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Highlights

- Provisional tolerable intake value was derived for intravenous exposure to nanoparticles released from medical devices.
- Critical study selection was based on the Annapolis Accords principles and Toxicological Data Reliability Assessment Tool.
- Modifying factor of 1,000 was determined by scientific review and analysis accounting for uncertainties.
- Provisional tolerable intake for *i. v.* exposure to silver nanoparticles was calculated to be 0.14 µg/kg bw/day.
- Methodology presented is appropriate for deriving provisional tolerable intake value for nanoparticles in general.

Abstract

Silver nanoparticles (AgNP) are incorporated into medical devices for their anti-microbial characteristics. The potential exposure and toxicity of AgNPs is unknown due to varying physicochemical particle properties and lack of toxicological data. The aim of this safety assessment is to derive a provisional tolerable intake (pTI) value for AgNPs released from blood-contacting medical devices. A literature review of *in vivo* studies investigating critical health effects induced from intravenous (*i. v.)* exposure to AgNPs was evaluated by the Annapolis Accords principles and Toxicological Data Reliability Assessment Tool (ToxRTool). The point of departure (POD) was based on an *i. v.* 28-day repeated AgNP (20 nm) dose toxicity study reporting an increase in relative spleen weight in rats with a 5% lower confidence bound of the benchmark dose ($BMDL_{05}$) of 0.14 mg/kg bw/day. The POD was extrapolated to humans by a modifying factor of 1,000 to account for intraspecies variability, interspecies

differences and lack of long-term toxicity data. The pTI for long-term *i. v.* exposure to 20 nm AgNPs released from blood-contacting medical devices was 0.14 μg/kg bw/day. This pTI may not be appropriate for nanoparticles of other physicochemical properties or routes of administration. The methodology is appropriate for deriving pTIs for nanoparticles in general.

Keywords

Silver nanoparticles, Safety assessment, Provisional tolerable intake, Medical devices, Intravenous, Uncertainty factors, Point of departure, Annapolis accords, ToxRTool

1. Introduction

Nanotechnology has many applications in medical devices; however, knowledge gaps exist inhibiting assessment of the risk of exposure and toxicity of nanoparticles released from medical devices to patients (Wijnhoven et al., 2009). Prediction of the toxic effects of nanoparticles could be calculated from the known toxicity of their bulk materials but is prevented due to fundamental physical and chemical properties that change as the particle size is decreased within the nanoscale range (Lai and Sayre, 2009, SCENIHR, 2009). Safety assessment of nanoparticles is further complicated by the vast number and variety of physicochemical properties produced differing widely by particle size, shape, agglomeration state, crystal structure, chemical composition, surface area and surface properties (Pang et al., 2016, Lai and Sayre, 2009, Warheit et al., 2007, Isakovic et al., 2006, Sayes et al., 2006a, Sayes et al., 2006b, Nemmar et al., 2003). A stringent battery of biological tests for each nanomaterial with varying physicochemical particle properties on a case-by-case basis would be costly, time-consuming and impractical (Lai, 2015, Oberdorster et al., 2005).

To address this complex problem, provisional tolerable intake (pTI) values can be determined for exposure to nanoparticles of specific physicochemical properties, routes of entry and durations of exposure. A pTI value is a dose estimate of the average daily intake of a chemical over a period of time based on body mass and is considered to be without appreciable harm to human health (ANSI/AAMI/ISO 10993-17:2002/(R)2012, 2003). A pTI is normally expressed in milligrams per kilogram of body mass per day (mg/kg bw/day) and derived as a part of establishment of an allowable limit for a leachable chemical in a medical device.

Silver nanoparticles (AgNPs) are incorporated into blood-contacting medical devices for their anti-microbial properties, such as in intravascular (*i. v.*) catheters (Wijnhoven et al., 2009). The toxic effects induced by AgNPs have been evaluated using *in vitro* and short-term *in vivo* studies (Dubey et al., 2015, Gaillet and Rouanet, 2015, Ge et al., 2014, Wijnhoven et al., 2009); however, the potential exposure and subsequent toxicity of AgNPs released from medical devices via *i. v.* exposure to patients is not completely understood. The aim of this safety assessment is to derive a pTI value for AgNPs released from blood-contacting medical devices. A comprehensive literature review of *in vivo* studies investigating critical health effects induced from *i. v.* exposure to AgNPs was reviewed (ANSI/AAMI/ISO 10993-17:2002/(R)2012, 2003) and evaluated by the Annapolis Accords principles as described by Gray et al. (2008). Key studies were further analyzed by the Toxicological Data Reliability Assessment Tool (ToxRTool) (Schneider et al., 2009) to determine the critical study for use in derivation of the pTI.

The point of departure (POD) is the most sensitive critical health effect reported in the critical study typically presented as a no-adverse-effect-level (NOAEL) or bench mark dose (BMD) value (FDA, 2015). The POD is then extrapolated to humans by a modifying factor (MF) determined based on scientific review and analysis to account for uncertainties including intraspecies variability, interspecies differences and lack of long-term toxicity data. The derivation of a pTI dose is similar to the methodology used in the safety assessment of di(2-ethylhexyl)phthalate (DEHP) (FDA, 2001) released from PVC medical devices and in Weldon et al. (2016) in the development of an occupational exposure limit for silver nanoparticles. The pTI derived in this work should be considered provisional because a limited number of critical toxicological studies are currently available and may not be appropriate for nanoparticles of other physicochemical properties or routes of AgNP administration. The methodology used was deemed appropriate for deriving pTIs for nanoparticles in general.

2. Literature review and selection of toxicological studies

2.1. Criteria for selection of toxicological studies

A comprehensive literature search using PubMed, Web of Science and Embase was conducted to identify *in vivo* studies investigated the critical health effects after *i. v.* exposure to AgNP that were published from August 31, 2006 to August 31, 2016. Studies were evaluated based on principles outlined in the Annapolis Accords on The Use of Toxicology in Risk Assessment and *Decision-Making* (Gray et al., 2008) and are presented in Table 1. Criteria for inclusion or exclusion of a study included: 1) administration route similar to the route of exposure to medical devices containing AgNPs, 2) relevance of the toxicological effects to human health, 3) clearly established critical health effects between a biomarker and functional endpoint, so that only studies with effects broadly considered to be adverse (histopathological or functional changes) would be used for pTI derivation; and 4) high quality of the published data based on rigor, power, corroboration, universality, proximity, relevance and cohesion (Gray et al., 2008).

