Alternative drinking-water disinfectants

Bromine, iodine and silver

Alternative drinking-water disinfectants: bromine, iodine and silver

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Part I

Bromine as a drinking-water disinfectant

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List of abbreviations and terms used in Part I

ADI	acceptable daily intake
Br^-	bromide
Br_2	elemental bromine
BrCl	bromine monochloride
bw	body weight
DBP	disinfection by-product
DMH	dimethylhydantoin
GDWQ	Guidelines for Drinking-water Quality
HAA	haloacetic acid
$HOBr^-$	hypobromous acid
HOCl	hypochlorous acid
HWT	household water treatment
IARC	International Agency for Research on Cancer
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LRV	log_{10} reduction value
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NTP	National Toxicology Program
OBr^-	hypobromite
OCl^-	hypochlorite
POU	point-of-use
PPM	parts per million
THM	trihalomethane

UK	United Kingdom
USA	United States of America
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

1. Introduction

Disinfection of water has greatly contributed to reducing risks to public health from microbiologically-contaminated drinking-water.

Numerous disinfection techniques have been developed over the centuries that are used in a wide range of applications, ranging from large and small public drinking-water plants to point-of-entry and point-of-use (POU) treatment devices.[1] Although chlorine has been used for more than 100 years, and several other disinfectants have been studied extensively, in many cases questions remain with respect to the optimization of biocidal effectiveness under a range of conditions (i.e. efficacy), the chemistry of the formation and toxicological significance of disinfection by-products (DBPs), interactions with other water components, and the biocidal effectiveness and toxicology of disinfectant residuals. Chemical disinfectants can react with natural organic matter or break down to produce unwanted by-products. Many newer products and applications are being developed and marketed for use, particularly in developing countries, however, the same unanswered questions exist about these, including their efficacy and potential for DBP formation.

Elemental bromine (Br_2), bromine monochloride (BrCl), hypobromous acid ($HOBr^-$) and bromodimethylhydantoin are used in swimming pools and marketed as a replacement for chlorine, with one advantage being that there are no asthma-related problems for individuals in contact with the disinfected water (e.g. swimmers and/or lifeguards).[2] Bromine in various chemical forms is also used in water fountains and cooling towers. In general, the use of bromine in potable water disinfection is very limited and is impeded by costs, concerns about brominated DBPs, as well as a lack of knowledge on its efficacy in certain areas. However, some applications do exist, as bromine is used to disinfect potable water in non-residential settings, for example, aboard ships and on oil and gas drilling/production platforms. Due to the safety risks of handling liquid bromine (i.e. burns to hands and eyes, release of toxic vapour), it is combined, for example, with dimethylhydantoin (DMH) to form bromodimethylhydantoin and other polymeric brominated hydantoins for disinfection applications. Bromodimethylhydantoin is provided as tablets or cartridges which dissolve slowly to release hypobromous acid. Hypobromous acid can also be generated on site by reaction between sodium bromide and chlorine. In addition, bromine is also combined with chlorine, both of which are hazardous and corrosive, to produce bromine monochloride, which is also classified as hazardous and corrosive.[3] Polymeric brominated hydantoins provide an immobilized controlled source of bromine release. For example, an immobilized bromine flow-through product is currently used in POU water treatment products (see section 2.2.3).

The emphasis of this literature review is to evaluate the available evidence on the biocidal efficacy and toxicity of bromine (Br_2, and other forms) as a water disinfectant. Information included in this review was obtained using a targeted literature search strategy, with inclusion dates up to November 2013 and further "ad hoc" searches were carried out up to the closing date for public review (16 December 2016). Further details of the search strategy are included in Appendix 1.

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[1] Point-of-use devices treat only the water intended for direct consumption (drinking and cooking), typically at a single tap or limited number of taps, while POE treatment devices are typically installed to treat all water entering a single home, business, school, or facility.

[2] Although there is some literature relating to health effects associated with dermal exposure of regular swimmers to bromine in swimming pools, this is beyond the scope of this document.

[3] See: http://echa.europa.eu/en/substance-information/-/substanceinfo/100.034.169

2. Disinfectant characteristics and efficacy

2.1 Chemistry basics

Bromine, chlorine, iodine, and fluorine belong to the halogen group of elements. All of the halogens share the common property of being oxidants with seven electrons in their outer shell. As oxidizing agents, halogens accept an electron to become the analogous halide ion. Different halogens vary in their oxidation potential. The halogen with the strongest oxidative power is fluorine, followed by chlorine, bromine, and iodine. Their reactivities are directly correlated with their electronegativities, which are as follows (based on the Pauling nomenclature of electronegativity values):[4]

fluorine (3.98) > chlorine (3.16) > bromine (2.96) > iodine (2.66).

The reactivities of the given halogens therefore decrease from left to right. Nevertheless, the usefulness of a particular halogen as a disinfectant is determined not only by its reactivity, but also by its manageability, selectivity, chemical stability, and other factors including the potential to form by-products. At ambient temperature, bromine is a brownish-red corrosive liquid. It is the only non-metallic element that is liquid under Standard Ambient Temperature and Pressure (SATP[5]), and evaporates easily under conditions slightly above SATP as a red vapour with a strong irritating odour resembling that of chlorine.

2.1.1 Water solubility, taste and odour

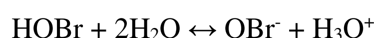
Bromine is more soluble in water than iodine, but less so than chlorine. Water solubility is reported to be 3.55 g/100 mL (West, 1984).

Free halogen residuals usually produce tastes and odours in potable water. Bryan et al. (1973) compared taste threshold determinations of chlorine, iodine and bromine residuals in water. The threshold taste values for chlorine residuals varied with pH: 0.075 mg/L at pH 5.0; 0.156 mg/L at pH 7.0; and 0.450 mg/L at pH 9.0. In contrast, threshold taste values for iodine and bromine did not vary appreciably with pH, ranging from 0.147 to 0.204 mg/L for iodine and 0.168 to 0.226 mg/L for bromine. Chlorine has a high vapour pressure (5100 mm Hg at 20 °C) and readily volatilizes, especially in the presence of sunlight or higher temperatures; iodine has a low vapour pressure (1 mm Hg at 38.7 °C) resulting in little loss by volatilization. Bromine has a vapour pressure between chlorine and iodine of 175 mm Hg at 20 °C with an odour threshold of 0.05 to 3.5 mg/L (IPCS, 1999).

2.1.2 Chemical speciation of bromine in water and corresponding disinfection powers

Elemental bromine (Br_2) disproportionates rapidly in water to give bromide (Br^-) and hypobromous acid (HOBr), which is in equilibrium with hypobromite (OBr^-) in a pH-dependent manner (Table 1).

$$Br_2 + 2H_2O \leftrightarrow HOBr + H_3O^+ + Br^-$$



$$HOBr + 2H_2O \leftrightarrow OBr^- + H_3O^+$$

Bromide can be further oxidized to form bromate (BrO_3^-) via a complex series of oxidation/reduction disproportionation oscillation processes. Bromate is typically associated with use of ozone in water

[4] The Pauling scale is a dimensionless relative quantity that describes the electronegativity of an atom in the periodic table.

[5] SATP: 298.15 K (25 °C) 0.987 atm.

treatment, but there are situations where it can be formed in chlorinated water systems. Bromate can also be present as a by-product in hypochlorite from the electrolytic production of chlorine.

The different chemical species vary in their disinfection power. In analogy to hypochlorous acid (HOCl) and hypochlorite (OCl^-), hypobromous acid and hypobromite compounds display antimicrobial activity, with hypobromous acid being the more effective disinfectant. The most effective pH range for bromine to operate as a disinfectant is therefore between pH 6.0 and 8.5, when hypobromous acid predominates (Table 1). As hypobromous acid does not dissociate at alkaline pH as much as hypochlorous acid does, the disinfection efficacy of bromine is not as pH sensitive as chlorine (most effective pH range between 6.0 and 7.5; Table 1). In addition, bromine and hypobromous acid react with ammonia and amines to produce bromoamines that are more effective biocides than the corresponding chloramines (World Health Organization [WHO], 2004a). Thus, bromine has the potential to be a much more effective disinfectant than chlorine in sewage treatment and in other waters containing ammonia and other reduced forms of nitrogen.

Table 1: pH-dependent speciation of bromine and chlorine in water (Russel, 2006)

pH	Bromine		Chlorine	
	% bromine as $HOBr^-$	% bromine as OBr^-	% chlorine as HOCl	% chlorine as OCl^-
6.0	100	0	90.0	10.0
6.5	99.4	0.6	80.0	20.0
7.0	98.0	2.0	70.0	30.0
7.5	94.0	6.0	37.5	62.5
8.0	83.0	17.0	25.0	75.0
8.5	57.0	43.0	12.5	87.5

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2.2 Disinfection efficacy of bromine

2.2.1 Microbiocidal efficacy of bromine

In the following section and in Table 2, results from selected studies on the efficacy of bromine are summarized.

Bromine is primarily used as an alternative disinfectant for swimming pools, spas and cooling tower water, but not for municipal drinking-water, partly due to cost and partly to concerns about the formation of brominated DBPs.

However, the disinfection of drinking-water with bromine has been recognized and allowed by the USEPA since 1976, although not for use as a municipal drinking-water disinfectant.[6] The use of

[6] In 2010, USEPA issued a Final Registration Review Decision for bromine (Case 4015) stating that the use of bromine to treat potable water does not trigger the need for a drinking-water assessment. In addition, the USEPA stated that “Bromine is registered for use to treat/disinfect potable water (examples of potable water system treatment sites include, but are not restricted to, aboard ships and on oil and gas drilling/production platforms).”

bromine to inactivate bacteria, viruses and protozoa has been reported in a number of laboratory-scale disinfection studies (Kim, 2014). Early studies on the germicidal action of bromine were reported by Tanner and Pitner (1939). The authors determined the concentration of free bromine (in the form of hypobromous acid) required to reduce aerobic spore-forming bacteria, mould spores, yeasts and non-spore forming bacteria to below detection limits within a set contact time (30 seconds) at room temperature; for aerobic spore-forming bacteria, the effect of neutral (pH 6.8–7.2) and low (pH 3.5–4.0) pH was also investigated (Table 2). The authors reported the resistance of the organisms to bromine to be in the following order (decreasing resistance):

bacterial spores > mould spores > yeasts and non-spore-forming bacteria.

For each aerobic spore-forming bacterium tested, bromine was 3–4 times more effective at the lower pH (Table 2).

Goodenough (1964) demonstrated the use of bromine as a disinfectant for swimming pool water. A residual of 0.8 mg/L greatly reduced (but did not eliminate) total bacterial counts; bactericidal activity was also shown to increase with decreasing pH within the range pH 7.8–7.0. Initial studies by Lindley (1966) on bromine efficacy against *Escherichia coli* and f2 coliphage, which were expanded on by Krusé et al. (1970), showed that free bromine at a level of 4 mg/L was able to bring about a 5 log_{10} reduction of *E. coli* and a 3.7 log_{10} reduction of f2 coliphage within 10 minutes at pH 7.0 and 0 ºC. Williams et al. (1988) showed that a water-soluble organic N-bromo oxazolidinone was 50 times more effective against *Staphylococcus aureus* than the N-chloro analog. A later study also highlighted the effectiveness of bromine against *Pseudomonas aeruginosa* at higher temperatures (38 ºC) (Clark and Smith, 1992).

Sharp et al. (1975) assessed the inactivation of reovirus by bromine (in the form of hypobromous acid) and reported a plateau of resistance after rapid inactivation of reovirus within the first three minutes (3 log_{10} reduction) using 0.75 mg/L bromine. As treatment continued, the disinfection rate decreased and finally did not result in further inactivation. Such resistance was not observed when subjecting reovirus suspensions to light centrifugation; this was probably due to the removal of aggregates (Sharp et al., 1975). Indeed, the negative impact of aggregates on the disinfection rate was confirmed in a follow-up study by the same authors, who reported first-order inactivation kinetics at a rate of 10^3 units/second for bromine (in the form of hypobromous acid) against reovirus (pH 7.2, 3.3 µM bromine; Sharp et al., 1976). Whereas this disinfection rate was measured with suspensions of single virus particles, disinfection efficacy decreased in the presence of small virus aggregates. It was suggested that aggregates strongly influenced viral resistance to bromine, as is the case with many disinfectants.

Further studies on the effects of virus particle aggregation on the effectiveness of bromine (in the form of hypobromous acid) disinfection kinetics have been reported by Floyd et al. (1976), using monodispersed poliovirus type 1 at pH 7.0 and a temperature range of 2 to 20 ºC. Greater inactivation was seen with increasing concentration of bromine (0.6–22 µM) and increasing temperature, with log_{10} reduction values (LRVs) ranging between 1 to 3.8 for contact times between 6 and 16 seconds. Use of monodispersed virus particles minimized virus aggregation, which is considered to provide a protective barrier against disinfection (Scarpino et al., 1972). For comparison, Floyd et al. (1978) demonstrated the efficacy of hypobromite and molecular bromine against poliovirus, with both bromine species able to achieve LRVs between 2 to 4 within 4 seconds at 4 ºC in buffered water.

The effectiveness of bromine against cysts of the protozoan parasite *Entamoeba histolytica* has been shown to be greater than chlorine or iodine (Stringer et al., 1975). In addition, evidence on bromine

inactivation of purified *Cryptosporidium parvum* oocyst infectivity in cell cultures has been reported (Kim, 2014). However, cystocidal studies assessing the inactivation of the waterborne protozoan parasite *Giardia lamblia* by bromine have not been identified.

Both bromine and chlorine will lose free residuals under similar conditions of oxidant demand. This has been reported for the use of bromine in cooling tower waters (with bromine concentrations of 400 mg/L and pH 7.7) where residual levels below 0.5 mg/L were reached within hours and were seen to be ineffective against *Legionella pneumophila* (Thomas et al., 1999). Further examples have been reported (Johnson & Overby, 1970; Mercado-Burgos et al., 1975). Bromine may be a superior disinfectant to chlorine when organic matter or ammonium is present as it provides a longer-term release of active bromine. Bromine has been reported to be more effective than chlorine to inactivate poliovirus in reclaimed water (derived from tertiary treated wastewater) containing dissolved organic matter and ammonia (Freund et al., 2010).

Table 2: Disinfection capabilities of bromine (HOBr)

Microorganism	Dose (mg/L)[a]	Comments	Log_{10} reduction value; contact time	Reference
Proteus vulgaris	40–60	pH 3.5–4.0	CK; 30 s	Tanner & Pitner (1939)
	170	pH 6.8–7.2	CK; 30 s	
		Temperature not stated		
Bacillus megatherium	28–35	pH 3.5–4.0	CK; 30 s	Tanner & Pitner (1939)
	110	pH 6.8–7.2	CK; 30 s	
		Temperature not stated		
Bacillus species (*mesentericus*, *subtilis* (565), *subtilis* (566))	160–220	pH 3.5–4.0	CK; 30 s	Tanner & Pitner (1939)
	> 450	pH 6.8–7.2	CK; 30 s	
		Temperature not stated		
Aspergillus niger	25–28	pH not stated; room temperature	CK; 15–30 s	Tanner & Pitner (1939)
Oöspora lactis	8.0	pH not stated; room temperature	CK; 15–30 s	Tanner & Pitner (1939)
Mucor species	30	pH not stated; room temperature	CK; 15–30 s	Tanner & Pitner (1939)
Penicillium species	1.0–5.0	pH not stated; room temperature	CK; 15–30 s	Tanner & Pitner (1939)
Yeasts (*Cryptococcus, Mycoderma, Monila albicans*)	0.25–0.5	pH not stated; room temperature	CK; 15–30 s	Tanner & Pitner (1939)
Saccharomyces species	3.0	pH not stated; room temperature	CK; 15–30 s	Tanner & Pitner (1939)
Staphylococcus species (*aureus* (92), *albus* (76), sp. (80), *aureus* (77), *aureus* (79), *aureus* (89))	0.10–0.25	pH not stated; room temperature	CK; 15–30 s	Tanner & Pitner (1939)
E. coli (252, 251)	0.15	pH not stated; room temperature	CK; 15–30 s	Tanner & Pitner (1939)
E. coli	4.0	pH 7.55; 0 ºC	2.7–4.5; 5–30 min	Lindley (1966)
	4.0	pH 6.0–8.0; 0 ºC	3.2–4.7; 10 min	Krusé et al. (1970)

Microorganism	Dose (mg/L)[a]	Comments	Log_{10} reduction value; contact time	Reference
Pseudomonas aeruginosa	0.2–1.5	pH 7.5; 38 ºC	> 2; 0.5–10 min	Clark & Smith (1992)
f2 coliphage	4.0	pH 7.55; room temperature	2.3–4.5; 1–30 min	Lindley (1966)
	4.0–8.0	pH 7.5; 0 ºC	3.3–5.0; 10 min	
	4.0	pH 6.0–8.0; 0 ºC	2.5–6.5; 10 min	Krusé et al. (1970)
Eberthella typhosa	0.03–0.06	pH not stated; room temperature	CK; 15–30 s	Tanner & Pitner (1939)
Reovirus	22.5–7.0	pH 7.0; 2 ºC	2.3–3.4; 3 min–30 s	Sharp et al. (1975)
Poliovirus (type 1)	00.6–22.0 µM	pH 7.0; 2 ºC	1–3.8; 16 s	Floyd et al. (1976)
	1.9–10.0 µM	pH 7.0; 10 ºC	1–3.4; 16–12 s	
	1.9–9.5 µM	pH 7.0; 20 ºC	2.3–3.2; 8–6 s	
Other				
Entamoeba histolytica (cysts)	1.5–4.0	pH 4.0–10.0; 4–10 ºC	3; 10 min	Stringer et al. (1975)
Cryptosporidium parvum	5	pH 7.5; 25 ºC	0.8[b]; 240 min	Kim (2014)
Giardia lamblia		No studies identified		

a–dose in mg/L unless stated otherwise; b–declining rates of inactivation over time to a maximum of 0.8 log_{10}; CK–complete kill.

2.2.2 Disinfection in the presence of impurities

Bromine combines with ammonia in water to form bromamines, commonly monobromamine (NH_2Br), dibromamine ($NHBr_2$) and tribromamine (NBr_3) (Johnson & Overby, 1970; Russell, 2006; Anderson et al., 1982).

In an early study, Johannesson (1958) demonstrated the effectiveness of monobromamine against *E. coli*, with 0.28 mg/L monobromamine achieving a 3.1 log_{10} reduction in 10 minutes at pH 8.2. Sollo et al. (1975) and Johnson & Sun (1975) confirmed the efficacy of monobromamine against total coliforms and *E. coli* respectively, with Sollo et al. (1975) also reporting increased effectiveness of monobromamine at higher pH.

Floyd et al. (1978) reported inactivation of poliovirus by tribromamine with > 3 log_{10} reduction occurring within seconds to 1 minute of contact time for concentrations between 3 and 50 µM. In contrast, monobromamine achieved LRVs of 2.3 to > 3 within 1 and 8 minutes of contact time at doses between 3 and 40 µM. These results indicate that monobromamine is much more effective than monochloramine for virus inactivation for which comparable LRVs require hundreds of minutes.

Mercado-Burgos et al. (1975) showed bromamines to be effective against *Schistosoma mansoni* ova, with a concentration of 25 mg/L (as bromine) achieving complete kill within 15 minutes.

In tertiary treated sewage (alum coagulated secondary effluent) with an ammonia concentration of 33.5 mg/L, poliovirus inactivation at 3 ^{o}C was 99% (2 log_{10} reductions) in 30 minutes by a dose of 3 mg/L bromine or 10 mg/L dose of chlorine. Estimated times for 2 log_{10} reductions of bromine at doses of 2, 3 and 5 mg/L were about 10, 30 and 70 minutes, respectively, at both pH 7 and 9. Poliovirus inactivation by bromine was equally effective at pH 7 and 9, but chlorine effectiveness was lower at pH 9 than 7 (Johnson & Sun, 1975).

Sollo et al. (1975) directly compared the use of bromine and chlorine (present as bromamines and chloramines) as disinfectants of wastewater effluents. Brominated effluents had consistently lower levels of total coliforms than the chlorinated effluents. The effectiveness of bromine treatment over chlorine increased with increasing pH which is considered to be due to the predominance of the more potent dibromamine species over monobromamine species at higher pH.

A further comparison of the use of bromine and chlorine for disinfection of highly contaminated water was reported by McLennan et al. (2009). Samples were prepared by mixing 9 volumes of potable non-disinfected well water with raw sewage with final turbidities averaging 7.5 ± 2.0 nephelometric turbidity units. When passing water through POU disinfection cartridges with a contact time of 30 minutes, log reductions for bromine and chlorine were comparable for total coliforms, *E. coli*, heterotrophic plate counts, *Enterococcus*, and *Clostridium*. However, bromine was shown to be more effective than chlorine for inactivating coliphages, with LRVs of 1.9 and 1.1, respectively.

2.2.3 Point of use water purification devices using bromine

Many communities in developing countries do not have sufficient funds or infrastructure to adequately protect drinking-water from faecal contamination, systematically treat water for drinking purposes or provide safe water at the tap (Coulliette et al., 2010). One option to reduce microbial (and chemical) threats is household water treatment (HWT) or POU devices comprised of physical (e.g. biosand filter, ceramic filter) or chemical (e.g. chlorine, flocculation/coagulation) barriers. A number of alternative systems are available, including some utilizing bromine.

Halogenated N-halamine media have been developed as part of a household water purification system which is commercially available and sold throughout India and other countries. Canisters containing chlorinated DMH or brominated DMH polymers are available; an important feature of these is their ability to be regenerated by consumers. These have been tested and found to produce minimal amounts of brominated species (Bridges et al., 2009). The primary function of this disinfection technology is a contact biocide that is used as a packed bed filter when incorporated into a treatment train. These polymers have been evaluated for disinfection efficacy; the N-bromamine version was found to be more effective than the N-chloroamine (Sun et al., 1995). To test the disinfection impact of the media only, Coulliette et al. (2010) used these polymers without the other toxin removal devices (e.g. filter). The authors reported that both units were effective against MS2 bacteriophage (with a mean ± standard error reduction of $2.98 \pm 0.26\ \log_{10}$ and $5.02 \pm 0.19\ \log_{10}$, respectively) and microcystin toxin (with reductions of 27.5% and 88.5% to overall mean ± standard error concentrations of 1600 ± 98 ng/L and 259 ± 50 ng/L, respectively). However, the mono-brominated media was seen to be most effective (Coulliette et al., 2010). Halogenated N-halamine derivatives conjugated on polystyrene beads have been reported to show broad antimicrobial activity affected by the covalently bound oxidative bromine, not free bromine. Tested materials have exhibited strong antimicrobial activity against *E. coli* and bacteriophages MS2 of 7 and approximately 4 log reduction, respectively (Farah et al., 2015). Such devices have also been shown to be effective with water of poor quality, that is, when contaminated with sewage (Coulliette et al., 2010; Enger et al., 2016).

2.2.4 Comparison of efficacy with chlorine

The disinfection properties of bromine and chlorine have been previously compared (Keswick et al., 1978; Keswick et al., 1982; Taylor & Butler, 1982). Although the properties of bromine and chlorine differ in a number of ways, as described below, they do have many performance characteristics in common.

The commonalities of bromine and chlorine include:

- different classes of microorganisms have different susceptibilities (activity against *Giardia lamblia* is unknown); the order of resistance to both bromine and chlorine disinfection from least to most resistant is:

  bacteria < viruses < bacterial spores < helminth ova and protozoan parasites; and
- the effectiveness of bromine and chlorine is impacted by temperature, disinfectant concentration, contact time, pH and organic and inorganic content.

With regards to the advantages of bromine over chlorine:

- bromine is more effective in disinfecting bacteria, viruses and protozoan parasites at higher pH levels (pH 9 or 9.5) and in the presence of ammonia;
- bromine provides greater protection across a wider pH range; and
- bromine is more effective for poor quality water.

The disadvantages of bromine over chlorine include:

- the safety of long-term consumption of bromine and its DBPs when used as a drinking-water disinfectant is not fully established. At present brominated DBPs are generally considered more toxic than chlorinated DBPs (see section 3.6.2); it should be noted that brominated DBPs are also produced in chlorinated water in the presence of bromide.

2.2.5 World Health Organization International Scheme to Evaluate Household Water Treatment Technologies

Assessment of the microbial effectiveness of disinfectants as a household-level water treatment option should, as far as possible, model actual use conditions in the field; e.g. water of varying quality, realistic contact times and testing of all three classes of pathogens which cause diarrhoeal disease. In order to comprehensively assess effectiveness, WHO has set tiered health based performance targets for HWT products based on reductions of bacteria, viruses and protozoa (WHO, 2011). These targets are based on microbial risk models using assumed levels of reference pathogens in untreated water. Since 2014, WHO has been testing products against those performance targets through the WHO International Scheme to Evaluate Household Water Treatment Technologies.[7] Box 1 gives further information on the Scheme and its three tiers of $\log_{10}$ performance targets for bacteria, viruses and protozoans. At the time of this report, bromine products have not been tested, but may be included in future rounds.

[7] http://www.who.int/water_sanitation_health/water-quality/household/scheme-household-water-treatment/en/

Box 1. WHO International Scheme to Evaluate Household Water Treatment Technologies

The objective of the Scheme is to independently and consistently evaluate the microbiological performance of household and POU water treatment technologies. The evaluation considers both turbid and non-turbid water, and is carried out to manufacturers' instructions for daily household use.[7] The results of the evaluation are intended to assist and inform Member States and procuring UN agencies in the selection of these technologies.

The performance targets define treatment requirements in relation to source water quality for each pathogen class as detailed below.

Performance target	**Bacteria** (log_{10} reduction required)	**Viruses** (log_{10} reduction required)	**Protozoa** (log_{10} reduction required)	**Classification** (assuming correct and consistent use)
★★★	**≥ 4**	**≥ 5**	**≥ 4**	Comprehensive protection (very high pathogen removal)
★★	**≥ 2**	**≥ 3**	**≥ 2**	Comprehensive protection (high pathogen removal)
★	Meets at least 2-star (★★) criteria for two classes of pathogens			Targeted protection
–	Fails to meet WHO performance criteria			Little or no protection

The performance of HWT products is classified as 3-star (★★★); 2-star (★★); and 1-star (★), denoting descending order of performance, based on log_{10} reductions of bacteria, viruses and protozoa from drinking-water. Performance that does not meet the minimum target is given no stars. Products that meet 3-star (★★★) or 2-star (★★) performance targets are classified as providing "Comprehensive protection" against the three main classes of pathogens which cause diarrhoeal disease in humans. The use of these products is encouraged where there is no information on the specific pathogens in drinking-water (and a prudent approach is to protect against all three classes), or where piped supplies exist but are not safely managed. Products that meet the performance targets for at least 2-star (★★) for only *two* of the three classes of pathogen are given one star (★) and are classified as providing "Targeted protection". In general, the use of these products may be appropriate in situations where the burden of diarrhoeal disease is high due to known classes of pathogens, such as a cholera outbreak.

3. Safety and toxicity of bromine

The toxicity of bromine has been reviewed by a limited number of international bodies, and opinions from the expert bodies on intake are described below. In addition, a detailed assessment of toxicological literature for bromine was undertaken (to November 2013, with further ad hoc searches to the closing date for public review [16 December 2016]) and the relevant findings are included here.

The reactivity of bromine in biological systems makes it difficult to separate the effects of bromine from those of bromine compounds and metabolites that are formed on contact with moisture in mucous membranes and with tissues of the respiratory and gastrointestinal system. Due to its reactivity, bromine does not persist as an element in living tissue, but quickly forms bromide and organobromine compounds. For a full narrative of the toxicity of bromide, the reader is referred to the background document prepared by the WHO (2009)[8] to inform the WHO Guidelines for Drinking-water Quality (GDWQ). However, for ease of reference, bromide exposure and toxicity data are summarized in the sections below.

3.1 Human exposure

Bromine occurs naturally as bromide in various chemical forms in the earth's crust and seawater. Bromide concentrations in seawater are generally in the range of 65 mg/L to in excess of 80 mg/L in some confined sea areas (WHO, 2009). Bromide levels in natural waters are highly variable (10–1000 µg/L), although typically range from trace amounts to approximately 0.5 mg/L (von Gunten, 2003). Groundwaters and reservoirs located near seawater have the potential to have higher levels of bromide related to the geology; desalinated seawater also has the potential to contain bromine (from 1 mg/L to several mg/L; WHO, 2017) depending upon the source water and the type of desalination being practiced.

Bromine is a volatile liquid at room temperature and, therefore, inhalation exposure is considered the most relevant route of exposure to humans. Minimal exposure may also occur through ingestion of food, for example, seafood has relatively high levels of bromide. The typical daily dietary intake of bromide is 2–8 mg in the USA, and 8.4–9.4 mg in the Netherlands (WHO, 2009). Fumigants containing bromide, mainly methyl bromide, are used for soil disinfection as well as postharvest treatment of plant products (PHE, 2009).

3.2 Guideline values

3.2.1 WHO drinking-water quality guidelines

The WHO Guidelines for Drinking-water Quality (GDWQ) have not evaluated bromine as it quickly forms hypobromous acid and bromide in water. A drinking-water guideline value has not been proposed for bromide as it occurs in drinking-water at concentrations well below those of health concern (WHO, 2017). However, to provide guidance to Member States, should the chemical be found in drinking-water or its sources, the GDWQ includes a health-based value of 6 mg/L for adults and 2 mg/L for children. For bromate, a provisional guideline value of 10 µg/L is included based on achievable analytical quantitation limits and treatment methods.

[8] The latest version of the background document is dated 2009 and so will undergo review. However, no new studies were identified up to December 2016 to amend the findings of the document.

3.2.2 Other values

At a joint meeting of the Food and Agriculture Organization of the United Nations/WHO in 1988, an acceptable daily intake (ADI) of 0–1 mg/kg bw (body weight) was established for bromide ion, based on a no-observed-effect level (NOEL) from a human study reported by Sangster et al. (1986).

The European Agency for the Evaluation of Medicinal Products (EMEA, 1997) also utilized the study by Sangster et al. (1986) to derive an ADI of 0.4 mg/kg bw based on marginal effect within normal limits of electroencephalograms in females at 9 mg/kg bw per day, including a safety factor of 10 for population diversity (see section 3.3.3).

The United Kingdom Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT, 2000) considered dietary intake of bromine. Intake was estimated from the 1997 total diet study as 3.6 mg/person per day (equivalent to 0.06 mg/kg bw per day). The Committee noted that the upper boundary of the ADI proposed by Food and Agriculture Organization of the United Nations/WHO of 1 mg/kg bw could be taken as a tolerable daily intake. Estimated dietary intakes were therefore well below the acceptable level, allowing for significant exposure from other routes (EA, 2005).

When used as a pesticide, the USEPA has defined bromide as "exempt from the requirement of tolerance" (USEPA, 2010).

Most recently, NSF International[9] has proposed a combined bromine/bromide action level of 10 mg/L for drinking-water. The derived maximum contaminant level applies specifically to elemental bromine and inorganic bromide ion and is considered protective of human health. The maximum contaminant level does not consider potential formation of bromate or DBPs (NSF, 2011).

3.3 Human toxicity data

3.3.1 Toxicokinetics

3.3.1.1 Absorption

Following inhalation, bromine is absorbed by the lungs (as bromide) and deposition in the lungs is primarily determined by the water solubility of bromine (IPCS, 1999). Following ingestion, bromide is rapidly and completely absorbed from the intestine by passive, paracellular transport (HCN, 2005). Bromide uptake and equilibrium concentrations are interrelated with chlorine levels: as chloride intake increases, the excretion of bromide increases (WHO, 2009).

No data could be located regarding absorption of bromine vapours via the ocular or dermal routes of exposure, however it is likely to react on the surface of the body immediately on contact (PHE, 2009).

[9] This document was prepared to allow toxicological evaluation of bromine/bromide in drinking-water, as an extractant from one or more drinking-water system components or as a contaminant in a drinking-water treatment chemical, evaluated under NSF/ANSI standards.

3.3.1.2 Distribution

Absorbed bromine (as bromide and organobromine chemicals by all routes of exposure) is distributed widely into various tissues and mainly into the extracellular fluid of the body (PHE, 2009).

3.3.1.3 Metabolism

No data could be identified regarding the metabolism of bromine. However, bromine has been shown to quickly form bromide in living tissue and is partitioned in the body similarly to chloride. Its presence is related to the amount of chloride intake (HCN, 2005).

3.3.1.4 Elimination

Bromide is excreted mainly by the kidneys and in small quantities in sweat, tears, and other body excretions. The biological half-life of bromide has been reported to be between 12 and 30 days in humans, with that in rats being markedly shorter, at approximately 3 days (HCN, 2005).

3.3.2 Acute toxicity

No median lethal dose (LD_{50})[10] values for bromine or bromide have been reported for humans.

Due to its water solubility, bromine generally produces effects on the upper respiratory tract. However, inhalation of high concentrations, for example, in confined spaces, may also cause marked effects on the lower airways. Acute inhalation exposure to bromine results in symptoms of respiratory irritation including, shortage of breath, cough, choking and wheezing, bronchoconstriction, inflammation of the oesophagus, and laryngeal spasm; respiratory distress has led to hypoxaemia, metabolic acidosis and death (DEFRA, 2006). Acute inhalation of high concentrations of bromine vapour has resulted in brown colouration of the eyes, tongue, and mucous membranes of the mouth as well as catarrh (thick phlegm or mucus in an airway), salivation, coughing, feeling of suffocation, glottis cramps, hoarseness, bronchitis and bronchial asthma (USEPA, 2009). Central nervous system effects documented following overdoses of bromide-containing medicines or fumigants include ataxia, slurred speech, tremor, nausea, vomiting, lethargy, dizziness, visual disturbances, headaches, impaired memory and concentration, disorientation, and hallucinations (IPCS).[11]

Accidental (acute) ingestion of liquid bromine has been associated with haemorrhagic nephritis, with oliguria or anuria, (reduced or increased urine production respectively) developing within 1 to 2 days (concentration not stated). Associated injuries due to corrosivity and inhalation of vapour were not described (PHE, 2009).

Bromine is highly irritating to the skin in both liquid and vapour form. Acute dermal exposure to bromine results in localized blister formation, brownish discolouration of the skin and slow-healing ulcers. Appearance of injury is often delayed (Sagi et al., 1985).

Ocular effects following exposure to bromine vapour (0.5 parts per million [ppm]) include stinging and burning of the conjunctiva and lacrimation, and at higher levels (not stated) photophobia and blepharospasm (i.e. forcible closure of the eyelids) have been reported (PHE, 2009).

The irritating properties of bromine vapour act to prevent prolonged exposure at high concentrations. Exposure for 50 minutes at levels of 0.006 ppm (0.04 mg/m^3) is associated with some irritation of the

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[10] The dose required to kill half the members of a test population after a specified test duration.
[11] http://www.inchem.org/documents/pims/chemical/pim080.htm

eyes; at levels of 0.2 ppm (1.3 mg/m^3), clear irritation of eyes, nose, and throat occurs; and levels of 0.5 ppm (3.3 mg/m^3) and above cannot be tolerated due to the severity of these symptoms (Rupp & Henscler, 1967).

Doses of bromide giving plasma levels of 12 mmol/L (96 mg/L) produce bromism (the chronic state of bromide intoxication), and plasma levels greater than 40 mmol/L (320 mg/L plasma) are sometimes fatal. The signs and symptoms of bromism relate to the nervous system, skin, glandular secretions and gastrointestinal tract (WHO, 2009).

3.3.3 Repeat dose toxicity

Data relating to the effects in humans following chronic exposure (by all routes) to bromine could not be identified.

The key repeat dose toxicity study in humans for bromide reported by Sangster et al. (1986) and described in the WHO background document (WHO, 2009), determined a conservative NOEL of 4 mg sodium bromide/kg bw per day based on marginal effect within normal limits of electroencephalograms in females at 9 mg/kg bw per day.

3.3.3.1 Systemic effects

No data could be located regarding systemic effects in humans following repeated exposure to bromine or bromide.

3.3.3.2 Neurotoxicity

No data could be located regarding neurotoxic effects in humans following repeated exposure to bromine or bromide.

3.3.3.3 Reproductive and developmental toxicity

No data could be located regarding reproductive and developmental effects in humans following repeated exposure to bromine or bromide.

3.3.3.4 Immunotoxicity

No data could be located regarding immunotoxic effects in humans following repeated exposure to bromine or bromide.

3.3.3.5 Genotoxicity

No data could be located regarding genotoxic effects in humans following repeated exposure to bromine or bromide.

3.3.3.6 Carcinogenicity

Bromine and bromide are not listed as carcinogens by the International Agency for Research on Cancer (IARC). No data are available to assess the carcinogenicity of bromine or bromide in humans.

3.4 Animal toxicity studies

The predominant route of exposure reported in experimental studies to date relate to the inhalation of bromine. Although not directly relevant to drinking-water, except potentially in cases of aerosol

formation, these studies are included as the main experimental evidence available. In addition, once inhaled, bromine will be rapidly converted to bromide as for oral intake.

3.4.1 Toxicokinetics

No data could be located regarding the toxicokinetics of bromine or bromide in animals.

3.4.2 Acute toxicity

The 30-minute median lethal concentration (LC_{50})[12] of bromine (vapour) in female (NMRI) mice was reported to be 174 ppm (1158 mg/m^3) with an observation period of 10 days. Generally, mortality occurred in two distinct periods, either, within the first 4 days (the majority) or between days 8 and 10. The cause of death in the animals was reported to be either bronchospasm (spasm of bronchial smooth muscle producing narrowing of the bronchi) or lung oedema (fluid accumulation in the lung) in the early deaths and peribronchitis (a form of bronchitis consisting of inflammation and thickening of the tissues around the bronchi) with abscess formation during days 8 to10 (HCN, 2005).

An LC_{50} of 240 ppm (1569 mg/m^3) has been reported in mice (strain and sex not specified) exposed to bromine vapour for 2 hours (PHE, 2009).

Exposure to 22 and 40 ppm (147 and 266 mg/m^3) bromine vapour for 3 hours caused mortality in 0 of 10 and 3 of 10 mice, respectively, while 7 of 10 and 8 of 10 animals, respectively, died within 10 days following a 6-hour exposure. Bitron & Aharonson (1978) studied the delayed mortality (observation time: 30–45 days) following a single inhalation event of bromine (in comparison with formaldehyde, sulphur dioxide, and chlorine). Mice were exposed to bromine concentrations of 240 ppm (approximately 1600 mg/m^3) for 15–270 minutes or to 750 ppm (approximately 5000 mg/m^3) for 5–30 minutes. A 100-minute LC_{50} of 240 ppm and a 9 minute LC_{50} of 750 ppm were identified.

Cats, rabbits and guinea pigs (strain and sex not specified) exposed to 23 ppm (approximately 150 mg/m^3) bromine vapour for 7 hours showed slight irritation of the respiratory tract, whilst at 180 ppm (1176 mg/m^3) CNS function disturbances were seen; a lowest-observed-adverse-effect level (LOAEL) of 23 ppm (150 mg/m^3) was identified (Bingham et al., 2001).

Bromide is considered to have very low acute toxicity. Oral LD_{50} values of 3500 mg/kg bw have been reported for the rat and 5020–7000 mg/kg bw for the mouse (WHO, 2009).

3.4.3 Repeat dose toxicity

3.4.3.1 Systemic toxicity

A limited number of experimental studies on the effects of chronic exposure to excess bromine have been reported:

- Exposure to bromine vapours at 33 and 67 mg/m^3 (5 and 10 ppm) for 8 hours/day for 3 days did not cause mortality, but body weights were decreased; this was attributed to irritation of the upper respiratory tract. A LOAEL of 33 mg/m^3 (5 ppm) was identified from this study (Schlagbauer & Henschler, 1967).
- Rats, mice and rabbits (strain and sex not specified) were exposed via inhalation to bromine vapour continually for 4 months at doses of 0.13–1.31 mg/m^3 (approximately 0.02–0.2 ppm). At the highest dose, animals developed disturbances in respiratory, nervous and endocrine

[12] The concentration required to kill half the members of a test population after a specified test duration.

functions. No adverse effects were observed at the lowest dose employed. A no-observed-adverse-effect level (NOAEL) of 0.13 mg/m^3 (0.02 ppm) could be identified from this study (Schlagbauer & Henschler, 1967).

- Rats (strain and sex not specified) were fed liquid bromine (38%) at 20 mg/kg bw per day in a 28-day oral study. Clinical signs of salivation and decreased activity were observed, with increased red blood cell count, haemoglobin and packed cell volume, increased serum glucose and increased urinary volume with protein also being reported (USEPA, 2005a).

The key repeat dose toxicity study identified for bromide, as described in the WHO background document (WHO, 2009), determined a NOAEL for sodium bromide of 300 mg/kg diet (240 mg/kg diet as bromide; 12 mg/kg bw per day) based on effects on the thyroid in male Wistar rats. An important finding of this study was the increased toxicity of sodium bromide in rats fed a low-chloride diet, with toxicity being around 10 times higher than for rats on a diet containing standard chlorine levels.

3.4.3.2 Neurotoxicity

No data could be located regarding neurotoxic effects in animals following repeated exposure to bromine or bromide.

3.4.3.3 Reproductive and developmental toxicity

Ivanov et al. (1976) reported that a 4-hour exposure to bromine vapour at 15 ppm affected spermatogenesis in male mice; further details were not reported.

In a three-generation reproduction study (two litters per generation), Wistar rats fed sodium bromide at 19 200 mg/kg bw showed complete infertility. Fertility and offspring viability were also reduced at 4800 mg/kg diet. No treatment-related effects were observed in reproductive performance, viability or body weight of the offspring in the second and third generations bred only from the groups dosed of 0, 75, 300 and 1200 mg/kg diet. Relative adrenal weight was significantly reduced in adult (F0) females at 4800 and 1200 mg/kg feed but effects on other organs did not show a clear pattern of dose-response (WHO, 2009).

No experimental studies relating to the developmental toxicity of bromine were identified.

3.4.3.4 Immunotoxicity

No data could be located regarding immunotoxic effects in animals following repeated exposure to bromine or bromide.

3.4.3.5 Genotoxicity *(in vivo)*

No data could be located regarding genotoxic effects in animals following repeated exposure to bromine or bromide. (See 3.4.4 for *in vitro* genotoxicity studies.)

3.4.3.6 Carcinogenicity

No experimental studies relating to the carcinogenicity of bromine or bromide were identified. Studies are underway to assess the role of bromide in the cancers of the thyroid and testes mesothelium from the metabolism of bromate to bromide in high dose tests (J. Cotruvo, personal communication; 1 April 2016).

3.4.4 *In vitro* toxicity studies

Liquid bromine, tested at a concentration of 38 % and a volume of 10 µg/plate, was positive in the *Salmonella typhimurium* microsome reverse mutation assay with strains TA 1537 and TA 100 in the absence of S9 and with strain TA 1537 in the presence of S9 activation. As would be expected from its reactivity, bromine was cytotoxic for all strains with and without metabolic activation at more than 3333 µg/plate (USEPA 2005a).

Sodium and ammonium bromide were studied in an Ames test with *Salmonella typhimurium* strains TA98 and TA100. At dose levels of 0.001–10 mg/plate, both with and without metabolic activation, no mutagenic effect was observed (WHO, 2009).

3.5 Vulnerable populations

No information on the possible impact of bromine or bromide on vulnerable populations was identified.

3.6 Toxicity of brominated disinfection by-products

3.6.1 Formation and occurrence of brominated disinfection by-products

When present in water, either as part of the ambient conditions or when used as a disinfectant, bromine and bromide have the ability to form brominated DBPs. The common source of brominated DBPs is chlorination of water containing bromide. The bromide is oxidized by chlorine to hypobromous acid which rapidly halogenates organic matter, producing the following brominated and mixed halogenated DBPs:

- bromoform;
- dibromoacetic acid;
- tribromoacetic acid;
- bromoacetic acid;
- bromochloroacetic acid;
- bromodichloroacetic acid
- dibromochloroacetic acid;
- dibromoacetonitrile;
- 2-bromo-2-methylpropanal;
- 2,3,5-tribromopyrrole;
- bromoacetone;
- bromoalkanes;
- bromohydrins; and
- brominated trihalomethanes (including bromodichloromethane, chlorodibromomethane, and tribromomethane (bromoform)).

Many of the DBPs listed above are generally present at very low concentrations (fractional parts per billion levels), although elevated levels are possible.

It has been suggested that one of the main DBPs of concern in high bromide-containing waters is bromate (WHO, 2005), particularly when the water is ozonated or a low-quality hypochlorite is used. Brominated trihalomethanes (THMs) may occur at concentrations exceeding those of chloroform when source waters with elevated bromide levels are chlorinated (Krasner et al., 1989) while ozonation prior to chlorination can further enhance the formation of brominated THMs (Shukairy et al., 1994). Among

the most prevalent brominated THMs are chlorodibromomethane, bromodichloromethane and tribromomethane (bromoform). In recent years, there has been a growing concern of public water systems facing higher bromide levels in their source waters from anthropogenic contamination through coal-fired power plants, conventional oil and gas extraction, textile mills, and hydraulic fracturing (McTigue et al., 2014; States et al., 2013).

Evaluation of the formation of brominated DBPs from use of bromine containing HWT and/or POU devices remains to be fully investigated.

3.6.2 Toxicological evaluations of brominated by-products

Epidemiology studies indicate increased risk of bladder cancer associated with increased THM concentrations in drinking-water, with brominated DBP species, or other co-occurring DBPs (including chlorinated DBPs) being potentially significant contributing factors (Cantor et al., 2010). This has been highlighted as a possible issue in recent literature, linked to a potential increase in bromide levels in drinking-water sources in the USA as a result of anthropological contamination (Regli et al., 2015).

Several brominated DBPs have been shown in animal studies to be more carcinogenic than their chlorinated analogs (Richardson, 2003a). Richardson (2007) has summarized the relative occurrence and genotoxicity of a wide variety of DBPs including brominated compounds.

WHO (2009) reports that bromate is mutagenic both *in vitro* and *in vivo*. The IARC has classified potassium bromate in Group 2B (possibly carcinogenic to humans) concluding that there is inadequate evidence for carcinogenicity in humans but sufficient evidence of carcinogenicity in animals. The IARC has classified dibromochloromethane and bromoform in Group 3 (not classifiable as to its carcinogenicity to humans) and dibromoacetonitrile in Group 2B. The USEPA has also classified bromate as a probable human carcinogen by the oral route based on data from male and female rats, bromoform and bromodichloromethane as likely to be carcinogenic to humans by all routes of exposure, and dibromochloromethane as having suggestive evidence of carcinogenicity (USEPA, 2005a). Health Canada also considers bromate to be carcinogenic to humans.[13] Although classified as probably carcinogenic to humans, WHO (2009) states that there is insufficient information to conclude the carcinogenic mode of action of potassium bromate. Later studies have reported that the mode of action of bromate, at levels well above those found in drinking-water, does not involve genotoxicity in rats (Bull & Cotruvo, 2013; Yamaguchi et al., 2008).

The carcinogenicity of brominated THMs were assessed in a series of older studies carried out by the National Toxicology Program (NTP) using corn oil as the vehicle (NTP, 1985; NTP, 1987; NTP, 1989a; NTP, 1989b). Due to concerns surrounding a possible corn oil vehicle effect, where available, studies utilizing drinking-water as the vehicle are described below.

Limited reports of a two-year feeding study using SPF Wistar rats administered chlorodibromomethane have been identified. The authors observed no increase in gross tumours in male rats treated with chlorodibromomethane at doses of 10, 39, or 210 mg/kg per day, or in female rats treated at doses of 17, 66, or 350 mg/kg per day (Tobe et al., 1982).

In a 2-year oral study, CBA x C57B1/6 mice were administered chlorodibromomethane in drinking-water at concentrations of 0, 0.04, 4.0 or 400 mg/L (equivalent to doses of 0.008, 0.76 or 76 mg/kg per

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[13] http://www.healthycanadians.gc.ca/health-system-systeme-sante/consultations/bromate/document-eng.php

day); controls were untreated. Survival time was not related to dose in either male or female animals. No increase in tumour incidence was observed in treated animals in comparison with controls (Voronin et al., 1987).

Male Wistar rats were administered bromodichloromethane in drinking-water at concentrations of 0, 175, 350 or 700 mg/L (equivalent to 0, 6, 12 or 25 mg/kg per day) for 102 weeks. Body weights of treated groups remained similar to those of control animals. An increased incidence of inflammation in the liver was noted at the two higher doses, however the relevance of this is uncertain. There were no increased incidences of neoplasms that were attributable to bromodichloromethane (NTP, 2006).

In a 2-year study in female B6C3F1 mice, bromodichloromethane was administered in drinking-water at concentrations of 0, 175, 350, or 700 mg/L (equivalent to 0, 9, 18 or 36 mg/kg per day) Mean body weights were lower in treated groups when compared to controls from week 4 of the study. The incidence rates of hepatocellular carcinoma or adenoma (combined) or hemangiosarcoma in all organs in treated animals were not statistically significantly different from those in controls. The authors concluded that under the conditions of the assay, bromodichloromethane was not carcinogenic (NTP, 2006).

No oral study utilizing water as a vehicle for administration of bromoform could be identified and therefore the study using corn oil as the vehicle is described. Bromoform was administered for 103 weeks in corn oil by gavage for 5 days per week to groups of F344/N rats and B6C3F1 mice. Daily doses of 0, 100 or 200 mg/kg were administered to rats and female mice, and 0, 50 or 100 mg/kg to male mice. In comparison to controls, decreased body weights were noted in male rats in the high (12–28%) and low (5–14%) dose groups, with a decrease in female rats in the high dose group only (10–25%). Female mice also showed a decrease in body weight in the high (5–16%) and low (6–11%) dose groups relative to controls; body weights of male mice were not decreased. Adenomatous polyps or adenocarcinomas were noted in the large intestine (colon and rectum) of male rats at the highest dose (3 of 50) and female rats at both doses (1 of 50 and 8 of 50) in comparison to controls (0 of 50 in both sexes) but this was not considered significant. No tumours were apparent in mice at either dose of bromoform. The authors concluded that under the conditions of the study, there was clear evidence of carcinogenicity for female rats, some evidence for male rats and no evidence for male and female mice (NTP, 1989). The IARC concluded that there was limited evidence for the carcinogenicity of bromoform in animals, and inadequate evidence in humans, with an overall evaluation of Group 3 (not classifiable as to its carcinogenicity to humans).

The NTP (1989b) has also assessed the reproductive and developmental toxicity of bromoform in CD-1 mice following administration at doses of 0, 50, 100 or 200 mg/kg per day by oral gavage. Varying degrees of hepatocellular degeneration were seen in all treated animals, however no changes in reproductive parameters were noted at levels below significant hepatotoxicity.

Prevalent brominated acetic acids include monobromoacetic acid, dibromoacetic acid and bromochloroacetic acid. These DBPs have been covered in a background document from the WHO (WHO, 2004b) to support the GDWQ. In brief, the following toxicities were identified for these brominated acetic acids:

- Monobromoacetic acid has an oral LD_{50} in rats of 177 mg/kg bw, (Linder et al., 1994), with observed clinical symptoms of excess drinking-water intake, hypomobility, laboured breathing and diarrhoea following acute exposure. Chronic studies were not identified. Monobromoacetic acid

was mutagenic in *Salmonella typhimurium* (NTP, 2000a) and positive with microsomal activation in the Ames fluctuation test using *S. typhimurium* strain TA100 (Giller et al., 1997). Monobromoacetic acid produced DNA strand breaks in L-1210 mouse leukaemia cells (Stratton et al., 1981).

- Dibromacetic acid has a reported oral LD_{50} in rats of 1737 mg/kg bw, with observed clinical symptoms of excess drinking-water intake, hypomobility, laboured breathing, diarrhoea and ataxia following acute exposure. Spermatotoxicity was also apparent on histopathological examination (Linder et al., 1994). Sub-chronic and chronic exposure studies have identified liver toxicity, immunotoxicity, and spermatotoxicity. Dibromoacetic acid was mutagenic in *S. typhimurium* (NTP, 2000b) and in the Ames fluctuation test with *S. typhimurium* tester strain TA100, with and without metabolic activation (Giller et al., 1997). Dibromoacetic acid is associated with DNA repair in the SOS chromotest, with and without metabolic activation (Giller et al., 1997) and DNA damage (Austin et al., 1996).
- Chronic exposure to bromochloroacetic acid has been associated with induced liver toxicity and reproductive changes (decreased implants and decreased number of live fetuses per litter). Bromochloroacetic acid was mutagenic in *S. typhimurium* in the standard Ames assay (NTP, 2009). DNA damage has been reported (Austin et al., 1996).

In USEPA's health criteria document for brominated acetic acids (USEPA, 2005a); monobromoacetic acid, bromochloroacetic acid, and dibromoacetic acid were all identified as "not classifiable as to human carcinogenicity" under the 1986 Carcinogen Risk Assessment Guidelines, and "inadequate for an assessment of human carcinogenic potential" under the 1999 Draft Guidelines for Carcinogen Risk Assessment. The IARC has classified bromochloroacetic acid and dibromoacetic acid as Group 2B (possibly carcinogenic to humans).

Genotoxicity and cytotoxicity studies of brominated and chlorinated haloacetic acids (HAAs) have been described by Plewa et al. (2008). Brominated HAAs were found to be more cytotoxic than their chlorine analogs, with a rank order of:

bromoacetic acid = dibromoacetic acid > chloroacetic acid > tribromoacetic acid > dichloroacetic acid > trichloroacetic acid.

Brominated HAAs were also more genotoxic than their chlorine analogs, with a rank order of:

bromoacetic acid = chloroacetic acid > dibromoacetic acid > tribromoacetic acid.

3.7 Summary of the safety and toxicity of bromine

Due to its reactivity, bromine, as with chlorine does not persist as an element in living tissue, but quickly forms bromide and brominated organic chemicals, making the study of the toxicokinetics difficult. This is reflected in the very limited toxicokinetic data available for bromine from human and animal studies. Many reports have utilized data from toxicity studies for sodium bromide in place of bromine. Bromide and chloride are always present in body fluids in animals in steady state at levels dependent upon intake, and both are excreted readily. Increased chloride intake will increase the excretion of bromide.

Symptoms of acute bromine toxicity via the inhalation route include respiratory irritation/distress and central nervous system effects (all dependant on concentration). Bromine is highly irritating to the skin

in both liquid and vapour form, with appearance of injury in the form of often delayed blister formation. Ocular irritation following exposure to bromine vapour is reported. Although rare, ingestion of liquid bromine is associated with haemorrhagic nephritis, with oliguria or anuria, developing within 1 to 2 days. Where comparisons can be made, the findings from human studies are supported by those from animal studies. The acute toxicity of bromide is considered to be very low.

Sub-chronic and chronic bromine toxicity studies in humans were not identified from available literature. Animal studies are also very limited but suggest chronic exposure to bromine may have adverse effects on reproduction. Repeat dose oral toxicity studies with bromide in rats indicate adverse effects on the thyroid, with toxicity being enhanced by a low chloride diet. Reproductive and developmental toxicity of sodium bromide were also noted in a three-generation study in rats.

Bromine and bromide are not classifiable as human carcinogens. Bromine has shown positive results in reverse mutation assays with and without metabolic activation. Bromide has not shown evidence of mutagenicity in similar assays.

Among the most prevalent brominated THMs are chlorodibromomethane, bromodichloromethane and tribromomethane (bromoform). These are not carcinogenic when tested by the NTP protocol (WHO, 2017). There are large toxicological data gaps across all DBPs, however where available, brominated DBPs have been shown in general to be more genotoxic than the chlorinated analogs.

Prevalent brominated acetic acids include monobromoacetic acid, dibromoacetic acid and bromochloroacetic acid.

Brominated HAAs have been found to be more cytotoxic and genotoxic than their chlorine analogs (see Section 3.6.2 for the rank order).

The potential for formation of brominated DBPs from the use of bromine as an alternative disinfectant is unknown, although more brominated DBPs would be formed based upon dose levels compared to chlorine.

4. Environmental considerations

Environmental considerations are largely beyond the scope of this report however, as noted in Table 3, the impact of release of bromine into the environment to "non-target" organisms should be considered.

Table 3: Environmental toxicity of bromine to "non-target" species[14]

Group of organisms	Common name (scientific name)	Median lethal dose (LD_{50})	Acute toxicity rating
Fish (freshwater)	Bluegill sunfish (*Lepomis macrochirus*)	0.52 mg/L (24 h)	Highly toxic
	Rainbow trout (*Oncorhynchus mykiss*)	0.31 mg/L (24 h)	Highly toxic
Invertebrates	Water flea (*Daphnia magnamagna*)	1.5 mg/L (24 h)	Moderately toxic
	Water flea (*Daphnia magnamagna*)	1 mg/L (48 h)	Moderately toxic

[14] Source: Kegley SE, Hill BR, Orme S, Choi AH, PAN Pesticide Database, Pesticide Action Network, North America (Oakland, CA, 2016), http://www.pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PC35462; visited September 2017.

