Silver in Drinking-water

Background document for development of
WHO Guidelines for Drinking-water Quality

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Preface

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Abbreviations used in the text

AgNPs	Silver nanoparticles
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (French agency for food, environmental and occupational health & safety)
ATSDR	Agency for Toxic Substances and Disease Registry (USA)
bw	body weight
CAS	Chemical Abstracts Service
DNA	deoxyribonucleic acid
FDA	Food and drug administration (USA)
IARC	International Agency for Research on Cancer
i. p.	intra peritoneal
LD_{50}	median lethal dose
LOAEL	lowest-observed-effect-level
NAS	National Academy of Science
NOAEL	no-observed-adverse-effect-level
NTP	National Toxicology Program (USA)
PND	Postnatal day
POD	Point of departure
PVP	Polyvinylpyrrolidone
ROS	Reactive oxygen species
SD	Sprague Dawley
spICPMS	single particle inductively coupled plasma mass spectrometry
TEM	Transmission electron microscopy
USA	United States of America
USEPA	United States Environment Protection Agency

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1 EXECUTIVE SUMMARY

To be written after public review

2 GENERAL DESCRIPTION

2.1 Identity

Silver (Chemical Abstracts Service [CAS] no. 7440-22-4) is a transition metal that is present in silver compounds. Silver ions primarily occur in the +1 oxidation state. The silver compounds that are most relevant to drinking-water are silver nitrate ($AgNO_3$, CAS no. 7761-88-8) and silver chloride (AgCl, CAS no. 7783-90-6).

2.2 Physicochemical properties

The silver, silver nitrate, silver chloride, silver(I)oxide (Ag_2O) and silver acetate ($Ag(C_2H_3O_2)$) are summarized in Table 1.

Table 1. Physicochemical properties of silver and silver compounds

Property	Ag	$AgNO_3$	AgCl	Ag_2O	$Ag(C_2H_3O_2)$
Colour	Silver-white	White	White, darkens when exposed to light	Brown-black	White
Melting point (°C)	962	212	455	230	Not reported
Water solubility at 20 or 25 °C (g/L)	Insoluble	2 150	0.001 86	0.025	10

Sources: Holleman & Wiberg (2017), ChemID

Silver can also occur as nanoparticles (AgNP) of between 1 nm and 100 nm in size. While frequently described as being 'silver' some are composed of a large percentage of Ag_2O due to their large ratio of surface-to-bulk silver atoms. In addition, AgNPs can possess different coatings like bovine serum albumin, tubulin or ubiquitin (Durán et al., 2015). According to the literature, the coating or corona as it is also called, interacts with the cells and not the bare AgNP itself (Durán et al., 2015). Due to their surface energy nanoparticles tend to be aggregated and form larger particles with reduced surface energy (Shresta et al., 2020).

2.3 Organoleptic properties

Silver ions and AgNP have no impact on taste, colour or odour at concentrations (maximum of 100 µg/L) used in drinking water treatment systems (Butkus et al., 2004; Heidarpour et al., 2011). However, colloidal silver chloride concentrations above 150 µg/L cause opalescence in water (NAS, 1982).

2.4 Major uses and sources

Silver has the highest electrical and thermal conductivity of all metals (Hammond, 1994). It is used in alloys with copper, mercury and other metals. Since the onset of digital photography, the importance of silver salts in photography has declined substantially. Nevertheless, silver and its salts, oxides and halides are still part of our daily lives, as they are used in alkaline batteries, electrical equipment, hard alloys, mirrors, chemical catalysts, coins, table silver and jewelery. Silver has antibacterial bacteriostatic or possibly bactericidal properties against gram negative and gram-positive bacteria alike (Marin et al., 2015; WHO, 2018). Consumer products such as clothing with AgNP as an antimicrobial agent have become more popular in recent years (Carlson et al., 2008).

Because of its bacteriostatic and/or bactericidal properties, silver is used in domestic water filters to possibly reduce biofilm growth within the filter, or with some claims, as an additional level of antimicrobial treatment (Barillo & Marx, 2014). More than 100 consumer products coated with metallic silver and intended for water treatment are commercially available (De Gusseme et al., 2010). AgNP are currently being tested in some experimental point-of-use treatment systems and are contained in consumer products such as ceramic filters. Another popular filter system, comprised of cartridges with silver-spiked activated carbon for use in table-top filters, releases silver into the drinking-water at concentrations less than 25 to 50 µg/L (Garbos & Swiecicka, 2013; WHO, 2018). These applications are evaluated in section 7.4.

3 ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Water

In surface water and groundwater, silver concentrations are usually below 2 µg/L (ATSDR, 1990). Average silver concentrations in these natural waters have been reported at 0.2–0.3 µg/L (USEPA, 1980). In the 1988 US National Inorganics and Radionuclides Survey, 982 of 989 (99.3%) randomly selected groundwaters had concentations less than 4 µg/L and 4 of 989 (0.4%) had concentrations between 18 and 20 µg/L. In river water, silver ions form complexes with chloride and humic matter (Whitlow & Rice, 1985). AgNP can be mobile and enter ground and drinking-water supplies when released to the environment via wastewater or industrial discharges. The final thermodynamic sink for AgNPs is believed to be insoluble AgS (Schaumann et al., 2014).

Silver concentrations in drinking-water in the USA that were not treated with silver for disinfection purposes varied between "non-detectable" and 5 µg/L (USEPA, 1980). In a survey of Canadian tap water, only 0.1% of the samples contained Silver at concentrations above 1–5 µg/L (Neri et al., 1974).