Table 1. Criteria for selection of toxicological studies based on Annapolis Accords principles.[a]

Principle	Criteria for Inclusion in Derivation of a Provisional Tolerable Intake Value
Rigor	− Evaluated for proper conduct and analysis. − Greater weight given to more rigorous studies. − Studies with poor methods discounted.
Power	− Statistical power of the experimental design examined for ability to detect effects of a given magnitude. − For example, some "negative" studies may misinterpret a low level of response as a lack of response.
Corroboration	− Determine if effects are replicated in similar studies or under varied conditions to predict if effects would be seen under conditions of human exposure as well. − Conversely, lack of corroboration of effects provides a basis for doubting either the validity of single experimental results or their applicability to other species or conditions of exposure.
Universality	− Effect seen in multiple species by various routes of exposure gives confidence that the effect may apply to humans.

Principle	Criteria for Inclusion in Derivation of a Provisional Tolerable Intake Value
	− If an effect is restricted to a certain species, strain, or route of administration, there is less confidence in the ability to generalize the response to other species or routes.
Proximity	− When effects exist in a species taxonomically-related to humans or at exposure doses similar to those expected in humans, such results weigh more heavily than effects found in taxonomically less related species by less relevant routes or at markedly different doses.
Relevance	− Knowledge of the underlying biologic basis for toxicity in animals can assist in determining whether similar metabolism, mechanisms of damage and repair and molecular targets of toxic action occur in humans. Accordingly, confidence in applicability to humans can increase or decrease.
Cohesion	− Extent to which all data are consistent and subject to a single, biologically plausible explanation increases the weight-of-evidence when comparing situations where inconsistencies require ad hoc explanations and exceptions to general patterns.

aPrinciples outlined as in (Gray et al., 2008).

Studies that met the Annapolis Accords principles were furthered analyzed by the ToxRTool. The ToxRTool was developed by the European Commission's Joint Research Center in 2009 (Schneider et al., 2009) and built upon Klimisch categories (Klimisch et al., 1997) to evaluate peer-reviewed publications providing criteria and guidance for accessing the reliability of toxicological studies. The methodology of how the tool assesses data reliability from a toxicological study is previously described in Schneider et al. (2009). For our use, the *in vivo* spreadsheet of the ToxRTool was used to determine if 21 criteria were met in the following 5 areas: (1) test substance identification, (2) test organism characterization, (3) study design description, (4) study results documentation and (5) plausibility of study design and data. To minimize rater bias during analysis and provide a more objective screening (Segal et al., 2015), three raters were employed to score each study independently while blinded to the other's ratings. Ratings were jointly reviewed to conclude the score of the study. Studies that are categorized as reliable (scores of 1 or 2) are deemed appropriate for use in derivation of the pTI.

2.2. Summarization of in-vivo toxicological data

The comprehensive literature review identified eighteen (18) *in vivo* studies investigating the critical health effects after *i. v.* AgNP exposure. These studies are summarized in Table 2 and in more detail in the Supplemental Material considering study methodology including animal model used, characterization of AgNPs employed, AgNP treatment dose, exposure and duration, toxicological health effects seen, POD reported and appropriateness of the study for derivation of the pTI based on the Annapolis Accords. A study assessed to be lacking in any of the Annapolis Accords criteria does not mean the study lacked scientific merit, but does reduce its appropriateness for deriving a pTI (Table 1).

Table 2. Summarization of *In vivo* studies investigating the toxicity and health effects of intravenous silver nanoparticle exposure.

Species (strain, sex)	AgNP Treatment Dose[a]	Exposure/Duration	Toxicological Health Effect	Point of Departure[a]	Appropriate-ness of Study for pTI Derivation[b]	Reference
			Mouse studies			
Mouse (C57BL 6, wild type (WT) and 8-oxo-guanine DNA gly-cosylase knockout (KO) male)	5 mg/kg bw/day (20 and 200 nm AgNP dispersed in dH_2O/10X BSA/10X PBS in 8:1:1 ratio) n = 6 per treatment	Single injection and euthanized 1 and 6 days after treatment	Genotoxicity: ↑ DNA-SB and alkali sites in KO lung (200 nm, 7 days). ↑ Fpg-ss lesions in lung (200 nm, 7 days) and testis of WT (20 and 200 nm, 7 days) Gene expression: ↑ *Atm*, *Rad51*, *Sod1*, *Fos* and *Mmp3* in KO lung (200 nm, 1 and 7 days). ↑ Atr and Rad51 in KO testis (20 and 200 nm, 7 days).	NOAEL/LOAEL: None	Lacks Power	Asare et al., 2016
Mouse (CD-1, female)	0.66 mg/kg bw/day (10 nm AgNP in CT buffer) n = 8–10 pregnant females per treatment	3 daily injections of 2.2 mg/ml on GDs 7, 8, 9 and euthanized 1 day after treatment	Histopath: No abnormalities Reprod: ↑ number of smaller-sized GD10 embryos	NOAEL/LOAEL: None	Lacks Power, Corroboration and Universality	Austin et al., 2016
Mouse (CD-1, female)	1.2 and 2.2 mg/kg bw/day (50 nm AgNP in CT buffer) n = 6–12 pregnant females per treatment	3 day treatments on GDs 7, 8, 9 and euthanized 1 day after treatment	Histopath: No abnormalities Reprod: No gross abnormalities to embryo	NOAEL: 2.2 mg/kg bw/day	No adverse effect[b]	Austin et al., 2012
Mouse (Balb/c, unknown gender)	0.2, 2 and 5 mg/kg bw/day (20 nm AgNP maintained in 4% polyoxy-ethylene glycerol trioleate	Single injection and euthanized 8 h after treatment	Toxicity: No effect on body weight Hepatic: ↑ ER stress marker levels (5 mg/kg bw/day)	NOAEL: 2 mg/kg bw/day	Lacks Power and Universality	Chen et al., 2016

Species (strain, sex)	AgNP Treatment Dose[a]	Exposure/Duration	Toxicological Health Effect	Point of Departure[a]	Appropriate-ness of Study for pTI Derivation[b]	Reference
	and 4% Tween 20) n = 3 per treatment		Blood: ↓ lymphocyte percentage and ↑ IL-6 Histopath: Thickened alveolar walls, multifocal consolidation and infiltration of focal inflammatory cells in lungs. Disorganized hepatic cords, damaged hepatic lobules, edema cytoplasm and ballooning-like tissue changes in the liver. No effects in the brain, heart, spleen and kidneys. ↑ apoptotic cells in the lung, liver, spleen and kidneys (5 mg/kg bw/day).			
Mouse (Balb/c, female)	0.25 mg/kg bw/day (10, 75 and 110 nm AgNP in 5% isotonic glucose solution) n = 3 per treatment	Single injection and euthanized 4 h or 1, 3, 7 days after treatment. 3 injections on days 1, 4 and 10 and euthanized 7 days later	Toxicity: Liver, kidney and lung had inflammation (greatest after 75 or 100 nm)	NOAEL/LOAEL: None	Lacks Power and Universality	Guo et al., 2016
Mouse (CD-1, male)	1.0 mg/kg bw/day (14 nm, citrate-coated AgNP) in	Injections on 0, 3, 6, 9, and 12 days and	Toxicity: No effect on body weight Histopath: ↑	NOAEL/LOAEL: None	Lacks Power	Garcia et al., 2014

