FINAL REPORT

DATA GAP ANALYSIS SILVER AND SILVER COMPOUNDS

GENERAL OVERVIEW

A joint orientating proposal for "consulting activities in preparation of a risk assessment for human health and environment, to be conducted in analogy to the EU Existing Substances Regulation framework and in consideration of possible future implications of the REACH programme" was submitted by EBRC and EURAS to the Silver Task Force at the beginning of February 2006. In this proposal, a tentative time line was given, assuming a 6 - 7 month period for the draft report.

EBRC/EURAS were commissioned beginning of June 2006 by the Silver Task Force, represented by Johnson Matthey, to address the following initial project phases:

(I) data collection phase (literature searching and collecting, compilation of environmental emission data, as well as information on occupational and consumer exposure)
(II) hazard characterisation - data evaluation and identification of data gaps

It was agreed that all primary literature, other reports, reviews and documents held by the Silver Task Force would be made available to EBRC. Furthermore, literature searches were initiated separately by EBRC and EURAS for human health and environment, respectively.

DATA SOURCES

Data transfer from sponsors:

EBRC received 26 articles and studies from Umicore in June 2006. Furthermore, EBRC received a Notox-report on the data gap analysis on silver nitrate and the booklet "World Silver Survey 2004, GFMS Limited" from Johnson Matthey also in June 2006.

Literature searches conducted by EBRC on HH aspects:

EBRC conducted a literature search covering toxicity data from animal testing and human clinical and epidemiological data. For this search, the database TOXCENTER was used, which comprises of all toxicologically relevant literature from several major bibliographical databases, e.g. CAPLUS, BIOSIS, MEDLINE. After a preview search resulted in an unmanageable amount of unspecific hits (>2000), the search was then narrowed (silver, silver nitrate and silver (I) oxide) and resulted in approx. 340 hits. For these, the bibliographical information and the abstracts were obtained. These abstracts were manually assessed for relevance and assigned to one or more of the TGD data requirements for human health. A documentation of the literature search on STN is given in Annex 2.

In total, 134 articles were assessed as being relevant for assessment of effect on human health. Of these 134 articles, approx. 35 deal with medical applications of silver compounds, which are currently not considered further for the risk assessment of silver chemicals (out of scope of REACH). However, these 35 references (bibliographical information + abstracts, not the full articles) have been archived separately and could be further considered, when necessary.

In addition to the literature search, further sources like the Notox-Report on the data gap analysis on silver nitrate (Notox, 2003), the IUCLID on Silver and Silver nitrate (2000) were also evaluated, and yielded another potentially relevant 98 articles.

Overall extent of the data base as a result of data transfer and literature searches

Altogether about 240 articles/reports are currently available for the HH part (status 14/02/07), as given in Annex 1. Any comments on this allocation and the completeness of the information are most welcome.

DATA EVALUATION PROCEDURE

Data allocation scheme

All incoming data were allocated on a preliminary basis to one or more individual data requirements as specified by the TGD RAR reporting template and agreed upon between the sponsor and the consultant prior to the conduct of this work. The basis for the structure of the data allocation is given overleaf. In Annex 1 to this summary report, a tabular evaluation of data sorted by data requirement is presented.

Secondary literature

Reviews and international summary reports (WHO etc.) were screened for references that were not identified by the literature search where they clearly appeared to contain relevant information. These are identified endpoint-by-endpoint in the tabular completeness check provided further below. All these documents received a reliability code of "4" since they do not represent peer-reviewed primary sources of information or GLP reports. However, further potentially relevant primary literature was identified upon screening of these reviews. Some of this literature was already available as a result of our searches, whereas others would still need to be obtained. A list of this primary literature is given in Annex 3.

However, further procurement and subsequent evaluation of this data is made dependant on a decision by the sponsors, since the bulk of these sources are older than 25 years. Despite that they may provide supportive information, it is unlikely that they correspond to current guideline standards.

Procedure for literature screening and data gap analysis

Each literature item relevant for human health was screened by EBRC in detail to verify or reject our preliminary assignment, or the need to re-assign any document to another data requirement. For the majority of literature items, a brief summary was obtained from this screening procedure and a preliminary reliability rating was given where applicable. Summaries and reliability ratings are included in the table attached to this report. The preliminary quality screening procedure was applied according to the scoring scheme of Klimisch[1] as follows:

1 = reliable without restrictions:
"Studies or data ... generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to GLP) or in which the test parameters documented are based on a specific (national) testing guideline ... or in which all parameters described are closely related/comparable to a guideline method."

2 = reliable with restrictions:
"Studies or data ... (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable."

3 = not reliable:
"Studies or data...in which there were interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure (e.g., non physiological pathways of application) or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for assessment and which is not convincing for an expert judgment."

4 = not assignable:
"Studies or data....which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature (books, reviews, etc.)."

[1] Klimisch et al.: A systematic approach for evaluating the quality of experimental and ecotoxicological data, *Reg.Tox. and Pharm.* 25, 1-5 (1997)

SCOPE OF THE PROJECT

The scope of the project extends to currently only 3 silver compounds, as agreed in February 2006. A list of these 3 compounds is given in the table below:

Substance	CAS	EC	Formula	MW [g/mo]	Ag valency	Water solubility	EU-Classification
Silver (metallic)	7440-22-4	231-131-3	Ag	107.86	0	Insoluble [1]	Not classified
Silver nitrate	7761-88-8	231-853-9	$AgNO_3$	169.87	+ I	2160 g/L (at 20 °C)[2]	C, R34 N, R50-53
Silver (I) oxide	20667-12-3	243-957-1	Ag_2O	231.74	+ I	0.022 g/L [1]	Not classified

[1] Toxicological Profile for Silver. Agency for Toxic Substances and Disease Registry. U.S. Public Health Service, December 1990.
[2] Concise International Chemical Assessment Document 44, International Programme on Chemical Safety (IPCS), 2002

INITIAL CONSIDERATIONS ON POSSIBLE READ-ACROSS

In the table above, merely silver metal and two mono-valent compounds are addressed, one of high and the other of very low water solubility.

Water solubility is a poor surrogate for bioavailability, so that we propose the conduct of „bioaccessibility" testing for the given compounds in various physiologically relevant media, in order to provide basic data for any read-across considerations and/or in support of derogation from testing.

However, generally this document assumes that testing of the soluble nitrate (assumed to be the most bioavailable form of silver) would allow read-across to the poorly soluble oxide and ultimately metallic silver as well, although realising at the same time that such extrapolation would constitute a worst-case in view of the (assumed) lesser bioavailability of the metal and the oxide. Thus, considerations should be given on an endpoint-by-endpoint basis on whether or not such read-across may be disadvantageous, so that testing of a poorly soluble form of silver may also have to be considered at a later stage.

Finally, we note that particle size considerations play a key role in our understanding of read-across with respect to inhalation toxicity and systemic uptake, and particularly for subsequent risk assessment under occupational circumstances. Appropriate laboratory (non-animal) testing is recommended further below, where appropriate.

EXPOSURE ASSESSMENT

For the sake of completeness, we explicitly note here that searching, collecting and evaluating data on consumer, occupational and indirect exposure via the environment at this point was not part of this project as commissioned by the Silver Task Force. Whereas such data were recovered as a "by-product" of our literature searches, their evaluation has been put aside for the time being.

RESULTS OF LITERATURE SCREENING AND DATA GAP ANALYSIS

Physico-chemical data

This section briefly addresses all information requirements on physico-chemical properties for substances >=1000 t/a (according to points 7.1-7.17 in Annexes VII - X of the REACH regulation, dated 30.12.2006). For metals and their inorganic compounds, the following phys-chem. parameters are considered essential for risk assessment and/or identification purposes:

REACH No.	Requirement	Comments
7.1.	State of the substance at 20°C and 101,3 kPa	available
7.2.	Melting/freezing point	available from secondary literature
7.3.	Boiling point	derogation anticipated
7.4.	Relative density	available from secondary literature
7.7	Water solubility	partly available from secondary literature
7.14	Granulometry / Particle Size information	data gap, studies required

The following parameters formally need to be addressed, but are of minor or no relevance for risk-assessment of metals and inorganic metal compounds, and may therefore be addressed predominantly by way of derogation for lack of relevance:

REACH No.	Requirement	Comments
7.5	Vapour pressure	derogation anticipated
7.6	Surface tension	derogation anticipated
7.8	Partition coefficient n-octanol/water	derogation anticipated
7.9	Flash point	derogation anticipated
7.10	Flammability	derogation anticipated
7.11	Explosive properties	derogation anticipated
7.12	Self-ignition temperature	derogation anticipated
7.15	Stability in organic solvents and identity of relevant degradation products. Only required if stability of the substance is considered to be critical.	derogation anticipated
7.17	Viscosity (registrant obliged to make testing proposal)	derogation anticipated

The following data requirements may have to be addressed either on a case-by-case basis (oxidising properties), or it may be assumed to be partly solved by handbook data and/or exp. investigations (dissociation constant):

REACH No.	Requirement	Comments
7.13	Oxidising properties	to be discussed on a case-by-case basis, derogation foreseen in most cases
7.16	Dissociation constant	registrant obliged to make testing proposal

Conclusions on data gaps, physico-chemical data

The melting/boiling point data and the data concerning rel. density and partly water solubility were extracted from handbook sources. However, the quality and reliability of these data may be considered questionable, since the origin of these data and the test methods used are usually not documented.

In view of the relative importance for read-across based on assumed bioavailability, it should therefore be considered on a case-by-case basis to conduct experimental investigations on water solubility to address this endpoints for all compounds.

For lack of reliable data concerning the endpoints relative density and particle size, and considering their importance for inhalation exposure risk assessment, the conduct of experimental investigations on these parameters is strongly recommended.

Either a valid scientific argumentation for not providing data on oxidising properties of silver nitrate, or a test should be provided. We also suggested determining the dissociation constant for silver nitrate, if this can not be retrieved otherwise.

4.1.2.1 - Toxicokinetics:

Approximately 50 articles were identified, some of which contain information that is only remotely connected to toxicokinetics. For an initial screen as to their relevance and reliability for risk assessment purposes, they were grouped into the following categories

(i) reviews and toxicological summaries (n=11)

These were not evaluated in full detail at this stage because they represent secondary literature, and not primary sources of information, as required. However, for the sake of completeness and in order not to miss any sources identified as valuable data in such previous toxicological reviews, they were screened carefully whether or not relevant data are cited therein which has not been recovered in our literature searches, recovering the following publications:

literature cited in EBRC #24: Durbin (1960), Kehoe et al. (1940), Kent & McCance (1941), Tipton et al. (1966), McKee & Wolf (1963), Natusch et al. (1974), Standler & Vonnegut (1972),
literature cited in EBRC #31: East et al. (1980), McIntyre (1978), Snyder et al. (1975),

In a second step, the above mentioned literature was additionally acquired and subjected to the same data screening procedure as others previously.

(ii) inhalation absorption data (n=3)

With the exception of one article (on ultra-fine particles < 20 nm, dated 2001), the other sources are quite dated (older than30 years), and their relevance may be questioned. Nevertheless, they provide supportive data on absorption and clearance after inhalation of radio-labelled Silver particles (0.5 µm size). However, the (ultra-fine) particle sizes investigated are unlikely to be relevant for the assessment of inhalation absorption under most occupational circumstances, where aerosol diameters are usually much higher. Therefore, for the assessment of inhalation absorption of commercially produced Silver compounds, we propose the following testing programme which is in accordance with the guidance developed under HERAG:

- collection of particle size data on all grades of silver powder, nitrate and oxide

- selection of one or more representative test materials for each compound, and conduct of particle size and dustiness measurements (note: reliable rel. density data are essential for this approach)

- deviation of aerodynamic diameters for these material, and calculation predictions of deposition patterns in the respiratory tract (MPPD model) for the derivation of substance-specific inhalation absorption factors

(iii) dermal absorption data (n=6)

One review article (Hostynek1993) exists which contains only one reference to published silver-related investigations (Wahlberg, 1965; Skog & Wahlberg, 1964), in which a percutaneous absorption experiment with ^{110m}Ag as tracer in guinea pigs *in-vivo is* described. Whereas the authors conclude on a dermal absorption rate of < 1%, the study by current standards has considerable methodological drawbacks, and does not represent a robust, risk-assessment compliant data source.

All other available data relate to either non-standard test systems, or absorption through wounded or burnt skin, and therefore are not relevant to the assessment of percutaneous absorption through intact skin, as required for RA purposes. In conclusion, no reliable dermal absorption data exist, so that the conduct of a dermal absorption study should be considered.

(iv) oral absorption data (n=1)

There are no robust data available to establish a definitive figure for the oral absorption rate for any of the three Silver compounds.

Thus, the conduct of a relative oral bioavailability study with all three compounds should be considered, involving at least a single low and a single high dose, as well as repeated dosing. The dose levels should reflect approx. dietary intake level (general population), as well as low and high occupational exposure levels.

(v) comprehensive toxicokinetic studies incl. distribution and elimination (n=10)

The distribution among organs for various routes of administration including elimination rates has been studied reasonably well. In particular for the phenomenon of argyria, detailed investigations are available.

Further, detailed studies on the biliary elimination pathway ultimately via faeces also exist, which address both dietary and “excess” oral exposure levels.

Notwithstanding the results of a more extensive validation of the available studies, this particular end-point is not an obvious data gap.

However, relative bioavailability of poorly soluble forms (silver metal and oxide) in comparison to the soluble nitrate remains an open issue; instead of in-vivo studies, perhaps less expensive in-vitro bioacessibility testing may be a way forward.

(vi) studies investigating the mechanism of argyria (n=16)

A large number of published studies exist in which the pattern of deposition in body tissues linked to argyria is described. Whereas these investigations do not necessarily address RA-specific endpoints, their abundance and detail nevertheless appear to describe this silver-specific pathological circumstance adequately.

For lack of direct relevance for risk assessment purposes, further evaluation of these studies is suggested to be deferred to the dossier evaluation phase.

4.1.2.2 - Acute toxicity:

42 literature items were assigned to this section, 5 of them are review articles (secondary literature) which still need to be followed up by acquisition of potentially relevant, additional primary literature, if required by the client.

Animal studies:

acute toxicity -oral (n=7):
Silver (I) oxide: one GLP study (#5, data holder Umicore), LD50 > 2000 mg/kg bw
Silver powder: one GLP study (#13, data holder Umicore), LD50 > 2000 mg/kg bw
(a study summary of a test on a colloidal silver solution (#16) is also available)
Silver nitrate: one published study in rats and rabbits (#104), 200 mg/kg bw < LD50 < 2000 mg/kg bw and one published very old study in mice (#231), LD50: 50mg/kg bw
Other silver compounds: two published study (#153and #231)

Notwithstanding a final complete validation, read-across between silver compounds is currently not required since data for all three substances are available - no data gaps at this point identifiable.

acute toxicity -dermal (n= 1):
Silver nitrate: one published study with percutaneous and intraperitoneal application in guinea pigs (#41) exists, which is not a guideline-conform test, and only uses a single, inconclusive dose (LD50 > 216 mg/kg bw).

However, no information on acute dermal toxicity was identified for silver metal and silver (I) oxide at this stage of the project.

A state-of-the art percutaneous toxicity in rats with silver nitrate should be considered, with read-across (worst-case) to the other two substances.

acute toxicity -inhalation (n=3):
Silver powder: two inhalation studies are available; one study in dogs (#3) which is however principally a toxicokinetic study, and one study in rats which deals with ultra-fine silver particles (#91) – both studies do not provide data reliable for risk assessment purposes. Silver nitrate was tested in rats by intra-tracheal instillation (#91) – not relevant because of the route of administration.

There is no information on acute inhalation toxicity for silver (I) oxide.

In conclusion, an acute inhalation toxicity study in rats with silver nitrate as a soluble substance should be performed in the first instance. In the case that no toxicity is observed up to the limit dose, then no further testing is considered to be required, and derogation by way of read-across can be applied. Should however toxicity be observed, then a second study with a poorly soluble form of silver should be considered.

acute toxicity – other routes of administration (n=5):
Silver nitrate: three studies with i.p. administration (#41, #104, #200)
Other silver compounds: one studies with i.p., or s.c. administration in mouse (#159, #153)

This is not a core data requirement, and therefore these studies are not considered further at this point.

Other data

Apart from the animal studies discussed in more detail above, 5 human case reports and 21 reports on in-vitro cytotoxicity were also identified, which are not further addressed here since they do not address a formal data requirement under REACH.

4.1.2.3 - Skin/Eye irritation:

21 literature items were assigned to this section; 2 of them are review articles (secondary literature) with no relevant information on irritation; however, one review (#220) still needs to be followed up by acquisition of potentially relevant, additional primary literature (if required).

Skin irritation (n=4):
Silver (I) oxide: one GLP study (#6, data holder: Umicore): non-irritating
Silver powder: one GLP study (#12, data holder: Umicore) non-irritating
Silver (II) oxide: one GLP study (#14, data holder: Umicore) non-irritating
Silver nitrate: 1 published in-vitro study exists, which is not a guideline-conform test, but an indication for a skin irritation potential is given (#198)

Data for silver (I) oxide and silver powder are available - no data gaps at this point identifiable.

Notwithstanding a final complete validation, a state-of-the art dermal irritation study with silver nitrate is not likely to be required. According to OECD 404, an in vivo test is not required if an accepted in vitro test leads to an irritant or corrosive response, and irritancy or corrosivity will be assumed in vivo correspondingly. Furthermore, in the light of the existing classification C, R34 for silver nitrate, further experimental testing would appear redundant.

The basis for this classification needs to be explored, and a discussion of whether this was done on a sound basis or whether the classification should be challenged needs to be made.

Eye irritation (n=11):
Silver (I) oxide: one GLP study (#7, data holder: Umicore): corrosive
Silver powder: one GLP study (#11, data holder: Umicore): non-irritating
Silver (II) oxide: one GLP study (#15, data holder: Umicore): irritating
Silver nitrate: 5 published studies exist, which are not guideline-conform tests, but indication for an eye irritation potential is given (#77, #175, #176, #197, #217)

Data for silver (I) oxide and silver powder are available - no data gaps at this point identifiable.

A definitive statement on silver nitrate is not yet possible, for the following reasons: A state-of-the art eye irritation study with silver nitrate does not exist, neither in vitro nor in vivo. However, since according to OECD 405, in vivo testing is not required if an accepted in vitro test leads to an irritant or corrosive response, a more detailed evaluation of the data is required.

Respiratory tract (n=3)
One identified published article is not relevant for this data point (#221). One published article exists, where the ultra structure of the epithelium in rabbits after inhalation exposure to colloidal silver solution is described with an indication of respiratory irritation (#233); not considered relevant under this project. One published mechanistic study on pleurodesis in rabbits using silver nitrate is available, which is not particularly relevant for risk assessment purposes (#73), despite indications of irritation effects.

In conclusion, there is some indication of respiratory irritation which needs to be further addressed in follow-up evaluations, but no immediate testing requirement.

Other data

Apart from the animal studies discussed in more detail above, 1 human case report (#144) was identified, giving an indication of irritation potential for silver nitrate and silver oxide which requires more detailed review. Furthermore, one article describing a special investigation (#196) was not further addressed here since this article does not address a formal data requirement under REACH. One published article on the interpretation of Draize scoring in general (#209) was identified as not relevant.

4.1.2.4 - Corrosivity:

No documents were assigned to this section (4.1.2.4). However, we assume that this data requirement is already addressed in section 4.1.2.3 (Irritation).