A more recent report on "Naturally Occurring Groundwater Contamination in Texas" prepared for the Texas Water Development Board detected silver in 73 of 5420 groundwater samples (1.3%) with only one detection (112 µg/L) exceeding the USEPA secondary (aesthetics-based) standard of 100 µg/L (Reedy et al., 2011). Maximum values in the other aquifers ranged from not detected to 67 µg/L with a median value of 1.1 µg/L. The French agency for food, environmental and occupational health & safety (ANSES) estimated the mean silver concentration in drinking-water to be between 8 and 49 µg/L (ANSES, 2011).

3.2 Food

It has been known for decades that food contains trace amounts of silver (Kent & McCance, 1941; Murthy & Rhea, 1968). The Second French Total Diet Study found the highest concentrations of silver in crustaceans and offal, with mean concentrations of 6.48 mg/kg and 0.45 mg/kg, respectively (ANSES, 2011). In most other food, the silver concentration was below 0.1 mg/kg. The results of this total diet study are, largely, in accordance with a study by Gibson & Scythes (1984), who found that most foods contain traces of silver in the 10–100 µg/kg range.

3.3 Air

According to the Agency for Toxic Substances and Disease Registry of the United States of America (USA), naturally occurring concentrations of silver in ambient air are in the nanogram per cubic metre range (ATSDR, 1990).

Occupational exposure is the primary source for the inhalation of silver dusts or fumes by humans (Drake & Hazelwood, 2005). In comparison to the inhalation of silver as aerosols in occupational

settings, the amount of silver possibly inhaled during bathing or showering would be negligible.

3.4 Estimated total exposure and relative contribution of drinking-water

Most silver and its salts are naturally-occurring, and the trace element content of food is influenced by geographic origin, soil type, fertilizers and processing methods (Gibson & Scythes, 1984). In France, the mean dietary exposure to silver was estimated to be between 1.29 and 2.65 µg/kg bw in adults and between 1.60 and 3.47 µg/kg bw in children (ANSES, 2011). For an adult with a body weight of 60 kg, this would result in an overall exposure from food of between 77 and 160 µg/person per day. Estimates of daily intake of silver vary widely from about 0.4 µg/person in Italy and 10–44 µg/person in the United Kingdom (Warrington, 1996). The disparity in these exposure estimates may be related to differences in exposure assessment methods as well as the intake sources considered.

The contribution of drinking-water to overall silver exposure varies considerably. In Canada, it is estimated that about 1% of silver exposure is from drinking-water (Warrington, 1996), whereas estimates from France range from 8% to 20% (ANSES, 2011). Where silver salts are used as bacteriostatic agents in water treatment (up to 100 µg/L), the daily intake of silver from drinking-water probably constitutes the major source of exposure.

4 TOXICOKINETICS AND METABOLISM IN ANIMALS AND HUMANS

Silver can be found either in its ionic or (nano)particulate form. In addition, AgNP also release silver ions (Kittler et al., 2011). It is therefore difficult to distinguish whether an effect seen after exposure to AgNP is caused by the particles or the ions released.

4.1 Absorption

Silver can be absorbed via the gastrointestinal tract, lungs, mucous membranes and skin lesions (Loeschner et al., 2011; Munger et al., 2014; Pelkonen et al., 2003; USEPA, 1980; van der Zande et al., 2012). The absorption of colloidal silver after oral exposures can be as high as 5% (USEPA, 1980). The absorption from the GI tract of ionic silver, as $AgNO_3$ or silver acetate, is higher than that from AgNPs (Loeschner et al., 2011; Van der Zande et al., 2012). Data on fecal elimination in rats exposed to approximately 9 mg/kg-day AgAc or polyvinylpyrrolidine coated AgNP (Loeschner et al., 2011) suggests that silver acetate has a higher bioavailability than AgNP, as 63% of the administered dose of AgNP was eliminated in the faeces over a 24-hour period vs. only 49% of the administered dose of silver acetate. Another study in rats showed that the bioavailability of colloidal AgNP in a protein matrix from water is between 1 and 4 % when animals were treated with 1 and 10 mg/kg bw, respectively (Park et al., 2011). Water hardness may reduce the bioavailability of AgNPs by increasing their aggregation and by competition with the physiological transport mechanism, an effect that may vary with the particle coating (Stoiber et al., 2015). A case study by East et al. (1980) reported a silver retention rate of 18% after oral ingestion of lozenges containing silver acetate as an anti-smoking remedy.

4.2 Distribution

Most of the silver ion transported in blood is bound to globulins (USEPA, 1980). In tissues, it is present in the cytosolic fraction, bound to metallothionein (Nordberg & Gerhardsson, 1988). Silver is stored mainly in liver and skin with smaller amounts in other organs (Furchner, Richmond & Drake, 1968; USEPA, 1980). Silver is also deposited in the skin epidermis, renal glomeruli and intestine in nanosized particles, regardless of whether exposure occurred as ions or AgNP (Hadrup & Lam, 2014).

Silver crosses the blood–brain barrier in rats after exposure in drinking-water (Pelkonen & Hanninen

2003; Van der Zande et al. 2012). Deposition of AgNPs of different sizes (22, 42 and 71 nm) was observed in brain, lung, liver, kidney and testis after 14 days of oral administration of 1 mg/kg bw per day in mice (Park et al., 2010). Larger particles (323 nm) were not found in those tissues.