Species (strain, sex)	AgNP Treatment Dose[a]	Exposure/Duration	Toxicological Health Effect	Point of Departure[a]	Appropriate-ness of Study for pTI Derivation[b]	Reference
	PBS n = 6 per treatment	euthanized 15, 60, and 120 days	lumen volume and tubule diameter and ↓ seminiferous epithelium volume density in the testis (15 and 60 days). ↑ % of apoptotic germ cells in the testis. ↑ cytoplasm and size of Leydig cells in the testis (15 and 60 days). Hormone: No effect in serum levels of LH or FSH. ↑ serum and intratesticular testosterone at 15 days Reprod: No effects on sperm concentration and motility (15–20 days)			
Mouse (B6C3F1, male)	0.5, 1.0, 2.5, 10, 20 mg/kg bw/day (5 nm, PVP-coated AgNP) 25 mg/kg bw/day (15–100 nm, PVP-coated AgNP) 25 mg/kg bw/day (10–80 nm, silicon-coated AgNP) n = 5 per treatment	Single injection (5 nm, PVP-coated) Single injection and 3 day repeat dose (15–100 nm, PVP-coated and 10–80 nm, silicon-coated)	Gentox: Cytotoxicity of reticulocytes (PVP-coated) and presence of oxidative damage (Comet Assay) in liver (PVP and silicon-coated AgNPs). No increase in mutation frequencies in the *Pig*-a gene or the	NOAEL (5 nm PVP-coated): 20 mg/kg bw/day	Lacks Corroboration and Universality	Li et al., 2014

Species (strain, sex)	AgNP Treatment Dose[a]	Exposure/Duration	Toxicological Health Effect	Point of Departure[a]	Appropriate-ness of Study for pTI Derivation[b]	Reference
			percent of micronucleated (MN) reticulocytes.			
Mouse (CD-1, male)	10 mg/kg bw/day (10, 40 and 100 nm, CT- or PVP-coated, spherical AgNP suspended in Milli-Q water) n = 3 per treatment	Single injection and euthanized 1 day after treatment	Toxicity: 2 mortalities. ↓ body weight, ↑ spleen weight. Midzonal hepatocellular necrosis and gall bladder hemorrhage (10 nm).	NOAEL/LOAEL: None	Lacks Power and Corroboration	Recordati et al., 2016
Mouse (ICR, male and female)	7.5, 30, and 120 mg/kg bw/day (15 nm AgNP suspended in saline) n = 5 per sex per treatment	Single injection and euthanized at 6, 12 h and 1, 7, 14 days	Toxicity: No significant changes in body or relative organs weights were observed. Histopath: Infiltration of focal inflammatory cells and thickened alveolar walls in the lungs at day 7 but diminished by day 14 (120 mg/kg bw/day). Liver edema and loose interstitial cytoplasm in hepatic cells (120 mg/kg bw/day). None in brain, heart, spleen, kidneys, testicles or ovaries	NOAEL:120 mg/kg bw/day	No adverse effect[c]	Xue et al., 2012

Species (strain, sex)	AgNP Treatment Dose[a]	Exposure/Duration	Toxicological Health Effect	Point of Departure[a]	Appropriate-ness of Study for pTI Derivation[b]	Reference
Mouse (ICR, female)	1.0 mg/kg bw/day (8 nm, spherical AgNP) n = 20 per treatment	Single injection at 6.5 dpc and euthanized at 13.5, 15.5 and 17.5 dpc	Reprod: ↑ progression of meiotic prophase I of female fetal germ cells Gene expression: ↑ meiosis-specific genes, Stra8, Daz1, Scp1, Scp3 and Dmc1 and ↓ development-related genes, Cx37, ZP glycoprotein 1, 2 and 3, and Figla. ↑ imprinted genes, *H19, Zac1, Ascl2, Snrpn, Kcnq1ot1, Peg3, Zac1, H19, Igf2r* and *Igf2*. DNA methylation: ↓ *Zac1* and ↑ *Igf2r*	NOAEL/LOAEL: None	Lacks Power, Corroboration and Universality	Zhang et al., 2015a
			Rat Studies			
Rat (Wistar WU, male and female)	0.0082, 0.0025, 0.074, 0.22, 0.67, 2.0 and 6.0 mg/kg bw/day (20 and 100 nm AgNP suspended in PB) n = 2–4 per sex per treatment	Daily injections for 28 days and euthanized 1 day after final treatment	Toxicity: ↓ thymus and ↑ spleen wt. Histopath: enlarged, brown-colored spleen, liver, and lymph nodes Immunol: ↓ cytokine production including interferon-γ, IL-10, and IL-6, as well as increased serum	BMDL05: 0.14 mg/kg bw/day ↑ relative spleen weight and 0.001 mg/kg bw/day for ↓ thymus weight	Chosen as a critical study. Used to derive the pTI.	De Jong et al., 2013

Species (strain, sex)	AgNP Treatment Dose[a]	Exposure/Duration	Toxicological Health Effect	Point of Departure[a]	Appropriate-ness of Study for pTI Derivation[b]	Reference
			IgM, IgE and increased blood neutrophilic granulocytes			
Rat (Wistar, male)	5 and 10 mg/kg bw/day (20 nm, spherical AgNP in NaCl solution) 5 mg/kg bw/day (200 nm; spherical AgNP in NaCl solution) n = 7 per treatment	Single injection and euthanized 1, 7 and 28 days after treatment	At 10 mg/kg bw/day (20 nm) significantly higher frequency of micronuclei after 4 weeks (possible bone marrow toxicity)	NOAEL:10 mg/kg bw/day (20 nm)	Lacks Power, Rigor, Corroboration and Universality	Dobrzynska et al., 2014
Rat (Sprague Dawley, male)	10 mg/kg bw/day (117 nm AgNP dispersed in dH_2O) n = 7 per treatment and n = 5 for controls	Single injection and euthanized 2 days after treatment	Urine: Proteinuria Blood: ↑ creatinine and urea in serum Nephro: accumulation of glycosaminoglycan, hemorrhage in renal cortex and ↑ thickness of the parietal layer in Bowman's capsule.	NOAEL/LOAEL: None	Lacks Power, Corroboration and Universality	Feng et al., 2015
Rat (Wistar Kyoto)	0.238 mg/kg bw/day (17.3 nm AgNP dispersed in H2O containing 4% each of PGT and Tween 20) n = 3 per treatment	Single injection and euthanized 1 day after treatment	No changes in glutathione, ↑ in TNF-α, IL-1R1, and MIP-2 gene expression (24 h)	NOAEL/LOAEL: None	Lacks Power and subtle effects not considered critical	Gaiser et al., 2013

