent (leading to almost total cell death from 5.0 µg/ml upwards). Cytotoxicity in <u>embryoid body drop culture</u> was reduced; Ag <sup>+</sup> did not cause significant cytotoxicity at <2.0 µg/ml, nor did AgNP at <5.0 µg/ml, and lethality indices at greater concentrations were less than in mono-layers. Transcriptome changes were detected following a 24 h exposure of differentiating Day 3 ESC to either AgNP at 5 µg/ml or Ag <sup>+</sup> (1 or 5 µg/ml; ≡ 9 and 46 µM). At 5 µg/ml, circa 100 differentially expressed genes (upregulated or down-regulated) were identified in AgNP-treated cells, whereas 400 genes responded to Ag <sup>+</sup> treatment. Considerable overlaps were noted in the affected gene classes for AgNP and Ag <sup>+</sup> , but the effects of Ag <sup>+</sup> were more potent at equimolar concentration.   |
| <b>Klimisch (KL) Rank</b>                        | The study was investigative (a OECD TG for the assay does not exist). The model (C57BL/6-derived mouse ESC) is appropriate for in vitro study of early stage embryotoxicity, and is known to be sensitive to a variety of developmental toxicants. AgNP characterisation encompassed size, shape, PSD, zeta potential (manufacturer data), aggregation/agglomeration state, and Ag dissolution characteristic; but data on porosity, surface area and surface properties were absent. In respect of OECD recommendations, this equates to tolerably acceptable characterisation. Endotoxin contamination was controlled (an often-neglected parameter). Achieved Ag concentrations in culture and AgNP suspension stability were confirmed. Other experimental procedures including cytotoxicity assays and gene expression profiling appear robust based on the methodology and datasets given in the publication. The study main findings were considered to be biologically plausible. <b>Assigned KL Rank = 2.</b>  |
| <b>Significance / contribution to Ag dataset</b> | Cultured mouse ESCs can model early embryo developmental stages and have been used to study embryotoxicity of chemical substances in vitro <sup>21</sup> . The embryonic stem cell test (EST) has been validated by the European Committee for the Validation of Alternative Methods (ECVAM) but does not yet have an OECD TG. Due to dosimetry which is nearer to the in vivo situation, more weight should be given to the cytotoxicity results with embryoid bodies. As would be predicted, Ag <sup>+</sup> generally exhibited relatively greater cytotoxicity to ESC. This study represents an enhanced version of the EST coupled with toxicogenomics and is the first of its kind on AgNP/Ag <sup>+</sup> . Based on differential gene expression analysis, both AgNPs and Ag <sup>+</sup> impacted multiple groups of genes involved in general development, morphogenesis, embryonic development, cell differentiation, and metabolic pathways – suggesting potential for embryotoxicity. Whilst these findings are of interest, it is important to note the inherent limitations of an in vitro model for a developmental toxicity endpoint (e.g. TK and dosimetric divergence from in vivo), and that interpretation of toxicogenomic outcomes, especially adversity contexts, is challenging. However, external reviewers may conclude that this in vitro study adds support to the in vivo developmental findings on AgAc reported by Sprando et al. (2017) <sup>22</sup> . The researchers also presented partial evidence that AgNP effects were not solely due to liberated Ag <sup>+</sup> , though the data did show that gene expression differences were highly influenced by ionic Ag <sup>+</sup> concentrations. In support of their hypothesis, AgNP caused differentially higher expression of stress response proteins. |
| <b>Recommendation</b>                            | This study is significant in its findings and should feature in any PMC commentary/rebuttal point assembly covering Ag developmental toxicity.  |

<sup>20</sup> Team from Center for Food Safety and Applied Nutrition, US Food and Drug Administration, including researchers involved in the US FDA-sponsored OGRS on silver acetate.

<sup>21</sup> Tandon S and Jyoti S. Embryonic stem cells: An alternative approach to developmental toxicity testing. J Pharm Bioallied Sci. 2012 Apr; 4(2): 96-100.

<sup>22</sup> Sprando RL et al. Silver acetate exposure: Effects on reproduction and post natal development. Food Chem Toxicol. 2017 Aug;106(Pt A):547-557.