4.1.2.5 - Sensitisation:

Merely 5 literature items were assigned to this section:

2 review articles (#28, #31) needed to be followed up by acquisition of potentially relevant, additional primary literature as follows: Heyl et al. 1979 and Marks 1966. In a second step, the above mentioned literature was acquired and subjected to the same data screening procedure as others previously. However, no further valuable information was obtained from these articles.

One published human sensitisation study on silver nitrate (#112) exists, where however the results are only sparsely reported, and therefore this article can only be used as supplementary data (a moderate allergic reaction was observed for silver nitrate). One published human case report (#210) with silver amalgams, which can only be used as supplementary data. One published article on the biocompatibility of implanted alloys was rated as not relevant (#113)

At this stage, no reliable data could be assigned to this section, which adequately address the issue of skin sensitisation potential of silver nitrate, silver (I) oxide or silver metal. Thus, the conduct of a guideline-conform skin sensitisation study with silver nitrate should be considered, with subsequent potential read-across from this soluble form to the other two substances.

4.1.2.6 - Repeated dose toxicity:

A rather large number of studies (approx. 50) were initially allocated to this endpoint. However, upon closer inspection, some of these (n=9) do not actually represent toxicity studies, but instead mechanistic studies investigating the course and effects of argyria. These were subsequently shifted to a separate chapter (4.1.2.1.4 - Mechanistic studies on argyria). The available studies (including human data) can therefore be broken down as follows:

- 4 studies were regarded as not relevant, because of the route of administration or endpoint
- 9 studies with information about deposition of silver in different tissues and no information on repeated dose toxicity (shifted to other chapter)
- 4 studies with information on deposition and some limited information for repeated dose toxicity
- Two studies in mice which are limited because of the study design (1 dose level) and the endpoints evaluated
- One behavioural study in rats which is limited because of the design (1 dose level) and the endpoint
- Two Russian study for which the information is taken from the IUCLID dataset; studies performed in the 1970
- Two Russian studies for which the information is taken from the IUCLID dataset, but limited because only one specific endpoint evaluated
- Two studies in chickens which are not relevant for human (probably more interesting for environmental toxicity)
- One dermal study in guinea pigs, but only effects on the skin evaluated and not systemic toxicuty
- One study with 2 dose levels in pigs
- One French publication with information (taken from IUCLUD) on dermal, oral and i.p toxicity
- One study in close compliance with OECD guidelines, but the route of administration was via swabbing of the oral cavity
- 8 human case reports
- 8 human clinical/field studies

Three further studies were regarded as “other” toxicity studies, addressing in a wider context endpoints such as immunotoxicity and neurotoxicity.

In view of the extent of the available primary literature, several existing review articles (n=7) were not evaluated comprehensively because of time constraints. However, since the selected sources above already do not meet the requirements, it is debatable whether other sources may provide any additional, reliable information (further procedure to be discussed with the sponsor).

Whereas there are several studies with repeated oral administration, none of these studies except for one provide reliable information, because only a limited number of endpoints and/or effects were investigated. There is only one acceptably conducted and documented study (#104), but the route of administration (swabbing of the oral cavity) is not relevant for evaluation of the repeated dose toxicity following oral treatment, because an exposure correlation can not be done.

In conclusion, a sub-chronic oral toxicity study in rats should be conducted to provide a minimal basis for deriving no-effect levels for subsequent risk assessment purposes. Tentatively, it is therefore proposed to conduct such a study on a soluble silver compound (i.e. nitrate) and to read-across to the oxide and the metal, although recognising that this will be a worst-case read-across in view of the anticipated lower bioavailability of these two forms (subject to further review/confirmation).

4.1.2.7 - Mutagenicity:

24 articles were available for this endpoint, of which three represent reviews (secondary literature).

- Three publication were regarded as not relevant (one cytotoxicity test, 2 with irrelevant exposure scenarios)
- Two UDS tests are available (one in accordance with existing guidelines)
- 9 bacterial mutation assay with different endpoints (reverse mutation, forward mutation, photogenotoxicity, bioluminescence, SOS chrom) of variable quality
- One viral transformation assay in SCH cells
- Two chromosomal malsegregation assays in fungi
- One Russian publication about chromosomal aberration in root tips
- One HGPRT test with silver diaminotetraborate (of questionable relevance) and one in which it was stated that AgNO3 was positive in a preliminary assay.
- One drosophila melanogaster test
- Three reviews: some references stated in this reviews are not available and need to be checked.

The overall result from the tests above is equivocal, with some negative (11) as well as some positive (7) conclusions. However, except for a small selection of bacterial mutation tests, none of the above in-vitro studies on different endpoints document information from reliable, guideline-conform test systems, except for one UDS test in accordance with existing guidelines. Apart from this, other relevant in-vivo data were not identified.

In conclusion, it should be considered in a first step to conduct a standard package of in-vitro state-of-the-art tests on cytogenicity and gene mutation, and depending on the outcome, prepare for testing corresponding endpoints in-vivo as well.

4.1.2.8 – Carcinogenicity:

Only very little data (13 published items, including one review) at all are available, none of which qualify for this endpoint because of the route of administration. In conclusion, there are no classical or special short-term carcinogenicity studies available.

- Two publications were regarded as not relevant (lack of information related to carcinogenicity and silver)
- Three publications about implantation of silver foil in rats
- One publication with i.m. and one with a combination of s.c. and i.v. administration
- Three in-vitro cytotoxicity studies in alveolar macrophages, which are not relevant for this endpoint
- Two in-vivo studies with single intra-abdominal administration to investigate the fibrogenic potential

The lack of a guideline-conform, combined chronic toxicity/carcinogenicity study constitutes a formal data gap. However, a more detailed evaluation of all available animal and human data together with the anticipated “new” genotoxicity data should be conducted either to explore the possibility of derogating from testing, or in the definition of the testing proposal that needs to be developed under REACH.

4.1.2.9- Reproduction toxicity

Only very few studies on reproduction toxicity exist, which except for one are older than 25 years, and correspondingly lack guideline-compliance and relevance, although some information about effects on male and female fertility can be extracted:

- One review article
- Two publications with some information about effects on the male/female reproductive tract (repeated dose tox)
- One developmental study in monkeys, in which the effect of a single intrauterine dose on the progress of pregnancy was evaluated; however, no classical developmental toxicity endpoints were evaluated
- One developmental study in rats (dated 1995) with one high embryotoxic dose; designed as a mechanistic study to evaluate the effect of ceroluplasmin on the embryotoxicity of silver; may be used as supportive data on embryotoxicity and gross visceral effects
- One in-vitro study with human sperm
- One publication with an invalid study design (intratesticular injection)

Developmental toxicity: Whereas there is some information available on embryotoxicity and teratogenicity (visceral malformations) in rats, the available study does not provide information on skeletal effects and only involves a single embryotoxic dose. Thus, the conduct of a guideline-conform teratogenicity study in rats should be considered to address this endpoint properly.

Reproduction toxicity: No reproduction toxicity study available, which constitutes a formal data gap.

Annex I: Tabular evaluation of data sorted by data requirement

4	Human Health

Section	Description	Pub/GLP	Key findings	Rating/Comments	Source	EBRC#
4.1.2	**Effects assessment – reviews (general interest and review articles)**					
4.1.2	**Review** Test substance: Guideline:	Pub.: Y GLP: N	Rather brief review on several aspects of silver. The following cited primary literature on human health is not available: Polachek et al. 1960, Eichhorn 1973, Petering 1976, Smith and Carson 1977, Hill et al. 1964, Bunyan et al. 1968, Shaver and mason 1951	RL = 4 Secondary literature	Petering, H.G. (1984): Chapter II.20: Silber. In: Metalle in der Umwelt - Verteilung, Analytik und biologische Relevanz, 555-560	21
4.1.2	**Review** Test substance: Silver nitrate Guideline:	Pub.: Y GLP: N	Comprehensive overview of published data on silver nitrate.	RL = 4 (secondary literature) followed up by checking on cited prim. literature	Humphreys, S.D.; Routledge, P.A.. (1998): The toxicology of silver nitrate. Adverse Drug React. Toxicol. Rev. 17, 115-143	49
4.1.2	**Review** Test substance: Guideline:	Pub.: Y GLP: N	Most recent and comprehensive overview of published data The following cited primary literature on toxicology is not available: Sue et al. 2001, Juberg and Hearne 2001, Brooks 1981, Wobling et al. 1988, Williams and Gardner 1995, Grabowski and Haney 1972, Fung and Bowen 1996, Rongioletti 1992, Buckley et al. 1965, Egli 2000.	RL = 4 (secondary literature) followed up by checking on cited prim. literature	Drake, P.L.; Hazelwood, K.J. (2005): Exposure-related health effects of silver and silver compounds: a review. Ann. Occup. Hyg. 49, 575-585	70
4.1.2	**Review** Test substance: Guideline:	Pub.: Y GLP: N	only very brief summary of silver toxicity, source rather out of date (1979)	RL = 4 (secondary literature)	Weir, F.W. (1979): Health hazard from occupational exposure to metallic copper and silver dust. Am. Ind. Hyg. Assoc. J. 40, 245-247	167

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01	Review documents considered either relevant or useful					
4.1.2.01	**Toxicokinetics, in vivo, absorption** Test substance: Silver (unspecified) Guideline: Not applicable	Pub.: Y GLP: N	Once deposited in the layers of the ski of humans, silver accumulates throughout the ageing process.	RL = 4 (secondary literature)	Hostynek, J.J.; et al. (1993): Metals and the skin. Crit. Rev. Toxicol. 171-235	190
4.1.2.01	Test substance: Silver (unspecified) Guideline: Not applicable	Pub.: Y GLP: N	Interaction of Silver with other metals - the potential of Silver to induce Selenium deficiency is discussed	RL = 4 (secondary literature)	Ganther, H.E. (1980): Interactions of vitamin E and selenium with mercury and silver. Ann. N.Y. Acad. Sci. 355, 212-226	165
4.1.2.01	Test substance: Silver compounds (various) Guideline: Not applicable	Pub.: Y GLP: N	only brief overview of Ag body burdens; inhal. absorption given with 50-80% for fine particles; other abs. data not described with adequate precision	RL = 4 (secondary literature) followed up by checking on cited prim. literature	Klein, D.A. (1978): Chapter 12: Effects on humans. Klein, D.A. (ED.): Environmental Impacts of Artificial Ice Nucleating Agents, Chap. 12, 169-175	24
4.1.2.01	Test substance: Silver metal Guideline: Not applicable	Pub.: Y GLP: N	EU criteria document; most recent and comprehensive overview of published data oral abs. 10-18%, tissue burdens of occ. non-exposure very low, clearance predominantly via liver and subsequent faecal elimination	RL = 4 (secondary literature) followed up by checking on cited prim. literature	Jongerius, O.; Jongeneelen F.J. (1990): Occupational exposure limits. Criteria Document for metallic silver. Commission of European Communities	222
4.1.2.01	Test substance: Silver and Silver nitrate Guideline:	Pub.: Y GLP: N	There is evidence that inhalation of silver nitrate can irritate the upper respiratory pathway and the gastrointestinal tract; however, these effects may be due to the caustic properties of the substance rather than the presence of silver. Silver absorbed into the body by inhalation and ingestion is mainly deposited in liver, kidneys, pancreas, skin and conjunctiva. There is no evidence that the silver deposits impair the functioning of these organs.	RL = 4 (secondary literature) followed up by checking on cited prim. literature	Anonymous (1990): Toxicological profile for silver. ATSDR - Agency for Toxic Substances and Disease Registry	31

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01	Review documents of limited benefit					
4.1.2.01	Test substance: Silver and its compounds Guideline: Not applicable	Pub.: Y GLP: N	only very brief summary of toxicokinetics, little useful information source rather out of date (1978)	RL = 4 (secondary literature)	Venugopal, B.; Luckey, T.D. (Eds.) (1978): Cap. 1 – Toxicity of group I metals. Metal Toxicity in Mammals, vol. 2, cap. 1, pp. 1-39	232
4.1.2.01	Test substance: Silver (unspecified) Guideline: Not applicable	Pub.: Y GLP: N	ICRP-Publication; reference intakes for humans given; otherwise, only few statements on toxicokinetics provided	RL = 4 (secondary literature)	Snyder, W.S.; et al. (Eds.) (1975): Chapter 3 – Physiological data for reference man. Report of the Task Group of Reference Man, Pergamon Press	237
4.1.2.01	Test substance: Not applicable Guideline: Not applicable	Pub.: Y GLP: N	Document acquired because mentioned in other secondary literature. Upon detailed inspection, no information on silver whatsoever contained therein.	RL = 4 (secondary literature)	Natusch, D.F.S.; et al. (1974): Toxic trace elements: Preferential concentration in respirable particles. Science 183, 202-204	247
4.1.2.01	Test substance: Silver iodide Guideline: Not applicable	Pub.: Y GLP: N	Orientating risk assessment for the use of silver iodide as a cloud-seeding agent. Upon detailed inspection, no relevant information on toxicokinetics of silver contained therein.	RL = 4 (secondary literature)	Standler, R.B.; Vonnegut, B. (1972): Estimated possible effects of AgI cloud seeding on human health. J. Appl. Meteorol. 11, 1388-1391	248
4.1.2.01	Test substance: Silver (unspecified) Guideline: Not applicable	Pub.: Y GLP: N	No useful information for current RA contained in thus review document.	RL = 4 (secondary literature)	McKee, J.E.; Wolf, H.W. (Eds.) (1963): Water quality criteria. Water Quality Criteria, 2nd Ed., Calif. State Water Qual. Control Board Publ. 3A, 246-257	246
4.1.2.01	Test substance: Silver metal Guideline: Not applicable	Pub.: N Data holder: Umicore GLP: N	only fragmentary records; no valuable information on toxicokinetics	RL = 4 (secondary literature)	Anonymus (1993): Silver (metallic). AIDA Grunddatensatz, pp.33	28

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.1	Toxicokinetics, metabolism and distribution – studies in animals					
4.1.2.01.1	**Toxicokinetics, in vivo, retention and excretion** Test substance: Silver (110Ag) nitrate Guideline: Not applicable species: rats, mice, dogs, primates i.v., i.p. and oral administration excretion monitoring	Pub.: Y GLP: N	More than 90% of an oral dose was not absorbed; excretion was predominantly (>90%) via faeces.	RL = 2 reasonably well-documented publication	Furchner, J.E.; et al. (1968): Comparative metabolism of radionuclides in mammals-IV. Retention of silver-110m in the mouse, rat, monkey, and dog. Health Physics 15, 505-514	186
4.1.2.01.1	**Toxicokinetics, in vivo, tissue distribution** Test substance: Silver nitrate Guideline: Not applicable species: mouse drinking water study, repeated exposure	Pub.: Y GLP: N	Considering the background of the WHO drinking water limit of 0.1 mg/l, this study investigated tissue retention and distribution after 1and 2weeks of exposure via drinking water (0.03mg Ag/l). Significant deposition of silver in barn and muscle were observed at this exposure level.	RL = 2 reasonably well-documented publication	Pelkonen, K.H.O.; et al. (2003): Accumulation of silver from drinking water into cerebellum and musculus soleus in mice. Toxicology 186, 151-157	78
4.1.2.01.1	**Toxicokinetics, in vivo, inhalation absorption** Test substance: Silver metal (ultra-fine particles) Guideline: Not applicable species: rats (female)	Pub.: Y GLP: N	Rats were exposed (whole body) for 6h to a silver aerosol of 15 nm particle diameter; exposure concentration 133 µg/m³; estimated cumulative dose 7.2 g. persistence in the lungs was investigated by intratracheal instillation (AgNO3). Silver was cleared rapidly from lungs, with only 4% of dose remaining after 7 days.	RL = 2 well-documented publication	Takenaka, S.; et al. (2001): Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ. Health Persp. 109, 547-551	91
4.1.2.01.1	**Toxicokinetics, in vivo, tissue distribution and excretion** Test substance: Silver metal Guideline: Not applicable species: rats i.v. administration, 0.01-0.3 mg/kg bw	Pub.: Y GLP: N	Comparative investigation of 18 metals. Rapid and almost total excretion of silver, mostly via faeces; biliary pathway verified; highest tissue concentrations in liver.	RL = 2 reasonably well-documented publication	Gregus, Z.; Klaassen, C.D. (1986): Disposition of metals in rats: a comparative study of fecal, urinary, and biliary excretion and tissue distribution of eighteen metals. Toxicol. Appl. Pharm. 85, 24-38	191

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.1	**Toxicokinetics, in vivo, tissue distribution** Test substance: Silver nitrate Guideline: Not applicable species: rats	Pub.: Y GLP: N	The tissue distribution of 110Ag was measured after intramuscular injection; the overwhelming does 1 day p.a. was found in liver (66%). Follow-up by reference to the source publication (Hamilton, 1951) not considered to be required in view of the lack of relevance of the route of administration.	RL = 4 inadequate detail of reporting	Durbin, P.W. (1960): Metabolic characteristics within a chemical family. Health Phys. 2, 225-238	245
4.1.2.01.1	**Toxicokinetics, in vivo, tissue distribution** Test substance: Silver nitrate Guideline: Not applicable species: rats Route of administration: i.p.	Pub.: Y GLP: N	The i.p. administration of radioactive silver nitrate to tumour-bearing rats led to higher liver and blood level in tumour-bearing animals due to the impaired elimination via liver and biliary excretion. An increase in radioactivity was also observed in the tumour tissue. Of little use in a risk assessment context.	RL = 2 reasonably well-documented publication	Habighorst, L.V.; Buchwald, W. (1971): Der Metabolismus radioaktiver Silber- und Quecksilberverbindungen bei Tumorträgern im Tierexperiment. Nucl. Med. 9, 35-38	219
4.1.2.01.1	**Toxicokinetics, in vivo, distribution** Test substance: Silver nitrate Guideline: Not applicable species: guinea pig; uptake of silver ions after antiseptic treatment of burns or wounds with silver nitrate	Pub.: Y GLP: N	Continuous application of a 0.5 % silver nitrate (radiolabel: 111 Ag) solution to a square area of removed full thickness skin yielde dteh following results: - the majority of radioactivity remained in the skin - significant amounts of silver appeared in the liver and less in kidneys - no significant uptake in the reticulo-endothelial system - less than 40 % of radioactivity remained in the liver after 1 week, less than 25 % after 2 weeks	RL = 2 reasonably well-documented publication	Constable, J.D. et al. (1967): Absorption pattern of silvernitrate from open. Plast. Reconstruct. Surgery 39, 342-348	218
4.1.2.01.1	**Toxicokinetics, in vivo, dermal absorption** Test substance: Silver nitrate Guideline: Not applicable guinea pig study, “disappearance analysis”	Pub.: Y GLP: N	Less than 1 % of an applied dose of silver nitrate was absorbed across intact skin of guinea pigs within 5 hours of exposure. Test system not according to current standards (i.e., no mass balance, no analysis of actual percutaneous transfer or residual Ag in skin layers). To be considered as supporting data only	RL = 2 reasonably well-documented publication, despite major methodological deficiencies	Wahlberg, R.C.; et al. (1965): Percutaneous toxicity of metal compounds. A comparative investigation in guinea pigs. Arch. Environ. Health 11, 201-204	41