5. Discussion and conclusions

The decision on whether bromine can serve as a disinfectant for drinking-water and wastewater treatment is likely to be a balance between the dose required to achieve efficacy (see section 2.2), its advantages over other disinfectants, particularly chlorine (see section 2.2.4), aesthetic impacts, preventing potential adverse health effects (see section 3) from chronic exposure, and cost. Any potential risk of adverse effects should be considered in context of the benefit of water disinfection which should always take precedence.

Disinfection with bromine and the comparison of its efficacy with chlorine appears greatly understudied. Compared to the wealth of literature available for chlorine, a very limited number of studies have investigated the effect of alternative halogens including bromine. Bromine has demonstrated effectiveness in removing several pathogens in laboratory settings, but has not been tested against many protozoan parasites including *Giardia*.

Bromine disinfection is superior to chlorine for microbiological inactivation when applied to low-quality water containing ammonia and other nitrogenous components (McLennan et al., 2009). This may give support for the use of bromine as a potential alternative to chlorine in disaster relief scenarios, however, further investigations would be required. Also, these potential benefits should be balanced with the significant issues surrounding the ease and safety of bromine generation and its subsequent use for water purification purposes. Practical handling of free bromine is a safety issue; it is usually combined with DMH, an organic carrier. Other chemical forms of bromine are currently used for disinfection of non-drinking-waters including swimming pools and cooling towers.

At the household level, there are a number of additional considerations beyond efficacy, for determining whether any water treatment product, including bromine, will protect against adverse health effects. Achieving health gains from household water treatment requires products to be used correctly and consistently, and thus, clear product information and use instructions are important. In addition, user preferences, supply chains and availability, and cost are important factors to consider. Products such as bromine which require a reliable supply chain can be problematic in resource-scare settings where such systems are not in place.

Toxicity studies in humans or animals for bromine *per se* via ingestion are very limited; this is mostly due to the corrosiveness and high reactivity of bromine; it quickly forms bromide in living tissues. Human studies with sodium bromide have allowed derivation of an ADI for bromide of 0.4 mg/kg bw based on the most sensitive toxicological endpoint relating to changes within electroencephalograms. However, a drinking-water guideline value has not been proposed for bromide in the WHO GDWQ as it occurs in drinking-water at concentrations well below those of health concern. However, the GDWQ includes a health-based value of 6 mg/L for adults and 2 mg/L for children (WHO, 2017). For bromate, a provisional guideline value of 10 µg/L is recommended as a pragmatic value based on difficulties in removing bromate once it is formed.

The greatest potential concern to humans from using bromine as a drinking-water disinfectant may stem from the generation of brominated DBPs. The formation of brominated DBPs during water disinfection with chlorine has been well studied. There are toxicity data in some of these studies that indicate that brominated DBPs may be more toxic in some respects than their chlorinated analogs. Currently the potential for formation of brominated DBPs from the use of bromine as an alternative drinking-water disinfectant in HWT and POU devices has not been comprehensively addressed, although some devices have been shown to produce minimal amounts of brominated products (Bridges et al., 2009).

In summary, the current evidence is sufficient to indicate that:

- In a similar way to chlorine, as a drinking-water disinfectant, bromine can be most effective against bacteria, effective to a somewhat lesser extent against viruses, and least effective against some protozoan parasites; however, the evidence base is more limited in comparison to chlorine;
- Bromine appears to be effective against cysts of the protozoan parasite *Entamoeba histolytica,* and there is some evidence of limited effectiveness against oocysts of *Cryptosporidium parvum*; studies on the efficacy of bromine against *Giardia* cysts were not available; and
- Somewhat similar to chlorine and iodine, disinfection efficacy is impacted by the temperature, bromine concentration, contact time, pH and organic and inorganic content; however, bromine is much less affected by pH and ammonia.

In general, the use of bromine in wastewater disinfection is promising and warrants further study, and reasons for particular consideration have been outlined above. However, active bromine would not be recommended for use as a primary disinfectant at the current time due to the concerns with the formation and potential toxicity of organobromine and organobromine DBPs and the availability of widely used, well-characterized disinfectants. Although the evidence base indicates that it may be a superior disinfectant to chlorine in several respects, there is a need for additional data on the range of microorganisms against which it is effective and under what conditions.

POU devices that provide contact disinfection may be appropriate under targeted circumstances (such as when pathogenic bacteria and viruses are the organisms of concern) or when combined with another barrier that is effective against protozoa, provided that there is little release of bromine into the finished water to minimize DBP formation. The use of POU devices should be appropriately approved or certified to ensure efficacy and safety.

6. References

Anderson AC, Reimers RS, DeKernion P (1982). A brief review of the current status of alternatives to chlorine disinfection of water. Am Public Health. 72(11):1290–93.

Austin EW, Parrish JM, Kinder DH, Bull RJ (1996). Lipid peroxidation and formation of 8-hydroxydeoxyguanosine from acute doses of halogenated acetic acids. Fundam Appl Toxicol. 31:77–82.

Bingham E, Cohrssen B, Powell CH (2001). Patty's Toxicology. 5th ed. Vol. 1–9. New York (NY) [cited by PHE (2009)].

Bitron MD, Aharonson EF (1978). Delayed mortality of mice following inhalation of acute doses of CH_2O, SO_2, Cl_2 and Br_2. Am Ind Hyg Assoc J. 39:129–38.

Bridges M, Reckhow D, Liu B, Cotruvo J (2009). Analysis and comparisons of disinfection by-products from brominated hydantoinylated cross-linked polystyrene to traditional chlorination. In: Proceedings. Water Environment Federation Disinfection Conference, Atlanta GA, 2 October 2009: (1):440.

Bryan PE, Kuzminski LN, Sawyer FM et al. (1973). Taste thresholds of halogens in water. J Am Water Works Assoc. 65:363–68.

Bull RJ, Cotruvo JA (2013). Nongenotoxic mechanisms involved in bromate-induced cancer in rats. J Am Water Works Assoc. 105(12):E709–20.

Cantor KP, Villanueva CM, Silverman DT, Figueroa JD, Real FX, Garcia-Closas R et al. (2010). Polymorphisms in *GSTT1, GSTZ1*, and *CYP2E1*, disinfection by-products, and risk of bladder cancer in Spain. Environ. Health Perspect. 118:1545–5.

Clark C, Smith P (1992). The survival of *Pseudomonas aeruginosa* during bromination in a model whirlpool spa. Lett Appl Microbiol. 14(1):10–12 [cited by Kim J, Sobsey M (2013)].

Clasen TF, Parra GG, Boisson S et al. (2005). Household-based ceramic water filters for the prevention of diarrhea: a randomized, controlled trial of a pilot program in Columbia. Am J Trop Med Hyg. 73:790–95 [cited by Coulliette et al. (2010)].

Clasen TF, Brown J, Collin SM (2006). Preventing diarrhea with household ceramic water filters: assessment of a pilot project in Bolivia. Int J Environ Health Res. 16:231–239 [cited by Coulliette et al. (2010)].

COT (2000). Committee on Toxicity of Chemicals in Food Consumer Products and the Environment, Statement on the 1997 total diet study - fluorine, bromine and iodine, in COT statement 2000/05; pp. 26–28.

Coulliette AD, Peterson LA, Mosberg JAW et al. (2010). Evaluation of a new disinfection approach: Efficacy of chlorine and bromine halogenated contact disinfection for reduction of viruses and microcystin toxin. Am J Trop Med Hyg. 82(2):279–88.

DEFRA (2006). Bromine. Expert panel on air quality standards. United Kingdom Department for Food, Environment and Rural Affairs [cited by PHE (2009)].

Enger KS, Leak E, Aw TG et al. (2016). Antibacterial and antiviral effectiveness of two household water treatment devices that use monobrominated hydantoinylated polystyrene. J Water Health. 14(6):950–60.

EA (2005). A review of the toxicity and environmental behaviour of bromine in air. United Kingdom Environment Agency (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/290734/scho0105bimu-e-e.pdfa, accessed February 2015).

EMEA (1997). Bromide, sodium salt. Summary report. London, European Agency for the Evaluation of Medicinal Products, Committee for Veterinary Medicinal Products, March (EMEA/MRL/182/97-Final (http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500011113.pdf, accessed November 2017).

Farah S, Aviv O, Laout N, Ratner S, Domb AJ (2015). Antimicrobial N-brominated hydantoin and uracil grafted polystyrene beads. J Control Release. 216:18–29.

Floyd R, Johnson JD, Sharp DG (1976). Inactivation by bromine of single poliovirus particles in water. Appl Environ Microbiol. 31:298–303.

Floyd R, Sharp D, Johnson J (1978). Inactivation of single poliovirus particles in water by hypobromite ion, molecular bromine, dibromamine and tribromamine. Environ Sci Technol. 12(9):1031–35.

Freund A, Johnson JD, Sobsey M (2010). Comparative inactivation of poliovirus in reclaimed water by bromine and chlorine. In: Proceedings. International Water Association Biennial Meeting, Montreal QC, January 2010.

Giller SF, Le Curieux F, Erb F, Marzin D (1997). Comparative genotoxicity of halogenated acetic acids found in drinking water. Mutagenesis. 12(5):321–28.

Goodenough RD (1964). Variables on the bactericidal activity of bromine in water. Swimming Pool Age [cited by Kim J, Sobsey M (2013)].

HCN (2005). Health-based reassessment of administrative occupational exposure limits; Bromine. Health Council of the Netherlands (http://www.gr.nl/en/publications/gezonde-arbeidsomstandigheden/health-based-reassessment-of-administrative-occupatio-123, accessed February 2015).

IARC (1999). Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. Lyon, International Agency for Research on Cancer; pp. 481–496 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 73).

IPCS (1999). Bromine. Poisons Information Monograph. PIM 080. Geneva, World Health Organization, International Programme on Chemical Safety [cited by PHE (2009)].

Ivanov NG, Klyachkina AM, Germanova AL (1976). Experimental materials on hygienic regulation of bromine and hydrogen bromide in the air of the working zone. Gig Tr Prof Zabol *20*(3):36–39 (in Russian) [cited by USEPA (2005)].

Johannesson JK (1958). Anomalous bactericidal action of bromamine. Nature. 181:1799–1800 [cited by Kim J, Sobsey M (2013)].

Johnson J, Overby R (1970). Bromine and bromamine disinfection chemistry. In: Proceedings. National Specialty Conference on Disinfection, Amherst. American Society of Civil Engineers; pp. 37–60 [cited by Kim J, Sobsey M (2013)].

Johnson JD, Sun W (1975). Bromine Disinfection of Wastewater. In: Johnson J, ed. Disinfection: Water and Wastewater. Ann Arbor: Ann Arbor Science Publishers, Inc.; 179–91 [cited by Kim J, Sobsey M (2013)].

Keswick BH, Fujioka RS, Burbank NC et al. (1978). Comparative disinfection efficiency of bromine chloride and chlorine for poliovirus. J Am Water Works Assoc. 70(10):573–77.

Keswick BH, Fujioka RS, Loh PC et al. (1982). Comparative Disinfection by Bromine Chloride and Chlorine of Viruses and Bacteria in Wastewater. J Water Pollut Control Fed. 52(10): 2581–88.

Kim J (2014). Critical review of studies on bromine disinfection of water and wastewater: Literature review. The University of North Carolina at Chapel Hill, Department of Environmental Sciences and Engineering (https://cdr.lib.unc.edu/search/uuid:35ed8d6a-85a0-4569-ae0b-b72a18157c1b, accessed February 2016).

Krasner SW, McGuire MJ, Jacangelo JG et al. (1989). The occurrence of disinfection by-products in US drinking water. J Am Water Works Assoc. 81:41–53.

Krusé CW, Hsu Y-C, Griffiths AC et al. (1970). Halogen action on bacteria, viruses, and protozoa. In: Proceedings. National Specialty Conference on Disinfection, Amherst: American Society of Civil Engineers. pp. 113–36 [cited by Kim J (2014)].

Linder RE et al. (1994). Acute spermatogenic effects of bromoacetic acids. Fundam Appl Toxicol. 22:422–30.

Lindley AC (1966). The activity of bromine against f2 bacteriophage and its host *Escherichia coli* K-12 under various aqueous conditions [thesis]. Baltimore: Johns Hopkins University, School of Hygiene and Public Health [cited by Kim J (2014)].

McTigue NE, Cornwell DA, Graf K, Brown R (2014). Occurrence and consequences of increased bromide in drinking water sources. J Am Water Works Assoc. 106 (11):E492–E508.

Mercado-Burgos N, Hoehn RC, Holliman RB (1975). Effect of halogens and ozone on *Schistosoma* ova. J Water Pollut Control Fed. 47(10): 2411–19.

McLennan SD, LA Peterson, JB Rose (2009). Comparison of point-of-use technologies for emergency disinfection of sewage-contaminated drinking water. Appl Environ Microbiol. 75(22):7283–86.

NSF (2011). Bromine/bromide (CAS# 7726-95-6/24959-67-9) - Oral risk assessment document (http://www.techstreet.com/standards/nsf-bromine-bromide-2011?product_id=1848270, accessed February 2015).

NTP (1985). NTP Technical report on the toxicology and carcinogenesis studies of chlorodibromomethane (CAS NO. 124-48-1) in F344/N rats and B6C3F1 mice (gavage studies) (http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr282.pdf, accessed February 2015).

NTP (1987). NTP Technical report on the toxicology and carcinogenesis studies of bromodichloromethane (CAS NO. 75-27-4) in F344/N rats and B6C3F1 mice (gavage studies) (http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr321.pdf, accessed February 2015).

NTP (1989a). NTP Toxicology and carcinogenesis studies of tribromomethane (bromoform) (CASRN 75-25-2) in F344/N rats and B6C3Fi mice (gavage studies). Technical Report Series No. 350. Research Triangle Park (NC): National Toxicology Program (http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr350.pdf, accessed February 2015).

NTP (1989b). Bromoform. Reproduction and fertility assessment in Swiss CD-1 mice when administered by gavage. Research Triangle Park (NC): National Toxicology Program.

NTP (2000a). Water disinfection byproducts (bromoacetic acid). Research Triangle Park (NC): National Toxicology Program (http://ntpserver.niehs.nih.gov/htdocs/Results_Status/Resstatb/M920034.html, accessed February 2015).

NTP (2000b). Water disinfection byproducts (dibromoacetic acid). Research Triangle Park (NC): National Toxicology Program (http://ntpserver.niehs.nih.gov/htdocs/Results_Status/Resstatw/M960093.html, accessed February 2015).

NTP (2006). Toxicology and carcinogenicity studies of bromodichloromethane (CASRN 75-27-4) in male F344/N rats and female B6C3F1 mice (Drinking water studies). Technical Report Series No. 532. Research Triangle Park (NC): National Toxicology Program (https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr532.pdf, accessed February 2015).

NTP (2009). Studies of bromochloroacetic acid (CAS No. 5589-96-8) in F344/N rats and B6C3F1 mice (drinking water studies). Research Triangle Park (NC): National Toxicology Program.

NTP (2013). Draft OHAT approach for systematic review and evidence integration for literature-based health assessments-February 2013. Research Triangle Park (NC): National Toxicology Program (https://ntp.niehs.nih.gov/ntp/ohat/evaluationprocess/draftohatapproach_february2013.pdf, accessed April 2015).

PHE (2009). Bromine: Toxicological overview. Public Health England (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/337537/Bromine_Toxicological_Overview_phe_v1.pdf, accessed February 2015).

Plewa MJ, Muekkner M, Richardson, S (2008). Occurrence, synthesis, and mammalian cell cytotoxicity and genotoxicity of haloacetamides: An emerging class of nitrogenous drinking water disinfection byproducts. Environ Sci Technol. 42: 955–61.

Regli S, Chen J, Messner M, Elovit M, Letkiewicz F, Pegram R et al. (2015). Estimating potential increased bladder cancer risk due to increased bromide concentrations in sources of disinfected drinking waters. Environ Sci Technol. 49(22):13094–102.

Richardson SD (2011). In: Disinfection by-products: Formation and occurrence in drinking water. In Encyclopedia of Environmental Health; Nriagu, JO, ed. Elsevier: Burlington; Vol. 1; pp. 110–136.

Richardson SD, Plewa M J, Wagner ED, Schoeny R, DeMarini DM (2007). Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. Mutat Res. 636:178−242.

Richardson S (2003a). Disinfection by-products and other emerging contaminants in drinking water. Trends Analyt Chem. 22(10): 666–84.

Richardson SD, Thruston AD Jr, Rav-Acha C et al. (2003b). Tribromopyrrole, brominated acids, and other disinfection byproducts produced by disinfection of drinking water rich in bromide. Environ Sci Technol. 37(17): 3782−93.

Rupp H, Henschler D (1967). Wirkungen geringer chlor- und bromkonzentrationen auf den menschen. Int Arch Gewerbepathol Gewerbehyg. 23:79−90 [cited by HCN, 2005].

Russel DL (2006). Practical wastewater treatment. Hoboken (NJ): John Wiley & Sons.

Sagi A, Baruchin AM, Ben-Yakar Y, Kon M, Eyal A, Mahler D et al. (1985). Burns caused by bromine and some of its compounds. Burns 11:343−50.

Sangster B, Blom JL, Baas C, Loeber JG, Rauws A (1986). Onderzoek naar de invloed van natriumbromide bij menselijke vrijwilligers: III. Bilthoven, Rijksinstituut voor de volksgezondheid en milieuhygiene (RIVM), October (Report No. 348301001) [cited by WHO, 2009].

Scarpino P, Berg G, Chang S, Dahling D, Lucas M (1972). A comparative study of the inactivation of viruses in water by chlorine. Water Res. 6(8):959–65 [cited by Kim J, 2014].

Schlagbauer M, Henschler D (1967). Toxizität von chlor und brom bei einmaliger und wiederholter inhalation. Int Arch Gewerbepathol Gewerbehyg. 23:91–8 [cited by HCN, 2005].

Sharp DG, Floyd R, Johnson JD (1976). Initial fast reaction of bromine on reovirus in turbulent flowing water. Appl Environ Microbiol. 31(2):173–81.

Sharp DG, Floyd R, Johnson JD (1975). Nature of the surviving plaque forming units of reovirus in water containing bromine. Appl Microbiol. 29(1):94–101.

Shukairy HM, Miltner RJ, Summers RS (1994). Bromide's effect on DBP formation, speciation and control: Part I, Ozonation. J Am Water Works Assoc. 86(6):72–87.

Sollo F, Mueller H, Larson T, Johnson J (1975). Bromine disinfection of wastewater effluents. In: Johnson J, ed. Disinfection: Water and Wastewater. Ann Arbor: Ann Arbor Science Publishers, Inc.; 163–77 [cited by Kim J (2014)].

States S, Cyprych, G, Stoner M, Wydra F, Kuchta J, Monnell, J et al. (2013). Marcellus shale drilling and brominated THMs in Pittsburgh, Pa., drinking water. J Am Water Works Assoc. 105:E432–48.

Stauber CE, Ortiz GM, Loomis DP, Sobsey MD (2009). A randomized controlled trial of the concrete biosand filter and its impact on diarrheal disease in Bonao, Dominican Republic. Am J Trop Med Hyg. 80:286–93 [cited by Coulliette et al 2010].

Stratton CE, Ross WE, Chapman S (1981). Cytotoxicity and deoxyribonucleic acid damage associated with bromoacetate. Biochem Pharmacol. 30:1497–1500.

Stringer R, Cramer W, Krusé C (1975). Comparison of bromine, chlorine and iodine as disinfectants for amoebic cysts. In: Johnson J, ed. Disinfection: Water and Wastewater. Ann Arbor: Ann Arbor Science Publishers, Inc.; pp. 193–209 [cited by Kim J (2014)].

Sun G, Allen LC, Luckie EP, Wheatley WB, Worley SD (1995). Disinfection of water by N-halamine biocidal polymers. Ind Eng Chem Res. 34(11):4106–09.

Tanner F, Pitner G (1939). Germicidal action of bromine. Proceedings of the Society for Experimental Biology and Medicine. 143–45 [cited by Kim J (2014)].

Taylor GR, Butler M (1982). A comparison of the virucidal properties of chlorine, chlorine dioxide, bromine chloride and iodine. J Hyg. 89(2):321–328.

Thomas WM, Eccles J, Fricker C (1999). Laboratory observations of biocide efficiency against *Legionella* in model cooling tower systems. ASHRAE Trans 105(SE-99-3); pp. 607–26.

Tobe M, Suzuki Y, Aida K et al. (1982). Studies on the chronic oral toxicity of tribromomethane, dibromochloromethane and bromodichloromethane. Unpublished intra-agency report to the National Institute of Hygienic Sciences. Tokyo Medical and Dental University, Tokyo, Japan; 8–43 [cited by USEPA IRIS, http://www.epa.gov/iris/subst/0222.htm#carc].

USEPA (2010). Bromine Final Registration Review Decision. Case 4015. EPA-HQ-OPP-2009-0167. February. Washington (DC): United States Environmental Protection Agency. Office of Prevention, Pesticides, and Toxic Substances.

USEPA (2005a). Bromine/Bromide. EPA-HQ-OPP-2006-0143-0005. Washington (DC): United States Environmental Protection Agency. Office of Prevention, Pesticides and Toxic Substances (http://www.epa.gov/pesticides/reregistration/bromine/, accessed February 2015).

USEPA (2005b). Drinking water criteria document for brominated trihalomethanes. EPA-822-R-05-011. November 2005. Washington (DC): United States Environmental Protection Agency. Office of Science and Technology (https://nepis.epa.gov/Exe/ZyPDF.cgi/P1006GVD.PDF?Dockey=P1006GVD.PDF, November 2017).

USEPA (2005c). Economic analysis for the final stage 2 Disinfectants and disinfection byproducts rule. EPA-815-R-05-010. December 2005. Washington (DC): United States Environmental Protection Agency.

USEPA (2005d). Drinking water criteria document for brominated acetic acids. EPA-822-R-05-007. November 2005. Washington (DC): United States Environment Protection Agency. Office of Science and Technology.

USEPA (2006). National primary drinking water regulations: Stage 2 Disinfectants and disinfection byproducts rule. Washington (DC): United States Environmental Protection Agency. Office of Science and Technology. Fed Regist. 71:387–493.

USEPA (2009). Bromine summary document: Registration review preliminary work plan. Washington (DC): United States Environmental Protection Agency.

USEPA (2010). Bromine final registration review decision. Case 4015. EPA-HQ-OPP-2009-0167. Washington (DC): United States Environmental Protection Agency.

von Gunten U (2003). Ozonation of drinking water: Part II. Disinfection and by-product formation in presence of bromide, iodide or chlorine. Water Res. 37:1469–87.

Voronin VM, Donchenko AI, Korolev AA (1987). Experimental study of the carcinogenicity of dichlorobromomethane, and dibromochloromethane formed during chlorination of water (Russ.). Gig Sanit. 1:19–21 [cited by USEPA IRIS: https://www.epa.gov/iris#carc, accessed February 2015).

West RC, editor (1984). Handbook of Chemistry and Physics. Boca Raton (FL): CRC Press.

Westerhoff P, Chao P, Mash M (2004). Reactivity of natural organic matter with aqueous chlorine and bromine. Water Res. 38:1502–13.

WHO (2004a). Disinfectants and disinfectant by-products. Environmental Health Criteria 216. Geneva: World Health Organization (http://www.who.int/ipcs/publications/ehc/ehc_216/en/, accessed February 2015).

WHO (2004b). Brominated acetic acids in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. Geneva: World Health Organization (http://www.who.int/water_sanitation_health/dwq/chemicals/brominatedaceticacids.pdf, accessed February 2015).

WHO (2005). Bromate in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. Geneva: World Health Organization (http://www.who.int/water_sanitation_health/dwq/chemicals/bromate260505.pdf, accessed February 2015).

WHO (2009). Bromide in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. Geneva: World Health Organization (http://www.who.int/water_sanitation_health/dwq/chemicals/Fourth_Edition_Bromide_Final_January_2010.pdf, accessed February 2015).

WHO (2011). Evaluating household water treatment technologies: Health based targets and microbiological performance standards. Geneva: World Health Organization (http://www.who.int/water_sanitation_health/publications/2011/household_water/en/, accessed February 2015).

WHO (2017). Guidelines for drinking-water quality: Fourth edition. Geneva: World Health Organization (http://www.who.int/water_sanitation_health/publications/drinking-water-quality-guidelines-4-including-1st-addendum/en/, accessed September 2017).

Williams DE, Elder ED, Worley SD (1988). Is free halogen necessary for disinfection? Appl Environ Microbiol. 54(10):2583–85.

Yamaguchi T, Wei M, Hagihara N, Omori M, Wanibuchi H, Fukushima S (2008). Lack of mutagenic and toxic effects of low dose potassium bromate on kidneys in the big blue rat. Mutat Res. 652(1):1–11.

Appendix A: Methodology

Two initial literature searches were conducted in November 2013 as follows:

i) to update toxicity assessment; and
ii) to update efficacy assessment.

The search strategy and terms are outlined in Box 1 and 2 respectively, below.

Box 1- Search strategy for updating toxicity assessment for bromine

((KEY(human OR animal) OR TITLE-ABS-KEY({in vitro} OR {in vivo})) AND DOCTYPE(ar OR re) AND PUBYEAR > 2004) AND ((TITLE-ABS-KEY(toxicokinetic OR irritation OR sensitisation) OR TITLE-ABS-KEY(genotoxicity OR mutagenicity OR carcinogenicity) OR TITLE-ABS-KEY({Acute toxicity} OR {Repeat dose toxicity} OR {Chronic toxicity}) OR TITLE-ABS-KEY({Reproductive toxicity} OR {Developmental toxicity})) AND DOCTYPE(ar OR re) AND PUBYEAR > 2004) AND (((CASREGNUMBER(7726-95-6) AND DOCTYPE(ar OR re) AND PUBYEAR > 2004))

Box 2- Search strategy for updating efficacy assessment for bromine

(TITLE-ABS-KEY(bromine) AND TITLE-ABS-KEY({drinking water} OR {potable water}) AND TITLE-ABS-KEY(disinfection OR microorganism OR bacteria OR virus OR protozoa OR antimicrobial OR bactericidal OR bacteriostatic)) AND PUBYEAR > 2004.

Searches were carried out using Scopus and Web of Knowledge databases. Titles and abstracts of journal articles identified from the initial literature searches included 24 papers relating to bromine toxicity and 195 papers relating to bromine efficacy, which were reviewed to inform on their potential relevance to the project. For those titles selected, which were included in the document, papers were obtained in full for review to extract key data. Additional searches were carried out up to the closing date for public review (16 December 2016), particularly for identification of “grey” literature and earlier studies.

Part II

Iodine as a drinking-water disinfectant

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List of abbreviations and terms used in Part II

AI	adequate intake
bw	body weight
Ct	product of disinfectant concentration and contact time
DBP	disinfection by-product
GI	gastrointestinal
HAV	hepatitis A virus
HOCl	hypochlorous acid
HIO	hypoiodous acid
HWT	household water treatment
I_2	elemental iodine
IO_3^-	iodate
I^-	iodide
NOAEL	no-observed-adverse-effect level
NTU	nephelometric turbidity units
OI^-	hypoiodite
PBI	protein bound iodine
POU	point-of-use
PPM	parts per million
T4	thyroxine
T3	triiodothyronine
THM	trihalomethane
TRH	thyrotropin releasing hormone
TSH	thyroid stimulating hormone

UL	tolerable upper intake limit
UK	United Kingdom
USA	United States of America
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

1. Introduction

Disinfection of water has greatly contributed to reducing risks to public health from microbiologically-contaminated drinking-water.

Over the centuries numerous water disinfection techniques have been developed that are used in a wide range of applications ranging from large and small public drinking-water treatment plants to point-of-entry and point-of-use (POU) treatment devices.[15] Chlorine has been used for more than 100 years and several other disinfectants have been studied extensively, but in many cases, questions remain with respect to optimization of biocidal effectiveness under a range of conditions (i.e. efficacy), the chemistry of the formation and toxicological significance of disinfection by-products (DBPs), interactions with other water constituents, and the effectiveness and toxicology of disinfectant residuals. Most chemical disinfectants can react with natural organic matter or breakdown to produce unwanted by-products. Many newer products and applications are being developed and marketed for use, particularly in developing countries, and even more unanswered questions exist about some of those products, including efficacy and DBP formation.

Iodine is an essential nutrient. In addition, it has been used generally as an antiseptic for skin wounds, as a disinfecting agent in hospitals and laboratories, and in the production of pharmaceuticals. In terms of the disinfection of drinking-water, iodine is commonly used in the form of tablets or solutions during emergencies and by travellers (Ongerth et al., 1989; Backer & Hollowell, 2000). At regular intervals, there is renewed interest in the use of iodine as an alternative disinfectant to chlorine (and other disinfectants) for drinking-water.

Iodine-based disinfection of water has a long history: iodine in concentrations between 2.5–7 mg/L (equivalent to parts per million [ppm]) has been used for potable water treatment since the early 1900s, especially for military operations (Hitchens, 1922; Vergnoux, 1915). Tablet formulations have been used since the 1940s to ensure the microbiological safety of drinking-water for military personnel deployed in the field (Chang & Morris, 1953). Also, in more recent times, iodine (and bromine) has become attractive for particular applications. Elemental iodine is used, for example, as a drinking-water disinfectant aboard space vessels at a residual concentration of approximately 2 ppm (Atwater et al., 1996). The more general use of iodine is impeded, however, by the potential for excess iodine intake, cost and the possibility of the formation of toxic DBPs.

The emphasis of this literature review is to evaluate available evidence on the efficacy and toxicity of iodine as a water disinfectant. Information included in this review was initially obtained using a targeted literature search strategy, with inclusion dates up to November 2013 and further "ad-hoc" searches were carried out up to the closing date for public review (16 December 2016). Additional details of the search strategy are included in Appendix 1.

[15] Point-of-use devices treat only the water intended for direct consumption (drinking and cooking), typically at a single tap or limited number of taps, while point-of-entry treatment devices are typically installed to treat all water entering a single home, business, school, or facility.

2. Disinfection characteristics and efficacy

2.1 Chemistry basics

Iodine, chlorine, bromine and fluorine belong to the halogen group of elements in the periodic table. All of the halogens share the common property of being oxidants with seven electrons in their outer shell. As oxidizing agents, halogens accept an electron to become the analogous halide ion. Different halogens vary in their oxidation potential and the halogen with the strongest oxidative power is fluorine, followed by chlorine, bromine, and iodine. Their reactivities are directly correlated with their electronegativities, which are as follows (based on the Pauling nomenclature of electronegativity values):[16]

fluorine (3.98) > chlorine (3.16) > bromine (2.96) > iodine (2.66).

The reactivities of the given halogens therefore decreases from left to right. Nevertheless, the usefulness of a particular halogen as a disinfectant is determined not only by its reactivity, but also by its selectivity, chemical stability and other factors including the potential to form by-products. Fluorine, the most reactive of all elements of the periodic table, is so unstable that it reacts with surrounding water molecules in a violent reaction forming hydrogen fluoride and oxygen. The reactivity of other halogens is more selective, making them more suitable for practical applications. Among the three halogens used for disinfection purposes (chlorine, iodine and bromine), iodine has the highest atomic weight and is the only one to exist as a solid at room temperature.

2.1.1 Water solubility, taste and odour

Elemental iodine is less soluble in water than chlorine or bromine. Water solubility depends on pH and temperature and is reported to be 0.03 mg/L at 20 ºC, 0.78 mg/L at 50 ºC and 4.45 mg/L at 100 ºC (West, 1984). A saturated aqueous solution of iodine can be produced by passing water through a column of crystalline iodine. The iodine concentration achieved will be approximately 200 mg/L at 10 ºC and 400 mg/L at 30 ºC (Chang, 1968). This concentrated solution can be diluted to achieve the desired concentration of iodine.

Free halogen residuals usually produce tastes and odours in potable water. Bryan et al. (1973) compared taste threshold determinations of chlorine, iodine and bromine residuals in water. The threshold taste values for chlorine residuals varied with pH: 0.075 mg/L at pH 5.0; 0.156 mg/L at pH 7.0; and 0.450 mg/L at pH 9.0. In contrast, threshold taste values for iodine did not vary appreciably with pH, ranging from 0.147 to 0.204 mg/L. In contrast to chlorine, which has a high vapour pressure and readily volatilizes, especially in the presence of sunlight or higher temperatures, iodine has a low vapour pressure resulting in little loss by volatilization (Black et al., 1970).

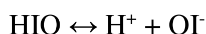
2.1.2 Chemical speciation of iodine in water and corresponding disinfection powers

Once elemental iodine (I_2) is added to water, it hydrolyses in a pH-dependent manner to form hypoiodous acid (HIO) and iodide (I^-). The overall stoichiometry of iodine hydrolysis between pH 2 and 7 is given below; for a more detailed description of iodine hydrolysis, the reader is referred to Lengyel et al. (1993):

$$I_2 + H_2O \leftrightarrow HIO + I^- + H^+$$

[16] The Pauling scale is a dimensionless relative quantity that describes the electronegativity of an atom in the periodic table.

Similar to hypochlorous acid (HOCl), hypoiodous acid can deprotonate to form hypoiodite (OI^-) according to the following general reaction:



$$HIO \leftrightarrow H^+ + OI^-$$

The different chemical species of iodine vary in their disinfection power. The active disinfectants are elemental iodine and hypoiodous acid (Backer & Hollowell, 2000). Other species including iodide, iodate (IO_3^-) and hypoiodite have mild or little antimicrobial activity (Chang, 1958). Comparing the two disinfection-active chemical species, the oxidizing power of hypoiodous acid is nearly twice that of elemental iodine (West, 1984). A comparison of oxidizing potentials with the equivalent chlorine species is shown in Table 1.

Table 1: Comparison of oxidizing potentials of iodine and chlorine species (West, 1984)

Chemical species	Oxidizing potential (in volts)
I_2	0.535
HIO	0.987
Cl_2	1.358
HOCl	1.482

Cl_2–chlorine

The disinfection efficacy of the different chemical species depends not only on oxidizing potential, but also on penetration power. Elemental iodine has higher penetrating power than hypoiodous acid (White, 1992).

A comprehensive study of disinfection efficacy was performed by Chang & Morris (1953). Iodine concentrations in the range 5–10 ppm were found to be effective against different types of microorganisms within 10 min at room temperature. Organisms tested included enteric bacteria, amoebic cysts, cercariae, leptospira and viruses. Overall, different classes of microorganisms have different susceptibilities to iodine: vegetative bacteria tend to be most sensitive, whereas viruses have an intermediate sensitivity and protozoa tend to be more resistant (Backer & Hollowell, 2000). Moreover, elemental iodine and hypoiodous acid contribute to different extents to the disinfection efficacy against different microbes. Chemical speciation is highly pH dependent (addressed in the next section). Disinfection sometimes follows first-order kinetics with the primary determinants of effectiveness being disinfectant concentration and time of exposure of the microorganism (expressed as Ct in mg·min/L i.e. the product of disinfectant concentration (C in mg/L) and contact time (t in min). Departures from first-order kinetics can occur due to such phenomena as declines in iodine concentration over time, microbial aggregation and microbial protection by other particles.

2.1.2.1 Effect of pH

Both the hydrolysis and the subsequent equilibrium between elemental iodine and hypoiodous acid are pH-dependent, but the effect is not as pronounced as with chlorine. Table 2 shows the proportions of

elemental iodine, hypoiodous acid and hypoiodite for a pH range between pH 5 and 9, in comparison with the chlorine equivalents.

Table 2: Effect of pH on the speciation of iodine and chlorine (0.5% titratable iodine)

pH	Iodine			Chorine		
	I_2 (%)	HIO (%)	OI^- (%)	Cl_2 (%)	HOCl (%)	OCl^- (%)
5	99	1	0	0.5	99.5	0
6	90	10	0	0	99.5	0.5
7	52	48	0	0	96.5	3.5
8	12	88	0.005	0	21.5	78.5
9	-	-	-	0	1	99

Cl_2–chlorine; OCl^-–hypochlorite
Adapted from Chang (1958), Black et al. (1970) and Ellis & van Vree (1989).

Higher pH results in a progressive decline in elemental iodine with a shift in the equilibrium toward hypoiodous acid. As the active disinfectants are elemental iodine and hypoiodous acid, to use iodine most effectively as a disinfectant, the pH should be near neutral to mildly alkaline (pH 7–7.5) to allow adequate levels of both elemental iodine and hypoiodous acid (Table 2). At ≥ pH 8.0, hypoiodous acid was reported to be unstable and to slowly decompose into iodate and iodide (Ellis & van Vree, 1989). However, in the absence of a stronger oxidant such as chlorine, iodate formation does not occur readily (Black et al., 1970). More detailed information about speciation and its pH dependence is available in Gottardi (1999). Overall, the disinfection effectiveness of iodine is not as heavily influenced by pH as chlorine is. Hypochlorite formation increases at values > pH 7. Strong evidence has been provided that hypochlorite has less disinfection power that can be influenced by concentrations of anions, such as sodium and potassium (Chang & Morris, 1953; Keirn & Putnam, 1968; Haas et al., 1986; Jensen et al., 1980).

The progressive decline of free iodine residual and increasing proportion of hypoiodous acid with increasing pH also has fundamental consequences for the disinfection power of iodine. Different effects occur along this gradient and different iodine species have different disinfection efficacies for different groups of microbes (Ellis et al., 1993). In generalized terms, elemental iodine is primarily effective against bacterial spores and protozoan cysts, whereas hypoiodous acid is known to be an effective bactericide and virucide (Ellis et al., 1993). For example, Chang (1966) reported elemental iodine to be 2–3 times more effective against *Entamoeba histolytica* cysts than hypoiodous acid, whereas hypoiodous acid was found to be approximately 40 times more effective than elemental iodine against viruses. Hypoiodous acid also had greater germicidal activity against vegetative bacteria than elemental iodine (e.g. hypoiodous acid was found 3–4 times more effective than elemental iodine against *Escherichia coli*).

The effect of pH on speciation of iodine and the resulting effect on disinfection efficacy is exemplified in a study by Taylor & Butler (1982). The authors reported that iodine was more effective against

poliovirus at pH 9 than at lower pH values, probably due to the fact that at this pH most iodine will exist in the form of hypoiodous acid, which has greater virucidal activity than elemental iodine (Chang, 1966). The virucidal efficacy of hypoiodous acid and elemental iodine was reported to be respectively 4–5 times and 200 times less than hypochlorous acid (Clarke et al., 1964).

As the prevalent iodine species varies with pH, the most suitable pH range for the disinfection of different microbial groups will also vary. Overall trends in disinfection power of the various iodine species for different groups of microorganisms are summarised in Table 3 below.

Table 3: Trends in disinfection power for different iodine species when applied to different microbial groups and pH range where the most effective disinfectant prevails (Taylor and Butler, 1982)

Microbial group	Disinfection effectiveness of different iodine species	Suitable pH range for disinfection
Bacteria (vegetative)	HIO > I_2	pH 5–8
Bacteria (spores)	I_2 > HIO	pH 5–7
Viruses	HIO > I_2	pH 8–9
Protozoa[a]	I_2 > HIO	pH 5–7

Information about suitable pH is based on abundance of the most effective iodine species for a certain microbial group. Overall, vegetative bacteria are most susceptible.
[a] elemental iodine is effective against *Giardia* cysts but not effective against *Cryptosporidium* oocysts.

While these tendencies hold true for microorganisms suspended in clean water, disinfection efficacies can be altered in the presence of turbidity and in poor quality water. The underlying reason is that although hypoiodous acid is more reactive and has a higher oxidation potential, it has less penetrating power than elemental iodine. If microorganisms are sheltered in particles, as found in turbid and poor quality water, the enhanced penetrating power becomes more important than overall reactivity (Ellis et al., 1993). Even if microorganisms are not attached to particles, the higher oxidation potential of hypoiodous acid (prevalent at pH 8–9) might lead to preferential reaction with oxidizable organic matter (e.g. in the case of turbid water with elevated total organic content) leaving less residual available for disinfection (Ellis et al., 1993). More information on the effects of turbidity can be found in Section 2.2.2. Karalekas et al. (1970) reported the effect of 1 ppm iodine on 6 different bacterial species. A noticeable reduction of the germicidal effect was reported when increasing the pH from 5 to 9 (while noticing little difference between pH 5 and 7). A slight decline in disinfection efficacy against *E. coli* and faecal streptococci was also observed by Ellis & van Vree (1989) when increasing the pH from pH 7 to 8.5. The reduced efficacy of iodine at higher pH was explained by Ellis et al. (1993) by the progressive decline of free iodine residual. In studies on the effects of water quality and pH on inactivation of hepatitis A virus (HAV), poliovirus 1 and echovirus 1 by 8 and 16 mg/L doses of iodine, HAV was inactivated more efficiently by iodine than were the other two test viruses, and the order of virus inactivation was:

HAV > echovirus 1 > poliovirus 1 (Sobsey et al, 1991).

Virus inactivation was generally more effective at higher pH, in cleaner water, at higher temperature and at higher iodine dose.

The partitioning into different chemical species with different disinfection power is not only dependent on pH, but also the initial concentration of titratable iodine (Chang, 1966). The lower the iodine concentration, the higher the relative percentage of hypoiodous acid at a given pH.

2.1.2.2 Effect of temperature

For iodine, the pH effect on disinfection efficacy is more noticeable at lower temperatures (Ellis et al., 1993). In general, higher doses of iodine are required at lower temperatures to achieve the same degree of disinfection (Chambers et al., 1952). As with chlorine, the reason can be found in the lower reactivity of the disinfectant at lower temperature as the reaction rate is negatively correlated with the temperature via the reaction constant. At low temperatures near the freezing point, a higher contact time is therefore required to compensate for the loss in reactivity. Chang & Morris (1953) reported a doubling of required contact time when decreasing the temperature from 25 ºC to near freezing temperature (2–3 ºC). Temperature dependence of disinfection efficacy is however not fully understood as different effects might counter-act each other. Ellis et al. (1993) found better germicidal performance at 5 ºC than at 20 ºC, whereas an increase to 35 ºC further reduced the effectiveness. This contradicts previous findings but was explained by an increase in the oxidation potential at higher temperatures leading to faster inactivation and less residual. The authors concluded that higher temperatures favour increased hydrolysis leading to higher concentrations of hypoiodous acid which, in turn, has a higher oxidation potential than elemental iodine.

2.2 Efficacy of iodine

2.2.1 Bactericidal efficacy of iodine

Chang & Morris (1953) investigated the bactericidal effects of a number of Ct combinations of iodine on different bacterial pathogens. Tests conducted with *E. coli* showed that iodine concentrations of ≥ 0.05 ppm consistently reduced the culturability of 10^4 bacteria cells/mL to less than 1 cell/mL within 10 min (25 ºC, pH 8.1–8.5). Other results obtained when exposing 10^6 *E. coli* cells/mL to different iodine concentrations are shown in Table 4 below.

Chang & Morris (1953) reported stronger inactivation than seen with *E. coli* for other enteric bacteria including, *Salmonella typhimurium*, *Shigella dysenteriae* and *Vibrio cholera*, with initial iodine concentrations of 7–8 ppm and pH 4.5–8.1. The authors reported that there was no effect of pH on bactericidal efficacy of iodine in the range pH 4.5–8.1. Similar (but declining) results were obtained with values up to pH 10, which is in sharp contrast to the strong pH dependence of chlorine.

Table 4: Bactericidal efficacy of iodine (adapted from Chang & Morris, 1953)

Iodine concentration (in ppm)		Viable cells/100 mL (log_{10} reduction)			
Initial	30 min	5 min	10 min	20 min	30 min
1.0	0	1600 (2.80)	540 (3.27)	240 (3.62)	130 (3.89)
2.0	1.2	4.6 (5.34)	1.0 (6.00)	1.0 (6.00)	2.2 (5.66)
3.0	1.6	4.6 (5.34)	4.6 (5.34)	4.6 (5.34)	< 1 (6.00)
4.0	2.6	24 (4.62)	6.9 (5.16)	2.5 (5.60)	1.0 (6.00)
5.0	3.3	< 1 (6.00)	< 1 (6.00)	< 1 (6.00)	< 1 (6.00)

Bactericidal efficacy of different iodine concentrations to reduce viability of *E. coli* spiked into tap water at an initial concentration of 10^6 cells/mL. A standard iodine dose of between 7 to 9 ppm was used to meet the iodine demand in the tap water and to obtain a bactericidal residual of 1 to 5 ppm. The experiment was performed at 25 °C and a pH between pH 8.1 to 8.5.

Adapted with permission from: Chang S, Morris J (1953). Elemental iodine as a disinfectant for drinking water. Ind Eng Chem. 45: 1009–12. Copyright (1953) American Chemical Society.

2.2.2 Disinfection in the presence of turbidity

There is limited information discussing the effects of turbidity on the disinfection capability of iodine. When testing the effect of ammonium and urea on the efficacy of iodine disinfection, concentrations up to 5 ppm were not found to have any measurable effect on the inactivation of *E. coli* (Chang & Morris, 1953). The same held true when adding different clays in concentrations of up to 500 ppm. It is generally accepted that iodine shows less reactivity with organic nitrogenous impurities compared to chlorine (Punyani et al., 2006) but does react to produce iodamines. The organic colour of water (due to the presence of natural organic substances) is associated with iodine demand and reduced efficacy. At organic colour concentrations > 70 ppm, a doubling of the dose was found to be required for disinfection. A study by Ellis & van Vree (1989) found that when supplementing water with sediments from a natural stream, the stepwise increase in turbidity up to a maximum of 1000 nephelometric turbidity units (NTU) reduced the germicidal effectiveness of iodine.

In general, the disinfection capability of iodine, as with all disinfectants is reduced with increasing turbidity as microorganisms can be protected from the iodine by adsorption to, or enmeshment in, solid particles in water. In addition, there may be an increasing disinfectant demand due to reactions between organic particles and the disinfectant. Sobsey et al. (1991) reported that the inactivation of HAV by iodine at doses of 8 and 16 mg/L was less effective in "dirty water" (i.e. 10 mg/L of a 1:1 mixture of humic and fulvic acids and 5 NTU of bentonite clay turbidity). Ellis et al. (1993) applied iodine to water supplemented with stream sediments to achieve three different turbidity ranges (5–7, 50–54 and 93–97 NTU). Water was additionally adjusted to values of pH 6, 7.5, and 9 and different temperatures (5, 20, and 35 °C). Under all conditions tested, a dose of 3 mg/L iodine with a contact time of 30 min was found sufficient to inactivate *E. coli*. When supplementing water with digested sludge (up to the highest turbidity range) or raw sludge (5–7 NTU), doses of 8 or 10 mg/L iodine were necessary to achieve inactivation for the same contact time. The authors argued that the nature of the turbidity was more important than its density. It was hypothesized that the higher organic and nitrogen content of the sludge

compared to the stream sediments was responsible. When comparing disinfection efficacy with chlorine at 1.0 mg/L, chlorine was reported to be slightly more effective for water containing stream sediments (e.g. at 20 ˚C and pH 7.5 the percentage inactivation of *E. coli* ranged from 99.52–100% for chlorine at turbidities between 94–5 NTU, with the corresponding values for iodine ranging between 98.58–99.97%). However, iodine was found more efficient in cases where sludge was added, particularly at the higher temperature and pH values (e.g. at 20 ˚C and pH 7.5, percentage inactivation of *E. coli* ranged from 21.7–38.74% for chlorine at turbidities between 97–5 NTU, with the corresponding values for iodine ranging between 42.40–50.70% [Ellis et al., 1993]).

2.2.3 Iodine-based disinfection products

Iodine-based disinfection products available today can be divided into two categories; iodine solutions and iodine resins. A summary of the disinfection capabilities of each is given in Table 5.

2.2.3.1 Iodine solutions

Iodine solutions are made by adding iodine (e.g. tincture of iodine, a 2% iodine solution), or by adding a tablet containing iodine, a carrier, and stabilizing agents to enhance dissolvability (e.g. tetraglycine hydroperiodide, sodium acid pyrophosphate and talc) to the water to be disinfected. The United States Army has utilised iodine as a drinking-water disinfectant since 1952, issuing iodine-based tablets to American soldiers. The United States Army continues to provide iodine-based tablets in addition to other emergency field drinking-water products. Today, there are several commercial off the shelf individual water purification devices that use iodine for disinfection.

For non-drinking-water disinfectant applications, iodine has been compared with chlorine and bromine as alternative disinfectants for swimming pools. Although not directly related to the use of iodine as a drinking-water disinfectant, these studies provide useful evidence of the efficacy of iodine for water disinfection and the tolerance of individuals to residual concentrations of iodine. Typical of the now dated studies, Black et al. (1959) investigated the effectiveness of iodine solutions for disinfecting public and domestic swimming pools in Florida, USA. The solutions were added in the form of potassium iodide over three weeks (twice weekly) at a dose equivalent to 1–2 ppm of iodine. The crystalline potassium iodide was either spread over the surface of the pool together with a small amount of chlorine to release free iodine, or uniformly distributed through a recirculation system. Iodine was found to be fully effective in meeting bacteriological standards. The amount of iodine required for public pools with high bathing activity was reported to be only slightly higher than required for pools with low bathing load (domestic pools), suggesting that the iodine residual appeared to be less sensitive to bathing load than the chlorine residual. The authors considered that this was due to iodine not reacting with ammonium, as does chlorine. However, the reason is more likely to be due to the direction of the equilibrium between iodine and ammonium. The study concluded that a daily dosage of 1 or 2 ppm of iodine would suffice to disinfect domestic or public pools. This translates into a residual concentration of approximately 0.2 ppm (Black et al., 1959).

Available evidence indicates that iodine solutions can be effective disinfectants against bacteria and, to a lesser extent, viruses. Recommended dosages range from between 4 and 16 mg/L with contact times ranging from 20–35 min, resulting in Ct values of 80–560 mg·min/L to achieve a 6 $\log_{10}$ reduction/inactivation of bacteria and a 4 $\log_{10}$ reduction/inactivation of viruses. Iodine is least effective against protozoa and in particular, ineffective against *Cryptosporidium parvum* oocysts, where the doses and contact times required are impractical for drinking-water disinfection (Gerba et al., 1997).

2.2.3.2 Resins

Iodine resins are solid-phase iodine disinfectants through which water is passed, with disinfection occurring through direct contact of the microorganisms and the iodine sorbed onto the resin as exchangeable ions. Iodine resins are generally considered demand-release disinfectants as iodine is released to the microorganism after coming into contact with the resin, and generally produce a dilute iodine residual. As is the case with iodine solutions, available data on iodine resins indicates they are effective disinfectants against bacteria, viruses, and some protozoa (Punyani et al., 2006; Vasudevan & Tandon, 2010). However, the resins have not, to date, been proven effective against *Cryptosporidium* oocysts.

Resin-based iodine release systems comprise (1) organic iodide compounds, (2) iodophors (iodine in combination with non-ionic surfactants) and (3) iodine incorporated resins (Punyani et al., 2006). Iodine resins used in individual water purification devices are generally combined with other treatment processes, such as filtration, to remove iodine residuals and iodine-resistant microorganisms. Modern applications of resins have resulted in an increase in their use. Devices used by National Aeronautics and Space Administration for space flights are prominent examples. Controlled release of iodine on board the International Space Station Alpha is achieved through a flow-through device (referred to as Microbial Check Valve) containing an iodinated polymer (Atwater et al., 1996; Gibbons et al., 1990). The iodine residual concentration released into the water stream flow is a maximum of approximately 2 mg/L. The released dissolved iodine undergoes a series of hydrolytic reactions resulting in the formation of iodide, triiodide, hypoiodous acid and hypoiodide, with different biocidal capabilities associated to each inorganic species (Punyani et al., 2006; Venkobachar & Jain, 1983). Another resin employed by National Aeronautics and Space Administration consists of the iodine-polyvinyl pyrrolidone (iodine-PVP) complex which releases iodine and iodide at concentrations of 2–3 mg/L and 1.5 mg/L, respectively (Punyani et al., 2006). Again, the dissolved iodine speciates into a variety of different inorganic compounds. Greatest biocidal activity can be attributed to iodine and hypoiodous acid (Gazda et al., 2004; Gottardi, 1991).

A number of other resins have been developed with some promising results. Considering the potential health impact of released aqueous iodine, Punyani et al. (2006) proposed the development of resins that do not release iodine, but inactivate microorganisms during flow through by contact. Whereas resins loaded with iodate did not exhibit a germicidal effect, polyiodide resins were reported to be efficient for drinking-water disinfection (Vasudevan & Tandon, 2010).

Table 5: Disinfection capabilities of iodine solutions and resins[a]

Parameter	Iodine solutions	Iodine resins
General	Cysts most resistant. Achieving *Giardia* cyst inactivation will ensure adequate bacteria and virus inactivation.	Cysts most resistant. Achieving *Giardia* cyst inactivation will ensure adequate bacteria and virus inactivation.
Bacteria	4 log_{10} reduction at Ct values < 10 mg·min/L.[b]	Triiodide and pentaiodide resins can potentially provide a 6 log_{10} reduction under most natural water quality conditions.
Viruses	2 log_{10} reduction at Ct values of 15–75 mg·min/L.[c] 4 log_{10} reduction for HAV, poliovirus 1 and echovirus 1 by doses of 8 and 6 mg/L in 60 min or less, depending on water quality, pH and temperature.	Triiodide and pentaiodide resins can potentially provide a 4 log_{10} virus reduction under most natural water quality conditions.
Giardia cysts	3 log_{10} reduction at Ct values of 45–241 mg·min/L at > 20 °C. Provide additional contact time and higher Ct values at < 20 °C to achieve 3 log inactivation.	3 log_{10} reduction at 25 °C and 4°C using pentaiodide resin compared with 0.2–0.4 log reduction with triiodide resin. Additional contact time after passing through resin needed compared to iodine solutions.
Cryptosporidium oocysts	Not effective at practical Ct values.[d]	Not effective at practical Ct values.[d]
Effect of temperature	Major effect. Increase contact time and/or dose at colder temperatures. Ct values up to 720 mg·min/L recommended for *Giardia* cyst inactivation in colder waters (< 5 °C).	Major effect.[e] Increase contact time after passing through pentaiodide resin at colder temperatures. Allow up to 40 min additional contact time for *Giardia* cysts inactivation in colder waters (< 5 °C).
Effect of pH	Minor effect. Generally effective over typical pH levels for natural waters.	Minor effect. Generally effective over pH range typical for natural waters.
Effect of Turbidity	Affects disinfection capability. Provide additional contact time and/or increase iodine dose in more turbid waters.	Affects disinfection capability. Heavy organic matter loading can significantly reduce disinfection capability.

Source: Adapted from Technical Information Paper #-31-005-0211 (2011).

[a] Testing was carried out using iodinated resins only, with no filter applied, as would normally be found in individual water purification devices. Whilst bacteria and viruses are not physically filtered by the resin, due to electrostatic interactions, *Giardia* cysts and *Cryptosporidium* oocysts are filtered by the resin bed. However, subsequent use of the resin leads to release or flushing of cysts and oocysts, which could remain viable.

[b] Assuming a contact time of 20 min, a 0.5 mg/L iodine residual would be necessary to provide 4 log_{10} reduction of *E. coli* at near neutral pH at any temperature encountered in natural waters (20 min x 0.5 mg/L = 10 mg·min/L).

[c] 2 log_{10} reduction at near neutral to alkaline pH levels (pH 6–10) and various water temperatures (5–30 °C) at Ct values of 15–75 mg·min/L with the higher Ct values occurring at lower pH levels and colder water temperatures.

[d] Gerba et al. (1997).

[e] Temperature dependence of disinfection efficacy is not fully understood as different effects might counter-act each other. Current “best-practice” is given.

The residual iodine concentration with iodine resins is much less than concentrations from the recommended doses of tablet or liquid forms of iodine (Table 6).

Table 6: Residual iodine in demand-free water using recommended doses of available product

Iodine products	Recommended dose per litre of water[a]	Residual concentration of iodine
Iodine tablets (tetraglycine hydroperiodide)	1–2 tablets	8–16 mg/L
Iodine solution (tincture; 2%)	0.25–0.5 mL	4–8 mg/L
Providone-iodine solution (10%)	0.35–0.70 mL	4–8 mg/L
Saturated iodine crystals in water	13–26 mL	4–8 mg/L
Pentaiodide resin (room temp)	-	1–2 ppm[b]
Triiodide resin (room temp)	-	0.2 ppm
Triiodide resin at 42 ºC	-	1 ppm
Triiodide resin at 71 ºC	-	6–10 ppm
Triiodide resin and granular activated carbon	-	0.01 ppm

Modified from Backer & Hollowell (2000).

