Testing proposal:
EOGRTS (TG 443) – Ag soluble compounds

Testing proposal for Reproductive toxicity endpoint (extended one-generation reproductive toxicity study; EOGRTS; TG 443). The testing proposal is based on concern identified by the registrant.

Dossier fields

Administrative Data

Study result type: Experimental study planned.

Study period: Dependent upon feedback from ECHA.

Materials and methods

Test type: Extended one-generation reproductive toxicity study (EOGRTS).

Test guideline: According to OECD Guideline 443; revision October 2012 (Extended One-Generation Reproductive Toxicity Study).

GLP compliance: Yes.

Test materials; Identity of test material same as for substance defined in section 1 (if not read-across): No.

The proposed test article is silver(1+) lactate [CAS number 128-00-7]. Silver lactate is proposed instead of silver nitrate as the latter compound is highly irritant to mammalian tissues due to the properties of the anion. Silver lactate is readily soluble in water and possesses approximately equivalent solubility to silver nitrate (CRC, 2005). It has been previously utilised for read-across purposes in the dossier, and is known to exhibit satisfactory bioavailability via the oral route.

Test animals; Species: Rat.

Sex: Male/female.

Administration / exposure; Route of administration: Oral: unspecified.

Applicant's summary and conclusion:

In relation to toxicokinetics, experimental studies have demonstrated that absorbed silver as Ag^+ readily binds to multiple extracellular proteins and is distributed to a wide range of tissues including those of the reproductive organs in both males and females (as reviewed by Drake and Hazlewood, 2005; Landsdown, 2010; ATSDR, 1990). After oral administration and a first pass effect through the liver subsequent distribution of silver is similar to that following doses of silver absorbed via other routes (ASTDR, 1990) – in rats, parentally administered silver (including radiolabelled silver nitrate) was distributed such that the highest concentrations were found, in decreasing order, in the GI tract, liver, blood, kidney, muscle, bone, and skin (Scott and Hamilton, 1950). Following intravenous injection the highest concentrations were found, in decreasing order, in the liver, pancreas, spleen, and plasma (Klaassen 1979). Hence, the reproductive organs are not commonly described as the principle target tissues for distribution and then silver deposition (argyria). The silver selenide and sliver sulphide complexes described in systemic argyria are very insoluble and inert depots which typically have not been linked to reactive local pathological change (Drake and Hazlewood, 2005; Lansdown, 2010). However, it is acknowledged that for toxicokinetics studies directly relevant to reproductive toxicity, the available dataset is fragmentary with some uncertainties, e.g. there is a paucity of reliable information as to whether Ag^+ passes transplacentally to accumulate in the fetus.

Repeated dose studies focused on general toxicology conducted in rodents on ionic silver compounds, such as silver acetate, and elemental silver (in particular silver nanoparticles), have not demonstrated significant toxic effects on the reproductive organs (Kim et al., 2008; Hadrup et al., 2012; Boudreau, 2012; Williams et al. 2014; ECHA, 2015a). These investigations, of up to 90-days duration, have typically included organ weights and histopathology assessments of the reproductive system.