Species (strain, sex)	AgNP Treatment Dose[a]	Exposure/Duration	Toxicological Health Effect	Point of Departure[a]	Appropriate-ness of Study for pTI Derivation[b]	Reference
Rat (Wistar, male)	5 and 10 mg/kg bw/day (20 nm, spherical AgNP dispersed in 0.9% NaCl solution) 5 mg/kg bw/day (200 nm, spherical AgNP dispersed in 0.9% NaCl solution) n = 24 per treatment	Single injection and euthanized 1, 7 and 28 days after treatment	20 nm:Reprod: ↓ sperm count (5 mg/kg bw/day; 1 and 28 days) and germ count (5 mg/kg bw/day). DNA damage in germ cells (5 and 10 mg/kg bw/day at 24 h). 200 nm:Morphological changes in testes (5 mg/kg bw/day)	NOAEL (200 nm): 5.0 mg/kg bw/day	Lacks Power	Gromadzka-Ostrawska et al., 2012
Rat (Wistar, male)	4, 10, 20 and 40 mg/kg bw/day AgNP (13 nm dispersed in ethylene glycol) n = 6 per treatment	5 day intervals for 32 days and euthanized after treatment	Toxicity: ↓ in body weight (20 and 40 mg/kg bw/day after 15 d exposure). No effect in organ weight. Hematol: ↓ platelet counts and ↑ white blood cells (20 and 40 mg/kg bw/day) Hepatotox: ↑ ALT and AST (20 and 40 mg/kg bw/day) and ↑ ALP and GGTP (40 mg/kg bw/day). Blood: ↑ ROS and DNA damage. Histopath: No inflammation, damage or morphological	NOAEL: 10 mg/kg bw/day	Lacks Power	Tiwari et al., 2011

Species (strain, sex)	AgNP Treatment Dose[a]	Exposure/Duration	Toxicological Health Effect	Point of Departure[a]	Appropriate-ness of Study for pTI Derivation[b]	Reference
			changes in the liver or kidney.			
Rat (Wistar WU, male)	0.0082, 0.0025, 0.074, 0.22, 0.67, 2.0 and 6.0 mg/kg bw/day AgNP (20 nm,CT-coated dispersed in CT) n = 5 per treatment	Daily injections for 28 days and euthanized 21 days after treatment	Toxicity: ↓ body and thymus weight and ↑ spleen weight and cell number. Immunol: ↑ spleen monocytes and ↓ KLH-specific IgG	BMD ($BMDL_{05}$): 0.98 (0.76) mg/kg bw/day ↑ spleen wt. 1.3 (0.76) mg/kg bw/day ↓ thymus wt.	Chosen as a critical study but was not used to derive the pTI.	Vandebriel et al., 2014
Rat (Sprague Dawley, male)	5, 10 and 45 mg/kg bw/day AgNP (7.2 nm, spherical) in H_2O n = 6 per treatment and 12 per control	Single injection or daily for 3 days	Toxicity: ↓ body weight Neurotox/Behavioral: Locomotor activity appeared to be sensitive and rearing freq. ↓ (45 mg/kg bw/day)	LOAEL: 45 mg/kg bw/day	Lacks Power, Corroboration and Universality	Zhang et al., 2013
			Rabbit Studies			
Rabbit (SPF New Zealand White, male)	0.6 mg/kg bw/day AgNP (45 nm in dH_2O and PVP (<1%)) n = 8 per treatment	Single injection with one euthanized from each group at 21, 42, 84 and 105 days and all euthanized at 126 days	Toxicity: No effect on body weight Histopath: No effect in testes Reprod: Lower % of motile, vigor and oxygen consumption of sperm cells. Sperm had acrosome and mitochondrial damage.	NOAEL/LOAEL: None	Lacks Power	Castellini et al., 2014
Rabbit (SPF New Zealand White, male)	0.5 and 5.0 mg/kg bw/day AgNP (7.9 nm, citrate-coated in isotonic 5% glucose solution)	Single injection with tissue sampling at 1, 7 and 28 days	Histopath: Pigmentation in liver, increased inflammatory cell infiltration levels in liver, lung and	LOAEL: 5.0 mg/kg bw/day	Lacks Power	Lee et al., 2013

Species (strain, sex)	AgNP Treatment Dose[a]	Exposure/Duration	Toxicological Health Effect	Point of Departure[a]	Appropriate-ness of Study for pTI Derivation[b]	Reference
	n = 4 per treatment		kidneys (5.0 mg/kg bw/day)			

↑: increased; ↓: decreased; ALP: alkaline phosphatase; ALT: alanine transaminase; AST: aspartate aminotransferase; *Atm*: Ataxia telangiectasia mutated; BMD: bench-mark dose; $BMDL_{05}$: BMD 95% lower confidence limit; BSA: bovine serum albumin; CT: citrate; dH_2O: distilled water; Ddb2: damage specific DNA binding protein 2; dpc: days post conception; DNA-SB: DNA strand breaks; ER: endoplasmic reticulum; Fpg-ss: Formamidopyrimidine DNA glycosylase sensitive sites; GD: gestational day; KO: 8-oxoguanine DNA glycosylase knock-out; LOAEL: lowest-observed-adverse-effect-level; Mmp3: matrix metallopeptidase 3; n: sample size; NaCl: sodium chloride; NOAEL: no-observed-adverse-effect level; PB: phosphate buffer; PBS: phosphate buffered saline; PGT: polyoxyethylene glycerol trioleate; PVP: polyvinylpyrrolidone; ROS: reactive oxygen species; *Sod1*: superoxide dismutase 1.

amg/kg bw/day.

bBased on the Annapolis Accords principles (Gray et al., 2008).

cStudy has no adverse effect so can be excluded from further evaluation by the Annapolis Accords principles.

2.3. Identification of the point-of-departure (POD)

Two *i. v.* 28-day repeated AgNP dose toxicity studies in rats by De Jong et al. (2013) and Vandebriel et al. (2014) were deemed appropriate key studies for derivation of the pTI value for AgNPs released from blood-contacting medical devices (Table 2 and Supplemental Material). De Jong et al. (2013) (Table 3) and Vandebriel et al. (2014) met the principles outlined in the Annapolis Accords (Gray et al., 2008) (Table 1). The ToxRTool was used to further verify the study quality and reliability of data of these two studies, and both were assigned a category of reliable with restrictions (score of 2) confirming their appropriateness for derivation of the pTI. Both studies were of similar experimental design with minor differences as Vandebriel et al. (2014) was a follow-up study to De Jong et al. (2013). The study by De Jong et al. (2013) analyzed the toxic effects of repeated 28-day dosing of 20 and 100 nm AgNP with no recovery period prior to evaluating critical health effects. Vandebriel et al. (2014) analyzed the toxic effects of repeated 28-day dosing of 20 nm, citrate-coated AgNPs for 28 days with a 28-day recovery period prior to evaluating critical health effects (Table 2). Both studies report $BMDL_{05}$, the lower limit of the 95% confidence interval surrounding the BMD, for multiple toxicological parameters including body, spleen, thymus and liver weight, blood chemistry, hematology parameters and immune parameters. The $BMDL_{05}$ was determined from fitting a dose-response curve to the dataset over the entire dose range studied (De Jong et al., 2013, Vandebriel et al., 2014).