[a] Lower dose in clear, warm water (> 15 ºC), higher dose in very cold or cloudy water. Disinfection activity is a function of iodine, contact time and water temperature.

[b] mg/L equivalent to ppm.

2.2.4 Comparison of efficacy with chlorine

The properties of iodine and chlorine differ in several important ways. Although speciation of iodine is pH dependent, a notable property of iodine is that it provides protection across a wider pH range than chlorine (Black et al., 1965; Ellis et al., 1993). Compared with chlorine, iodine has also greater chemical stability and shows less reactivity with organic nitrogenous contaminants, leaving a higher free residual; the reduced reactivity with organic contaminants leads to a reduction in iodine demand (Backer & Hollowell, 2000). On the negative side, less is known about iodine in regard to disinfection performance on some important pathogens in waters of different quality and above all on potential negative health impacts. In addition, the lower reactivity of iodine compared to chlorine requires the use of higher doses. A comparison with chlorine is given below:

Commonalities with chlorine:

- Different classes of microorganisms have different susceptibilities (e.g. neither are effective against *Cryptosporidium* oocysts); and
- Effectiveness is impacted by temperature, concentration, contact time, pH and organic content.

Advantages of iodine over chlorine:

- Provides protection across a wider pH range;

- Greater chemical stability;
- Less disinfection demand through reduced reactivity with organic nitrogenous impurities;
- Germicidal action of iodine occurs over a wider range of water quality conditions than chlorine; and
- Works better for water of poor quality.

Disadvantages of iodine compared to chlorine (these relate mainly to potential health concerns, as discussed fully in Section 3):

- The safety of long-term consumption of iodine when used as a drinking-water disinfectant is not established;
- Excess iodine intake is not safe for people with thyroid disease; and
- Higher concentrations are required as compared to chlorine to achieve comparable disinfection efficacy.

2.2.5 World Health Organization International Scheme to Evaluate Household Water Treatment Technologies

Existing evidence pertaining to the effectiveness of disinfectants against all three classes of pathogens which cause diarrhoeal disease, may not reflect actual use conditions in the field (e.g. water of varying quality, shorter contact times). In order to comprehensively assess effectiveness, WHO has set tiered health-based log_{10} reduction performance targets for household water treatment (HWT) products for the removal and/or inactivation of bacteria, viruses and protozoa (WHO, 2011). These performance targets are based on microbial risk models using assumed levels of reference pathogens in untreated water. Since 2014, WHO has been evaluating products against those performance targets through the WHO International Scheme to Evaluate Household Water Treatment Technologies.[17] Box 1 gives further information on the Scheme. At the time of this report, iodine has not been tested but may be included in future rounds.

[17] http://www.who.int/water_sanitation_health/water-quality/household/scheme-household-water-treatment/en/

Box 1 WHO International Scheme to Evaluate Household Water Treatment Technologies

The objective of the Scheme is to independently and consistently evaluate the microbiological performance of household and POU water treatment technologies. The evaluation considers both turbid and non-turbid water, and is carried out to manufacturers' instructions for daily household use.[17] The results of the evaluation are intended to assist and inform Member States and procuring UN agencies in the selection of these technologies.

The performance targets define treatment requirements in relation to source water quality for each pathogen class as detailed below.

Performance target	**Bacteria** (log_{10} reduction required)	**Viruses** (log_{10} reduction required)	**Protozoa** (log_{10} reduction required)	**Classification** (assuming correct and consistent use)
★★★	**≥ 4**	**≥ 5**	**≥ 4**	Comprehensive protection (very high pathogen removal)
★★	**≥ 2**	**≥ 3**	**≥ 2**	Comprehensive protection (high pathogen removal)
★	Meets at least 2-star (★★) criteria for two classes of pathogens			Targeted protection
–	Fails to meet WHO performance criteria			Little or no protection

The performance of HWT products is classified as 3-star (★★★); 2-star (★★); and 1-star (★), denoting descending order of performance, based on log_{10} reductions of bacteria, viruses and protozoa from drinking-water. Performance that does not meet the minimum target is given no stars. Products that meet 3-star (★★★) or 2-star (★★) performance targets are classified as providing "Comprehensive protection" against the three main classes of pathogens which cause diarrhoeal disease in humans. The use of these products is encouraged where there is no information on the specific pathogens in drinking-water (and a prudent approach is to protect against all three classes), or where piped supplies exist but are not safely managed. Products that meet the performance targets for at least 2-star (★★) for only *two* of the three classes of pathogen are given one star (★) and are classified as providing "Targeted protection". In general, the use of these products may be appropriate in situations where the burden of diarrhoeal disease is high due to known classes of pathogens, such as a cholera outbreak.

3. Safety and toxicity of iodine

The health effects of iodine have been reviewed by a number of international bodies:

- European Food Safety Authority (EFSA, 2014);
- Council for Responsible Nutrition (CRN, 2013);
- USEPA, 2006;
- Agency for Toxic Substances and Disease Registry (ATSDR, 2004);
- World Health Organisation /Food and Agriculture Organisation (WHO/FAO, 2004);
- World Health Organisation (WHO, 2003);
- Expert Group on Vitamins and Minerals (EVM, 2003);
- European Commission Scientific Committee on Food (EC, 2002);
- Institute of Medicine (IOM, 2001); and
- Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1988).

In this section, opinions from expert bodies on intake of iodine, as detailed above, are described. In addition, a detailed assessment of recent[18] toxicological literature for iodine was undertaken and the relevant findings are included here.

3.1 Human exposure

Iodine is an essential dietary element for mammals. It is required for the synthesis and function of the thyroid hormones thyroxin (T4) and triiodothyronine (T3), as well as being the precursor of iodotyrosines. Through these hormones, iodine has an important role in energy-yielding metabolism and on the expression of genes that impact many physiological functions, from embryogenesis to growth and development, neurological and cognitive functions (EFSA, 2014).

The only natural sources of iodine for humans and animals are the iodides in food and water. The use of iodine and iodophors for sanitizing purposes has been reported to result in significant amounts of iodine entering the food chain (Phillips, 1997). The iodine content of foods is highly variable both between food categories as well as within each category. Marine products such as shellfish and molluscs, and eggs and milk are the richest sources of dietary iodine (Phillips, 1997). In Japan, iodine intake exceeds that of most other countries, primarily due to substantial seaweed consumption. Zava & Zava (2011) utilized information from a number of sources including dietary records, food surveys, urine iodine analysis (both spot and 24-hour samples) and seaweed iodine content, to estimate daily Japanese iodine intake. The authors estimated that the Japanese iodine intake averages 1000–3000 µg/day (1–3 mg/day). The iodine content of drinking-water is also highly variable. In Denmark, tap water concentrations of iodine from a number of locations were reported to contain between < 1.0–139 µg/L (median 2.6 µg/L) (Pedersen et al., 1993). Drinking-water in the USA has a reported mean concentration of total iodine of 4 µg/L, with a maximum concentration of 18 µg/L (Andersen et al., 2008). Chronic excessive iodine intake has been linked to development of goitre (enlarged thyroid gland) (Zhao et al., 2000), early onset of sub-clinical thyroid disorders, hyperthyroidism (excessive production and/or secretion of thyroid hormones) and hypothyroidism (diminished production of thyroid hormones), an increased incidence of autoimmune thyroiditis (inflammation of the thyroid gland) and increased risk of thyroid cancer (Laurberg et al., 1998; Teng et al., 2006).

[18] To November 2013, with further ad hoc searches were carried out up to the closing date for public review (16 December 2016).

In contrast, iodine deficiency remains a major public health concern in many countries, including some European countries (WHO/UNICEF/ICCIDD, 2007; Zimmermann & Andersson, 2011; Andersson et al., 2012). Chronic deficiency has been linked with compensatory thyroid hyperplasia with goitre, with an associated increase in risk of thyroid cancer. In an attempt to counteract the deficiency, iodine fortification of salt is recommended by WHO and has been implemented in approximately 120 countries worldwide (WHO/UNICEF/ICCIDD, 2007). Of these, 40 are European countries:[19] it is mandatory in 13 countries, voluntary in 16 and not regulated in the remaining countries. The amount of iodine added varies from 10–75 mg/kg salt with a majority of values in the range 15–30 mg/kg.

3.2 Guideline values

3.2.1. WHO Guidelines for Drinking-water Quality

The WHO Guidelines for Drinking-water Quality (WHO, 2017) did not formally establish a guideline value for iodine. Iodine was last reviewed by WHO for the Guidelines for Drinking-water Quality in 1993, when it was concluded that available data suggested that derivation of a guideline value for iodine on the basis of information on the effects of iodide was inappropriate and there were few relevant data on the effects of iodine. Also, because iodine is not recommended for long-term disinfection, lifetime exposure to iodine concentrations such as might occur from drinking-water disinfection is unlikely (WHO, 2003).

3.2.2 Other values

In 1988, the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1988) set a Provisional Maximum Tolerable Daily Intake for iodine of 1 mg/day (17 µg/kg body weight [bw] per day) from all sources. This upper limit was reaffirmed by WHO in 1994 (WHO, 1994). This was based on the tolerance of high doses of iodine in healthy iodine-replete adults and did not include neonates and young infants. In light of the recognition that excess iodine could lead to hypothyroidism, hyperthyroidism and thyroid autoimmunity in vulnerable individuals, in 2004 the WHO/FAO recommended the following nutrient intakes for iodine (WHO/FAO, 2004):

- Infants and children 0–59 months: 90 µg/day
- Children 6–12 years: 120 µg/day
- Adolescents and adults, from 13 years of age through adulthood: 150 µg/day
- Pregnant women: 200 µg/day
- Lactating women: 200 µg/day

In 2001, the Food and Nutrition Board at the United States National Institute of Medicine recommended the following dietary intakes for iodine (IOM, 2001):

- Infants
  - 0–6 months: 110 µg/day
  - 7–12 months: 130 µg/day
- Children
  - 1–3 years: 90 µg/day
  - 4–8 years: 90 µg/day
  - 9–13 years: 120 µg/day
- Adolescents and Adults
  - Males age 14 and older: 150 µg/day

[19] List of countries available from: http://www.who.int/nutrition/publications/VMNIS_Iodine_deficiency_in_Europe.pdf

  - Females age 14 and older: 150 µg/day

- Pregnant women: 220 µg/day
- Lactating women: 290 µg/day

The same group derived tolerable upper intake levels of between 200 and 1100 µg/day (children between 1–3 years and all adults respectively) from all sources. Recommendations for adults were based on changes in serum thyrotropin concentrations in response to varying levels of ingested iodine in adults, with children's levels obtained by extrapolation from adult levels with adjustment on the basis on body weight (IOM, 2001).

In 2002, the European Commission Scientific Committee on Food (EC, 2002) provided a Tolerable Upper Intake Limit (UL) for iodine of 600 µg/day for adults (including pregnant and lactating women). This value was based on dose-response studies of short duration (two weeks) in small numbers of subjects (n=10–32). An increased response of thyroid stimulating hormone (TSH) to thyrotropin-releasing hormone (TRH) at intakes of 1700–1800 µg/day was reported by Gardner et al. (1988) and Paul et al. (1988) but changes were not associated with any adverse clinical outcome. In a five-year study, Stockton & Thomas (1978) also reported an absence of clinical thyroid pathology following similar intakes. An uncertainty factor of 3 was applied to the highest intake assessed in these studies (1800 µg/day) to derive the UL for adults. For children, an adjustment based on body weight was applied to the adult value. The report concluded that dietary intakes are unlikely to exceed 500 µg/day, since the 97.5 percentile intake in European men is 434 µg/day.

The UK's Expert Group on Vitamins and Minerals (EVM, 2003) set a guidance level for iodine intake, concluding that neither human nor animal data were sufficient to set a UL value. Following assessment of the findings from several clinical studies of supplemental iodine, the author's concluded that 500 µg/day of supplemental iodine "would not be expected to have any significant adverse effects in adults." This led to recommendation of guidance levels of 500 µg/day for supplemental iodine and 930 µg/day for total intake from all sources (EVM, 2003).

The Council for Responsible Nutrition (CRN, 2013) recommended an upper limit for iodine intake of 500 µg/day based on the absence of adverse effects in healthy adults following daily oral intake of iodine supplements of 500 µg. In 2014, the European Food Safety Authority published Adequate Intake (AI) levels for iodine in different age groups (including pregnant and breast-feeding women), based in part on a large epidemiological study in European school-aged children (EFSA, 2014). The study showed that goitre prevalence is lowest for a urinary iodine concentration <100 µg/L, associated with iodine intakes of 150 µg/day in adults.

From this, the following AI levels were recommended:

- Infants (< 1 year of age): 70 µg/day
- Children
  - Aged between 1–3, 4–6 and 7–10 years: 90 µg/day
  - Aged between 11–14 years: 120 µg/day
  - Aged between 15–17 years: 130 µg/day
- Adults: 150 µg/day
- Pregnant or breast-feeding women: 200 µg/day

For individuals from countries with long-standing iodine deficiency disorder, the Expert Committee on Human Nutrition of the Agence Française de Sécurité Sanitaire des Aliments has suggested a provisional maximal tolerable daily intake of 500 µg/day to avoid the occurrence of hyperthyroidism (AFSSA, 2001).

3.3 Human toxicity data

3.3.1 Toxicokinetics

3.3.1.1 Absorption

Iodine is readily absorbed through inhalation and ingestion, with dermal absorption being extremely low (< 1% of applied dose). Human volunteers exposed to radioactive elemental iodine vapour by inhalation showed clearance with a half-life of 10 minutes, with the majority of iodine being removed by mucociliary clearance to the gastrointestinal (GI) tract (Black & Hounam, 1968; Morgan et al., 1968). Iodine ingested in the form of water-soluble salts shows 100% absorption from the GI tract (Fischer et al., 1965). Absorption of iodine from the GI tract has been shown to be similar in adults, adolescents, children and older infants, however, uptake in newborns is reported to be between 2–20% lower (Ogborn et al., 1960; Morrison et al., 1963).

Iodine ingested in forms other than iodide is reduced to iodide in the gut prior to absorption by the small intestine (Fisher et al., 1965; Fish et al., 1987; Hays, 2001) with an efficacy of 92% (IOM, 2001; Jahreis et al., 2001; Aquaron et al., 2002). Iodide absorption is reduced in the presence of humic acids in drinking-water (Gaitan, 1990), and of thiocyanates, isothiocyanates, nitrates, fluorides, calcium, magnesium and iron in food and water (Ubom, 1991).

3.3.1.2 Distribution

In human volunteers exposed to radiolabelled iodine via ingestion, between 20–30% of the dose was distributed to the thyroid within 10 hours, with between 30–60% being excreted in urine (Morgan et al., 1967a, b). Of total body iodine typically 70–90% is concentrated in the thyroid gland. Maternal exposure to iodine results in exposure of the fetus to thyroid hormones, with accumulation of iodine in the fetal thyroid gland commencing at around 70–80 days gestation (Evans et al., 1967; Book & Goldman, 1975).

3.3.1.3 Metabolism

As noted above, iodine undergoes rapid conversion to iodide which is then transported by the sodium iodide symporter to the thyroid and utilised for the production of T4 and T3 hormones (Morgan et al., 1967a, b; Black & Hounam, 1968). Competition with sodium iodide symporter transport of iodine occurs from exposure to numerous anions including perchlorate, chlorate, nitrate and thiocyanate.

3.3.1.4 Elimination

Around 97% of iodine is excreted in the urine as iodide, with faecal elimination of between 1–2% (Larsen et al., 1998; Hays, 2001). Absorbed iodine can also be excreted in breast milk, saliva, sweat, tears and exhaled air (Cavalieri, 1997). The elimination half-life of absorbed iodine is considerably variable between individuals, and has been estimated as 31 days for healthy adult males (Van Dilla & Fulwyler, 1963; Hays, 2001).

3.3.2 Acute toxicity

Several biological mechanisms protect against acute iodine toxicity; these include reduced iodine uptake and preferential production of the more heavily iodinated thyroid hormones. Not all exposed subjects will react to excess iodine. Clinical features of acute iodine toxicity that have been produced following accidental or deliberate ingestion, or medical procedures such as wound irrigation, include GI disturbance (vomiting and diarrhoea), metabolic acidosis, seizure, stupor, delirium and collapse. Sensitivity reactions, such as iodide mumps, iododerma and iodide fever may also occur following treatment with iodine-containing drugs, or the use of radiographic contrast media (EVM, 2003).

Deaths (usually within 48 hours) in humans have occurred for iodine ingested in tinctures at doses ranging from 1200–9500 mg (17–120 mg/kg). Acute oral toxicity is primarily due to irritation of the GI tract, marked fluid loss and shock occurring in severe cases (ATSDR, 2004).

3.3.3 Repeat dose toxicity

A large number of human experimental, clinical, and epidemiological studies on the effects of repeat doses of excess iodine on human health has been reported.

3.3.3.1 Systemic effects

Both sub-acute (≤ 30 days) and sub-chronic (30–90 days) exposure studies for iodine intake have been reported:

Men who drank iodized water providing iodine doses of 0.17–0.27 mg/kg bw per day for 26 weeks reported no adverse effects (Morgan & Karpen, 1953).

The ingestion of about 3 mg iodine/day for 6 months during daily mouth-rinsing with an iodine-containing mouthwash had no effect on thyroid function (Ader et al., 1988).

A study on the effects of doses of 250, 500 or 1500 µg iodide/day for 14 days on thyroid function was carried out in 9 euthyroid men (normal thyroid function; mean age 34 years) and 23 euthyroid women (mean age 32 years) with 5 age-matched controls (Paul et al., 1988). The parameters examined were protein bound iodine (PBI) of the thyroid total serum iodine, T4, T3, TSH, integrated 1-hour serum TSH response to an intravenous dose of 500 µg TRH, and 24-hour urinary iodine excretion. The dietary intake of iodine was estimated from the urinary iodine excretion to be approximately 200 µg/person/day making the total ingested doses approximately 450, 700 and 1700 µg iodide/day. The estimated dose of 1700 µg/day was associated with an increase in total serum iodine without affecting the PBI, a significant decrease in serum T4 and T3 levels and an increase in TSH levels. Administered doses of 700 and 450 µg/day did not significantly affect the measured parameters. Only 1700 µg/day increased the TSH response to TRH (in women more than in men). The TSH response to TRH was also increased, though not significantly, in the individuals receiving 700 µg iodide/day. No biochemical effects were detected with 450 µg of iodide/day. However, this study used only small groups, extended over only 2 weeks and the dietary iodine intake was not determined analytically but was estimated.

In another study, 10 males (mean age 27 years) were treated for 2 weeks with either 500, 1500 or 4500 µg iodide/day (Gardner et al., 1988). The dietary intake was estimated from urine iodine excretion to have been approximately 300 µg/person/day making the total ingested doses approximately 800, 1800 or 4800 µg iodide/day. Serum levels of T4, T3, TSH, PBI, and total iodide, the TSH response to intravenous TRH and 24-hour urinary excretion of iodide were measured before treatment and again on day 15. Serum T4 levels decreased significantly after ingestion of 1800 µg and 4800 µg/day but did not

change with 800 µg/day. Serum T3 levels did not change following administration of any of the doses. Serum TSH levels remained unchanged in those receiving 800 µg/day but increased in those receiving 1800 µg and 4800 µg/day. The TSH response to TRH was significantly enhanced with all iodide doses administered. No adverse effects were reported and no significant symptoms of thyroid dysfunction were noted. Again, only small groups of males were studied, exposure was rather short and the actual dietary intake of iodine was not determined analytically but estimated.

Chow et al. (1991) assessed the effect of supplementing normal dietary intakes of iodide to give a total iodide intake of approximately 750 µg iodide/day, or a placebo for a period of 28 days. Volunteers were groups of women aged 25–54 years. They were either thyroid antibody positive (subclinical Hashimoto's thyroiditis) (n=20), antibody negative (n=30), aged 60–75 years and from an area with adequate dietary iodine supply (n=29) or from an area that was previously iodine deficient (n=35). In all iodine-supplemented groups, mild biochemical hypothyroidism was present, evidenced by decreases in T4 levels and increases in TSH levels. None of the groups on supplemental iodide showed any incidence of hyperthyroidism. Following iodide supplementation TSH levels increased above the normal level of 5 milli-international units (mU)/L in 3 of the 60–75 year-old subjects, while the raised TSH levels increased even further in 2 antibody-positive subjects.

Chronic (> 6 months) exposure through ingestion of iodine at levels > 0.03 mg/kg bw is considered to be associated with adverse health effects (ATSDR, 2004). The introduction of iodized bread in The Netherlands raised the daily intake by 120–160 µg iodine resulting in an increase in the incidence of hyperthyroidism (Van Leeuwen, 1954). The consumption of winter milk[20] in the UK raised the iodine intake of women to 236 µg/day and of men to 306 µg/day and was also associated with a peak incidence of hyperthyroidism (Nelson & Phillips, 1985). In 32 young Swiss adults with simple goitre (and urinary iodine excretion of 32 µg/day) administered 200 µg iodine/day, only one case of transient hyperthyroidism appeared which showed a serum T4 of 14 µg/100 mL, a serum T3 of 293 ng/100 mL, suppressed TSH, tachycardia and weight loss (Baltisberger et al., 1995).

Peace Corps volunteers in Niger, West Africa using iodine-resin water purification devices for 32 months during the period 1995–1998, showed an increased incidence (42%) of thyroid abnormality but effects were reversed when iodinated water consumption ceased (Pearce et al., 2002). The purification devices delivered a mean concentration of 10 mg iodine/L to the drinking-water, which with a daily consumption amongst volunteers of 5–9 L resulted in consumption of 50 mg/iodine per day (300 times the recommended dietary allowance for the USA at that time). The adjusted odds ratio for thyroid dysfunction (abnormal thyrotropin) adjusted for age, sex, and other potential confounding factors, was 3.9 (95% CI 1.1–14.3) ($p < 0.04$) for the devices, with a positive relation with duration of exposure (adjusted odds ratios 4.6 and 10.9 at 6 and 12 months, respectively).

In a 5-year study using iodinated drinking water (1 mg/L) supplied to 750 male and female prison in-mates, no hyper- or hypothyroidism sensitization reactions and iodism (symptoms provided in the following paragraph) were noted (Stockton and Thomas, 1978). The average dose was 30 µg/kg bw per day. There was a statistically significant decrease in iodine uptake and an increase in PBI of the thyroid. One hundred and seventy-seven women in-mates delivered 181 infants showing no thyroid-related adverse effects. In four women who were already hyperthyroid, their symptoms became even more severe. The difficulties with this study were the imprecise estimates of intakes from the diet and fluid

[20] Seasonal differences in the iodine content of milk are apparent and vary directly in relation to farming practices.

consumption of the participating individuals as well as the variable exposure time but the group size and duration of exposure were adequate.

Although most individuals who ingest large amounts of iodine remain euthyroid (i.e. have normal thyroid gland function) some will develop hypothyroidism with or without goitre or hyperthyroidism which can manifest as thyrotoxicosis (inflammation of the gland), and changes in the incidence and types of thyroid malignancies. Very large amounts of iodide may cause iodism, the symptoms of which resemble rhinitis as well as salivary gland swelling, GI irritation, acneform dermatitis, metallic taste, gingivitis, increased salivation, conjunctivitis and oedema of eye lids (ATSDR, 2004; Leung & Braverman, 2014). In children aged between 5–15 years of age, 10 µg/kg bw per day is considered to be a no-observed-adverse-effect level (NOAEL) based on thyroid effects (subclinical hypothyroidism with thyroid gland enlargement) (Boyages et al., 1989; Chow et al., 1991).

It has been proposed that excess iodide intake may be a contributing factor in the development of autoimmune thyroiditis in people who are vulnerable (Brown and Bagchi 1992; Foley 1992; Rose et al., 1997; Safran et al., 1987); however, evidence to support this in humans is incomplete.

3.3.3.2 Neurotoxicity

Iodine-induced hypothyroidism in sensitive populations (including fetuses, newborn infants, and individuals who have thyroiditis) has the potential to produce neurological effects (Boyages, 2000b). This is particularly applicable to fetuses and newborn infants as thyroid hormones are essential to the development of the neuromuscular system and brain. An iodine-induced hypothyroid state can result in delayed or deficient brain and neuromuscular development of the newborn. Iodine-induced hypothyroidism in an older child or adult would be expected to have little or no deleterious effects on the neuromuscular system.

Iodine-induced hyperthyroidism presenting as thyrotoxicosis in sensitive individuals (including those who are initially iodine deficient; those who have thyroid disease; including nodular goitre; Graves' disease; those who have been previously treated with antithyroid drugs; and those who have developed thyrotoxicosis from amiodarone or interferon-alpha treatments [Roti and Uberti, 2001]) may experience neuromuscular disorders, including myopathy (muscular weakness), periodic paralysis, myasthenia gravis (weakness in skeletal muscles), peripheral neuropathy, tremor, and chorea (involuntary movement disorder) (Boyages, 2000a).

3.3.3.3 Reproductive and developmental toxicity

Chronic exposure to excess iodine has been shown to disrupt reproductive function secondary to thyroid gland dysfunction. Induced changes in the menstrual cycle, including menorrhagia (excessive uterine bleeding) and anovulation (no ovulation); spontaneous abortions, stillbirths, and premature births have also been associated with hypothyroidism (Longcope, 2000a).

Reproductive impairments associated with hyperthyroidism include amenorrhea (no uterine bleeding), alterations in gonadotropin release and sex hormone-binding globulin, and changes in the levels and metabolism of steroid hormones in both females and males (Longcope, 2000b).

Exposure to iodine may give rise to developmental defects secondary to thyroid gland dysfunction (Boyages, 2000a, b). As noted in Section 3.3.3.2, hypothyroidism may be associated with impairment in neurological development of the fetus or growth retardation (Boyages, 2000a, b; Snyder, 2000a).

Hyperthyroidism has been associated with accelerated growth linked to accelerated pituitary growth hormone turnover or a direct effect of thyroid hormone on bone maturation and growth (Snyder, 2000b).

3.3.3.4 Immunotoxicity

No data could be located regarding immunotoxic effects in humans following repeated exposure to iodine.

3.3.3.5 Genotoxicity

No data could be located regarding genotoxic effects in humans following repeated exposure to iodine.

3.3.3.6 Carcinogenicity

The American Conference of Governmental Industrial Hygienists has classified iodine as A4 - not classifiable as a human carcinogen (ATSDR, 2004). The International Agency for Research on Cancer has not classified non-radioactive iodine (ATSDR, 2004).

The results from several epidemiology studies suggest that increased iodide intake may be a risk factor for thyroid cancer in certain populations, in particular, those that are iodine-deficient (Bacher-Stier et al., 1997; Harach & Williams, 1995; Franceschi, 1998; Franceschi & Dal Maso, 1999). Studies of populations in which iodine intakes are sufficient have not found significant associations between iodine intake and thyroid cancer (Horn-Ross et al., 2001; Kolonel et al., 1990).

A lowest-observed-no-effect level of 3.5 µg/kg bw per day has been identified based on thyroid cancer prevalence in Salta, an endemic goitre area in Argentina (Harach & Williams, 1995; Bacher-Stier et al., 1997).

3.4 Animal toxicity studies

Laboratory animals, poultry, pigs and cattle have a high tolerance to large iodine intakes. Animal data are of limited value to humans because of species differences in basal metabolic rate and in iodine metabolism (IOM, 2001).

3.4.1 Toxicokinetics

Rapid absorption of iodine vapour following inhalation exposure observed in humans is supported by studies in rats, mice, dogs and sheep (Willard & Bair, 1961; Bair et al., 1963). Compounds of iodine were also seen to be rapidly absorbed in monkeys when inhaled as vapours or aerosols, with a half-life of 10 min (Thieblemount et al., 1965; Perrault et al., 1967).

Absorption, distribution, metabolism and excretion data from animal studies for iodine exposure via the GI tract, were not apparent from the reviews identified during the literature search.

3.4.2 Acute toxicity

Due to the rapid conversion of iodine to iodide *in vivo*, the acute toxicity of iodine has been poorly studied. Conversely, the acute toxicity of iodides and iodates have been well studied and can be used to estimate the acute toxicity of iodine.

The acute oral median lethal dose (LD_{50})[21] for potassium iodide in rats was 3320 mg iodide /kg bw and in mice, 1425 mg iodide /kg bw (Stokinger, 1981).

3.4.3 Repeat dose toxicity

3.4.3.1 Systemic toxicity

A number of experimental studies on the effects of chronic exposure to excess iodine or iodide, particularly on thyroid function have been reported, with representative studies from different species summarized below:

- Two strains of chickens (CS and OS), genetically vulnerable to autoimmune thyroiditis, were given either 20 or 200 mg potassium iodide/L in their drinking water for the first 10 weeks of their lives. At both levels the incidence of the disease was increased as shown histopathologically, and also by measurements of, T4, T3 and thyroglobulin antibody titres (Bagchi et al., 1985).

- In female Wistar rats administered diets containing iodine concentrations between 0.015 and 0.23 mg/kg bw per day for 10 weeks, significantly enlarged thyroids were found at all doses, with a dose-dependent increase at all doses (Fischer et al., 1989).

- Newton and Clawson (1974) reported a dose-dependent increase in thyroid weights of pigs administered iodine at concentrations between 3 and 218 mg/kg bw per day.

- Female calves fed iodine at concentrations between 0.011 and 3.96 mg/kg feed twice daily for 5 weeks from day 4 of age showed a significant decrease in body weight gain at the highest dose; food intake was also decreased. Haematological changes (decreased packed cell volume) and clinical signs of nasal discharge were noted in the highest dose group and lacrimation was noted in the two highest dose groups (Jenkins & Hidiroglou, 1990).

- A NOAEL of 10 mg/L has been proposed for the most sensitive endpoint of thyroid hormone imbalance in rats. This was based upon a decrease in T3 levels and an increase in T4/T3 ratio after 100 days of iodine treatment (Sherer et al., 1991). When considering the use of rat models, it should be noted that rats are much more sensitive to thyroid hormone imbalance than humans (requiring around 10 times more T4/kg than humans).

3.4.3.2 Neurotoxicity

No data could be located regarding neurotoxic effects in animals following repeated exposure to iodine.

3.4.3.3 Reproductive and developmental toxicity

Arrington and colleagues (Arrington et al., 1965) investigated the reproductive and developmental toxicity of iodine in a series of studies:

- Iodine administered to pregnant Long-Evans rats at a concentration of 2500 mg/kg in the diet for 12 days in the latter part of gestation was associated with an increased incidence of death in the neonates, < 10% of the neonates survived for more than 3 days. Length of labour (parturition) was also increased.

[21] The dose required to kill half the members of a test population after a specified test duration.

- Syrian hamster pups from mothers fed iodine at 2500 mg/kg in the diet for 12 days in the latter part of gestation showed decreased feed intake (10%) and weaning weights at 21 days were significantly less than controls.

- Pups from pregnant rabbits (Dutch and New Zealand) fed iodine at concentrations between 250 and 1000 mg/kg feed for 2–5 days before parturition showed decreased survival rates.

- Pregnant pigs receiving diets containing 1500 or 2500 mg iodine/kg feed for the 30 days prior to parturition delivered litters that were unaffected by dietary levels of iodine that were toxic to rabbits and rats.

In female rats administered 0, 500, 1000, 1500 and 2000 mg potassium iodide/kg diet throughout gestation, lactation and weaning, pup survival was reduced from 93% in controls to 16% in rats given the highest dose; milk secretion was also diminished. There were no adverse effects on ovulation rate, implantation rate and fetal development (Ammermann et al., 1964). Brain enzymes of pups from pregnant rats administered 11 mg potassium iodide/day in their drinking water (37 mg/kg bw per day) showed transient increases in glutamate dehydrogenase and transient decreases in succinate dehydrogenase. Phosphofructokinase and malate enzymes were increased; however, hexokinases were unaffected. Serum T4 levels were also unchanged compared to controls (Morales de Villalobos et al., 1986).

In further studies a NOAEL of 10 mg/kg bw per day has been derived for reproductive and developmental toxicity in rats administered iodine by oral gavage (based on no observed toxicity at any dose level). A NOAEL for parental toxicity of 10 mg/kg bw per day was also established (based on no supported changes at any dose level) (EC, 2002).

Mares given 48–432 mg iodine/day during pregnancy and lactation produced foals with disturbed metabolism. The long bones of the legs of the foals showed osteopetrosis (hard, dense bones). Serum phosphate and alkaline phosphatase levels were increased (Silva et al., 1987).

3.4.3.4 Immunotoxicity

No data could be located regarding immunotoxic effects in animals following repeated exposure to iodine.

3.4.3.5 Genotoxicity (in vivo)

No data could be located regarding genotoxic effects in animals following repeated exposure to iodine. (See 3.4.4 for *in vitro* genotoxicity studies.)

3.4.3.6 Carcinogenicity

Metaplasia of the thyroid was reported in rats given potassium iodide in their drinking water for two years (dose not quoted by authors). This was thought to occur through a non-genotoxic proliferation dependent mechanism (EVM, 2003)

3.4.4 *In vitro* toxicity studies

The mutagenicity data for iodine are generally negative; iodine has been shown to be non-mutagenic using the mouse (TK +/-) lymphoma assay and no induction of unscheduled deoxyribonucleic acid synthesis was seen in Syrian Hamster Embryo cells (ATSDR, 2004).

3.5 Vulnerable populations

Individuals identified as most vulnerable to iodine-induced toxicity in the form of hypothyroidism are shown in Table 7.

Table 7: Risk groups for iodine-induced hypothyroidism (WHO/UNICEF/ICCIDD, 2007)

Risk group / subgroup	
No underlying thyroid disease	
Fetus and neonate, mostly preterm	Secondary to transplacental passage of iodine or exposure of neonate to topical or parenteral iodine-rich substances
Infant	Occasionally reported in infants drinking iodine-rich water (China)
Adult	In Japanese subjects with high iodine intake where Hashimoto thyroiditis has been excluded
Elderly	Reported in elderly subjects with and without possible defective organification (incorporation of iodine into thyroglobulin to produce thyroid hormone) and autoimmune thyroiditis
Chronic non-thyroid illness	Cystic fibrosis Chronic lung disease Chronic dialysis treatment Thalassaemia major Anorexia nervosa
Underlying thyroid disease	
	Hashimoto thyroiditis Euthyroid patients previously treated for Graves' disease with ^{131}I, thyroidectomy, or antithyroid drugs Subclinical hypothyroidism (particularly the elderly) After transient postpartum thyroiditis After subacute painful thyroiditis After hemithyroidectomy for benign nodules Euthyroid patients with a previous episode of amiodarone-induced destructive thyrotoxicosis Euthyroid patients with a previous episode of interferon-induced thyroid disorders Patients receiving lithium therapy

3.6 Toxicity of iodinated disinfection by-products

3.6.1 Formation and occurrence of iodinated disinfection by-products

When present in water, either at background levels or when used as a disinfectant, iodine has the ability to form iodinated DBPs. These have been identified in some chloraminated drinking-water in countries including the USA (Weinberg et al., 2002) and include:

- iodoacetic acid;
- bromoiodacetic acid;
- (*Z*)-3-bromo-3-iodopropenoic acid;
- (*E*)-3-bromo-3-iodopropenoic acid; and
- (*E*)-2-iodo-3-methylbutenedioic acid.

In addition, iodinated trihalomethanes (THMs) identified in chlorinated and chloraminated drinking water (Richardson et al., 2007) have been identified as:

- dichloroiodomethane;
- bromochloroiodomethane;
- dibromoiodomethane;
- chlorodiiodomethane;
- bromodiiodomethane; and
- iodoform.

When chloramine or chlorine is used as a disinfectant, these compounds are usually present in very low concentrations (fractional parts per billion) due to the low background presence of iodide in natural waters.

Smith et al. (2010) compared the formation of DBPs from a number of iodine-based disinfectants (used at the manufacturer's recommended levels) to chlorination and chloramination under overdosing conditions. The authors reported the following findings:

- the predominant THM formed during iodination was iodoform; chloroform predominated during chlorination or chloramination;
- THM formation increased with pH during chlorination but was only slightly elevated at neutral pH during iodination;
- use of iodine tincture was associated with higher levels of iodoform than with iodine tablets;
- iodoform formation with iodine tincture was 20–60% (on a molar basis) of chloroform formation during chlorination;
- total organic iodide formation was twice that of total organic chlorine;
- iodoacetic acid, diiodoacetic acid, and other iodoacids were also formed with iodine tincture treatment, but at levels < 11% of iodoform formation;
- a POU device combining an iodinated anion exchange resin with activated carbon post-treatment, indicated minimal formation of iodinated DBPs, no iodine residual and N-nitrosamine formation below 4 ng/L after the first few flushes of water.

3.6.2 Toxicological evaluations of iodinated disinfection by-products

Concern has arisen regarding iodinated DBPs as they are considered, on current evidence, to be of greater toxicological concern than their brominated and chlorinated analogues (Richardson et al., 2007).

However, it should be noted that this view is predominantly based on findings from a very limited dataset of *in vitro* cytotoxicity and genotoxicity assays, which are described below; the applicability of findings from *in vitro* cytotoxicity and genotoxicity assays to humans has not been established at present. A dataset of basic toxicological information on DBPs, as presented for iodine, is not available at the current time. An exception to this is that iodoform has been tested in National Toxicology Program bioassays and was not carcinogenic under test conditions (NCI, 1978).

Following the identification of iodoacids and iodinated THMs in chloraminated and chlorinated drinking waters in the USA (section 3.6.1), Richardson et al. (2008) assessed the cytotoxicity and genotoxicity of five iodoacids (iodoacetic acid, bromoiodoacetic acid, (*Z*)-3-bromo-3-iodo-propenoic acid, (*E*)-3-bromo-3-iodo-propenoic acid, and (*E*)-2-iodo-3-methylbutenedioic acid) and two iodinated THMs (dichloroiodomethane and bromochloroiodomethane) using *in vitro* assays with Chinese Hamster Ovary cells.

The chronic cytotoxicity of the compounds measured in the study were ranked and compared to other iodinated compounds by the authors. This resulted in a ranking order as follows:

iodoacetic acid > (*E*)-3-bromo-2-iodopropenoic acid > iodoform > (*E*)-3-bromo-3-iodo-propenoic acid > (*Z*)-3-bromo-3-iodo-propenoic acid > diiodoacetic acid > bromoiodoacetic acid > (*E*)-2-iodo-3-methylbutenedioic acid > bromodiiodomethane > dibromoiodomethane > bromochloroiodomethane ~ chlorodiiodomethane > dichloroiodomethane.

With the exception of iodoform, the iodinated THMs were much less cytotoxic than the iodoacids.

Of the iodo-compounds analysed, 7 were genotoxic; their rank order was:

iodoacetic acid >> diiodoacetic acid > chlorodiiodomethane > bromoiodoacetic acid > (*E*)-2-iodo-3-methylbutenedioic acid > (*E*)-3-bromo-3-iodo-propenoic acid > (*E*)-3-bromo-2-iodopropenoic acid.

The authors reported that, in general, compounds containing an iodo-group had enhanced mammalian cell cytotoxicity and genotoxicity as compared to their brominated and chlorinated analogues.

In the study described previously (section 3.6.1), Smith et al. (2010) compared the cytotoxicity of THMs in four natural waters treated with different disinfectants (free chlorine, 20mM monochloramine, 20mM iodine tincture, 72 mM elemental iodine, 172mM potassium iodide as iodine tablets, and a personal POU treatment unit). THMs formed following treatment with iodine tincture were associated with between 19–92 times higher cytotoxicity than for chlorination, with toxicity being driven by total organic iodine content of the water samples. The cytotoxicity of THMs formed with the iodine tablet treatment was around 40% lower than for treatment with iodine tincture. The authors estimated that from an exposure perspective, chlorination may be preferable to iodination for long-term disinfection, where comparable degrees of disinfection are achieved. Use of the personal POU treatment unit was also associated with THM formation, with associated cytotoxicity approximately 10% of that with iodine tincture, but 6-fold higher than for chlorination, with no iodine residuals apparent.

The authors highlight the importance of considering all iodinated DBPs when evaluating potential risks, with measurement of iodoacids, and iodoforms as the dominant DBPs, following iodination. Diiodoacetic acid and iodoacetic acid were formed at levels < 10% of iodoform following treatment with iodine tincture. However, iodoacetic acid has greater cytotoxicity (> 2 times) in mammalian cells than iodoform, and distinct from iodoform is genotoxic.

3.7 Summary

- Limited data (both from human and animal studies) suggest that the bioavailability of iodine from foods and water is high, with inorganic iodine (usually in the form of iodide) being readily absorbed (92%) from the small intestine. Iodine is rapidly distributed, including across the placenta, and is stored in the thyroid gland for the synthesis of thyroid hormones (T4 and T3). Excess iodine is mainly excreted in the urine, with very small amounts excreted in sweat, faeces and exhaled air and secreted into human breast milk.
- In humans, several mechanisms help regulate iodine levels, to protect against toxicity; these include reduced iodine uptake and preferential production of more heavily iodinated thyroid hormones. Symptoms of acute iodine toxicity include vomiting and diarrhoea, metabolic acidosis, seizure, stupor, delirium, and collapse. Sensitizing reactions include iodine mumps, iododerma, and iodine fever.
- Chronic and sub-chronic iodine toxicity in humans includes disruption of thyroid function, leading to hypothyroidism which can present with or without goitre, hyperthyroidism, and changes in the incidence and types of thyroid malignancies. Responses of this type are associated with a general high iodine intake or where intervention has taken place to compensate for iodine deficiency. Measures of serum thyroid hormone levels (T4, T3 and TSH) are used as indicators of iodine disturbances in humans.
- Iodine-induced hypothyroidism in humans has the potential to produce neurological effects (delayed or deficient brain and neuromuscular development) in sensitive populations, particularly in fetuses and new-born infants. Hyperthyroidism in humans has been associated with accelerated growth.
- Dysfunction of the thyroid in humans has also been associated with reproductive disruptions including changes in the menstrual cycle, menorrhagia, anovulation, spontaneous abortions, stillbirths, and premature births.
- Iodine is not classifiable as a human carcinogen. Chronic iodine exposure has been associated with metaplasia of the thyroid, considered to occur via a non-genotoxic mechanism. Mutagenicity data for iodine are generally negative.
- Acute, sub-chronic, and chronic toxicity studies in animals support the findings from human studies.
- The adverse effects associated with high levels of iodine intake are linked to the disruption of thyroid hormone metabolism, the thyroid-pituitary axis, and the compensatory mechanisms that exist to protect such metabolism against low or high levels of iodine intake. Previous exposures to iodine and the complex effects of pre-existing thyroid conditions also influence the effects of subsequent exposure.
- A threshold level for inducing thyrotoxicosis has not been established and available data are inadequate to establish a dose-response relationship.
- Vulnerable members of the general population to iodine toxicity include pregnant and lactating women, and neonates.

Due to limited available evidence, there are uncertainties regarding both the potential for formation of iodinated DBPs and likely adverse effects at the concentrations predicted to be formed from use of iodine as a drinking-water disinfectant. The applicability of findings from *in vitro* cytotoxicity and genotoxicity assays to humans has not been established at present.

4. Environmental considerations

Environmental considerations are largely beyond the scope of this report; however, as noted in Table 8, the impact of release of iodine into the environment to 'non-target' organisms should be considered.

Table 8: Environmental toxicity of iodine to 'non-target' species (USEPA, 2006)

Group of organisms	Common name (scientific name)	Test compound	$L(E)C_{50}$[22]	NOEC[23]	Acute toxicity rating
Fish (freshwater)	Bluegill sunfish (*Lepomis macrochirus*)	Iodine (99.8%)	0.61 mg/L	0.16 mg/L	Highly toxic
Invertebrates	Water flea (*Daphnia magna*)	Iodine (99.8%)	0.33 mg/L	0.09 mg/L	Very highly toxic

[22] LC_{50} – median lethal concentration; the dose required to kill half the members of a test population after a specified test duration.
EC_{50} – half maximal effective concentration; the effective concentration of a chemical that causes half of the maximum response in a test population after a specified test duration.
[23] No-observed-effect concentration.

5. Discussion and conclusions

The use of iodine for drinking-water disinfection, as with all water disinfectants, should be considered in terms of risk versus benefit. Known issues of water quality in many parts of the world necessitate additional measures to ensure potability. The risk of enteric infection should therefore be weighed against the risk for, and severity of, acquiring thyroid disease from exposure to iodine over a short- and long-term period of exposure, as well as alternative disinfection options.

Ideally a water treatment product (or combination of products) should be effective against all three classes of pathogens, i.e. bacteria, viruses and protozoa. The evidence presented in this review indicates that iodine is most effective against bacteria, has some effectiveness against viruses (particularly iodinated resins) and comparatively less effectiveness against certain species of protozoa. Higher dosages and longer contact times will be required when used as a disinfectant against protozoan cysts such as *Giardia*. Iodine is not effective against *Cryptosporidium* oocysts at practical Ct values. At the time of this publication, iodine has not been tested against WHO HWT performance targets and no evaluations have been carried out on the health impacts in low-income settings with microbiologically contaminated drinking-water.

From a disinfection perspective, iodine offers some advantages over chlorine:

- Water disinfection process requires less supervision, is simple and cost effective (although more expensive than chlorine); and
- Iodine may provide superior disinfection to chlorine for water of poor quality. The reduced overall reactivity of iodine prompts slower reactions with organic material and thus a lower disinfectant demand. The low reactivity with organic nitrogenous contaminants results in improved maintenance of residual iodine concentrations (Backer and Hollowell, 2000).

At the household level, there are a number of additional considerations beyond efficacy for determining whether any product, including iodine, will protect health. Achieving health gains from HWT requires products to be used correctly and consistently, and thus clear product information and use instructions are important. In addition, user preferences, supply chains and availability, and cost are important factors to consider. Products such as iodine and other drinking-water disinfectants which require a reliable supply chain can be problematic in resource-limited settings where such systems are not in place.

The lack of knowledge on long-term toxic effects of iodine consumption impedes the use of iodine for disinfection of municipal or community supplies. Considerable controversy exists about the maximal "safe" dietary dose of iodine (in the range of 500 to 1000 µg/day in healthy adults) and the maximum "safe" period of consumption for iodine treated water. Although a number of studies have been carried out, the data are not adequate to establish a linear and temporal dose response between iodine intake and altered thyroid function (Backer & Hollowell, 2000).

Current POU water disinfection devices that are both effective in terms of disinfection and can achieve low residual levels (0.01 ppm) of iodine (such as triiodinated resins including a granular activated carbon filter), are considered to be "safe" from a toxicological perspective to use for long periods of time in euthyroid individuals (see Table 6). Assuming drinking water consumption in an adult of 2 L per day, residual iodine at this level would result in intakes of approximately 0.02 mg/day. This is well below the low-end range of the recommended upper limit of 0.5 mg/day (CRN, 2013) even allowing for greater consumption of drinking-water and/or intake of iodine from other sources. It is also low in

comparison to the AI of 0.15 mg/day for an adult recommended by EFSA (2014). However, for those disinfection devices/methods that produce higher residual iodine levels (> 1 mg/L; such as iodine tablets, which leave residual concentrations from 8–16 mg/L), intake of 2 L of purified water per day would result in intakes of up to 32 mg/day, exceeding the recommended upper limit. Advice is given to limit the use of such devices to a few months (Backer & Hollowell, 2000; WHO, 2011b). Current evidence (outlined in section 3.3) suggests that intake at levels of 18 mg/day and above are associated with changes to serum T4 and TSH levels and TSH response to TRH (Gardner et al., 1988). Although no significant symptoms of thyroid dysfunction were associated with these biochemical changes, this study was conducted over a two-week period; hence, it is unclear if thyroid dysfunction would become apparent with prolonged exposure. Supporting evidence from a study of Peace Corps volunteers (Pearce et al., 2002), which showed a positive relationship between thyroid dysfunction and intake of iodine at 50 mg/day over 32 months, suggests that this would occur.

Iodine use for water disinfection is therefore not recommended for high-risk members of the population including:

- Infants and young children;
- Pregnant women (the fetus is vulnerable to goitre);
- Individuals with known hypersensitivity to iodine;
- Individuals with a history or strong family history of thyroid disease; and
- Individuals from areas of severe iodine deficiency (may lead to hyperthyroidism).

In summary, the current evidence indicates that:

- As a drinking-water disinfectant, iodine can be most effective against bacteria. Iodine is less effective against viruses and least effective against protozoa. Specifically, based on the information presented in Table 5, iodine solutions are less effective against these two pathogen classes compared to iodine resins.
- Effectiveness of iodine is impacted by the temperature, concentration, contact time, pH and organic content of water; however, this is to a lesser extent than for chlorine. In addition, the effectiveness of individual disinfectant products will vary according to manufacturing processes and related quality management.
- Higher dosages and longer contact times for iodine will be required when used as a disinfectant against protozoan cysts; iodine shows some effectiveness against *Giardia* cysts, but does not appear to be effective against *Cryptosporidium* oocysts.

Iodine would not be recommended for use as a primary disinfectant due to the lack of knowledge on long-term toxic effects and the availability of widely used, well-characterized disinfectants.

Use of POU applications of iodine as a water disinfectant may be appropriate under certain circumstances. In POU applications, the potential toxicity associated with iodine consumption from drinking-water will be variable depending on the method employed for disinfection and individual susceptibility. When considering to use iodine as a drinking-water disinfectant compared to other water disinfectants, recommendations should be considered in the context of overall benefits versus harm from potential iodine toxicity and ingestion of contaminated water, as outlined below:

- For euthyroid individuals using resin-based disinfection devices that result in low residual concentrations of iodine (e.g. those using resins with carbon filters), few adverse effects are anticipated. Although there is insufficient evidence to support long-term use of devices

containing resin-based disinfectants and carbon filters, it is anticipated that these devices could be used over extended periods of time. However, activated carbon filters should be replaced at frequencies recommended by the manufacturer. In addition, care should be taken to ensure the treated water is safely stored to prevent recontamination as the finished water will have no residual disinfectant.

- For euthyroid individuals using other iodine disinfection techniques that result in higher residual concentrations of iodine (e.g. solutions or tablets and resins without carbon filters), use should be restricted to as short a period of time as possible. If longer term use of a disinfectant is needed, another disinfectant should be utilized.
- For high-risk members of the population (noted on the previous page), water disinfection with iodine is not recommended and an alternative disinfectant should be utilized. However, disinfection should not be compromised due to the public health significance of microbiologically unsafe water, and therefore if iodine is the only disinfectant available, use should be limited to as short a time as possible, and an alternative disinfectant sought.

On the basis of limited effectiveness against viruses and particularly protozoa, as well as uncertainties around the safety and toxicity, the use of iodine products may be appropriate for short-term use for euthyroid individuals in targeted situations where the causative agent of disease is known. However, where the causative disease agent is unknown, use by euthyroid individuals should ideally be combined with another HWT method (e.g. with a filter) to provide comprehensive protection. The use of POU devices should be appropriately approved or certified to ensure efficacy and safety.

6. References

Ader AW, Paul TL, Reinhardt W et al. (1988). Effect of mouth rinsing with two polyvinylpyrrolidoneiodine mixtures on iodine absorption and thyroid function. J Clin Endocrinol Metab. 66(3):632–35.

AFSSA (2001). Agence Française de Sécurité Sanitaire des Aliments. Report from the AFSSA Expert Committee on Human Nutrition. Vitamin and mineral fortification of commonly eaten foods: meeting the nutritional and safety needs of the consumer. 8 November 2001 (transcripted version – 15 January 2002). Case No. 2000-SA-0239. Annex 4b, p 15. [cited by EFSA, 2014].

Ammermann CB et al. (1964). Reproduction and lactation in rats fed excessive iodine. J Nutr. 84:107–12. [cited by EC 2002].

Andersen S, Pedersen KM, Iversen F et al. (2008). Naturally occurring iodine in humic substances in drinking water in Denmark is bioavailable and determines population iodine intake. Br J Nutr. 99:319–25.

Andersson M, Karumbunathan V, Zimmermann MB (2012). Global iodine status in 2011 and trends over the past decade. J Nutr. 142:744–750.
(http://jn.nutrition.org/content/early/2012/02/28/jn.111.149393.full.pdf, accessed February 2016).

Aquaron R, Delange F, Marchal P, Lognone V, Ninane L 2002. Bioavailability of seaweed iodine in human beings. Cell Mol Biol. 48:563–69.

Arrington LR, Taylor RN, Ammerman CB et al. (1965). Effects of excess dietary iodine upon rabbits, hamsters, rats and swine. J Nutr. 87:394–98.

ATSDR (2004). Agency for Toxic Substances and Disease Registry. Toxicological profile for iodine. (http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=479&tid=85, accessed February 2015).

Atwater JE, Sauer RL, Schultz JR (1996). Numerical simulation of iodine speciation in relation to water disinfection aboard manned spacecraft I. Equilibria. J Environ Sci Health. A3:1965–79.

Bacher-Stier C, Riccabona G, Totsch M et al. (1997). Incidence and clinical characteristics of thyroid carcinoma after iodine prophylaxis in an endemic goiter country. Thyroid. 7(5):733–41.

Backer H, Hollowell H (2000). Use of iodine for water disinfection: Iodine toxicity and maximum recommended dose. Environ Health Perspect. 108:679–84.

Bagchi N, Brown TR, Urdanivia E et al. (1985). Induction of autoimmune thyroiditis in chickens by dietary iodine. Science. 230:325–27.

Bair WJ, Synder MD, Walters RA, Keough RF (1963). Effect of I^{127} on thyroid uptake of inhaled I^{131}. Health Phys. 9:1399–1410.

Baltisberger BL, Minder CE, Burgi H (1995). Decrease of incidence of toxic nodular goitre in a region of Switzerland after full correction of mild iodine deficiency. Eur J Endocrinol.132:546–49.

Black A, Hounam RF (1968). Penetration of iodine vapour through the nose and mouth and the clearance and metabolism of the deposited iodine. Ann Occup Hyg.11:209–25.

Black AP, Kinman RN, Keirn MA et al. (1970). The disinfection of swimming pool waters. Part I: Comparison of iodine and chlorine as swimming pool disinfectants. Am J Public Health Nations Health. 60(3):535–45.

Black A, Kinman R, Thomas W et al. (1965). Use of iodine for disinfection. J Am Water Works Assoc. 57:1401–21.

Black AP, Lackey JB, EW Lackey (1959). Effectiveness of iodine for the disinfection of swimming pool water. Am J Public Health Nations Health. 49(8):1060–68.

Book SA, Goldman M (1975). Thyroidal radioiodine exposure of the fetus. Health Phys. 29:874–77 [cited by ATSDR, 2004].

Boyages SC (2000a). The neuromuscular system and brain in hypothyroidism. In: Braverman LE, Utiger RD, eds. Werner and Ingbar's the thyroid: A fundamental and clinical text. 8th ed. Philadelphia, PA: Lippincott-Raven; pp. 803–10.

Boyages SC (2000b). The neuromuscular system and brain in thyrotoxicosis. In: Braverman LE, Utiger RD, eds. Werner and Ingbar's the thyroid: A fundamental and clinical text. 8th ed. Philadelphia, PA: Lippincott-Raven; pp. 631–33.

Boyages SC, Bloot AM, Maberly GF et al. (1989). Thyroid autoimmunity in endemic goitre caused by excessive iodine intake. Clin Endocrinol. 31:452–65.

Brown TR, Bagchi N (1992). The role of iodine in the development of autoimmune thyroiditis. Int Rev Immunol. 9:167–82.

Bryan PE, Kyzminski LN, Sawyer FN, Feng TH (1973). Taste thresholds of halogens in water. J Am Water Works Assoc. 54(5):363–68.

Cavalieri RR (1997). Iodine metabolism and thyroid physiology: Current concepts. Thyroid. 7(2):177–181.

Chambers CW, Kabler PW, Malaney G, Bryant A (1952). Iodine as a bactericide. Soap Sanit Chem. 28(10):149–51.

Chang S, Morris J (1953). Elemental iodine as a disinfectant for drinking water. Ind Eng Chem. 45: 1009–12.

Chang SL (1968). Waterborne viral infections and their prevention. Bull. Org. mond Santé. 38:401–14.

Chang SL (1966). Iodination of water (Yodacion del agua). Bol Ofic Sanit Panem. 6:317–24.

Chang SL (1958). The use of active iodine as a water disinfectant. J Am Pharmac Assoc. 47:417–23.

Chow CC, Phillips DIW, Lazarus JH et al. (1991). Effect of low dose iodide supplementation on thyroid function in potentially susceptible subjects: Are dietary iodide levels in Britain acceptable? Clin Endocrinol. 34:413–16. [cited by ATSDR, 2004].

Clarke NA, Berg G, Kabler PW et al. (1964). Human enteric viruses in water: source, survival, and removability. In: Proceedings. International Conference on Adv Water Pollut Res. London. 2:523–36.