One developmental study in the rat conforming to OECD TG 414 (prenatal development toxicity) has been conducted in the rat with a soluble silver compound (Price and George, 2002). Silver acetate was administered on gestational days 6-19 at doses of 0, 10, 30 and 100 mg silver acetate per kg bw/d (corresponding to 0, 6.5, 19.4 or 64.6 mg silver/kg bw/day). No differences between groups were observed in the number of implantations or in preimplantation loss rates per litter, and no toxicologically relevant differences were observed in the incidences of fetal malformations or variations. This study established a maternal LOAEL of 30 mg/kg/day, a maternal NOAEL of 10 mg/kg/day, and a developmental NOAEL of 100 mg/kg/day. Other developmental toxicity studies on silver compounds have been reported, though these are considered to be of a lower reliability ranking, and due to design limitations are not fully conformant with the above TG. Embryotoxic effects have been described in rats following dietary administration of a high dose of silver chloride (250 mg/kg bw/d which is equal to 188 mg of silver/kg bw/d) throughout the entire period of gestation (Shavlovski et al., 1995). However, this effect is considered to be due to a secondary non-specific consequence of a disruption of maternal copper homeostasis. Copper is a vital trace element in prenatal development (Keen et al., 1998), and copper deficiency is known to lead to a disturbance of normal embryonic development. In mechanistic terms, in the study conducted by Shavlovski et al., it is postulated that disturbed copper homeostasis in dams is accompanied by the formation of a silver-modified, functionally inactive ceruloplasmin lacking a copper transport function (Pribyl et al., 1989; Hirasawa et al. 1997), thereby reducing copper availability to the placenta and fetus. Though it is often referenced as a key investigation, it should be noted that the study by Shavlovski et al. suffers from a number of limitations which hinder its ability to serve as a substitute for a prenatal developmental toxicity study (OECD TG 414), including: (a) only one dose was tested and therefore no dose-response relationship can be derived – thus NOAELs for both maternal toxicity and embryo-fetal toxicity cannot be established; (b) actual dietary intake was not reported; (c) a lack of data relating to potential maternal effects, e.g. body weight change, food intake, water consumption and reporting of evident maternal toxicity including clinical signs; (d) data on litter size and individual fetal body weights were absent. In summary, whilst some developmental toxicity studies with silver compounds have demonstrated disturbances of embryonic development, it is thought that secondary non-specific mechanisms involving disruption of maternal homeostasis are involved rather than a direct effect of silver ions on embryogenesis. It is concluded that given the existence of a fully TG 414 conformant and reliable study (Price and George, 2002), there is no formal data gap in this area of reproductive toxicology.

In relation to potential effects on fertility, no two-generation reproduction toxicity study (TG 416) is known to have been conducted for any simple silver compound. A two-generation study in the rat is available for a silver zinc zeolite, Type AK (ECHA, 2015b), but the relevance of this investigation to ionic silver *per se* is unclear. In the study there were no statistically significant or clearly dose-related effects on fertility parameters. However, the percentage of abnormal sperm was higher in treated animals compared to controls (though not at the level of statistical significance). Overt toxicity was observed in the high-dose group, but the percentage of females delivering litters with stillborn pups was increased in the mid-dose group. Several adverse effects were reported in the F1 and F2 generation pups. One combined repeated dose toxicity study with reproduction / developmental toxicity screening in the rat (to OECD TG 422) exists on citrate-capped silver nanoparticles (ECHA, 2015a). Parental reproductive performance was normal and no statistically significant differences were observed in parameters including gestation period, number of corpora lutea and implantation, delivery rate, percentage of live and dead pups, preimplantation loss or post-implantation loss. However, it should be noted that this investigation did not involve a soluble silver salt, and that accompanying toxicokinetics sufficient to allow the extrapolation of its outcome more generally to ionic silver were not part of its design. Therefore for simple soluble silver compounds it is considered that a formal data gap exists in relation to reproductive performance in males or females (in respect of REACH Annex IX and X requirements).

In final conclusion, when taking into account available investigational toxicology assessments (see also ECHA, 2015a) at least partial data gaps exist in respect of the reproductive toxicity potential of ionic silver in the following areas: (a) comprehensive evaluations of the integrity and performance of the male and female reproductive systems, with explicit focus on fertility; (b) investigations of pre- and post-natal stages; (c) an in-depth assessment of systemic toxicity in both pregnant female and lactating female animals; (d) systemic and general developmental toxicity during neonatal and adolescent stages. As previously outlined, no two-generation study has previously been performed on an ionic silver form suitable for read-across purposes. Furthermore the precise relevance of an existing two-generation study on a complex silver zinc zeolite (SZZ) to that of simple silver compounds is uncertain, e.g. due to the potentially confounding influences of the zinc moiety in the SZZ, and possible interaction of the zeolite with essential metals. In recognition of these data gaps and uncertainties, it is proposed to conduct an extended one-generation reproductive toxicity study (EOGRTS) on a simple soluble silver compound known to liberate the Ag^+ ion (viz. silver lactate). With due consideration of the existing reproductive toxicology dataset in rodents, and background data including previous general toxicity assessments in the rat, the proposed test species for the EOGRTS is the rat (Wistar or Sprague Dawley strain).