Studies on the ability of AgNPs to cross the blood-placenta boundary give equivocal results. Ema et al. (2017) reviewed studies that found AgNPs in foetal tissues of mice and rats treated with 0.2 to 1000 mg/kg bw. Other studies using orally applicated AgNPs with a size of 8.8 nm did not induce reproductive, developmental, or repeated dose toxicity at 62.5–250 mg/kg/day. (Ema et al., 2017). Reasons for those conflicting results might relate to the different time points of application during pregnancy, different intervals between application and necropsy or size differences of the AgNP used.

Silver was identified in human brain tissues at concentrations of up to 5 µg/kg wet weight (Drasch et al., 1995) with the level of silver in brain tissue shown to be correlated with the number of dental amalgam fillings in human subjects (Drasch et al., 1995; Skare & Enqvist, 1994). Lyon et al. (2002) attributed the origin of significant amounts of silver in the livers of children 6 years of age or younger to maternal dental amalgams because of exposures during pregnancy and lactation.

A US FDA study evaluated tissue accumulation and distribution of silver in SD rats exposed by oral gavage to AgNP or ionic silver for 13 weeks (Boudreau et al., 2016). Treatment groups included those receiving citrate-coated AgNP (10, 75, or 110 nm) at 9, 18, or 36 mg/kg bw per day; silver acetate (AgAc) at 100, 200, or 400 mg/kg bw per day; and controls (2 mM sodium citrate or water). Significant dose-dependent and AgNP size-dependent accumulations were detected in tissues. Sex differences in silver accumulations were noted for many tissues and organs, with accumulations being significantly higher in female rats, especially in the kidney, liver, jejunum, and colon.

After gavage of AgNP (70 nm diameter) in rats at 1 and 2 mg/kg bw per day for 30 days, damages were seen in the tissues of liver, kidneys and spleen (Sardari et al., 2012). Since no AgNP were seen in these tissues, the authors concluded that silver ions were interfering with the intercellular redox balance.

In mice exposed to 0.03 mg/L as silver nitrate in drinking water for 1 or 2 weeks, silver was found in brain, muscle, spleen and other tissues at concentrations between 1 and 29 µg/kg wet weight (Pelkonen et al., 2003). The dose was adjusted for silver ion concentration (5.7 µg/kg bw/day) with an assumption of a 0.03 kg bw and 0.0057 L/day intake. The 0.03 mg/L concentration used in this study is well below the maximum concentration of 0.1 mg/L silver allowed in many countries.

Rats were exposed to ionic silver at 9 mg/kw bw per day or AgNP at 90 mg/kg bw per day (PVP-coated or uncoated with a diameter of 15 or 20 nm, respectively) for 28 days via gavage (Van der Zande et al., 2012). Samples of liver, spleen, testis, kidney, brain, lungs, blood, bladder and heart, plus the wall of stomach, small and large intestine were evaluated for ionic silver and AgNP content either on day 29 or after a wash out period of 1 or 8 weeks. When these large doses were adjusted for concentration, the total amount of ionic silver found in different tissues was always higher than the amount of AgNPs. After 8 weeks, brain and testis were the only organs from which silver was not washed out.

4.3 Metabolism

After uptake, metallic silver is converted into its ionic form by moisture and body fluids such as saliva and stomach fluid. Silver ions are biologically active and bind to sulphydryl groups and other anionic ligands present in proteins and other cell constituents such as glutathione and cysteine (Hadrup & Lam, 2014).

4.4 Elimination

The liver plays a key role in silver excretion; most absorbed silver is excreted with the bile in the faeces. The biological half-life of silver in humans (liver) ranges from several to 50 days (Nordberg & Gerhardsson, 1988). Silver that is not eliminated is ultimately oxidized to silver sulphide. This compound is responsible for the grey-bluish discoloration of the skin of humans, referred to as argyria (Drake & Hazelwood, 2005). In mice, rats, monkeys and dogs, cumulative silver excretion was in the range 90–99% of the intake. Silver retention was about 10% in the dog, <5% in the monkey and <1% in rodents (Furchner et al., 1968). In humans, under conditions where silver exposure occurs daily, retention rates between 0% and 10% have been observed (USEPA, 1980).

Two studies performed by Loeschner et al. (2011) and Van der Zande et al. (2012) showed that most silver (between 50 and 99 %) is eliminated via the faeces, both for ionic silver and AgNP. Since a higher percentage of AgNP are found in the faeces compared to ionic silver, their bioavailability seems to be lower.

Both ionic silver and AgNPs are cleared from the blood stream in less than one week (Van der Zande et al., 2012).

5 EFFECTS ON HUMANS

5.1 Acute exposure

The estimated acute lethal dose of silver nitrate in humans is at least 10 g (Hill & Pillsbury, 1939). More recent data were not identified.

Irritation of the upper and lower respiratory tract observed after inhalation of silver nitrate is probably attributable to nitrate rather than the silver itself (Drake & Hazelwood, 2005). There is one case report of severe but reversible respiratory problems due to occupational exposure associated with processing of molten silver (ATSDR, 1990).

5.2 Short-term exposure

In a study by Munger et al. (2014), 60 healthy subjects of both sexes aged 18 – 80 years were treated with one single daily dose of elemental AgNP coated with silver oxide for 3, 7 or 14 days. The hydrodynamic diameter of the particle was 59.8 ± 20 nm. The applied dose was either 2.5 µg/kg bw or 7.9 µg/kg bw based on a 60 kg adult. No clinically relevant changes in body weight, blood pressure, metabolic markers or cellular composition of blood were observed in any group.