Table 3. *In vivo* Critical Study (De Jong et al., 2013) Chosen to Derive the Provisional Tolerable Intake Value for Intravenous Silver Nanoparticle Exposure Met the Annapolis Accords Criteria.

Principle	Criteria for Inclusion in Derivation of a Tolerable Intake Value
Rigor	The selected study properly conducted methods and reporting during their analysis of AgNP toxicity leading to increased confidence: − Followed OECD Guideline 407, for the testing of chemicals − Used a wide dose range (0, 0.0082, 0.0025, 0.074, 0.22, 0.67, 2.0, 6.0 mg/kg bw/day) as opposed to the conventional three dose group exposure design (low, mid and high dose) − Treated with repeated dose exposures as opposed to a single injection − Use of two different sizes of AgNPs (20 and 100 nm) − Incorporated both male and female animals − Provided toxicity data on general (*e.g*. body and target organ weight change) as well as specific (immunological) endpoints − Measured levels of biochemical parameters in blood serum − Provided histopathological analysis of targeted organs including spleen, thymus, liver, and lymph nodes
Power	Statistical power of the study was appropriate to have the ability to detect effects of a given magnitude including: − Sample size per treatment was small, but the increase in the number of dose groups improved the characterization of the dose response. − Provided a robust and adequately conducted statistical analysis for the calculation of the BMD_{05} for several parameters
Corroboration	Similar effects in immunologically functional tissues were reported in multiple studies[a,b].
Universality	Similar effects are reported in different species[a].
Proximity	Critical health effects shown in a species taxonomically related to humans such as rodents[a,b].
Relevance	Toxic response in animal models include metabolism, mechanisms of damage and repair, and molecular targets of toxic action is expected to be the same in humans.
Cohesion	A similar plausible biological explanation is seen across studies[b].

aRecordati et al., 2016.

bVandebriel et al., 2014.

The most sensitive critical health effect reported in the critical study is selected as the POD for derivation of a pTI value. When multiple critical health effects are reported in the critical study, or between multiple studies, selection of the POD is based on the lowest POD reported, with the highest magnitude of response (e.g. percent change or change in standard deviation from the control). The use of a NOAEL has limitations due to its determination being based on one experimental dose tested, dependence on doses and dose spacing chosen by the study authors, and the sample size of the animals per each dose group (Filipsson and Victorin, 2003). More advanced procedures such as benchmark dose (BMD) analysis can identify a POD value by including dose response data from the entire study, based

on selection of the response level by the investigator (Weldon et al., 2016, EFSA Scientific Committee, 2012, FDA, 2001, Crump, 1984) reducing the variability of the POD to ≤10% from a possible ≥20% when using a NOAEL (Gaylor and Kodell, 2000). The BMD includes calculation of the variability in the dose–response data as the 90 or 95% confidence limit of the BMD is calculated and presented as the $BMDL_{10}$ or $BMDL_{05}$, respectively (Weldon et al., 2016).

We applied this critical effect selection concept to De Jong et al. (2013) and Vandebriel et al. (2014). The critical health effects reported from exposure to 20 nm AgNP by De Jong et al. (2013) was an increase in spleen weight, $BMDL_{05}$ of 0.14 mg/kg bw/day (maximal response of +150%) and a decrease in thymus weight, $BMDL_{05}$ of 0.001 mg/kg bw/day (maximal response of −17.4%). Vandebriel et al. (2014) confirmed these findings reporting an increase in spleen weight and a decrease in thymus weight with a $BMDL_{05}$ of 0.76 mg/kg bw/day for both endpoints. The $BMDL_{05}$ of 0.14 mg/kg bw/day for increased spleen weight reported in De Jong et al. (2013) qualified to serve as the critical study for derivation of the pTI based on the $BMDL_{05}$ being the lowest critical health effect with the highest response. The increased $BMDL_{05}$ values reported in Vandebriel et al. (2014) may have been due to the 28-day recovery period after the final treatment used in the study; whereas, De Jong et al. (2013) did not have a recovery period.

3. Derivation of a provisional tolerable intake

3.1. Evaluation of uncertainties

The uncertainty factor (UF) concept is integral to safety assessment to ensure when extrapolating the POD derived in animal models to human health that the value yields a no-adverse-effect dose for the greater majority of the human population including sensitive subpopulations (Dankovic et al., 2015). UFs considered are interindividual variability among the human population (UF_1), interspecies variability in response to exposure when extrapolating data from animal models to humans (UF_2) and lack of chronic toxicity exposure data (UF_3) (FDA, 2001). Use of a default of 10 for each UF employed is standard when data is lacking; however, UFs should be derived on a case-by-case basis ranging from 1 to 10 based on chemical-biological specific adjustment factors when available or with scientific-support based on data in literature (EFSA Scientific Committee, 2012, Dankovic et al., 2015). The rationale for assigning uncertainty factors (UF) in the derivation of the provisional pTI in our study was in accordance to guidelines from the International Organization for Standardization (ANSI/AAMI/ISO 10993–17:2002/(R)2012, 2003).

3.1.1. Interindividual variability in human population (UF_1)

An UF_1 accounts for interindividual variability among the human population. When data assessing human variation is lacking, a default of 10 is typically assigned to account for the range of human variability when the safety assessment has been based on animal studies (ANSI/AAMI/ISO 10993-17:2002/(R)2012, 2003). If animal studies suggest that variation among humans may be significant, an UF_1 of or approaching 10 is selected (ANSI/AAMI/ISO 10993-17:2002/(R)2012, 2003). Sex-related differences were found in a mouse study reporting a significant difference in the elimination of Ag from blood during the 24 h after *i. v.* AgNP (15 nm) treatment, with a half-life of 29.9 h in females compared to 15.6 h in males. Additionally, the lungs and kidneys showed a sex-dependent accumulation of Ag with higher concentrations in females compared to males (Xue et al., 2012) (Table 4). Other exposure routes have also found sex-related differences after AgNP exposure. A 28-day oral toxicity study in rats

reported a 2-fold increase in AgNP in female kidneys compared to males with higher accumulation found in all kidney regions including the cortex, medulla, inner medulla and cortical glomeruli compared to males (Kim et al., 2008, Kim et al., 2009). A 90-day inhalation study in rats found statistically significant increases ($p < 0.01–0.05$) in parameters of lung inflammation in females compared to males (Sung et al., 2008). Additionally, interindividual differences in the excretion of Ag in urine and feces between rats have been reported after exposure with 20 and 200 nm AgNP (Dziendzikowska et al., 2012). These animal model studies suggest that sex-related and interindividual differences in AgNP toxicokinetics may exist in humans. Due to the lack of *i. v.* studies characterizing individual variability in humans and animal model data indicating the potential for interindividual variability between sexes, a default UF_1 of 10 was assigned.