CRN (2013). Vitamin and Mineral Safety 3rd Edition. Council for Responsible Nutrition (http://www.crnusa.org/safety/CRN-SafetyBook-3rdEdition-2014-fullbook.pdf, accessed February 2015).

EC (2002). Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Iodine. European Commission Scientific Committee on Food (https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out146_en.pdf, accessed February 2015).

EFSA (2014). Scientific opinion on dietary reference values for iodine. EFSA Panel on Dietetic Products, Nutrition and Allergies. European Food Safety Authority. EFSA Journal. 12(5):3660 (http://www.efsa.europa.eu/en/efsajournal/pub/3660.htm, accessed February 2015).

Ellis KV, van Vree HBRJ (1989). Iodine used as a water disinfectant in turbid waters. Wat Res. 23(6):671–76.

Ellis KV, Cotton AP, Khowaja MA (1993). Iodine disinfection of poor quality waters. Wat Res. 27(3):369–75.

Evans TC, Kretzschmar RM, Hodges RE et al. (1967). Radioiodine uptake studies of the human fetal thyroid. J Nucl Med. 8:157–65.

EVM (2003). Risk assessments: Iodine. Expert Group on Vitamins and Minerals (http://cot.food.gov.uk/sites/default/files/vitmin2003.pdf, accessed February 2015).

Fish LH, Schwartz HL, Cavanaugh J, Steffes MW, Bantle JP and Oppenheimer JH, 1987. Replacement dose, metabolism, and bioavailability of levothyroxine in the treatment of hypothyroidism. Role of triiodothyronine in pituitary feedback in humans. N Engl J Med. 316:764–770.

Fischer PWF, Campbell JS, Giroux A (1989). Effect of dietary iodine on autoimmune thyroiditis in the BB Wistar rat. J Nutr. 119:502–07.

Fischer WD, Voorhess ML, Gardner LI (1965). Congenital hypothyroidism in infant maternal I^{131} therapy: With a review of hazards of environmental radioiosotope contamination. J Pediatr. 62:132–46.

Foley TP (1992). The relationship between autoimmune thyroid disease and iodine intake: A review. Endokrynol Pol. 43(Suppl 1):53–69.

Franceschi S (1998). Iodine intake and thyroid carcinoma-a potential risk factor. Exp Clin Endocrinol Diabetes. 106(Suppl 3):S38–S44.

Franceschi S, Dal Maso L (1999). Hormonal imbalances and thyroid cancers in humans. In: Capen CC, Dybing E, Rice JM et al. eds. Species differences in thyroid, kidney and urinary bladder carcinogenesis. Lyon, France: International Agency for Research on Cancer; pp. 33–43.

Gardner DF, Centor RM, Utiger RD (1988). Effects of low dose oral iodide supplementation on thyroid function in normal men. Clin Endocrinol. 28:283–88.

Gaitan E (1990). Goitrogens in food and water. Annu Rev Nutr. 10:21–39.

Gazda DB, Lipert RJ, Fritz JS et al. (2004). Investigation of the iodine-poly (vinylpyrrolidone) interaction employed in the determination of biocidal iodine by colorimetric solid-phase extraction. Analytica Chemica Acta. 510:241–47.

Gerba CP, Johnson DC, Hasan MN (1997). Efficacy of iodine water purification tablets against *Cryptosporidium* oocysts and *Giardia* cysts. Wilderness and Environ Med. 8:96–100.

Gibbons RE, Flanagan DT, Schultz JR et al. (1990). Recent experiences with iodine water disinfection in shuttle. In: 20th Intersoc Conf Environ Syst. Williamsburg, VA.

Gottardi W (1999). Iodine and disinfection: theoretical study on mode of action, efficiency, stability, and analytical aspects in the aqueous system. Arch Pharm. 332:151–56.

Gottardi W (1991). Iodine and iodine compounds. In: Block S, ed. Disinfection, sterilization and preservation. Philadelphia (PA): Lea & Febiger; pp. 152–67.

Harach HR, Williams ED (1995). Thyroid cancer and thyroiditis in the goitrous region of Salta, Argentina, before and after iodine prophylaxis. Clin Endocrinol. 43:701–06.

Haas CN, Kerallus, MG, Brncich DM et al. (1986). Alteration of chemical and disinfectant properties of hypochlorite by sodium, potassium, and lithium. Environ. Sci. Technol. 20:822–26.

Hays MT (2001). Estimation of total body iodine content in normal young men. Thyroid. 11(7):671–75.

Hitchens AP (1922). The emergency treatment of water for drinking purposes. J Mil Surg. 51:657–63.

Horn-Ross PL, Morris JS, Lee M et al. (2001). Iodine and thyroid cancer risk among women in a multiethnic population: the Bay Area Thyroid Cancer Study. Cancer Epidemiol Biomarkers Prev. 10(9):979–85.

IOM (2001). Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Institute of Medicine. Food and Nutrition Board. Washington (DC): National Academy Press (http://www.nap.edu/download.php?record_id=10026#, accessed September 2015).

Jahreis G, Hausmann W, Kiessling G et al. (2001). Bioavailability of iodine from normal diets rich in dairy products-results of balance studies in women. Exp Clin Endocrinol Diabetes. 109(3):163–67.

Jenkins KJ, Hidiroglou M (1990). Effects of elevated iodine in milk replacer on calf performance. J Dairy Sci. 73:804–07.

Jensen H, Thomas K, Sharp DG (1980). Inactivation of coxsackieviruses B3 and B5 in water by chlorine. Appl Environ Microbiol. 40(3):633–40.

JECFA (1989). Toxicological evaluation of certain food additives and contaminants. Joint FAO/WHO Expert Committee on Food Additives. FAS 24:267–94. Geneva: World Health Organization.

Karalekas PC, Kuzminski LN and Feng TH (1970). Recent developments in the use of iodine for water disinfection. J N Engl Water Works Assoc. 84:152–88.

Keirn MA, Putnam HD (1968). Resistance of *Staphylococci* to halogens as related to a swimming pool environment. Health Lab Sci. 5:180.

Kolonel LN, Hankin JH, Wilkens LR et al. (1990). An epidemiologic study of thyroid cancer in Hawaii. Cancer Causes Control. 1:223–34.

Larsen PR, Davies TF, Hay ID (1998). The thyroid gland. In: Wilson JD, Foster DW, Kronenberg HM, et al. eds. Williams textbook of endocrinology. Philadelphia (PA): W.B. Saunders Company; pp. 390–515.

Laurberg P, Pedersen KM, Hreidarsson A et al. (1998). Iodine intake and the pattern of thyroid disorders: a comparative epidemiological study of thyroid abnormalities in the elderly in Iceland and in Jutland, Denmark. J Clin Endocrinol Metab. 83:765–69.

Lengyel I, Epstein IR, Kustin K (1993). Kinetics of iodine hydrolysis. Inorg Chem. 32(25):5880–82.

Leung AM, Braverman LE (2014) Consequences of excess iodine. Nat Rev Endocrinol., 10(3):136–42.

Longcope C. 2000a. The male and female reproductive systems in hypothyroidism. In: Braverman LE, Utiger RD. eds. Werner and Ingbar's the thyroid: A fundamental and clinical text, eighth ed. Philadelphia (PA): Lippincott-Raven; pp. 824–27.

Longcope C. 2000b. The male and female reproductive systems in thyrotoxicosis. In: Braverman LE, Utiger RD, eds. Werner and Ingbar's the thyroid: A fundamental and clinical text eighth ed. Philadelphia (PA): Lippincott-Raven pp. 653–58.

Morales de Villalobos LM, Campos G, Ryder E (1986). Effect of chronic ingestion of iodide during pregnancy and lactation on pup brain enzymes. Enzyme 35:96–107. [cited by EC, 2002].

Morgan A, Morgan DJ, Arkell GM (1967a). A study of the retention and subsequent metabolism of inhaled methyl iodide. In. Davies CN, ed. Inhaled Particles and Vapours II. Oxford: Pergamon Press; pp. 309–21.

Morgan A, Morgan DJ, Evans JC et al. (1967b). Studies on the retention and metabolism of inhaled methyl iodide-II: Metabolism of methyl iodide. Health Phys. 13:1067–74.

Morgan A, Morgan DJ, Black A et al. (1968). A study on the deposition, translocation and excretion of radioiodine inhaled as iodine vapour. Health Phys. 15:313–22.

Morrison RT, Birkbeck JA, Evans TC et al. (1963). Radioiodine uptake studies in newborn infants. J Nucl Med. 4:162–66.

NCI (1978). Carcinogenesis Technical Report Series No. 110 Bioassay of iodoform for possible carcinogenicity. National Cancer Institute (https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr110.pdf).

Nelson M, Phillips DIW (1985). Seasonal variations in dietary iodine intake and thyrotoxicosis. Hum Nutr Appl Nutr. 39A:213–16.

Newton GL, Clawson AJ (1974). Iodine toxicity: Physiological effects of elevated dietary iodine on pigs. J Anim Sci 39(5):879–84. [cited by ATSDR, 2004].

Ogborn RE, Waggener RE, VanHove E (1960). Radioactive-iodine concentration in thyroid glands of newborn infants. Pediatrics. 771–76.

Ongerth JE, Johnson RL, MacDonald SC et al. (1989). Backcountry water treatment to prevent giardiasis. Am J Pub Health. 79:1633–37.

Paul T, Meyers B, Witorsch RJ et al. (1988). The effect of small increases in dietary iodine on thyroid function in euthyroid subjects. Metabolism. 37:121–24.

Pearce EN, Gerber AR, Gootnick DB et al. (2002). Effect of chronic iodine excess in a cohort of long-term American workers in West Africa. J Clin Endocrinol Metab. 87(12):5499–502.

Pedersen KM, Laurberg P, Iversen E et al. (1993). Amelioration of some pregnancy-associated variations in thyroid function by iodine supplementation. J Clin Endocrinol Metab. 77:1078–83.

Perrault G, Thieblemont P, Pasquier C, et al. (1967). Cinétique du passage du radioide soluble à travers les épithéliums respiratoires, après inhalation. Health Phys 13:707–18. [cited by ATSDR, 2004].

Phillips DI (1997). Iodine, milk, and the elimination of endemic goitre in Britain: the story of an accidental public health triumph. J Epidemiol Community Health. 51:391–93.

Punyani S, Narayanan P, Singh H, Vasudevan P (2006). Iodine based water disinfection: A review. J Sci Ind Res: 65:116–20.

Richardson S, Plewa, MJ, Wagner ED et al. (2007). Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. Mutat Res. 636(1-3):178–242.

Richardson SD, Fasano F, Ellington JJ et al. (2008). Occurrence and mammalian cell toxicity of iodinated disinfection byproducts in drinking water. Environ Sci Technol. 42:8330–38.

Rose NR, Saboori AM, Rasooly L et al. (1997). The role of iodine in autoimmune thyroiditis. Crit Rev Immunol 17:511–17.

Roti E and Uberti E (2001). Iodine excess and hyperthyroidism. Thyroid. 11(5):493–500.

Safran M, Paul TL, Roti TL et al. (1987). Environmental factors affecting autoimmune thyroid disease. Endocrinol Metab Clin N Am 16(2):327–42.

Sherer TT, Thrall KD, Bull RJ (1991). Comparison of toxicity induced by iodine and iodide in male and female rats. J Toxicol Environ Health. 31(1):89–101.

Silva CA, Merkt H, Bergamo PN, Barros SS, Barros CS, Santos MN, Hoppen HO, Heidemann P, Meyer H (1987). Consequences of excess iodide supply in a Thoroughbred stud in southern Brazil. J Reprod Fertil. 35(Suppl):529–33. [cited by EC 2002].

Smith EM, Plewa MJ, Lindell C, Richardson S, Mitch WA (2010). Comparison of by product formation in waters treated with chlorine and iodine: relevance to point-of-use treatment. Environ Sci Technol. 44:8446–52.

Snyder PJ (2000a). The pituitary in hypothyroidism. In: Braverman LE, Utiger RD, eds. Werner and Ingbar's the thyroid: A fundamental and clinical text. eighth ed. Philadelphia (PA): Lippincott-Raven; pp. 836–40.

Snyder PJ (2000b). The pituitary in thyrotoxicosis. In: Braverman LE, Utiger RD, eds. Werner and Ingbar's the thyroid: A fundamental and clinical text. 8th ed. Philadelphia (PA): Lippincott-Raven; pp. 634–636.

Stokinger HE. The halogens and the non-metals, boron and silicon. In: Clayton GD, Clayton FE, eds. *Patty's industrial hygiene and toxicology,* Vol, 2B, 3rd rev. ed. New York, NY, John Wiley, 1981: 2937-3043.

Stockton LK, Thomas Jr WC (1978). Absence of neonatal goiter during maternal use of iodinated water. Clin. Res. 26:586A.

Sobsey MD, Oldham CE, McCall DE (1991). Comparative inactivation of hepatitis A virus and other enteroviruses in water by iodine. Wat Sci Technol. 7(2):331–37.

Taylor GR, Butler MA (1982). A comparison of the viracidal properties of chlorine, chlorine dioxide, bromine chloride and iodine. J Hyg (Camb). 89:321–28.

Technical Information Paper # -31-005-0211 (2011). Iodine disinfection in the use of individual water purification devices (http://phc.amedd.army.mil/PHC%20Resource%20Library/Iodine%20Disinfection%20in%20the%20Use%20of%20Individual%20Water%20Purification%20Devices.pdf, accessed February 2015).

Teng W, Shan Z, Teng X et al. (2006). Effect of iodine intake on thyroid diseases in China. N Eng J Med. 354:2783–93.

Thieblemont P, Marble G, Perrault G et al. (1965). Évaluation de la retention respiratoire et de l'élimination du radioiode après contamination aérienne du singe. Int J Radiat Biol. 9(3):219–31[cited by ATSDR, 2004].

Ubom GA (1991). The goitre-soil-water-diet relationship: Case study in Plateau State, Nigeria. Sci Total Environ. 107:1–11.

USEPA (2006). Reregistration eligibility decision for iodine and iodophor complexes. (http://www.epa.gov/opp00001/reregistration/REDs/iodine-red.pdf, accessed February 2015).

Van Dilla MA, Fulwyler MJ (1963). Thyroid metabolism in children and adults using very small (nanocurie) doses of iodine^{125} and iodine^{131}. Health Phys. 9:1325–31.

Van Leeuwen E (1954). Een vorm van genuine hyperthyreose (M. Basedow zonder exophthalmos) na gebruik van gejodeerd brood. Tijdschr Geneesk 98:81 [cited by ESFA, 2014].

Vasudevan P, Tandon M (2010). Antimicrobial properties of iodine based products. J Sci and Ind Res. 69:376–383.

Venkobachar C, Jain RK (1983). Principles and practice of disinfection and sterilization of water. Water Supply; pp. 193–204.

Vergnoux (1915). Examen rapide et stérilization des eaux pour les troupes en champagne. L'Union Pharmaceutique. 194–201.

Weinberg HS, Krasner SW, Richardson SD, Thruston AD (2002). The occurrence of disinfection by-products (DBPs) of health concern in drinking water: Results of a nationwide DBP occurrence study. EPA/600-02/068 (http://www.epa.gov/athens/publications/reports/EPA_600_R02_068.pdf, accessed February 2015).

West RC (1984). Handbook of Chemistry and Physics. Boca Raton (FL): CRC Press.

White G (1992). Handbook of chlorination. New York: Van Nostrand Reinhold [cited by Ellis et al. 1993].

WHO (1994). Indicators for assessing iodine deficiency disorders and their control through salt iodization. WHO/NUT/94.6. World Health Organization (WHO)/ International Council for the Control of Iodine Deficiency Disorders, Geneva.

WHO (2003). Iodine in Drinking-water: Background document for development of Guidelines for Drinking-water Quality. Geneva: World Health Organization (http://www.who.int/water_sanitation_health/dwq/chemicals/iodine.pdf, accessed February 2015).

WHO (2011). Evaluating household water treatment technologies: health based targets and microbiological performance standards. Geneva: World Health Organization (http://www.who.int/water_sanitation_health/publications/2011/household_water/en/ accessed February 2015).

WHO (2017). Guidelines for drinking-water quality, fourth edition incorporating the first addendum. Geneva: World Health Organization (http://www.who.int/water_sanitation_health/publications/drinking-water-quality-guidelines-4-including-1st-addendum/en/, accessed October 2017).

WHO/FAO (2004). Vitamin and mineral requirements in human nutrition, second edition. Geneva: World Health Organization/Food and Agriculture Organization (http://apps.who.int/iris/bitstream/10665/42716/1/9241546123.pdf, accessed September 2015).

WHO/UNICEF/ICCIDD (2007). Assessment of iodine deficiency disorders and monitoring their elimination. A guide for programme managers. World Health Organization/United Nations Children's Fund/International Council for Control of Iodine Deficiency Disorders (http://apps.who.int/iris/bitstream/10665/43781/1/9789241595827_eng.pdf, accessed February 2016).

Williard DH, Bair WJ (1961). Behaviour of I^{131} following its inhalation as a vapour and as a particle. Acta Radiol. 55:486–96.

Zava TT, Zava DT (2011). Assessment of Japanese iodine intake based on seaweed consumption in Japan: A literature-based analysis. Thyroid Res. 4:14.

Zhao J, Wang P, Shang L, Sullivan KM, van der Haar F, Maberly G (2000). Endemic goiter associated with high iodine intake. Am J of Pub Health. 90:1633–35.

Zimmermann MB, Andersson M (2011). Prevalence of iodine deficiency in Europe in 2010. Ann d'endocrinol. 72:164–66.

Appendix A: Methodology

Two initial literature searches were conducted in November 2013 as follows:

iii) to update the toxicity assessment; and
iv) to update the efficacy assessment

The search strategy and terms are outlined in Box 1 and 2 respectively, below.

Box 1- Search strategy for toxicity assessment for iodine

((KEY(human OR animal) OR TITLE-ABS-KEY({in vitro} OR {in vivo})) AND DOCTYPE(ar OR re) AND PUBYEAR > 2004) AND ((TITLE-ABS-KEY(toxicokinetic OR irritation OR sensitisation) OR TITLE-ABS-KEY(genotoxicity OR mutagenicity OR carcinogenicity) OR TITLE-ABS-KEY({Acute toxicity} OR {Repeat dose toxicity} OR {Chronic toxicity}) OR TITLE-ABS-KEY({Reproductive toxicity} OR {Developmental toxicity})) AND DOCTYPE(ar OR re) AND PUBYEAR > 2004) AND (((CASREGNUMBER(7553-56-2) AND DOCTYPE(ar OR re) AND PUBYEAR > 2004))

Box 2- Search strategy for efficacy assessment for iodine

(TITLE-ABS-KEY(iodine) AND TITLE-ABS-KEY({drinking water} OR {potable water}) AND TITLE-ABS-KEY(disinfection OR microorganism OR bacteria OR virus OR protozoa OR antimicrobial OR bactericidal OR bacteriostatic)) AND PUBYEAR > 2004.

Searches were carried out using Scopus and Web of Knowledge databases. Titles and abstracts of journal articles identified from the initial literature searches included 62 papers relating to iodine toxicity and 155 papers relating to iodine efficacy, which were reviewed to inform on their potential relevance to the project. For those titles selected, which were included in the document, papers were obtained in full for review to extract key data. Additional searches were carried out as needed, particularly for identification of “grey” literature, earlier studies and during the period of document preparation (up to 16 December 2016).

Part III

Silver as a drinking-water disinfectant

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List of abbreviations and terms used in Part III

Ag	elemental silver
Ag^{+}	silver ion
AgAc	silver acetate
AgCl	silver chloride
$AgNO_3$	silver nitrate
AgNP	silver nanoparticles
bw	body weight
cfu	colony-forming units
DLS	dynamic light scattering
DNA	deoxyribonucleic acid
EC_{50}	half maximal effective concentration
GSH	glutathione
HRT	hydraulic retention time
IC_{50}	half maximal inhibitory concentration
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect level
LRV	log_{10} reduction value
MIC	minimum inhibitory concentration
NO	nitric oxide
NOAEL	no-observable-adverse-effect level
NTU	nephlomentric turbidity unit
pfu	plaque-forming unit

POU	point-of-use
PVP	polyvinylpyrrolidone
ROS	reactive oxygen species
SOD	superoxide dismutase
UK	United Kingdom
USA	United States of America
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

1. Introduction

The emphasis of this literature review is to evaluate available evidence on the efficacy and toxicity of silver as a water disinfectant. The report considers both ionic silver (Ag^+) and silver nanoparticles (AgNP) and also examines the effectiveness of copper-silver disinfection in plumbing systems for the control of *Legionella* spp. The initial review was written in autumn 2013, but some aspects of the report (specifically the efficacy of silver in household water treatment devices, *in vivo* oral toxicity studies, *in vitro* studies on primary mammalian cell lines and genotoxicity) have been updated to account for literature published until September 2015.

1.1 Antimicrobial properties

Silver has been known to have antibacterial properties since Roman times. However, the increased use of nanosilver in a range of (as yet largely) experimental drinking-water treatment systems, its use in conjunction with ceramic filters, and its perceived potential to be a water disinfectant that does not result in disinfection by-products in the treated water, have raised the profile of this chemical.

Silver has been shown to have general (i.e. not specifically water disinfection related) antibacterial properties against a range of both Gram-negative (e.g. *Acinetobacter*, *Escherichia*, *Pseudomonas*, *Salmonella* and *Vibrio*) and Gram-positive bacteria (e.g. *Bacillus*, *Clostridium*, *Enterococcus*, *Listeria*, *Staphylococcus* and *Streptococcus*) (Wijnhoven et al., 2009). Some researchers have also demonstrated that fungi, such as *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisia*, are sensitive to silver (reviewed by Marambio-Jones & Hoek, 2010). In addition, a number of studies have suggested a biocidal action of silver nanoparticles against hepatitis B virus (Lu et al., 2008), HIV-1 (Elechiguerra et al., 2005), syncital virus (Sun et al., 2008) and murine norovirus (De Gusseme et al., 2010 – see Sections 2.1.1. and 2.2). There is also a suggestion that silver nitrate ($AgNO_3$) and some silver nanoparticles may reduce the infectivity of *Cryptosporidium* oocysts (Abebe et al., 2015).

1.2 Nanoparticles

According to a review issued by the European Commission (2013) and cited by Bondarenko et al. (2013), nanomaterial is defined as "*a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more of the external dimensions is in the size range 1–100 nm*." In the scientific literature, nanoparticles are usually defined as particles having one or more dimensions in the order of 100 nm or less (Moore, 2006). Although the terminology may be relatively new, the use of silver nanoparticles is not (Nowack et al., 2011), with Lea (1889) reporting on the synthesis of a citrate-stabilized silver colloid (which has an average particle size between 7 and 9 nm).

The most common method of producing silver nanoparticles is the chemical reduction of a silver salt (often silver nitrate) dissolved in water with a reducing compound such as sodium borohydride, citrate, glucose, hydrazine and ascorbate (Marambio-Jones & Hoek, 2010). There are, however, numerous different manufacturing methods (including spark discharging, electrochemical reduction, solution irradiation and cryochemical synthesis) some of which have been outlined by Marambio-Jones & Hoek (2010). In addition to different manufacturing methods, different capping or stabilizing agents may be used; these are generally used to prevent the silver nanoparticles from aggregating or agglomerating (Ema et al., 2010) and common examples include polyvinylpyrrolidone (PVP) and citrate (Völker et al., 2013). The different methods employed in the manufacturing process result in silver nanoparticles with different sizes (typically < 50 nm), shapes (e.g. spheres, rods and cubes) and other characteristics.

1.3 Water-related applications

In terms of water disinfection-related applications, silver is most commonly used in domestic water filters (allegedly to reduce the level of biofilm growth within the filter or, hypothetically, as an additional level of treatment); it is used in both granular and powdered activated carbon filters and also domestic ceramic water filters. It is also quite commonly used in conjunction with copper ionization as a preventative measure against colonization by a variety of bacteria (especially *Legionella* spp.) in plumbing hot water systems. Silver nanoparticles are currently being tested in a number of experimental point-of-use (POU) treatment systems and ionic silver has been investigated for its potential use as a secondary disinfectant in drinking-water supplies. Silver ions (in combination with both copper and chlorine) have also been investigated for use in swimming pool disinfection. This report focuses on both established and experimental use of silver for drinking-water disinfection and, briefly, the use of copper-silver ionization for *Legionella* spp. control within plumbing systems.

2. Disinfection efficacy of silver

Numerous studies have been conducted on the disinfection efficacy of silver and silver nanoparticle applications against a range of microorganisms found in water. Although the majority of these have focused on bacterial disinfection (often using indicator bacteria), some have also looked at the impact on bacteriophages, viruses and protozoa. In addition to the material below, which focuses on water disinfection, there is also a short section (Appendix A) on the general disinfectant mode of action of silver and silver nanoparticles.

2.1 Ionic silver applications

2.1.1 Efficacy of ionic silver for disinfection of potable water

In the studies outlined below, silver ion (ionic silver) efficacy (generated from silver salts [silver nitrate, silver chloride (AgCl)] or produced electrolytically) was tested against a range of bacteria; the inactivation was principally assessed by the log_{10} reduction in bacterial numbers. Initial bacterial concentrations ranged from 3.5 cells/mL up to 1.5 x 10^7 cells/mL. Single studies examined the impact of silver nitrate on bacteriophage (De Gusseme et al., 2010) and *Cryptosporidium* oocysts (Abebe et al., 2015).

Hwang et al. (2007) looked at the efficacy of silver ions (up to 100 µg/L), derived from silver nitrate, against *Legionella pneumophila*, *Pseudomonas aeruginosa* and *Escherichia coli* (all at 1.5 x 10^7 cells/mL) in synthetic drinking-water (pH 7, temperature 25 °C – defined chemical composition outlined in Hwang *et al.*, 2006). After a three-hour contact time with the highest concentration of silver the following log_{10} reductions were reported:

- 2.4 log_{10} reduction – *L. pneumophila*;
- 4 log_{10} reduction – *P. aeruginosa*;
- 7 log_{10} reduction – *E. coli*.

Similar work was conducted by Huang et al. (2008), where the efficacy of silver ions, derived from silver chloride, against 3 x 10^6 colony-forming units (cfu)/mL of *P. aeruginosa*, *Stenotrophomonas maltophilia* and *Acinetobacter baumannii* was investigated. A 5 log_{10} reduction in *P. aeruginosa* was seen with 80 µg/L silver (the highest concentration used) after 12 hours. *S. maltophilia* was more

sensitive to Ag, with a 5 $\log_{10}$ reduction seen after 6 hours when exposed to 80 µg/L. For *A. baumannii*, however, a 5 $\log_{10}$ reduction was only seen after 72 hours exposure to 80 µg/L Ag.

Silvestry-Rodriguez et al. (2007) investigated the inactivation of *P. aeruginosa* and *Aeromonas hydrophila* by silver in tap water, with a view to assessing the possibility of using silver as a secondary disinfectant to replace or reduce the level of chlorine. Dechlorinated municipal water (obtained from a groundwater source) was seeded with 10^6 cfu/mL bacteria and silver nitrate added to a concentration of 100 µg/L. Experiments were performed at pH 7 and pH 9 at 24 °C for both bacterial species and at 4 °C for *P. aeruginosa*. In addition, 3 mg/L of humic acid was added to the dechlorinated tap water (to simulate a surface water source). Inactivation of the bacteria was time and temperature dependent; after 8 to 9 hours of exposure to 100 µg/L silver at 24 °C, there was more than a 6 $\log_{10}$ reduction in both bacteria (at 4 °C a 4.5 $\log_{10}$ reduction in *P. aeruginosa* was seen only after 24 hours). Silver was found to be almost as effective in reducing bacteria in the presence of humic acid (5.5 $\log_{10}$ reduction in *P. aeruginosa* at pH 7, 24 °C after 8 hours in the presence of 3 mg/L humic acid). This group also looked at the potential for exposure to silver (100 µg/L) to reduce biofilm formation in drinking-water distribution systems (Silvestry-Rodriguez et al., 2008). In this role, silver was found to be ineffective, and there was no difference seen between the silver treatment and the control.

Cunningham et al. (2008) used flow cytometry to examine the minimum inhibitory concentration (MIC) of silver nitrate on *E. coli*, with a view to the methodology being used to examine water and wastewater disinfection. They reported a 24 hour MIC of between 60 and 80 µg/L for silver. A 4 $\log_{10}$ reduction (approximately) was seen at 100 µg/L after 24 hours of exposure.

Pathak & Gopal (2012) evaluated the efficacy of silver ions against *E. coli*. Bacteria (concentration – 1.75 x 10^3 cfu/mL) were exposed to various concentrations of silver ions (1, 2, 5, 10 and 20 µg/L), produced from silver electrodes, for up to 60 minutes. A 3 $\log_{10}$ reduction was seen at neutral pH and ambient temperature after a 20 minute period for the 20 µg/L concentration. A 3 $\log_{10}$ reduction was also seen for the other silver concentrations (with the exception of 1 µg/L), although a longer contact time was required (10 µg/L – 40 minutes; 5 µg/L – 50 minutes; 2 µg/L – 60 minutes). Disinfection was most efficient at pH values between pH 8 and 9 and at temperatures greater than 20 °C.

Nawaz et al. (2012) looked at the efficacy of silver (silver nitrate) in inactivating *P. aeruginosa* and *E. coli* in rooftop harvested rainwater supplies. Prior to disinfection, samples were found to contain between 350–440 cfu/100 mL *P. aeruginosa* and 740–920 cfu/100 mL *E. coli*. The disinfection rate and residual effect of silver was determined using final silver concentrations between 10–100 µg/L over a period of up to 168 hours. Samples were taken for microbial analysis every two hours for 14 hours after the application of silver and then daily for one week, to examine regrowth. At higher concentrations (80–100 µg/L) complete inactivation ($\log_{10}$ reduction values [LRVs] between 2.5 and 2.9) of both microorganisms was seen in 10 hours, with no regrowth of *E. coli* seen after 168 hours. Inactivation was slower at lower silver concentrations (LRVs between 1.3 and 2 for silver concentrations between 10–40 µg/L after 14 hours) and regrowth was also observed (e.g. 7.5% survival of *P. aeruginosa* exposed to 10 µg/L silver for 168 hours compared to approximately 4.5% survival at 14 hours). Thus, at the lower concentrations, silver only seemed to delay bacterial reproduction and did not cause permanent damage or loss of ability to increase in number. There were, however, a number of methodological issues with this study, including a lack of follow-up investigation to document regrowth.

Adler et al. (2013) also looked at the effectiveness of silver disinfection as part of rainwater harvesting treatment. Ten rainwater harvesting systems in Mexico, equipped with silver electrodes, were evaluated

for a number of water quality parameters. The silver electrodes were located in line with the filtering system (after a mesh filter, designed to remove large particles, and before an activated carbon filter). On average, the ionizers reduced the level of total coliforms by approximately 1 log_{10} and *E. coli* by approximately 0.4 log_{10} and resulted in a silver concentration of approximately 0.01 mg/L in the final water. The systems, as a whole, delivered water containing 0/100 mL *E. coli* and less than 10/100 mL total coliforms.

In a comparative study of disinfectants, the potency of silver ions, derived from silver nitrate, was examined in a batch disinfection test of ground water using 10^6 cfu/mL *E. coli* (Patil et al., 2013). It was found that for a 6 log_{10} reduction (i.e. complete inactivation), the minimum concentration of silver required was 10 mg/L with a contact time of 3 hours. The bacterial studies are summarized in Table 1 below.

De Gusseme et al. (2010) in a study of ionic silver and silver nanoparticles (outlined in more detail in Section 2.2) found that silver nitrate, at a concentration of 5.4 mg/L, added to UZ1 bacteriophage-spiked (2 x 10^6 plaque-forming units (pfu)/100 mL) bottled water, produced a 3.1 log_{10} reduction after 2 hours and a > 4 log_{10} reduction after 5 hours.

The infectivity of *Cryptosporidium parvum* to mice following exposure of the oocysts to high levels of silver ions (100 mg/L ionic silver from silver nitrate for 30 minutes) was investigated by Abebe et al. (2015). Infectivity was determined by the effect on animal weight and the number of parasites shed in the stool, relative to those exposed to untreated oocysts. Mice receiving silver nitrate treated oocysts demonstrated 3% weight loss at 3 days post infection, compared with 12% weight loss in those mice exposed to untreated oocysts. Parasite shedding was also significantly lower in the animals receiving treated oocysts.

It can be seen from these studies that LRVs varied widely, with some bacteria being more sensitive to silver (i.e. more easily killed or inactivated) than others. Generally, relatively long contact times were required to effectively reduce bacterial concentrations (e.g. 3 hours or longer), the exception being the study of Pathak & Gopal (2012), where silver ions were generated electrolytically (rather than from silver salts), and a 3 log_{10} reduction was seen after 20 minutes at a relatively low silver concentration (20 µg/L). In contrast to the laboratory-spiked samples, where generally higher LRVs were reported, relatively LRVs were seen in harvested rainwater samples (with a low initial bacterial concentration) used by Nawaz et al. (2012). The bacteriophage study of De Gusseme et al. (2010), while suggesting that silver nitrate can result in 3 log_{10} reduction of UZ1, used a high silver concentration (5 mg/L), which would not be relevant as a drinking-water application. The one study on *C. parvum* (Abebe et al., 2015) indicates that silver may potentially be effective for protozoa reduction, but more research is needed.

Table 1: Summary of ionic silver bacterial disinfection studies by microorganism

Organism	Silver type	Concentration (µg/L)	Medium and conditions	Initial concentration	Duration	Log $_{10}$ reduction value	Reference
A. baumannii	AgCl	80	-	3 x 10^6 cfu/mL	72 h	5	B
A. hydrophila	$AgNO_3$	100	Dechlorinated tap water, pH 7, 24 °C	1 x 10^6 cfu/mL	9 h	> 6	C
	$AgNO_3$	100	Dechlorinated tap water, pH 9, 24 °C	1 x 10^6 cfu/mL	9 h	> 6	C
E. coli	$AgNO_3$	100	Synthetic drinking water, pH 7, 25 °C	1.5 x 10^7 cells/mL	3 h	7	A
	$AgNO_3$	100	-	2 x 10^4 cells/mL	24 h	4	D
	$AgNO_3$	100	Harvested rainwater, pH 7–8, 25–27 °C	740–920 cfu/100 mL	10 h	2.8–2.9	F
	$AgNO_3$	40	Harvested rainwater, pH 7–8, 25–27 °C	740–920 cfu/100 mL	14 h	1.3–2[a]	F
	$AgNO_3$	10 000	Ground water	10^6 cfu/mL	3 h	6	G
	Ag^+ from electrodes	20	Autoclaved tap water	1.75 x 10^3 cfu/mL	20 min	3	E
	Ag^+ from electrodes	10	Autoclaved tap water	1.75 x 10^3 cfu/mL	40 min	3	E
	Ag^+ from electrodes	5	Autoclaved tap water	1.75 x 10^3 cfu/mL	50 min	3	E
	Ag^+ from electrodes	2	Autoclaved tap water	1.75 x 10^3 cfu/mL	60 min	3	E
	Ag^+ from electrodes	No data	Harvested rainwater	Max. 275 cfu/mL	No data	0.4	H
L. pneumophila	$AgNO_3$	100	Synthetic drinking water, pH 7, 25 °C	1.5 x 10^7 cells/mL	3 h	2.4	A
P. aeruginosa	$AgNO_3$	100	Synthetic drinking water, pH 7, 25 °C	1.5 x 10^7 cells/mL	3 h	4	A
	AgCl	80	-	3 x 10^6 cfu/mL	12 h	5	B
	$AgNO_3$	100	Dechlorinated tap water, pH 7, 24 °C	1 x 10^6 cfu/mL	8 h	> 6	C
	$AgNO_3$	100	Dechlorinated tap water, pH 9, 24 °C	1 x 10^6 cfu/mL	8 h	> 6	C
	$AgNO_3$	100	Dechlorinated tap water, pH 7, 4 °C	1 x 10^6 cfu/mL	24 h	4.5	C
	$AgNO_3$	100	Dechlorinated tap water, pH 7, 4 °C	1 x 10^6 cfu/mL	24 h	5	C
	$AgNO_3$	100	Harvested rainwater, pH 7–8, 25–27 °C	350–440 cfu/100 mL	10 h	2.5–2.6	F
	$AgNO_3$	40	Harvested rainwater, pH 7–8, 25–27 °C	350–440 cfu/100 mL	14 h	1.3–2[a]	F
S. maltophilia	AgCl	80	-	3 x 10^6 cfu/mL	6 h	5	B

A–Hwang et al., 2007; B–Huang et al., 2008; C–Silvestry-Rodriguez et al., 2007; D–Cunningham et al., 2008; E–Pathak & Gopal, 2012; F–Nawaz et al., 2012; G–Patil et al., 2013; H–Adler et al., 2013; [a]Regrowth observed

2.1.2 Copper/silver ionization

Copper/silver is generally applied to water as an ionization process, with the electrolytic generation of copper and silver ions. Sometimes it is used in combination with a halogen (e.g. chlorine, iodine), although it may also be applied as copper and silver salts. Copper/silver systems are generally used for *Legionella* control (typically in hospital hot water systems), where there are long contact times within the plumbing system. They have been investigated for the treatment of swimming pool water (which is beyond the scope of this report).

2.1.3 Hospital water systems

Copper/silver ionization is often used for *Legionella* control in hot water distribution systems especially in hospital environments. The studies outlined in this sub-section typically relate to systems that are in use and so tend to assess samples for the presence/absence of the organism of interest, rather than using quantitative tests to determine $\log_{10}$ reduction. It is generally considered that ion levels should be regularly monitored and remain within prescribed concentrations (USEPA, 2015; WHO, 2007); published studies suggest levels of between 0.2 to 0.8 mg/L for copper and 0.01 to 0.08 mg/L silver are needed to maximize efficacy (Cachafeiro et al., 2007; Lin et al., 2011).

Liu et al. (1998) looked at the intermittent use of a single copper/silver ionization system in the hot water systems of two buildings. Twenty distal sites in each building were examined for *Legionella* before the start of ionization and then monthly after installation. The elimination of *Legionella* took between 4 and 12 weeks. After cessation of disinfection (16 weeks), re-colonization did not occur for between 6 to 12 weeks (depending on the sampling site) in the first building, and 8 to 12 weeks in the second building. The control building (no ionization) remained positive for *Legionella* throughout the study period.

In 2003, Stout & Yu (2003) reported on surveys of the first 16 hospitals in the USA to install copper/silver ionization systems for *Legionella* control. Prior to installation, all of the hospitals had reported cases of hospital-acquired Legionnaires' disease and 75% had attempted other disinfection methods. Two postal surveys (1995 and 2000) gathered information on environmental monitoring of *Legionella*, identification of hospital-acquired legionellosis, and monitoring and maintenance of the copper/silver ionization systems. *Legionella* monitoring was conducted at 15 out of the 16 hospitals at both time points, although the frequency of monitoring was markedly lower at the second survey (9/16 hospitals reported monthly or quarterly monitoring in 1995, compared to only 4/16 hospitals reporting quarterly monitoring in 2000). Regular monitoring (undefined) of copper/silver concentrations was reported by 15/16 hospitals in 1995; no information is presented for the 2000 survey. Colonization of distal water sites with *Legionella* was much less frequent after installation of the copper/silver ionization (with between 7 and 8 of the hospitals reporting zero positivity of monitoring sites, and the remaining hospitals reporting 30% positivity or lower). A single case (shortly after installation) of hospital-acquired Legionnaires' disease was reported from the surveyed hospitals after implementation of copper/silver ionization.

In Switzerland, Blanc et al. (2005) found that at a water temperature of 50 °C, copper/silver ionization was not effective at reducing *Legionella* in their hospital hot water system (90% of water samples were positive for *Legionella* before treatment, 93% were positive after the introduction of ionization), although they acknowledged that the low concentration of ions (copper 0.3 mg/L, silver not reported) and the high pH (7.8–8.0) of the hot water may have explained the poor results. High pH had previously been shown to have a detrimental effect on the ability of copper ions to kill *Legionella* (Lin et al., 2002). In addition, trisodium phosphate was used within the hospital hot water system to protect against

corrosion; copper ions are known to bind to phosphate (Lin & Vidic, 2006), which will reduce their efficacy against *Legionella*. Blanc et al. (2005) found that ionization in conjunction with increased temperature (65 °C) was more effective, with the number of *Legionella* positive samples falling to 39%, and the level of *Legionella* in the positive samples also decreasing (mean of 7.6 cfu/mL with ionization alone, compared to a mean of 0.23 cfu/mL with ionization and a raised temperature).

In Spain, Mòdol et al. (2007) looked at hospital-acquired Legionnaires' disease following introduction of a copper/silver ionization system. Prior to installation, hospital-acquired Legionnaires' disease was 2.45 cases/1000 patient discharges. After installation, the level dropped to 0.18 cases/1000 patient discharges and, after increasing ion levels, no further cases of Legionnaires' disease were reported up to the end of the study period (19 months). Prior to installation, 57% of water samples were positive for *L. pneumophila* compared to 16% after installation, when the system was running consistently with copper/silver ion levels greater than 0.3 and 0.03 mg/L, respectively.

Pedro-Botet et al. (2007) investigated the impact of copper/silver ionization on fungal colonization of a number of health-care centre water systems after noticing that the number of consultations regarding fungal infections in their centre had dropped markedly since the installation of an ionization system (for *Legionella* control). Samples from ionized water distribution systems (nine health care centres) were compared with non-ionized systems (seven health care centres). The prevalence of fungi was significantly lower in the samples of ionized water (29% compared to 77%) in both hot and cold water systems – with the most marked difference seen in the cold water samples (14% compared to 88%). A decrease in fungal colonization following the implementation of copper/silver ionization was also reported by Chen et al. (2013). They found a 40% reduction in fungal colonization during ionization treatment, with fungi isolated from only 2% of samples during this period.

Chen et al. (2008) looked at the efficacy of a point-of-entry copper/silver ionization system (designed to treat both hot and cold water) against *L. pneumophila* in a hospital water distribution system. Prior to installation, typically between 32% and 50% of samples were positive for *L. pneumophila*. In the first three months (when ion levels were well below recommended levels: average copper and silver levels of 0.095 and 0.012 mg/L respectively), no change was seen in the number of positive samples. However, when ion concentrations were reportedly increased (average copper and silver levels of 0.135 and 0.011 mg/L respectively) in months 4 to 7, the number of positive samples decreased significantly to between 5 and 16%. Rates of *Legionella* positivity dropped further to between 0 and 5% after month 7. Mean positivity remained at 50% in the control (non-treated) sites. The ion concentrations varied between sampling sites and over the course of the monitoring. Mean levels (between months 4 to 12) were 0.132 mg/L copper and 0.012 mg/L silver, below the target concentrations of 0.2 and 0.02 mg/L respectively. The authors note that, while the system was not operating at the required ion concentrations and did not completely eradicate *L. pneumophila*, no cases of hospital-acquired Legionnaires' disease were reported during the year-long study.

According to Lin et al. (2011) emergence of *L. pneumophila* with resistance to copper/silver ions has been documented in some cases, usually several years after installation of the ionization system, although hospitals where ion concentrations and *Legionella* positivity were monitored were less likely to report resistance problems.

In 2012, five confirmed and 16 probable hospital-acquired cases of Legionnaires' disease were identified in Pittsburgh, USA at one of the first hospitals to adopt copper/silver ionization for *Legionella* treatment in 1993. The system was successful for several years. Reports suggest that prior to the outbreak and until the outbreak was formally identified the system had not been subject to appropriate

monitoring and control and the system had not been properly run (with issues such as an incorrect amperage being used and staff not appreciating the importance of maintaining the correct pH range).[24] A Centre for Disease Control study noted that the outbreak coincided with construction work at the hospital, which may have introduced organic matter to the water system, increasing consumption of chlorine leading to amplification of *Legionella*.[25]

Typically, it would seem that copper/silver ionization reduces the number of *Legionella* (and fungal) positive samples in treated systems; however, it may not completely eradicate the pathogen. This point was made by Cachafeiro et al. (2007) who, following a review of the literature, noted that eradication cannot be achieved by any method in isolation and that maintaining high temperatures in hot water systems maximizes the effectiveness of the ionization approach. Despite the fact that complete eradication of the pathogen is not achieved, a number of studies have suggested that the implementation of copper/silver ionization markedly reduces the number of cases of hospital-acquired Legionnaires' disease.

2.2 Silver nanoparticle applications

The potential of silver nanoparticles for household POU drinking-water disinfection is currently being extensively explored, principally in conjunction with filtration. The medium or matrix utilized for the nanoparticles varies widely and includes coating on polyurethane foams (Jain & Pradeep, 2005), fibreglass (Nangmenyi et al., 2009), copolymer beads (Gangadharan et al., 2010), paper (Dankovich & Gray, 2011), polystyrene resin beads (Mthombeni et al., 2012), alginate composite beads (Lin et al., 2013), ceramic (Lv et al., 2009), titiania (Liu et al., 2012), activated carbon composite incorporating magnetite (Valušová et al., 2012) and bacterial carriers (De Gusseme et al., 2010; 2011). As the focus here is on the efficacy of silver in water disinfection, only studies where this can be distinguished from, for example, the filtration effect, have been considered below. In addition to considering the LRVs of microorganisms exposed to the test material, a number of studies also conducted zone of inhibition tests.[26]

Jain & Pradeep (2005) coated polyurethane foam with citrate-stabilized silver nanoparticles. The antibacterial efficacy was assessed by adding small pieces of Ag-treated or untreated foam to *E. coli* suspensions (10^5–10^6 cfu/mL) and assessing bacterial growth after a 5- or 10-minute exposure period. No bacterial growth was seen in the samples exposed to Ag-treated polyurethane, while the untreated polyurethane samples showed "substantial growth". In addition, no growth of *E. coli* was detected on agar plates beneath pieces of silver nanoparticle-treated foam in a zone of inhibition test. A prototype filter was created using the treated foam, which was found to be effective at eliminating *E. coli* growth, but equivalent data are not available for untreated foam, making the contribution of the silver treatment difficult to determine.

Nangmenyi et al. (2009) looked at the performance of silver nanoparticle (< 30 nm) impregnated fibreglass during immersion and during filtration. For the immersion test, a silver-impregnated mat (1% silver by weight) was added to a 100 mL *E. coli* suspension (10^6 cfu/mL). After an hour of immersion, *E. coli* could not be detected in the suspension. Using an *E. coli* concentration of 10^{12} cfu/mL, the silver nanoparticle fibreglass mat (1.8% silver by weight) resulted in a 7 $\log_{10}$ reduction in concentration in

[24] http://www.post-gazette.com/local/city/2014/03/11/Studies-blame-VA-outbreak-on-employees-errors/stories/201403110229

[25] http://www.cdc.gov/washington/testimony/2013/t20130205.htm

[26] The zone of inhibition is the area on an agar plate containing a lawn of bacteria where the growth of the microorganisms is prevented by the antimicrobial activity of the test material placed on the agar surface.

five minutes. Antibacterial filters (5% silver by weight) were fabricated and a bacterial solution (10^6 cfu/mL *E. coli*) was pumped through the filter at a flow rate of 20 mL/min. *E. coli* were not found in the treated water, amounting to a 6 log_{10} reduction. The untreated fibreglass accounted for only an approximately 1 log_{10} reduction.

Lv et al. (2009) examined the efficacy of silver nanoparticle-coated porous ceramic tiles. The ceramic was modified (using a coupling agent) to ensure that the silver nanoparticles were fixed to the material (rather than relying on weak forces of attraction). There was no obvious loss of silver nanoparticles when the tiles were exposed to water. Antibacterial action was assessed by exposing a solution of *E. coli* (10^4–10^5 cfu/mL) to pieces of the treated and untreated (control) ceramic, followed by conducting a zone of inhibition test and a flow test. After 24 hours, no bacteria could be grown from the samples exposed to silver-treated ceramic and, in the zone of inhibition test, there was a clear zone where no bacteria grew on the agar plate after 24 hours of exposure to the ceramic. In the flow test using an experimental water filter (flow rate 10 mL/min), no bacteria were detected in the filtered water. Substantial (unquantified) concentrations of bacteria, however, were detected in water filtered through untreated ceramic. The authors suggest two possible antimicrobial mechanisms, namely: (a) the bacteria are killed by ionic silver released from the ceramic; and/or (b) the bacteria flowing from the ceramic are contaminated with silver, which prevents their subsequent growth. Silver measurements, however, were not reported from the filtered water.

Gangadharan et al. (2010) investigated the antibacterial effectiveness of polymer microspheres containing non-leaching silver nanoparticles by incubating various bacteria (*E. coli*, *P. aeruginosa*, *B. subtilis* and *Staphylococcus aureus*, with concentrations of between 10 x 10^6–300 x 10^6 cfu/mL), with the beads for up to 24 hours. The beads were found to be effective against both Gram-negative and Gram-positive bacteria, with bacterial counts reduced to zero for all strains tested, with the exception of *B. subtilis* (where a 3 log_{10} reduction was seen). Zones of inhibition were seen around agar plated beads for all of the bacteria tested. There was no bacterial adsorption or adhesion to the silver-containing beads.

Heidarpour et al. (2011) investigated the ability of silver nanoparticle-coated polypropylene filters to remove *E. coli* from water. Fifteen litres of distilled water containing 10^3 cfu/mL *E. coli* was passed through either uncoated or silver-coated filters at a flow rate of 3 litres per hour. After 7 hours of filtration and re-circulation, the *E. coli* level from the silver-treated filter was zero, while the concentration from the untreated filter remained at 10^3 cfu/mL. Scanning electron micrographs demonstrated *E. coli* cells attached to the surface of the silver nanoparticle-coated filter. No silver nanoparticles were detected in the treated water. The reported bacterial removal is likely to be a combination of the bacteriostatic/bactericidal impact of the silver and the decreased pore size of the silver-treated polypropylene in comparison with the untreated material (pore size of 1.3 µm and 9.9 µm, respectively).

Dankovich & Gray (2011) investigated the efficacy of nanosilver impregnated paper for reducing bacterial contamination in water. The silver nanoparticles were produced *in situ* by the reduction of silver nitrate in the paper sheet. The bactericidal impact was assessed by passing model bacterial suspensions (*E. coli* and *Enterococcus faecalis*) through the paper and analysing the effluent water for viable bacteria. The average percolation time for 100 mL of bacterial solution was 10 minutes. Plate counts showed up to a 7.6 and a 3.4 log_{10} reduction of viable *E. coli* and *E. faecalis* (respectively) in the effluent compared to the initial concentration of bacteria (10^9 cfu/mL) at the highest silver concentration (5.9 mg silver/dry g paper). Of this reduction, less than 1 log_{10} was attributed to the filtration of the paper. The average silver content of the effluent water was 50 µg/L. Dankovich (2014) has done further

work, examining the effect of producing the nanosilver paper using a safer technique. Filtration through the paper, produced using glucose as a reducing sugar in combination with a domestic microwave oven, produced similar results to the earlier work, with an 8.1 $\log_{10}$ reduction for *E. coli* (initial concentration 1 x 10^9 cfu/mL) and a 2.3 $\log_{10}$ reduction for *E. faecalis* (initial concentration 2 x 10^8 cfu/mL), with a 1 $\log_{10}$ reduction attributed to the paper alone. It was thought, based on filtration time, that there was a greater physical retention of *E. coli* compared to *E. faecalis*. The average silver content in the treated water was 105 ± 36.3 µg/L.

Mpenyana-Monyatsi et al. (2012) compared the bacterial removal by a number of low-cost filter materials coated with silver nanoparticles. Various concentrations of silver nanoparticles were deposited on zeolite, sand, fibreglass, anion resin and cation resin substrates. In the first phase of analysis, the substrates were tested using *E. coli* spiked water samples (10^6 cfu/100 mL), to determine the optimal silver loading (0.1 mM). In the second phase, each of the substrates (with the optimal silver loading) was tested against *E. coli*, *Salmonella typhimurium*, *S. dysenteriae* and *Vibrio cholerae* in groundwater samples (all bacteria present at 10^3 cfu/100 mL). The silver/cation resin filter was found to be the best performing, achieving 3 $\log_{10}$ reduction of all the targeted bacteria, with no regrowth over 2 hours. The silver/zeolite filter was found to have the worst performance, with $\log_{10}$ reduction rates between 0.5 and 2. The amount of silver ions eluted from the filter material varied according to material type and time, with high concentrations released from zeolite, sand, fibreglass and anion resin substrates within the first 10 minutes (maximum concentration 1.8 mg/L). The cation resin filter released the lowest concentration of silver (less than 100 µg/L) in the eluent and, thus was found to be the best performing in terms of bacterial reduction and silver loss.

Lin et al. (2013) synthesized and studied the efficacy of three types of silver nanoparticle-alginate composites for application as a POU technology for water disinfection. Alginate was chosen as the immobilization/delivery material because of both its natural abundance and biocompatibility. The finished beads were used to create porous columns and the bacterial removal abilities of the different beads were compared using *E. coli* (approx. 10^5 cfu/mL). Two of the three bead types consistently produced a 5 $\log_{10}$ reduction during filtration, even with a short hydraulic retention time (HRT) and the third bead type produced a 2 $\log_{10}$ reduction. Silver was also measured in the filtered water, and again the three bead types produced different results ranging from 11–98 µg/L to 4–22 mg/L (depending on the type and HRT). The authors speculate that the disinfection efficacy, despite the short retention time, is probably due to released silver ions or silver nanoparticles in the effluent, which continue to exert an influence over the test bacteria even after plating for culture assay, although one bead type produced both low silver concentration in the filtered water and excellent removal of *E. coli*. The authors concluded that the results suggest that the beads show promise, but note that long-term breakthrough studies are needed. Other researchers have noted that the validity of efficacy studies could be biased because they did not correct for the presence of toxic contaminants. Samberg et al. (2011), for example, investigated the efficacy of silver nanoparticles against a number of bacteria in culture medium and found that washed and unwashed silver nanoparticles had notably different MICs (e.g. for the 20 nm silver nanoparticles against *E. coli* J53, MICs were 64 µg/mL and < 4 µg/mL, respectively). The additional toxicity of the unwashed particles was attributed to the presence of formaldehyde.

Loo et al. (2013) explored the use of silver nanoparticles in cryogels as a possible POU treatment. The silver nanoparticle-treated gels were added to water containing 10^8 cfu/mL of *E. coli* or *B. subtilis*. After 15 seconds, to allow swelling, the gel was removed from the bacterially spiked (bulk) water and squeezed to recover the absorbed water. Gels with different silver contents (0 and approximately 20, 90 and 170 mg/g) were assessed. Significantly higher disinfection efficacies (5.4–7 $\log_{10}$ reduction) were seen for the “squeezed” water compared to the remaining bulk water (maximum 2 $\log_{10}$ reduction). The

highest $\log_{10}$ reduction was seen from the gel with the greatest silver nanoparticle content (approximately 6.5 and 7 $\log_{10}$ reduction for *E. coli* and *B. subtilis,* respectively). Untreated gel was capable of less than 1 $\log_{10}$ reduction. The silver content of the squeezed water was assessed and found to range between 36.4 to 76.6 µg/L (with the lower concentrations being from the 90 and 170 mg/g gels; 59.6 and 36.4 µg/L, respectively). The squeezed water contained both ionic silver (45–56%) and silver nanoparticles.

De Gusseme et al. (2010; 2011) have investigated the possibility of using biogenic silver for water disinfection, where bacteria are used as reducing agents for the production of nanosized elemental silver particles. In comparison with chemically produced silver nanoparticles, the biogenic particles were found to be far more effective at disinfection. In a spiking experiment using a bacteriophage (10^6 pfu/mL), biogenic silver produced a 4 $\log_{10}$ reduction after three hours, while the chemically produced elemental silver particles showed no inactivation. The biogenic particles were also found to be effective against murine norovirus, with a greater than 4 $\log_{10}$ reduction after only 30 minutes. The capacity of biogenic silver for use in continuous disinfection was assessed following coating of an electropositive cartridge filter. Addition of the biogenic silver increased the reduction of virus from 1.5 $\log_{10}$, with the filter alone, to 3.8 $\log_{10}$. Low concentrations of ionic silver (3 µg/L) were initially detected in the filtrate (up to 5 minutes); thereafter, none was detected (De Gusseme et al., 2010). This group has also looked at the immobilization of biogenic silver to microporous membranes (De Gusseme et al., 2011). The system was found to be capable of achieving at least a 3.4 $\log_{10}$ reduction in bacteriophage concentration (compared to a less than 1 $\log_{10}$ reduction by the membrane alone). Silver was found to leach out of the system; initially levels of 271 µg/L were recorded, but these soon dropped to below 100 µg/L.