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.1	**Toxicokinetics, in vivo, inhalation absorption** Test substance: Metallic silver (30µCi of 110mAg) Guideline: Not applicable Species beagle dogs (female) Route of admin.: acute inhalation Exposure period: 7-15 min Frequency of treatment: once Doses: 1 mg/kg bw (averaged absolute deposition)	Pub.: Y GLP: N	Up to 90 % of silver particles (mean aerodynamic diameter = 0.5 µm) deposited in the lungs of dogs were absorbed into the systemic circulation 6 hours after exposure. Six hours after intra-tracheal administration of metallic silver to dogs, 96.9, 2.4, and 0.35% of the initially deposited dose was detected in the lungs, liver, and blood, respectively. The remaining silver was detected in the gall bladder and bile, intestines, and stomach. After 225 days, the distribution in tissue type was similar, with most of the silver found in the liver. Dogs excreted approximately 90% of an inhaled dose of metallic silver particles in faeces within 30 days of exposure.	RL = 2 reasonably well-documented publication	Phalen, R.F.; Morrow, P.E. (1973): Experimental inhalation of metallic silver. Health Physics 24, 509-518	3
4.1.2.01.1	**Toxicokinetics, in vivo, absorption and excretion** Test substance: Silver nitrate, Silver sulphate Guideline: Not applicable species: rats route of administration: i.v., i.m. and oral elimination and organ distribution of radiolabelled silver	Pub.: Y GLP: N	At low (close to dietary) intake levels, 93 % of i.v. or orally administered silver was eliminated via after 4 days. Only traces remained in the body after 16 days. Excretion was predominantly via bile into faeces. At high intake level, body retention was much higher. Mild, chloroform-induced liver malfunction reduced liver clearance to a level of less than 0.1% of normal.	RL = 2 reasonably well-documented publication	Scott, K.G.; Hamilton, J.G. (1950): The metabolism of silver in the rat with radio-silver used as an indicator. Univ. Calif. Publ. Pharmacol. 2, 241-262	39
4.1.2.01.1	**Toxicokinetics, in vivo, absorption and excretion** Test substance: Silver nitrate (110Ag) Guideline: Not applicable rats, rabbits, dogs	Pub.: Y GLP: N	Following i.v. administration, silver is excreted predominantly via faeces, as verified by monitoring of secretion via bile. The highest Ag tissue concentrations were observed in liver.	RL = 2 reasonably well-documented publication	Klaassen, C.D. (1979): Biliary excretion of silver in the rat, rabbit, and dog. Toxicol. Appl. Pharmacol. 50, 49-55	23

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.1	**Interaction of metals (Selenium)** Test substance: Silver nitrate Guideline: Not applicable Repeated oral treatment – distribution to kidneys Species: Wistar rats Exposure period: 8 months Frequency of treatment: continuously Doses: 0.1 % (w/v) in drinking water Dose groups: A: Silver nitrate1 % in drinking water for up to 8 months B: Silver nitrate 1 % in drinking water with additional SeO2 injections i.p. (0.5 mg/kg 3 times per week). C: SeO2 alone as B D: controls receiving saline injections i.p. 3 times per week	Pub.: Y GLP: N	Group A (silver nitrate only treatment): ultrastructural changes in the basal membranes of glomerular capillaries and mesangial cells; localised grains of silver sulphide increased in number with dosing time; deposits in mesanglial cells localised as dense or elongated clumps in lysosome-type structures increased in number with time. Group B (silver nitrate plus SeO2 treatment): Increase in depositions in glomerular structures and proximal tubules occurring at an earlier time (2 weeks); after 4 months necrosis in the cytoplasm of proximal convoluted tubules. Group C (SeO2 alone): No significant ultra-structural changes in the glomeruli; after 8 months necrosis of the cytoplasm of proximal convoluted tubules. Sulphide replaced by Selenide in the granules. Group D (controls): no significant changes observed in the kidneys. Conclusion: Selenium accelerates and intensifies the formation of silver deposits in rat kidney.	RL = 2 The study was conducted according to state of the art methodology at that time; methods are described briefly and results are presented in detail and are visualised in figures.	Berry, J.P.; Galle, P. (1982): Selenium and kidney deposits in experimental argyria. Electron microscopy and microanalysis. Pathologie-Biologie 30, 136-140	160
4.1.2.01.1	**Toxicokinetics (unspecified)** Test substance: Silver (unspecified) Guideline: Not applicable species: rat	Pub.: Y GLP: N	study reliability and adequacy not further assignable because of language	RL = 4 not assignable (Russian language, English abstract of poor quality)	Kazimov, M.A. (1990): Correlations of the processes of metabolism of metals after their separate administration and their hygienic significance. Gig. Truda I Prof. Zabol. No. 7, 22-26	134

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.1	**Toxicokinetics, in vivo, distribution** Test substance: Silver nitrate Guideline: Not applicable Species: Wistar rat Route of administration: i.v. tissue distribution of radiolabelled silver (111-Ag) after i.v. injection 24 hr, 1 and 2 weeks after dosing	Pub.: Y GLP: N	The distribution of radioactivity was measured in whole body, testes, epididymis, liver, kidney, brain and blood. Testes were additionally examined histologically by light and electron microscopy. After 24 hr the highest concentrations were observed in liver and blood. After 7 and 14 days the highest concentrations were found in the liver. The concentration of radioactive silver in the testis was about 5 % of the concentration in the liver and remained constant after 1 and 2 weeks, and was detected in interstitial macrophages and in the basal membrane.	RL = 2 reasonably well-documented publication	Ernst, E.; et al. (1991): Ultrastructural localization of silver in rat testis and organ distribution of radioactive silver in the rat. J. Appl. Toxicol. 11, 317-321	129
4.1.2.01.1	**Interaction of metals (Cobalt/Silver)** Test substance: Silver (unspecified) Guideline: Not applicable	Pub.: Y GLP: N	study reliability and adequacy not further assignable because of language	RL = 4 not assignable (Russian language, English abstract of poor quality)	Kazimov, M.A. (1992): The metabolic interaction of cobalt and silver in the body during their combined action. Gig. Truda I Prof. Zabol. No. 11-12, 23-27	126
4.1.2.01.1	**Toxicokinetics, in vivo, distribution** Test substance: Silver nitrate Guideline: Not applicable rats and mice tissue and intracellular distribution monitored; effects of induction of lipid peroxidation and induction of metallothionein also studied	Pub.: Y GLP: N	The organ distribution of radioactive silver ions (110AgNO3) in rats after single i.p. injection 24hr after dosing yielded the highest concentrations in liver, pancreas and spleen, lowest concentrations were observed in brain and muscle. Uptake in liver and kidney was increased by Cd-pretreatment (for metallothionein induction). Silver ion content was highest in the mitochondriae, nucleus, microsomes and membranes. The elimination half life of silver ions from blood was reduced in Cd-pretreated mice from 1.4 to 0.7 d. The elimination half life from liver was increased from 13 to 22 d.	RL = 2 reasonably well-documented publication	Shinogi, M.; Maeizumi, S. (1993): Effect of preinduction of metallothionein on tissue distribution of silver and hepatic lipid peroxidation. Bio. Pharm. Bullet. 16, 372-374	123

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.1	**Interaction of metals (Selenium)** Test substance: Silver (unspecified) Guideline: Not applicable species: rats	Pub.: Y GLP: N	Mechanistic study on the interaction of selenium with silver and the intracellular deposition pattern.	RL = 2 reasonably well-documented publication	Berry, J.P.; et al. (1995): Interaction of selenium with copper, silver, and gold salts. Electron microprobe study. J. Submicro. Cytol. Pathol. 27, 21-28	118
4.1.2.01.1	**Toxicokinetics, in vivo, dermal absorption** Test substance: Silver nitrate Guideline: Not applicable guinea pig study	Pub.: Y GLP: N	Less than 1 % of an applied dose of silver nitrate was absorbed across intact skin of guinea pigs within 5 hours of exposure. Test system not according to current standards (i.e., no mass balance, no analysis of actual percutaneous transfer or residual Ag in skin layers). To be considered as supporting data only	RL = 2 reasonably well-documented publication, despite major methodological deficiencies	Skog, E.; Wahlberg, J.E. (1964): A comparative investigation of the percutaneous absorption of metal compounds in the guinea pig by means of the radioactive isotopes: 51Cr, 58Co, 65Zn, 110mAg, 115mCd, 203Hg*. J. Invest. Derm. 43, 187-192	230
4.1.2.01.2	Toxicokinetics, metabolism and distribution – studies in humans					
4.1.2.01.2	**Absorption and excretion, humans** Test substance: Silver (unspecified) Guideline: Not applicable dietary balance study	Pub.: Y GLP: N	Two individuals were monitored for 30 days in succession for regular dietary intake via food (duplicate diet analysis) plus excretion pattern; Despite the lack of statistical significance (N=2), this study nevertheless provides valuable insight into dietary intake levels, and the absorption/excretion pattern plus a balance assessment.	RL = 2 reasonably well-document human toxicokinetic study	Tipton, I.H.; et al. (1966): Trace elements in diets and excreta. Health Phys. 12, 1683-1689	241
4.1.2.01.2	**Tissue distribution, humans** Test substance: Silver acetate Guideline: Not applicable human (single) case report (argyria from oral anti-smoking remedy)	Pub.: Y GLP: N	Note: same case report as described above (ref # 234).	RL = 4 (not assignable) only very briefly described human case report, inadequate detail	MacIntyre, D.; et al. (1978): Silver poisoning associated with an antismoking lozenge. Br. Med. J. 2, 1749-1750	235

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.2	**Absorption and excretion, humans** Test substance: Silver (unspecified) Guideline: Not applicable human (single) case report (argyria from use of silver nose-wash)	Pub.: Y GLP: N	The silver-in-food intake and excretion pattern of a female argyria patient were monitored in this study. Only little information extractable.	RL = 4 only very briefly document human case report	Kent, N.L.; McCance, R.A. (1941): The absorption and excretion of 'minor' elements by man. I. Silver, gold, lithium, boron and vanadium. Biochem. J. 35, 837-844	243
4.1.2.01.2	**Toxicokinetics, tissue distribution** Test substance: Silver (unspecified) Guideline: Not applicable spectrochemical analysis in biological samples	Pub.: Y GLP: N	Silver concentrations are reported in samples of human tissue, human excreta, food and environmental samples.	RL = 2 reasonably well-documented publication; however, relevance possibly questionable in view of publication date	Kehoe, R.A.; et al. (1940): A spectrochemical study of the normal ranges of concentrations of certain trace metals in biological materials. J. Nutri. 19, 579-592	242
4.1.2.01.2	**Human serum level biomonitoring** Test substance: Silver acetat Guideline: Not applicable human clinical trial 21 human (11 males/10 females); treatment with anti-smoking chewing gum for 12 weeks; up to 6 pieces per day; chewing for 15-30 min.	Pub.: Y GLP: N	Serum concentrations of silver clearly rose after chewing gum use had started, and concentrations quickly returned to normal after use had ceased. The number of silver granules in skin biopsies increased. No participant developed any clinical signs of argyria.	RL = 2 reasonably well-documented publication; methods are described in detail and results are presented adequately in the text and tables	Jensen, E.J.; et al. (1988): Serum concentrations and accumulation of silver in skin during three months treatment with an anti-smoking chewing gum containing silver acetate. Human Toxicol. 7, 535-540	139
4.1.2.01.2	**Absorption, humans** Test substance: Silver nitrate Guideline: Not applicable human prospective study	Pub.: Y GLP: N	A study with 11 human volunteers was conducted with AgNO3 in order to assess silver absorption from the nasal septum resulting from cauterisation of nose bleed. Significant increases of Ag blood concentrations were observed already 3h after administration. However, this information is of little use in conventional risk assessment.	RL = 2 reasonably well-document human case report	Nguyen, R.C.; et al. (1999): Argyremia in septal cauterization with silver nitrate. J. Otolaryng. 28, 211-216	100

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.2	**Tissue distribution, humans** Test substance: Silver (unspecified) Guideline: post-mortem investigation on silver release from amalgam	Pub.: Y GLP: N	Tissue samples (liver, kidney, brain) from a total of 173 deceased humans were analysed for silver, and a causal relationship established with the number of teeth containing amalgam fillings.	RL = 2 reasonably well-documented publication	Drasch, G.; et al. (1995): Silver concentrations in human tissues. Their dependence on dental amalgam and other factors. J. Trace Elem. Med. Bio. 9, 82-87	117
4.1.2.01.2	**Permeability, human blood-brain barrier** Test substance: Silver nitrate Guideline: Not applicable human case report	Pub.: Y GLP: N	Silver deposits were found in neurons of the central nervous system of a female who used silver nitrate nasal drops over an extended period, but without any other (adverse) effects being reported.	RL = 2 reasonably well-documented human case report	Landas, S.; et al. (1985): Demonstration of regional blood-brain barrier permeability in human brain. Neurosci. Letters 57, 251-256	152
4.1.2.01.2	**Biomonitoring study in smelter workers** Test substance: Silver (unspecified) Guideline: Not applicable human biomonitoring study	Pub.: Y GLP: N	Biomonitoring study in smelter workers, on tissue levels of metals including silver. Silver compounds are absorbed into the body by inhalation and ingestion and are mainly deposited in the liver, kidneys, pancreas, skin and conjunctiva. There is no evidence that the silver deposits impair the functioning of these organs.	RL = 2 reasonably well – documented investigation, including tabulate draw Ag data in several key organs	Nordberg, G.F.; Wester, P.O.; Brune, D. (1978): Tissue levels of 25 elements in smelter workers – a preliminary communication. Proc. Int. Symp. Control Air Pollution Working Environment, Libertryck, Stockholm, 261-272	221
4.1.2.01.2	**Toxicokinetics, in vivo, absorption/distribution** Test substance: Silver nitrate Guideline: Not applicable human case report	Pub.: Y GLP: N	Two human case reports on silver tissue deposition after wound treatment with Silver nitrate: (1) Silver was absorbed into body tissues and fluids following treatment of burns with 0.5 % silver nitrate; silver levels ranged from 0.12ppm (blood, urine) to 1250ppm (skin). (2) The skin application of silver nitrate for burn treatment resulted in silver being distributed to muscle (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).	RL = 2 reasonably well-documented human case report	Bader, K.F. (1966): Organ deposition of silver following silver nitrate therapy of burns. Plas. Reconstr. Surg. 37, 550-551	43

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.2	**Toxicokinetics, in vivo, irritation/distribution** Test substance: Silver nitrate Guideline:	Pub.: Y GLP: N	There is evidence that inhalation of silver nitrate can irritate the upper respiratory pathway and the gastrointestinal tract. However, these effects may be due to the caustic properties of this compound rather than the presence of silver. Silver compounds are absorbed into the body by inhalation and ingestion and are mainly deposited in the liver, kidneys, pancreas, skin and conjunctiva. There is no evidence that the silver deposits impair the functioning of these organs.	RL = not rated	Greene, R.M.; Su, W.P.D. (1987): Argyria. Am. Fam. Phys. 36, 151-154	33
4.1.2.01.2	**Toxicokinetics, in vivo, absorption/distribution/excretion** Test substance: Silver metall Guideline: Not applicable human case report	Pub.: Y GLP: N	Absorption from the lung was documented in a case of accidental exposure to radiolabelled silver metal dust. Silver was confined mainly to the liver of a worker who had accidentally inhaled radiolabelled silver metal; a biological half-life of 52 days was estimated. Following oral or inhalation exposure to silver compounds, humans excrete silver primarily in the faeces and only very minor amounts in the urine.	RL = 2 reasonably well-documented human case report	Newton, D.; Holmes, A. (1966): A case of accidental inhalation of zinc-65 and silver-110m. Radiat. Res. 29, 403-412	187
4.1.2.01.2	**Tissue distribution, humans** Test substance: Silver acetate Guideline: Not applicable human (single) case report (argyria from oral anti-smoking remedy)	Pub.: Y GLP: N	Tissue distribution of silver measured in a subject suffering from argyria, who had consumed a silver acetate containing oral anti-smoking remedy on a long-term basis. Measurements (non-invasive) by neutron activation analysis; total body burden estimated at 6.4 g; major deposits in skin (dermis). Note (!): the retention of a single oral dose of silver over 30 weeks was also monitored, including urinary excretion pattern.	RL = 2 reasonably well-document human case report	East, B.W.; et 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, 305-311	234

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.2	**Toxicokinetics, in vivo, tissue distribution** Test substance: Néocarbagol, Carbagol, Collargent-Acétarsol, Collargol Guideline: Not applicable human case reports, chronic exposure	Pub.: Y GLP: N	Results from 4 cases (human females; age: 46, 58, 72, 78 years) are described. The fate of silver in the dermis of chronic argyria was evaluated. Silver was detected in tissue as irregular aggregates of elementary granules. After recent intoxication, the main location is intracellular (elementary particles in lysosomes). After more earlier intoxication, silver is found either in fibrillar components of connective tissue or in the basal material of sweat glands. In summary, silver is first phagocytised by macrophages, which are not able to degenerate silver completely; silver is finally found on connective fibres (on sulphated glycoproteins).	RL = 4 French publication; only English abstract available; information presented here was taken from the abstract.	Reymond, J.L.; et al. (1980): Cutaneous argyria: an electron microscopic study of four eases with microanalysis X study of one case (author's transl) Original Title: Etude en microscopie electronique et en microanalyse X de 4 cas. Ann. Dermatol. Venereol. 107, 251-255	164
4.1.2.01.4	Mechanistic and other studies related to argyria – reviews and other studies					
4.1.2.01.4	**In vitro mechanistic study, tissue binding** Test substance: Silver nitrate Guideline: Not applicable kinetics of silver binding by tissue components	Pub.: Y GLP: N	In vitro incubation of sections of human spinal cord with silver nitrate (1 g/l at pH 7.5 or pH 9) and radioactive labelled silver (111Ag); a time-dependent binding of silver ions followed by a reduction to metallic silver was observed.	RL = 2 reasonably well-documented publication	Gallyas, F. (1979): Kinetic of formation of metallic silver and binding of silver ions by tissue components. Histochemistry 64, 87-96	212
4.1.2.01.4	**Biomonitoring study in workers** Test substance: Silver compounds (various) Guideline: Not applicable	Pub.: Y GLP: N	Based on detailed biomonitoring data, the authors concluded that (4) workers would excrete 0.3 mg of silver when working at a TWA of 0.03 mg/m³ daily, and (ii) a risk if generalised argyria was unlikely to occur at such exposure levels.	RL = 4 secondary literature	DiVincenzo, G.D.; et al. (1985): Biologic monitoring of workers exposed to silver. Int. Arch. Occup. Environ. Health 56, 207-215	20

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.4	**Review document, argyria** Test substance: Silver metal Guideline: Not applicable dermal absorption	Pub.: Y GLP: N	This review summarises potentially relevant data from two primary sources: (4) Norgaardt (1954), indicating a max. dermal absorption of 4% (ii) Skog & Wahkberg (1964), cited in this report elsewhere, indicate a dermal abs. <1% Given the publication date and the nature and precision of the study ("disappearance assay), it is not considered worthwhile to further follow-up on this publication.	RL = 4 (secondary literature)	Breitstadt, R. (1995): Occupational exposure limits for metallic silver. 2nd Eur. Precious Metals Conference, Lisbon, 1-13	29
4.1.2.01.4	**Review document, methodological aspects** Test substance: Silver (unspecified) Guideline: Not applicable	Pub.: Y GLP: N	An overview of methodological aspects of trace levels of silver and other heavy metals in tissue samples is given.	RL = 4 (secondary literature)	Danscher, G. (1991): Applications of autometallography to heavy metal toxicology. Pharm. Toxicol. 68, 414-423	128
4.1.2.01.4	**Review document, argyria** Test substance: Silver (unspecified) Guideline: Not applicable	Pub.: Y GLP: N	Only very brief summary article on argyria.	RL = 4 Secondary literature	Greene, R.M.; Su, W.P.D. (1987): Argyria. Am. Fam. Phys. 36, 151-154	33
4.1.2.01.4	Mechanistic and other studies related to argyria - studies in animals					
4.1.2.01.4	**Mechanistic study, argyria** Test substance: Silver lactate or silver nitrate Guideline: Not applicable Repeated oral treatment - distribution in CNS Species: Spraque Dawley and Wistar rats Exposure period: 4 months Frequency of treatment: continuously Doses: 0.01 % silver lactate or silver nitrate in drinking water	Pub.: Y GLP: N	Silver penetrated the blood brain barrier and accumulated in neurones and glia. The distribution of silver in the CNS was heterogeneous. Silver accumulated in large motoneurons in the brain stem and spinal cord and neurones in the cerebellar nuclei. Electron microscopy showed that silver was located intracellularly in the lysosomes and extracellularly in basement membranes and elastic fibres of vessels.	RL = 2 The study was conducted according to state of the art methodology at that time; methods are described in detail and results are presented appropriately in the text and are visualised in figures.	Rungby, J.; Danscher, G. (1983): Localization of exogenous silver in brain and spinal cord of silver exposed rats. Acta Neuropathol. 60, 92-98	56