Table 4. Pharmacokinetic or Biodistribution Studies of Intravenous Exposure to 20 ± 5 nm Silver Nanoparticles.

Species (strain, sex)	AgNP Treatment[a]	Duration	Tissue Distribution and Excretion[b]	Half-life	Reference
Mouse (BALB/c, male and female)	1.3 mg/kg bw/day (25 nm, PVP-coated AgNP spheres suspended in PBS) Injected 2x a week for 28 days n = 3–5 per treatment	15, 39 and 78 days (females) 120 days (males)	Sp > Li > Ki » Lu > H (females, levels ↓ over time) Te > Sp > Li » Ki > H > Ln > M (120 days)	NR	Wang et al., 2013
Mouse (ICR, male and female)	120 mg/kg bw/day (15 nm AgNP dispersed in saline) Single injection n = 6 per sex per treatment	10, 20, 30 min; 1, 3, 6, 12 h; 1, 7, 14 days	Sp > Li > Lu > Ki (females had higher silver levels in the Lu and Ki than males)	NR	Xue et al., 2012
Rat (Wistar, male)	5 mg/kg bw/day (20 nm, spherical AgNP dispersed in NaCl solution) Single injection n = 8 per treatment	1, 7, 28 days	Li » Sp > Ki > Lu > Br (1 day) Lu > Li > Sp > Ki > Br (7 days) Ki » Li > Sp > Lu > Br (28 days)	NR	Dziendzikowska et al., 2012
Rat (Wistar, male)	0.0238–0.0276 mg/kg bw/day (20 nm, spherical AgNP dispersed in PB) Single injection and 5-day repeat treatment n = 3 per treatment	2, 3, 5, 6, 8, 11 and 17 day	Li » Ki > Te > Sp > Lu > Br > H (single injection; Day 2) Li » Ki > Te > Sp > Lu > Br > H (5-day repeat treatment; Day 6) Li » Ki > Sp > Te > Br > Lu, H (5-day repeat treatment; Day 17)	NR[c]	Lankveld et al., 2010

NaCl: sodium chloride; NR: Not reported; PB: phosphate buffer; PBS: phosphate buffered saline; PVP: polyvinylpyrrolidone.

aSize (nm) and/or coating of particles, number of treatments and concentration (mg/kg bw/day).

bBr, brain; Fe, feces; H, heart; K, kidneys; Li, liver; Ln, lungs; M, muscle; Se, serum; Sp, spleen; Te, testis; Th, thymus; Ur, urine.

cAccumulation of AgNP occurred in all organs with most in kidneys (factor 5.5), liver (factor 5) and brain (factor 4).

3.1.2. Interspecies variability (UF_2)

UF_2 accounts for uncertainty in extrapolating data from animal models to humans (ANSI/AAMI/ISO 10993-17:2002/(R)2012, 2003). Traditionally, a default of 10 has been applied to account for inherent differences between animals and humans, who may be more sensitive to chemical critical health effects (Lehman and Fitzhugh, 1954). If the toxicity and toxicokinetics are known and similar between animals and humans, a smaller uncertainty factor may be used with justification (ANSI/AAMI/ISO 10993-17:2002/(R)2012, 2003). There is currently limited data on the pharmacokinetics, pharmacodynamics and toxicity mechanisms of AgNP to evaluate the relevance of animal data for human responses (Lin et al., 2015, Sweeney et al., 2015, Bachler et al., 2013, Lankveld et al., 2010, Faustman, 1996). The pharmacokinetic and biodistribution studies available do not report the half-life of AgNPs after *i. v.* exposure to 20 ± 5 nm AgNPs (Table 4). Studies in smaller AgNPs by Park et al. (2011) and Lee et al. (2013) report the half-life of AgNPs (7.9 nm, citrate coated) is species-dependent with 4.1 days in rats and 11.7–16.3 days in rabbits, respectively, after injection. The half-life was approximately 3–4 fold higher in rabbits compared to rats. This increase in half-life for the larger mammal is consistent with a lower metabolic rate and longer circulation time allowing for development of a more stable NP protein corona before distribution to tissue or elimination from the body (Sahneh et al., 2015, Riviere, 2013). The NP protein corona is a collection of selectively adsorbed biomolecules as the NP comes into contact with complex biological fluids lowering surface energy, promoting dispersion and defining the biological interaction of the NP (Monopoli et al., 2012). The formation of the protein corona decreases the extracellular dissolution of AgNPs into ionic Ag leading to the cellular uptake of the particles (Shannahan et al., 2015). Additionally, the binding of opsonins could induce a rapid clearance of NP from the vascular system or the binding of a polyethyleneglycol coating can decrease the uptake by macrophage cells (Pozzi et al., 2014).

Additionally, studies indicate that human male germ cells exhibit a lower capacity to repair some types of DNA oxidative lesions including 8-oxo-7,8-dihydroguanine (although 8-oxoguanine-DNA glycosylase-1 (hOGG1) was present) and showed poor removal of formamidopyrimidine-DNA glycosylase (Fpg)-sensitive lesions in general, which was not seen in rat male germ cells (Olsen et al., 2003). Asare et al. (2016) reported that injection of 5 mg/kg bw/day 200 nm AgNPs into hOGG1 knockout mice (proposed as an appropriate model for humans) induced DNA single stranded breaks, oxidative DNA lesions including Fpg-sensitive lesions and key DNA damage response and repair genes, *Atm*, *Rad51*, *Sod1*, *Fos and Mmp3*, in the lung and testis.

These interspecies differences potentially cause extrapolation from animal models to humans to be difficult (Sahneh et al., 2015). An UF_2 was assigned a 10 to account for the interspecies differences between rodents and humans after exposure to AgNPs.