Patil et al. (2013) conducted a comparative study of disinfectants for use in household water treatment systems and considered both silver ions (outlined in Section 2.1.1) and silver nanoparticles, using a batch disinfection test of ground water spiked with *E. coli* (10^6 cfu/mL). Silver nanoparticles (synthesized from silver nitrate using citrate as a reducing agent) at 1 mg/L required a 3 hour contact time for a 6 $\log_{10}$ reduction.

Mecha & Pillay (2014) investigated the efficacy of silver nanoparticle-impregnated woven fabric microfiltration membranes. Turbidity removal was examined using water with up to 700 nephlometric turbidity units (NTU) and disinfection performance was assessed using three concentrations of *E. coli*, based on river water (2500 and 10 000 cfu/100 mL) and one on synthetic feed water (77 000 cfu/100 mL). The silver nanoparticle impregnated membranes, after an initial priming period, reduced turbidity to below 1 NTU, irrespective of the turbidity of the feed water. *E. coli* removal for the membrane alone was between 84–91%, while the coated membrane completely removed *E. coli* (i.e. up to 5 $\log_{10}$ reduction). Silver eluted from the coated filters was below 0.02 mg/L. A long-term study showed that the silver-coated membrane was effective at removing *E. coli* for at least 2 months of continuous operation (Mecha et al., 2014).

Liu et al. (2014) examined the use of nanosilver textile fixed to a plastic tube as a POU disinfection kit. The kit is immersed in the water to be treated and then used to stir the water (for 1, 5 or 10 minutes). The system produced minimal reduction in bacteria naturally present in rainwater (LRVs between 0.61 and 0.96 depending upon contact time) and also spiked concentrations of *E. coli* (1.65 $\log_{10}$ reduction after 5 minutes).

In mice infectivity tests (outlined in Section 2.1.1), Abebe et al. (2015) found that treatment of *Cryptosporidium* oocysts with proteinate-capped or PVP-capped silver nanoparticles resulted in a minor reduction (statistical significance not stated) in the level of weight loss (12% reduction in body weight

in mice receiving untreated oocysts compared with an 8% and 6% loss [respectively] for the mice receiving silver nanoparticle-treated oocysts). Some reduction was also seen in the amount of oocyst shedding in the stool, with the proteinate-capped silver nanoparticles resulting in a marked (but non-statistically significant) reduction in shedding.

The majority of studies considering silver nanoparticles for drinking-water treatment applications tested efficacy against bacteria, typically *E. coli*, with the exception being the work of De Gusseme et al. (2010, 2011), who considered the effects against bacteriophage and murine norovirus and Abebe et al. (2015) who considered *Cryptosporidium* infectivity. Typically, good bacterial LRVs were reported (as summarized in Table 2), with values up to 7 $\log_{10}$ reduction (depending upon the spiking concentration) for *E. coli*. However, in many cases, contact times were generally long. Generally, the silver nanoparticle test materials were effective in both test tube trials (where the silver-treated material is immersed in microbially spiked water) and, where tested, following filtration. Where reported, levels of silver in the filtered water were usually below 50 µg/L.

Table 2: Summary of silver nanoparticle bacterial disinfection studies by microorganism

Organism	Medium/matrix	Conditions	Initial concentration	Duration or volume	Log_{10} reduction value	Reference
B. subtilis	Copolymer microspheres	Immersion (200 mg AgNP)	24 x 10^6 cfu/mL	4 h	3.3	D
	AgNP-decorated cryogels	Gel (170 mg Ag/g) added to spiked water for 15 s and then removed and squeezed	10^8 cfu/mL	15 s	7	I
E. coli	Coated polyurethane foam	Immersion of foam into bacterial suspension	10^5 cfu/mL	5–10 min	5	A
	Impregnated fibreglass	Immersion of fibreglass (1% Ag by weight) in bacterial suspension	10^6 cfu/mL	1 h	6	B
	Impregnated fibreglass	Filter created with fibreglass (5% Ag by weight), flowrate 20 mL/min	10^6 cfu/mL	3 L	6	B
	Porous ceramic tiles	Immersion of tiles in bacterial suspension	10^4–10^5 cfu/mL	24 h	4-5	C
	Porous ceramic tiles	Filtration of *E. coli* solution, flow rate of 10 mL/min	10^5 cfu/mL	500 mL	5	C
	Copolymer microspheres	Immersion (200 mg AgNP)	7 x 10^6 cfu/mL	4 h	6	D
	Polypropylene filters	Filtration of 15 L (at 3 L/h)	10^3 cfu/mL	7 h	3	E
	Paper	Filtration (5.9 mg Ag/dry g of paper), flow rate 10 mL/min	10^9cfu/mL	10 min	7.6	F
	Cation resin filter substrate	Filtration of bacterially spiked groundwater, flow rate 0.12 L/h	10^3 cfu/100 mL	10 min	3	G
	AgNP-alginate composites, created using different methodologies	Filtration through adsorption reduction beads	10^5 cfu/mL	HRT of 1 min	5	H
	AgNP-alginate composites, created using different methodologies	Filtration through AgNP incorporation beads	10^5 cfu/mL	HRT of 1 min	2	H
	AgNP-alginate composites, created using different methodologies	Filtration through simultaneous gelation-reduction beads	10^5 cfu/mL	HRT of 1 min	5	H
	AgNP-decorated cryogels	Gel (170 mg Ag/g) added to spiked water for 15 s and then removed and squeezed	10^8 cfu/mL	15 s	6.4	I

Organism	Medium/matrix	Conditions	Initial concentration	Duration or volume	Log_{10} reduction value	Reference
E. coli contd.	AgNP	AgNP solution (varying concentrations) added to bacterial suspension	10^6 cfu/mL	1 mg/L at 3 h	6	J
	AgNP impregnated woven fabric microfiltration membranes	Filtration through membrane	Up to 7 x 10^4 cfu/100 mL	5 min	4	K
	AgNP immobilized onto cotton textile	Immersion and stirring of bacterial suspension	1.9 x 10^5 cfu/mL	5 min	1.65	L
E. faecalis	Paper	Filtration (5.9 mg Ag/dry g of paper), flow rate 10mL/min	10^9 cfu/mL	10 min	3.4	F
P. aeruginosa	Copolymer microspheres	Immersion (200 mg AgNP)	22 x 10^6 cfu/mL	4 h	6	D
S. aureus	Copolymer microspheres	Immersion (200 mg AgNP)	46 x 10^6 cfu/mL	4 h	6	D
S. dysenteriae	Cation resin filter substrate	Filtration of bacterially spiked groundwater. Flow rate 0.12 L/h	10^3 cfu/100mL	10 min	3	G
S. typhimurium	Cation resin filter substrate	Filtration of bacterially spiked groundwater. Flow rate 0.12 L/h	10^3 cfu/100mL	10 min	3	G
V. cholerae	Cation resin filter substrate	Filtration of bacterially spiked groundwater. Flow rate 0.12 L/h	10^3 cfu/100mL	10 min	3	G

A–Jain & Pradeep, 2005; B–Nangmenyi et al., 2009; C–Lv et al., 2009; D–Gangadharan et al., 2010; E–Heidarpour et al., 2011; F–Dankovitch & Gray, 2011; G–Mpenyana-Monyatsi et al., 2012; H–Lin et al., 2013; I–Loo et al., 2013; J–Patil et al., 2013; K–Mecha & Pillay, 2014; L–Liu et al., 2014

2.3 Silver-coated ceramic filter applications

A number of different types of silver-coated or silver-impregnated ceramic filters (using
nanoparticles or silver nitrate) have been used as POU devices, typically in developing co
household treatment of drinking-water. Much of the literature on ceramic filter studies, h
been designed to look at the effectiveness of the filters, rather than the impact of the si
effectiveness (e.g. Baumgartner et al., 2007; Brown et al., 2008; Clasen et al., 2004, 2005;
al., 2008; Salsali et al., 2011; Abebe et al., 2014). In addition to filters employing silver
microbial removal, domestic (and travel) filters may also incorporate silver into the filte
biofilm formation, however, no published literature (in terms of silver efficacy) was fo
application.

Van Halem et al. (2007) reported results from filter challenge studies with *E. coli* (K12),
spores and MS2 bacteriophage for six silver nanoparticle-coated and six uncoated Nicaragu
filters (Table 3). Although the silver-coated filters slightly outperformed the uncoated filter
removal, the difference was not statistically significant. There was very little difference in p
for *Clostridium* spore removal and the silver-free filters outperformed the coated filters a
points for MS2 bacteriophage removal.

**Table 3: Log_{10} reduction values from coated and uncoated ceramic filters (van Halem
2007)**

Microbe	Challenge doses	Log_{10} reduction values	
		Silver-coated (n=6)	Silver-free (
E. coli	10^5 to 10^7 cfu/100 mL	4.7 to 7.2	2.6 to 5.
Clostridium spores	10^3 to 10^5 n/100 mL	3.6 to 5.3	2.7 to 5.
MS2 (1)	10^4 to 10^6 pfu/mL	0.5 to 0.7	0.8 to 1.
MS2 (2)	10^4 to 10^6 pfu/mL	0.8 to 1.4	1.8 to 2.

The two MS2 experiments were done at different points in a long-term study, namely week 5 and week 13. The a
that the improvement in performance between the two time points may be due to biofilm formation.

Wubbels et al. (2008) looked at the bacterial removal efficiency of silver (unspecified) i
ceramic filters in extensive laboratory-based testing. Ceramic, candle-type filters with a
silver were compared for their ability to remove *E. coli* WR1 (10^6 cfu/L) spiked into drinkin
further details available), at two different flow rates, over time. Over 8000 litres of drinking
passed through each of the filters and samples were periodically spiked with *E. coli* an
reduction efficacy assessed (Table 4). Initially, there was little difference between the filter
generally, between 5 and 6 log_{10} reduction seen in all cases. After passage of almost 5500 litr
however, the silver filters started to out-perform the non-silver filters. After over 8000
reduction rates had dropped for both silver (log_{10} reduction 2.2–3.2) and non-silver (log_{10} red
1.2) filters. The silver concentration in the effluent from the silver filters ranged between 11
μg/L at the start of the experiment to 1.72 to 3.65 μg/L at the end.

Table 4: Log_{10} removal values for *E. coli* from two untreated and two silver-impregnated ceramic filters (Wubbels et al., 2008)

Volume filtered (L)	Reference filter Flow – 6 L/h	Silver-coated filter Flow – 6 L/h	Reference filter Flow – 3 L/h	Silver-coated filter Flow – 3 L/h
Start	5.1	> 5.4	> 5.4	> 5.4
1000	4.4	5.5	5.5	5.5
2067	5.2	> 6.0	6.0	> 6.0
3452	5.5	> 5.8	5.8	> 5.8
4487	5.1	> 5.6	> 5.6	> 5.6
5469	3.8	5.5	4.9	> 5.8
6411	3.7	> 6.4	4.5	> 6.4
7390	2.7	4.5	2.9	5.6
8389	1.1	2.2	1.2	3.2

Bielefeldt et al. (2009) showed that the disinfection efficacy of ceramic filters could be variable. In tests of untreated (2 filters), or previously heavily used silver nanoparticle-treated filters, *E. coli* (K12) LRVs varied between < 1 to > 4 for both the untreated and heavily used filters. The initial filter run tended to show the best reduction, with LRVs of between 3.7 and 4 in the untreated filters, and 2.9 to 4.1 in the heavily used filters. Re-coating of the previously heavily used filters improved LRVs slightly, but filters still showed high variability between filter runs and the improved removal efficiencies were not maintained. As with the initial tests, the first filter run was generally the one with the best reduction (3.5 log_{10} to 4.5 log_{10}). It is not clear from the paper whether the short-term improvement with re-coating was statistically significant. Silver, however, was found to be important in preventing contamination of subsequent batches of un-spiked dechlorinated water passing through the filter (levels of < 20 to 41 cfu/mL in recoated filters compared to 10^3–10^5 cfu/mL prior to re-recoating).

Bloem et al. (2009) looked at *E. coli* and MS2 bacteriophage removal from filters with and without silver (silver nitrate). Water was spiked with 10^3–10^6 cfu/mL *E. coli* or 10^3–10^4 pfu/mL MS2 and passed through the filters. Twenty litres of water was treated daily for up to six months. Over the lifetime of the experiment, the silver-treated filters outperformed the untreated ones with mean *E. coli* LRVs of 5.9 and 3.1, respectively. No difference was seen between treated and untreated filters in MS2 removal, with removal being uniformly low (0.5 log_{10}). As reported in some other studies, high variability in removal efficiencies were seen, with *E. coli* LRVs in silver-treated filters ranging between 3.82 and 7.65 compared to 2.01 and 4.3 in untreated filters.

Brown & Sobsey (2010) found no significant difference in the removal of *E. coli* – CN13 (challenge dose 10^4–10^7 cfu/mL) or MS2 (challenge dose 10^5–10^8 pfu/mL) between silver-treated (silver nitrate) and untreated filters. *E. coli* LRVs were between 2.2 and 2.3 in the silver-treated filters, compared to 2.1 in the untreated filters. MS2 removal was between 1.3–1.5 log_{10} in the silver-treated filters, compared to 1.6 to 1.7 log_{10} in the untreated filters.

Kallman et al. (2011) looked at *E. coli* (wild strain) removal and compared untreated and silver nanoparticle-treated filters made with different percentages of sawdust. Although the authors comment that silver improves the LRVs for the filters (Table 5), probably the only significant improvement is that seen for the 17% sawdust filter.

Table 5: *E. coli* log_{10} reduction values for silver-treated and untreated filters with different sawdust content (Kallman et al., 2011)

Percentage sawdust	Log_{10} reduction value	
	Untreated	Silver-treated
4%	4.56	4.74
9%	3.52	3.81
17%	2.55	4.91

Zhang & Oyanedel-Craver (2013) compared *E. coli* (wild strain) log_{10} reduction values (challenge dose 10^{10}–10^{11} cfu/mL) in ceramic disks with or without silver nanoparticle treatment. Silver treatment did not noticeably improve the performance of the disks (4.2–4.3 log_{10} reduction by the untreated disks compared to 4.4 log_{10} reduction by the treated disk).

Rayner et al. (2013) investigated the impact of the type of silver (ionic or silver nanoparticles) on bacterial removal efficacy using ceramic disks. Using different silver concentrations (0.003, 0.03 and 0.3 mg of silver/g of disk), bacterial removal performance was assessed using 10^6 cfu/mL *E. coli* continuously fed to the disks at a flow rate of 0.5 mL/minute. Samples were taken daily for 10 days, with the concentration of bacteria measured in both the influent and effluent. In addition, viable bacterial retention on and within the disks was also examined. A sharp decrease in LRVs was seen from day 1 to day 4, with a levelling off seen thereafter, so the LRVs are based on samples taken from days 5 to 10. The results varied according to the source of the clay used for the disks. For the Indonesian and Tanzanian clays, 0.3 mg/g silver nanoparticles was found to be the most effective, with > 4 log_{10} reduction seen on day 10 (1 to 1.7 log_{10} reduction improvement over the control disks without silver). Disks coated with other concentrations of silver either produced no improvement or a less than 1 log_{10} reduction improvement over the control. Silver desorption was greater for the disks coated with silver nitrate compared to silver nanoparticles. Effluent silver concentration, bacterial removal and viable bacteria retention were dose-dependent on the amount of silver applied. The authors recommend that, based on the results, ceramic filter factories should use silver nanoparticles rather than silver nitrate, to improve silver-filter retention and that silver nanoparticle application should be increased to 0.3 mg/g to maximise microbiological performance without compromising the effluent quality.

Although Ren & Smith (2013) did not measure microbial reduction, they compared the retention of silver nanoparticles in a ceramic porous medium following different application methods (paint-on, dipping and fire-in methods). The fire-in method appeared to significantly improve silver nanoparticle retention and even where the amount of silver applied to the disk was increased by a factor of 10 (27.3 mg silver per disk), the effluent silver concentrations did not exceed 0.02 mg/L.

Bielefeldt et al. (2013) looked at the impact of various water quality parameters on the detachment of silver nanoparticles from a solid silica surface (representative of ceramic pot filters). Over typical ranges of pH, ionic strength, turbidity and dissolved organic matter, minimal impact was seen on the rate of release and dissolution of silver nanoparticles. Free chlorine (added as sodium hypochlorite), however, rapidly removed silver (whether applied as ionic silver or silver nanoparticles) even at drinking-water levels, leading to the suggestion that contact between ceramic pot filters and pre-chlorinated water and cleaning with bleach should be avoided. Mittelmann et al. (2015) also examined the impact of different water chemistries on silver dissolution and release from ceramic water filters (coated with casein-coated silver nanoparticles or silver nitrate) and concluded that saline, hard or acidic waters should be avoided to minimize eluent silver concentrations and preserve silver treatment integrity.

Van der Laan et al. (2014) looked at the role of silver (silver nitrate) during both filtration and storage, comparing different silver applications (non-silver-treated, silver-treated either on the outside or on both sides of the filter) in long-term loading experiments using *E. coli* (K12 and WR1 strains) and MS2 bacteriophage. Comparison of samples taken within 5 minutes of filtration and after 11 hours of storage showed that, for silver nitrate coated filters, there is very little inactivation during the filtration phase, with no significant difference found immediately after filtration between the filters, with or without silver treatment (median LRVs between ~ 0.7 and 1.1 for *E. coli* and an average of 0.6 for MS2). Storage time, post filtration, was found to be the dominant parameter in *E. coli* inactivation (no post-storage data presented for MS2); after 11 hours of storage the median LRVwas approximately 4 in the silver treated filters.

Simonis et al. (2014) tested silver-treated ceramic filters (with silver applied either by sputter coating or through dipping [silver impregnation] and drying in different atmospheres to coat the filters with ionic silver or silver oxide) against F-specific and somatic phages. Initial spiking levels were 2.5×10^5 pfu/mL for F-specific phage and 2.1×10^3 pfu/mL for the somatic phage. The LRVs varied according to both the coating method and the phage type. For the F-specific phage, LRVs were between < 0.1 and 0.56. Greater inactivation was seen for the somatic phage (LRVs between 1.2 and 1.84). In both cases the sputter coated filter was the most effective.

Matthies et al. (2015) looked at bacterial and bacteriophage removal using silver-coated (silver nitrate) Indonesian filters. Bacterial LRVs from spiked samples were between 3.4 and 5 (*E. coli* 5.1–5.2 $\log_{10}$ reduction; *Enterococcus faecium* 3.4–> 4.5 $\log_{10}$ reduction; *P. aeruginosa* 3.4–5.0 $\log_{10}$ reduction). Log_{10} reduction values for bacteriophages MS2 and øX174 were considerably lower and, typically, were between 0.5 and 0.6.

Although some studies have suggested that silver treatment improves the *E. coli* removal performance of filters, others have shown only small benefits, short-term improvements or negligible impact (beyond the filtration effect) as a result of silver, with no clear pattern of removal in relation to the type of silver used (i.e. ionic or nanoparticles) – see the summary of LRVs in Table 6.

Work directly comparing filters using ionic silver versus silver nanoparticles, however, suggest that silver nanoparticles may be as effective at bacterial reduction but shows better retention within the filter (Rayner et al., 2013). The silver concentration used to treat the filter is likely to be important reducing bacterial numbers. Rayner et al., (2013) suggest that this can be increased to 0.3 mg/g silver nanoparticles without high silver leaching. The use of differing concentrations and application methods may go some way to explaining the contradictory results. Studies which looked at the impact on bacteriophage concentrations, typically found that silver treatment has a limited effect, with LRVs often below 1.

Table 6: Summary of ceramic filter log_{10} reduction values

Organism	Comments	Log_{10} reduction values				Reference
		AgNP coated	$AgNO_3$ coated	Ag other	Uncoated	
E. coli	-	4.7–7.2	-	-	2.6–5.7	A
	Ag unspecified; flow 6 L/h; initial measurements	-	-	> 5.4	5.1	B
	Ag unspecified; flow 6 L/h after > 8000 L throughput	-	-	2.2	1.1	B
	Ag unspecified; flow 3 L/h; initial measurements	-	-	> 5.4	> 5.4	B
	Ag unspecified; flow 3 L/h after > 8000 L throughput	-	-	3.2	1.2	B
	LRV in freshly coated filters	3.2–4.2	-	-	< 1–> 4	C
	-	-	Mean 5.9 (3.8–7.6)	-	Mean 3.1 (2.0–4.3)	D
	-	-	2.2–2.3	-	2.1	E
	4% sawdust	4.74	-		4.56	F
	9% sawdust	3.81	-	-	3.52	F
	17% sawdust	4.91	-	-	2.55	F
	-	4.4	-	-	4.2–4.3	G
	Tanzanian clay; day 10; 0.003 mg/g Ag	~ 3	~ 3	-	2.5	H
	Tanzanian clay; day 10; 0.03 mg/g Ag	~ 3.5	~ 3.5	-	2.5	H
	Tanzanian clay; day 10; 0.3 mg/g Ag	~ 4.4	~ 5	-	2.5	H
	Ag coating inside and outside	-	1.1	-	0.7	I
	Ag coating outside only	-	1.1	-	0.7	I
	-	-	5.1–5.2	-	-	K
E. faecium	-	-	3.4–> 4.5	-	-	K

Organism	Comments	Log_{10} reduction values				Reference
		AgNP coated	$AgNO_3$ coated	Ag other	Uncoated	
P. aeruginosa	-	-	3.4–5.0	-	-	K
Clostridium spores	-	3.6–5.3	-	-	2.7–5.3	A
MS2	-	0.5–1.4	-	-	0.8–2.4	A
MS2	-	-	0.5	-	0.5	D
MS2	-	-	1.3–1.5	-	1.6–1.7	E
MS2	Ag coating inside & outside	-	0.59	-	0.4	I
MS2	Ag coating outside only	-	0.65	-	0.4	I
MS2	-	-	0.5–0.6	-	-	K
øX174	-	-	0.5–0.6	-	-	K
F-specific phage	Sputter coated	-	-	0.56	0.03	J
F-specific phage	Ag^+	-	-	0.25	0.03	J
F-specific phage	Ag_2O	-	-	0.09	0.03	J
Somatic phage	Sputter coated	-	-	1.84	0.02	J
Somatic phage	Ag^+	-	-	1.32	0.02	J
Somatic phage	Ag_2O	-	-	1.2	0.02	J

A–van Halem et al., 2007; B–Wubbels et al., 2008; C–Bielefeldt et al., 2009; D–Bloem et al., 2009; E–Brown & Sobsey, 2010; F– Kallman et al., 2011; G–Zhang & Oyandel-Craver, 2013; H–Rayner et al., 2013; I–van der Laan et al., 2014; J–Simonis et al., 2014; K–Matthies et al., 2015; Ag_2O–silver oxide

2.4 World Health Organization International Scheme to Evaluate Household Water Treatment Technologies: Performance evaluations of silver

Assessment of the microbial effectiveness of silver as a household-level water treatment option should, as far as possible, model actual use conditions in the field, for example, water of varying quality, realistic contact times and testing of all three classes of pathogens which cause diarrhoeal disease. In order to comprehensively assess effectiveness, the World Health Organization (WHO) has set health-based performance targets for household water treatment products based on the removal of bacteria, viruses and protozoa (WHO, 2011). These targets are based on microbial risk models using assumed levels of reference pathogens in untreated water. Since 2014, WHO has been testing products against those targets through the WHO International Scheme to Evaluate Household Water Treatment Technologies.[27] Box 1 gives further information on the Scheme and its three tiers of $\log_{10}$ performance targets for bacteria, viruses and protozoans. Two silver products were evaluated in the first round of testing (WHO, 2016); a silver coated ceramic filter and a liquid colloidal silver suspension designed to be added in the form of drops to drinking-water to be treated. Testing of the filter had to be discontinued because of unacceptably low filtration rates and the colloidal silver product demonstrated no reduction against viruses and only a mean LRV of 2 against bacteria (the efficacy against protozoa was not evaluated). Therefore, the two products containing silver failed to meet the WHO performance criteria.

[27] http://www.who.int/water_sanitation_health/water-quality/household/scheme-household-water-treatment/en/

Box 1. WHO International Scheme to Evaluate Household Water Treatment Technologies

The objective of the Scheme is to independently and consistently evaluate the microbiological performance of household and POU water treatment technologies. The evaluation considers both turbid and non-turbid water, and is carried out to manufacturers' instructions for daily household use.[4] The results of the evaluation are intended to assist and inform Member States and procuring UN agencies in the selection of these technologies.

The performance targets define treatment requirements in relation to source water quality for each pathogen class as detailed below.

Performance target	**Bacteria** (log_{10} reduction required)	**Viruses** (log_{10} reduction required)	**Protozoa** (log_{10} reduction required)	**Classification** (assuming correct and consistent use)
★★★	**≥ 4**	**≥ 5**	**≥ 4**	Comprehensive protection (very high pathogen removal)
★★	**≥ 2**	**≥ 3**	**≥ 2**	Comprehensive protection (high pathogen removal)
★	Meets at least 2-star (★★) criteria for two classes of pathogens			Targeted protection
–	Fails to meet WHO performance criteria			Little or no protection

The performance of HWT products is classified as 3-star (★★★); 2-star (★★); and 1-star (★), denoting descending order of performance, based on log_{10} reductions of bacteria, viruses and protozoa from drinking-water. Performance that does not meet the minimum target is given no stars. Products that meet 3-star (★★★) or 2-star (★★) performance targets are classified as providing "Comprehensive protection" against the three main classes of pathogens which cause diarrhoeal disease in humans. The use of these products is encouraged where there is no information on the specific pathogens in drinking-water (and a prudent approach is to protect against all three classes), or where piped supplies exist but are not safely managed. Products that meet the performance targets for at least 2-star (★★) for only *two* of the three classes of pathogen are given one star (★) and are classified as providing "Targeted protection". In general, the use of these products may be appropriate in situations where the burden of diarrhoeal disease is high due to known classes of pathogens, such as a cholera outbreak.

3. Safety and toxicity of silver

This section outlines the potential for human exposure to silver and silver nanoparticles through all routes (i.e. it is not confined to ingestion) and outlines opinions from various expert bodies on intake. In addition, an assessment of recent (up to autumn 2015) toxicological literature was undertaken and relevant findings are included here. It should be noted that as the study of silver nanoparticles toxicity is relatively new, it is likely that methods of assessment will continue to develop as different challenges posed by nanoparticles are identified (Doak et al., 2012).

3.1 Human exposure

Silver is not considered an essential metal and therefore any exposure is unwanted (Lansdown, 2010). Since ancient times, silver has been used in a variety of products such as jewellery, utensils and coins. More recently, however, largely due to its antimicrobial properties, its uses (and hence potential for human exposure) have expanded rapidly and it can now be found in food packaging materials, babys' bottles and pacifiers, cleaning products, food supplements, cosmetics, medical devices and products, electronics, odour-resistant textiles (e.g. socks and shirts) and household appliances, such as washing machines and refrigerators (Wijnhoven et al., 2009). Dietary intake of silver is estimated to be in the range of 7–90 µg/day (Winjhoven et al., 2009; Lansdown, 2010), although this does not take into account some of the more recent forms of exposure, or non-dietary ingestion of silver (e.g. Tulve et al., 2015). Inhalation of silver dust and fumes may occur in some occupational settings and skin contact can occur in occupational settings, from contact with jewellery and application of topical creams for burns (Wijnhoven et al., 2009).

3.2 Guideline values

3.2.1. WHO drinking-water quality guidelines

There is currently no health-based guideline value for silver in the WHO Guidelines for Drinking-water Quality (WHO, 2017). Silver was last reviewed by WHO in 1993 (WHO, 1993), when it was concluded that, on the basis of epidemiological and pharmacokinetic knowledge at the time, a total lifetime oral intake of about 10 g of silver could be considered as the human no-observable-adverse-effect level (NOAEL). It was felt that the contribution of drinking-water to this NOAEL would normally be negligible and so it was not deemed necessary to establish a health-based guideline value. However, it was suggested that, where silver salts are used for drinking-water treatment, a concentration of 0.1 mg/L could be tolerated without risk to health (a concentration that would give a total dose over a 70-year period of half of the NOAEL outlined above). The 0.1 mg/L level is thus a health advisory rather than a guideline value, a distinction that is rarely appreciated by researchers (e.g. Pelkonen et al., 2003) who often refer to 0.1 mg/L as a guideline or allowable amount.

3.2.2 Other values

In Germany, the drinking-water regulations (Trinkwasserverordnung) set an allowable maximum for silver of 0.08 mg/L.

In the 2012 edition of the USEPA drinking-water standards and health advisories document (USEPA, 2012) silver has the following health advisory[28] values:

[28] A USEPA health advisory is defined as an estimate of acceptable drinking-water levels for a chemical substance based on health effects information; it is not a legally enforceable Federal standard, but serves as technical guidance.

- 10 kg child one-day (mg/L): 0.2
- 10 kg child ten-day (mg/L): 0.2
- Reference dose (mg/kg/day): 0.005 (based on a cosmetic effect)
- Drinking-water equivalent level (mg/L): 0.2
- Life time health advisory (mg/L): 0.1 (based on a cosmetic effect)

3.3 Human toxicity data

3.3.1 Toxicokinetics

3.3.1.1 Absorption

Absorption following oral administration in humans has been described qualitatively in several case studies (Chang et al., 2006; Mirsattari et al., 2004; Ohbo et al., 1996). The occurrence of generalized argyria[29] in a woman who repeatedly applied silver nitrate solution to her gums has been reported as evidence to show that absorption of silver can occur across oral mucosa (Marshall & Schneider 1977).

Quantitative data has been reported by East et al. (1980) who estimated an absorption of 18% of the administered dose in a 47-year-old woman who already suffered from argyria. Transit time in the gastrointestinal tract has been shown to affect absorption, with a faster transit time leading to lower absorption (Furchner et al. 1968).

Evidence of the absorption of silver in humans following inhalation exposure is limited to occupational studies. Inhalation of silver nitrate and silver oxide in the range 0.039 to 0.378 mg silver/m^3 was associated with detectable blood silver levels in workers (Rosenman et al., 1979). Di Vincenzo et al. (1985) reported detectable silver levels in the blood and faeces of workers exposed to time weighted average levels of 0.001 to 0.1 mg/m^3 insoluble silver in a photographic materials manufacturing facility.

Several silver compounds appear to be absorbed through the intact skin of humans, although the degree of absorption is thought to be low.

3.3.1.2 Distribution

Silver and silver nanoparticles have been reported to be distributed to a wide range of organs in the human body following oral administration, however some organs are suggested to be more prone to silver deposition than others (Hadrup & Lam, 2014). Human data relating to silver ingestion is largely limited to a number of case reports where people have ingested varying amounts of colloidal silver, generally over a protracted period (Chung et al., 2010). The most common presenting feature is argyria[29] (Brandt et al., 2005; Wadhera & Fung, 2005). Silver has also been found to cross the placental barrier in humans. Lyon et al. (2002) looked at liver samples, collected at autopsy, and found significant levels of silver (median 15.5 ng/g wet weight) in livers of children under 6 years old. It was speculated that silver is accumulated from the mother (probably from maternal mercury amalgam fillings) during pregnancy and lactation.

Very limited information was identified concerning the distribution of silver in humans following inhalation of elemental silver or silver nanoparticles. Newton & Holmes (1966) estimated that 25% of

[29] Argyria is where tissues become impregnated with silver sulfide, which forms a complex in elastic fibres; large amounts of this complex under the skin give it a bluish, grey-blue or (in extreme cases) a black colour. Generalized argyria results from increased serum silver levels and silver granules can be detected in all body tissues, with the highest concentrations found in the skin, liver, spleen and adrenal glands (Brandt et al., 2005).

a dose of radioactive silver (dose unknown) was distributed to the liver between 2 and 6 days after exposure.

Following the topical application of silver nitrate for the treatment of burns in two humans, silver was distributed to the muscles (0.03–2.3 ppm), liver (0.44 ppm), spleen (0.23 ppm), kidney (0.14 ppm), heart (0.032–0.04 ppm), and bones (0.025 ppm) (Bader 1966).

3.3.1.3 Metabolism

Metallic silver is inert and absorption through any route is determined by ionization (under oxidizing conditions) to release the biologically active ionic silver. Ionic silver subsequently binds to sulfydryl groups and other anionic ligands of proteins, cell membranes, and tissue debris (Hadrup & Lam, 2014). Controversies exist on the predominant routes of silver metabolism in the human body (Wan et al., 1991; East et al., 1980).

3.3.1.4 Excretion

Following absorption via any route, ionic silver can be excreted in bile, urine, hair and nail, with the biliary route predominating over the urinary route. However, urinary silver measurement provides a convenient index of silver absorption by all routes. At higher concentrations, patterns of urinary excretion are irregular (Lansdown, 2010).

Following oral exposure to silver acetate (AgAc) in humans, silver is eliminated primarily in the faeces, with only minor amounts eliminated in the urine. The rate of excretion is most rapid within the first week, after a single oral exposure (East et al., 1980).

Accidental inhalation of silver isotope (110m) resulted in rapid removal from the lungs (primarily by ciliary action), with subsequent ingestion and ultimate elimination in the faeces, with biological half-lives of 1 and 52 days (Newton & Holmes 1966).

No studies were identified concerning the excretion of silver or silver nanoparticles by humans following dermal exposure. Once absorption through the skin and distribution to bodily tissues occurs, it can be expected that elimination would be similar to that of silver ions absorbed via oral or inhalation exposure, that is, primarily via the faeces, with minimal amounts excreted in the urine.

3.3.2 Acute toxicity

No information concerning toxicity in humans following acute exposure to silver or silver nanoparticles through oral or dermal routes could be identified. Acute exposure through inhalation during work with molten silver has been linked to acute respiratory failure in one worker. However, quantitative measurements of exposure and history of pre-exposure are unavailable (ATSDR, 1990).

3.3.3 Repeat dose toxicity

3.3.3.1 Systemic effects

Munger et al. (2013) conducted a study looking at human exposure (60 healthy subjects) to commercial nanoscale silver colloid in a single dose, blinded, cross-over, intent-to-treat design. Two commercial silver nanoparticle (colloid) solutions were used, one with particle sizes between 5–10 nm (10 ppm solution) and one with particles of sizes between 25–40 nm (32 ppm solution). With the 10 ppm solution, subjects were dosed for 3, 7 or 14 days (150 µg/day – equivalent to 2.1 µg/kg body weight [bw] – assuming 70 kg adult), while for the 32 ppm solution all subjects were dosed for 14 days (480

µg/day – equivalent to 6.8 µg/kg bw). No clinically important changes in human metabolic, haematologic and urinalysis were noted. Physical findings and imaging morphology of organs was also unchanged.

Occupational inhalation exposure to silver nitrate and/or silver oxide at estimated exposure levels of between 0.039 and 0.378 mg silver/m^3 for less than 1 to greater than 10 years, has been linked to upper and lower respiratory tract irritation. The same exposure levels can also cause gastric discomfort in humans. Occupational exposure to silver compounds has not been observed to affect blood counts or the cardiovascular system (ATSDR, 1990).

No studies could be identified to assess the potential systemic toxicity of silver or silver nanoparticles in humans following dermal exposure.

3.3.3.2 Neurotoxicity

No studies could be identified to assess the potential neurotoxicity of silver or silver nanoparticles in humans by any route of exposure.

3.3.3.3 Reproductive and developmental toxicity

No studies could be identified to assess the potential reproductive and developmental toxicity of silver or silver nanoparticles in humans by any route of exposure.

3.3.3.4 Immunotoxicity

No studies could be identified to assess the potential immunotoxicity of silver or silver nanoparticles in humans by any route of exposure.

3.3.3.5 Genotoxicity (in vivo)

A single cross-sectional study in Turkey (Aktepe et al., 2015) has examined DNA (deoxyribonucleic acid) damage in peripheral mononuclear leucocytes (measured using the comet assay) in 35 silver jewellery workers (mean age 31.7 ± 8.4 years) exposed to silver particles or silver nanoparticles and 41 non-exposed healthy subjects (mean age 29.42 ± 7.4 years). The exposed group were reported to work for at least 4 hours a day, however, no further information is given by the authors on exposure conditions. A statistically significant increase in DNA damage, measured as endogenous mononuclear leukocyte DNA strand breaks, was reported in the silver workers (mean 15.4 versus 7.48 [arbitrary units] in the control group). Workers were also significantly more likely to have an increased oxidative stress index, increased ceruloplasmin levels and decreased total thiol measurements. As an acute phase reactant, ceruloplasmin concentration increases during periods of inflammation, infection and trauma. In addition, thiols play an important role in mediating oxidative stress, and reduced levels in the workers was shown to correlate with an increased oxidative stress index. The authors conclude that exposure of workers to silver particles increases oxidative stress, leading to inflammation and decreased levels of thiols; the subsequent DNA damage results from both the direct interaction of silver and the overproduction of reactive oxygen species (ROS). The findings of this study, however, should be interpreted with care as there are a number of study limitations, particularly in terms of the small number of participants, and the lack of direct measurements or estimate of exposure for either the workers or the control population.

3.3.3.6 Carcinogenicity

No studies could be identified regarding the possible carcinogenic activity of silver or silver nanoparticles in humans. The USEPA has determined that silver is not classifiable as to human carcinogenicity.

3.4 Animal toxicity studies

3.4.1 Toxicokinetics

3.4.1.1 Absorption

Quantitative absorption data are available for silver from a limited number of oral studies using radiolabelled ^{110}Ag (as silver nitrate or silver acetate) in monkeys, dogs, rats and mice (Furchner et al., 1968). Absorption has been estimated to be around 6% in monkeys, 10% in dogs, 2% in rats and < 0.5% in mice. As for humans, absorption is also linked to transit time in these species (Furchner et al., 1968).

A study in dogs exposed by inhalation to metallic silver particles (median aerodynamic diameter of approximately 0.5 µm) estimated absorption to be 3.1% (0.8 µg) (Phalen & Morrow 1973).

Absorption of silver nitrate across intact skin has been demonstrated in guinea pigs and is similar to that of intact human skin, with approximately 1% of the applied dose being absorbed within 5 hours (Wahlberg 1965).

3.4.1.2 Distribution

It is clear that silver (largely irrespective of the route of exposure or form) can distribute widely within the body and has been shown to cross the placenta, and potentially the blood-brain barrier in experimental animals. In addition to skin, silver has been detected in liver, kidneys, brain, spleen, eyes, muscles, blood, small intestine, stomach, lungs, bladder, prostate, tongue, teeth, salivary glands, thyroid, parathyroid, heart, pancreas and duodenum (Hadrup & Lam, 2014).

Particular targets for silver deposition are the small and large intestines, stomach, liver and kidneys (Loeschner et al., 2011). Of particular note is deposition of silver within the glomerular basement membrane in kidneys, and potentially within glial cells and neurons in some brain regions including the hippocampus and pons (Hadrup & Lam, 2014). Other lines of evidence suggest that silver is deposited within the lining of the blood-brain barrier and does not cross over (Hadrup & Lam, 2014).

Ionic silver and silver nanoparticles administered through the oral route are distributed in a similar way. Van der Zande et al. (2012) compared an oral dose of 9 mg of ionic silver per kg bw per day (administered as silver nitrate) with an oral dose of 90 mg of nanoparticulate silver per kg bw per day for 28 days (the nanoparticle sizes were 15 and 20 nm). The authors reported the majority of the silver in the stomach and small and large intestines, followed by (in descending order) the liver, spleen, testes, kidneys, brain, lungs, blood, bladder and heart. In the same study, less deposition was observed following silver nanoparticle administration than following ionic silver administration. However, the differences were pronounced for all organs.

Silver nanoparticle inhalation (in various doses and exposure periods) studies in rats have shown that silver is distributed to the lungs, liver, kidney, brain, heart, nasal cavity, olfactory bulb, eyes, spleen, ovaries, testes and blood (Ji et al., 2007; Song et al., 2013; Sung et al., 2009; Takenaka et al., 2001). In mice (in a single study) only the lungs were found to exhibit elevated silver concentrations after

exposure (Stebounova et al., 2011). In addition, a study of intra-nasal administration of silver nanoparticles in natal rats reported concentrations of almost 20 µg total silver/g in the cerebellum (the only tissue investigated) following administration of 1 mg silver nanoparticles (20–30 nm) per kg bw for 21 consecutive days (Yin et al., 2013). Silver was also found to be widely distributed following intravenous administration (Lankveld et al., 2010; Dziendzikowska et al., 2012).

3.4.1.3 Metabolism

Silver deposition in cells is caused by precipitation of insoluble silver salts, such as silver chloride and silver phosphate which are transformed to soluble silver sulfide albuminates (Berry & Galle 1982). The sulfides can bind to or complex with amino or carboxyl groups in ribonucleic acid, DNA, and proteins, or can be reduced to metallic silver by ascorbic acid or catecholamines (Danscher 1981).

3.4.1.4 Excretion

Faecal and urinary levels of silver, post-exposure, were measured in two ingestion studies. Loeschner et al. (2011) found very low levels in the urine (< 0.1%) and reported slightly different levels in the faeces, depending upon the nature of the original challenge, with higher faecal levels from silver nanoparticles compared to silver acetate (63% and 49% of the daily dose, respectively). The faecal excretion levels reported by Loeschner et al. (2011) are notably lower than those reported by van der Zande et al. (2012), who reported that over 99% of the daily dose was excreted in faeces. Based on the higher faecal excretion and lower absolute levels seen in organs in animals orally exposed to ionic silver or silver nanoparticles, it would seem that silver nanoparticles are less bioavailable than ionic silver (Loeschner et al., 2011; van der Zande et al., 2012; Hadrup & Lam, 2014).

Van der Zande et al. (2012) reported a generally rapid reduction in tissue silver concentrations following 28 days of ingestion. In most tissues, silver concentrations were already significantly reduced (to below 50% of the immediate post-exposure levels) just one week following cessation of exposure and approached a return to control levels in most samples within 12 weeks. There were, however, four exceptions, namely brain, testis, kidney and spleen, where silver concentrations were still elevated after 12 weeks, with the brain retaining over 90% of the original post-exposure levels. Lee et al. (2013) looked at clearance of tissue-accumulated silver from rats administered 10 or 25 nm citrate-stabilized silver nanoparticles administered (by gavage) either 100 or 500 mg/kg per day for 28 days, followed by up to 4 months recovery. While the clearance half-times differed according to dose and gender; liver, spleen and kidney elimination showed similar clearance trends. Silver concentrations in the testes and brain (i.e. tissues with a biological barrier), however, did not decrease to control levels, even after a 4-month recovery period.

Following inhalation of metallic silver particles (average aerodynamic diameter of 0.5µm) in dogs, the predominant route of clearance was reported as dissolution of the silver and transport through the blood to the liver. A proportion of silver particles were also cleared by the mucociliary escalator and swallowed. Approximately 90% of the inhaled dose was excreted in the faeces within 30 days of exposure (Phalen & Morrow 1973).

No studies could be identified assessing the excretion of silver or silver nanoparticles by animals following dermal exposure. However, once absorption through the skin and distribution to bodily tissues occurs, it can be expected that elimination would be similar to that via oral or inhalation exposure, that is, primarily via the faeces, with minimal amounts excreted in the urine.

3.4.2 Acute toxicity

A number of studies have reported no adverse acute effects as a result of silver or silver nanoparticle ingestion.

Tamimi et al. (1998) investigated the acute toxicity of an anti-smoking mouthwash containing silver nitrate as the active ingredient. The oral median lethal dose (LD_{50})[30] in rats was found to be 280 mg of silver per kg bw and in rabbits, 800 mg of silver per kg bw.

Orally administered nanoparticulate silver was not toxic to mice or guinea pigs at acute doses of up to 5000 mg/kg bw (Maneewattanapinyo et al., 2011). The authors found no mortality or signs of toxicity throughout a 14-day post treatment observation period. In addition, there was no difference in the percentage of body weight gain between the treatment and control groups or a significant difference in haematological parameters.

Following a large single dose (2.5 g) of silver nanoparticles (13 nm) or silver microparticles (2–3.5 µm) administered by gavage, Cha et al. (2008) reported focal lymphocyte infiltration in the mouse liver portal tracts, suggesting the induction of inflammation. They also reported nonspecific focal haemorrhages in the heart, focal lymphocyte infiltration in the intestine and nonspecific medullary congestion in the spleen in the mice exposed to silver nanoparticles.

Korani et al. (2011) conducted an acute dermal toxicity study in guinea pigs, exposed to either 1000 or 10 000 µg silver nanoparticles/mL, with observations following exposure for 14 days. A dose-dependent reduction in thickness in the epidermis and papillary layers of skin was observed.

3.4.3 Repeat dose toxicity

3.4.3.1 Systemic effects

In a drinking water study, Sprague-Dawley rats were administered silver nitrate at doses of 6, 12 and 24 mM for 60 weeks. Death occurred in 3 of 12 rats at the highest dose within 2 weeks and this group was discontinued; the group receiving 6 mM silver nitrate was also discontinued after 12 weeks, although the reasons for this are not clearly stated by the author. A decreased body weight gain was observed in the remaining group and a NOAEL of 181.2 mg/kg bw per day has been reported by the ATSDR (1990) for this study. In a further study, albino rats were administered silver (as silver nitrate) as a 0.25% solution (in drinking water; equivalent to a dose of 222.2 mg/kg bw per day) for up to 8.5 months. Deaths were recorded between 23 and 37 weeks, which the authors propose may have been related to decreased weight gain during the same period. A lowest-observed-adverse-effect level (LOAEL) of 222.2 mg/kg bw per day has been reported by the ATSDR (1990) for this study.

Patlolla et al. (2015) conducted an oral study in which rats were administered silver nanoparticles at doses between 5–100 mg/kg bw per day in deionized water for 5 days. A dose-related increase in ROS concentration was seen, with the two highest doses being statistically significant when compared with controls. Statistically significant increases in activity (at the two highest doses) were also seen for alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. Lipid hydroperoxide (a marker of cellular injury and death) in serum also increased in a dose-dependent manner when compared to the control group. Histopathological damage was seen to the liver at doses of 25 mg/kg bw per day and above.

[30] The dose required to kill half the members of a test population after a specified test duration.

Kulthong et al. (2012) administered silver nanoparticles to rats at concentrations between 0 and 100 mg/kg bw per day for two weeks. No differences were seen in body weight or liver weight between groups and no effect was seen on plasma levels of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase nor in hepatic cytochrome P450 enzyme activity. It should be noted, however, that while the silver nanoparticles were purchased as being < 100 nm in diameter, analysis by the authors showed an average size of 181 nm. Similarly, Kim JS et al. (2013) found no difference in body weight gross findings at necropsy or mortality in rats treated with up to 2000 mg/kg bw per day of citrate-coated silver nanoparticles (10 nm), and Van der Zande et al. (2012) reported that there was no hepatotoxicity or immunotoxicity in a 28-day feeding study in rats exposed to silver nanoparticles (< 20 nm non-coated; < 15 nm PVP-coated) at 90 mg/kg bw per day or silver nitrate at 9 mg/kg bw per day.

Conversely, Kim et al. (2008) reported significant dose-dependent changes in alkaline phosphatase and cholesterol values in male and female rats in a 28-day feeding study with silver nanoparticles administered at doses of 30, 300 or 1000 mg/kg bw per day (60 nm, suspended in carboxymethyl-cellulose). This led the authors to suggest that exposure to levels of silver nanoparticles greater than 300 mg/kg may result in slight liver damage. Hadrup et al. (2012a) examined the oral sub-acute toxicity of 14 nm silver nanoparticles (stabilised with PVP) and silver acetate in rats. Doses of 2.25, 4.5 or 9 mg/kg bw per day of silver nanoparticles or 9 mg/kg bw per day of silver acetate were given daily, by gavage, for 28 days. The authors found no toxicological effects following silver nanoparticle administration. Following silver acetate administration, however, they found lower body weight gain, increased plasma alkaline phosphatase, decreased plasma urea and lower absolute and relative thymus weight. The authors also conducted a metabolomics investigation of the rat urine obtained on day 18 of the study. The analysis revealed differences in the urine composition of female (but not male) rats when compared to the control group. Differences were found in the levels of uric acid and its degradation product, allantoin. Silver nanoparticle ingestion led to an increase in both metabolites, while silver acetate only increased allantoin levels. As both silver nanoparticles and silver acetate altered urine composition this suggests that female rat physiology was affected by silver ingestion (Hadrup et al., 2012b).

Jeong et al. (2010) and Shahare et al. (2013) reported adverse effects of silver nanoparticles on the intestinal mucosa following consumption. In rats administered with silver nanoparticles up to 1000 mg/kg bw per day (60 nm in carboxymethylcellulose) by oral gavage for 28 days, a dose-dependent increase in silver nanoparticles in the lamina propria (connective tissue under the epithelia) in both the small and large intestine and also in the tip of the upper villi in the ileum and protruding surface of the fold in the colon were found (Jeong et al., 2010). In addition, silver nanoparticle-treated rats showed higher numbers of goblet cells that had released their mucus granules resulting in more mucus materials in the crypt lumen and ileal lumen; they also showed an abnormal mucus composition in the intestinal goblet cells.

In mice administered silver nanoparticles (3–20 nm) up to 20 mg/kg bw per day by oral gavage for 21 days, a significant decrease in body weight was seen in all treatment groups compared to the control on days 14 and 21 (despite no difference in food consumption between the groups). The maximum weight loss was observed in the 10 mg/kg bw per day group. In this treatment group (results are not given for the others) there was damage to the microvilli in the small intestine. The authors suggest that silver nanoparticles interact with the protective layer of the glycocalyx and other structural elements of the microvilli of the intestinal absorptive cells causing structural changes, which results in the alteration of membrane permeability and the destruction of the microvilli. In addition, it was also suggested that the

epithelial cells of the gastrointestinal tract are destroyed leading to the observed decrease in body weight, although they do not comment as to why the effects are more pronounced in the 10 mg/kg group compared to the 20 mg/kg group (Shahare et al., 2013).

In a 28-day silver biodistribution study, rats were administered two sizes (10 and 25 nm) of citrate-stabilized silver nanoparticles by oral gavage at doses of 100 or 500 mg/kg bw per day (Section 3.4.1.4). The authors reported evidence of liver toxicity, based on an increase in cholesterol in male rats at both doses for the 10 nm partices and at the lowest dose for the 25 nm particles. In females, an increase in alkaline phosphatase and aspartate aminotransferase was reported for both doses of the 10 nm particles and at the highest dose of the 25 nm particles. One case of bile duct hyperplasia was observed among the male rats treated with the 10 nm silver nanoparticles, with a non-significant increase in inflammatory cell infiltration (Lee et al., 2013).

Sardari et al. (2012) reported adverse effects on liver, spleen and kidney in rats fed 1 and 2 mg of silver nanoparticles (70 nm) per kg bw per day by gavage for 30 days. Within the spleen, red pulp was decreased in rats treated with high doses of silver nanoparticles, while the number of lymphocytes (white pulp) was increased. The authors reported pathological changes to the kidney including necrosis of glomerular cells, bowman capsule, and proximal tubules. Inflammation of the parenchymal cells was seen in the liver and intracellular space enlargement was observed in the hepatic lobules, in addition apoptosis (programmed cell death) was reported around the central vein.

A study exposing rats to two sizes of silver nanoparticles (14 and 36 nm) in water by oral administration (ad libitum–535 µg/mL) over 55 days was conducted by Espinosa-Cristobal et al. (2013). Daily ingestion was found to average 157 mg/kg bw per day and no changes were seen in in individual behaviour, body weight, sociability and food consumption between the test groups and the control. The clinical chemistry and haematology conducted only showed a significant difference (at 55 days) in blood urea nitrogen concentration for the smaller silver nanoparticles (14 nm) tested, leading the group to suggest that the smaller silver nanoparticles altered the normal glomerular filtration from the kidneys. A number of other parameters were found to be different from the control group but only after 25 days of exposure and were within normal values at the end of the study.

Ebabe Elle et al. (2013) suggested that silver nanoparticles led to liver damage by altering the regulation of lipid metabolism. Rats fed 500 mg/kg bw per day of silver nanoparticles (20 nm) by gavage for 81 days were found to have significantly elevated cholesterolemia and LDL-cholesterol and lowered triglycerides. They also found increased liver and cardiac superoxide anion production and raised liver inflammatory cytokines.

In a sub-chronic oral study in rats (Kim et al., 2010) using 30, 125 and 500 mg/kg doses of silver nanoparticles (60 nm) over a 90-day exposure period, the group found that there were significant differences in the body weights of the males exposed to the mid and high dose of silver nanoparticles compared to the control animals. As in the short-term study (Kim et al., 2008), the group also found significant dose-dependent changes in alkaline phosphatase and cholesterol for male and female rats. In addition, histopathologic examination revealed a higher incidence of bile duct hyperplasia (enlargement).

Yun et al. (2014) administered citrate stabilized silver nanoparticles (< 20 nm) to rats by gavage daily over a 13-week period (highest dose of 1030.5 mg/kg bw per day). They reported increases in serum alkaline phosphatase and calcium as well as lymphocyte infiltration in the liver and kidney, suggesting liver and kidney toxicity.

Thakur et al. (2014) investigated the impact of 5–20 nm spherical silver nanoparticles (20 µg/kg bw per day) administered by repeated gavage (90 days) on male rats. No overt signs of toxicity recorded as deaths, changes in body weight or behavioural changes were seen.

In a 14-day feeding study, 20 nm silver nanoparticles (0.1 and 0.2 mg/kg bw per day) were administered to mice by oral gavage and the effects on erythrocytes and tissues examined (Shrivastava et al., 2014). The group found a significant decrease in body weight in both test groups compared to the control (despite the relatively low doses given) and also found a number of statistically significant differences in various blood and urinary biochemical variables indicative of oxidative stress, including elevated ROS, blood gluathione (high dose only), glutathione peroxidase, glutathione-S-transferase and urinary 8-hydroxy-2'-deoxyguanosine (a biomarker of DNA damage). The levels of ROS increased significantly in all of the tissues examined (brain, liver, kidney and spleen) at the higher concentration and in all except brain at the lower concentration. Hepatic and renal toxicity was evident from liver and kidney function tests.

In a 28-day feeding study in mice, Park et al. (2010a) found that silver nanoparticles (42 nm) at the highest dose given (1 mg/kg per day) resulted in some changes in serum biochemistry, with increased levels of alkaline phosphatase and aspartate transaminase in both male and female mice. Levels of alanine transaminase were also increased following high dose administration, but only in female mice. They also found that pro-inflammatory cytokines were increased in a dose-dependent manner. Minor histopathological changes were seen in the kidney (slight cell infiltration in the cortex), but not in the liver or small intestine following high dose administration.

A number of inhalation studies have been performed with silver nanoparticles (typically 12–18 nm in size) in rats and mice via whole body inhalation chambers. The degree of toxicity observed is considered to be dependent on the duration of exposure. With short-term exposure (up to 28 days) no significant adverse effects have been reported (Ji et al., 2007; Hyun et al., 2008; Sung et al., 2011; Stebounova et al., 2011). In contrast, some longer-term exposure studies (≥ 90 days) suggest a dose-dependent toxicity of silver nanoparticles to lungs and liver in rats exposed via whole body inhalation to silver nanoparticles (18–19 nm) at low (49 $\mu g/m^3$), medium (133 $\mu g/m^3$) or high (515 $\mu g/m^3$) doses for six hours a day, five days a week for 13 weeks (Sung et al., 2008, 2009). Although a small degree of recovery of lung function following a 12-week rest period was apparent, an exposure-related lung function decrease in males (exposed to the highest silver nanoparticle dose) persisted during the recovery period (Song et al., 2013).

There are relatively few *in vivo* animal studies that assess the potential dermal toxicity of silver application (Samberg et al., 2010; Korani et al., 2011; Maneewattanapinyo et al., 2011; Kim JS et al., 2013). In the main, reported studies have evaluated the use of silver-impregnated wound dressings. Kim JS et al. (2013) conducted dermal toxicity/irritation tests using citrate-coated 10 nm silver nanoparticles. Rats were exposed for 24 hours to up to 2000 mg/kg bw per day and then observed for 15 days; no toxicity was observed. Similarly, no skin reaction was seen in three rabbits subjected to the same form of silver nanoparticles. In a skin sensitization test using 20 guinea pigs, a single animal showed some erythema, suggesting that the tested silver nanoparticles could be classified as a weak skin sensitizer. In pigs dosed topically with a solution of silver nanoparticles (0.34–34 µg/mL) for 14 consecutive days, microscopic observations showed intracellular and intercellular epidermal oedema at the lowest dose, and severe intracellular and intercellular epidermal oedema with focal dermal inflammation and the highest dose (Samberg et al., 2010). Korani et al. (2011) assessed dermal toxicity in guinea pigs exposed

to either 1000 or 10 000 µg/mL of silver nanoparticles, five times a week for 13 weeks. Toxic skin responses were noted which were dose and time-dependent.