5.3 Long-term exposure

The only known clinical picture of chronic silver intoxication is that for argyria, a condition where silver is deposited on skin and hair and in various organs following occupational or iatrogenic exposure to metallic silver and its compounds. Pigmentation of the eye is considered the first sign of generalized argyria (Hill & Pillsbury, 1939). Striking discoloration, which occurs particularly in areas of the skin exposed to light, is attributed to the photochemical reduction of silver in the accumulated silver compounds, mainly silver sulphide. Melanin production has also been stimulated in some cases (East et al., 1980; Westhofen & Schäfer, 1986). As there is no effective treatment for argyria, the effect is permanent, even if the uptake of silver is discontinued (Drake & Hazelwood, 2005).

It is difficult to determine the lowest dose that may lead to the development of argyria. A patient who developed grey pigmentation on the face and neck after taking an unknown number of anti-smoking pills containing silver ethanoate had a total body silver content of 6.4 ± 2 g (East et al., 1980). Localized argyria (i.e. discoloration of the nail bed) was found in a patient who ingested 1.5 g of silver, and generalized argyria can be induced by total amounts of silver as low as 3 g (Kim et al., 2009). Intravenous administration of 4.1 g of silver arsphenamine (about 0.6 g of silver) can lead to argyria

(Gaul & Staud, 1935). Other investigators concluded that the lowest intravenous dose of silver arsphenamine causing argyria in syphilis patients was 6.3 g (about 0.9 g of silver) (Hill & Pillsbury, 1939). It should be noted that syphilis patients suffering from argyria were already in a compromised state of health and were treated with bismuth, mercury or arsphenamine in addition to silver.

Kim et al. (2009) presented a case report of a woman, with diffuse blue-gray discoloration of the skin, who ingested about 34 mg colloidal silver per day for approximately 16 months (0.6 mg/kg assuming 60 kg bw). Her serum copper level was about one third of the lower normal range, her ceruloplasmin level about 50% of the lower normal range suggesting that silver may alter copper metabolism by decreasing serum copper concentrations and ceruloplasmin oxidase activity.

Inhalation exposure to silver is usually occupational. Workers exposed to silver nitrate and/or silver oxide at concentrations of 0.039 to 0.378 mg silver/m³ for less than one to more than 10 years developed irritation of the upper and lower respiratory tract and occasional gastric discomfort without effects on the cardiovascular system or blood counts (ATSDR, 1990).

5.4 Neurological effects

The literature research did not identify any studies on potential neurotoxic effects of silver in humans.

5.5 Reproductive and developmental effects

The literature research did not identify any studies on potential reproductive or developmental effects of silver in humans.

5.6 Immunological effects

Hypersensitivity to silver-containing compounds was reported in individuals previously sensitized by working as silver miners, jewellers or photographers (Sterling, 2014). Hypersensitivity to silver sulfadiazine was reported however, it is not clear whether the hypersensitivity was caused by silver or sulfadiazine (Sterling, 2014).

5.7 Genotoxicity and carcinogenicity

The United States Environmental Protection Agency states that "No evidence of cancer in humans has been reported despite frequent therapeutic use of the compound over the years" (USEPA, 2014). No monograph on silver is available from the International Agency for Research on Cancer (IARC, 2018). In addition, silver is not listed in NTP's 14th Report on Carcinogens (NTP, 2016).

Significantly increased DNA damage in peripheral leucocytes was reported in Turkish jewelry workers exposed to airborne silver particles of unspecified size for at least four hours per day for an unknown duration (Aktepe et al., 2015). The authors proposed that direct interaction of (nano-)particles with DNA and excessive reactive oxygen species (ROS) were responsible for the damage; however, no data or further details were included. Due to the limited number of participants (35 and 41 for exposure and control groups, respectively), confounding factors such as cigarette smoking, co-exposure to other genotoxic metals, and unknown exposure concentrations, the reported results should be interpreted with caution (WHO, 2018).

Epidemiological studies investigating other health effects including the carcinogenicity of silver were not identified.

6 EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS[1]

6.1 Acute exposure

Median lethal doses (LD_{50} values) of 50 mg/kg bw for $AgNO_3$ and silver dinaphthylmethane disulphonate and 100 mg/kg bw for colloidal silver were determined in mice (Goldberg et al., 1950). A more recent study by Maneewattanapinyo et al. (2011) found no acute effects in mice after oral administration of 5,000 mg/kg bw colloidal AgNP. One reason for the different LD_{50} values might be the higher bioavailability of ionic silver compared to AgNP as shown in the paper by Van der Zande et al. (2012).

6.2 Short-term and subchronic exposure

Death was observed in rats following ingestion of 1680 mg/kg bw colloidal silver after oral dosing for 4 days (Dequidt et al., 1974). A drinking-water concentration of 2.6 g/L (364 mg/kg assuming 0.35 kg bw and 0.049 L water intake) silver was reported to be fatal for rats (Warrington, 1996); however, no further details regarding the chemical form(s) of silver were available.

Rats receiving silver nitrate daily for 2 weeks in their drinking-water survived at 181 mg/kg bw per day, but 3 of 12 died at 362 mg/kg bw per day (Walker, 1971).