3.1.3. Route-to-route extrapolation (UF_3)

A UF_3 accounts for the quality and relevance of the study data and can range from 1 to 100 considering but not limited to the study having only LOAEL data instead of NOAEL or BMD data; absence of supporting studies; inappropriate route of exposure; and lack of chronic study data (ANSI/AAMI/ISO 10993-17:2002/(R)2012, 2003). In our literature review, no chronic studies were found for *i. v.* exposure to AgNP. A TI that is protective of critical health effects resulting from chronic exposure should be based on long-term repeated dosing (30–90 days) or a chronic dosing study (90 days–2 years) (U.S. EPA, 1996, OECD, 1995). A value of 3–10 is typically assigned to account for the possibility of identifying a lower POD for chronic toxicity when extrapolating from a subchronic animal study. Assessing the appropriate value is commonly determined by evaluating if the critical effect that is the basis of the POD could be expected to increase in incidence, severity or occur at a lower dose given longer exposure time (Dankovic et al., 2015). Following *i. v.* injection, one of the primary sites of 20 nm AgNP accumulation has been consistently demonstrated to be in the spleen (Table 4). The localization of particles within the spleen can be accounted for by their uptake by the abundant number of resident macrophage populations (Lankveld et al., 2010). The marginal zone and red pulp macrophages are the major particle scavengers in the spleen followed by the peritoneal macrophages and dendritic cells (Recordati et al., 2016, Xue et al., 2012). Phagocytosis of AgNPs stimulates inflammatory signals through the generation of reactive oxygen species in macrophage cells (Martinez-Gutierrez et al., 2012, Park et al., 2010a, Park et al., 2011). *In vitro* studies indicate AgNP induces oxidative stress resulting in endoplasmic reticulum stress (Chen et al., 2016) and apoptosis in spleen cells (Xue et al., 2012, Lankveld et al., 2010).

Additionally, De Jong et al. (2013) examined the organ weight and histology of the testes and brain, which have been shown to be sensitive health effect endpoints, but reported no effects. AgNPs can cross the blood-testis and blood-brain barrier accumulating over time in these organs (Zhang et al., 2015a, Wang et al., 2013, Lee et al., 2013, Dziendzikowska et al., 2012, van der Zande et al., 2012, Lankveld et al., 2010, Sharma et al., 2010, Kim et al., 2009). *In vivo i. v.* studies in multiple species report testes toxicity after short-term AgNP treatment (Table 2) (Asare et al., 2016, Gromadzka-Ostrowska et al., 2012, Castellini et al., 2014). AgNP-induced toxicity in on the male reproductive system and spermatozoa was seen after other routes of exposure (Lafuente et al., 2016, Zhang et al., 2015a, Sleiman et al., 2013, Miresmaeili et al., 2013, Kim et al., 2009). Adverse effects induced by AgNPs in the brain has been reported including neurotoxicity (Bagheri-Abassi et al., 2015, Shanker Sharma and Sharma, 2012, Sharma et al., 2010, Tang et al., 2009). To account for the possibility that the critical study might have not examined the most sensitive health effect endpoint, a UF_3 of 10 was assigned.

3.2. Calculation of the tolerable intake

A modifying factor (MF) of 1,000, which is the mathematical product of the three UFs ($UF_1 \cdot UF_2 \cdot UF_3 = MF$), was applied to account for uncertainties including intraspecies variability ($UF_1 = 10$), interspecies differences ($UF_2 = 10$) and lack of chronic toxicity data ($UF_3 = 10$). The pTI for long-term *i. v.* exposure to 20 nm, uncoated AgNPs was determined to be 0.14 μg/kg bw/day derived from the POD of 0.14 mg/kg bw/day for immunotoxicological effects as calculated below:

4. Discussion

In this safety assessment, a pTI value was derived for *i. v.* exposure to 20 nm AgNP. The critical health effect study appropriate for deriving the pTI was determined to be an *i. v.* 28-day repeated-dose toxicity study in rats performed by De Jong et al. (2013) who investigated the immunotoxicological effects of AgNP. The POD was based on the critical health effect of increased relative spleen weight in rats with a $BMDL_{05}$ of 0.14 mg/kg bw/day. Histological analysis of the spleen revealed inflammation and a brownish pigment in the red pulp indicative of red blood cell degradation, as well as, a decrease in NK lymphocyte activity, as notable immunotoxic effects (De Jong et al., 2013). T, B and NK cell populations were increased in the spleen after treatment with 20 nm AgNP, and the authors suggest this increase in cell number may be responsible for the increase in spleen weight (De Jong et al., 2013). Such effects may be in part due to the preferential accumulation of AgNP in the spleen. *In vitro* studies investigating the toxicity of AgNP in spleen cells report AgNP induces oxidative stress of the endoplasmic reticulum (Chen et al., 2016) and apoptosis (Xue et al., 2012, Lankveld et al., 2010).

Supporting studies report toxic effects in the testes and sperm, which are considered more sensitive health effects compared to increase in spleen weight because this effect occurs at lower treatment doses than what was reported in the critical study by De Jong et al. (2013). De Jong et al. (2013) found no changes in testes weight or histology after 6.0 mg/kg bw/day AgNP (20 nm) 28-day repeated *i. v.* exposure in rats. In contrast, Gromadzka-Ostrawska et al. (2012) reported decreased sperm and germ count and DNA damage in germ cells after *i. v.* exposure to 5 mg/kg bw/day AgNP (20 nm); however, Asare et al. (2016) reported a single *i. v.* injection of 5 mg/kg bw/day AgNPs (20 nm) did not significantly increase single strand breaks in the testis 7 days after treatment in mice (Table 2). Castellini et al. (2014) investigated the toxic effects of a single *i. v.* injection of 0.6 mg/kg bw AgNPs (45 nm) on the sperm quality of rabbits throughout a 126-day study reporting sperm cells with a lower percent of motility, vigor and oxygen consumption and acrosome and mitochondrial damage (Table 2). AgNPs were seen in the spermatids and ejaculated sperm; however, no effect was seen morphologically in the testes nor was libido, serum testosterone, sperm concentration or semen volume affected (Castellini et al., 2014). Although these supporting studies did not meet the Annapolis Accords criteria and not used to derive the pTI; the scientific merit of these studies were used in determining the level of uncertainty in deriving the pTI.

Other routes of exposure, albeit at higher doses, collaborate with the observed toxic effects induced in the testes and sperm after *i. v.* AgNP exposure. Miresmaeili et al. (2013) reported oral gavage exposure to AgNP (70 nm) for 48 days induced a dose-dependent decrease in the number of primary spermatocytes, spermatids and spermatozoa with a NOAEL of 25 mg/kg bw/day and a LOAEL of 50 mg/kg bw/day. Lafuente et al. (2016) investigated the subchronic toxic effects of polyvinyl pyrrolidone (PVP)-coated AgNPs (average particle core size of 25 nm) by oral gavage on epididymal sperm rat parameters and found sperm morphology abnormalities after 90 days of repeated dose treatment with 100 mg/kg bw/day PVP-AgNP. Based on the NOAEL (25 mg/kg bw day) and LOAEL (100 mg/kg bw/day) reported in Miresmaeili et al. (2013) and Lafuente et al. (2016), respectively, and a BAF_{oral} of 4% to account for oral bioavailability (Bachler et al., 2013, Loeschner et al., 2011, Kim et al., 2010), the estimated *i. v.* NOAEL is calculated to be ranging from 1.0 to 4.0 mg/kg bw/day. Abdominal subcutaneous injection of AgNP (15 nm) in five doses over 13 days in mice induced reduction of the average testis weight (5 mg/kg bw/day; postnatal day PND42); reduction in the diameter of the convoluted tubules (1 and 5 mg/kg bw/day; PND28 and PND42); increase in the rate of abnormal sperm

(5 mg/kg bw/day; PND42 and PND63) and decreased sperm concentration (5 mg/kg bw/day; PND100) (Zhang et al., 2015b). Furthermore AgNPs are known to accumulate in the testes (Wang et al., 2013, Lankveld et al., 2010) (Table 4). These testicular toxicity data observed at lower *i. v.* equivalent AgNP doses as compared to De Jong et al. (2013) suggests that immunotoxicity of the spleen may not be the most sensitive toxicological endpoint. To account for the uncertainty that the POD may have not been the most sensitive critical health effect, a default value of 10 was used for UF_3. Use of default values for UF_1, UF_2, and UF_3 results in a conservative MF of 1,000 to be applied to the POD pTI of 0.14 μg/kg bw/day. This threshold dose represents acceptable exposure for non-cancer health effects resulting from particles leached from medical devices containing 20 nm, uncoated AgNP.