3.4.3.2 Neurotoxicity

Evidence to show that silver crosses the blood brain barrier remains equivocal, however, even in absence of silver in the extracellular fluid of the brain, silver-induced neurotoxic effects may occur via secondary molecules that are released from the periphery (Hadrup & Lam, 2014). Silver appears to have some neurotoxic effects in rats. Rungby & Danscher (1983) reported hyperactivity in rats administered 0.01% silver nitrate in drinking water for 4 months. Hypoactivity was found to be induced in mice following withdrawal of silver nitrate in drinking water at a dose of 14 mg/kg bw per day, that had been previously administered for 125 days (Rungby & Danscher, 1984).

Hadrup et al. (2012c) assessed the effect of silver nanoparticles (14 nm stabilized with PVP) and silver acetate on the levels of brain neurotransmitters. The authors reported that silver nanoparticles (4.5 and 9 mg/kg bw per day) and silver acetate (9 mg/kg bw per day) increased the brain dopamine concentration after 28 days of oral administration. In contrast to the results seen after a 28-day exposure period, after 14 days of exposure, dopamine concentration was decreased by silver nanoparticles (at concentrations of 2.25 and 4.5. mg/kg bw per day), leading to the suggestion that there are differential effects of silver on dopamine depending on the length of exposure. In the 28-day exposure, brain noradrenaline levels were significantly increased only by silver acetate (9 mg/kg bw per day) and brain 5-hydroxytryptamine was increased only by silver nanoparticles (9 mg/kg bw per day).

Skalska et al. (2015) administered rats with silver nanoparticles (10 nm stabilized in sodium citrate, 0.2 mg/kg bw per day), silver citrate (0.2 mg/kg bw per day) or saline, over a 14-day period,. Both types of silver were found to result in ultrastructural pathological changes in the forebrain cortex and hippocampus of the treated animals, with the synaptic degeneration being greater in the hippocampus region.

No studies could be identified to address potential neurotoxic effects of silver or silver nanoparticles in animals following inhalation or dermal exposure.

3.4.3.3 Reproductive and developmental toxicity

Pregnant female rats administered 50 mg of silver chloride per animal (corresponding to 190 mg of silver per kg bw per day) orally during gestation days 1–20 showed increased post-implantation lethality. The incidence of visceral damage in the offspring was considerably higher compared to the control group and all offspring died within 24 hours of birth (Shavlovski et al., 1995).

Pregnant rats were treated with silver nanoparticles (< 10 nm suspended in carboxymethylcellulose, maximum concentration 1000 mg/kg per day) for 14 days during their pregnancy and the impact on fetal development determined on day 20 of gestation. There were no significant differences between the groups during the feeding stage. On *post mortem* examination, there were signs of oxidative stress in maternal hepatic tissues at 100 mg/kg and above (decrease in liver catalase and glutathione reductase activities), but no evidence of developmental toxicity (Yu et al., 2013). Hong et al. (2014) also failed to find any impacts of reproduction/development (mating, fertility, implantation, delivery and fetuses) in rats fed up to 250 mg/kg bw per day of citrate-stabilized silver nanoparticles (7.9 nm) for up to 52 days. They also did not show any differences in haematology, serum biochemical investigation or histopathological analysis.

In a study by Mathias et al. (2015), prepubertal male rats were fed either 15 or 30 µg/kg bw per day of silver nanoparticles for 35 days from postnatal day 23 to postnatal day 58 and then sacrificed 44 days later. While no changes were seen in growth and the animals showed no changes in sexual behaviour and serum hormone concentrations, silver nanoparticle exposure delayed the onset of puberty and reduced the acrosome and plasma integrities, reduced the mitochondrial activity and increased the abnormalities of the sperm in both treatment groups.

In male rats administered silver nanoparticles at 20 µg/kg bw per day by oral gavage for 90 days, histopathological changes in the testes were seen. These were reported as: disorganization of the normal appearance of the testis with varying degrees of atrophy in the seminiferous tubules; depletion of germ cells and germinal cell necrosis in spermatogonia; degenerative changes in form of necrosis and severe vacuolisation in sertoli cell cytoplasm; and vacuolated Leydig cells (Thakur et al., 2014). As only one dose of silver nanoparticles was utilised in this study, a LOAEL of 20 µg/kg bw per day only can be derived.

Kovvuru et al. (2015) reported that in mice, maternal ingestion of 500 mg/kg bw per day of silver nanoparticles (PVP-coated) for 5 days during the post-coitum period induced DNA deletions in developing embryos. They also reported irreversible chromosomal damage in bone marrow and double strand breaks and oxidative DNA damage in peripheral blood and/or bone marrow.

In mice administered 0.03% silver nitrate in the drinking water for 1 month (corresponding to 23 mg of silver/kg bw per day), changes in ovarian nuclear and cytoplasmic cell morphology were reported (Hadek, 1966).

No studies could be identified to address potential reproductive or developmental effects of silver or silver nanoparticles in animals following inhalation or dermal exposure.

3.4.3.4 Immunotoxicity

Reported effects of silver or silver nanoparticles on the immune system following oral administration are variable. Lymphocyte infiltration was reported in mice fed a single, very high dose (125 g/kg bw per day) of 13 nm nanoparticulate or 2–3.5 µm micro-particulate silver (Cha et al., 2008). Silver induced an autoimmune condition in the genetically susceptible H-2s mouse strain following the administration of 0.5 g of silver nitrate/L in the drinking water (which corresponded to 47 mg of silver per kg bw per day) for 10 weeks (Havarinasab et al., 2009). In a 28-day oral study, administration of nanoparticulate and ionic silver at does up to 9 mg/kg bw per day were associated with a decreased thymus weight (Hadrup et al., 2012b). Park et al. (2010a) observed increases in plasma concentrations of interleukin 1 (high dose), interleukin 4 (high dose), interleukin 6 (middle and high dose), interleukin 10 (all doses), interleukin 12 (middle and high dose) transforming growth factor b (middle and high dose) and immunoglobulin E (high dose), as well as an increase in cell infiltration in the kidney cortex (high dose) following oral exposure of mice to 42 nm silver nanoparticles at doses of 0.25, 0.5 and 1 mg/kg bw per day.

Conversely, Van der Zande et al. (2012) observed no immunotoxicity following the oral administration of silver nanoparticles (15 and 20 nm) at 90 mg/kg bw per day or ionic silver at 9 mg/kg bw per day for 28 days.

No studies could be identified to address potential immunotoxic effects of silver or silver nanoparticles in animals following inhalation or dermal exposure.

3.4.3.5 Genotoxicity (in vivo)

A number of *in vivo* genotoxicity studies have been carried out with ionic silver and silver nanoparticles in rats and mice. Those using the comet assay, micronucleus assay and chromosome aberration test are summarized in Table 7 below. With regards to genotoxicity testing, the the Organisation for Economic Co-operation and Development has produced a series of test guidelines for genetic toxicology which includes the follow battery of tests:

- bacterial reverse mutation test (e.g. the Ames test) TG 471;
- *in vivo* mammalian alkaline comet assay (single cell gel electrophoresis assay) TG 489;
- mammalian micronucleus assay (*in vivo* TG 474; *in vitro* TG 487);
- mammalian chromosome aberration test (*in vitro* TG 473, *in vivo* TG 475, TG 483); and
- mammalian gene mutation assay (*in vitro* TG 476, *in vivo* TG 488).

Many of the test guidelines have recently been updated and a new genetic toxicology guidance document is in draft form (OECD, 2015). While it is acknowledged that some substances, including nanomaterials, may require special modifications to the test guidelines, no guidance is provided on this within the test guidelines. It is becoming increasingly clear, for example, that the Ames test is not effective at assessing the genotoxic potential of nanoparticles as the results following challenge with a variety of nanoparticles have predominantly been negative, although nanoparticles have been shown to produce positive genotoxic responses from *in vitro* mammalian cell test systems (Landsiedel et al., 2009; Doak et al., 2012). In addition, the comet assay is an "indicator" test which detects primary DNA damage, but not the consequences of the damage. The DNA measured in the comet assay may, for example, lead to cell death or it may result in DNA repair (which can result in return the DNA to its original state or may result in a mutation). The micronucleus assay and chromosome aberration test both test for chromosomal aberrations resulting from exposure to the test chemical.

Of the 11 studies outlined in Table 7, five used rats and six used mice and, most studies analysed bone marrow samples. Doses of silver nanoparticles ranged from 0.01 mg/kg bw per day (Taveres et al., 2012) to 1000 mg/kg bw per day (Kim et al., 2008; Kim JS et al., 2011). The comet assay was used in eight of the studies. In rats, Patolla et al. (2015) found a dose-dependent increase in DNA damage in the comet assay (significant at 50 mg/kg bw per day and above) in a 5-day oral feeding experiment, while Dobrzynska et al. (2014) saw no significant effect on bone marrow leukocytes after a single intravenous injection (maximum concentration 10 mg/kg bw). In mice, the comet assay gave more consistent results with five out of the six studies showing an increase in DNA damage following exposure to silver nanoparticles using a variety of routes of adminstration. Asare et al. (2015) found no effect in mouse liver, lung or testes following a single intravenous dose (5 mg/kg) of 20 nm silver nanoparticles. The micronucleus assay gave mixed results, where two of the five tests were positive. Both of the positive tests were in bone marrow tissue from rats; one using a 5-day oral administration and one using a single intravenous injection. There is some suggestion that different cells within the same tissue (Dobrzyńska et al., 2014) and different tissues (Li Y et al., 2013) may display different susceptibility to genotoxic effects. Dobrzyńska et al. (2014), for example, found a statistically significant increased frequency of micronuclei in erythrocytes from bone marrow, following exposure to silver nanoparticles, but not reticulocytes; although other authors have reported negative results in rat bone marrow erythrocytes (e.g. Kim JS et al., 2011). Chromosome aberrations were seen in each of the three studies that employed this test; two in rats (Patolla et al., 2015; El Mahdy et al., 2014) and one in mice (Ghosh et al., 2012). The most frequently noted abberations varied by study. One study (Li Y et al., 2013) used the Pig-a assay (a relatively new *in vivo* gene mutation test – not shown in Table 7)

in mice exposed to 5 nm PVP silver nanoparticles by intravenous injection and reported no effect over the control.

There was no evidence of genetic toxicity in male or female rats based on an analysis of micronucleus induction from bone marrow, following inhalation exposure of rats to silver nanoparticles at levels up to 515 µg/m3 over a 90-day period (Kim JS et al., 2011). Dong et al. (2013) exposed rats to silver nanoparticles at an inhalation level of 381 µg/m^3 for 12 weeks. Although they found a change in gene expression in the kidneys with, overall, male rat kidneys showing a higher expression of genes involved in xenobiotic metabolism and the female rat kidneys showing a higher expression of genes involved in extracellular signalling, this was not considered to be of toxicological significance.

No studies could be identified to assess genotoxic effects of silver of silver nanoparticles following dermal exposure in animals.

3.4.3.6 Carcinogenicity

Silver is not classified as a human carcinogen. Fibrosarcomas have been induced in rats following subcutaneous imbedding of silver foil; imbedded silver metal foil appeared to produce fibrosarcomas with a latent period of 275 days in 32% of implantation sites (Oppenheimer et al., 1956). However, the relevance of this to humans is uncertain and may reflect solid-state carcinogenesis in which even insoluble solids such as plastic have been shown to result in local fibrosarcomas (Coffin & Palekar, 1985). Both positive (Schmahl & Steinhoff, 1960) and negative (Furst & Schlauder, 1977) results for tumorigenesis have been reported following subcutaneous and intramuscular injection, respectively, of colloidal silver in rats. However, the relevance of these routes of exposure to humans is, again, unclear (ATSDR, 1990). No studies on carcinogenicity from silver nanoparticles were identified.

Table 7: Genotoxicity testing of silver nanoparticles

Reference	Animal	Tissue/cell type	Silver type	Exposure duration	Dose	Comet assay	Micronucleus assay	Chromosome aberration test
Kim et al., 2008	Rat	Bone marrow	CMC 60 nm, NP	28 days, oral (unstated admin.)	30, 100, 1000 mg/kg bw/day	-	No significant effect. A small, dose-related, increase in polychromatic erythrocytes was seen in male rats. A small increase was also seen in polychromatic erythrocytes in female rats for 2 of the tested doses. None of the increases, however, were statistically significant when compared to the control.	-
Patolla et al., 2015	Rat	Bone marrow	10 nm, naked NP	5 days, oral (using feeding needles)	5, 25, 50, 100 mg/kg bw/day	All doses caused a dose-dependent increase in DNA damage, the 50 and 100 mg/kg doses produced statistically significant increases.	A dose-dependent increase in micronucleus frequencies was seen. The 50 and 100 mg/kg doses gave statistically significant increases.	A dose-dependent increase in chromosome aberrations was observed. Chromatid gaps and breaks were the most frequently noted aberrations.
Kim JS et al., 2011	Rat	Bone marrow	18 nm, naked NP	90 days inhalation	30, 300, 1000 mg/kg bw/day	-	No statistically significant differences were seen in the erythrocytes.	
El Mahdy et al., 2014	Rat	Bone marrow	9 nm, PVP NP	28 days ip injection	1, 2, 4 mg/kg bw/day	-	-	Statistically significant chromosome aberrations were seen at all tested concentrations. Centromeric attenuations were the most frequent structural aberration observed.

Reference	Animal	Tissue/cell type	Silver type	Exposure duration	Dose	Comet assay	Micronucleus assay	Chromosome aberration test
Dobrzynska et al., 2014	Rat	Bone marrow	20 nm, naked NP	Single iv injection, animals killed at 24 h, 1 and 4 weeks	5, 10 mg/kg bw	No significant effect was seen on bone marrow leukocytes.	A significantly increased frequency of erythrocyte micronuclei was seen 24 hours after exposure to both 5 and 10 mg AgNP/kg bw. The enhanced frequency was also seen at 1 and 4 weeks post-exposure. No impact was seen on reticulocytes.	-
Awasthi et al., 2015	Mouse	Liver	5 nm, NP	Single oral dose (by oral intubation) (animals killed at 3 and 24 h)	50, 100 mg/kg bw	A significant difference in all comet assay parameters at both 3 and 24 h for the single 100 mg/kg dose.	-	-
				Weekly (5 weeks) oral dose (by oral intubation)	10, 20 mg/kg bw/day	For the multiple exposure mice, significant damage was seen for both 10 and 20 mg/kg doses.	-	-
Ghosh et al., 2012	Mouse	Bone marrow	120 nm (ave), NP	Single ip injection	10, 20, 40, 80 mg/kg bw	An increase in DNA damage (over the control) was seen. There was no clear dose-response relationship.	-	A significant increase (cf. control) in the frequency of aberrant cells and number of breaks per cell was seen.
Tavares et al., 2012	Mouse	Blood	19 nm (ave), citrate NP	Single ip injection, blood taken at 1, 6, 12 and 24 h	10, 25, 50 µg/kg bw	Limited effects, with only the lowest dose (10 µg/kg) producing a significant increase in DNA damage cf. the control.	-	Aberrations were mainly chromatid breaks. No clear dose-response relationship.

Reference	Animal	Tissue/cell type	Silver type	Exposure duration	Dose	Comet assay	Micronucleus assay	Chromosome aberration test
Al Gurabi et al., 2015	Mouse	Liver	44 nm (ave), NP	Single ip dose (animals killed at 24 or 72 h)	26, 52, 78 mg/kg bw	Significant damage seen at all doses. At the lowest dose (26 mg/kg) damage was only significant after 72 h.	-	-
Li Y et al., 2013	Mouse	Bone marrow	5 nm, PVP NP	Single iv injection	0.5, 1, 2.5, 10, 20 mg/kg bw	-	No effect seen on reticulocytes.	-
Li Y et al., 2013	Mouse	Bone marrow, liver	15-100 nm, PVP NP, 10-80 nm, silicon NP	Single or 3 day iv injection	25 mg/kg bw	Following a 3-day exposure, no increase in liver DNA damage was seen in the standard comet assay. A significant increase in DNA damage, however, was seen in the enzyme modified assay for both PVP and silicon AgNP, suggesting that AgNP can cause oxidative DNA damage.	No effect seen on reticulocytes.	-
Asare et al., 2015	Mouse	Liver, lung, testes	20 nm, NP	Single iv dose (animals killed at 1 and 7 days)	5 mg/kg bw	No significant effect in any tissue.	-	-

ave–average; bw-body weight; ip–intraperitoneal; iv–intravenous; NP–nanoparticles

3.4.4 *In vitro* toxicity

There has been a marked increase in the number of *in vitro* studies investigating the potential toxicity of ionic silver and silver nanoparticles in recent years. A wide range of cell types from human, rat, mouse, hamster and porcine origin have been investigated, including cells derived from: blood (e.g. Zhang et al., 2013); brain (e.g. Haase et al., 2012); bone (e.g. Hardes et al., 2007); cervix (e.g. Mukherjee et al., 2012); immune system (e.g. Pratsinis et al., 2013); intestine (e.g. Gopinath et al., 2010); kidney (e.g. Kermanizadeh et al., 2013); liver (e.g. Gaiser et al., 2013); lung (e.g. Suliman et al., 2013); skin (e.g. Samberg et al., 2010); and testes (e.g. Ema et al., 2010). It is possible that secondary cells (i.e. cancer-derived or immortalized cell lines) may not provide useful information in terms of silver or silver nanoparticle toxicity on normal undifferentiated cells, which are most relevant to human exposure scenarios, as these may have multiple molecular pathways that are deregulated (Franchi et al., 2015). Thus, the emphasis in this document is data derived from toxicological studies that have utilized primary cells.

The interpretation of *in vitro* data in terms of its relevance to human exposure scenarios is unknown and so, for clarity, a summary of findings is provided below, with full details provided in Appendix B.

- Ionic silver and silver nanoparticles cause toxic effects in a wide variety of cell types from a range of organs and tissues. The degree of toxicity varies according to the form of silver (ionic silver or silver nanoparticles), the silver nanoparticle size and coating and the sensitivity of individual cell types.
- In the liver, although toxic effects are reported, primary cells seem to be much more resilient to the effects of silver than secondary cell lines.
- Silver is cytotoxic to lung macrophages and fibroblasts and also brain cells, including astrocytes and neurons. Based on work using cells derived from the rat adrenal medulla it has been suggested that both ionic silver and silver nanoparticles are developmental neurotoxicants.The majority of studies identified relating to the gut are based on secondary cancer-derived cell lines and have suggested that silver is cytotoxic to colon epithelial cells (Caco-2, HT29 and SW480), although there is a suggestion that uptake of silver by intestinal cells (HT29) is less than other cells and that the production of mucin may be protective. Normal colon mucosal epithelial cells (NCM460) seem to be less susceptible to the impact of silver nanoparticles than the secondary cell lines.
- Baby hamster kidney cells and human embryo kidney cells (HEK293) are sensitive to silver nanoparticles, with DNA damage seen in HEK293 cells detected following exposure to 1µg silver nanoparticles per mL.
- In the blood, silver nanoparticles can cause platelet aggregation *in vitro* (human platelets) and *in vivo* (rats following intravenous or intratracheal administration) and has been shown to result in rupture of human red blood cells. Silver nanoparticles have also been shown to be cytotoxic to human blood mononuclear cells at concentrations as low as 1µg/mL.
- Silver shows cytotoxicity in macrophages and keratinocytes and also affects zinc and selenium metabolism in keratinocytes and skin fibroblasts.
- Potential reproductive effects of silver have been shown, *in vitro* with a decrease in oocyte maturation and inhibition of the proliferation of spermatogonial stem cells.
- The comet assay and micronucleus test, in particular, indicate that silver nanoparticles (and ionic silver) may be genotoxic, with results dependent upon both the size and type of silver nanoparticles and also the sensitivity of the cell type although, as noted earlier, there is no regulatory approved comet assay for *in vitro* use and damage can be reversible.

- Generally, exposure of cells to silver (ionic silver and silver nanoparticles) results in oxidative stress; in susceptible cells this can result in a range of toxic effects including apoptosis, changes in gene expression and DNA damage. Different cell sensitivity is likely to be due to a range of factors and includes protection produced by mucin (e.g. HT29 cells) and intracellular antioxidant levels.

3.4.5 *In vitro* to *in vivo* extrapolations

Monteiro-Riviere et al. (2013) looked at the impact that pre-incubation of silver nanoparticles with a number of different proteins (albumin, IgG and transferrin – to form protein-complexed nanoparticles) had on the uptake of silver nanoparticles by human epidermal keratinocytes. Silver nanoparticle association with serum proteins significantly modulated silver uptake compared to native silver nanoparticle uptake. Shannahan et al. (2015) also examined how the formation of a protein corona as a result of exposure to a biological environment can impact on the uptake and also toxicity of silver nanoparticles; this group used citrate-coated silver nanoparticles (20 nm) and two rat cell types. The silver nanoparticles were incubated with human serum albumin, bovine serum albumin, high-density lipoprotein or water (control) to form a protein corona. Silver nanoparticles readily associated with human serum albumin, bovine serum albumin and high-density lipoprotein and, in each case, there was an increase in the hydrodynamic size of the silver nanoparticles. The addition of the protein corona decreased cellular uptake of silver nanoparticles and, at higher concentrations (25 and 50 µg/mL), reduced cytotoxicity.

3.5 Vulnerable populations

No information on the possible impact of silver or silver nanoparticles on vulnerable populations was identified.

3.6 Summary of the safety and toxicity of silver

The findings from identified *in vivo* studies relating specifically to the oral route of exposure are summarized below.

- Metallic silver is inert, and absorption is determined by ionization (under oxidizing conditions) to release the biologically active ionic silver, which is absorbed into the systemic circulation. Absorption rates of 18% have been reported for orally administered silver in humans, and between 0.4 and 18% in other mammals. Ionic silver binds strongly to metallothionein, albumins, and macroglobulins and is distributed to all tissues in the descending order of: stomach and small and large intestines, liver, spleen, testes, kidneys, brain, lungs, blood, bladder and heart. Silver deposition can occur through precipitation of insoluble silver salts, which are transformed to soluble silver sulfide albuminates. Excretion occurs via the bile and urine.
- There are a limited number of toxicity studies in humans relating to the toxicity of ionic silver or silver nanoparticles following exposure by any route. Of those available, no substantial toxicity has been reported.
- There are a limited number of experimental oral toxicity studies for silver. In the rat, silver (as silver nitrate) has moderate acute toxicity and is slightly toxic following acute exposure in the rabbit. Silver nanoparticles are considered to have slight to no acute toxicity via the oral route.
- There are a limited number of animal studies relating to the toxicity of silver following repeated exposures. A NOAEL of 181.2 mg/kg bw per day has been determined for silver based on reduced body weight gain in Sprague-Dawley rats.

- There are a large number of animal studies in rats (principally), mice and guinea pigs, relating to the toxicity of silver nanoparticles following repeated oral exposure. These have shown evidence of a dose dependent toxicity of silver nanoparticles related to a number of endpoints, namely: death, decreases in body weight, hypoactivity, altered neurotransmitter levels, altered liver enzymes, altered blood values, enlarged hearts, immunological effects, increased sperm abnormalities; delayed onset of puberty. Histopathological changes to liver, kidney, spleen, intestine, epidermis and brain tissue have also been reported.
- Of the studies identified, the most sensitive toxicological endpoint relates to histopathological changes in the testes in male rats. A LOAEL of 20 µg/kg bw per day has been derived for this endpoint.
- No *in vivo* genotoxicity studies carried out with silver in humans or animals could be identified. Although *in vitro* findings suggest that ionic silver may be genotoxic, the relevance of these findings to humans is unknown.
- Genotoxicity studies have been carried out *in vivo* with silver nanoparticles in the rat and in mice, at repeated oral doses between 5 and 1000 mg/kg bw per day for up to 35 days. The findings suggest that silver nanoparticles administered by the oral route may induce DNA damage at the chromosomal level, becoming clastogenic at higher levels. However, there is no indication of direct mutagenicity.
    - In the rat and mouse, a dose-dependent increase in DNA damage was seen using the comet assay, which was significant at 50 and 10 mg/kg bw per day and above in rats and mice respectively.
    - A dose-dependent increase in micronucleus formation has been reported in rats, which reached significance at a dose of silver nanoparticles of 50 mg/kg bw per day and above.
    - A dose-dependent increase in chromosome aberrations has also been observed in rats administered silver nanoparticles at doses between 5 and 100 mg/kg bw per day. Chromatid gaps and breaks were the most frequently noted aberrations.
    - The mode of action for silver nanoparticle toxicity is at present undefined, however it is closely related to its transformation in biological and environmental media:
        - nanosilver particles can interact with membrane proteins and activate signalling pathways, leading to inhibition of cell proliferation;
        - nanosilver particles can enter the cell through diffusion or endocytosis to cause mitochondrial dysfunction, generation of ROS, leading to damage to proteins and nucleic acids inside the cell, and finally inhibition of cell proliferation; and
        - both the ionic and nano-form of silver can interact with sulfur containing macromolecules such as proteins.

4. Environmental considerations

Environmental considerations are largely beyond the scope of this report; however, it has been noted that release of silver and silver nanoparticles (from whatever source) into the environment may pose a threat to “non-target” organisms (such as natural microbes and aquatic organisms). Bondarenko et al. (2013) reviewed the toxicity of silver salts and silver nanoparticles to selected environmentally relevant test organisms as well as target organisms. Table 8 shows the median $L(E)C_{50}$ [31] or MIC data for silver nanoparticles and silver salts.

Table 8: Median $L(E)C_{50}$ for all organisms except bacteria and median MIC for bacteria for silver nanoparticle and silver salts (adapted from Bondarenko et al., 2013)

Group of organisms	Median $L(E)C_{50}$/Minimum inhibitory concentration			
	AgNP (mg/L)	Number of data	Ag salts (mg/L)	Number of data
Target				
Algae	0.36	17	0.0076	10
Bacteria	7.10	46	3.3	27
Protozoa	38	7	1.5	3
Non-target				
Crustaceans	0.01	17	0.00085	8
Fish	1.36	17	0.058	4
Nematodes	3.34	21	4.8	4
Mammalian cells *in vitro*	11.3	25	2	18

The most sensitive organisms to both silver salts and silver nanoparticles are crustaceans (non-target organisms). Based on the lowest median $L(E)C_{50}$ value of the key environmental organisms both silver salt and silver nanoparticles would be classified as “very toxic to aquatic organisms” under EU Directive 93/67/EEC (CEC, 1996).

[31] LC50 – median lethal concentration: the concentration required to kill half the members of a test population after a specified test duration.
EC50 – half maximal effective concentration: the effective concentration of a chemical that causes half of the maximum response in a test population after a specified test duration.

5. Discussion

A review of the recent literature reveals that there is a considerable interest in silver and silver nanoparticles, in particular, both in terms of potential applications and toxicity.

5.1 Efficacy

In many of the studies reported in Section 2, it is often difficult to determine the efficacy of the silver component (especially in the studies outlined in Sections 2.2 and 2.3) as the impact of filtration alone is often not reported. In a number of cases, silver measurements in the treated water are not reported (meaning that it is not possible to assess human exposure to silver via this route).

5.1.1 Copper/silver ionization in hospital water systems

Copper/silver ionization is often used for microbial control (especially against *Legionella* spp.) particularly in hospital hot water distribution systems. In well-run systems (where ion concentrations are monitored and kept at recommended levels) most studies have shown that *Legionella* spp. are reduced to low levels and that implementation of the ionization system markedly reduces the number of cases of nosocomial Legionnaires' disease.

5.1.2 Ionic silver in drinking water

Studies on the bacterial inactivation resulting from ionic silver added to water have shown that LRVs vary widely (generally between 3 and 7), with some bacteria being more sensitive (i.e. more easily killed or inactivated) than others. Generally, long contact times are required to reduce bacterial concentrations. In addition, the majority of studies spiked water samples with laboratory grown bacteria. Studies using silver on harvested rainwater, for example, typically showed poorer LRVs (0.4–2.9). The use of laboratory grown bacteria, (which tend to be “less virulent and hearty than wild microbial consortiums”– Madrigan et al., 2000) may, thus, overstate the effectiveness of treatment.

Two non-bacterial studies were identified. De Gusseme et al. (2010) showed a 3 $\log_{10}$ reduction in bacteriophage after 2 hours of exposure to a high silver concentration (5 mg/L). Abebe et al. (2015) found that silver nitrate-treated *C. parvum* oocysts were significantly less infective in mice than untreated oocysts.

The role of water chemistry is also likely to be important in determining the efficacy of silver as a disinfectant in real world conditions. Silver forms numerous salts with low water solubility and silver ions are easily sequested by anions commonly found in natural waters including chloride, bromide, carbonate and phosphate. Even at low concentrations which do not induce silver precipitation, chloride and phosphate have been shown to hinder the bioavailability and mitigate the antibacterial activity of silver ions (Xiu et al., 2011).

5.1.3 Silver nanoparticle applications

The exploratory drinking-water applications (principally employing filtration) identified used a wide range of media/matrices (e.g polyurethane foam, paper and polystyrene beads). The majority of studies tested efficacy against bacteria (typically *E. coli*), with values up to 7 $\log_{10}$ reduction being reported (range 1.6–7.6). De Gusseme et al. (2010) showed that biogenic silver nanoparticles produced a 4 $\log_{10}$ reduction in bacteriophage after three hours and murine norovirus after 30 minutes; this was in stark contrast to chemically-produced silver nanoparticles, which showed no inactivation. Abebe et al. (2015)

found that silver nanoparticle-treated *Cryptosporidium* oocysts showed some reduction in mouse infectivity compared to non-treated oocysts.

5.1.4 Silver-coated ceramic filter applications

A number of studies have considered the impact of silver coating on ceramic filters. In terms of the efficacy of the silver within silver-coated ceramic filters, it seems likely that the type of silver employed (silver nitrate or silver nanoparticles), how it is applied (painted on, dipped or fired in) and in what concentration the silver is applied affects both the bacterial removal and the effluent silver concentration. Overall, however, many of the studies which compared silver-coated and non-coated filters have not shown convincing benefits of silver nitrate or silver nanoparticle application, as shown in Table 9.

Table 9: Comparison of bacterial removal efficiencies of silver-coated and non-coated ceramic filters

Silver type	Bacterial removal cf. non-coated filter	Reference
AgNP	No significant difference	van Halem et al., 2007
Unspecified	Significant improvement only after filtration of 5000+ litres	Wubbels et al., 2008
AgNP	Variability of results for both coated and non-coated filters was too great to reach a conclusion	Bielefeldt et al., 2009
$AgNO_3$	Overall, silver-coated filters outperformed non-coated filters, however, individual results were very variable	Bloem et al., 2009
$AgNO_3$	No significant difference	Brown & Sobsey 2010
AgNP	Slight improvement over non-coated filters, although only likely to be statistically significant at 17% sawdust content	Kallman et al., 2011
AgNP	No significant difference	Zhang & Oyanedel-Craver, 2013
AgNP/$AgNO_3$	AgNP was found to be more effective than $AgNO_3$, although source of clay was also an important factor	Rayner et al., 2013

While some studies (e.g. Bloem et al., 2009) have shown that silver application can improve microbial removal (e.g. by between 1.8 and 3.3 log_{10} reduction in *E. coli*) compared to non-treated ceramic filters, silver-coated ceramic filters (even from the same manufacturer) seem to show great variability in bacterial removal. A number of studies have shown that water chemistry may greatly affect the longevity of the treatment effect (Bielefeldt et al., 2013; Mittelmann et al., 2015). Of the silver-coated ceramic filter studies which considered virus reduction, viruses were poorly removed and did not meet the 3 log_{10} reduction required for 2-star performance classification (WHO, 2016).

5.1.5 General points for potential drinking-water applications

Although an initial glance at the results suggests that silver may reduce microbial contamination in water (in some circumstances), there are a number of limiting factors that need to be considered, including:

- There is an emphasis on bacterial testing (this has been highlighted above).
- Few studies have tried to assess the silver applications in field conditions, using both turbid and non-turbid water and realistic contact times (and this may account for the diversity of reported results and the higher reported LRVs seen in the laboratory compared to field studies). Of the two reported silver-containing water treatment products tested against the WHO Scheme, one using colloidal silver and one using silver-treated ceramic filters, neither met requirements for effective household water treatment performance (i.e. providing comprehensive protection).
- There is a lack of consideration that silver may be acting as a bacteriostat and the impacts of silver leaching on the mid- to long-term performance of the product. Few studies have looked at regrowth (i.e. the possibility that silver is acting as a bacteriostatic rather than bactericidal agent in the low concentrations required for drinking-water applications – i.e. ≤ 100 µg/L), or the presence of silver in stored filtered water and the mode of action of silver at low concentrations is unclear.
- There is no clear accounting for the presence of potentially toxic contaminants in applications using silver nanoparticles (which may, at least in some instances, be the cause of microbial inactivation). Silver nanoparticles can be synthesized in a variety of ways, some of which use toxic reagents. It is often not clear from the studies on silver nanoparticle applications whether adequate steps were taken to remove these contaminants before efficacy testing.

5.2 Toxicity

It is clear that silver (largely irrespective of route of exposure or form) can distribute widely within the mammalian body and is capable of crossing the blood-brain and placental barriers. Tissue distribution varies between studies but the liver and kidneys seem to be target organs following silver ingestion. Animal *in vivo* study results suggest a range of toxic effects including decreases in body weight, histopathological changes to a number of organs and tissues, alterations to serum enzymes and neurotransmitter levels, increased sperm abnormalities, delayed onset of puberty and indications of genotoxicity. *In vivo* and *in vitro* studies have, however, produced an array of often conflicting information, which means that drawing clear conclusions about silver toxicity is difficult, although there are a number of reasons for the conflicting results as illustrated in the following sections.

5.2.1 Silver nanoparticles

There are numerous different methodologies for the synthesis of silver nanoparticles; they can be produced in a wide range of sizes and shapes and stabilized with a variety of capping agents, and these factors alone make generalizations difficult.

5.2.1.1 Synthesis and capping

Chernousova & Epple (2013) have noted that the reproducible laboratory synthesis of silver nanoparticles is “more difficult than expected”. They relate this to the initial formation of the nuclei of metallic silver, which develop different morphologies and crystal sizes when reaction conditions (such as concentrations, reduction agent, temperature or presence of additives) change. In addition, a number

of studies have shown that the choice of capping or stabilizing agent can change the toxicity of silver nanoparticles.

5.2.1.2 Size

There are a number of techniques for determining silver nanoparticle size; those most commonly used are transmission electron microscopy and dynamic light scattering. Transmission electron microscopy is useful to capture the size of the individual (or primary) particle, but it is limited as it can only be used to measure particles after they have been suspended and then dried (it may also be affected by the solvent used for silver nanoparticle dispersion prior to drying). Dynamic light scattering captures the hydrodynamic size and is performed in solution, but may be affected by the suspension media and how the sample was mixed, for example, sonication intensity and duration (Choi et al., 2011). The size of the silver nanoparticles also depends on the medium in which they are suspended, with Bouwmeester et al. (2011), for example, finding larger hydrodynamic sizes for silver nanoparticles when they were suspended in cell culture medium, compared to water. In the review sections and below, usually only the primary size of the silver nanoparticles has been reported to avoid over complicating the text.

Some studies have suggested that smaller silver nanoparticles are more toxic to mammalian cells than larger nanoparticles and microparticles. Carlson et al. (2008), for example, found that 15 nm carbon-coated silver nanoparticles caused more toxicity than 50 nm carbon-coated silver nanoparticles in rat alveolar macrophages and Li et al. (2012) reported similar results for PVP-coated silver nanoparticles (25, 35, 45, 60 and 70 nm) in human lung fibroblasts. Liu et al. (2010) found that small PVP-coated silver nanoparticles (5 nm) were more toxic to four different cell lines than both ionic silver (silver nitrate) and larger particles, as shown in Table 10.

Table 10: Half maximal effective concentration for cell mortality in four different cell lines (Liu et al. 2010)

Cell line	**Half maximal effective concentration (EC_{50})**			
	$AgNO_3$ (µg/mL)	AgNP–5 nm (µg/mL)	AgNP–20 nm (µg/mL)	AgNP–50 nm (µg/mL)
A549	3.62	1.02	9.96	14.31
HepG2	1.11	0.59	25.35	33.57
MCF-7	1.81	0.51	14.33	47.64
SGC-7901	3.23	0.92	50.94	112.03

Adapted with permission from Liu W et al., Impact of silver nanoparticles on human cells: effect of particle size, Nanotoxicology. Copyright 2010 Taylor and Francis.

Dasgupta et al. (2015) reported that 60 nm silver nanoparticles were more toxic to both A549 (lung carcinoma cells) and HCT116 (colon carcinoma cells) than 85 nm silver nanoparticles, manufactured using the thermal co-reduction. However, this does not seem to be universally the case as Powers et al. (2011), for example, found that larger PVP-coated silver nanoparticles (50 nm) had greater effects on DNA synthesis and caused a higher degree of oxidative stress in PC12 cells than the smaller PVP-coated particle (10 nm). Park et al. (2010b) reported greater cytotoxicity of 70 nm silver nanoparticles

in mouse macrophages than Shavandi et al. (2011), although it has been suggested that this may have been an artefact of the preparation method, which could have led to high ionic silver concentration, but reduced silver nanoparticle concentration (Pratsinis et al., 2013). In a review of toxicity data on mammalian cell lines, Bondarenko et al. (2013) found that when plotting $L(E)C_{50}$ data for PVP-coated silver nanoparticles (to avoid coated versus non-coated toxicity issues) against the primary size of the silver nanoparticles, no correlation was seen (R^2=0.1); plotting the data from Liu et al. (2010) resulted in a correlation of R^2=0.4, while plotting data from just one study on A549 cells (Liu et al., 2010) revealed a correlation of R^2=0.81. This demonstrates how difficult it is to make generalizations about the toxicity of silver nanoparticles to mammalian cells.

5.2.1.3 Experimental quality

There are numerous pitfalls that await the unwary silver nanoparticle researcher. These include lack of characterization of the silver nanoparticles, gradual release of silver ions from the dissolved silver nanoparticles following preparation, toxicity of the capping agent or suspending solvent, presence of biological contaminants, failure to account for possible contaminants remaining after the manufacture of the silver nanoparticles, and interference of silver nanoparticles with the toxicity tests.

In order to improve comparability between studies it is important that the silver nanoparticles used are adequately characterized. It has been suggested that complete characterization of silver nanoparticles may include measurements of size distribution, shape and other morphological features, solubility, surface area, state of dispersion, surface chemistry and other physico-chemical properties (Park et al., 2010b). Studies which go to those lengths are rare, but silver nanoparticle characterization is increasingly being reported, and it is clear that where commercial silver nanoparticles are utilized, it is not always adequate to rely on the manufacturers claims (Choi et al., 2011).

Kittler et al. (2010) examined the toxicity of freshly prepared silver nanoparticle and previously stored silver nanoparticles on human mesenchymal cells. The aged silver nanoparticles were found to be considerably more toxic than those that were freshly prepared, with the silver nanoparticles that had been prepared for 1 or 6 months causing 100% loss of cell viability, compared with a 70% loss of viability seen in the cells treated with freshly prepared silver nanoparticles. The difference in toxicity was attributed to differing amount of released silver ions. The authors comment that some of the published discrepancies in reported toxicity studies may be explained by this observation. Oostingh et al. (2011) investigated a number of possible issues relating to toxicity testing of nanoparticles. Some of the agents used to stabilize silver nanoparticles may have a toxic effect in their own right; with citrate, for example (a common capping agent) exerting a dose-dependent cytotoxic effect on BEAS-2B human primary lung cells. They also looked at biological contamination which may be important when studying immunomodulating/immunotoxic effects. Although the nanoparticles they used were sterile (i.e. devoid of live microbiological contamination), they found that both the nanoparticles and their solvents contained variable levels of endotoxin (to which many immune cells are especially sensitive). As noted above, some of these aspects may also impact on disinfection efficacy studies.

A number of traditional measures of cytotoxicity rely on optically based tests, but it has been shown that nanoparticles can interfere with these tests. Small nanoparticles (4–15 nm) have been shown to absorb at the wavelengths typically used in most biological assay readouts (this could suggest improved viability), while some nanoparticles can inhibit colour formation – which would mimic a cytotoxic effect (Oostingh et al., 2011).

5.2.2 *In vivo* toxicity

The studies outlined in Section 3.4, largely focus on the effects of silver nanoparticles (with some comparisons with silver salts). A number of dose-dependent animal toxicity findings have been reported including death, weight loss, hypoactivity, altered neurotransmitter levels, altered liver enzymes, altered blood values, enlarged hearts and immunological effects (Hadrup & Lam, 2014). While many studies show no negative impacts, toxicological effects (histopathogical changes in the testes) following chronic oral administration in rats were seen at a silver nanoparticle concentration of 20 µg/kg bw per day.

There has been a recent increase in interest in the possible genotoxic effects of silver. Although a number of studies have been reported only three of the *in vivo* studies looked at oral exposure. Two of these found possible genotoxic effects (Awasthi et al., 2015; Patolla et al., 2015) of which one reported a dose-dependent increase in chromosome aberrations at 5 mg/kg bw (the lowest dose examined) and above. A human study of silver jewellery workers (Aktepe et al., 2015) found evidence of possible DNA damage (based on the comet assay), but gave no information about working conditions or exposure to silver and other possible contaminants.

5.2.3 *In vitro* toxicity

Primary cells are more representative of tissue. They can be expected to reproduce the normal response of normal individuals (Oostingh et al., 2011) and therefore are ideal for *in vitro* toxicity studies. The use of primary cells, however, is not always feasible as they may be difficult to obtain (e.g. human lung epithelial cells) and they have limited cellular life spans, which means that fresh cells (probably obtained from different donors) are required for each assay, making standardization difficult (Oostingh et al., 2011). Thus, secondary cell lines (transformed or tumour cells with unrestrained proliferative capacity), which are easier to maintain and produce reproducible results, are preferred in many toxicity studies (Arora et al., 2008). There may, however, be a number of issues related to the widespread use of secondary cell lines in *in vitro* toxicity testing. Oostingh et al. (2011) make the point that particular caution should be used when testing the cytotoxic and anti-proliferative effects of nanoparticles on secondary cells as they have different cell cycle regulation and cell survival compared to primary cells. Indeed, it has been reported (e.g. Arora et al., 2009) that secondary cells are more susceptible to the impacts of silver nanoparticles than primary cells and this has led to the exploration of silver nanoparticles as a possible cancer treatment (e.g. Sriram et al., 2010).

As noted by Samberg et al. (2012), there is currently no consensus on the cytotoxicity of silver nanoparticles; however, the majority of publications do show reduced cell viability and increased ROS generation following silver nanoparticle exposure. Some however, clearly show that ROS are not always produced (e.g. Xiu et al., 2011). Zanette et al. (2011) point out that while many studies consider evidence for the induction of oxidative stress and apoptosis in cells exposed to silver nanoparticles, less investigate the intracellular pathways involved in the processes. While such details are beyond the scope of this review, Zanette et al. (2011) suggest that silver nanoparticles may act on different cellular targets and may differentially affect specific intracellular pathways depending on the cell types used. Chernousova & Epple (2013) in their review of silver as an antimicrobial agent comment that, given the different possibilities for silver to disturb biological processes, a general statement about the origin of the toxic action of silver is not possible.

6. Conclusions

It is difficult to draw any strong conclusions about the efficacy of silver (ionic silver and silver nanoparticles) in drinking-water treatment because of the wide range of approaches used in the various studies reviewed. The studies have used different types of silver (silver salts versus silver nanoparticles; capped silver nanoparticles versus bare silver nanoparticles; differently sized silver nanoparticles; silver nanoparticles created using different synthesis methods), different methodologies, different cells and microorganisms, different concentrations of test organisms and exposure for different time periods.

In drinking-water treatment applications, silver (ionic silver, experimental silver nanoparticle applications and silver-coated ceramic filters [ionic silver and silver nanoparticles]) has generally only shown to be effective against bacteria (i.e. 1.6 to 7.6 $\log_{10}$ reductions), most notably *E. coli*, with relatively long contact times. Based on the current available evidence, which is particularly limited for viruses and protozoa, silver does not appear to meet the WHO minimum performance recommendations for POU treatment products, which require effectiveness for two of the three pathogen classes. This is, partly, because of the paucity of data documenting performance efficacy against these classes of microbes in water. In the one study on protozoan parasite reduction by silver, there was only limited effectiveness on *Cryptosporidium* infectivity and a $\log_{10}$ reduction was not documented. For silver ions and nanoparticles, only one study on bacteriophage reduction in water has been reported, with effective $\log_{10}$ reduction (i.e. 3–4 $\log_{10}$ reductions) by ions and "biogenic" silver (zerovalent silver nanoparticles on a bacterial carrier matrix) but not by chemically-produced nanoparticles. It should also be noted that two silver containing products have failed the WHO evaluation scheme for household water treatment products, one a colloidal silver added to water and the other a silver-treated ceramic filter. Furthermore, it should be noted that relatively long contact times were required for effectiveness, which would reflect conditions where water would need to be stored.

Silver in combination with copper (copper/silver ionization) has been used to successfully supress the growth of *Legionella* bacteria in plumbing (principally hospital) systems.

The body of evidence on safety seems to suggest that silver (in ionic form or as silver nanoparticles) is toxic to mammalian cells, although the sensitivity of the cells varies according to the cell type and the type of silver to which it is exposed. Most of the evidence on the toxicity of silver comes from *in vitro* studies. However, there is accumulating evidence from mammalian *in vivo* data, especially with silver nanoparticles, that suggest that exposure to silver may result in toxic effects in exposed subjects, given sufficient dosage and lengths of exposure. In particular, available data indicate that silver nanoparticles have potential to damage DNA, although the potential for genotoxicity or DNA damage with silver nanoparticles requires further investigation as to its significance for humans.

In summary, the current evidence is sufficient to indicate that:

- Silver has not demonstrated significant capability to be considered a candidate for primary disinfection of drinking water.
    - There are insufficient data to document that it acts against a broad spectrum of pathogenic organisms. Performance efficacy has been adequately documented only for some bacteria and not for viruses and protozoan parasites. The impact of water chemistry is often neglected in efficacy studies, and further, long contact times are generally required.
- Silver/copper continuous ionization systems can be effective supplemental disinfectants to control *Legionella* regrowth and reduce legionellosis risks in hospital hot water plumbing

systems at concentrations well below current recommended drinking-water concentrations. The hot water system is not considered to be drinking water, so human exposure from consumption is minimal. This approach is favoured by the long contact times that are achieved in those systems. Proper operation and maintenance and periodic monitoring is required to assure continued performance.

- In some studies, at least, silver may be toxic to mammalian cells *in vitro*, and there is an indication that some toxic effects can also be seen from *in vivo* animal studies.

It should also be noted that in its current applications in POU household water treatment devices, as a supplement or amendment to microporous filters, it is difficult to determine if silver is acting as a bacteriostat or bacteriocide.

On the basis of the significant data and performance gaps in disinfection efficacy as a primary disinfectant of water, the limited data on the range of microorganisms against which it is effective and under what conditions, and the availability of widely used, well-characterized disinfectants, silver is not recommended for use as a primary disinfectant in drinking-water supplies at this time. There are also uncertainties around the toxicology, particularly with regard to human health end points. While there is no evidence that the use of silver in household water filters has either caused adverse health effects or leached excessive levels of silver into filtered water, the overall evidence base does not indicate that such supplemental use of silver in water filters improves the microbiological quality and safety of the filtered water.

7. References

Abebe LS, Smith JA, Narkiewicz S, Oyanedel-Craver V, Conaway M, Singo A et al. (2014). Ceramic water filters impregnated with silver nanoparticles as a point-of-use water-treatment intervention for HIV-positive individuals in Limpopo Province, South Africa: a pilot study of technological performance and human health benefits. J Water Health. 12:288–300.

Abebe LS, Su Y-H, Guerrant RL, Swami NS, Smith JA (2015). Point-of-use removal of *Cryptosporidium parvum* from water: independent effects of disinfection by silver nanoparticles and silver ions and by physical filtration in ceramic porous media. Environ Sci Technol. 49:12958–967.

Adler I, Hudson-Edwards KA, Campos LC (2013). Evaluation of a silver-ion based purification system for rainwater harvesting at a small-scale community level. J Water Supply Technol – AQUA. 62:545–51.

Aktepe N, Kocyigit A, Yukselten Y, Taskin A, Keskin C, Celik H (2015). Increased DNA damage and oxidative stress among silver jewelry workers. Biol Trace Elem Res. 164(2):185–91.

Al Gurabi MA, Ali D, Alkahtani S, Alarifi S (2015). *In vivo* DNA damaging and apoptotic potential of silver nanoparticles in Swiss albino mice. OncoTargets Ther. 8:295–302.

Arora S, Jain J, Rajwade JM, Paknikar KM (2008). Cellular responses induced by silver nanoparticles: in vitro studies. Toxicol Lett. 179:93–100.

Arora S, Jain J, Rajwade JM, Paknikar KM (2009). Interactions of silver nanoparticles with primary mouse fibroblasts and liver cells. Toxicol Appl Pharmacol. 236: 310–18.

Asare N, Duale N, Slagsvold HH, Lindeman B, Olsen AK, Gromadzka-Ostrowska J et al. (2015). Genotoxicity and gene expression modulation of silver and titanium dioxide nanoparticles in mice. Nanotoxicol. 10(3):312–21.

AshaRani PV, Hande MP, Valiyaveettil S (2009a). Anti-proliferative activity of silver nanoparticles. BMC Cell Biol. 10:65.

AshaRani PV, Mun GLK, Hande MP, Valiyaveettil S (2009b). Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano. 3(2):279–90.

ATSDR (1990) Toxicological profile for silver. Atlanta (GA): Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services, Public Health Service.

Avalos A, Haza AI, Morales P (2015). Manufactured silver nanoparticles of different sizes induced DNA strand breaks and oxidative DNA damage in hepatoma and leukaemia cells and in dermal and pulmonary fibroblasts. Folia Biol. 61:33–42.

Awasthi KK, Verma R, Awasthi A, Awasthi K, Soni I, John PJ (2015). *In vivo* genotoxic assessment of silver nanoparticles in liver cells of Swiss albino mice using comet assay. Adv Mater Lett. 6:187–93.

Bader KF (1966). Organ deposition of silver following silver nitrate therapy of burns. Plast Reconstr Surg. 37:550–51.

Baumgartner J, Murcott S, Ezzati M (2007). Reconsidering 'appropriate technology': the effects of operating conditions on the bacterial removal performance of two household drinking-water filter systems. Environ Res Lett. 2:2.

Berry JP, Galle P (1982). Selenium and kidney deposits in experimental argyria. Electron microscopy and microanalysis. Pathol Biol. (Paris) 30:136–40.

Bielefeldt AR, Kowalski K, Summers RS (2009). Bacterial treatment effectiveness of point-of-use ceramic water filters. Water Res. 43:3559–565.

Bielefeldt AR, Stewart MW, Mansfield E, Summers RS, Ryan JN (2013). Effects of chlorine and other water quality parameters on the release of silver nanoparticles from a ceramic surface. Water Res. 47:4032–039.

Blanc DS, Carrar Ph, Zanetti G, Francioli P (2005). Water disinfection with ozone, copper and silver ions, and temperature increase to control *Legionella*: seven years of experience in a university teaching hospital. J Hosp Infect. 60:69–72.

Bloem SC, van Halem D, Sampson ML, Huoy L, Heijman B (2009). Silver impregnated ceramic pot filter: flow rate versus removal efficiency of pathogens. WEF Disinfection 2009, Atlanta 28.2.09-3.3.09.

Böhmert L, Girod M, Hansen U, Maul R, Knappe P, Niemann B et al. (2014). Analytically monitored digestion of silver nanoparticles and their toxicity for human intestinal cells. Nanotoxicol. 8:631–42.

Bondarenko O, Juganson K, Ivask A, Kasemets K, Mortimer M, Kahru A (2013). Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: a critical review. Arch Toxicol. 87:1181–1200.

Bouwmeester H, Poortman J, Peters RJ, Wijma E, Kramer E, Makama S et al. (2011). Characterization of translocation of silver nanoparticles and effects on whole-genome gene expression using an in vitro intestinal epithelium coculture model. Nano. 5(5):4091–103.

Brandt D, Park B, Hoang M, Jacobe HT (2005). Argyria secondary to ingestion of homemade silver solution. J Am Acad Dermatol. 53:S105–07.

Brown J, Sobsey MD (2010). Microbiological effectiveness of locally produced ceramic filters for drinking-water treatment in Cambodia. J Water Health. 8(1):1–10.

Brown J, Sobsey MD, Loomis D (2008). Local drinking-water filters reduce diarrheal disease in Cambodia: a randomized, controlled trail of the ceramic water purifier. Am J Trop Med Hyg. 79:394–400.

Cachafeiro SP, Naveira IM, Garcia IG (2007). Is copper-silver ionisation safe and effective in controlling legionella? J Hosp Infect. 67:209–16.

Carlson C, Hussain SM, Schrand AM, Braydich-Stolle LK, Hess KL, Jones RL et al. (2008). Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. J Phys Chem. B 112:13608–619.

Castiglioni S, Caspani C, Cazzaniga A, Maier JAM (2014). Short- and long-term effects of silver nanoparticles on human microvascular endothelial cells. World J Biol Chem. 5:457–64.

CEC (1996) Commission of the European Communities technical guidance document in support of Commission Directive 93/67/EEC on risk assessment for new notified substances. Part II, Environmental Risk Assessment. Luxembourg: Office for official publications of the European Communities [cited by Bondarenko et al., 2013].

Cha K, Hong HW, Choi YG, Lee MJ, Park JH, Chae HK et al. (2008). Comparison of acute responses of mice livers to short-term exposure to nano-sized or micro-sized silver particles. Biotechnol Lett. 30:1893–899.

Chang AL, Khosravi V, Egbert B (2006). A case of argyria after colloidal silver ingestion. J Cutan Pathol. 33:809–11.

Chen CH, Lin LC, Chang YJ, Liu CE, Soon MS, Huang CS (2013). Efficacy of copper-silver ionization for controlling fungal colonization in water distribution systems. J Water Health. 11:277–80.

Chen YS, Lin YE, Liu Y-C, Huang WK, Shih HY, Wann SR et al. (2008). Efficacy of point-of-entry copper-silver ionization system in eradicating *Legionella pneumophila* in a tropical tertiary care hospital: implications for hospitals contaminated with *Legionella* in both hot and cold water. J Hosp Infect. 68:152–58.

Chernousova S, Epple M (2013). Silver as antibacterial agent: ion, nanoparticle, and metal. Angew Chem. 52:1636–653.

Choi J, Reipa V, Hitchins VM, Goering PL, Malinauskas RA (2011). Physiocochemical characterization and in vitro hemolysis evaluation of silver nanoparticles. Toxicol Sci. 123:133–43.

Chung IS, Lee MY, Shin DH, Jung HR (2010). Three systemic argyria cases after ingestion of colloidal silver solution. Int J Dermatol. 49:1175–77.

Clasen T, Garcia Parra G, Boisson S, Collin S (2005). Household-based ceramic water filters for the prevention of diarrhea: a randomized, controlled trial of a pilot program in Columbia. Am J Trop Med Hyg. 73:790–95.

Clasen TF, Brown J, Collin S, Suntura O, Cairncross S (2004). Reducing diarrhea through the use of household-based ceramic water filters: a randomized, controlled trail in rural Bolivia. Am J Trop Med Hyg. 70(6):651–57.

Coffin DL, Palekar LD (1985). Bioassays for asbestos and other solid materials. In: Milman HA and Weisburger EK, ed. Handbook of Carcinogen Testing. Park Ridge (NJ): Noyes Publications; pp. 384–419.

Cunningham JH, Cunningham C, van Aken B, Lin LS (2008). Feasibility of disinfection kinetics and minimum inhibitory concentration determination on bacterial cultures using flow cytometry. Water Sci Technol. 55(4):937–44.

Dankovich TA, Gray DG (2011). Bactericidal paper impregnated with silver nanoparticles for point-of-use water treatment. Environ Sci Technol. 45:1992–1998.

Dankovich TA (2014). Microwave-assisted incorporation of silver nanoparticles in paper for point-of-use water purification. Environ Sci: Nano. 1:367–78.

Danscher G (1981). Light and electron microscopic localization of silver in biological tissue. Histochem. 71:177–86.

Dasgupta N, Ranjan S, Rajendran B, Manickam V, Ramalingam C, Avadhani GS et al. (2015). Thermal co-reduction approach to vary size of silver nanoparticle: its microbial and cellular toxicology. Environ Sci Pollut Res. 23:4149–63.

De Gusseme B, Hennebel T, Christiaens E, Saveyn H, Verbeken K, Fitts JP et al. (2011). Virus disinfection in water by biogenic silver immobilized in polyvinylidene fluoride membranes. Water Res. 45:1856–64.

