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# Silver acetate exposure: Effects on reproduction and post natal development☆

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ABSTRACT

Effects of oral silver acetate exposure were assessed in P generation and F generation post-natal day 26 rats. Male and female Sprague Dawley rats (n = 20 each) were exposed to silver acetate at 0.4, 4.0 or 40.0 mg/kg bw in their drinking water for 10 weeks prior to and during mating. Females were exposed to silver acetate throughout gestation and lactation. Clinical signs, body weight, feed and fluid consumption were recorded regularly. Decreased mean daily fluid consumption was observed in male and female animals during the 10 week pre mating period and during gestation in the 40 mg/kg bw dose group. Decreased fertility was observed in the 40 mg/kg bw dose group. Decreased feed consumption was observed across all dose groups and decreased mean daily fluid consumption was observed in the 4.0 mg/kg dose group during lactation. Decreased implant numbers, mean numbers of pups born/litter and numbers of live pups born/litter was observed in the 40 mg/kg bw dose group. Pup weight was reduced on lactation days 0, 4 and 7 (males) and 4, 7 and 21 (females) in the 4.0 mg/kg bw dose group and in males at lactation day 21 (40 mg/kg bw dose group). Runting was observed in males (Lactation Day; LD 4) and female (LD 4 and 7) animals in the 4.0 mg/kg bw dose group. Reduced postnatal-day 26 pup weight was observed in male pups in the 40 mg/kg bw dose group and female pups in the 4.0 mg/kg bw dose group.

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## 1. Introduction

Silver has been used for centuries for medicinal purposes as well as to prevent or retard microbial growth in both foods and beverages. In 400 B.C. Hippocrates documented its use to enhance wound healing. Historically sustained potability of milk, water or wine was attributed to these beverages being stored in silver lined vessels or submerging silver coins or silver eating utensils in the storage casks. Today silver is recognized for its antimicrobial activity and it has been formulated into nanoparticles which have been incorporated into many medicinal (Chaloupka et al., 2010; Murphy and Evans, 2012) as well as food contact items including water purification filters (Mpenyana-Monyatsi et al., 2012), toothpaste, plastics, food packaging material, storage containers, cutting boards (Martinez-Abad et al., 2012) and food supplements (silver hydrosol; Gaiser et al., 2013). As a consequence of the increasing use of silver in its many forms the potential exists for intentional or unintentional exposure to ionic silver.

Silver compounds can be absorbed orally (East et al., 1980), dermally (Snyder, 1975; Hostynek et al., 1993) and by inhalation (Phalen and Morrow, 1973; U.S. Public Health Service , 1990) and are distributed to a wide range of organs. A recently published work by van der Zande et al. (2012) reported that the main target organs for silver distribution upon oral exposure to nanosilver and ionic

*Abbreviations:* ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; bw, body weight; BUN, Blood Urea Nitrogen; A/G RATIO, albumin/globulin ration; B/C RATIO, BUN/Creatinine Ratio; F, pups first generation; P, parental generation; ISI, ionic strength adjuster; LD, lactation day; PN, postnatal.

☆ The findings and conclusions presented in this article are those of the authors and do not necessarily represent the views and opinions of the U.S. Food and Drug Administration.

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silver (silver nitrate) are the liver and spleen, followed by the testis, kidney, brain, and lungs. Silver concentrations were highly correlated to the amount of ionic silver in the silver nanoparticle suspension, indicating that mainly ionic silver, and to much lesser extent silver nanoparticles, passed the intestines in rats exposed to ionic silver and silver nanoparticles. Additionally, it was determined that silver was cleared from most organs after 8 weeks post dosing but not cleared from the brain and testis (van der Zande et al., 2012). Melnik et al. (2013) have reported that silver nanoparticles, and as a consequence ionic silver, can be transferred across the placenta to the developing fetus and through breast milk to the neonate.