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.4	**Mechanistic study, argyria** Test substance: Silver nitrate Guideline: Not applicable Repeated oral treatment of rats – deposition in eyes Exposure period: 10 weeks + 6 month Frequency of treatment: continuously, daily Doses: 0.25 % in water (222.2 mg/kg bw/d) Route of administration: drinking water Rats were given silver nitrate via drinking water for 10 weeks. Two animals were sacrificed and the remaining animals were divided in two groups. One group received this treatment for another 6 months followed by 6 months drinking water and the other received drinking water for 12 months. Animal were weighed regularly and at monthly intervals one rat from each group was killed an its eyes were examined by electron microscopy.	Pub.: Y GLP: N	There was no effect on body weight gains in the first 10 weeks of exposure. However, body weight losses in comparison to controls first appeared about 23 weeks after start of the experiment. Several animals that lost weight rapidly died. Body weight in the surviving experimental animals was an average of 50% less than that of control rats. Subsequent substitution of silver nitrate by water resulted in body weight increases, approaching that of untreated control rats within 13 weeks of cessation of exposure. Silver deposits were detected by electron microscopy in the eyes of the animals. Number and size of granules increased with exposure time. In rats receiving silver nitrate for 10 weeks, deposition of silver particles in the eye persisted for 3 months after cessation of exposure, but number and size had decreased 6 months, and after 12 months only fine granular deposits remained. Silver particles were not seen in the basement membranes of endothelial cells or cytoplasm.	RL = 2 The study was conducted according to state of the art methodology at that time; methods are described briefly and results are presented in reasonable in the text and figures.	Matuk, Y.; et al. (1981): Distribution of silver in the eyes and plasma proteins of the albino rat. Can. J. Ophthalmol. 16, 145-150	177
4.1.2.01.4	**Mechanistic study, argyria** Test substance: Silver nitrate Guideline: Not applicable Repeated oral treatment - deposition in various organs Species: Sprague-Dawley rats Frequency of treatment: continuously Doses: 12 mM in drinking water 1. Study eExposure period: 81 weeks 2. Study exposure period: 10 weeks	Pub.: Y GLP: N	1. Study: After 76 to 81 weeks of treatment, clinical condition was impaired (no further data). Iterim sacrifice at 4, 6, 12, 25 weeks: time related increase in silver deposits (number and size). Major organs with silver deposits: kidney (glomerulum), colon, liver (after 6 weeks); choroid plexus, thyroid acinar cells, skin appendages (after 12 weeks); skin surface, urinary bladder, prostatic acinar basement membranes (after more than 25 weeks); intracellular location: basement membranes and phagocytes. 2. Study: Silver deposition continued for further 4 weeks after cessation of treatment.	RL = 3 Very old study; methods and dose regime are described very poorly.	Walker, F. (1971): Experimental argyria: a model for basement membrane studies. Br. J. Exp. Pathol. 52, 589-593	174

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.4	**Mechanistic study, argyria** Test substance: Silver nitrate, Silver chloride Guideline: Not applicable Repeated oral treatment - distribution throughout the body species: rats Route of administration: drinking water Exposure period: life time Frequency of treatment: continuously Dose levels: 1, 0.4 or 0.1% silver salt (solution of silver nitrate or silver chloride dissolved in 0.3% sodium thiosulphate)	Pub.: Y GLP: N	Animals given 1% of silver salts did not survive. Two rats given 0.4% silver salts were kept alive for over 500 days. Survival of the animals treated with 0.1% was not affected by silver salt treatment. In skin no silver deposits were found in the dermis but in small numbers in the corium. Silver granules were observed in the tongue, salivary glands, thyroid, parathyroid, heart, blood vessels, lymph nodes, liver, kidneys, pancreas, intestines, spleen, adrenal glands, pituitary, choroid layer of the eye and bladder. No silver deposits could be detected in the lungs, testes, uterus, bone marrow, joints, striated muscles, brain except choroid plexus. No other pathological changes compared to controls were observed.	RL = 3 Very old study, highly deficient in description of methodology, but results well documented in the text and figures.	Olcott, C.T. (1948): Experimental argyrosis. IV. Morphologic changes in the experimental animal. Am. J. Path. 24, 813-833	36
4.1.2.01.4	**Mechanistic study, argyria** Test substance: Silver nitrate Guideline: Not applicable Repeated oral treatment of mice - deposition in kidneys Exposure period: 12 weeks Frequency of treatment: continuously Dosing: 0.25 % in drinking water	Pub.: Y GLP: N	After 1 week of dosing, fine silver granules could be detected randomly distributed throughout the glomerular basal membrane, increasing with time. At 16 months post exposure, most capillary loops had a single row of large, irregular subendothelial granules beneath the endothelium some extending into the endothelium. Large silver aggregates were observed in the axial matrix. After cessation of dosing the granules are displaced to the endothelial side of the basement membrane, enlarge, become fewer and eventually disappear. Wide variations in kidney deposition of silver between individual animals were reported. After 3 months of dosing, oedema in epithelial cells, increased amount of cytoplasmatic RNA and organelles were observed but these changes were not consistent. After 3 months of dosing, livers contained 6-7 mg silver/g liver; kidneys contained 3.7 to 9.8 mg/kg after 3 months.	RL = 2 The study was conducted according to state of the art methodology at that time; results are described in detail and are visualised in figures.	Ham, K.N.; Tange, J.D. (1972): Silver deposition in rat glomerular basement membrane. Aust. J. Exp. Biol. Med. Sci. 50, 423-424	34

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.4	**Mechanistic study, argyria** Test substance: Silver nitrate Guideline: Not applicable Repeated oral treatment – deposition in hypothalamic neuron Species: Wistar-Kyoto rats Exposure period: 62 days Frequency of treatment: continuously Doses : 0.1 % in drinking water	Pub.: Y GLP: N	Silver was found in the basal membranes, elastic membranes and fibres of large vessels. It penetrated the blood brain barrier and silver grains were observed in glia cells and neurons in all parts of the brain. Extracellular silver was primarily bound to the basement membrane of the vessel walls, intracellular silver was located exclusively in the lysosomes of neurons and glia cells. Distribution of silver in the hypothalamus was highly heterogeneous most prominent deposits were described in the supraoptic and paraventricular hypothalamic nuclei. No abnormal behaviour was observed in the treated rats.	RL = 2 The study was conducted according to state of the art methodology at that time; methods are described in detail and results are presented appropriately in the text and are visualised in figures.	Stoltenberg, M.; et al. (1994): Autometallographic detection of silver in hypothalamic neurons of rats exposed to silver nitrate. J. Appl. Toxicol. 14, 275-280	57
4.1.2.01.4	**Methodological investigation** Test substance: Silver lactate Guideline: Not applicable localisation of silver in biological tissue	Pub.: Y GLP: N	A methodology is described to visualise trace amounts of silver in tissues after i.p. administration of silver lactate in rats.	RL = 2 reasonably well-documented publication	Danscher, G. (1981): Light and electron localization of silver in biological tissue. Histochemistry 71, 177-186	188
4.1.2.01.4	**Mechanistic study, argyria** Test substance: Silver nitrate Guideline: Not applicable Repeated oral treatment – deposition in kidneys species: rats, rabbits Exposure periods: 4-20 weeks (rats), 8, 11, 15 weeks (rabbits) Frequency of treatment: continuously Dosing: 0.15 % solution in drinking water	Pub.: Y GLP: N	Rats: heavy deposits of silver were found in the glomerular basement membrane around the vessels of the vascular bundles of the outer medulla and around the vessels and loops of Hemle in the inner medulla. Rabbits: minimal amount of silver was found in the glomeruli and none in the vascular bundles; in the inner medulla, silver was found only in relation to those parts of the vessels and loops of Henle which lay nearest to collection ducts. Rats and rabbits: silver was found in the medullary interstitial tissue and in interstitial cells, which showed signs of degeneration. Conclusion: It is suggested that the species difference is due to differences in permeability and or in the constitution of the basal membranes.	RL = 2 The study was conducted according to state of the art methodology at that time; results are described briefly in the text and are visualised in figures	Moffat, D.B.; Creasey, M. (1972): The distribution of ingested silver in the kidney of the rat and of the rabbit. Acta Anat. 83, 346-355	54

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.4	**Mechanistic study, argyria** Test substance: Silver nitrate Guideline: Not applicable Repeated oral treatment - deposition in kidney species: mice Exposure period: 6 weeks Frequency of treatment: continuously Doses: 12 mmol, groups of 6-8 rats Route of administration: drinking water	Pub.: Y GLP: N	Strong silver labelling of glumerular basement membranes; some silver granules were seen in mesangial immune deposits; silver was usually deposited in electron-lucent areas around pre-existing deposits or formed a boundary to an expanded mesangium when immune deposits subsequently developed. In conclusion, there was no correlation with immune deposits in the kidney.	RL = 2 The study was conducted according to state of the art methodology at that time; methods are described briefly and results are presented in detail in the text and are visualised in figures. The study was not intended to investigate repeated dose toxicity.	McGiven, A.R.; et al. (1977): Glomerular lesions in argyria NZB/NZW mice. Brit. J. Exp. Path. 58, 57-62	53
4.1.2.01.4	**Mechanistic study, argyria** Test substance: Silver nitrate Guideline: Not applicable Repeated oral treatment - deposition in kidneys species: mice Exposure period: a) 12 d and b) 14 weeks Frequency of treatment: continuously Post treatment period: b) 21 weeks Doses: 6 mM in drinking water	Pub.: Y GLP: N	No morphological abnormalities were detected by light microscopy in mice treated for 14 weeks despite some darkening of the glomeruli after 14 weeks of dosing. a) by electron microscopy, granules could be identified in the basement membrane of the glomeruli and the mesangium after 12 days. b) after exposure between 2 and 14 weeks deposits increased in the basement membrane of capillary loops, but Bowman's capsule and tubular basement membrane were not significantly involved; dense clusters of deposits were observed in the mesangium. Until 21 weeks after cessation of exposure, no significant change in silver deposit distribution was observed.	RL = 2 The study was conducted according to state of the art methodology at that time; methods and results are described briefly in the text and are visualised in figures.	Day, W.A.; et al. (1976): Silver deposition in mouse glomeruli. Pathology 8, 201-204	45

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.4	**Mechanistic study, argyria** Test substance: Silver nitrate Guideline: Not applicable repeated administration, distribution of silver in eyes species: rat exposure via drinking water (0.25%) duration: 10 weeks	Pub.: Y GLP: N	The biological half life for the removal of intraperitoneal injected silver nitrate from blood, plasma, kidney and liver of rats was 40 hours. It took 70 and 84 hours to achieve a 50% reduction of the spleen- and brain-levels.	RL = 2 reasonably well-documented publication	Matuk, Y. (1983): Distribution of radioactive silver in the subcellular fractions of various tissues of the rat and its binding to low molecular weight proteins. Can. J. Physiol. Pharmacol. 61, 1391-1395	51
4.1.2.01.4	**Mechanistic study, argyria** Test substance: Silver nitrate Guideline: Not applicable Repeated oral treatment of rats - deposition in kidneys	Pub.: Y GLP: N	The development of experimental argyria is described with deposition of silver particles in the kidneys, mainly in glomerular basal membranes. Silver granules were found to a minor extent in the mesangioma, podocytes and endothelial cells in the glomerulum and in tubular basal membranes, proximal tubule epithelium and walls of small vessles.	RL = 4 only brief abstract in English available	Chorvath, D.; et al. (1976): Morphology of experimental argyrosis in kidneys. Ceskoslovenska Patologie 12, 161-165	170

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.4	**Mechanistic study, argyria** Test substance: Silver lactate and silver nitrate Guideline: Not applicable Repeated oral/i.p. treatment - deposition in eyes Species: Wistar rats a) Exposure period:45 days Frequency of treatment: continuously Doses:0.02 % silver lactate or nitrate administration: drinking water b) Frequency of treatment: 3 successive days Post treatment period: 4, 14 and 45 days Doses: 12 mg silver lactate administration: i.p. d) Exposure period:3 months Post treatment period: 15 months Frequency of treatment: continuously Doses:0.02 % silver nitrate (180 mg per rat) administration: drinking water	Pub.: Y GLP: N	The eyes of rats which received silver nitrate for 3 months developed a greyish discoloration of the cornea which did not disappear after cessation of treatment. Silver deposits were observed in the conjunctivae (basal lamina, occasionally in epithelial cells), Bowman's membrane, keratocyte extensions, corneal stroma, descemet's membrane, iris (stromal connective tissue cells and fibres) and the anterior part of the lens capsule. Lysosomes of retinal pigment epithelium were found to contain occasionally silver. In the optic nerve, silver was seen in fibres, cells and vessel walls. In nerve tissue, silver was located in astrocytes.	RL = 3 The methods are described very poorly and the results of the different experiments are not presented in adequate detail.	Rungby, J. (1986): Experimental argyrosis: ultrastructural localization of silver in rat eye. Exp. Mol. Pathol. 45, 22-30	151
4.1.2.01.4	Mechanistic and other studies related to argyria - studies in humans					
4.1.2.01.4	**Mechanistic study, argyria** Test substance: Silver (unspecified) Guideline: Not applicable	Pub.: N Data holder: Umicore GLP: N	50 workers of silver-processing industries were biomonitored for skin content of silver. A correlation with the exposure concentration at the workplace could not be established.	RL = 2 reasonably well-document human biomonitoring study	Wölbling, R.H.; et al. (1988): Silberablagerung in der Haut von Beschäftigten der silberverarbeitenden Industrie - Dermatologische Untersuchungen und quantitative Messungen mittels Atomabsorptionsspektrometrie. Johann Wolfgang Goethe-Universität Frankfurt am Main	22

4.1.2.01	Toxicokinetics, metabolism and distribution					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.01.5	Toxicokinetics, metabolism and distribution - other studies including in-vitro investigations					
4.1.2.01.5	**In-vitro bioavailability in physiological media** Test substance: Silver chloride, Silver sulfadiazine Guideline: Not applicable	Pub.: Y GLP: N	The solubility of both silver compounds in serum electrolyte as well as natural and synthetic serum (among others) was measured. Despite the assumption that chloride anions present on most physiological media would precipitate silver as the poorly soluble chloride and thus reduce bioavailability, this investigation shows that other serum components such as amino acids can enhance the solubility of silver.	RL = 2 reasonably well-documented publication	Tsipouras, N.; et al. (1995): Solubility of silver sulfadiazine in physiological media and relevance to treatment of thermal burns with silver sulfadiazine cream. Clinical Chemistry 41, 87-91	116
4.1.2.01.5	**Methodological investigation, controlled release** Test substance: Silver (acetate ?) Guideline: Not applicable implantation of Ag-loaded cation exchange resin beads in rats	Pub.: Y GLP: N	At test system is described that allows local, controlled release of Silver cations from implanted cation exchange resin beads. Of little use in a risk assessment context.	RL = 2 reasonably well-documented publication	Ellender, G.; Ham, K.N. (1988): Cationic radioisotope delivery to loose connective tissue in vivo using ion-exchange resin beads. Nucl. Med. Comm. 9, 403-409	140
4.1.2.01.5	**In-vitro membrane permeation studies** Test substance: Silver nitrate, Silver sulfadiazine Guideline: Not applicable	Pub.: Y GLP: N	The penetration of silver through synthetic membranes was found to be limited, indicating in the opinion of the authors that absorption of silver through healing wounds would be reduced by binding of silver to the growing skin membrane network. non-standardised test system, limited usefulness of reported data	RL = 2 reasonably well-documented publication	Tsipouras, N.; et al. (1997): Passage of silver ions through membrane-mimetic materials, and its relevance to treatment of burn wounds with silver sulfadiazine cream. Clinical Chemistry 43, 290-301	108

4.1.2.02	Acute toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.02	**Acute toxicity - Reviews**					
4.1.2.02	**Case study - human** Test substance: Guideline: Case study (one 17-year-old boy): argyria like symptons observed after treatment of burn wounds with a silver-coated wound dressing for one week.	Pub.: Y GLP: N	Argyria like symptons were observed after treatment of burn wounds with a silver-coated wound dressing. The possibility of a toxic silver effect from use of silver-coated wound dressing should be kept in mind.	RL = not relevant	Trop, M.; et al. (2006): Silver-coated dressing acticoat caused raised liver enzymes and argyria-like symptoms in burn patient. J. Trauma 60, 648-652	66
4.1.2.02	**Review document** Test substance: Silver compounds (various) Guideline: not applicable Review on effects of silver on humans, argyria, silver in tissues, absorption and excretion, and chronic deseases-	Pub.: Y GLP: N	Refers to the US Registry of Toxic Effects of Chemical Substances RTECS (as of 1975) for details on toxicty. Cites LD50 values for colloidal silver (100 mg/kg, oral mucosa) and silver nitrate (50 mg/kg, oral mucosa). Further detail on these LD50 values is missing.	RL = 4 Secondary literature. followed up by checking on cited prim. Literature	Klein, D.A. (1978): Chapter 12: Effects on humans. Klein, D.A. (ED.): Environmental Impacts of Artificial Ice Nucleating Agents, Chap. 12, 169-175	24
4.1.2.02	**Review** Test substance: Guideline:	Pub.: N Data holder: Umicore GLP: N		RL = 4 Review (from around mid of 20th century); no useful information available.	Zobrist, F.; et al. (0): Die Gesundheitsunschädlichkeit von gesilbertem Trinkwasser. not applicable	27
4.1.2.02	**Data collection on metallic silver** Test substance: Guideline: not applicable	Pub.: N Data holder: Umicore GLP: N	Few relevant acute toxicity data (LD50 values). The following primary literature is cited: two studies in mice (oral, #231, #232)), one in rat (oral, EBRC #16), one in rabbit (inhalation, #233).	RL = 4 Data set	Anonymus (1993): Silver (metallic). AIDA Grunddatensatz, pp.33	28

4.1.2.02	Acute toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.02	**Acute toxicity, mouse, i.p.** Test substance: silver nitrate (and other metal salts) Guideline: not stated Route of administration: i.p. Animals: male, ICR, swiss origin mice Groups: five dose levels, 4 mice each (more dose levels or more mice if statistical variation initially too high)	Pub.: Y GLP: N	LD50 12.5 mg/kg bw (after 72 hours) and 13.2 (24h). The toxicity decreased if animals were pretreated with 40% of the LD50 dose 8 hours prior to the study.	RL = 3 No guideline compliance, no controll group, non-standards route of administration, no raw data on dose levels or observations.	Jones, M.M.; Schoenheit, J.E.; et al. (1979): Pretreatment and heavy metal LD50 values. Tox. Appl. Pharmacol. 49, 41-44	200
4.1.2.02.1	**Acute toxicity – Studies in animals**					
4.1.2.02.1	**Acute toxicity, mouse, i.p.** Test substance: several silver salts of sulfonamides Guideline: not stated Route: intraperitoneal injection Species: male CD1 mice Number: controll and dosed groups between 5 and 10 animals each Observation period: 21d	Pub.: Y GLP: N	LD50 values for the tested compounds: AgAc: 0.22 mmol/kg AgMCP: 0.13 mmol/kg AgNH3DBS: 0.10 mmol/kg AgP(OCH3)3DBS: 0.15 mmol/kg	RL = not relevant Methods and results are described in rather good datail. However, the route of administration is not relevant for HH risk assessment.	Horner, H.C.; et al. (1983): Acute toxicity of some silver salts of sulfonamides in mice and the efficacy of penicillamine in silver. Drug Chem. Tox. 6, 267-277	159