De Gusseme B, Sintubin L, Baert L, Thibo E, Hennebel T, Vermeulen G et al. (2010). Biogenic silver for disinfection of water contaminated with viruses. Appl Environ Microbiol. 76:1082–87.

Di Vincenzo GD, Giordano CJ, Schriever LS (1985). Biologic monitoring of workers exposed to silver. Int Arch Occup Environ Health. 56:207–15.

Doak SH, Manshian B, Jenkins GJS, Singh N (2012). In vitro genotoxicity testing strategy for nanomaterials and the adaptation of current OECD guidelines. Mutat Res. 745:104–11.

Dobrzyńska MM, Gajowik A, Radzikowska J, Lankoff A, Dušinská M, Kruszewski M (2014). Genotoxicity of silver and titanium dioxide nanoparticles in bone marrow cells of rats *in vivo*. Toxicol. 315:86–91.

Dong MS, Choi JY, Sung JH, Kim JS, Song KS, Ryu HR et al. (2013). Gene expression profiling of kidneys from Sprague-Dawley rats following 12-week inhalation exposure to silver nanoparticles. Toxicol Mech Methods. 23(6):437–48.

du Preez M, Conroy RM, Wright JA, Moyo S, Potgieter N, Gundry SW (2008). Short report: Use of ceramic water filtration in the prevention of diarrheal disease: a randomized controlled trial in rural South Africa and Zimbabwe. Am J Trop Med Hyg. 79(5):696–701.

Dziendzikowska K, Gromadzka-Ostrowska J, Lankoff A, Oczkowski M, Krawczynska A, Chwastowska J et al. (2012). Time-dependent biodistribution and excretion of silver nanoparticles in male Wistar rats. J Appl Toxicol. 32:920–928.

East BW, Boddy K, Williams ED, Macintyre D, Mclay AL (1980). Silver retention, total body silver and tissue silver concentrations in argyria associated with exposure to an anti-smoking remedy containing silver acetate. Clin Exp Dermatol. 5:305–11.

Ebabe Elle R, Gaillet J, Vidé J, Romain C, Lauret C, Rugani N et al. (2013). Dietary exposure to silver nanoparticles in Sprague-Dawley rats: effects on oxidative stress and inflammation. Food Chem Toxicol. 60:297–301.

Elechiguerra J, Burt J, Morones J, Camacho-Bragado A, Gao X, Lara H et al. (2005). Interaction of silver nanoparticles with HIV-1. J Nanobiotechnol. 3(6).

El Mahdy MM, Eldin TAS, Aly HS, Mohammed FF, Shaalan MI (2014). Evaluation of hepatotoxic and genotoxic potential of silver nanoparticles in albino rats. Exp Toxicol Pathol. 67:21–9.

Ema M, Kobayashi N, Naya M, Hanai S, Nakanishi J (2010). Reproductive and developmental toxicity studies of manufactured nanomaterials. Reprod Toxicol. 30:343–52.

Espinosa-Cristobal LF, Martinez-Castañon GA, Loyola-Rodriguez JP, Patiño-Marin N, Reyes-Macías JF, Vargas-Morales JM et al. (2013). Toxicity, distribution, and acculmulation of silver nanoparticles in Wistar rats. J Nanopart Res. 15:1702.

Franchi LP, Manshian BB, de Souza TAJ, Soenen SJ, Matsubara EY, Rosolen JM et al. (2015). Cyto- and genotoxic effects of metallic nanoparticles in untransformed human fibroblast. Toxicol in Vitro. 29:1319–31.

Furchner JE, Richmond CR, Drake GA (1968). Comparative metabolism of radionuclides in mammals-IV. Retention of silver-110m in the mouse, rat, monkey, and dog. Health Phys. 15:505–514.

Furst A, Schlauder MC (1977). Inactivity of two noble metals as carcinogens. J Environ Pathol Toxicol. 1:51–57.

Gaiser BK, Hirn S, Kermanizadeh A, Kanase N, Fytianos K, Wenk A et al. (2013). Effects of silver nanoparticles on the liver and heptocytes in vitro. Toxicol Sci. 131: 537–47.

Gangadharan D, Harshvardan K, Gnanasekar G, Dixit D, Popat KM, Anand PS (2010). Polymeric microspheres containing silver nanoparticles as a bactericidal agent for water disinfection. Water Res. 44:5481–87.

Ghosh M, Manivannan J, Sinha S, Chakraborty A, Mallick SK, Bandyopadhyay M et al. (2012). In vitro and *in vivo* genotoxicity of silver nanoparticles. Mutat Res. 749:60–69.

Giovanni M, Tay CY, Setyawati MI, Xie J, Ong CN, Fan R et al. (2015). Toxicity profiling of water contextual zinc oxide, silver, and titanium dioxide nanoparticles in human oral and gastrointestinal cell systems. Environ Toxicol. 30(12):1459–69.

Gopinath P, Gogoi SK, Sanpui P, Paul A, Chattopadhyay A, Ghosh SS (2010). Signalling gene cascade in silver nanoparticle induced apoptosis. Colloids Surf B Biointerfaces. 77:240–45.

Haase A, Rott S, Mantion A, Graf P, Plendl J, Thunemann AF et al. (2012). Effects of silver nanoparticles on primary mixed neural cell cultures: uptake, oxidative stress and acute calcium responses. Toxicol Sci. 126:457–68.

Hadek R (1966). Preliminary report on the cellular effect of intravital silver in the mouse ovary. J Ultrastruct Res. 15:66–73.

Hadrup N, Loeschner K, Bergstrom A, Wilcks A, Gao X, Vogel U et al. (2012a). Subacute oral toxicity investigation of nanoparticulate and ionic silver in rats. Arch Toxicol. 86:543–51.

Hadrup N, Lam HR, Loeschner K, Mortensen A, Larsen EH, Frandsen H (2012b). Nanoparticulate silver increases uric acid and allantoin excretion in rats, as identified by metabolomics. J Appl Toxicol. 32:929–33.

Hadrup N, Loeschner K, Mortensen A, Sharma AK, Qvortrup K, Larsen EH et al. (2012c). The similar neurotoxic effects of nanoparticulate and ionic silver *in vivo* and *in vitro*. NeuroToxicol. 33:416–23.

Hadrup N, Lam HR (2014) Oral toxicity of silver ions, silver nanoparticles and colloidal silver – a review. Regul Toxicol Pharmacol. 68:1–7.

Hardes J, Streitburger A, Ahrens H, Nusselt T, Gebert C, Winkelmann W et al. (2007). The influence of elementary silver versus titanium on osteoblasts behaviour in vitro using human osteosarcoma cell lines. Sarcoma. 26539.

Havarinasab S, Pollard KM, Hultman P (2009). Gold- and silver-induced murine autoimmunity-requirement for cytokines and CD28 in murine heavy metal-induced autoimmunity. Clin Exp Immunol. 155:567–576.

Heidarpour F, Ghani WA, Fakhru'l-Razi A, Sobri S, Heydarpour V, Zargar M et al. (2011). Complete removal of pathogenic bacteria from drinking-water using nano silver-coated cylindrical polypropylene filters. Clean Technol Environ Policy. 13:499–507.

Hong JS, Kim S, Lee SH, Jo E, Lee B, Yoon J et al. (2014). Combined repeated-dose toxicity study of silver nanoparticles with the reproduction/developmental toxicity screening test. Nanotoxicol. 8:349–62.

Huang HI, Shih HY, Lee CM, Yang TC, Lay JJ, Lin YE (2008). In vitro efficacy of copper and silver ions in eradicating *Pseudomonas aeruginosa, Stenotrophomonas maltophila* and *Acinetobacter baumannii*: Implications for on-site disinfection for hospital infection control. Water Res. 42:73–80.

Hudecová A, Kusznierewicz B, Runden-Pran E, Magdolenova Z et al. (2012). Silver nanoparticles induce premutagenic DNA oxidation that can be prevented by phytochemicals from *Gentiana asclepiadea*. Mutagen. 27:759–69.

Hwang MG, Katayama H, Ohgaki S (2006). Effect of intracellular resuscitation of *Legionella pneumophila* in *Acanthamoeba polyphage* cells on the antimicrobial properties of silver and copper. Environ Sci Technol. 40:7434–39.

Hwang MG, Katayama H, Ohgaki S (2007). Inactivation of *Legionella pneumophila* and *Pseudomonas aeruginosa*: Evaluation of the bactericidal ability of silver cations. Water Res. 41:4097–104.

Hyun JS, Lee BS, Ryu HY, Sung JH, Chung KH, Yu IJ (2008). Effects of repeated silver nanoparticles exposure on the histological structure and mucins of nasal respiratory mucosa in rats. Toxicol Lett. 182:24–8.

Jain P, Pradeep T (2005). Potential of silver nanoparticles-coated polyurethane foam as an antibacterial water filter. Biotechnol Bioeng. 90(1):59–63.

Jeong GN, Jo UB, Ryu HY, Kim YS, Song KS, Yu IJ (2010). Histochemical study of intestinal mucins after administration of silver nanoparticles in Sprague-Dawley rats. Arch Toxicol. 84:63–9.

Ji JH, Jung JH, Kim SS, Yoon JU, Park JD, Choi BS et al. (2007). Twenty-eight-day inhalation toxicity study of silver nanoparticles in Sprague-Dawley rats. Inhal Toxicol. 19:857–71.

Kallman EN, Oyanedel-Craver VA, Smith JA (2011). Ceramic filters impregnated with silver nanoparticles for point-of-use water treatment in rural Guatemala. J Environ Eng. 137:407–15.

Kermanizadeh A, Vranic S, Boland S, Moreau K, Baeza-Squiban A, Gaiser BK et al. (2013). An in vitro assessment of panel of engineered nanomaterials using a human renal cell line: cytotoxicity, pro-inflammatory response, oxidative stress and genotoxicity. BMC Nephrol. 14:96.

Kim JS, Song KS, Sung JH, Ryu HR, Choi BG, Cho HS et al. (2013). Genotoxicity, acute oral and dermal toxicity, eye and dermal irritation and corrosion and skin sensitisation evaluation of silver nanoparticles. Nanotoxicol. 7:953–60.

Kim JS, Sung JH, Ji JH, Song KS, Lee JH, Kang CS et al. (2011). *In vivo* genotoxicity of silver nanoparticles after 90-day silver nanoparticle inhalation exposure. Saf Health Work. 2:34–8.

Kim YS, Kim JS, Cho HS, Rha DS, Kim JM, Park JD et al. (2008). Twenty-eight-day oral toxicity, genotoxicity and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. Inhal. Toxicol. 20:575–83.

Kim YS, Song MY, Park JD, Song KS, Ryu HR, Chung YH et al. (2010). Subchronic oral toxicity of silver nanoparticles. Part Fibre Toxicol. 7:20.

Kittler S, Greulich C, Diendorf J, Koller M, Epple M (2010). Toxicity of silver nanoparticles increases during storage because of slow dissolution under release of silver ions. Chem Mater. 22:4548–54.

Korani M, Rezayat SM, Gilani K, Arbabi Bidgoli S, Adeli S (2011). Acute and subchronic dermal toxicity of nanosilver in guinea pig. Int J Nanomedicine. 6:855–62.

Kovvuru P, Mancilla PE, Shirode AB, Murray TM, Begley TJ, Reliene R (2015). Oral ingestion of silver nanoparticles induces genomic instability and DNA damage in multiple tissues. Nanotoxicol. 9:162–71.

Kruszewski M, Gradzka I, Bertlomiejczyk T, Chwastowska J, Sommer S, Grzelak A et al. (2013) Oxidative DNA damage corresponds to the long term survival of human cells treated with silver nanoparticles. Toxicol Lett. 219:151–59.

Kulthong K, Maniratanachote R, Kobayashi Y, Fukami T, Yokoi T (2012). Effects of silver nanoparticles on rat hepatic cytochrome P450 enzyme activity. Xenobiotica. 42:854–62.

Landsiedel R, Kapp MD, Schulz M, Wiench K, Oesch F (2009). Genotoxicity investigations on nanomaterials: methods, preparation and characterization of test material, potential artifacts and limitations--many questions, some answers. Mutat Res. 681:241–58.

Lankveld DPK, Oomen AG, Krystek P, Neigh A, Troost-de Jong A, Noorlander CW et al. (2010). The kinetics of the tissue distribution of silver nanoparticles of different sizes. Biomater. 31:8350–61.

Lansdown ABG (2010). A pharmacological and toxicological profile of silver as an antimicrobial agent in medical devices. Adv Pharmacol Sci. 910686.

Lea MC (1889). On allotropic forms of silver. Am J Sci. 37:476–91 [cited by Nowack et al., 2011].

Lee JH, Kim YS, Song KS, Ryu HR, Sung JH, Park JD et al. (2013). Biopersistence of silver nanoparticles in tissues from Sprague-Dawley rats. Part Fibre Toxicol. 10: 36.

Li Y, Bhalli JA, Ding W, Yan J, Pearce MG, Sadiq R et al. (2013). Cytotoxicity and genotoxicity assessment of silver nanoparticles in mouse. Nanotoxicol. 8 Suppl 1:36–45.

Lichtenstein D, Ebmeyer J, Knappe P, Juling S, Böhmert L, Selve S et al. (2015). Impact of food components during in vitro digestion of silver nanoparticles on cellular uptake and cytotoxicity in intestinal cells. Biol Chem. 396:1255–64.

Lin YE, Vidic RD (2006). Possible phosphate interference with copper-silver ionization for *Legionella* control. J Hosp Infect. 62:119.

Lin YE, Vidic RD, Stout JE, Yu VL (2002). Negative effect of high pH on biocidal efficacy of copper and silver ions in controlling *Legionella pneumophila.* Appl Environ Microbiol. 68:2711–15.

Lin YE, Stout JE, Yu VL (2011). Controlling *Legionella* in hospital drinking water: an evidence-based review of disinfection methods. Infect Control Hosp Epidemiol. 32(2):166–73.

Lin S, Huang R, Cheng Y, Liu J, Lau BLT, Wiesner MR (2013). Silver nanoparticle-alginate composite beads for point-of-use drinking-water disinfection. Water Res. 47:3959–65.

Liu Z, Stout JE, Boldin M, Rugh J, Diven WF, Yu VL (1998). Intermittent use of copper-silver ionization for *Legionella* control in water distribution systems: a potential option in buildings housing individuals at low risk of infection. Clin Infect Dis. 26:138–40.

Liu W, Wu Y, Wang C, Li HC, Wang T, Liao CYet al. (2010). Impact of silver nanoparticles on human cells: effect of particle size. Nanotoxicol. 4:319–30.

Liu J, Wang Z, Luo Z, Bashir S (2012). Effective bactericidal performance of silver-decorated titania nano-composites. Dalton Trans. 42:2158–66.

Liu H, Tang X, Liu Q (2014). A novel point-of-use water treatment method by antimicrobial nanosilver textile material. J Water Health. 12:670–77.

Loeschner K, Hadrup N, Qvortrup K, Larsen A, Gao X, Vogel U et al. (2011). Distribution of silver in rats following 28 days of repeated oral exposure to silver nanoparticles or silver acetate. Part Fibre Toxicol. 8:18

Loo SL, Fane AG, Lim TT, Krantz WB, Liang YN, Liu X et al. (2013). Superabsorbent cryogels decorated with silver nanoparticles as a novel water technology for point-of-use disinfection. Environ Sci Technol. 47:9363–71.

Lu L, Sun R, Chen R, Hui C, Ho C, Luk J et al. (2008). Silver nanoparticles inhibit hepatitis B virus replication. Antivir Ther. 13:253–62 [cited by Marambio-Jones and Hoek, 2010].

Luther EM, Koehler Y, Diendorf J, Epple M, Dringen R (2011). Accumulation of silver nanoparticles by cultured primary brain astrocytes. Nanotechnol. 22(37):375101.

Luther EM, Schmidt MM, Diendorf J, Epple M, Dringen R (2012). Upregulation of metallothioneins after exposure of cultured primary astrocytes to silver nanoparticles. Neurochem Res. 37:1639–48.

Lv Y, Liu H, Wang Z, Liu S, Hao L, Sang Y et al. (2009). Silver nanoparticles-decorated porous ceramic composite for water treatment. J Memb Sci. 331:50–6.

Lyon TDB, Patriarca M, Howatson AG, Fleming PJ, Blair PS, Fell GS (2002). Age dependence of potentially toxic elements (Sb, Cd, Pb, Ag) in human liver tissue from paediatric subjects. Journal of Environ Monit. 4:1034–39.

Madrigan MT, Martinko JM, Parker J (2000). Brock Biology of Microorganisms, 9th edition. Upper Saddle River (NJ): Prentice-Hall, Inc. [cited by Cunningham et al., 2008].

Maneewattanapinyo P, Banlunara W, Thammacharoen C, Ekgasit S, Kaewamatawong T (2011). An evaluation of acute toxicity of colloidal silver nanoparticles. J Vet Med Sci. 73:1417–23.

Marambio-Jones C, Hoek EMV (2010). A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. J Nanopart Res. 12:1531–51.

Marshall JP II, Schneider RP (1977). Systemic argyria: Secondary to topical $AgNO_3$. Arch Dermatol. 113:1077–79.

Mathias FT, Romano RM, Kizys MM, Kasamatsu T, Giannocco G, Chiamolera MI et al. (2015). Daily exposure to silver nanoparticles during prepubertal development decreases adult sperm count and reproductive parameters. Nanotoxicol. 9:64–70.

Matthies K, Bitter H, Deobald N, Heinle M, Diedel R, Obst U et al. (2015). Morphology, composition and performance of a ceramic filter for household water treatment in Indonesia. Water Pract Technol. 10:361–70.

Mecha CA, Pillay VL (2014). Development and evaluation of woven fabric microfiltration membranes impregnated with silver nanoparticles for potable water treatment. J Memb Sci. 458:149–56.

Mecha AC, Otieno FAO, Pillay VL (2014). Long-term disinfection performance of silver nanoparticles impregnated membranes. Desal Water Treat. 57(11):4906–12.

Mirsattari SM, Hammond RR, Sharpe MD, Leung FY, Young GB (2004). Myoclonic status epilepticus following repeated oral ingestion of colloidal silver. Neurol. 62:1408–10.

Mittelmann AM, Lantagne DS, Rayner J, Pennell KD (2015). Silver dissolution and release from ceramic water filters. Environ Sci Technol. 49:8515–22.

Mòdol J, Sabria M, Reynaga E, Pedro-Botet ML, Sopena N, Tudela P et al. (2007). Hospital-acquired Legionnaires' disease in a university hospital: impact of the copper-silver ionization system. Clin Infect Dis. 44:263–5.

Monteiro-Riviere NA, Samberg ME, Oldenburg SJ, Riviere JE (2013). Protein binding modulates the cellular uptake of silver nanoparticles into human cells: implications for in vitro and *in vivo* extrapolations? Toxicol Lett. 220:286–93.

Moore MN (2006). Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environ Int. 32:967–76.

Mpenyana-Monyatsi L, Mthombeni NH, Onyango MS, Momba MNB (2012). Cost-effective filter materials coated with silver nanoparticles for the removal of pathogenic bacteria in groundwater. Int J Environ Res Public Health. 9: 244–71.

Mthombeni NH, Mpenyana-Monyatsi L, Onyango MS, Momba MNB (2012). Breakthrough analysis for water disinfection using silver nanoparticles coated resin beads in fixed-bed column. J Hazard Mater. 217–218:133–40.

Mukherjee SG, O'Claonadh N, Casey A, Chambers G (2012). Comparative in vitro cytotoxicity study of silver nanoparticle on two mammalian cell lines. Toxicol in Vitro. 26:238–51.

Munger MA, Radwanski P, Hadlock GC, Stoddard G, Shaaban A, Falconer J et al. (2013). *In vivo* human time-exposure study of orally dosed commercial silver nanoparticles. Nanomedicine. 10(1).

Nangmenyi G, Xao W, Mehrabi S, Mintz E, Economy J (2009). Bactericidal activity of Ag nanoparticles-impregnated fibreglass for water disinfection. J Water Health. 7(4):657–63.

Nawaz M, Han MY, Kim T, Manzoor U, Amin MT (2012). Silver disinfection of *Pseudomonas aeruginosa* and *E. coli* in rooftop harvested rainwater for potable purposes. Sci Total Environ. 431:20–5.

Newton D, Holmes A (1966). A case of accidental inhalation of zinc-65 and silver-110m. Radiat Res. 29:403–12.

Nowack B, Krug HF, Height M (2011). 120 years of nanosilver history: implications for policy makers. Environ Sci Technol. 45:1177–83.

Nowrouzi A, Meghrazi K, Golmohammadi T, Golestani A, Ahmadian S, Shafiezadeh M et al. (2010). Cytotoxicity of subtoxic AgNP in human hepatoma cell line (HepG2) after long-term exposure. Iran Biomed J. 14:23–32.

Nymark P, Catalán J, Suhonen S, Järventaus H, Birkedal R, Clausen PA et al. (2013). Genotoxicity of polyvivylpyrrolidone-coated silver nanoparticles in BEAS 2B cells. Toxicol. 313:38–48.

OECD (2015). Guidance document on revisions to OECD genetic toxicology test guidelines. November 30th, 2015, Paris: Organisation for Economic Co-operation and Development. (http://www.oecd.org/env/ehs/testing/Draft Guidance Document on OECD Genetic Toxicology Test Guidelines.pdf, accessed March 2016).

Ohbo Y, Fukuzako H, Takeuchi K, Takigawa M (1996). Argyria and convulsive seizures caused by ingestion of silver in a patient with schizophrenia. Psychiatry Clin. Neurosci. 50:89–90.

Oostingh GJ, Casals E, Italiani P, Colognato R, Stritzinger R, Ponti J et al. (2011). Problems and challenges in the development and validation of human cell-based assays to determine nanoparticle-induced immunomodulatory effects. Part Fibre Toxicol. 8:8.

Oppenheimer BS, Oppenheimer ET, Danishefsky I et al. (1956). Carcinogenic effect of metals in rodents. Cancer Res. 16:439–41.

Park EJ, Bae E, Yi J, Kim Y, Choi K, Lee SH et al. (2010a). Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles. Environ Toxicol Pharmacol. 30:162–8.

Park EJ, Yi J, Kim Y, Choi K, Park K (2010b). Silver nanoparticles induce cytotoxicity by a Trojan-horse type mechanism. Toxicol in Vitro. 24:872–8.

Pathak SP, Gopal K (2012). Evaluation of bactericidal efficacy of silver ions on *Escherichia coli* for drinking-water disinfection. Environ Sci Pollut Res. 19: 2285–90.

Patil RA, Kausley SB, Balkunde PL, Malhotra CP (2013). Comparative study of disinfectants for use in low-cost gravity driven household water purifiers. J Water Health. 11(3):443–56.

Patlolla AK, Hackett D, Tchounwou PB (2015). Silver nanoparticle-induced oxidative stress-dependent toxicity in Sprague-Dawley rats. Mol Cell Biochem. 399: 257–68.

Pedro-Botet ML, Sanchez I, Sabria M, Sopena N, Mateu L, García-Núñez M et al. (2007). Impact of copper and silver ionization on fungal colonization of the water supply in health care centres: implications for immunocompromised patients. Clin Infect Dis. 45:84–6.

Pelkonen KHO, Heinonen-Tanski H, Hanninen OOP (2003). Accumulation of silver from drinking water into cerebellum and musculus soleus in mice. Toxicol. 186:151–7.

Phalen RF, Morrow PE (1973). Experimental inhalation of metallic silver. Health Phys. 24: 509–18.

Powers CM, Badireddy AR, Ryde IT, Seidler FJ, Slotkin TA (2011). Silver nanoparticles compromise neurodevelopment in PC12 cells: critical contributions of silver ion, particle size, coating and composition. Environ Health Perspect. 119:37–44.

Pratsinis A, Hervella P, Leroux J, Pratsinis SE, Sotiriou GA (2013). Toxicity of silver nanoparticles in macrophages. Small 9:2576–84.

Rayner J, Zhang H, Schubert J, Lennon P, Lantagne D, Oyanedel-Craver V (2013). Laboratory investigation into the effect of silver application on bacterial removal efficacy of filter material for use on locally produced ceramic water filters for household drinking water treatment. ASC Sustain Chem Eng. 1:737–45.

Ren D, Smith JA (2013). Retention and transport of silver nanoparticles in a ceramic porous medium used for point-of-use water treatment. Environ Sci Technol. 47:3825–32.

Rosenman KD, Moss A, Kon S (1979). Argyria: Clinical implications of exposure to $AgNO_3$ and silver oxide. J Occup Med. 21:430–5.

Rungby J, Danscher G (1983). Localization of exogenous silver in brain and spinal cord of silver exposed rats. Acta Neuropathol. 60:92–8.

Rungby J, Danscher G (1984). Hypoactivity in silver exposed mice. Acta Pharmacol Toxicol. 55:398–401.

Sahu SC, Zheng J, Yourick JJ, Spando RL, Gao X (2015). Toxicogenomic responses of human liver HepG2 cells to silver nanoparticles. J Appl Toxicol. 35(10):1160–68.

Salsali H, McBean E, Brunsting J (2011). Virus removal efficiency of Cambodian ceramic pot water purifiers. J Water Health. 9(2):306–11.

Samberg ME, Loboa EG, Oldenburg SJ, Monteiro-Riviere NA (2012). Silver nanoparticles do not influence stem cell differentiation but cause minimal toxicity. Nanomedicine. 7:1197–1209.

Samberg ME, Oldenburg SJ, Monteiro-Riviere NA (2010). Evaluation of silver nanoparticle toxicity in skin *in vivo* and keratinocytes *in vitro*. Environ Health Perspect. 118:407–13.

Samberg ME, Oldenburg SJ, Monteiro-Riviere NA (2011) Antibacterial efficacy of silver nanoparticles of different sizes, surface conditions and synthesis methods. Nanotoxicol. 5, 244-253.

Sardari RRR, Zarchi SR, Talebi A, Nasri S, Imani S, Khoradmehr A et al. (2012). Toxicological effects of silver nanoparticles in rats. Afri J Microbiol Res. 6:5587–93.

Schmahl D, Steinhoff D (1960). [Experimental carcinogenesis in rats with colloidal silver and gold solutions]. Z Krebsforsc. 63:586–91(in German).

Shannahan JH, Podila R, Aldossari AA, Emerson H, Powell BA, Ke PC et al. (2015). Formation of a protein corona on silver nanoparticles mediated cellular toxicity via scavenger receptors. Toxicol Sci. 143:136–46.

Shahare B, Yashpal M, Singh G (2013). Toxic effects of repeated oral exposure of silver nanoparticles on small intestine mucosa of mice. Toxicol Mech Methods. 23:161–7.

Shavandi Z, Ghazanfari T, Moghaddam KN (2011). In vitro toxicity of silver nanoparticles on murine peritoneal macrophages. Immunopharmacol Immunotoxicol. 33:135–40.

Shavlovski MM, Chebotar NA, Konopistseva LA, Zakharova ET, Kachourin AM, Vassiliev VB et al. (1995). Embryotoxicity of silver ions is diminished by ceruloplasmin-further evidence for its role in the transport of copper. BioMetals. 8:122–8.

Shrivastava R, Kushwaha P, Bhutia YC, Flora SJS (2014). Oxidative stress induced following exposure to silver and gold nanoparticles in mice. Toxicol Ind Health. 32(8):1391–1404.

Silvestry-Rodriguez N, Bright KR, Slack DC, Uhlmann DR, Gerba CP (2008). Silver as a residual disinfectant to prevent biofilm formation in water distribution systems. Appl Environ Microbiol. 74:1639–41.

Silvestry-Rodriguez N, Bright KR, Uhlmann DR, Slack DC, Gerba CP (2007). Inactivation of *Pseudomonas aeruginosa* and *Aeromonas hydrophila* by silver in tap water. J Environ Sci Health Part A. 42:1579–84.

Simmons SO, Fan C-Y, Yeoman K, Wakefield J, Ramabhadran R (2011). NRF2 oxidative stress induced by heavy metals is cell type dependent. Curr Chem Genom. 5:1–12.

Simonis J, Ndwandwe M, Basson A, Selepe T (2014). Removal of selected microorganisms using silver-impregnated and coated, low-cost, micro-porous, ceramic water filters. J Sanit Hyg Dev. 4:37–42.

Skalska J, Frontczak-Baniewicz M, Strużyńska L (2015). Synaptic degeneration in rat brain after prolonged oral exposure to silver nanoparticles. Nanotoxicol. 46:145–54.

Song KS, Sung JH, Ji JH, Lee JH, Lee JS, Ryu HR et al. (2013). Recovery from silver-nanoparticle-exposure-induced lung inflammation and lung function changes in Sprague-Dawley rats. Nanotoxicol. 7:169–89.

Sriram MI, Kanth SBM, Kalishwaralal K, Gurunathan S (2010). Antitumor activity of silver nanoparticles in Dalton's lymphoma ascites tumor model. Int J Nanomedicine. 5:753–62.

Stebounova LV, Adamcakova-Dodd A, Kim JS, Park H, O'Shaughnessy PT, Grassian VH et al. (2011). Nanosilver induces minimal lung toxicity or inflammation in a subacute murine inhalation model. Part Fibre Toxicol. 8(5).

Stout JE, Yu VL (2003). Experiences of the first 16 hospitals using copper-silver ionization for *Legionella* control: implications for the evaluation of other disinfection modalities. Infect Control Hosp Epidemiol. 24:563–68.

Suliman YAO, Ali D, Alarifi AH, Mansour L, Alwasel SH (2013). Evaluation of cytotoxic, oxidative stress, proinflammatory and genotoxic effect of silver nanoparticles in human lung epithelial cells. Environ Toxicol. 30(2):149–60.

Sun L, Singh A, Vig K, Pillai S, Singh S (2008). Silver nanoparticles inhibit replication of respiratory syncytial virus. J Biomed Nanotechnol. 4:149–58 [cited by Marambio-Jones & Hoek, 2010].

Sung JH, Ji JH, Song KS, Lee JH, Choi KH, Lee SH et al. (2011). Acute inhalation toxicity of silver nanoparticles. Toxicol Ind Health. 27:49–54.

Sung JH, Ji JH, Yoon JU, Kim DS, Song MY, Jeong J et al. (2008). Lung function changes in Sprague-Dawley rats after prolonged inhalation exposure to silver nanoparticles. Inhal Toxicol. 20:567–74.

Sung JH, Ji JH, Park JD, Yoon JU, Kim DS, Jeon KS et al. (2009). Subchronic inhalation toxicity of silver nanoparticles. Toxicol Sci. 108:452–61.

Takenaka S, Karg E, Roth C, Schulz H, Ziesenis A, Heinzmann U et al. (2001). Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ Health Perspect. 109:547–51.

Tamimi SO, Zmeili SM, Gharaibeh MN, Shubair MS, Salhab AS (1998). Toxicity of a new antismoking mouthwash 881010 in rats and rabbits. J Toxicol Environ Health. A53:47–60.

Tavares P, Balbinot F, de Oliverira HM, Fagundes GE, Vanancio M, Ronconi JVV et al. (2012). Evaluation of genotoxic effect of silver nanoparticles (Ag-Nps) in vitro and *in vivo*. J Nanopart Res. 14:791.

Thakur M, Gupta H, Singh D, Mohanty IR, Maheswari U, Vanage G et al. (2014). Histopathological and ultra structural effects of nanoparticles on rat testis following 90 days (chronic study) of repeated oral administration. J Nanobiotechnol. 12: 42.

Tulve NS, Stefaniale AB, Vance ME, Rogers K, Schwegler-Berry D, Willis R et al. (2015). Characterization of silver nanoparticles in selected consumer products and its relevance for predicting children's potential exposure. Int J Hyg Environ Health. 218:345–57.

USEPA (2012) 2012 Edition of the Drinking Water Standards and Health Advisories. Washington (DC): United States Environmental Protection Agency. (http://water.epa.gov/action/advisories/drinking/upload/dwstandards2012.pdf, accessed November 017).

USEPA (2015) Draft – Technologies for *Legionella* control: scientific literature review. Office of Water EPA 815-D-15-001. Washington (DC): United States Environmental Protection Agency.

Valušová E, Vandžurová A, Pristaš P, Antalik M, Javorský P (2012). Water treatment using activated carbon supporting silver and magnetite. Water Sci Technol. 66: 2772–78.

Van der Laan H, van Halem D, Smeets PWMH, Soppe AIA, Kroesbergen J, Wubbels G et al. (2014). Bacteria and virus removal effectiveness of ceramic pot filters with different silver applications in a long term experiment. Water Res. 51:47–54.

van der Zande M, Vandebriel RJ, van Doren E, Kramer E, Herrera Rivera Z, Serrano-Rojero CS et al. (2012). Distribution, elimination and toxicity of silver nanoparticles and silver ions in rats after 28-day oral exposure. ACS Nano. 6(8):7427–42.

van Halem D, Heijman SGJ, Soppe AIA, van Dijk JC, Amy GL (2007). Ceramic silver-impregnated pot filters for household drinking-water treatment in developing countries: material characterization and performance study. Water Sci Technol. 7:9–17.

Völker C, Oetken M, Oehlmann J (2013). The biological effects and possible modes of action of nanosilver. Rev Environ Contam Toxicol. 223:81–106.

Wadhera A, Fung M (2005). Systemic argyria associated with ingestion of colloidal silver. Dermatol Online J. 11(1):12.

Wahlberg JE (1965). Percutaneous toxicity of metal compounds. A comparative investigation in guinea pigs. Arch Environ Health. 11:201–4.

Wan T, Conyers RAJ, Coombs CJ, Masterton JP (1991). Determination of silver in blood, urine, and tissues of volunteers and burn patients. Clin Chem. 37:1683–87.

WHO (1993). Silver. In: Guidelines for drinking-water quality. Second edition. Volume 2. Health criteria and other supporting information. Geneva: World Health Organization: 338-343. (http://www.who.int/water_sanitation_health/publications/gdwq2v1/en/, accessed September 2017.)

WHO (2007). *Legionella* and the prevention of legionellosis. Geneva: World Health Organization. (http://www.who.int/water_sanitation_health/publications/legionella/en/, accessed September 2017.)

WHO (2016) Results of Round I of the WHO international scheme to evaluate household water treatment technologies. Geneva: World Health Organization. (http://www.who.int/water_sanitation_health/publications/household-water-treatment-report-round-1/en/, accessed September 2017.)

WHO (2017). Guidelines for drinking-water quality. Fourth edition incorporating the first addendum. Geneva: World Health Organization. (http://www.who.int/water_sanitation_health/publications/drinking-water-quality-guidelines-4-including-1st-addendum/en/, accessed September 2017.)

Wijnhoven SWP, Peijnenburg WJ, Herberts CA, Hagens WI, Oomen AG, Heugens E et al. (2009). Nano-silver – a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicol. 3(2):109–38.

Xiu Z-M, Ma J, Alvarez PJJ (2011). Differential effect of common ligands and molecular oxygen on antimicrobial activity of silver nanoparticles versus silver ions. Environ Sci Technol. 45:9003–8.

Yin N, Liu Q, Liu J, He B, Cui L, Li Z et al. (2013) Silver nanoparticle exposure attenuates the viability of rat cerebellum granule cells through apoptosis coupled to oxidative stress Small. 9:1831–41.

Yu WJ, Son JM, Lee J, Kim SH, Lee IC, Baek HS et al. (2013). Effects of silver nanoparticles on pregnant dams and embryo-fetal development in rats. Nanotoxicol. 8 (Supp1):85–91.

Yun JW, Kim SH, You JR, Kim WH, Jang JJ, Min SK et al. (2014). Comparative toxicity of silicone dioxide, silver and iron oxide nanoparticles after repeated oral administration to rats. J Appl Toxicol. 35:681–93.

Zanette C, Pelin M, Crosera M, Adami G, Bovenzi M, Larese FF et al. (2011). Silver nanoparticles exert a long-lasting antiproliferative effect on human keratinoctye HaCaT cell line. Toxicol. in Vitro. 25:1053–60.

Zhang H, Oyanedel-Craver V (2013). Comparison of the bacterial removal performance of silver nanoparticles and a polymer based quaternary amine functionalized silsesquioxane coated point-of-use ceramic water filters. J Hazard Mater. 260:272–77.

Zhang S, Du C, Wang Z, Han X, Zhang K, Liu L (2013). Reduced cytotoxicity of silver ions to mammalian cells at high concentration due to the formation of silver chloride. Toxicol. in Vitro. 27:739–44.

Appendix A: Disinfectant mode of action

This short section outlines the disinfectant mode of action of silver ions and silver nanoparticles.

Silver ions are believed to impact on bacteria in a number of ways, including:

- extracellular binding or precipitation of silver to bacterial cell walls (Bellatone et al., 2002);
- the inhibition of essential enzymatic functions via interaction of the ions with the thiol-group (sulfhydryl group) of L-cysteine (Liau et al., 1997);
- the production of ROS (Park et al., 2009); and
- interaction with DNA (Thurman & Gerba, 1989).

Feng et al. (2000) conducted a mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphyococcus aureus*. Following treatment with silver nitrate, silver ions were detected inside the cells and both types of bacteria showed similar morphological changes, with the cytoplasmic membrane detaching from the cell wall. In addition, an electron-light region appeared in cells, with condensed DNA molecules within the centre of this region. DNA in a condensed form is unable to replicate.

Thurman & Gerba (1989) showed that silver binds to DNA, with the metal displacing the hydrogen bonds between adjacent nitrogens of purine and pyrimidine bases.

Dibrov et al. (2002) investigated the antimicrobial activity of silver ions on *Vibrio cholerae* and found that, at low concentrations of ionic silver, massive proton leakage through the cell membrane could be observed, which resulted in complete de-energization and, probably, cell death.

In their study, Park et al. (2009) found that almost half of the $\log_{10}$ reduction, caused by the silver ion disinfection in the bacteria they studied (*E. coli* and *S. aureus*), could be attributed to reactive oxygen species ROS-mediated activity, with the major form of ROS generated being the superoxide radical. The authors comment that the silver ions are likely to generate superoxide radicals by impairing enzymes in the respiratory chain and that this impairment may be caused by the thiol-interaction mechanism (as mentioned above).

The antimicrobial mode of action of silver nanoparticles is not fully understood (Wijnhoven et al., 2009), although some of the mechanisms may be the same as those for ionic silver or, as increasingly seems likely (e.g. Xiu et al., 2012), may result from the release of ionic silver from the silver nanoparticles (Li et al., 2008). A number of authors have shown that silver nanoparticles can anchor to and then penetrate the cell walls of Gram-negative bacteria (Sondi and Salopek-Sondi, 2004; Morones et al., 2005). Such damaged cell walls enhance cell permeability and inhibit appropriate regulation of transport through the plasma membrane.

Sondi and Salopek-Sondi (2004) looked at the biocidal effect of silver nanoparticles on *E. coli* using both scanning electron microscopy and transmission electron microscopy. The bacteria were cultured in a liquid medium supplemented with silver nanoparticles (50 $\mu g/cm^3$) for 4 hours before electron microscopy. The silver-treated cells were significantly changed in comparison with untreated *E. coli* and showed major damage, which was characterized by the formation of pits in the cell walls. The analysis showed that the silver nanoparticles were incorporated into the cell walls and accumulated in

the membrane, with some penetrating the cells. As a result, intracellular substances were found to be leaking from the affected bacteria.

As with ionic silver, it has been suggested that silver nanoparticles may cause free-radical generation, leading to subsequent cell damage. Kim et al. (2007) looked at the free-radical generation effect of silver nanoparticles on microbial growth inhibition using electron spin spectroscopy. The group showed that free-radicals were generated by the silver nanoparticles and that addition of an antioxidant reduced the bactericidal efficacy of the silver nanoparticles. They suggested that the free-radicals may be derived from the surface of the silver nanoparticles.

Shrivastava et al. (2007) studied the impact of silver nanoparticles on *E. coli*, *S. aureus* and *Salmonella typhus*. The silver nanoparticles were found to be more effective against the Gram-negative bacteria. The group found that the principal antimicrobial mechanisms were silver nanoparticles anchoring and penetration of the cell wall, along with modulation of cellular signalling (leading to growth inhibition).

Hwang et al. (2008) performed a study on stress-specific bioluminescent bacteria, based on which they proposed a synergistic toxic effect between the silver nanoparticles and the silver ions that they produce. The stress-specific bacterial strains used were designed to respond to protein/membrane, oxidative stress and DNA damage. They found that the silver nanoparticles caused toxicity via protein/membrane and oxidative damage. In their study, the silver nanoparticles released silver ions and subsequently superoxide radicals.

References

Bellatone M, Williams HD, Hench LL (2002). Broad-spectrum bactericidal activity of AgO_2-doped bioactive glass. Antimicrobial Agents and Chemotherapy. 46:1940–45.

Dibrov P, Dzioba J, Gosink KK, Häse CC (2002). Chemiosmotic mechanism of antimicrobial activity of Ag^+ in *Vibrio cholerae*. Antimicrobial Agents and Chemotherapy. 46:2668–70.

Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO (2000). A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. J Biomed Mater Res. 52:662–68.

Hwang ET, Lee JH, Chae YJ, Kim YS, Kim BC, Sang B et al. (2008). Analysis of the toxic mode of action of silver nanoparticles using stress-specific bioluminescent bacteria. Small. 4:746–50.

Kim JS, Kuk E, Yu KN, Kim J-H, Park SJ, Lee HJ et al. (2007). Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine. 3:95–101.

Li Q, Mahendra S, Lyon DY, Brunet L, Liga MV, Li D et al. (2008). Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications. Water Res. 42:4591–4602.

Liau SY, Read DC, Pugh WJ, Furr WJ, Russell AD (1997). Interaction of $AgNO_3$ with readily identifiable groups: relationship to the antibacterial action of silver ions. Lett Appl Microbiol. 25:279–83 [cited by Park et al., 2009].

Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT et al. (2005). The bactericidal effect of silver nanoparticles. Nanotechnol. 16(10):2346–53.

Park HJ, Kim JY, Kim J, Lee JH, Hahn JH, Gu MB et al. (2009). Silver-ion-mediated reactive oxygen species generation affecting bactericidal activity. Water Res. 43: 1027–32.

Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D (2007). Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnol. 18:225103.

Sondi I, Salopek-Sondi B (2004) Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for Gram-negative bacteria. J Colloid Interface Sci. 275: 177–82.

Thurman RB, Gerba CP (1989). The molecular mechanisms of copper and silver ion disinfection of bacteria and viruses. CRC Critical Reviews in Environmental Control. 18:295–315.

Wijnhoven SWP, Peijnenburg WJ, Herberts CA, Hagens WI, Oomen AG, Heugens E et al. (2009). Nano-silver – a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicol. 3(2):109–38.

Xiu Z, Zhang Q, Puppala HL, Colvin VL, Alvarez PJJ (2012). Negligible particle-specific antibacterial activity of silver nanoparticles. Nano Lett. 12:4271–75.

Appendix B: *In vitro* toxicity of silver and silver nanoparticles

There has been a marked increase in the number of studies looking at the *in vitro* toxic effects of silver (principally silver nanoparticles) in recent years, with a wide range of cells investigated, including cells derived from:

- blood (e.g. Zhang et al., 2013);
- brain (e.g. Haase et al., 2012a);
- bone (e.g. Hardes et al., 2007);
- cervix (e.g. Mukherjee et al., 2012);
- immune system (e.g. Pratsinis et al., 2013);
- intestine (e.g. Gopinath et al., 2010);
- kidney (e.g. Kermanizadeh et al., 2013);
- liver (e.g. Gaiser et al., 2013);
- lung (e.g. Suliman et al., 2013);
- skin (e.g. Samberg et al., 2010); and
- testes (e.g. Ema et al., 2010).

These cells came from a variety of different sources including human, rat, mouse, hamster and porcine cells. These are described in detail below and the genotoxicity studies are summarised in Table B1.

In vitro studies covering exposure to cells derived from many of the target organs identified from *in vivo* studies are outlined below. It is likely that secondary cells (i.e. cancer-derived or immortalized cell lines) may not provide useful information in terms of silver nanoparticle toxicity on normal undifferentiated cells, which are most relevant to human exposure scenarios. For example, molecular pathways in cancer-derived cells are potentially deregulated (Franchi et al., 2015). Thus, in the 2015 literature update, the emphasis is on toxicological studies using primary cells.

B1. Liver

In the studies outlined below, researchers tested different silver nanoparticles against a variety of liver cell types although, generally, these were secondary cells (i.e. cancer-derived or immortalized cell lines). Different tests were used to assess toxicity but, usually, at least one test of cytotoxicity was included. Results were expressed in a variety of ways and include measures of the half maximal inhibitory concentration (IC_{50}) and LC_{50}.

Cha et al. (2008) exposed Huh-7 (hepatoma) cells to silver nanoparticles (13 nm) and found little impact on mitochondrial activity or glutathione production. DNA contents in the treated cells, however, decreased by 15% and the expression of genes related to apoptosis and inflammation were altered.

Kim et al. (2009) compared the cytotoxicity of silver nanoparticles (5–10 nm) and silver nitrate to human hepatoma (HepG2) cells using three different measures of cell viability. The MTT[32] (a tetrazolium dye) and Almar Blue tests assess cell metabolic activity (through mitochondrial function), while the lactate dehydrogenase (LDH) tests assesses membrane integrity. The IC_{50} values for the LDH tests in both silver nanoparticles and silver nitrate were markedly lower than the other tests, suggesting

[32] 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

that in HepG2 cell membrane integrity is more readily affected by silver than the tested metabolic activities (which is in contrast to the results of Hussain et al., 2005).

The finding that the cytotoxicity seen in all three tests could be prevented by the addition of N-acetylcysteine (a precursor for the synthesis of glutathione, and thus, an important antioxidant) suggests that the cytotoxicity may be due to oxidative stress. Nowrouzi et al. (2010) reported an IC_{50} value (tests based on the tetrazolium dyes MTT and XTT[33]) for HepG2 cells exposed to silver nanoparticles (5–10 nm) of between 2.75 to 3 mg/L, very similar to that reported by Kim et al. (2009). They went on to subject HepG2 cells to 0, 1, 4 and 8% of the IC_{50} value, and found significant impacts on indicators of oxidative stress at levels of 4% and above (increases in the activity of LDH, alanine aminotransferase and aspartate aminotransferase activity; increase in nitric oxide (NO) concentration; increases in lipid peroxidation and cytochrome c content; decrease in glutathione (GSH) content and a decrease in superoxide dismutase [SOD] activity). Kawata et al. (2009) investigated the effects of silver nanoparticles (7–10 nm and stabilized with polyethylenimine) and silver carbonate on HepG2 cells at concentrations below those resulting in cytotoxicity. As silver nanoparticles were found to result in significant toxicity above 1 mg/mL (although silver carbonate still appeared to be non-cytotoxic at that dose), a concentration of 1 mg/mLwas used in further experiments. At that concentration, silver nanoparticles were found to significantly increase the frequency of micronucleus formation, indicating DNA damage and chromosome aberrations (silver carbonate did not increase levels above those seen in the control). In addition, exposure to silver nanoparticles also altered gene expression, including the up-regulation of stress-related genes. Sahu et al. (2015) evaluated gene expression profiles in HepG2 cells exposed to 2.5 mg/L of 20 or 50 nm silver nanoparticles for 4 and 24 hours, and found that exposure to 20 nm silver nanoparticles resulted in a transient upregulating of stress response genes (such as metallothioniens and heat shock proteins). A number of cellular pathways, including the p53 signalling pathway and NRF2-mediated oxidative stress response pathway, were also impacted by silver nanoparticle exposure.

Gaiser et al. (2013) looked at the impact of silver nanoparticles (mean 17.5 nm) on C3A cells. The silver nanoparticles were found to be highly toxic to the cultured cells (LDH LC_{50} of 2.5 µg/cm^3; Almar Blue LC_{50} of 20 µg/cm^3). It was also shown that hepatocyte homeostasis was affected, with a decrease in albumin release.

In 2005, Hussain et al. showed that silver nanoparticles (15 nm and 100 nm) were toxic to immortalized rat liver (BRL 3A) cells. Silver nanoparticles resulted in a concentration-dependent increase in LDH leakage and showed significant cytotoxicity at 10–50 µg/mL. The MTT assay also showed that silver nanoparticles caused significant cytotoxicity above 5 µg/mL. In addition, the level of ROS was found to increase in a concentration-dependent manner and a significant depletion of GSH was observed relative to control cells.

Arora et al. (2009) also looked at the toxicity of silver nanoparticles (7–20 nm) to mouse liver cells but, in contrast to other studies (e.g. Hussain et al., 2005; Kim et al., 2009), used primary cells. Exposure of the liver cells to up to 100 µg/mL for 24 hours did not alter cell morphology. The onset of apoptosis was seen at 12.5 µg/mL, which was much lower than the necrotic concentration (500 µg/mL). The primary cells seemed to be more resistant to the cytotoxic effects of silver nanoparticles, with an IC_{50} for the XTT assay of 449 µg/mL (although, not strictly comparable, the IC_{50} for the MTT test [similar to XTT] in human hepatoma HepG2 cells reported by Kim et al., 2009 was < 3.5 µg/mL). Exposure of the cells to silver nanoparticles at half of the IC_{50} value resulted in increased levels of SOD and GSH

[33] (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide).

as compared to unexposed cells suggesting that antioxidant defence mechanisms were triggered by silver nanoparticles exposure.

Kulthong et al. (2012) looked at the impact of silver nanoparticles on rat liver microsomes and specifically any changes in activity in hepatic cytochrome P450 (CYP) enzyme activity. The silver nanoparticles strongly inhibited CYP2C and CYP2D activities, but had no, or less, effect on other CYP activities. The impact in microsomes was in contrast to the *in vivo* study where no toxic effects were seen and where no change in CYP activity was observed.

The potential differences between primary and secondary cells is illustrated by Faedmaleki et al. (2014), who compared the impact of silver nanoparticles on mice primary liver cells in comparison to human HepG2 cells. Cell viability was measured using MTT and HepG2 were found to be significantly more sensitive to silver nanoparticles than the primary liver cells, with an IC_{50} value of 2.7 µg/mL (HepG2) compared to 121.7 µg/mL.

B2. Lung

A number of studies have been conducted on the toxicity of various types of silver nanoparticles (different sizes and coatings) to lung cells *in vitro*. Typically, either A549 cells (a lung carcinoma alveolar epithelial cell) or, less frequently, other cells have been used as test systems. Generally, authors have found impacts on cell viability and demonstration of oxidative stress (Carlson et al., 2008; Foldbjerg et al., 2011; Li et al., 2012: Suliman et al., 2013). Other studies have also considered impacts on the cell cycle (AshaRani et al., 2009a; Lee et al., 2011; Chairuangkitti et al., 2013). As with the results from studies on liver cells, there is an indication that size and coating of the silver nanoparticles impacts on toxicity with smaller silver nanoparticles typically being more toxic than larger particles (Carlson et al., 2008; Li et al., 2012; Gliga et al., 2014).

Foldbjerg et al. (2011) compared the toxic effects of silver nanoparticles (PVP-coated, 69 nm, up to 20 µg/mL) and silver nitrate (up to 10 µg/mL) on A549 cells. Both silver nanoparticles and silver nitrate were cytotoxic (as determined by impact on mitochondrial activity), although the cytotoxic impacts of silver nitrate (EC_{50} – 6 µg/mL) were seen at lower doses than those following silver nanoparticle exposure (EC_{50} – 12.5 µg/mL). The measured toxicity of both types of silver could be significantly reduced by pre-treating cells with antioxidant. It was found that cell death was primarily due to a dose-dependent increase in necrosis/late apoptosis, whereas only a minor increase in early apoptosis was detected. The silver nanoparticles were found to induce a greater increase in ROS than the silver nitrate. In comparison to the control, ROS levels were increased almost 16-fold at 10 µg silver nanoparticles/mL, but only approximately 8-fold by the same concentration of silver from silver nitrate. This group (Foldbjerg et al., 2012) also looked at the effects of silver nanoparticles (16 nm) and silver nitrate at low (non-cytotoxic) doses on gene expression in A549 cells. Exposure to silver nanoparticles altered the regulation (2-fold difference or greater) of more than 1000 genes, compared to only 133 genes following exposure to silver ions.

Suliman et al. (2013) investigated the toxicity of silver nanoparticles (56 nm, 10–100 µg/mL) on A549 cells using a wide array of methods. Morphological changes were clearly seen in cells exposed to 25 µg silver nanoparticles/mL for 48 hours. The silver nanoparticles caused cytotoxicity, as measured by mitochondrial function (MTT assay) and membrane permeability (LDH assay). Silver nanoparticles induced the generation of ROS and induced oxidative stress (shown by a depletion of GSH and increases in lipid peroxidation, SOD and catalase concentrations). Increased apoptosis following exposure to

silver nanoparticles was seen, the expression of pro-inflammatory cytokines was up-regulated and a concentration and a time-dependent increase in DNA damage was also observed.

In addition to changes in mitochondrial activity, membrane permeability and increases in ROS generation etc., a number of authors have shown that silver nanoparticles modulate the cell cycle in A549 cells. Lee et al. (2011) showed that silver nanoparticles (hydrodynamic diameter 480 nm) with an IC_{50} of 106 µg/mL for cell viability caused accumulation of cells at G2/M and sub-G1 (apoptosis) following exposure to 50 µg/mL for 4 hours. Chairuangkitti et al. (2013) showed that silver nanoparticles increased the proportion of cells in the sub-G1 population, increased S phase arrest and caused down-regulation of the cell cycle associated proliferating cell nuclear antigen (PCNA) protein. Pre-treatment with an antioxidant, while decreasing some of the effects, did not change the silver nanoparticle-mediated impact on S phase arrest or down-regulation of proliferating cell nuclear antigen protein, leading the authors to suggest that the *in vitro* toxic effects on A549 cells are mediated via a ROS-dependent (cytotoxicity) and a ROS-independent (cell cycle arrest) pathway. AshaRani et al. (2009a) looked at the anti-proliferative activity of silver nanoparticles (6–20 nm, starch coated) in normal human lung fibroblasts (IMR-90). Electron micrographs showed that silver nanoparticles were taken up by the cells and showed a uniform distribution both in cytoplasm and nucleus. Although the silver nanoparticle-treated lung fibroblasts exhibited chromosome instability and mitotic arrest, the cells recovered completely from the proliferation arrest.

Sur et al. (2010) looked at the impact on toxicity of modifying silver nanoparticles with glucose, lactose, oligonucleotides and combinations of these ligands in comparison with bare silver nanoparticles on A549 cells. While the modification seemed to increase the uptake of the silver nanoparticles into the cells it also acted to decrease the toxicity, with the bare silver nanoparticles being cytotoxic at a lower dose than the modified particles.

Li et al. (2012) treated human lung fibroblasts (unspecified) with five different sized PVP-coated silver nanoparticles (25, 35, 45, 60 and 70 nm) at the same doses (31.75, 62.5, 125, 250 µg/mL). Both tests of cell viability (MTT and LDH assay) showed size-dependent cytotoxicity which decreased with increasing silver nanoparticle size. Gliga et al. (2014) also examined the size-dependent cytotoxicity of silver nanoparticles, using 10, 40 and 75 nm citrate-coated, 10 nm PVP-coated and 50 nm uncoated silver nanoparticles. Using BEAS-2B cells (immortalized bronchial epithelial cells), they found that only the 10 nm silver nanoparticles were cytotoxic irrespective of coating; the reason for the greater toxicity of the smaller particles was believed to be due to the release of significantly more ionic silver compared with the other silver nanoparticles.

Carlson et al. (2008) explored the possible toxicity of inhaled silver nanoparticles using rat alveolar macrophages. The toxicity of three silver nanoparticles (coated in hydrocarbon) of different sizes (15 nm, 30 nm, 55 nm) was assessed at various doses. In general, the 15 nm silver nanoparticles showed the greatest toxicity and the 55 nm silver nanoparticles showed the least toxicity (e.g. the EC_{50} for increased LDH leakage was 27 µg/mL for the 15 nm silver nanoparticles and > 75 µg/mL for the larger particles). The authors also found a significant increase in ROS and a correlated decrease in levels of GSH following exposure to silver nanoparticles (15 nm) and increased secretion of inflammatory cytokines/chemokines.

B3. Brain and the blood-brain barrier

The brain is essentially made up of two key cell types – neurons and glial cells (including microglia, astrocytes/astroglia and oligodendrocytes). A number of recent toxicity studies focusing on brain cell

cultures, a model system for neuronal differentiation (PC12 cells) and cells involved in the blood-brain barrier have been identified in the literature.