The toxicity of silver is variable depending on the animal model. In rats, ionic silver is found to be moderately toxic (oral route; LD50 = 280 mg of silver/kg bw, Tamimi et al., 1998; water bottle 308 mg/kg bw, Walker, 1971); in rabbits it is found to be slightly toxic (LD50 = 800 mg of silver/kg bw/day; Tamimi et al., 1998) and practically not toxic in guinea pigs (nanosilver; LD50 = 5000 mg/kg of bw/day; Maneewattanapinyo et al., 2011). At present EPA's regulation on contaminants permit up to 0.1 mg/L of Ag+ in water (EPA, 2012) and it has been estimated that the mean daily intake from the diet including drinking water is 7.1 μg/p/d and 4.6 μg/p/d for the mean daily intake from drinking water alone (WHO, 2003). The most characterized effect from high dose silver exposure is argyria – an irreversible discoloration of the skin (Albers, 1816; Lansdown, 2006). Adverse effects induced as a consequence of silver ion and silver nanoparticle exposure have been observed in both *in vivo* and *in vitro* test systems and the available data suggest that ionic silver is more toxic than silver nanoparticles (Hunt et al., 2012; Beer et al., 2012). It has been observed that forms of silver or silver containing compounds with observed antimicrobial/cytotoxic properties are, in one way or another, sources of silver ions (Kumar and Munstedt, 2005; Lok et al., 2006, 2007; Rai et al., 2009) and the available information suggests that the toxicity of silver nanoparticles is a function of the amount of ionic silver released from these particles (Loeschner et al., 2011; van der Zande et al., 2012). Adverse reproductive effects from exposure to ionic silver administered via water bottle have previously been reported. In mice exposed to ionic silver at a concentration of 23 mg/kg bw/day (Hadek, 1966; Shavlovsky et al., 1995) effects included perturbations of ovarian mitochondria and endoplasmic reticulum. Shavlovsky et al. (1995) reported that in rats exposure to silver chloride (190 mg silver/kg bw/day) can induce embryonic copper deficiency as a consequence of silver-induced alterations in ceruloplasmin-mediated placental copper transport resulting in early (24 h) post-natal mortality. Aschengrau et al. (1993) reported, in a case controlled epidemiology study using women who delivered from 1977 to 1980 in Brigham and Women's Hospital in Massachusetts, that the frequency of ear, face and neck abnormalities increased in relation to detectable silver levels in the community drinking water. The authors did indicate that the associations were not "statistically stable" and further research was needed to corroborate the findings. Neither a decrease in fertility nor changes in sperm morphology were observed in male rats exposed (2 years) to 88.9 mg silver/kg/day as silver nitrate or silver chloride in drinking water (Olcott, 1948). However, studies are not available in which fertility was evaluated when both parents were exposed to ionic silver.

While a number of studies have assessed the effects of silver mixtures, amalgams and nanoparticles on reproduction and development, few have specifically addressed the effects of ionic silver on these same endpoints. As mentioned above, the available literature suggests that 1) upon exposure, ionic silver is distributed to all organs of the body; 2) once exposure is discontinued it is rapidly cleared from most organs except the brain and testis; and 3) exposure to silver ions can induce adverse effects in the ovary as well as in the developing fetus. The present study was conducted to assess the effects of long term ionic silver (silver acetate) exposure on reproduction and development over 1 generation. This one generation reproduction study in the Sprague-Dawley rat model was designed and conducted according to the current protocols for testing foods and food additives (FDA CFSAN Redbook, 2000). Dosage by water bottle was selected to mimic the route of human exposure.

## 2. Methods

### 2.1. Test material

The test article, silver acetate (purity = 99%; CAS – 563-63-3; KSCN %Ag – 63.7–65.5%), was obtained from Sigma-Aldrich and stored according to the conditions (dry, at room temperature) detailed in the MSDS Sheets supplied by the manufacturer.

### 2.2. Preparation and analysis of control and test dosing solutions

The target concentrations of the dosing solutions utilized in this study were 0.0 mg/kg body weight (bw), 0.4 mg/kg bw, 4.0 mg/kg bw and 40.0 mg/kg bw. These dosages were selected in order to achieve the equivalent of approximately 0, 0.25, 2.5 and 25 Ag+ mg/kg bw/day. Deionized double distilled HydroPure water (Hydro-Pico Systems, Inc., Research Triangle Park, NC, USA) was used as the control and all dosing solutions were formulated in ultra-pure water from the same source as the vehicle controls.(Hydro-Pico Systems, Inc., Research Triangle Park, NC, USA). Analysis of the Hydropure water indicated that the concentration of silver in the Hydropure water was not detectable. Dosing solution concentrations were verified using an Orion Star pH/ISE meter (Thermo) and Silver/Sulfide electrode with corresponding double-junction reference electrode (Thermo) using direct calibration method following the manufacturer's recommendation in Electrode User Guide. Briefly, the electrode was calibrated by preparing 4–5 calibration standards from a 1000 ppm AgNO3 stock solution using serial dilution. 1 mL of ionic strength adjuster (ISA) was added to each standard for every 50 mL of solution and Stable readings were taken while stirring. To verify electrode calibration, a 19.4 ppm Ag + standard was prepared from stock and analyzed in the same manner. For dosing solution analysis, (ionic strength adjuster) ISA was added to each aliquot in the same manner as the calibration standards, 3 stable measurements were taken and recorded in corresponding dosing solution analysis sheet. Since the meter only measures Ag + concentration, measured Ag + values were divided by 0.6463 (silver acetate is 64.63% Ag) to obtain final silver acetate concentration. The mean, Percent (%) RSD, and Percent Error were calculated and recorded on the same sheet. Analysis of the dosing solutions must indicate that they are within 10% of the target dose or these dosing solutions will not be utilized in the study. Stability of the test material was determined to be greater than 21 days at room temperature.

### 2.3. Test animals

#### 2.3.1. Animal husbandry

Sprague-Dawley rats [Crl:CD@ (SD) IGS BR] were obtained from Charles River Laboratories. Twenty male and twenty female rats, 4 weeks of age, were used for each dose group. Males and females weighed 119.5 ± 2.2 g and 104.8 ± 2.3 g respectively, at the start of the study. Upon receipt, all animals were identified by ear tag. The animals were acclimatized for approximately 1 week during which time they were observed daily for clinical signs of disease. All rats