There are few experimental investigations of specific immune or neurological system damage or significant dysfunction due to ionic silver compound exposure in animals, or in humans exposed to various forms of silver in occupational or clinical contexts:-

Some equivocal effects on WBC parameters have been described in humans treated with silver sulphadiazine and silver nitrate (Lansdown, 2010). Reduced thymus weights were observed in the aforementioned two-generation study on a silver zinc zeolite (ECHA, 2015b), but the applicability of this study in relation to ionic silver has not been established. Reports have recently emerged that silver nanoparticles (Ag-NP) administered to rats via the intravenous route in sub-acute studies caused alterations in thymus and spleen weights and perturbations in immune response, including reduced NK cell activity (De Jong et al., 2013) and also a suppression of T-cell dependent antibody response (Vandebriel et al., 2014). This contrasts with a previous finding (van der Zande et al., 2012) that both Ag-NP and silver nitrate were devoid of effect on humoral and cell-mediated immune parameters when administered orally to rats for 28-days (with target tissue distribution being confirmed). Therefore the relevance, if any, of the observations on the immune system effects of Ag-NP to ionic silver is currently undefined.

Very few investigations have examined the developmental neurotoxicity of silver, particularly in vivo. One report has been made of CNS damage in neonatal rodents (Rungby et al. 1987) though this study had methodological limitations such that it was not optimised to differentiate true neurodevelopmental effects. Previous critical review of the published literature has claimed that despite some claims of neurological damage in clinical and experimental studies covering adults and neonates, silver is not well absorbed into the brain and central or peripheral nervous systems (Lansdown, 2007). This assertion is supported by a toxicokinetics assessment (Shinogi and Maeizumi, 1993) in mice and rats using radiolabelled silver nitrate which determined that the brain was amongst the tissues with the lowest distributed silver concentrations. In a more recent TK study in rats using percutaneous application of a partially soluble silver compound to traumatised skin, only limited silver concentrations were detected in the brain (Pfurtscheller et al., 2014). Some other reports have set out the contrary position that silver does readily cross the blood-CNS barriers. Stoltenberg et al. (1994) administered silver nitrate to adult rats via the oral route and determined that silver penetrated the blood brain barrier and was found in glial cells and neurons in all parts of the brain. However, no evidence of CNS lesions or abnormal behaviour was observed in the treated rats. Soluble silver when parenterally administered to pregnant rats has been reported to lead to some deposition in the neurons and glial cells of the brain of progeny (Rungby and Danscher 1983a), but this investigation was not designed to examine whether there was attendant pathological change or CNS dysfunction. The same researchers reported comparable findings in adult animals dosed with soluble silver compounds (Rungby and Danscher 1983b). In another adult animal study, to assess whether silver nanoparticles influence spatial cognition and adult hippocampal neurogenesis, male ICR mice received intraperitoneal administration of Ag-NP (10, 25, and 50 mg/kg/bw) for 7 days (Liu et al., 2013). Neither reference memory nor working

memory were impaired in Ag-NP exposed groups, and hippocampal progenitor cell proliferation, cell survival and differentiation were reportedly unaffected. It is acknowledged that this particular study was not on a form of ionic silver, and that it was a sub-acute design which did not conform to an applicable OECD TG. A post-mortem investigation in humans exposed to silver from dental amalgams did detect low ppb concentrations of Ag in various brain regions (Drasch et al., 1995).

In summary, whilst some experimental investigations have demonstrated silver penetration into the CNS, actual associations with overt toxicity, including developmental neurotoxicity, have only been isolated reports. Overall a strong rationale for the inclusion of either the EOGRTS developmental immunotoxicity or developmental neurotoxicity cohorts is lacking, and it is proposed to omit these optional investigations from the EOGRTS study design.

Further remarks

It should be noted that a potential complicating factor in the interpretation of reproductive toxicity studies on silver compounds, particularly in respect of pre- and postnatal stages of exposure, relates to the biocidal activity of the silver ion. Oral exposure of rats to high doses of a soluble inorganic silver substance (silver acetate) caused significant disturbances in intestinal lumen microbiota (Williams et al. 2014), as would be expected from the microbiocidal effect of silver (Ag^+) ion. This depletion of enterobacterial populations may cause a severe gastroenteritis which would be expected to have a major impact on homeostasis. For instance, preliminary reports of a subchronic (90-day) study in rats conducted at the US National Center for Toxicological Research (NCTR) have shown that oral administration of silver acetate at doses of 200 and 400 mg/kg bw/d resulted in significant decreases in body weight gain and an increased morbidity at 400 mg/kg bw/d due to severe gastroenteritis, whereas a sparingly soluble silver form of Ag-NP did not cause similar effects (Boudreau, 2012). Any EOGRTS design will need to take due consideration of this secondary adverse effect, and its potential indirect influences on homeostasis.

Literature:

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