B3.1 Astrocytes

The ability of astrocytes to withstand silver seems to depend upon the form of the silver and the silver nanoparticle coating. Luther et al. (2011), for example, exposed primary cultures of rat astrocytes to PVP-coated silver nanoparticles (70 nm) for up to 24 hours (approximately 10 µg silver/mL) and found that, while incubation led to a time- and concentration-dependent accumulation of silver in the cells, it did not affect the cell viability or lead to a reduction in cellular glutathione level. In contrast, exposure to a similar concentration of silver nitrate, was found to severely compromise cell viability. This group found that the silver nanoparticles taken up by the astrocytes remained sequestered in the cells following 7 days of incubation in silver nanoparticle-free medium (Luther et al., 2012). The same robustness to silver nanoparticle toxicity was not seen when rat astrocytes were exposed to smaller, peptide coated silver nanoparticles (20 and 40 nm), where the silver nanoparticles were seen to induce a strong size-dependent cytotoxicity and an increase in ROS formation (Haase et al., 2012a). In secondary astrocyte cells lines, derived from human glioblastomas, silver nanoparticles (starch coated, 6–20 nm) were found to result in cytotoxicity and genotoxicity in U251 cells (AshaRani et al., 2009a, b) and silver chloride was found to cause oxidative stress in A172 cells (Simmons et al., 2011).

B3.2 Neurons

Some studies show that silver nanoparticles seem to be particularly toxic to neurons (Yin et al., 2013; Xu et al., 2013), although Haase et al. (2012a) found in their study that astrocytes were more sensitive to peptide coated silver nanoparticles than neurons. In rat cerebellum granule cells, commercial silver nanoparticles (sized 20–30nm) were found to cause cytotoxicity, based on an alcian blue staining assay, at very low doses – with a reported IC_{50} of 0.96 µg/mL. Cell-body shrinkage was seen after 24-hour exposure to 1 µg/mL silver nanoparticles and the silver nanoparticles were seen to cause oxidative stress (Yin et al., 2013). Xu et al. (2013) found that 20 nm silver nanoparticles caused cytotoxicity in rat cortical cell cultures at the lowest concentration examined (1 µg/mL) in developing cells and at 5 µg/mL in more mature cultures. The silver nanoparticles were found to inhibit not only the sprouting of neuronal branches and elongation of neurites, but also, they caused fragmentation and degeneration of mature neurons. In contrast, Haase et al. (2012a) found that a significant cytotoxic effect of peptide stabilized 20 nm silver nanoparticles was not seen until ≥ 50 µg/mL on their rat neuronal-enriched cultures.

B3.3 Neurodevelopment and neurogenesis

The possible impacts of silver on neurodevelopment have been examined using PC12 cells. PC12 cells, which are derived from rat adrenal medulla, stop dividing and terminally differentiate when treated with nerve growth factor. They are used as a model for neuronal differentiation. Powers et al. (2010, 2011) have looked at the impact of silver nitrate and silver nanoparticles on these cells. A one-hour exposure of undifferentiated PC12 cells to 10µM ionic silver was found to inhibit DNA synthesis and protein synthesis. Longer exposure resulted in oxidative stress and loss of viability. Ionic silver directly inhibited mitotic activity. The same concentration of ionic silver was found to elicit even stronger effects with the onset of cell differentiation, with greater DNA synthesis inhibition and greater levels of oxidative stress. In addition, selectively impaired neurite formation was seen and there was suppressed development of the acetylcholine phenotype in favour of the dopamine phenotype (Powers et al., 2010). This group has also looked at the effects of silver nanoparticles (citrate- and PVP-coated) in PC12 cells. In undifferentiated cells, citrate-coated silver nanoparticles (10 nm) impaired DNA and

protein synthesis, but did not result in significant oxidative stress or loss of cell viability. In differentiating cells, however, the citrate-coated silver nanoparticles caused oxidative stress and impaired differentiation into the acetylcholine phenotype. In undifferentiated cells, PVP-silver nanoparticles (10 nm and 50 nm) reduced DNA synthesis; the 50nm particle size had a greater effect. All three silver nanoparticles significantly suppressed the acetylcholine phenotype, but the small PVP-silver nanoparticles enhanced differentiation into the dopamine phenotype (Powers et al., 2011). The authors suggest that their results point to the likelihood that silver and silver nanoparticles are developmental neurotoxicants.

Cooper & Spitzer (2015) used rat neuroblastoma cells (B35) and cultured adult neural stem cells from the subventricular zone from Sprague-Dawley rats to assess the sublethal effects of silver nanoparticles (1 µg/mL) on neural function. Silver nanoparticle exposure in differentiating NSC induced the formation of f-actin inclusions (indicating a disruption of actin function). The silver nanoparticle exposure in B35 cells resulted in a decrease in neurite extension and branching, thus interfering with cytoskeleton-mediated processes that are vital to neurogenesis (which is thought to play a key role in cognitive functions such as learning and memory).

B3.4 Brain endothelial cells

Two recent studies have examined the impact of silver nanoparticles on rat brain endothelial cells (Trickler et al., 2010; Grosse et al., 2013). Trickler et al. (2010) used cultured rat brain microvessel endothelial cells as a model to examine cellular accumulation, changes in pro-inflammatory mediators and changes in morphology and permeability following exposure to PVP-coated silver nanoparticles (25, 40 and 80 nm in size). Silver nanoparticles were found to accumulate in the cells in a size-dependent manner (with less accumulation seen for the 80 nm silver nanoparticles). The cellular association of silver nanoparticles led to significant cytotoxicity and caused the release of cytokines and other inflammatory mediators from the cell monolayers. The changes in the pro-inflammatory mediators correlated with morphological changes and increased cell permeability.

Grosse et al. (2013) investigated the impact of citrate-coated silver nanoparticles (10, 50 and 100 nm) on rat brain endothelial cells (RBE4). Based on the neutral red uptake assay (membrane permeability as an indicator of cytotoxicity), toxicity was seen for all of the silver nanoparticles examined, with the smaller particles being more toxic (effects seen at lower concentrations and after a shorter period of time). Exposure of the cells to silver nitrate, suggested that the ionic form was less toxic to the endothelial cells than silver nanoparticles.

B4. Gut

A number of studies have looked at the impact of silver nanoparticles on intestinal cells, some of which have attempted to account for the likely effects of digestion or have used synthetic drinking-water as a medium for silver nanoparticles, rather than cell culture medium, to try and more closely simulate *in vivo* conditions.

Bouwmeester et al. (2011) used an *in vitro* model of the human intestinal epithelium (consisting of Caco-2 and M-cells) to study the passage of four different preparations of silver nanoparticles (nominal sizes 20, 34, 61 and 113 nm) and silver ions (from silver nitrate). Concentrations of silver nanoparticles of up to 50 µg/mL (irrespective of size) reduced metabolic activity in the Caco-2 cells by less than 20%, while a concentration of 5 µg/mL silver nitrate resulted in a 70% reduction in metabolic activity. Translocation of silver derived from either silver nanoparticle suspensions or silver nitrate was clearly

shown and the authors speculate that the translocation of silver is likely to be in the ionic and not the particulate form.

Walczak et al. (2013) studied the likely impact of digestion on 60 nm silver nanoparticles (citrate) and silver ions (silver nitrate). The model comprised artificial saliva, gastric, duodenal and bile juice, simulating digestion in the oral, gastric and intestinal compartments with salt and protein composition, pH differences, and transit times similar to human *in vivo* digestion. The silver nanoparticles, in the presence of proteins, were found to survive gastric digestion and reach the intestine where they were present in large clusters and co-localized with chlorine. The chlorine was thought to be involved in connecting separate silver nanoparticles inside clusters with "chlorine inter-particle bridges". Following intestinal digestion, the silver nanoparticles were found to be present in, essentially, their original form. Silver ions were also found to reach the intestine, but they were generally present as complexes of silver, sulfur and chlorine (20–30 nm in size). The authors suggest that ingestion of silver nanoparticles and silver ions results in intestinal exposure to nanoparticles, albeit with different chemical compositions. Böhmert et al. (2014) conducted some similar work, subjecting silver nanoparticles to simulated digestion (both Böhmert et al., (2014) and Walczak et al., (2013) based their digestion model on the method described by Versantvoort et al., 2005) but then examined their toxicity to Caco-2 cells. Cells were exposed to primary and digested particles as well as a digestion fluids mixture without silver nanoparticles to act as a control. It was found that silver nanoparticles seemed to overcome gastrointestinal juices in their particulate form, without forming large quantities of aggregates, and there seemed to be only a slight reduction in their cytotoxic potential following digestion. This work has been extended by also including the main food components (i.e. carbohydrates, proteins and fatty acids) in the *in vitro* digestion process to further simulate realistic conditions (Lichtenstein et al., 2015). The uptake and cytotoxicity of digested and undigested polyacrylic acid-coated silver nanoparticles were investigated in Caco-2 cells. Silver nanoparticles digested with simulated food had a similar cellular uptake to undigested ones. However, silver nanoparticles digested in the absence of food simulants had a considerably lower cellular uptake, leading the authors to suggest that without the use of food components during *in vitro* digestion, uptake may be under estimated. Hsin et al. (2008) looked at the impact of two different commercially available preparations of silver nanoparticles (1 and 100 nm) on human colon cells (HCT116). One preparation (Ching-Tai) was found to result in significant decreases in cell viability after 24 hours at 50 µg/mL, while the other (Sun-Lan) at the same concentration did not result in significant cytotoxicity even after 72 hours. Compared to the other cells examined (mouse fibroblasts – NIH3T3 and rat vascular smooth muscle cells – A10), HCT116 cells were relatively insensitive to silver nanoparticles.

Gaiser et al. (2009) looked at the potential human exposure to silver nanoparticles via ingestion of contaminated food sources. They looked at both bare silver nanoparticles (35 nm) and "bulk" silver (0.6–1.6 µm) on secondary intestinal epithelial cells (Caco-2) and human hepatocytes (C3A). Cytotoxicity was only assessed on the hepatocytes, with silver nanoparticles being more cytotoxic (LDH assay) than bulk silver. Both silver nanoparticles and silver were, however, shown to be taken up by Caco-2 cells.

The impact of silver nanoparticles (18 nm) on gene expression in HT29 cells (and human kidney cells – see below) was explored by Gopinath et al. (2010). A concentration of 11 µg/mL (less than half of the concentration required to inhibit cell growth by 50% – Gopinath et al., 2008) resulted in changes in cell morphology and caused an 11% increase in early apoptotic population, 21% increase in late apoptotic population, and a 7% increase in necrotic population. Exposure to silver nanoparticles resulted in an up-regulation of apoptotic genes and a down-regulation of anti-apoptotic genes.

Kruszewski et al. (2013) looked at the impact of bare 20 nm and 200 nm silver nanoparticles on liver (HepG2), lung (A549) and gut (HT29) cells in terms of DNA damage and colony forming ability. They found a substantial difference in the cell uptake of silver nanoparticles, with uptake by the gut cells being markedly lower than the other cell lines. The authors suggest that this might be due to the production of mucin by HT29 cells which prevents nanoparticle uptake. The cellular uptake of silver nanoparticles was found to correspond to the formation of ROS and the subsequent pattern of DNA breakage and base damage induction was found to correspond to intracellular ROS formation.

Abbott Chalew & Schwab (2013) looked at the cytotoxic effects of uncoated silver nanoparticles (20–30 nm) on Caco-2 and SW480 intestinal cells. The silver nanoparticles were not found to be particularly toxic to the intestinal cells when dispersed in cell culture medium (with LC_{50} values for the two cell lines greater than 100 mg/L). Far greater cytotoxicity was seen for SW480 when the cells were exposed to silver nanoparticles in buffered synthetic water, with a significant drop in viability seen after exposure to 1 mg/L. The authors suggest that the lower toxicity in silver nanoparticles in cell culture media may be due to the stabilizing effect of foetal bovine serum in the cell culture medium.

Giovanni et al. (2015) looked at a wide range of silver nanoparticles concentrations and their impact on selected human cell models representative of tissues in oral and gastrointestinal systems (TR146 – buccal epithelial cells and NCN460 – colon mucosal epithelial cells). After 24 hours incubation, very little cytotoxicity was seen in either cell type at silver nanoparticle levels of 100 µg/mL.

B5. Kidney

A number of different kidney cell types have been subjected to silver, these include embryo kidney cells, which are a heterogeneous mix of almost all the types of cells present in the body (although most are endothelial, epithelial or fibroblasts), proximal tubule cells (HK 2) and renal epithelial cells (A498).

Hudecová et al. (2012) exposed human embryo kidney cells (HEK293) to 20 nm silver nanoparticles. Although there was clear agglomeration of the particles, the silver nanoparticles were still taken up by the cells and could be identified in vacuoles and cytoplasm. No cytotoxicity was reported after exposure of the cells to 100 µg/mL for 30 minutes (based on Trypan Blue exclusion), although there was a 48% reduction in proliferation activity and a 21% reduction in colony number at that concentration. No cytotoxicity (in any of the employed tests) was seen at concentrations up to 25 µg/mL, although DNA damage could be detected even after exposure to 1 µg/mL silver nanoparticles. Singh and Ramarao (2012) found that renal epithelial cells (A498) were sensitive to 44 nm silver nanoparticles, with a significant reduction in viability (MTT and Coomassie Blue assay) at 1 µg/mL. This group looked at five different cell lines; the kidney cells were the most sensitive. Kermanizadeh et al. (2013) looked at the impacts of a variety of nanomaterials on renal proximal tubule epithelial cells. The silver nanoparticles (< 20 nm, capped with polyoxylaurat Tween) were one of the more toxic nanomaterials examined, with an LC_{50} of between 4.5–10 µg/cm^2 (depending on the cell culture medium used). Silver nanoparticle exposure resulted in a significant increase in ROS, interleukins 6 and 8 and evidence of DNA damage. Ionic silver has also been found to be toxic; with Simmons et al. (2011) reporting that silver chloride caused an increase in the oxidative stress response in four out of five cell lines examined, including kidney cells – HEK293T.

Gopinath et al. (2010) used baby hamster kidney cells (BHK21) to investigate the impact of 18 nm silver nanoparticles on primary cells. Cells exposed to 11 µg/mL (a concentration below the IC_{50} value) showed altered morphology and a 9% increase in the early apoptotic population compared to control

cells. An examination of gene expression showed that silver nanoparticles induced the p53-mediated apoptotic pathway.

B6. Blood

The toxic effects of silver on blood have been studied by a number of groups, using a variety of different methodologies. Foldbjerg et al. (2009) looked at the toxicity of PVP-coated silver nanoparticles (69 nm) and silver ions (from silver nitrate) on the human monocytic leukaemia cell line (THP-1). Cells were exposed for up to 24 hours: it was found that both silver nanoparticles and ionic silver induced apoptosis and necrosis (depending upon the dose and exposure time) and caused increased ROS levels after six hours. In the cytotoxicity test (Annexin V/PI) silver ions were found to be four times more toxic than silver nanoparticles (EC_{50} of 0.62 µg/mL ionic silver compared to 2.44 µg silver nanoparticles/mL). Haase et al. (2012b) also looked at the toxicity of silver nanoparticles on THP-1 cells. They used two peptide coated silver nanoparticles (20 nm and 40 nm) and found that while both silver nanoparticles were toxic to the monocytes, the 20 nm silver nanoparticles were more toxic. The toxic effect was found to increase with time, thus, the IC_{50} for 20 nm silver nanoparticles at 24 hours was 110 µg/mL, compared to 18 µg/mL at 48 hours.

Jun et al. (2011) looked at the effect of silver nanoparticles on platelet aggregation. The group used washed platelets from humans as an *in vitro* test and rats as an *in vivo* test. In platelets, the silver nanoparticles (< 100 nm) were found to induce platelet aggregation:

- control–5.4% aggregation;
- 100 µg silver nanoparticles/mL–28% aggregation; and
- 250 µg silver nanoparticles/mL–54% aggregation.

The aggregation was potentiated by co-treatment with a sub-threshold concentration of thrombin. Consistent with the human platelet studies, *in vivo* exposure of rats to silver nanoparticles (0.05–0.1 mg/kg by intravenous administration or 5–10 mg/kg by intratrachael instillation) enhanced venous thrombus formation and platelet aggregation. The authors suggest that silver nanoparticles may increase the prothrombotic risk in susceptible patients with compounding cardiovascular diseases.

Choi et al. (2011) used heparinized human blood to look at the impact of silver on haemolysis. They used four different silver preparations (two nano and two micron sized particles). Both silver nanoparticle preparations (citrate stabilized and bare particles) were significantly more haemolytic than the micron sized particles (of equivalent mass concentration). The haemolysis was related to the release of silver ions (with the silver nanoparticles releasing considerably more than the micron preparations).

Silver nitrate at various concentrations (up to 33 µM) was added to human whole blood and levels of GSH measured at time intervals (Khan et al., 2011). The GSH level was found to decrease in a concentration- and time-dependent manner in both the plasma and cytosolic fraction, with the depletion suggesting that the silver nitrate penetrated the blood cells and resulted in oxidation of the reduced glutathione or the formation of a silver-glutathione complex.

Barkhordari et al. (2014) explored the impact of naked silver nanoparticles (1–1500 µg/mL) on human blood mononuclear cells. MTT assays were conducted after 6 or 24 hours incubation. The percentage cell death was higher after 24 hours than 6 hours, and all concentrations of silver nanoparticles resulted in significantly more cell death than in the control cells. The greatest impact seemed to be at 500 µg/mL,

although the authors do not report whether the differences between the cells incubated with 500 µg/mL silver nanoparticles and the higher concentrations are statistically significant.

Wang et al. (2013) used mouse erythroleukemia cells to study the impact of a range of PVP-coated silver nanoparticles (10, 25, 40, 45 and 110 nm) on mRNA transcription. At 1 µg/mL (a non-cytotoxic dose) a large reduction in alpha- and beta-globin was seen. The shape of the silver nanoparticles seemed to be important as the spherical silver nanoparticles showed a greater impact on globin expression compared to the plate form; it was speculated that spherical silver nanoparticles may have a greater capability to cross the plasma membrane. Small spherical silver nanoparticles (10, 25 nm) showed a greater inhibition of globin expression than the larger particles. The group demonstrated that silver nanoparticles caused a significant suppression of RNA polymerase activity and overall RNA transcription through direct silver binding to RNA polymerase.

B7. Skin

Most researchers have used cell lines (keratinocytes, dermal fibroblasts and skin epithelial cells) to look at the potential toxicity of skin application of silver, but the potential for skin penetration of silver nanoparticles has also been investigated using an *in vitro* system.

B7.1 Skin penetration

Larese et al. (2009) looked at the penetration of silver nanoparticles through human skin using an *in vitro* test system that utilised abdominal full thickness skin obtained as surgical waste. Skin was essentially bathed in silver nanoparticles (25 nm in size, dispersed in ethanol and diluted with synthetic sweat) for 24 hours. The experiments were conducted using both intact and abraded skin. Low, but detectable, silver nanoparticle absorption through intact skin was seen. As might be expected, penetration through damaged skin was five times greater than that through intact skin. Silver nanoparticles could be seen (using transmission electron microscopy) in the stratum corneum and upper layers of the epidermis.

B7.2 Skin cells

Arora et al. (2008) used secondary human skin epithelial cells (A431) to study cellular responses induced by spherical silver nanoparticles (7–20 nm). As the IC_{50} (XTT assay) was 11.6 µg/mL, cells were subsequently exposed to a dose roughly half of that value. At 6.25 µg/mL, cellular morphology was unchanged, but there were clear signs of oxidative stress, namely decreased GSH (~ 2 fold), decreased SOD (~ 3 fold) and increased lipid peroxidation (~ 2 fold). Comfort et al. (2011) also found indicators of oxidative stress in A431 cells after exposure to low levels of silver nanoparticles (10 nm). In addition to inducing high quantities of ROS, silver nanoparticles caused a disruption in the epidermal growth factor signalling response.

Cortese-Krott (2009) treated primary human skin fibroblasts with low levels of silver nitrate (below that impacting on proliferation, mitochondrial activity or cell viability) and found that subtoxic concentrations (5–10 µM) strongly increased the intracellular production of ROS (including superoxide anion radicals) and impacted on intracellular zinc homeostasis.

Samberg et al. (2010) looked at the cytotoxicity of bare silver nanoparticles (20, 50 and 80 nm) and carbon-coated silver nanoparticles (50 and 80 nm) to primary neonatal human epidermal keratinocytes. If silver nanoparticles were applied to the keratinocytes unwashed, a 24-hour exposure resulted in a significant dose-dependent decrease in viability. However, application of the carbon-coated silver

nanoparticles or washed silver nanoparticles did not cause a decrease in cell viability, suggesting that the toxicity seen in the unwashed silver nanoparticles is a result of residual contamination from the silver nanoparticle synthesis (in this case formaldehyde). Although washed silver nanoparticles did not result a decrease in viability, they were taken up and were found to be internalized into the membrane-bound vacuoles in the keratinocytes.

Zanette et al. (2011) found that PVP-coated silver nanoparticles (25–50 nm) caused a concentration- and time-dependent decrease in cell viability (based on mitochondrial function) in HaCaT cells at concentrations of 11 µg/mL and greater. A long-lasting inhibition in cell proliferation was seen as cell proliferation was still showing a concentration-dependent decrease 6 days after the silver nanoparticles had been washed out of the system.

Comparative silver nanoparticle (~ 65 nm) cytotoxicity tests using HaCaT and cervical cancer cells (HeLa) were conducted by Mukherjee et al. (2012). They used a wide range of cytotoxicity tests and found that, in both cell lines, a measure of mitochondrial function (MTT assay) was the most sensitive test (HaCaT LD_{50} at 24 hours of 51.8 mg/L). After 24 hours, the LD_{50} values for the MTT test for both cell types were similar. After 48 and 72 hours, however, HeLa cells were found to be much more sensitive (LD_{50} after 72 hours for HaCaT of 30.4 mg/L compared to HeLa of 0.04 mg/L). The authors note that a major difference between the two cell types is their natural antioxidant levels, with HaCaT having over 30 times more glutathione than HeLa; this could be an important factor in the different sensitivity to silver nanoparticles.

Srivastava et al. (2012) investigated the impact of silver nanoparticles (size unstated) and silver ions (silver sulfate) on selenium metabolism in keratinocytes (HaCaT). They found that, while there was no clear cytotoxic effect of silver nanoparticle (up to 10 µM) or silver sulfate (up to 1000 nM) exposure on the keratinocytes, silver nanoparticles and ionic silver led to a dose-dependent inhibition of selenium metabolism. The authors commented that the decrease in selenoprotein synthesis could have significant implications in the defence against oxidative stress in the event of long-term exposures.

B8. Macrophages

Macrophages constitute the first line of defence upon uptake of silver nanoparticles by humans and other mammals (Pratsinis et al., 2013). Macrophages function in both nonspecific defence (innate immunity) as well as helping to initiate specific defence mechanisms (adaptive immunity).

Shavandi et al. (2011) looked at the cytotoxicity of silver nanoparticles (18–34 nm) to murine peritoneal macrophages using an assessment of mitochondrial activity (MTT assay). A significant decrease in viability was seen at concentrations of 1 ppm and above after 24 hours of exposure. Significant reductions in NO production were seen at 0.4 ppm silver nanoparticles. Park et al. (2010) also used murine peritoneal macrophages (RAW 264.7) to examine the impact of silver nanoparticles. Silver nanoparticles with an average size of ~70 nm were dispersed in foetal bovine serum and cells were exposed for up to 96 hours to concentrations between 0.2 to 1.6 ppm. Cell viability (MTT assay) decreased in a concentration and time-dependent manner, with the lowest concentration causing significant cytotoxicity after 96 hours. The silver nanoparticles also significantly reduced levels of intracellular GSH at concentrations of 0.4 ppm and above. In contrast to Shavandi et al. (2011), Park and colleagues found that NO was significantly increased. Park et al. (2010) reported that silver nanoparticles were ingested by phagocytosis, but that they were not observed in the dead cells, suggesting that the particles were released back into the culture medium by the damaged cells where they were available for further biological responses.

Four different silver nanoparticles with a similar size (< 10 nm) and shape (spherical), but different coatings and surface charge were tested against two cells lines: mouse macrophage (RAW 264.7) and mouse lung epithelial cells. The same pattern of toxicity was seen in both cell lines with, essentially, the silver nanoparticles with the greater positive surface charge being more toxic. The macrophage cells were more sensitive to the silver nanoparticles than the lung epithelial cells (Suresh et al., 2012). Singh and Ramarao (2012) also found that RAW 264.7 macrophages were highly sensitive to silver nanoparticle (44 nm) toxicity, with a significant reduction in cell viability (MTT assay) seen after 72 hours exposure to 3 µg/mL. Of the six cell lines examined only renal epithelial cells (A498) were more sensitive. Interestingly, J774.1 macrophages were one of the more resistant cells line (significant cytotoxicity was seen at 30 µg/mL).

Pratsinis et al. (2013) synthesized uncoated silver nanoparticles (6 to 20 nm) supported on inert nanostructured silica and looked at the impact of silver ion release on the viability of murine macrophages (RAW 264.7). Small silver nanoparticles (< 10 nm) released or leached larger fractions of their mass as ionic silver upon dispersion in water and this strongly influenced the cytotoxicity.

B9. Reproductive system

Tiedemann et al. (2014) assessed the impact of silver (silver nanoparticles and silver nitrate) on porcine gametes. The 11 nm bovine serum albumin coated silver nanoparticles and silver nitrate led to a significant decrease in oocyte maturation or complete arrest of maturation respectively. The silver was found to accumulate mainly in the cumulus cell layer surrounding the oocyte. None of the sperm vitality parameters assessed (motility, membrane integrity and morphology) were significantly affected by silver.

Zhang et al. (2015) exposed male mouse somatic Leydig (TM3) and Sertoli (TM4) cells to two different sizes of silver nanoparticles (10 nm and 20 nm) and examined effects on cell viability, metabolic activity, oxidative stress and apoptosis. TM3 and TM4 cells which had been exposed to silver nanoparticles for 24 hours were then used as feeder cells for spermatogonial stem cells and the impact on gene expression examined. The silver nanoparticles inhibited the viability and proliferation of both TM3 and TM4 cells by damaging cell membranes and inducing the generation of ROS. The 10 nm silver nanoparticles were found to be more cytotoxic than the 20 nm silver nanoparticles. Silver nanoparticle exposure was found to significantly down-regulate the expression of genes related to testosterone synthesis (TM3) and tight junctions (TM4). In addition, exposure of the TM3 and TM4 cells to silver nanoparticles inhibited the proliferation and self-renewal of spermatogonial stem cells.

B 10. Genotoxicity

A total of 19 *in vitro* studies are outlined in Table B1, of these the majority (14) used human cells including stem cells, bronchial epithelial cells, lymphocytes, fibroblasts and keratinocytes. A range of different silver nanoparticles were examined including naked particles and also those stabilized with citrate, bovine serum albumin, PVP, polyethylenimine, polyoxylaurat tween and polyetherimide; 2 studies (Jiang et al., 2013 and Milić et al., 2015) also looked at silver nitrate in parallel to silver nanoparticles.

The comet assay was the most frequently employed test (14 of 19 studies) and, in each case, suggested that silver nanoparticles can cause DNA damage. The lowest concentration at which DNA damage was seen was 0.01 µg/mL following exposure of human bronchial epithelial cells to 59 nm naked silver nanoparticles (Kim HR et al., 2011) and exposure of hamster ovary cells to 40–59 nm naked silver

nanoparticles (Kim HR et al., 2013). As with the *in vivo* results, there is a suggestion that sensitivity to the genotoxic effects of silver nanoparticles is cell specific (e.g. Tomankova et al., 2015). Castiglioni et al. (2014), however, found that the DNA damage in human microvascular endothelial cells exposed to 35 nm naked silver nanoparticles was reversible (when silver nanoparticles were removed from the culture medium) suggesting, in this case, that no permanent modifications occurred. In line with other (non-genotoxicity) studies, cells seem to be more sensitive to smaller silver nanoparticles (e.g. Avalos et al., 2015).

The micronucleus assay was used in 7 of the studies and was found to result in increased micronuclei in six cases. Nymark et al. (2013) reported no increase in micronucleus formation after exposure of human bronchial epithelial cells to 42 nm PVP silver nanoparticles (although positive results were reported for the comet assay). Vecchio et al. (2014) found that silver nanoparticle-induced genotoxicity, measured using the micronucleus assay, was dependent on lymphocyte sub-type and was particularly pronounced in $CD2^+$ and $CD4^+$ cells.

Only three studies used the chromosome aberration test. Chromosome damage was reported in a single study, where chromatid deletions and exchanges were significantly elevated following exposure of mesenchymal stem cells to 46 nm bovine serum albumin silver nanoparticles (Hackenberg et al., 2011).

One study used a gene mutation assay. Huk et al. (2015) looked at silver nanoparticles, with different surface coatings, using the HPRT gene mutation test in hamster lung fibroblast cells (V79-4). All the tested silver nanoparticles induced HPRT gene mutation, but it was shown that the stabilizing agent could play a significant role in the degree of reported toxicity, with sodium citrate and Tween being found to be mutagenic in their own right.

Table B1: ***In vitro*** **genotoxicity studies**

Reference	Animal	Tissue/cell type	Silver type	Exposure duration	Dose	Comet assay	MN assay	Chromosome aberration test
Hackenberg et al., 2011	Human	Mesenchymal stem cells	46 nm, bovine serum albumin NP	1, 3, 24 h	0.01–10 µg/mL	A statistically significant dose-dependent increase in DNA damage at ≥ 0.1 µg/mL after 1 h exposure.	-	A significant increase in chromosome damage (mainly chromatid deletions and exchanges) seen at ≥ 0.1 µg/mL.
Kim HR et al., 2011	Human	Bronchial epithelial cells (BEAS-2B)	59 nm, naked NP	24 h	0.01–10 µg/mL	A dose-dependent increase in DNA damage was seen (≥ 0.01 µg/mL).	A dose-dependent increase in micronuclei was observed at ≥ 0.01 µg/mL.	-
Nymark et al., 2013	Human	Bronchial epithelial cells (BEAS-2B)	42.5 nm, PVP NP	4, 24, 48 h	2.5–240 µg/mL	A significant increase in DNA damage was seen at concentrations of ≥ 60.8 µg/mL.	No increase in micronucleus formation was seen even after 48 h exposure.	No increase in chromosome aberrations was observed.
Flower et al., 2012	Human	Peripheral blood cells	40–60 nm, naked NP	5 min, 3 h	50, 100 µg/mL	A significant increase in DNA damage was seen (≥ 50 µg/mL), with effects apparent after 5 min (leading to the suggestion that the damage is caused by the generation of free radicals).	-	-

Reference	Animal	Tissue/cell type	Silver type	Exposure duration	Dose	Comet assay	MN assay	Chromosome aberration test
Tavares et al., 2012	Human	Leucocytes	19 nm, (ave) citrate NP	1, 6, 12, 24 h	10, 25, 50 µg/mL	DNA damage varied according to dose and length of exposure. The greatest level of DNA damage was seen at ≤ 6 h. No significant differences were seen at 24 h, suggesting DNA repair.	-	-
Ghosh et al., 2012	Human	Lymphocytes	120 nm (ave), NP	3 h	25, 50, 100, 150, 200 µg/mL	DNA breakage was seen at the lowest concentration administered (25 µg/mL), there was no clear dose-response relationship (with significant responses seen at 25, 50 and 200 µg/mL).	-	-
Vecchio et al., 2014	Human	Lymphocytes	10 and 70 nm, citrate and PVP NP	24, 48, 72 h	0.1, 10, 50 µg/mL	-	Unsorted lymphocytes showed an increase in micronucleus frequency when exposed to 10 µg/mL of the 10 nm citrate, 10 nm PVP and 70 nm citrate AgNP. Different lymphocyte sub-types showed different AgNP sensitivity.	-

Reference	Animal	Tissue/cell type	Silver type	Exposure duration	Dose	Comet assay	MN assay	Chromosome aberration test
Ivask et al., 2015	Human	Lymphocytes	18 nm, citrate NP; 28 nm, branched polyethylenimine NP	24 h	0.1–25 µg/mL	-	In primary lymphoctyes a significant increase in micronucleus formation was seen following exposure to 12.5 µg/mL citrate NP and 0.8 µg/mL polyethylenimine NP. Both cell lines examined (B- lymphoctyes – WIL2-NS & T-lymphocytes – JURKAT) were more susceptible to AgNP, with increased micronucleus formation seen at 3.1 µg/mL citrate NP and 0.4 µg/mL polyethylenimine NP.	-
Kermanizadeh et al., 2013	Human	Renal proximal tubule HK-2	8–47 nm, polyoxylaurat Tween NP	4 h	1.25, 2.5, 5 µg/cm^3	DNA damage was observed (in both the standard and FPG modified comet assay) for all doses assessed.	-	-
Castiglioni et al., 2014	Human	Microvascular endothelial	35 nm, naked NP	24 h	0.05–25 µg/mL	A dose-dependent increase in DNA damage was seen. The damage was reversible on removal of AgNP from culture medium, suggesting that no permanent modifications occur.	-	-

Reference	Animal	Tissue/cell type	Silver type	Exposure duration	Dose	Comet assay	MN assay	Chromosome aberration test
Franchi et al., 2015	Human	Fibroblasts	50–82 nm, PVP NP	24 h	0.01, 0.1, 1, 10 µg/mL	A significant increase in DNA damage was seen at the highest concentration tested (10 µg/mL), which was well below the IC_{50} value of 42.5 µg/mL.	-	-
Avalos et al., 2015	Human	Fibroblasts (dermal & pulmonary)	4.7 nm, polyetherimide NP; 4.7 nm, PVP NP; 42 nm, naked NP	24 h	0.1–1.6 µg/mL (coated NP), 0.1–6.7 nm (naked NP)	A significant and dose-dependent increase in DNA damage was seen in both fibroblast types to both sizes of AgNP. The fibroblasts were more sensitive to the smaller, coated, AgNP (increased damage at 0.1 µg/mL) compared to the 42 nm naked NP (increased damage at 0.5 µg/mL).	-	-
Tomankova et al., 2015	Human Mouse	Fibroblasts (BJ), Keratinoctyes (SVK14) Fibroblasts (NIH3T3)	106 nm, naked NP; 48 nm, naked NP	6 h	1.3–2.3 mg/L (based on individual IC_{50}value for each AgNP and cell type)	Both types of AgNP used caused DNA damage in the human fibroblasts and keratinocytes. The mouse cells were less sensitive and there was no significant difference seen from the control.	-	-

Reference	Animal	Tissue/cell type	Silver type	Exposure duration	Dose	Comet assay	MN assay	Chromosome aberration test
Szmyd et al., 2013	Human	Keratinocytes	15 nm, PVP NP	24, 48 h	12.5, 25 µg/mL	A significant increase in DNA damage seen following exposure to 25 µg/mL AgNP. Damage levels were significantly greater after 48 h exposure.	-	-
Jiang et al., 2013	Hamster	Ovary cells (CHO-K1)	15 nm, bovine serum albumin NP; $AgNO_3$	24 h	1, 5, 10 µg/mL	-	Concentrations of ≥ 5 µg/mL significantly increased micronucleus formation above the control. 10 µg/mL Ag^+ resulted in a significantly greater increase (~5-fold cf. control) in micronucleus formation than AgNP (~3 fold cf. control).	-
Kim JS et al., 2013	Hamster	Ovary cells (CHO-K1)	Citrate NP	6, 24 h	0.48–31.25 µg/mL	-	-	No effect seen.
Kim HR et al., 2013	Hamster	Ovary cells (CHO-K1)	40–59 nm naked NP	24 h	0.01, 0.1, 1, 10 µg/mL	Dose-dependent increase in DNA damage seen at ≥ 0.01 µg/mL. 10 µg/mL AgNP caused an approximately 450% increase in DNA breakage compared to the control.	A significant increase in micronucleus formation was seen at concentrations of ≥ 0.1 µg/mL. Greater micronucleus formation was seen in tests conducted without the presence of cytochalsin B and/or S9.	-

Reference	Animal	Tissue/cell type	Silver type	Exposure duration	Dose	Comet assay	MN assay	Chromosome aberration test
Li X et al., 2013	Hamster	Embryo cells	Naked NP	24 h	10, 20, 40 µg/mL	-	An increase in micronucleus frequency was seen following exposure to ≥ 10 µg/mL AgNP. The lower concentrations increased micronucleus formation in a dose-dependent manner. The increase was not found to be dose-dependent for the highest concentration examined and it is speculated that this might have been due to cell toxicity.	-
Milić et al., 2015	Pig	Porcine kidney (PK15)	61 nm, citrate NP; $AgNO_3$	24, 48 h	1–100 mg/L AgNP 1 mg/L Ag^+	DNA damage was seen at 1 mg/L Ag^+ and at ≥ 10 mg/L AgNP (after 24 h) and ≥ 5 mg/L (after 48 h).	-	-

ave–average; bw-body weight; cf–compared with; FPG–formamidopyrimidine-DNA glycosylase; ip–intraperitoneal; iv–intravenous; NP–nanoparticles

B 11. References

Abbott Chalew TE, Schwab KJ (2013). Toxicity of commercially available engineered nanoparticles to Caco-2 and SW480 human intestinal epithelial cells. Cell Biol Toxicol. 29:101–16.

Arora S, Jain J, Rajwade JM, Paknikar KM (2008). Cellular responses induced by silver nanoparticles: in vitro studies. Toxicol Lett. 179:93–100.

Arora S, Jain J, Rajwade JM, Paknikar KM (2009). Interactions of silver nanoparticles with primary mouse fibroblasts and liver cells. Toxicol Appl Pharmacol. 236:310–18.

AshaRani PV, Hande MP, Valiyaveettil S (2009a). Anti-proliferative activity of silver nanoparticles. BMC Cell Biol. 10:65.

AshaRani PV, Mun GLK, Hande MP, Valiyaveettil S (2009b). Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano. 3(2):279–90.

Avalos A, Haza AI, Morales P (2015). Manufactured silver nanoparticles of different sizes induced DNA strand breaks and oxidative DNA damage in hepatoma and leukaemia cells and in dermal and pulmonary fibroblasts. Folia Biol. 61:33–42.

Barkhordari A, Barzegar S, Hekmatimoghaddam H, Jebali A, Moghadam SR, Khanjani N (2014). The toxic effects of silver nanoparticles on blood mononuclear cells. Int J Occup Environ Med. 5:164–68.

Böhmert L, Girod M, Hansen U, Maul R, Knappe P, Niemann B et al. (2014). Analytically monitored digestion of silver nanoparticles and their toxicity for human intestinal cells. Nanotoxicol. 8:631–42.

Bouwmeester H, Poortman J, Peters RJ, Wijma E, Kramer E, Makama S et al. (2011). Characterization of translocation of silver nanoparticles and effects on whole-genome gene expression using an in vitro intestinal epithelium coculture model. Nano. 5:4091–103.

Carlson C, Hussain SM, Schrand AM, Braydich-Stolle LK, Hess KL, Jones RL et al. (2008). Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. J Phys Chem. B 112:13608–619.

Castiglioni S, Caspani C, Cazzaniga A, Maier JAM (2014). Short- and long-term effects of silver nanoparticles on human microvascular endothelial cells. World J Biol Chem. 5:457–64.

Cha K, Hong HW, Choi YG, Lee MJ, Park JH, Chae HK et al. (2008). Comparison of acute responses of mice livers to short-term exposure to nano-sized or micro-sized silver particles. Biotechnol Lett. 30:1893–99.

Chairuangkitti P, Lawanprasert S, Roytrakul S, Aueviriyavit S, Phummiratch D, Kulthong K et al. (2013). Silver nanoparticles induce toxicity in A549 cells via ROS-dependent and ROS-independent pathways. Toxicol in Vitro. 27:330–38.

Choi J, Reipa V, Hitchins VM, Goering PL, Malinauskas RA (2011). Physiocochemical characterization and in vitro hemolysis evaluation of silver nanoparticles. Toxicol Sci. 123:133–43.

Comfort KK, Maurer EI, Braydich-Stolle L, Hussain SM (2011). Interference of silver, gold, and iron oxide nanoparticles on epidermal growth factor signal transduction in epithelial cells. ACS Nano. 5:10000–08.

Cooper RJ, Spitzer N (2015). Silver nanoparticles at sublethal concentrations disrupt cytoskeleton and neurite dynamics in cultured adult neural stem cells. NeuroToxicol. 48:231–38.

Cortese-Krott MM, Munchow M, Pirev E, Hessner F, Bozkurt A, Uciechowski P et al. (2009). Silver ions induce oxidative stress and intracellular zinc release in human skin fibroblasts. Free Radic Biol Med. 47:1570–77.

Ema M, Kobayashi N, Naya M, Hanai S, Nakanishi J (2010). Reproductive and developmental toxicity studies of manufactured nanomaterials. Reprod Toxicol. 30:343–52.

Faedmaleki F, Shirazi FH, Salarian A-A, Ashtiani HA, Rastegar H (2014). Toxicity effect of silver nanoparticles on mice liver primary cell culture and HepG2 cell line. Iran J Pharm Res. 13:235–42.

Flower NAL, Brabu B, Revathy M, Gopalakrishnan C, Raja SVK, Murugan SS et al. (2012). Characterization of synthesized silver nanoparticles and assessment of its genotoxicity potentials using the alkaline comet assay. Mutat Res. 742:61–65.

Foldbjerg R, Irving ES, Hayashi Y, Sutherland DS, Thorsen K, Autrup H et al. (2012). Global gene expression profiling of human lung epithelial cells after exposure to nanosilver. Toxicol Sci. 130:145–57.

Foldbjerg R, Dang DA, Autrup H (2011). Cytotoxicity and genotoxicity of silver nanoparticles in the human cancer cell line, A549. Arch Toxicol. 85:743–50.

Foldbjerg R, Olesen P, Hougaard M, Dang DA, Hoffmann HJ, Autrup H (2009). PVP-coated silver nanoparticles and silver ions induce reactive oxygen species, apoptosis and necrosis in THP-1 monocytes. Toxicol Lett. 190:156–62.

Franchi LP, Manshian BB, de Souza TAJ, Soenen SJ, Matsubara EY, Rosolen JM et al. (2015). Cyto- and genotoxic effects of metallic nanoparticles in untransformed human fibroblast. Toxicol in Vitro. 29:1319–31.

Gaiser BK, Fernandes TF, Jepson M, Lead JR, Tyler CR, Stone V (2009). Assessing exposure, uptake and toxicity of silver and cerium dioxide nanoparticles from contaminated environments. Environ Health. 8:S2.

Gaiser BK, Hirn S, Kermanizadeh A, Kanase N, Fytianos K, Wenk A et al. (2013). Effects of silver nanoparticles on the liver and heptocytes in vitro. Toxicol Sci. 131:537–47.

Ghosh M, Manivannan J, Sinha S, Chakraborty A, Mallick SK, Bandyopadhyay M et al. (2012). *In vitro* and *in vivo* genotoxicity of silver nanoparticles. Mutat Res. 749:60–69.

Giovanni M, Tay CY, Setyawati MI, Xie J, Ong CN, Fan R et al. (2015). Toxicity profiling of water contextual zinc oxide, silver, and titanium dioxide nanoparticles in human oral and gastrointestinal cell systems. Environ Toxicol. 30(12):1459–69.

Gliga AR, Skoglund S, Wallinder IO, Fadeel B, Karlsson HL (2014). Size-dependent cytotoxicity of silver nanoparticles in human ling cells: the role of cellular uptake, agglomeration and Ag release. Part Fibre Toxicol. 11:11.

Gopinath P, Gogoi SK, Chattopadhyay A, Ghosh SS (2008). Implications of silver nanoparticle induced cell apoptosis for in vitro gene therapy. Nanotechnol. 19:7

Gopinath P, Gogoi SK, Sanpui P, Paul A, Chattopadhyay A, Ghosh SS (2010). Signalling gene cascade in silver nanoparticle induced apoptosis. Colloids Surf B: Biointerfaces 77:240–45.

Grosse S, Evje L, Syversen T (2013). Silver nanoparticle-induced cytotoxicity in rat brain endothelial cell culture. Toxicol in Vitro. 27:305–13.

Haase A, Rott S, Mantion A, Graf P, Plendl J, Thunemann AF et al. (2012a). Effects of silver nanoparticles on primary mixed neural cell cultures: uptake, oxidative stress and acute calcium responses. Toxicol Sci. 126:457–68.

Haase A, Mantion A, Graf P, Plendl J, Thuenemann AF, Meier W et al. (2012b). A novel type of silver nanoparticles and their advantages in toxicity testing in cell culture systems. Arch Toxicol. 86:1089–98.

Hackenberg S, Scherzed A, Kessler M, Hummel S, Technau A, Froelich K et al. (2011). Silver nanoparticles: evaluation of DNA damage, toxicity and functional impairment in human mesenchymal stem cells. Toxicol Lett. 201:27–33.

Hardes J, Streitburger A, Ahrens H, Nusselt T, Gebert C, Winkelmann W et al. (2007). The influence of elementary silver versus titanium on osteoblasts behaviour in vitro using human osteosarcoma cell lines. Sarcoma. 26539.

Hsin YH, Chen CF, Huang S, Shih TS, Lai PS, Chueh PJ (2008). The apoptotic effect of nanosilver is mediated by a ROS- and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells. Toxicol. Lett. 179:130–39.

Hudecová A, Kusznierewicz B, Runden-Pran E, Magdolenova Z et al. (2012). Silver nanoparticles induce premutagenic DNA oxidation that can be prevented by phytochemicals from *Gentiana asclepiadea*. Mutagen. 27:759–69.

Huk A, Izak-Nau E, el Yamani N, Uggerud H, Vadset M, Zasonska B et al. (2015). Impact of nanosilver on various DNA lesions and HPRT gene mutations – effects of charge and surface coating. Part Fibre Toxicol. 12:25.

Hussain SM, Hess KL, Gearhart JM, Geiss KT, Schlager JJ (2005). In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol in Vitro. 19:975–83.

Ivask A, Voelcker NH, Seabrook SA, Hor M, Kirby JK, Fenech M et al. (2015). DNA melting and genotoxicity induced by silver nanoparticles and graphene. Chem Res Toxicol. 28:1023–35.

Jiang X, Foldbjerg R, Miclaus T, Wang L, Singh R, Hayashi Y et al. (2013) Multi-platform genotoxicity analysis of silver nanoparticles in the model cell line CHO-K1. Toxicol Lett. 222:55–63.

Jun E-A, Lim K-M, Kim K, Bae ON, Noh JY, Chung KH et al. (2011). Silver nanoparticles enhance thrombus formation through increased platelet aggregation and procoagulant activity. Nanotoxicol. 5:157–67.

Kawata K, Osawa M, Okabe S (2009). In vitro toxicity of silver nanoparticles at noncytotoxic doses to HepG2 hepatoma cells. Environ Sci Technol. 43:6046–51.

Kermanizadeh A, Vranic S, Boland S, Moreau K, Baeza-Squiban A, Gaiser BK et al. (2013). An in vitro assessment of panel of engineered nanomaterials using a human renal cell line: cytotoxicity, pro-inflammatory response, oxidative stress and genotoxicity. BMC Nephrol. 14:96.

Khan H, Khan MF, Asim-Ur-Rehman, Jan SU, Ullah N (2011). The protective role of glutathione in silver induced toxicity in blood components. Pak J Pharmacol Sci. 24:123–28.

Kim HR, Kim MJ, Lee SY, Oh SM, Chung KH (2011). Genotoxic effects of silver nanoparticles stimulated by oxidative stress in human normal bronchial epithelial (BEAS-2B) cells. Mutat Res. 726:129–35.

Kim HR, Park YJ, Shin DY, Oh SM, Chung KH (2013). Appropriate *in vitro* methods for genotoxicity testing of silver nanoparticles. Environ Health Toxicol: 28:e2013003.

Kim JS, Song KS, Sung JH, Ryu HR, Choi BG, Cho HS et al. (2013). Genotoxicity, acute oral and dermal toxicity, eye and dermal irritation and corrosion and skin sensitisation evaluation of silver nanoparticles. Nanotoxicol. 7:953–60.

Kim S, Choi JE, Choi J, Chung KH, Park K, Yi J (2009). Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. Toxicol in Vitro. 23:1076–84.

Kruszewski M, Gradzka I, Bertlomiejczyk T, Chwastowska J, Sommer S, Grzelak A et al. (2013). Oxidative DNA damage corresponds to the long term survival of human cells treated with silver nanoparticles. Toxicol Lett. 219:151–59.

Kulthong K, Maniratanachote R, Kobayashi Y, Fukami T, Yokoi T (2012). Effects of silver nanoparticles on rat hepatic cytochrome P450 enzyme activity. Xenobiotica. 42:854–62.

Larese FF, D'Agostin F, Crosera M, Adami G, Renzi N, Bovenzi M et al. (2009). Human skin penetration of silver nanoparticles through intact and damaged skin. Toxicol. 255: 33–37.

Lee YS, Kim DW, Lee YH, Oh JH, Yoon S, Choi MS et al. (2011). Silver nanoparticles induce apoptosis and G2/M arrest via PKC-dependent signalling in A549 lung cells. Arch Toxicol. 85:1529–40.

Li X, Xu L, Shao A, Wu G, Hanagata N (2013). Cytotoxic and genotoxic effects of silver nanoparticles on primary Syrian hamster embryo (SHE) cells. J Nanosci Nanotechnol. 13:161–70.

Lichtenstein D, Ebmeyer J, Knappe P, Juling S, Böhmert L, Selve S et al. (2015). Impact of food components during in vitro digestion of silver nanoparticles on cellular uptake and cytotoxicity in intestinal cells. Biol Chem. 396:1255–64.

Luther EM, Koehler Y, Diendorf J, Epple M, Dringen R (2011). Accumulation of silver nanoparticles by cultured primary brain astrocytes. Nanotechnol. 22(37):375101.

Luther EM, Schmidt MM, Diendorf J, Epple M, Dringen R (2012). Upregulation of metallothieneins after exposure of cultured primary astrocytes to silver nanoparticles. Neurochem Res. 37:1639–48.

Milić M, Leitinger G, Pavičić I, Avdičević MZ, Dobrović S, Goessler W et al. (2015). Cellular uptake and toxicity effects of silver nanoparticles in mammalian kidney cells. J Appl Toxiciol. 35:581–92.

Mukherjee SG, O'Claonadh N, Casey A, Chambers G (2012). Comparative in vitro cytotoxicity study of silver nanoparticle on two mammalian cell lines. Toxicol in Vitro. 26:238–51.

Nowrouzi A, Meghrazi K, Golmohammadi T, Golestani A, Ahmadian S, Shafiezadeh M et al. (2010). Cytotoxicity of subtoxic AgNP in human hepatoma cell line (HepG2) after long-term exposure. Iran Biomed J. 14:23–32.

Nymark P, Catalán J, Suhonen S, Järventaus H, Birkedal R, Clausen PA et al. (2013). Genotoxicity of polyvivylpyrrolidone-coated silver nanoparticles in BEAS 2B cells. Toxicol. 313:38–48.

Park EJ, Yi J, Kim Y, Choi K, Park K (2010). Silver nanoparticles induce cytotoxicity by a Trojan-horse type mechanism. Toxicol in Vitro. 24:872–8.

Powers CM, Badireddy AR, Ryde IT, Seidler FJ, Slotkin TA (2011). Silver nanoparticles compromise neurodevelopment in PC12 cells: critical contributions of silver ion, particle size, coating and composition. Environ Health Perspect. 119:37–44.

Powers CM, Wrench N, Ryde IT, Smith AM, Seidler FJ, Slotkin TA (2010). Silver impairs neurodevelopment in PC12 cells. Environ Health Perspect. 118:73–79.

Pratsinis A, Hervella P, Leroux J, Pratsinis SE, Sotiriou GA (2013). Toxicity of silver nanoparticles in macrophages. Small. 9:2576–84.

Sahu SC, Zheng J, Yourick JJ, Spando RL, Gao X (2015). Toxicogenomic responses of human liver HepG2 cells to silver nanoparticles. J Appl Toxicol. 35(10):1160–08.

Samberg ME, Oldenburg SJ, Monteiro-Riviere NA (2010). Evaluation of silver nanoparticle toxicity in skin *in vivo* and keratinocytes *in vitro*. Environ Health Perspect. 118:407–13.

Shavandi Z, Ghazanfari T, Moghaddam KN (2011). In vitro toxicity of silver nanoparticles on murine peritoneal macrophages. Immunopharmacol Immunotoxicol. 33:135–40.

Simmons SO, Fan C-Y, Yeoman K, Wakefield J, Ramabhadran R (2011). NRF2 oxidative stress induced by heavy metals is cell type dependent. Curr Chem Genom. 5:1–12.

Singh RP and Ramarao P (2012). Cellular uptake, intracellular trafficking and cytotoxicity of silver nanoparticles. Toxicol Lett. 213:249–59.

Srivastava M, Singh S, Self WT (2012). Exposure to silver nanoparticles inhibits selenoprotein synthesis and the activity of thioredoxin reductase. Environ Health Perspect. 120:56–61.

Suliman YAO, Ali D, Alarifi AH, Mansour L, Alwasel SH (2013). Evaluation of cytotoxic, oxidative stress, proinflammatory and genotoxic effect of silver nanoparticles in human lung epithelial cells. Environ Toxicol. 30(2):149–60.

Sur I, Cam D, Kahraman M, Baysal A, Culha M (2010). Interaction of multi-functional silver nanoparticles with living cells. Nanotechnol. 21(17):175104.

Suresh AK, Pelletier DA, Wang W, Morrell-Falvey JL, Gu B, Doktycz MJ (2012). Cytotoxicity induced by engineered silver nanocrystallites is dependent on surface coatings and cell types. Langmuir. 28:2727–35.

Szmyd R, Goralczyk AG, Skalniak L, Cierniak A, Lipert B, Filon FL et al. (2013). Effect of silver nanoparticles on human primary keratinocytes. Biol Chem. 394:113–23.

Tavares P, Balbinot F, de Oliverira HM, Fagundes GE, Vanancio M, Ronconi JVV et al. (2012). Evaluation of genotoxic effect of silver nanoparticles (Ag-Nps) in vitro and *in vivo*. J Nanopart Res. 14:791.

Tiedemann D, Taylor U, Rehbock C, Jakobi J, Klein S, Kues WA et al. (2014). Reprotoxicity of gold, silver, and gold-silver alloy nanoparticles on mammalian gametes. Analyst. 139:931–42.

Tomankova K, Horakova J, Harvanova M, Malina L, Soukupova J, Hradolova S et al. (2015). Cytotoxicity, cell uptake and microscopic analysis of titanium dioxide and silver nanoparticles in vitro. Food Chem Toxicol. 82:106–15.

Trickler WJ, Lantz SM, Murdock RC, Schrand AM, Robinson BL, Newport GD et al. (2010). Silver nanoparticle induced blood-brain barrier inflammation and increased permeability in primary rat brain microvessel endothelial cells. Toxicol Sci. 118:160–70.

Vecchio G, Fenech M, Pompa PP, Voelcker NH (2014). Lab-on-a-chip-based high-throughput screening of the genotoxicity of engineered nanomaterials. Small. 10:2721–34.

Versantvoort CHM, Oomen AG, van de Kemp E, Rompelberg CJM, Sips AJAM (2005). Applicability of an in vitro digestion model in assessing the bioaccessibility of mycotoxins from food. Food Chem Toxicol. 43:31–40.

Walczak A, Fokkink R, Peters R, Tromp P, Herrera Rivera ZE, Rietjens IMCM et al. (2013). Behaviour of silver nanoparticles and silver ions in an in vitro human gastrointestinal digestion model. Nanotoxicol. 7:1198–1210.

Wang Z, Liu S, Ma J, Qu G, Wang X, Yu S et al. (2013). Silver nanoparticles induced RNA polymerase-silver binding and RNA transcription inhibition in erythroid progenitor cells. ACS Nano. 7:4171–86.

Xu F, Piett C, Farkas S, Qazzaz M, Syed NI (2013). Silver nanoparticles (AgNps) cause degeneration of cytoskeleton and disrupt synaptic machinery of cultured cortical neurons. Mol Brain. 6:29.

Yin N, Liu Q, Liu J, He B, Cui L, Li Z et al. (2013). Silver nanoparticle exposure attenuates the viability of rat cerebellum granule cells through apoptosis coupled to oxidative stress. Small. 9:1831–41.

Zanette C, Pelin M, Crosera M, Adami G, Bovenzi M, Larese FF et al. (2011). Silver nanoparticles exert a long-lasting antiproliferative effect on human keratinoctye HaCaT cell line. Toxicol in Vitro. 25:1053–60.

Zhang S, Du C, Wang Z, Han X, Zhang K, Liu L (2013). Reduced cytotoxicity of silver ions to mammalian cells at high concentration due to the formation of silver chloride. Toxicol in Vitro. 27:739–44.

Zhang XF, Choi YJ, Han JW, Kim E, Park JH, Gurunathan S et al. (2015). Differential nanoreprotoxicity of silver nanoparticles in male somatic cells and spermatogonial stem cells. Int J Nanomedicine. 10:1335–57.

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