Ten SD rats per sex and dose received AgNP (52.7-70.9 nm, average 60 nm; ≥99.98% purity in 0.5% carboxymethylcellulose (unspecified whether coated or not) via gavage at 0, 30, 300, or 1,000 mg/kg-day for 28 days (Kim et al., 2008). At 300 mg/kg-day, mean absolute liver weight was increased ($p<0.05$) in female rats by an unspecified magnitude. At 1,000 mg/kg-day, mean absolute brain weight was increased ($p<0.05$) in male rats by an unspecified magnitude. The study authors also reported increased incidence of bile duct hyperplasia in AgNP-exposed rats, but the dose level(s) associated with this effect were not specified.

6.3 Long-term exposure

No standardized chronic studies for silver or AgNP were identified. Increased pigmentation of different organs, including the eye, was observed in Osborne-Mendel rats after a lifetime exposure to silver at approximately 60 mg/kg bw per day as silver nitrate or silver chloride from their drinking-water (Olcott, 1947). After 218 days of exposure (~31 weeks), albino rats receiving a silver dose of approximately 60 mg/kg bw per day from their drinking-water containing either silver nitrate or silver chloride as salts exhibited a slight greyish pigmentation of the eyes, which later intensified (Olcott, 1950).

In a 90-day gavage study, F344 rats were exposed to 0, 30, 125 or 500 mg/kg bw AgNPs with a median diameter of 56 nm (Kim et al., 2010). Increased incidence of bile duct hyperplasia of minimal severity as well as increased incidence of liver necrosis of minimal severity were observed in both sexes at all doses; no other histopathological effects deemed adverse by the study authors were reported. A dose-dependent increase in silver deposition was observed in the brain, liver, kidneys, lungs and blood, with a higher deposition in the kidneys of female rats. Based on the results of the study, the authors proposed a NOAEL and LOAEL of 30 and 125 mg/kg-day respectively, likely based on significantly reduced mean body weights and significantly increased mean serum cholesterol and bilirubin levels in the mid- and high-dose groups.

An OECD 408-compliant US FDA study evaluated nano-particulate and ionic forms of silver and

[1] Additional information on the effects of silver nanoparticles and ionic silver in experimental animals and humans may be found in WHO (2018); the information provided here on AgNP and ionic silver has been summarized from that report with the inclusion of additional studies.

particle size for differences in silver accumulation, distribution, morphology, and toxicity after daily oral gavage to 10 SD rats per sex and dose for 13 weeks (Boudreau et al., 2016). Treatment groups included citrate-coated AgNP (10, 75, and 110 nm) at 9, 18, or 36 mg/kg bw per day; silver acetate (AgAc) at 100, 200, or 400 mg/kg bw per day; and controls (2 mM sodium citrate or water). Terminal necropsy, histopathology, hematology, and serum chemistry were performed. Rats exposed to AgNP did not show significant changes in body weights or intakes of feed and water relative to controls. Blood levels were similar to controls. Histopathology of the following organs was performed after exposing the animals to AgNP (36 mg/kg bw/day) or AgAc (64.6 mg silver/kg bw/day): jejunum, ileum, colon, kidney, liver, and spleen. The authors considered the pigmentation to be more a measure of silver mobility rather than toxicity, because the pigmentation did not induce lesions that were visible by light microscopy. Therefore, Boudreau et al. did not identify a NOAEL for AgAc. However, it is recognized that argyria in humans is permanent even after exposure is discontinued because no treatment is available. Thus, the lowest dose tested of AgAc (100 mg/kg) could be classified as a LOAEL for argyria. For AgNP, 18 mg/kg/day is the NOAEL for pigment deposition with 36 mg/kg bw per day as the LOAEL, recognizing that no histopathological lesions were seen at this dose.

If the pigmentation is considered as nonadverse the dose of 200 mg/kg bw per day would be the NOAEL for AgAC while 400 mg/kg/day would be the LOAEL for AgAC based on the increased incidence and severities of histopathological lesions at the highest dose. Histopathological lesions included mucosal hyperplasia in the small and large intestine and thymic atrophy or necrosis. The authors considered the thymic response to be stress-related because of the observed gastrointestinal disturbances.

Weanling rats treated with drinking-water containing 1.6 g/L of silver given as silver nitrate for 37 weeks showed reduced weight gain compared with rats treated with the same water for 10 weeks and allowed 27 weeks of recovery. Some of the rats treated with silver-containing drinking-water started losing weight rapidly after 23 weeks and eventually died (Matuk et al., 1981). Assuming 0.011 L/day water intake and 0.05 kg bw for weanling rats, the rats received approximately 0.35 mg/kg bw per day

6.4 Neurological effects

Hypoactivity was observed in mice receiving a silver dose of 4.5 mg/kg bw per day provided as silver nitrate via drinking-water at 95 mg/L silver) for 125 days (Rungby & Danscher, 1984). A more in-depth review of possible neurological effects can be obtained from the WHO document "Silver as a drinking-water disinfectant" (WHO, 2018).

6.5 Reproductive and developmental effects

In a developmental toxicity study, Price and George (2002) administered gavage doses of 10, 30 or 100 mg silver acetate/kg bw per day to pregnant female SD rats during GD 6 to 19. The test substance used in the study contained approximately 65% silver by weight. In the maternal animals, the study authors reported a statistically significant trend ($p<0.05$) of reduced body weight on GD 12; however, the group pairwise comparisons to the control mean were not statistically significant. The study authors also reported increased frequency of rooting and piloerection in the mid- and high-dose dams. There were no treatment-related developmental effects reported in the offspring. Based on the results of the study, the authors identified a maternal NOAEL of 10 mg silver acetate/kg bw per day (6.5 mg silver/kg bw per day) and a developmental NOAEL of 100 mg/silver acetate/kg bw per day (65 mg silver/kg bw per day).