At the time of writing this paper, no patient exposure studies are known investigating AgNPs released/leached from medical devices. Roe et al. (2008) examined the toxic risk of catheters coated with 3–18 nm AgNPs *in vitro* and *in vivo*. *In vitro* studies examined Ag release from radioactive Ag-coated catheters (600 and 1000 μg/g) placed in saline solution over a period of 10 days. Ag released from the catheters was relatively constant with an average release of 45.1 ± 1.1 ng/cm for catheters coated with 1,000 μg/g Ag and 24.1 ± 2.4 ng/cm for catheters coated with 600 μg/g Ag. The release of Ag was higher on the first day than on the final day of the study, thereby resulting in a biphasic release of Ag over the time period. Biofilm inhibition and measurement of bactericidal activity was tested on 600 μg Ag-coated catheters in growth medium for 24, 48 or 72 h. The catheters demonstrated significant antimicrobial activity against all tested microorganism inhibiting cell growth and biofilm formation for 72 h. *In vivo* studies investigated the toxicity and biodistribution of Ag from radioactive Ag-coated catheters implanted in the dorsum of C57BI/6J mice and monitored for 10 days. Body weight decreased by 8% post treatment, but organ weight was unaffected. No other toxicity was reported. Ag excretion was higher in feces compared to urine with the highest fecal (4.50 ± 0.40 μg) amount on day 2 (approximately 2.1% of implanted Ag) with a decline and plateau of Ag concentration in feces (0.6–1.0 μg/day) by day 6. Silver urine excretion was low (0.02 μg/day) and accounted for 0.1% of the Ag implanted. Cumulative excretion of Ag in feces and urine over a 10 day period was 18.33 ± 0.99 μg and 0.22 ± 0.04 μg. By day 10, approximately 84% of Ag remained attached to the catheters with an Ag recovery rate at 96% on average. The 4% of unaccounted for Ag was reported by authors to be at the implantation site or along the borders of the insertion pockets where the catheter was inserted (Roe et al., 2008).

Using the AgNP catheter release information from Roe et al. (2008), a hypothetical patient exposure situation can be formulated. Assuming 25 μg AgNPs was released daily for 10 days (250 μg cumulative), this equates to 25% of AgNPs released from a catheter coated with 1,000 μg Ag/g of device weight. For a 70 kg patient (25 μg/day • 1 day/70 kg patient) (ANSI/AAMI/ISO 10993-17:2002/(R)2012, 2003), the patient exposure per day from release of AgNPs from a catheter is calculated to be 0.357 μg/kg bw/day. The resulting hypothetical exposure value is 2 fold higher than our pTI of 0.14 μg/kg bw/day with a toxicological risk that is equivocal.

Our pTI value for *i. v.* exposure to 20 nm AgNPs released from medical devices for *i. v.* applications can be compared to other risk assessment values. Weldon et al. (2016) derived an occupational exposure limit (OEL) of 0.19 $\mu g/m^3$ for AgNPs from BMDs from subchronic rat inhalation toxicity assessments with the liver identified as the critical target organ. This OEL can be calculated for a 70 kg adult with an adult air consumption of 20 m^3/day to be 0.05 μg/kg bw/day, which is 2.8 fold lower than the pTI calculated for long-term *i. v.* exposure to 20 nM AgNP. A Tolerable Daily Intake (TDI) value for oral

exposure to AgNP based on noncancer effects was reported by Hadrup and Lam (2014) to be 2.5 μg/kg bw/day. When a 4% value for oral bioavailability (4% BAF_{oral}) is taken into account, the health-based exposure limit equals to 0.1 μg/kg bw/day (Bachler et al., 2013, Loeschner et al., 2011, Kim et al., 2010). This TDI was based on a study by Park et al. (2010b) that reported a NOAEL of 0.25 mg/kg bw/day in mice after daily oral exposure to AgNP (42 nm) for 28 days in both males and females and consideration of a UF of 100 based on Nielsen et al. (2008) (Hadrup and Lam, 2014). Our pTI for *i. v.* exposure to 20 nm AgNPs released from medical devices is similar to the calculated TDI for oral exposure accounting for 4% BAF_{oral}.

5. Conclusion

In summary, this safety assessment derived a pTI value for *i. v.* exposure to 20 nm AgNPs released from blood-contacting medical devices. Criteria for selecting relevant studies to determine a benchmark dose was based on the principles from the Annapolis Accords and ToxRTool analysis. The De Jong et al. (2013) study, a 28-day study in rats investigating a series of immunotoxicological endpoints after exposure to 20 nm AgNP, qualified to serve as the critical study for the pTI derivation. De Jong et al. (2013) reported the lowest dose-dependent critical health effect, which was a $BMDL_{05}$ of 0.14 mg/kg bw/day for increased spleen weight. To derive the pTI, a modifying factor (MF) of 1,000 was applied to the POD to account for interindividual variability (10), potential interspecies difference in potency (10), and the lack of chronic toxicity study data (10) based on scientific review.

The pTI for long-term *i. v.* exposure to 20 nm AgNPs is the first non-cancer risk assessment performed for the *i. v.* exposure of AgNP-containing medical devices. This pTI is not necessarily protective for other sizes or coatings of AgNPs or other administration routes of exposure. The pTI may be used to complete a safety assessment once data is available to estimate the dose of AgNP that patients are exposed to following release from blood-contacting medical devices. The approach will enable toxicological risk assessors to further develop a general index of acceptable toxicological risk with regard to patient *i. v.* exposure to AgNP released from medical devices as additional toxicological data becomes available.

Conflict of interest statement

The authors declare that there are no conflicts of interests.

Note

The findings and conclusions in this paper have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any agency determination or policy. The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by Department of Health and Human Services.

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Appendix A. Supplementary data

The following is the supplementary data related to this article:

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Supplementary material.

Transparency document

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Online data.

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