4.1.2.02	Acute toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.02.1	**Acute inhalation study - dogs** Test substance: 110m Ag aerosol AMAD = 0.42 - 0.54µm, GSD = 1.5 Guideline: not stated Sex: females Strain: 6 Beagle dogs Route of admin.: acute inhalation Exposure period: 7-15 min Frequency of treatment: once Doses: 1 mg/kg bw (averaged absolute deposition) Anaesthetised dogs were tracheally intubated and exposed to airborne particles produced by exploding radioactive silver wires.	Pub.: Y GLP: N	The absolute deposition of Silver is given as about 1 mg/kg body weight. No deaths were reported. The fractional depositon in the respiratory tract was 0.17 +- 0.05. An acute toxicity NOAEL for inhalation in the dog may be set to > 1 mg/kg body weight (the total deposition of silver reported).	RL = 4 secondary literature only	Phalen, R.F.; Morrow, P.E. (1973): Experimental inhalation of metallic silver. Health Physics 24, 509-518	3
4.1.2.02.1	**Acute toxicity, oral, rat, limit test** Test substance: colloidal silver solution (8g Ag/L) Guideline: not stated single oral administration, by gavage Wistar rats, 10 males and 10 females volume: 30 mL/kg bw Dose: 240 mg Ag /kg bw observation period: 14 days	Pub.: N Data holder: Umicore GLP: N	Within a few hours after dosing the rats showed severe diarrhoea and black discolouration of the faeces. This pjhenomenon had disappeared after 24 hours. No other signs of intoxication were observed and no deaths occured. LD50 : > 30 mL /kg bw (colloidal silver solution) LD50 : > 240 mg /kg bw (Ag)	RL = 3 supplementary Data Only study summary available. No purity stated.	Spanjers, M.T.; Til, H.P. (1980): Determination of the acute oral toxicity of colloidal silver solution in rats. CIVO / TNO, Zeist, The Netherlands	16

4.1.2.02	Acute toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.02.1	**Acute toxicity, oral, rat** Test substance: Silver powder CAP 9 batch 08P108 purity: not stated particle size: < 40 µm Guideline: OECD 401 (1987), 84/449/EEC B.1 single oral administration, by gavage Sprague-Dawley rats, DRF: 3 males and 3 females main study: 15 males and 15 females vehicle: distilled water volume: 21.5 mL/kg bw Dose: 2000, 200 and 10 mg/kg bw observation period: 14 days	Pub.: N Data holder: Umicore GLP: Y	DRF-study: No death or clinical signs of toxicity. Main Study: No death or clinical signs of toxicity. All animals showed expected gain in bodyweight during the study. No abnormalities were noted at necropsy. LD50: > 2000 mg/kg bw.	RL = 2 No information on the purity stated.	Allen, D.J. (1993): Silver powder cap 9: acute oral toxicity (limit test) in the rat. Safepharm Laboratories Ltd., Derby, UK	13
4.1.2.02.1	**Acute toxicity, oral, rat** Test substance: Silver (I) oxide batch 18 and 41 purity: approx. 93% Ag Guideline: OECD 401 (1981) , 84/449/EC B.1 single oral administration Wistar Bor: WISW (SPFTNO), 15 males and 15 females vehicle: peanut oil volume: 21.5 mL/kg bw Dose: 2370, 3480 and 5110 mg/kg bw observation period: 14 days	Pub.: N Data holder: Umicore GLP: Y	LD50 (male) = 3970 mg/kg bw LD50 (female) = 3702 mg/kg bw LD50 (male + female) = 3804 mg/kg bw No mortality occured at the lowest dose of 2370 mg/kg bw.	RL = 1	Zechel, H.-J. (1989): Silver(I)-oxide - testing the acute toxicity after single oral administration in rats. ASTA Pharma AG, Bielefeld, Germany	5

4.1.2.02	Acute toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.02.1	**Acute toxicity, mice, oral, s.c. and i.p.** Test substance: silver sulfadiazine Guideline: no Strain:CD-1 mice Doses: 550- 1,050 mg/kg Route of administration: orally, i.p. and subcutaneously Control group: No	Pub.: Y GLP: N	As results, limit LD90-100 values are reported for acute toxicity for oral , s.c. and i.p. administration for silver sulfadiazine: LD90-100, oral.: >=1050 mg/kg LD90-100, s.c.: >=1050 mg/kg LD90-100, i.p.: <=550 mg/kg	RL = 3 The acute toxicity studies are not described in sufficient detail. E.g. dose levels, number of animals and raw observation data is missing.	Wysor, M.S. (1975): Orally-administered silver sulfadiazine: chemotherapy and toxicology in CF-1 mice; Plasmodium berghei (Malaria) and Pseudomonas aeruginosa. Chemotherapy 21, 302-310	153
4.1.2.02.1	**Acute toxicity, rats and rabbits, i.p. and oral administration** Test substance: silver nitrate (antismoking mouthwash solution, 0.5, 8 or 10 % silver nitrate dissolved in placebo). Guideline: compliance with OECD quoted Sex: males and females Strain: Fischer 344 rats and Californian rabbits Groups: 4-5 groups of 10 animals per sex per route Route of admin.: i.p or oral administration volume: 1mL (rat), 2mL (rabbit) Doses: rat oral: 10, 20 ,30 ,40 and 80 mg/kg bw rat i.p.: 200, 300, 400, 600 and 800 mg/kg bw rabbit oral.: 200, 800, 1000, 1800 and 4000 mg/kg bw rabbit i.p. injection: 50, 100 ,300 and 400 mg/kg bw Control Group: yes, rat: 1 ml placebo mouthwash rabbit: oral 10mL and i.p. injection 2mL placebo mouthwash 2 weeks observation priod	Pub.: Y GLP: N	LD50 values in mg/kg body weight for males and females LD50 (acute, i.p., rats): 35.7 (m) and 37.2 (f) LD50 (acute, i.p., rabbits): 113 (m) and 128 (f) LD50 (acute, oral, rats): 428 (m) and 433 (f) LD50 (acute, oral, rabbits): 1261 (m) and 1320 (f)	RL = 2 The study was conducted according to standard methods that comply with the OECD guidelines.	Tamimi, S.O.; et al. (1998): Toxicity of a new antismoking mouthwash 881010 in rats and rabbits. J. Toxicol. Environ. Health 53, 47-60	104

4.1.2.02	Acute toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.02.1	**Inhalation study, rats - pulmonary deposition and systemic absorption/distribution** Test substance: silver powder (ultrafine, inhalation) and silver nitrate (intratracheal instillation) Guideline: Rats were exposed (whole body) for 6h to a silver aerosol of 15 nm particle diameter; exposure concentration 133 µg/m³ (particle number concentration 3x10^6/cm³); estimated cumulative dose 7.2 g. Groups of 4 animals were sacrificed on days 0, 1, 4 and 7.	Pub.: Y GLP: N	No deaths following the inhalation exposure were observed.	RL = 2 supplementary Data well-documented publication However, focus is not on acute toxic affects but on pulmonary deposition and systemic absorption/ distribution.	Takenaka, S.; et al. (2001): Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ. Health Persp. 109, 547-551	91
4.1.2.02.1	**Review document** Test substance: silver and its compounds Guideline: not applicable	Pub.: Y GLP: N	Liste acute toxicity data for several silver compounds (page 35). source rather out of date (1978)	RL = 4 Secondary literature	Venugopal, B.; Luckey, T.D. (Eds.) (1978): Cap. 1 - Toxicity of group I metals. Metal Toxicity in Mammals, vol. 2, cap. 1, pp. 1-39	232
4.1.2.02.1	**Acute toxicity, mice, oral** Test substance: silver dinaphthyl-methane disulphonate and silver nitrate Guideline: not applicable Animals: mice Route of administration: oral, stomach tube vehicle: water number of animals: 4 mice per dosing group Doses: not stated Control group: not stated 14 days observation period	Pub.: Y GLP: N	Silver nitrate: LD0: 30 mg/kg LD50: 50 mg/kg LD100> 60 mg/kg Silver dinaphthyl-methane disulphonate: LD0: 25 mg/kg LD50: 50 mg/kg LD10> 70 mg/kg	RL = 3 Very old study (1950), highly deficient in description of methodology, results are briefly described .	Goldberg, A.A.; et al. (1950): Antiacterial collodial electrolytes: The potentiation of the activities of mercuric-, phenylmercuric- and silver ions by a collodial sulphonic anion. Pharm. Pharmacol. 20, 20-26	231

4.1.2.02	Acute toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.02.1	**Acute dermale toxicity, guinea pig, and i.p. administration** Test substance: Silver nitrate Guideline: Number of animals: 20 guinea pigs Route of administration: percutaneaous and i.p. Vehicle: water Doses: single dose 216 mg/kg bw (0.239 M aqueous solution) application volume: 2.0 mL (topically) Weight at study initiation: 374 - 380 g Area covered: 3.1 sq cm Occlusion: occlusive Control: water Removal of test substance: none Post dose observation period: 3 weeks Examinations: symptoms, food and water intake, mortality, body weight	Pub.: Y GLP: N	percutaneous application: At a dose corresponding to 216 mg/kg bw no mortalities were observed within the observation period of 4 weeks p.a. LD0 >= 216 mg/kg bw LD50 >216 mg/kg bw After i.p. administration of 216 mg/kg b.w. 6 of 10 animals died within 7 days.	RL = 2 supplementary Data acceptable, reasonably well documented publication However, only one dose. Study may provide some additional information. Study was designed to compare i.p. with dermal dosing to elucidate potential dermal absorption.	Wahlberg, R.C.; et al. (1965): Percutaneous toxicity of metal compounds. A comparative investigation in guinea pigs. Arch. Environ. Health 11, 201-204	41
4.1.2.02.2	**Acute toxicity – human toxicity data**					
4.1.2.02.2	**Case report - acute toxicity human** Test substance: Silver nitrate Guideline: Case report on a fatal accidental silver poisoning by instillation of a 7% silver nitrate solution into the uterine cavity for atempted abortion.	Pub.: Y GLP: N	Intrauterine administration of about 100 mL of a 7 % silver nitrate solution has been reported to be rapidly fatal.	RL = not relevant Not a common route of administration, not relevant for HH risk assessment.	Reinhart, G.; et al. (1971): Akute tödliche Vergiftung mit Silbernitrat als Folge eines Abtreibungsversuches. Arch. Krimonol. 148, 69-78	55

4.1.2.02	Acute toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.02.2	**Review on argyria** Test substance: Guideline: Review on argyria (history, mechanism, clinical manifestations, histopathology, diagnosis, treatment).	Pub.: Y GLP: N	No endpoints on acute toxicity.	RL = not relevant Review only, with no endpoint on acute toxicity.	Greene, R.M.; Su, W.P.D. (1987): Argyria. Am. Fam. Phys. 36, 151-154	33
4.1.2.02.2	**Review (ATSDR)** Test substance: Guideline: not applicable	Pub.: Y GLP: N		RL = 4 (secondary literature) followed up by checking on cited prim. literature	Anonymous (1990): Toxicological profile for silver. ATSDR - Agency for Toxic Substances and Disease Registry	31
4.1.2.02.2	**Case report** Test substance: Guideline: not applicable Case report: silver containing pigmentation observed 58 years after eye surgery using silver containing thread.	Pub.: Y GLP: N	A localised argyria 58 years after eye surgery using a silver-containing thread is reported as an ophthalmologic rarity.	RL = 3	Frei, J.; et al. (2001): Localized argyrosis 58 years after strabismus operation--an ophthalmological rarity Original Title: Lokalisierte Argyrose 58 Jahre nach Schieloperation--Eine ophthalmologische Raritat. Klinische Monatsblatter Augenheilkunde 218, 61-63	97
4.1.2.02.2	**Case report** Test substance: silver nitrate Guideline: not applicable human prospective study	Pub.: Y GLP: N	A significant increase in silver blood levels due to absorption is reported. Silver levels did not increase in hair sampled 3-month after application.	RL = not relevant Not a common route of administration, not relevant for HH risk assessment.	Nguyen, R.C.; et al. (1999): Argyremia in septal cauterization with silver nitrate. J. Otolaryng. 28, 211-216	100
4.1.2.02.3	**Acute toxicity – Studies in all lines**					
4.1.2.02.3	**Cytotoxicity, in vitro, gingival fibrolast cells** Test substance: amalgams, alloys and their constituent elements and phase Guideline: Comparative cytotoxicity test of several dental materials and their constituant materials.	Pub.: Y GLP: N	Pure copper and zinc showed intensive cytotoxicity, significantly greated than that of pure silver and mercury. Pure tin was non-cytotoxic. The gamma-one phase (Ag2Hg3) revealed moderate cytotoxicity.	RL = not relevant	Kaga, M.; et al. (1991): Cytotoxicity of amalgams, alloys, and their elements and phases. Dent. Mat. 7, 68-72	130

4.1.2.02	Acute toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.02.3	**Cytotoxicity, in vitro, human keratinocytes** Test substance: Silver nitrate Guideline: Cytotoxicity was tested in human keratinocytes in vitro using 3 different assays.	Pub.: Y GLP: N	Flow cytometry for the testing of cell survival resulted in 7.6 % survival after treatment with 0.05 % silver nitrate solution. Treatment with silver nitrate resulted in no regrowth of keratinocyte cell cultures. Radial migration of keratinocytes in dermal explants was observed 1 week after treatment.	RL = not relevant	Smoot, E.C.; Kucan, J.O.; et al. (1991): In vitro toxicity testing for antibacterials against human keratinocytes. Plastic Reconstructive Surgery 87, 917-924	194
4.1.2.02.3	**Cytotoxicity, in vitro, 3T3-L1 cells** Test substance: Silver nitrate (and other metal salts) Guideline: The FRAME in-vitro cytotoxicity test and a physicochemical parameter (softness) were tested as alternative methods for prediction of acute toxicity (possible replacement of in-vivo experiments). Cytotoxicity of silver nitrate was determined in 3T3-L1 cells. Linear regression analysis was performed on in-vivo vs. in-vitro results.	Pub.: Y GLP: N	Cytotoxicity of silver nitrate: The ID50 value (concentration required to reduce final total cellular protein to 50 % of solvent control level) was reported to be 0.01 mM. The cited LD50 value for silver nitrate is 0.13 mmol/kg for mouse i.p. (cited from RTECS 1980). In the regresssion analysis over all tested compounds, the in-vitro cytotoxicity test was found to be a better surrogate for acute toxicity of metal salts than the "softness" parameters. Correlation was better with mouse i.p. LD50 values than with rat oral LD50 values.	RL = not relevant	Hulme, L.M.; Reeves, H.L.; et al. (1987): Assessment of two alternative methods for predicting the in vivo toxicities of metallic compounds. Molecular Toxicology 1, 589-596	205
4.1.2.02.3	**Cytotoxicity, in vitro, human synovial cells** Test substance: silver nitrate Guideline: The dose-dependent effects of heavy metals on cell proliferation, collagen synthesis and non-collagen protein synthesis were studied in early passage cultures of human synovial cells exposed to 1 - 100 µM concentrations of gold, silver, mercury, cadmium or lead for 5 days.	Pub.: Y GLP: N		RL = not relevant	Goldberg, R.L.; et al. (1983): Effect of heavy metals on human rheumatoid synovial cell proliferation and collagen synthesis. Biochem. Pharm. 32, 2763-2766	158

4.1.2.02	Acute toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.02.3	**Cytotoxicity, in vitro, four different cell lines** Test substance: silver nitrate, purity >99.9% Guideline: Test on the cytotoxicity of several metal compounds (silver nitrate amongst them) on four different cellines: murine monocyte-macrophages J774A.1, human uterine cervic epitheloid carcinoma HeLa S3, human neuroblastoma IMR-32, human pulmonary diploid fibrolasts IMR-90. For the latter cell line, a WST -1 assay was used to evaluate metal salt toxicity, for the other cell lines, a colony formation assay was used.	Pub.: Y GLP: N	In general, the cytotoxicty of AgNO3 was superseeded only by potassium dichromate, with the 10 other metal salts tested compounds exhibiting lower cytotoxicity. Sensitity for metal salt toxicity differed with the used cell line. IMR-32 had the highest sensitivity.	RL = not relevant no formal data requirement under REACH	Yamamoto, A.; et al. (1999): Generic tendency of metal salt cytotoxicity for six cell lines. J. Biomed. Materials Res. 47, 396-403	61
4.1.2.02.3	**Cytotoxicity, in vitro, mouse peritoneal macrophages** Test substance: silver lactate Guideline: The aim of the present study was to describe the effects of silver on structure and viability of cultured mouse macrophages. Moreover, the peroxidative capacity of the mouse liver preparations after silver exposure was studied in order to investigate whether lipid peroxidation might be involved in silver toxicity, since vitamine E has been shown to prevent silver-induced liver necrosis.	Pub.: Y GLP: N	This study demonstrated that silver affects viability and structure of cultured macrophages, possibly due to inducion of lipid peroxidation, as demonstrated to occur in the liver of silver-exposed mice.	RL = not relevant	Rungby, J.; et al. (1987): Silver affects viability and structure of cultured mouse peritoneal macrophages and peroxidative capacity of whole mouse liver. Arch. Toxicol. 59, 408-412	143

4.1.2.02	Acute toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.02.3	**Cytotoxicity, in vitro, eukaryotic microorganisms and cell culture lines** Test substance: silver nitrate and metallic silver (low intensity direct current generated) Guideline: This study was undertaken to examine the effects of silver salts and low intensity direct current generated (LIDC) silver on a variety of eukaryotic cells lines. Previous literature had ambiguous results on whether LIDC Ag was more cytotoxic to this type of cells than silver salts.	Pub.: Y GLP: N	The results of this study suggest that LIDC Ag has an appropriate selective toxicity for prokaryotic cells. It appears that levels of this agent could be obtained clinically that would have marked bacteriostatic activity and yet have little or no effect on mammalian cells.	RL = not relevant	Hall, R.E.; et al. (1988): In vitro effects of low intensity direct current generated silver on eukaryotic cells. J. Oral Maxillofac. Surg. 46, 128-133	142
4.1.2.02.3	**Cytotoxicity, in vitro, rat hepatocytes** Test substance: silver nitrate and silver lactate Guideline: The purpose of this study was to determine the role of thiol modification in silver-induced toxicity to freshly isolated hepaocytes.	Pub.: Y GLP: N	Addition of silver nitrate or silver lactate to freshly isolated rat hepatocytes cause dose-dependant loss of cell viability observed by using the trypan blue exclusion method. No cytotoxic effect was seen at 10 µM. Cytotoxicity (> 50 %) of silver nitrate was observed at concentrations of 30-70 µM.. The cytotoxicity was accompanied by a decrease in thiol concentration and an increase in thiobarbituric acid reactive products. Cytotoxicity was increased by the addition of DNA depleting agents and decreased by addition of thiol reducing agent.	RL = not relevant	Baldi, C.; et al. (1988): Effects of silver in isolated rat hepatocytes. Toxicol. Letters 41, 261-268	141
4.1.2.02.3	**Dissolution testing and cytotoxicity** Test substance: eight silver based alloys Guideline:	Pub.: Y GLP: N		RL = 4 The exact experimental procedure and detailed results cannot be extracted from this paper because only the abstract and figures are in English language with the body of the text probably in Japanese language.	Yoshioka, S. (1989): Dissolution of silver-based alloys under dynamic conditions and its relation to cytotoxicity (in vitro). Shika zairyo, kikai = J. Japan. Soc. Dent. Mat. Dev. 8, 324-336	138