No loss of fertility was reported in either sex exposed to ionic silver in drinking-water (as silver nitrate or as silver chloride) at a dose of 89 mg/kg bw per day (Olcott, 1948; as summarized by ATSDR, 1990). Ionic silver was not deposited in the testes, and the spermatozoa appeared normal (Olcott, 1948).

Boudreau et al. (2016) tested ionic or AgNP for differences in sperm motility, testis sperm count, caudal sperm count, or sperm morphology in exposed male SD rats and differences in the estrous cycle in exposed female SD rats. Animals were 7 weeks old at the beginning of the treatment. No significant effects were found in any group given either citrate-coated AgNP (10, 75, and 110 nm diameter) at 9, 18, or 36 mg/kg bw per day or silver acetate (AgAc) at 100, 200, or 400 mg/kg bw per day for 13 weeks (Boudreau et al., 2016).

0, 15 or 50 µg/kg bw citrate-coated AgNP (60 nm diameter) were administered to male Wistar rats during the prepubertal period for 30 days starting at PND 23 by oral gavage. When examined as adults, impaired spermatogenesis, and reduced sperm count were observed at 15 µg/kg bw (Sleiman et al., 2013). This concentration seems to be extremely low compared to other AgNP studies, but since the publication provides no further information on the preparation of the suspensions or the experimental procedures, a re-calculation of the concentrations and confirmation of the administered dose is not possible. Also, the fact that treatments started at different points in development doesn't seem to account for the disparity in the observations.

In adult male rabbits, intravenous exposure to AgNP at 0.6 mg/kg bw decreased sperm velocity and mobility with AgNPs visible in the acrosome and the mitochondria of the sperm cells (Castellini et al., 2014).

In a 90-day study by Thakur et al. (2014) citrate-coated AgNPs with a diameter of 5- 20 nm were administered by oral gavage at 0 or 20 µg/kg bw per day to eight male rats. Structural damages observed through TEM included depletion of germ cells and germinal cells necrosis. Again, this dose is extremely low with no information to confirm the concentration of AgNP in the administered suspension.

The very limited available data on potential reproductive and developmental toxicity of AgNPs in rabbits, mice or rats has been critically reviewed by Ema et al. (2017). The authors concluded that further studies using state-of-the-art methodologies and relevant routes and doses for human exposure are required to substantiate the finding of (Thakur et al (2014).

6.6 Immunological effects

In a 28-day study, Park et al. (2010) tested for immunological effects of uncoated AgNPs with a diameter of 42 nm after oral gavage in mice at 0, 0.25, 0.5 and 1 mg/kg bw per day. Deposition in liver and kidney and an increase in cytokines (IL-1, IL-4, IL-6, IL-10, IL-12 and TGF-ß) were observed at 1 mg/kg bw per day. The NOAEL can be considered 0.5 mg/kg bw per day.

Increases in oxidative stress and cellular Zn^{2+} and decreases in nitric oxide, an immune effector, were reported in cultured mouse monocyte cells (RAW 264.7) exposed to polyester stabilized AgNP with 5 different mean sizes ranging from 2.0 to 34.7 nm or ionic silver (Haase et al., 2014). Silver concentration were 10 µM in all settings. The functional relevance of the responses observed in the cultured cells is unclear.

6.7 In vivo genotoxicity and carcinogenicity

In male and female SD rats, oral administration of AgNP up to 1000 mg/kg bw per day for 28 days (Kim et al., 2008), or up to 36 mg/kg bw per day AgNP for 90 days (Boudreau et al., 2016), did not induce statistically significant increases in micronucleus formation. The reason for the large disparity in the tested doses of AgNP between these two studies is unclear; however, Boudreau et al. (2016) conducted stability studies and determined that the tested dose was the maximum dose level that could be achieved with the sodium citrate stabilizing agent, whereas Kim et al. (2008) included limited discussion of AgNP characterization and stability.

A small but statistically significant increase in the frequency of micronucleated reticulocytes in peripheral blood sampled at week 4 was reported in both male and female rats given 400 mg/kg bw per day silver acetate (Boudreau et al., 2016); however, this increase was not observed at subsequent time points and is therefore of doubtful significance. Overall the weight of evidence indicates that silver is not a significant genotoxic concern.

No oral carcinogenicity studies for silver were identified. Fibrosarcomas have been induced in rats following subcutaneous imbedding of silver foil (Oppenheimer et al., 1956) while positive (Schmahl and Steinoff, 1960) and negative (Furst & Schlauder, 1978) results for tumorigenesis was reported in rats following subcutaenous and intramuscular injection, respectively of colloidal silver. Most of the tumours (6/7) were found at the injection side. However, these studies represent a very different exposure scenario compared to ingestion of drinking-water and such studies are not considered to be relevant in assessing hazards due to systemic exposure, such as from drinking-water and so the data are of very limited value.

6.8 In vitro genotoxicity

No mutagenic effects were reported in several in vitro bacteria reverse mutation assays after applying silver chloride or silver particles of different sizes, including the nano-size range (Nishioka, 1975, Asakura et al., 2009, Li et al. 2012). In an in vitro micronucleus study conducted in the Chinese hamster ovary cell line (CHO-1K) (Jiang et al., 2013), there was a concentration-dependent increase in the frequency of DNA adduct and micronucleus formation in cells treated with ionic silver and AgNP. However, no mechanism for this was proposed.