4.1.2.02	Acute toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.02.3	**Cytotoxicity, in vitro, agar overlay test** Test substance: titanium-silver alloy Guideline: Study focused on metallurgical / electochemical properties of a titanium-silver-alloy in comparison to titanium alone. In parallel, the cytotoxicit of both materials was compared.	Pub.: Y GLP: N	Titanium-Silver alloy showed better electrochemical properties than titanium alone. The cytotoxity was similar. The adoption of titanium-silver alloys by the biomedical and dental fields is recommended.	RL = not relevant	Oh, K.-T.; et al. (2005): Properties of titanium-silver alloys for dental application. J. Biomed. Mat. Res. Part B, Appl. Biomat. 74, 649-658	69
4.1.2.02.3	**Cytotoxicity in dependance of Pd content of the Ag-Pd alloy** Test substance: Ag-Pd alloys with varying composition Guideline:	Pub.: Y GLP: N		RL = 4 The exact experimental procedure and detailed results cannot be extracted from this paper because only the abstract and figures are in English language with the body of the text probably in Japanese language.	Takeda, S.; et al. (1990): Corrosion behavior of Ag-Pd binary alloys under dynamic conditions and its cytotoxicity (in vitro). Shika zairyo, kikai = J. Japan. Soc. Dent. Mat. Dev. 9, 825-830	131
4.1.2.02.3	**Cytotoxicity, in-vitro, chinese hamster ovary cells** Test substance: Silver nitrate Guideline: Cytotoxicity of sixteen metallic compounds in chinese hamster ovary cells was determined by measuring the cloning efficency.	Pub.: Y GLP: N	Cytotoxicity was determined as the inhibition of cellular clonal growth of CHO K1-BH4 cells after 16 hr incubation at 37 deg. C in the presence of silver nitrate. The concentration reducing cloning efficiency by 50 % (CE50) was 6.2 µM.	RL = not relevant	Tan, E.-L-; Williams, M.W.; et al. (1984): The toxicity of sixteen metallic compunds in chinese hamster ovary cells. Tox. Appl. Pharmacol. 74, 330-336	199
4.1.2.02.3	**Cytotoxicity, in vitro, CGL 1 and CGL 3 hybrid cells and human fibrolast cells** Test substance: silver impregnated collagen cuff material Guideline: Cytotoxicity study to examine the effects of silver-impregnated collagen cuff material from central venous catheters on human fibrolast growth.	Pub.: Y GLP: N	A marked local cytotoxic effect was observed in test flasks containing the silver-impregnated collagen cuff material. Cell-free zones surrounding the cuff material were demonstrated. No cytotoxic effect was seen in the silver-free controll group.	RL = not relevant	Hemmerlein, J.B.; et al. (1997): In vitro cytotoxicity of silver-impregnated collagen cuffs designed to decrease infection in tunneled catheters. Radiology 204, 363-367	107

4.1.2.02	Acute toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.02.3	**Cytotoxicity, in vitro, L-929 mouse fibrolasts and human gingival fibrolasts** Test substance: several endodontic materials, some of them containing silver Guideline: Comparison of the cytotoxicity of a range of endodontic materials (rood canal sealers and rood-end filling materials). MTT assay for mitochondrial enzyme activity and CV assay for cell number	Pub.: Y GLP: N	In this paper, the dental materials are assessed as "the whole material" and not discriminated with respect to the contained substances. From the reported results it can be concluded that the three dental materials containing silver (Ketac silver, a gallium alloy and Amalgam), all showed at least little or even higher cytotocity.	RL = not relevant	Osorio, R.M.; et al. (1998): Cytotoxicity of endodontic materials. J. Endodon. 24, 91-96	103
4.1.2.02.3	**Cytotoxicity, in vitro, human dermal fibrolasts** Test substance: silver nitrate Guideline: Human dermal fibrolasts were exposed to silver nitrate at concentrations of 4.12 to 82.4 µM for 8 to 24 hours.	Pub.: Y GLP: N	Silver ions greatly inhibited fibrolast proliferation and prolonged silver nitrate exposure produced ag dependent cell loss. Inhibitory action on DNA synthesis was the primary event in silver nitrate cytotocity, associated with significant loss of cell protein.	RL = not relevant	Hidalgo, E.; Dominguez, C. (1998): Study of cytotoxicity mechanisms of silver nitrate in human dermal fibroblasts. Toxicol. Letters 98, 169-179	102
4.1.2.02.3	**In vitro biocompatibility (fibrolasts and osteoblast-like cells)** Test substance: Silver-reinforced glass ionomer dental cement Guideline:	Pub.: Y GLP: N	Silver-reinforced glass ionomer dental cement was used.	RL = not relevant	Bosetti, M.; et al. (2002): Silver coated materials for external fixation devices: in vitro biocompatibility and genotoxicity. Biomaterials 23, 887-892	85

4.1.2.02	Acute toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.02.3	**Cytotoxicity, in vitro, various cells lines** Test substance: silver metal (not described in more detail!), in comparison with gold, copper and a palladium-alloy Guideline: Investigation of anti-proliferation activity of dental metals (gold, silver, copper, palladium-alloy) on human promyelocytic leukemic HL-60 cells and potential influence of antioxidants (such as sodium ascorbate and N-acetyl-L-cysteine) on cytotoxicity.	Pub.: Y GLP: N	Amongst the dental metal tested, copper showed the highest cytotoxicity against human oral squamous cell carcinoma and human submandibular gland carcinoma cells followed by palladium-alloy, gold and silver. Normal human cells (gingival fibrolasts, pulp cells, periodontal ligaments fibrolasts) were relativly resistant to these metals. It was also shown that antioxidants modify the biological activity of dental metals.	RL = not relevant no formal data requirement under REACH	Kinoshita, N.; et al. (2002): Interaction between dental metals and antioxidants, assessed by cytotoxicity assay and ESR spectroscopy. Anticancer Res. 22, 4017-4022	81
4.1.2.02.3	**Cytotoxicity, in vitro, human dermal fibrolasts** Test substance: antimicrobial tissue conditioner containing silver-zeolite Guideline: The purpose of this study was to determine the effects of incorporating antimicrobial silver-zeolite on the in vitro cytotoxicity of five tissue conditioners against the living dermal model, which consists of normal human dermal fibrolasts in a collagen lattice.	Pub.: Y GLP: N	Cell viabilities for four out of five pharmaceutical tissue conditioners decreased with increasing silver-zeolite content. For the fifth tissue conditioner, no significant difference in cell viability with degree of silver-zeolite incorporation and had higher values (of viability) than the other four.	RL = not relevant no formal data requirement under REACH	Abe, Y.; et al. (2003): Cytotoxicity of antimicrobial tissue conditioners containing silver-zeolite. Int. J. Prosthodontics 16, 141-144	79
4.1.2.02.3	**Cytotoxicity, in vitro, human keratinocytes and fibrolast cells** Test substance: silver nitrate and nanocrystalline silver Guideline: Cytotoxicity study (in the context of clinical wound care) with silver nitrate and nanocrystalline silver on human keratinocytes and fibrolast cells. The composition of the culture medium and the culture technique were modified to assess the effect of the culture environment on the susceptibility of the cells to the toxic action of silver.	Pub.: Y GLP: N	Silver is highly toxic to both keratinocytes and fibrolast cells. When using optimized and individualized culture the fibrolasts appear to be more sensitive to silver than keratinocytes. However, when both cell types were grown in the same medium their viability was the same. Using tissue culture models again indicated an "environmental effect" with decreased sensitivity of the cells to the cytotoxic effects of the silver.	RL = not relevant no formal data requirement under REACH	Poon, V.K.M.; Burd, A. (2004): In vitro cytotoxity of silver: implication for clinical wound care. Burns 30, 140-147	76

4.1.2.02	Acute toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.02.3	**Cytotoxity, in vitro** Test substance: silver nitrate Guideline: Methodologicyl paper on advantages of 3-dimensional (3D) cell cultures over 2D cell cultures in the context of dermatotoxicity in-vitro studies used in chemical or pharmaceutical testing.	Pub.: Y GLP: N	3D cell cultures showed a higher resistance to loss of viability from treament with hydrogen peroxide or silver nitrate. Further comparisons between cells in co-cultures and mono-cultures, suggested that using 3D cultures in dermatotoxicity testing might more likely be reflective of true physiological responses to xenobiotic materials than existing models using 2D cultures.	RL = not relevant	Sun, T.; et al. (2006): Culture of skin cells in 3D rather than 2D improves their ability to survive exposure to cytotoxic agents. J. Biotech. 122, 372-381	67
4.1.2.02.3	**Cytotoxicity, in vitro, fibrolast cells** Test substance: several endodontic materials Guideline: Methodological paper. Two cytotoxicity test systems were compared. One with and one without the presence of a compacted layer of dentine chips mimicking the periapical dentine plug.	Pub.: Y GLP: N	Cytotoxicity was generally reduced in the presence of the dentine chip material. It is concluded that the method is a satisfactory alternative to in-vivo implantation testing for dental material, which usually follows the standards in-vitro tests. Result with respect to silver cytotoxicity: One dental material (AH26) is available with or without silver and both types were tested resulting in the same cytotoxicity.	RL = not relevant	Meryon, S.D.; Brook, A.M. (1990): In vitro comparison of the cytotoxicity of twelve endodontic materials using a new technique. Int. Endodon. J. 23, 203-210	133

4.1.2.03	Irritation					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.03	**Irritation - Reviews**					
4.1.2.03	**Review - ATSDR 1990** Test substance: Guideline: Not applicable	Pub.: Y GLP: N	No skin or eye irritaion study cited	RL = 4 Secondary literature	Anonymous (1990): Toxicological profile for silver. ATSDR - Agency for Toxic Substances and Disease Registry	31
4.1.2.03	**Review** Test substance: Guideline:	Pub.: N Data holder: Umicore GLP: N	No further primary literature cited. Reference to EBRC # 31 (ATSDR)	RL = 4 Secondary literature with no relevance for skin or eye irritation.	Anonymus (1993): Silver (metallic). AIDA Grunddatensatz, pp.33	28

4.1.2.03	Irritation					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.03	**Case study - human** Test substance: Siver nitrate, silver oxide, silver chloride, and silver cadmium oxide powder Guideline: Not applicable 27 employees who had worked at least for 2 years in silver flake, silver nitrate, silver powder or the refinery area. Testing consisted of a detailed questionair, slit lamp examination by an ophthalmologist, measurement of weight etc.	Pub.: Y GLP: N	Overall, 15 of the 27 workers (56%) complained of mucosal irritation such as itchy, red, or water eyes, sneezing, runny or stuffy nose, or sore throat. Nine complained of lower respiratory symptoms such as cough, wheezing, or tightness in the chest. Complaints of upper or lower respiratory symptoms were most prevalent in the crystal powder, and melting departments. No significant association was found between smoking status and respiratory complaints. Eight out of 27 (30%) complained of nose bleeds, including four of the five individuals in the crystal area. Respiratory system complaints had no statistical relation with the results of biological monitoring. Conclusion: Silver compounds such as silver oxide and nitrate are irritating materials and probably account for these symptoms.	RL = 2 Well documented case report	Rosenman, K.D.; et al. (1987): Potential nephrotoxic effects of exposure to silver. Brit. J. Ind. Med. 44, 267-272	144
4.1.2.03.1	**Irritation - Skin**					
4.1.2.03.1	**Skin irritation (Patch test)** Test substance: Silver (I) oxide batch 41 purity: approx. 93% Ag Guideline: OECD 404 (1981), 84/449/EEC B.4 animals: 3 rabbits (white russian), 1 male and 2 females 0.5g test substance vehicle: demineralised water 4 hours occlusive patch 3 days observation period	Pub.: N Data holder: Umicore GLP: Y	During the observation period neither erythema nor edema could be detected. The treated skin area was yellow to brown discoloured 24 hours after removal of the patch until the end of the 14-day observation period. No systemic toxic effects could be observed. The primary irritation index was 0. Non-irritant	RL = 1	Zechel, H.-J. (1989): Silver(I)-oxide - Acute toxicity. Testing the primary irritancy after single application to the skin of the rabbit (patch test). ASTA Pharma AG, Bielefeld, Germany	6

4.1.2.03	Irritation					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.03.1	**Skin irritation (Patch test)** Test substance: Silver powder CAP 9 batch: 08P108 purity: not stated partcle size: < 40µm Guideline: OECD 404 (1981), 84/449/EEC B.4 animals: 3 rabbits (New Zealand White), 1 male and 2 females 0.5g test substance moistend with 0.5mL distilled water 4 hours semi-occlusive patch 72 hours observation period	Pub.: N Data holder: Umicore GLP: Y	Very slight erythema was noted at two treated skin sites one hour after patch removal and persisted at one treated skin site at the 24 and 48-hour observations. Very slight oedema was noted at one treated skin site one hour after patch removal. All treated skin sites appeared normal at the 72-hour observation. Non-irritant to rabbit skin according to EEC labelling regulations.	RL = 2 In principall well documented GLP-study, but no information on the purity was provided.	Allen, D.J. (1993): Silver powder cap 9: acute dermal irritation test in the rabbit. Safepharm Laboratories Ltd., Derby, UK	12
4.1.2.03.1	**Skin irritation (Patch Test)** Test substance: Silver (II) oxide batch 12-F-001 purity: not stated Guideline: OECD 404 (1981), 84/449/EEC B.4 animals: 3 rabbits (New Zealand White), 1 male and 2 females 0.5g test substance moistend with 0.5mL distilled water 4 hours semi-occlusive patch 72 hours observation period	Pub.: N Data holder: Umicore GLP: Y	Yellow/brown-coloured staining caused by the test material was noted at two treated skin sitesfollowing patch removal. Very slight erythema was noted at one treated skin site one hour after patch removal, at two treated skin sites at the 24-hour observation and at one tretaed skin site at the 48-hour observation. Very slight oedema was noted at one treated skin site at the 24-hour observation. No adverse skin reactions were noted 72 hours after treatment. Non-irritant to rabbit skin according to EEC labelling regulations.	RL = 2 In principall well documented GLP-study, but no information on the purity was provided.	Allen, D.J. (1992): Silver (II) oxide: acute dermal irritation test in the rabbit. Safepharm Laboratories Ltd., Derby, UK	14

4.1.2.03	Irritation					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.03.1	**Skin irritation, in vitro** Test substance: Silver nitrate Guideline: not applicable test system: epidermal side of skin explants from hairless mice concentration: 500-5000 ug/cm2 skin incubation: 24 hr parameters for skin damage: lactate dehydrogenase, glutamic-oxaloacetate transaminase activity as well as glucose utilisation of the cultured skin were determined. In addition the skin was examined histologically.	Pub.: Y GLP: N	Enzyme activities and glucose utilisation decreased with increasing AgNO3 concentration. Based on this experiment silver nitrate was regarded as moderate irritant.	RL = 2 Method is described in detail and results are presented adequately in the text and tables.	Bartnik, F.G.; Pittermann, W.F.; et al. (1990): Skin organ culture for the study of skin irritancy. Toxicology In Vitro 4, 293-301	198
4.1.2.03.2	**Irritation - Eyes**					
4.1.2.03.2	**Eye irritation, in vitro, cytotoxicity** Test substance: Silver nitrate Guideline: Cytotoxicity to cultured human corneal endothelial cells was tested as a proposed in vitro assay for eye irritation. For detection of cellular damage an isotopic (51Cr) release assay was used.	Pub.: Y GLP: N	For silver nitrate the ED50 for (51Cr) release was 6 x 10 exp.-4 M. The substance was described as non irritant to moderate irritant.	RL = 3 supplementary Data Silver nitrate was used as a positive reference substance for the evaluation of an in vitro eye irritation test.	Douglas, W.H.J.; Spilman, S.D. (1983): In vitro ocular irritancy testing. Alternative Methods in Toxicology, Mary Ann Liebert Inc., Publ., New York, 1, 207-230	217

4.1.2.03	Irritation					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.03.2	**Model of corneal inflammation and hyperalgesia in rat** Test substance: silver nitrate Guideline: Not applicable strain: adult male Sprangue-Dawley rats application: centers of the right corneas were cauterised with silver nitrate (75% silver nitrate, 25% potassium nitrate) the applicator was held in contact with the cornea for 2s, producing a discrete grayish-white lesion 1mm in diameter. Cauterised eye was rinsed several times with saline.	Pub.: Y GLP: N	silver nitrate produced acute corneal inflammation and hyperalgesia and may prove a useful model for the study of primary afferent nociceptors (specialized free nerve endings of primary afferent nerves).	RL = not rated supplementary Data Supplementary information on "eye irritation".	Wenk, H.N.; Honda, C.N. (2003): Silver nitrate cauterization: characterization of a new model of corneal inflammation and hyperalgesia in rat. Pain 105, 393-401	77
4.1.2.03.2	**Eye irritation, rabbit** Test substance: Silver (I) oxide batch 41 purity: approx. 93% Ag Guideline: OECD 405 (1987), 84/449/EEC B.5 animals: 3 rabbits (white russian), 1 male and 2 females 0.08g test substance as supplied observation period: 1 hour post application	Pub.: N Data holder: Umicore GLP: Y	Cornea: opacity of the whole corneal area, olive-green discolouration of the upper corneal area Iris: evaluation impossible conjunctiva: olive-green discolouration, nictitating membrane parchment-like thinned (one animal); slight or obvious swelling with partial eversion of lids additinal lesions: discharge with moistening of considerable parts around the eye (two animals) The findings were recorded one hour after application. Thereafter the animals were sacrificed. Histopathological findings were present in all animals. Systemic toxic effects could not be detected. Silver (I) oxide is corrosive on the eye of the rabbit.	RL = 1	Zechel, H.-J. (1989): Silver(I)-oxide - Acute toxicity. Testing the primary irritancy after single application to the eye of the rabbit. ASTA Pharma AG, Bielefeld, Germany	7

4.1.2.03	Irritation					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.03.2	**Eye irritation** Test substance: Silver (II) oxide batch12-F-001 purity: not stated Guideline: OECD 405 (1987), 84/449/EEC B.5 animals: 1 rabbit (New Zealand White), female 0.1g test substance as supplied observation period: 24 hours post application	Pub.: N Data holder: Umicore GLP: Y	Opaque corneal opacity was noted over the lower edge of the cornea in the treated eye one hour after treatment with opaque corneal opacity over approx. 75% of the cornea and translucent corneal opacity over the remaining area of the cornea. Iridial inflammation was noted in the treated eye one and 24 hours after treatment. Moderate conjunctival irritation was noted in the treated eye one hour after treatment with severe conjunctival irritation at the 24 hour observation. White appearance of the nititating membrane and whiote areas over the conjunctival membranes were also noted in the treated eye at the 24-hour observation. The animal was sacrificed 24 hours after treatment. Irritant to rabbit eye according to EEC labelling regulation. Requires classification as Xi and R41.	RL = 2 In principall well documented GLP-study, but no information on the purity was provided.	Allen, D.J. (1992): Silver (II) oxide: acute eye irritation test in the rabbit. Safepharm Laboratories Ltd., Derby, UK	15
4.1.2.03.2	**Eye irritation, rabbit** Test substance: Silver nitrate 1 % in water Guideline: Method: Griffith (EBRC # 176)	Pub.: Y GLP: N	see EBRC # 176	RL = 4 Review only.	Chan, P-K.; Hayes, A.W. (1985): Assessment of chemically induced ocular toxicity: A survey of methods. In: Hayes, A.W. (Ed.): Toxicology of the Eye, Ear, and other Special Senses, Raven Press, New York, 103-143	220

4.1.2.03	Irritation					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.03.2	**Eye irritation, rabbit** Test substance: Silversalt not further identified Guideline: not applicable 0.1 M solution dropped on scraped cornea for 10 minutes 1. Evaluation of injuriousness of metal cations to the corneal stroma in rabbit eyes. 2. Determination of the chemical "binding" of these cations by the stroma. 3. Measurement of "denaturing"effects on stromal structure. 4. Attempts as therapeutic reversal of binding, denaturation, and injury by means of special antidotes.	Pub.: Y GLP: N	corrosive Rapid complete blackening of the cornea. Silver ions had a 4 times greater tendency to bind to cornea than Calcium ions and the water absorption capacity of isolated cornea after treatment was reduced to 10 % of normal.	RL = not relevant not a standard protocol The interaction of metal ions with the corneal stroma were investigated	Grant, W.M.; Kern, H.L. (1956): Cations and the cornea - toxicity of metals to the stroma. Am. J. Ophthal. 42, 167-181	196
4.1.2.03.2	**Eye irritation, rabbit** Test substance: Silver nitrate Guideline: no strain: young adult albino rabbits administration: instillation of 1/5 or 1 drop of the solution into the lower cul-de-sac. 2-5 minutes after administration lavage of 1% boric acid in the eyes. Observation: at least once every 24 hours Negative control: right eye positive control: distilled water, 3% boric acid, 0.85% saline solution and ammonium hydroxide (0.44%, 0.88%, 1.75% and 3.5% ammonium) The following signs were evaluated: Hypermia, swelling, mucopurulent exudation, coagulation, scar tissue formation and corneal injury.	Pub.: Y GLP: N	Silver nitrate at concentrations of 1 to 12 % produced concentration dependent irritation. At 12 % silver nitrate concentration blindness and silver deposits were observed in one of 4 animals. Silver nitrate is iritating to the eyes.	RL = 2 The study was conducted according to state of the art methodology at that time; methods are well described and results are presented reasonable in the text and in tabular format.	Calvery, H.O.; et al. (1941): Effects of some silver salts on the eye. Arch. Ophthalmol. 25, 839-847	175

4.1.2.03	Irritation					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.03.2	**Eye irritation, rabbit** Test substance: Silver nitrate 1 % in water Guideline: Strain: New Zealand albino rabbit dose volume: 0.01, 0.03 and 0.1 mL 2 groups of three animals per dose, administration: directly to the central corneal surface one eye was treated one was untreated and used as control The eyelids were released immediately and not forcibly closed or further manipulated. observation period: up to 21 days scoring: according to Draize (1, 3, 7, 14 and 21 days after dosing)	Pub.: Y GLP: N	moderate irritant acc. to Draize mean scores: 0.01 mL, 2 (day 1), 0 (day 3) 0.03 mL, 3 (day 1), 1 (day 3) and 0 (day 7) 0.10 mL, 12 (day 1), 4 (day 3) and 1 (day 7) The effects were reversible after 1 to 3 days. No differentiation between effects on conjunctiva, Iris, Cornea	RL = 3 supplementary Data Methods are described in detail and but there are deficiencies in the presentation of the results.	Griffith, J.F. (1980): Dose-response studies with chemical irritants in the albino rabbit eye as a basis for selecting optimum testing conditions for predicting hazard to the human eye. Toxicol. Appl. Pharm. 55, 501-513	176
4.1.2.03.2	**Eye irritation** Test substance: Silver nitrate Guideline: 1. Draize test 2. low-volume eye irritation test (Griffith et al. 1980, EBRC #176) 3. HET-CA assay 4. neutral red uptake assay by SIRC cells.	Pub.: Y GLP: N	Silver nitrate was classified according to the Draize eye irritation test as extreme irritant.	RL = 3 supplementary Data The methods are described in detail. However, different eye irritation methods were performed with 20 different substances at different laboratories for the validation of alternative test methods. The results are only given in very summarised format.	Blein, O.; Adolphe, M.; et al. (1991): Correlation and validation of alternative methods to the draize eye irritation test (opal project). Toxicology In Vitro 5, 555-557	197
4.1.2.03.2	**Eye irritation, interpretation of scoring** Test substance: Guideline:	Pub.: Y GLP: Y		RL = not relevant General interpretation of the scoring according to Draize. No information on silver or silver compounds.	Kay, J.H.; Calandra, J.C. (1962): Interpretation of eye irritation tests. J. Soc. Cosmet. Chem. 13, 281-289	209

4.1.2.03	Irritation					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.03.2	**Eye irritation, rabbit** Test substance: Silver powder CAP 9 batch 08P108 purity: not stated partcle size: < 40µm Guideline: OECD 405 (1987), 84/449/EEC B.5 animals: 3 rabbits (New Zealand White), 1 male and 2 females 0.1g test substance as supplied observation period: 72 hours post application	Pub.: N Data holder: Umicore GLP: Y	No corneal or iridial effects were noted during the study. Minimal conjunctival irritation was noted in all treated eyes one hour after treatment. No ocular effects were noted 24 hours after treatment. Non-irritant according to EEC labelling regulations.	RL = 2 In principall well documented GLP-study, but no information on the purity was provided.	Allen, D.J. (1993): Silver powder cap 9: acute eye irritation test in the rabbit. Safepharm Laboratories Ltd., Derby, UK	11
4.1.2.03.3	**Irritation – Respiratory tract**					
4.1.2.03.3	**Biomonitoring study in smelter workers** Test substance: Silver (unspecified) Guideline: not applicable	Pub.: Y GLP: N		RL = not relevant No information on irritating potential.	Nordberg, G.F.; Wester, P.O.; Brune, D. (1978): Tissue levels of 25 elements in smelter workers - a preliminary communication. Proc. Int. Symp. Control Air Pollution Working Environment, Libertryck, Stockholm, 261-272	221
4.1.2.03.3	**Inhalation study,** Test substance: colloidal solution of silver Guideline: not aplicable 3 adult rabbit 8h inhalation For further detail on the method the author refers to an (in press) Czech publication.	Pub.: Y GLP: N	For the description of the ultrastructure of the epithelium of a control group the author refers to a previously published paper: Konradova, V. (1966): The ultrastructure of the tracheal epithelium in rabbit. Folia morphol. 14, 210-214	RL = not relevant supplementary Data Deficiencies on the method description. Results are described in detail in the text.	Konradova, V. (1968): The ultrastructure of the tracheal epithelum in rabbits following the inhalation of aerosols of collodial solutions of heavy metals. Folia Morphol. 16, 265-271	233

4.1.2.03	Irritation					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.03.3	**Experimental pleurodesis in rabbits** Test substance: Silver nitrate Guideline: no strain: white New Zealand male rabbits 6 groups of 6 rabbits administration: single intrapleural injection dose: 0.5% silver nitrate in enodoxin free water or 3 mL saline solution test period: 6, 24 and 48 hours control: 1 group collection of blood and pleural fluid Blood and pleural fluid were prepared for cytologic and cytokine analysis as well as LDH evaluation. The following parameters were evaluated: Leukocytes, neutrophils, LDH, IL-8 and VEGF	Pub.: Y GLP: N	The mean LDH and IL-8 levels were signif. Increased in the SN group. VEGF was undetectable in the preinjection serum and saline solution-injected animals, but was increased in the serum after the pleural injection of SN. SN elicited a intense acute pleural inflammation reaction , with high WBC count and IL-8 levels in the pleural fluid, mainly within the first 6 h. LDH and VEGF levels, and pleural liquid production were also high for SN, and increased with time. These findings suggest that the intrapleural injection of SN produces a systemic inflammatory response that may have a role in the pathogenesis of fever and ARDS, which occure with pleurodesis.	RL = 2 supplementary Data Well described study; results are well documented in the text and figures. The study was not intended to investigate the irritation of the respiratory tract but is rather a mechanistic study on pleurodesis.	Marchi, E.; et al. (2004): Talc and silver nitrate induce systemic inflammatory effects during the acute phase of experimental pleurodesis in rabbits. Chest 125, 2268-2277	73

4.1.2.04	Corrosivity
	No publications assigned

4.1.2.05	Sensitisation					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.05	**Sensitisation - Reviews**					
4.1.2.05	**Review** Test substance: silver Guideline: not applicable not aplicable	Pub.: N Data holder: Umicore GLP: N	see EBRC #210 Heyl et al. 1979 Marks (1966)	RL = 4 Secondary literature (AIDA Grunddatensatz)	Anonymus (1993): Silver (metallic). AIDA Grunddatensatz, pp.33	28
4.1.2.05	**Review** Test substance: Guideline: not applicable	Pub.: Y GLP: N	see EBRC #210 Heyl et al. 1979 Marks (1966)	RL = 4 Review only	Anonymous (1990): Toxicological profile for silver. ATSDR - Agency for Toxic Substances and Disease Registry	31
4.1.2.05.1	**Sensitisation – Studies in animals**					
4.1.2.05.1	**Biocompatibility of implanted alloys** Test substance: different silver alloys Guideline: not applicable Male rats Two different metals per animal each metal was tested seven times for each test period two animals per test period served as control Testperiod: 15, 30 or 60 days samples: disc shaped wax samples 5mm in diameter and 1.5mm in thickness	Pub.: Y GLP: N	Ranking of the tested alloys with respect to the histopathological criteria investigated at the end of the test period.	RL = not relevant	Kansu, G.; Aydin, A.K. (1996): Evaluation of the biocompatibility of various dental alloys: Part I-- Toxic potentials. Europ. J. Prosth. Restorat. Dent. 4, 129-136	113

4.1.2.05	Sensitisation					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.05.1	**Sensitisation, human (Patch Test)** Test substance: silver nitrate Guideline: 60 humans Group I: 20 humans with a history of sever metal allergy Group II: 20 humans with a history of moderate metal allergy Group III: 20 humans without a history of metal allergy 0.5% in aqueous solution on the skin for 72h	Pub.: Y GLP: N	Moderate allergic reaction was observed for silver nitrate.	RL = 3 supplementary Data well descibed study Reportig of the results is in view of ranking different metal salt solution for their allergic potential.	Kansu, G.; Aydin, A.K. (1996): Evaluation of the biocompatibility of various dental alloys: Part 2-- Allergenical potentials. Europ. J. Prosth. Restorat. Dent. 4, 155-161	112
4.1.2.05.2	**Sensitisation –Studies in humans**					
4.1.2.05.2	**Case report - sensitisation, human** Test substance: silver amalgams Guideline: Not applicable 52-year old white female patient with severe periodental problems.	Pub.: Y GLP: N	It was believed that the symptoms in this case were due to hypersnsitivity to silver. Furthermore, a patch test with various metals was done and severe hypersensitivity to silver was found.	RL = 2 supplementary Data case report	Catsakis, L.H.; Sulica, V.I. (1978): Allergy to silver amalgams. Oral Surg. 46, 371-375	210
4.1.2.05.2	**Case report** Test substance: 1% silver chloride (complexed with sodium thiosulphate) 1% silver nitrate Guideline: Patch test	Pub.: Y GLP: N	positive reaction towards 1% silver nitrate solution and 1% silver chloride solution (complexed with sodium thiosulphate) after 48 and 72hr. Indication of an allergic contact sensitivity to ionic silver.	RL = 3 Method and results are described scarcely.	Marks, R. (1966): Contact dermatitis due to silver. Br. J. Dermatol. 78, 606-607	240
4.1.2.05.2	**Short comunication -case report** Test substance: silver coat Guideline:	Pub.: Y GLP: N		RL = 4 Short comunication - case report	Heyl, T. (1979): Contact dermatitis from silver coat. Contact Derm. 5, 197	239

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06	**Repeated dose toxicity - Reviews**					
4.1.2.06	**Review article** Test substance: Silver nitrate Guideline: not applicable review of case reports of silver exposed workers in the chemicla industry	Pub.: Y GLP: N	Summary review of human case reports of workers in various chemical industries. Predominant symptoms apart from argyria included irritation of the eye, GI and respiratory tract.	RL = 4 secondary literature, review	Holzegel, K. (1969): Über die Argyrose der Haut und Schleimhäute in der chemischen Industrie. Z. Ges. Hyg. 6, 440-447	202
4.1.2.06	**Review article** Test substance: Guideline: Silver in pharmaceutical preparations and devices.	Pub.: Y GLP: N	Silver and topical antimicrobials: One of the widest applications of silver is in combination with sulfadiazine (1%) as a topical antibacterial agent for the treatment of burn wounds. Transient leukopenia is seen in patients receiving silver sulfadiazine. Delayed wound healing is often observed clinically following the use of silver containing topical antimicrobial agents. Silver deposition has been found in the skin, gingiva, cornea, liver, and kidney of patients treated with silver sulfadiazine producing corresponding argyria, ocular injury, leukopenia and toxicity in kidney, liver, and neurological tissues. Silver electrodes: metallic taste in the mouth has been reported by some patients when a silver electrode containing iontophoretic device was placed on the face. This symptom strongly suggests the migration of sufficient silver from the device into the oral cavity to exceed the taste threshold for the metal, which is in contrast to the general consideration that silver is fixed by tissues at the site of application (i.e. protein bound). Mechanism of action: The toxic effects produced by silver compounds have been attributed to the free silver cation released into solution and subsequent interaction of the ion with sulfhydryl, amino, imadazole, phosphate, or carboxyl groups of membrane or enzyme proteins.	RL = 4 Review The following in-vivo studies are available as primary literature: #135 #145 #132 #123 #89 Not available (in-vivo): Gamelli R:L. 1993: bone marrow toxicity by silver sulfadiazine.	Hollinger, M.A. (1996): Toxicological aspects of topical silver pharmaceuticals. Crit. Rev. Toxicol. 26, 255-260	111

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06	**Review article including repeated dose toxicity** Test substance: Guideline: Not applicable	Pub.: Y GLP: N	The following primary literature is cited: #36, #41, #174, # 177 and #181	RL = 4 secondary literature	Anonymous (1990): Toxicological profile for silver. ATSDR - Agency for Toxic Substances and Disease Registry	31
4.1.2.06	**Review** Test substance: Guideline:	Pub.: Y GLP: N		RL = 4 Review, some toxicological information cited from a secondary sources (e.g. *RTECS); no reference list available.	Klein, D.A. (1978): Chapter 12: Effects on humans. Klein, D.A. (ED.): Environmental Impacts of Artificial Ice Nucleating Agents, Chap. 12, 169-175	24
4.1.2.06	**Review** Test substance: Guideline:	Pub.: N Data holder: Umicore GLP: N		RL = 4 Review (from around mid of 20th century); no useful information available.	Zobrist, F.; et al. (0): Die Gesundheitsunschädlichkeit von gesilbertem Trinkwasser. not applicable	27
4.1.2.06	**Data set** Test substance: Guideline:	Pub.: N Data holder: Umicore GLP: N		RL = 4 Data set: 4.4. Repeated dose toxicity: one reference for oral treatment (#181); one reference for drinking water and diet (primary literature not available: Bunyan J. et al. 1968); two references for i.p. (#181 and one reference with incomplete citation); two references for dermal (#181 and one reference with incomplete citation).	Anonymus (1993): Silver (metallic). AIDA Grunddatensatz, pp.33	28

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06	**Review** Test substance: Guideline:	Pub.: Y GLP: N		RL = 4 Review article about generalised/localised argyria in human.	Hunter, D. (Ed.) (1969): Silver. The Diseases of Occupations, 4th Ed., Little Brown Boston (MA), 409-420	225
4.1.2.06	**Overview article on argyria in human** Test substance: Guideline:	Pub.: Y GLP: N		RL = 4 Overview article on argyria in human.	Greene, R.M.; Su, W.P.D. (1987): Argyria. Am. Fam. Phys. 36, 151-154	33
4.1.2.06.1	**Repeated dose toxicity – Studies in animals**					
4.1.2.06.1	**Repeated dose toxicity, rats, swabbing of the oral cavity** Test substance: Silver nitrate (antismoking mouthwash containing 0.5, 8 or 10 % silver nitrate dissolved in placebo) Guideline: In compliance with OECD guidelines Sex: males and females Strain: Fischer 344 rats and Californian rabbits Groups: 4 groups of 4 animals per sex Route of admin.: swabbing of the oral cavity Exposure period: 320 days Frequency of treatment: daily Doses: 1.5, 15 and 150 mg/kg (the high dose was reduced to 75 mg/kg after the first week for rabbits) Control Group: yes, 1.88 ml placebo mouthwash	Pub.: Y GLP: N	Rats: mild diarrhoea and teeth staining; no mortality; stat. signif. reduction of kidney and lung weight in high dose females; stat. signif. increased platelet counts in all groups; no effects on clinical chemistry; histopathological changes in high dose animals: mild inflammation in the gum, tongue and oesophagus most probably related to silver deposition in the tissues; mid and low dose: similar histopathological findings to a lesser extent. Rabbits: mild to severe diarrhoea, general weakness and staining of teeth in mid and high dose animals; three of 8 high dose rabbits died in week 1 after which the dose was reduced; 1 male and 1 female of the mid dose group died after 14 days; histopathology revealed stomach ulceration with pigment deposition in the mucosa and serosa, peritoneal fat necrosis, brain oedema, severe pneumonia, pleuritis and peritonitis. Results of organ weights, haematological and clinical chemistry data and histopathology were not evaluated statistically (death of animals) and are not presented.	RL = 2 The study was conducted according to standard methods that comply with the OECD guidelines.	Tamimi, S.O.; et al. (1998): Toxicity of a new antismoking mouthwash 881010 in rats and rabbits. J. Toxicol. Environ. Health 53, 47-60	104
4.1.2.06.1	Test substance: Guideline:	Pub.: Y GLP: N	The absorption of silver from surgically induced open wounds was investigated in Guinea pigs.	RL = not relevant	Constable, J.D. et al. (1967): Absorption pattern of silvernitrate from open. Plast. Reconstruct. Surgery 39, 342-348	218

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06.1	Test substance: Guideline:	Pub.: Y GLP: N	Single intrapleural injection; rabbit; lung damage.	RL = not relevant	Vargas, F.S.; et al. (2002): Lung damage in experimental pleurodesis induced by silver nitrate or talc: 1-Year follow-up. Chest 122, 2122-2126	60
4.1.2.06.1	**Repeated oral treatment of rats - distribution throughout the body** Test substance: Silver nitrate or silver chloride Guideline: Not applicable Animals: rats Route of administration: drinking water Exposure period: life time Frequency of treatment: continuously Doses: 1, 0.4 or 0.1% silver salt (solution of silver nitrate or silver chloride dissolved in 0.3% sodium thiosulfate) Route of administration: drinking water Control group: yes, water Macroscopic and light microscopy examination of various tissues.	Pub.: Y GLP: N	Animals given 1% of silver salts did not survive. Two rats given 0.4% silver salts were kept alive for over 500 days. Survival of the animals treated with 0.1% was not affected by silver salt treatment. In skin no silver deposits were found in the dermis but in small numbers in the corium. Silver granules were observed in the tongue, salivary glands, thyroid, parathyroid, heart, blood vessels, lymph nodes, liver, kidneys, pancreas, intestines, spleen, adrenal glands, pituitary, choroid layer of the eye and bladder. No silver deposits could be detected in the lungs, testes, uterus, bone marrow, joints, striated muscles, brain except choroid plexus. No other pathological changes compared to controls were observed.	RL = 3 Very old study, highly deficient in description of methodology, but results well documented in the text and figures. The study was not intended to investigate repeated dose toxicity.	Olcott, C.T. (1948): Experimental argyrosis. IV. Morphologic changes in the experimental animal. Am. J. Path. 24, 813-833	36