6.9 Other in vitro studies

There has been a recent increase in studies scrutinizing the toxicology of silver ions and AgNP. This is probably due to an ever-expanding market of applications for AgNPs. A wide variety of cells from different tissues have been tested, i.e. brain, blood, bone, liver, kidney, lung, cervix and testes (WHO, 2018). The cells were derived from humans, rats, mice hamsters or pigs. Besides being of non-human origin, many cells were secondary cells (i.e. cancer-derived or immortalised cell lines) reducing relevance to human exposures even further. In primary cells, exposure to silver (ionic or AgNP) resulted in oxidative stress. Silver was cytotoxic in lung macrophages and fibroblasts plus brain cells. A cytotoxic effect on human blood mononuclear cells was observed at concentrations as low as 1 µg/mL (WHO, 2018).

The relevance of these in vitro findings to exposure of humans from drinking-water is unknown and questionable because cell lines have different properties compared to whole organisms. First, since cell lines are directly exposed to the test compound, in vitro studies do not account for limited absorption of the substance in the whole organism. Secondly, since silver ions bind to serum globulins and metallothionein, in vivo exposure of cells in humans will be very different.

6.10 Mode of action

Since there are limited data on silver toxicity, it is difficult to define a mode of action. As discussed by Aktepe et al. (2015) the formation of ROS after inhalation of silver particles by jewellery workers is a possible explanation for silver toxicity. However, the relevance of this mode of action to exposure through drinking-water is unclear.

The formation of ROS was observed after exposure of cells from different tissues to silver, e.g. lung, liver, kidney, blood, skin and brain, both of human and animal origin in *in vitro* systems (WHO, 2018). Changes in the delicate redox balance of cells are often regarded as precursor events for cytotoxicity

and genotoxicity. However, translation from in vitro to in vivo exposures is difficult for the reasons described in section 6.9, Hadrup and Lam (2014) also noted that silver might be deposited as nano-sized granules, which results in mechanical disruption of anatomical structures. This property was described for both colloidal silver and AgNP after oral uptake (Hadrup & Lam, 2014).

7 OVERALL DATABASE AND QUALITY OF EVIDENCE

7.1 Summary of Health Effects

Silver is deposited in various organs (e.g. skin, kidney, liver) after oral ingestion in its ionic form and as AgNP. Silver that remains in the body is ultimately oxidized to insoluble Ag_2S, which is responsible for skin darkening characteristic for humans suffering from argyria. Respiratory problems induced by inhalation are not relevant for drinking-water and tend to result from occupational rather than general population exposure. Lansdown (2010) reviewed the safety of silver ions used in medical applications, including wound dressings and treating burns and concluded that health risks associated with systemic absorption of silver as ions are low.

7.2 Quality of Evidence

The limited studies on potential neurotoxicity and carcinogenicity of silver is a clear deficiency in the database, although there are no significant indications that these are likely to be an issue, particularly at low intakes in view of the propensity of silver ions to bind to sulphide groups and to form insoluble silver halides. Silver has been used by humans in various ways, including ways resulting in ingestion since about 3000 B.C., thus it seems reasonable to assume that severe effects from exposure would be historically evident (Holleman & Wiberg, 2017). However, the use of silver as nanoparticles in water treatment and other applications is recent. The age of AgNP studies varies considerably, spanning a time period of years not centuries while the reliability of the data from some AgNP studies is uncertain.

8 PRACTICAL CONSIDERATIONS

8.1 Monitoring

Routine monitoring of silver concentrations in drinking-water is not presently recommended. The primary source of silver in drinking-water is from the use of silver in point of use water treatment devices intended for disinfection purposes rather than as a disinfectant in water treatment plants. This application is not recommended, because its usefulness is not proven. When detected in drinking-water, the concentrations are usually in the low or sub µg/L range. When silver is added to these point of use devices, they should ideally be tested and certified to not allow silver concentrations in water to exceed 0.1 µg/l (see section 8.1) during their useful life. See section 7.4 for more information on use of silver as a drinking-water disinfectant.

Where silver/copper ions are used to control Legionella in the distribution system of buildings the dosing system should be calibrated to determine the amounts of silver and copper being released. Regular maintenance and occasional monitoring is necessary to ensure that the concentrations remain sufficient to exert control but not excessively high, particularly if cold water drinking-water systems are being treated. Frequently only the hot water side of the distribution system is treated for legionella management because -*Legionella* proliferate in warmer water, so monitoring copper or silver concentrations is less important in those cases, other than to ensure sufficient concentrations for efficacy.

8.2 Analytical methods

The limit of detection for silver using a spectrographic and colorimetric method with dithizone is 10

µg/L for a 20 mL sample. The limit of detection for silver using graphite furnace atomic absorption spectroscopy is 2 µg/L, and using neutron activation analysis, 2 ng/L (Fowler & Nordberg, 1986). For inductively coupled plasma mass spectroscopy, the Ag detection limit is 5 ng/L (USEPA, 2007). A new technique called asymmetric flow field-flow-fractionation (AF4) in combination with single particle inductively coupled plasma mass spectrometry (spICPMS) can differentiate between AgNPs and dissolved silver (Huynh et al., 2016). This is not yet a standard procedure (Huynh, et al., 2016). For AgNPs a detection limit of 1-5 ng/L is descripted by Hetzer et al. for spICPMS (2017). However, ionic silver can be released from AgNP (Kittler et al., 2011). Therefore, it may be difficult to determine whether silver dispersed in water is originating from the ionic or the particulate fraction.

8.3 Treatment methods and performance

Ionic silver is readily removed during water treatment by conventional coagulation and lime-softening techniques (USEPA, 1977). Ionic silver will precipitate and form complexes of low solubility, in the presence of halides such as chloride (Sousa & Teixeira, 2015), which is common in many water treatment environments. Alum and ferric sulfate coagulation achieve removal rates of approx. 80 percent in the pH range 6 to 8. Because of poor alum floc formation under alkaline conditions, this method is less effective above pH 8. Lime softening removes from 75 to 90 percent silver in the pH range 9 to 11.5 (USEPA, 1977).

A recently published paper by Salih et al. (2019) showed that conventional treatment could also be sufficient for the removal of coated AgNPs.

8.4 Efficacy as a disinfectant

Silver is used in some drinking-water treatment devices in both granular and powdered activated carbon filters and in domestic ceramic water filters. Although silver is widely used to reduce microbial growth on filter media, its efficacy as a disinfectant is doubtful. Drawing a conclusion about the efficacy of silver as a disinfectant is difficult, because researchers used many different approaches and a variety of devices. The type of silver tested and doses and times varied from ionic silver to AgNP which were either pure or capped with some secondary material. A comprehensive review of the literature concluded that in its current applications, silver is not an effective drinking-water disinfectant (WHO, 2018). One reason for this conclusion is that a limited number of different microorganisms has been evaluated with mixed results. It has generally been only been found to be effective against bacteria, particularly *Escherichia coli*) with long contact times relative to standard primary disinfectants like chlorine (WHO, 2018).

In most studies, it was not clear whether silver was bactericidal or merely bacteriostatic. The evidence is particularly limited for inactivation of protozoa and viruses, although some additional studies have been identified since the publication of the WHO, 2018 report. These more recent studies show limited inactivation of protozoa and viruses at long contact times. Thus, the overall weight of the evidence indicates that it is not an effective drinking-water disinfectant. It should be noted that two silver containing products have failed the WHO evaluation scheme for household water treatment products (WHO, 2018), one was a colloidal silver dispersion added to water and the other a silver-treated ceramic filter. WHO does not support the use of silver as a drinking-water disinfectant. Its efficacy is uncertain, and any effect requires higher concentrations and lengthy contact periods (WHO, 2018).The use of silver in combination with copper as a measure to reduce colonization and growth of *Legionella* spp. in water distribution systems in buildings has proven effective in many cases. The positive effects are based on long contact times in building water distribution systems (WHO, 2018)

9 CONCLUSION

9.1 Derivation of the health-based value and/or final guideline value

Silver is rarely found at notable concentrations in drinking-water except as a consequence of its use in point of use water treatment devices or in the distribution systems of buildings when applied for *Legionella* control in combination with copper ions. It is mostly found in the low µg/l range in drinking-water. Recognizing that silver has been used by humans for thousands of years, the data on toxic effects of silver in humans other than as the cause for argyria are very limited. Data obtained from studies in animals, including those for AgNP at very high concentrations hint at some toxic effects that are of unclear relevance to the silver concentrations usually found in drinking-water.

The efficacy data on silver as a disinfectant are not comprehensive and there are concerns for all applications other than its combination with copper for preventive Legionella management in building water distribution systems where very long, contact times are allowed

The toxicological database on silver is not adequate to support derivation of a formal Guideline value. Nevertheless, it is recognized that a "bounding value" may be useful. Striking diffuse, blue-gray skin discoloration was reported in a woman who ingested 1 L or about 34 mg colloidal silver per day for approximately 16 months (0.6 mg/kg bw per day assuming 60 kg bw; Kim et al., 2009). This may be the lowest chronic LOAEL in humans. An uncertainty factor of 100 (10 each for intraspecies variability and for limited data including use of a LOAEL) applied to the LOAEL of 0.6 mg/kg bw per day with an allocation factor of 80 % and intake of 2 litres of drinking-water daily and 60 kg body weight, results in a drinking-water concentration of approximately 0.1 mg/L. This concentration can be considered the provisional reference value for silver in drinking-water (i.e. maximum allowable concentration), particularly where silver/copper is used in the control of Legionella in the distribution systems of buildings or where silver is used in point of use water treatment devices. 80% was chosen as an allocation factor for such situations since these circumstances would result in drinking-water contributing to the major source of silver exposure. In respect of the use of silver/copper in Legionella management it is normal practice to only apply this to the hot water system but it is recognised that occasionally there are circumstances where this is not possible and it is also applied to both the hot and the cold water system.

This value is underpinned by the prior assessment that 10 g of ingested silver can be considered a human NOAEL (WHO, 1993, 1984a,b; EPA 1992). Assuming 2 litres of drinking-water intake per day, 0.1 mg/L is a concentration in drinking-water that would give a total dose over 70 years of half this NOAEL. However, as noted, there remains considerable uncertainties regarding the toxicity of silver (ions and NPs) and this makes the data inappropriate for deriving a formal guideline value. Further, a formal guideline value is considered unnecessary since the contribution of drinking-water to this NOAEL will normally be negligible and since WHO does not recommend the use of silver for the disinfection of drinking-water.

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