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06.1	**Repeated dose toxicity, mice, oral** Test substance: Silver sulfadiazine Guideline: No Strain:CD-1 mice Exposure period: 30 days Frequency of treatment: daily or every other day Doses: 1,050 mg/kg Route of administration: orally and subcutaneously Control group. No Body weight gain was monitored and kidney, intestines, liver, spleen were removed for histopathology	Pub.: Y GLP: N	No death occurred following oral and s.c. administration. Histopathology did not reveal any obvious changes related to treatment. No weight loss, behavioural changes or diarrhoea were observed. Animals treated s.c. had granuloma lesions at the injection sites.	RL = 3 Methods and results are briefly described; the study was not intended to investigate repeated dose toxicity; only a limited number of endpoints was investigated.	Wysor, M.S. (1975): Orally-administered silver sulfadiazine: chemotherapy and toxicology in CF-1 mice; Plasmodium berghei (Malaria) and Pseudomonas aeruginosa. Chemotherapy 21, 302-310	153
4.1.2.06.1	**Repeated dose toxicity, chick, oral** Test substance: silver nitrate Guideline: No Strain: Hubbard broiler chicks (1 day old) Dose: basal diet + 900 ppm silver Time: 28 days Route of administration: oral via diet Control group: basal diet Two experiments with either 20 (a) or 10 (b) chicks per group were conducted. Silver induced copper deficiency and the effect of dietary Cu supplementation was investigated in this study. a) Heart weight, body weight ratio and packed cell volume data were obtained at study termination; b) glycogen content in heart and aortic elastin content were determined, samples of blood, liver, spleen, kidney and excreta were analysed for copper content.	Pub.: Y GLP: N	a) Addition of 900 ppm silver nitrate to the diet of chicks for 4 weeks significantly depressed growth rate, increased relative wet and dry heart weight. Silver in the diet had no significant effect on packed cell volume, but resulted in a 35% mortality rate. Supplementing the diet with 50 ppm copper prevented cardiac enlargement and mortality, but only partially corrected the growth depression. b) Glycogen content was not affected, but aortic elastin content was significantly reduced by silver and restored to normal by copper supplementation. Dietary silver significantly reduced copper contents in blood, brain, spleen and liver. Except brain, copper levels were restored by supplementation.	RL = 3 Methods and results are briefly described; the study was intended to investigate the effects of sliver induced copper deficiency and not repeated dose toxicity; only a limited number of endpoints were investigated. Not relevant for human health risk assessment.	Peterson, R.P.; Jensen, L.S. (1975): Interrelationship of dietary silver with copper in the chick. Poultry Sci. 54, 771-775	171

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06.1	**Repeated dose toxicity, pig, oral** Test substance: Silver acetate Guideline: Not applicable Strain: weanling pigs (4 animals per group) Exposure period: 40 days Frequency of treatment: continuously Doses: 0.2 5and 0.5 % Route of administration: diet Control Group: yes, commercial diet Animals received either silver alone or in combination with Se or alpha-tocopherol via the diet. They were observed daily for clinical signs and weighed each week. Pathology and histopathology was conducted.	Pub.: Y GLP: N	Food consumption and body weight gain was dose-dependently lower in pigs fed silver supplemented diets in comparison to control animals. The pigs continuously showed anorexia and diarrhoea characterised by black watery faeces. At 0.5%, three of 4 pigs died and the animals of this group showed histopathological changes characteristic of Se deficiency (hepatic necrosis, cardiomyopathy and myodegeneration). Hepatic Se content was significantly increased in pigs receiving silver via the diet. Supplementation of the diet with alpha-tocopherol, but not with Se prevents mortality and histopathological lesions.	RL = 2 The study was conducted according to state of the art methodology at that time; methods are described briefly and results are presented in reasonable in the text, tables and figures.	Van Vleet, J.F. (1976): Induction of lesions of selenium-vitamin E deficiency in pigs fed silver. Am. J. Veterin. Res. 37, 1415-1420	169
4.1.2.06.1	**Repeated dose toxicity, rats, oral** Test substance: Silver nitrate Guideline: No Sex: male Strain: Sprague-Dawley (8 per group) Exposure period: 13 weeks Frequency of treatment: continuously Doses: 1000 ppm (elemental silver) Route of administration: drinking water Control Group: yes, deionised water Animals received either silver, selenium or a combination of both. Body weight, food and water consumption were measured and behavioural tests were conducted	Pub.: Y GLP: N	No death occurred. General health and body weight were not affected by treatment with silver. Water consumption was stat. signif. decreased. Diarrhoea, lacrimation or salivation, motor ataxia, irritability or gross activity changes were not observed. No effects on fore- and hindlimp grip strength were seen.	RL = 2 Methods are described in detail and results are presented appropriately in the text and figures. Not all relevant endpoints to investigate subchronic toxicity were measured.	Cabe, P.A.; et al. (1979): Effects of selenium, alone and in combination with silver or arsenic, in rats. Neurobehavioral Toxicology 1, 275-278	166

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06.1	**Repeated dose toxicity, mouse, oral** Test substance: Silver nitrate Guideline: No Sex: female Strain: NMRI, 20 animals per group Route of admin.: drinking water Exposure period: 125 days Frequency of treatment: continuously Post. treatment period: 10 days Doses: 0.015 % in water (0.09 mg/mouse) Control Group: yes, water 10 days after the last treatment, animals were examined in an open field cage on 5 successive days.	Pub.: Y GLP: N	Treated animals were hypoactive compared to untreated controls. The weight of animals was not affected and the appearance of the animals was normal.	RL = 2 Methods are described appropriately and results are presented briefly in the text and a table. Very limited relevance, because only one end point (open field) was investigated.	Rungby, J.; Danscher, G. (1984): Hypoactivity in silver exposed mice. Acta Pharm. Toxicol. 55, 398-401	154
4.1.2.06.1	Test substance: Guideline: Extensive evaluation of cytokine mRNA expression levels in special strains of mice, which were either genetically susceptible or resistant. Pathogenesis of systemic autoimmune diseases. No relevant information on repeated dose toxicity; very special mechanistic endpoint	Pub.: Y GLP: N		RL = not relevant	Haggqvist, B.; Hultman, P. (2001): Murine metal-induced systemic autoimmunity: baseline and stimulated cytokine mRNA expression in genetically susceptible and resistant strains. Clin. Exp. Immunol. 126, 157-164	95
4.1.2.06.1	Test substance: Guideline:	Pub.: Y GLP: N	Evaluation of the effects of silver nitrate and silver sulphadiazine on wound healing in rats under defined laboratory conditions. Incisional wounds were induced surgically and treated daily for 10 days with the test compounds.	RL = not relevant	Lansdown, A.B.; et al. (1997): Silver aids healing in the sterile skin wound: experimental studies in the laboratory rat. Brit. J. Derm. 137, 728-735	106

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06.1	**Repeated oral treatment of rats - deposition in eyes** Test substance: Silver nitrate Guideline: No Sex: male Strain: Wistar Number of animals: 40 Exposure period: 10 weeks + 6 month Frequency of treatment: continuously, daily Doses: 0.25 % in water (222.2 mg/kg bw/d) Route of administration: drinking water Control Group: yes, concurrent no treatment Rats were given silver nitrate via drinking water for 10 weeks. Two animals were sacrificed and the remaining animals were divided in two groups. One group received this treatment for another 6 months followed by 6 months drinking water and the other received drinking water for 12 months. Animal were weight regularly and at monthly intervals one rat from each group was killed and its eyes were examined by electron microscopy	Pub.: Y GLP: N	There was no effect on body weight gains in the first 10 weeks of exposure. However, body weight losses in comparison to controls first appeared about 23 weeks after start of the experiment. Several animals that lost weight rapidly died. Body weight in the surviving experimental animals was an average of 50% less than that of control rats. Subsequent substitution of silver nitrate by water resulted in body weight increases, approaching that of untreated control rats within 13 weeks of cessation of exposure. Silver deposits were detected by electron microscopy in the eyes of the animals. Number and size of granules increased with exposure time. In rats receiving silver nitrate for 10 weeks, deposition of silver particles in the eye persisted for 3 months after cessation of exposure, but number and size had decreased 6 months, and after 12 months only fine granular deposits remained. Silver particles were not seen in the basement membranes of endothelial cells or cytoplasm.	RL = 2 The study was conducted according to state of the art methodology at that time; methods are described briefly and results are presented in reasonable in the text and figures. The study was not intended to investigate repeated dose toxicity; only a very limited number of relevant endpoints were evaluated.	Matuk, Y.; et al. (1981): Distribution of silver in the eyes and plasma proteins of the albino rat. Can. J. Ophthalmol. 16, 145-150	177

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06.1	**Repeated dose toxicity, rat, oral** Test substance: Silver Guideline: No Exposure period: 9 months Frequency of treatment: continuously Doses: 0.5, 2, 20 mg/l Route of administration: drinking water Control Group: yes, water Effects on conditioned reflexes activity were investigated.	Pub.: Y GLP: N	A silver dose of 0.5 mg/l does not affect the proceeding of conditioned reflexes. After 4 months of dosing, disturbance of conditioned reflex response was reported at 2 mg/l. At 20 mg/l, inhibition of excitation with a rise of intersignal response quality was seen. After 6 months, the inhibition of positive conditioned reflexes increased. After 9 months 20 mg/l: accumulation of silver in brain and liver.	RL = 4 Russian publication; only English abstract available.	Kulsky, L.A.; et al. (1972): Dynamics of changes of cortical activity in Albino rats with chronic silver intoxication. Dopov. Akad. Nauk. RSR Ser. B Heol. Heofiz. Khim. Biol. 34, 660-662	178
4.1.2.06.1	**Pharmacokinetic, distribution and excretion following single inhalation exposure, dog** Test substance: Metallic silver (30µCi of 110mAg) Guideline: No Sex: females Strain: 6 Beagle dogs Route of admin.: acute inhalation Exposure period: 7-15 min Frequency of treatment: once Doses: 1 mg/kg bw (averaged absolute deposition) Anaesthetised dogs were tracheally intubated and exposed to airborne particles produced by exploding radioactive silver wires.	Pub.: Y GLP: N	The lung had biological clearance half-lives of 1.7, 8.4, and 40 days accounting respectively for about 59, 39, and 2% of the total amount deposited. Corresponding values for the liver were 9 (97%) and 40 days (3%). Excretion was predominantly faecal, the major portion of which was believed to have been secreted into the bile. Sacrifice measurements showed that the liver was a major site for the deposition of silver. Lung, brain and muscle also contained relatively large accumulations.	RL = 2 Methods are described in detail and results are presented appropriately in the text, tables and figures.	Phalen, R.F.; Morrow, P.E. (1973): Experimental inhalation of metallic silver. Health Physics 24, 509-518	3

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06.1	**Repeated dose toxicity, guinea pig, dermal** Test substance: Silver nitrate Guideline: Not applicable Strain: Guinea pigs (20 animals) Exposure period: 8 weeks Frequency of treatment: 1 time/day Doses: 2.0 ml (0.239 M aqueous solution) to 3.1 cm2 of skin Route of administration: dermal Control Group: yes, distilled water Animals were observed for mortality and clinical signs, body weight, food and water consumption.	Pub.: Y GLP: N	No deaths recorded. Animals ceased to gain weight and weighed about 10-20 % less than controls at the end of the 8 week test period.	RL = 3 Very old study; the methods are described very poorly (details are given in a previous paper) and the results are not presented in detail; only a limited number of relevant endpoints were determined.	Wahlberg, R.C.; et al. (1965): Percutaneous toxicity of metal compounds. A comparative investigation in guinea pigs. Arch. Environ. Health 11, 201-204	41
4.1.2.06.1	**Repeated dose toxicity, rat, oral** Test substance: Guideline: Exposure period: 3 months Frequency of treatment: continously Post. obs. period: 2 months Doses:0.2 and 20 mg/l (0.03 mg/kg/d and 3 mg/kg/d Route of administration: drinking water Control Group: yes, concurrent no treatment	Pub.: Y GLP: N	No significant changes of haematological parameters in both groups compared to controls. Changes in amino acid concentrations in blood of the high dose group. At 3 mg/kg/d increased levels of silver in blood and liver after 3 months but no impairment of liver function. Liver and body weights decreased in the high dose group.	RL = 4 Russian publication; no English abstract available; presented results were taken from published IUCLID dataset.	Savlyk, O.S.; Moroz. O.G. (1973): Reaction of rats to long-term intake of silver with the drinking water. Vodopodgotovka i Ochistka Prom. Stokov 10, 99-108	179

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06.1	**Repeated dose toxicity, rat, oral** Test substance: Guideline: Sex: male Exposure period: 6 to 12 months Frequency of treatment: continuously Doses: 0.5, 2, 20 mg/l Route of administration: drinking water Control Group: yes, concurrent no treatment Studied effects: behaviour (conditioned reflexes), body weight, brain weight, amount of nuclei in brain, nucleic acid content of brain.	Pub.: Y GLP: N	No adverse effects at 0.5 mg/l. At 2 mg/l tendency to elevated brain weight and increase in nucleic acid content of brain.	RL = 4 Russian publication; no English abstract available; presented results were taken from published IUCLID dataset.	Kharchenko, P.D.; et al. (1973): Change in the content of brain nucleic acids of rats following long-term intake of ionic silver with the drinking water. Vodopodgotovka i Ochistka Prom. Stokov 10, 108-117	180
4.1.2.06.1	**Repeated dose toxicity, rat, dermal, oral and i.p.** Test substance: Silver nitrate Guideline: No 1. Study Route of administration: dermal 2. Study Route of administration: oral Exposure period: 4 days Frequency of treatment: 1 time/day 3. Study Route of administration: i.p. Test substance: silver nitrate, silver proteinate	Pub.: Y GLP: N	1. Study Silver nitrate was non toxic when applied to the skin 2. Study LOEL = 1680 mg Ag/kg/day Large amounts of silver found in all organs especially the kidneys, spleen and liver. 3. Study Silver nitrate was less toxic than silver proteinate following intra–peritoneal treatment. Chronic treatment gave tissue silver contents that were proportionately higher than those from acute treatment. The test substances were more toxic by intraperitoneal than oral routes.	RL = 4 French publication; no English abstract available; information presented here was taken from IUCLID dataset.	Dequidt, J.; et al. (1974): Experimental toxicoloigical study of some silver derivates. Bull. Soc. Pharm. Lille 1, 23-35	181

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06.1	**Repeated dose toxicity, chick, oral** Test substance: Silver Guideline: Not applicable Strain: Hubbard broiler and white leghorn chicks Number of animals: 3 pens of 10 chicks assigned to each dietary treatment Exposure period: 2 weeks Frequency of treatment: continuously Doses: 1000 ppm Route of administration: diet Control Group: yes, basal diet Mortality and food consumption was recorded and chicks weighed at study termination. Livers were removed for Se content. Effects of high levels of silver and copper on chicks fed diets containing various levels of Se were investigated in two experiments. For our purpose, results for basal diet plus 1000 ppm silver were compared to basal diet without any supplementation.	Pub.: Y GLP: N	For our purpose, results for basal diet plus 1000 ppm silver were compared to basal diet without any supplementation. 1000 ppm silver in the diet stat. Signif. reduced body weight compared to basal diet in both experiments. 13 or 20% mortality in the silver treated group compared to 3 or 0% mortality in the basal diet group was observed, respectively, in the two experiments. Food consumption was stat. signif. lower in the group fed 1000 ppm silver in both experiments. Selenium levels were lower in livers from animals fed silver via the diet compared to diet only animals.	RL = 2 The study was conducted according to state of the art methodology at that time; methods are described briefly and results are presented adequately in the text and tables. Not relevant for human health risk assessment.	Jensen, L.S. (1975): Modification of a selenium toxicity in chicks by dietary silver and copper. J. Nutr. 105, 769-775	172
4.1.2.06.1	**Repeated dose toxicity, rat, oral** Test substance: Guideline: Exposure period: 5 months Frequency of treatment: continuously Doses: 0.05 mg/l and 20 mg/l Route of administration: drinking water	Pub.: Y GLP: N	20 mg/l caused a reduction in weight gain of the animals and toxic effects on the functional sate of the liver and morphologic structure of the stomach, liver and intestines (abstract). Clinical chemistry: increased activities of alanin transaminase and alanin-aminotransferase at 20 mg/l. Histopathological findings: hyperplasia of glandular stomach, hypertrophy of stomach mucosa thickening of connective tissue layer in stomach. Swelling, infection and necrosis of intestinal mucosa. Granular dystrophy in the kidney, hyperchromic nuclei (IUCLID).	RL = 4 Russian publication; only English abstract available; presented results were taken from the abstract and from published IUCLID dataset.	Maslenko, A.A. (1976): Effect of "silver water" and silver treated water on the digestive organs. Vrach. Delo 5, 88-90	211

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06.1	**Repeated oral treatment of rats - deposition in various organs** Test substance: Silver nitrate Guideline: Not applicable Sex: male Strain: Sprague-Dawley rats Frequency of treatment: continuously Doses: 12 mM in water Route of administration: drinking water Control Group: yes, drinking water Preliminary studies: Dose: 6, 12 and 24 mM in water 12 rats per group Exposure period: up to 60 weeks 1. Study: Exposure period: 81 weeks Animals were killed either singly or in pairs after 0, 2, 4, 6, 8, 10, 12, 16, 25 and 60 weeks. 2. Study Exposure period: 10 weeks Post obs. period: 12 weeks Animals were killed 2, 4, 6, 8, 10 and 12 weeks after cessation of dosing Light- and electronmicroscopic examination of various organs were conducted	Pub.: Y GLP: N	Preliminary studies: At 6 mM, animals developed brown stained muzzles and teeth, but did not otherwise (behaviour, appearance, water consumption) differ from controls. At 24 mM, 3 out of 12 rats died, water intake was reduced and animals were terminated after in the second week, because of poor conditions. At 12 mM, only slight weight reduction and no clinical signs observed in animals exposed for 60 weeks. 1. Study: After 76 to 81 weeks of treatment clinical condition was impaired (no further data). Interim kills at 4, 6, 12, 25 weeks: time related increase in silver deposits (number and size). Major organs with silver deposits: kidney (glomerulum), colon, liver (after 6 weeks); choroid plexus, thyroid acinar cells, skin appendages (after 12 weeks); skin surface, urinary bladder, prostatic acinar basement membranes (after more than 25 weeks); intracellular location: basement membranes and phagocytes. 2. Study: Silver deposition continued for further 4 weeks after cessation of treatment.	RL = 3 Very old study; methods and dose regime are described very poorly. The study was not intended to investigate repeated dose toxicity.	Walker, F. (1971): Experimental argyria: a model for basement membrane studies. Br. J. Exp. Pathol. 52, 589-593	174

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06.2	**Repeated dose toxicity – Studies in humans**					
4.1.2.06.2	**Case report, human, chronic exposure - generalised and localised argyria** Test substance: Guideline: Not applicable 4 cases (3 females and one male); one generalised and one localised; light- and electron microscopy and X-ray analysis of skin biopsies.	Pub.: Y GLP: N	In all 4 cases, deposits consisted mainly of silver, selenium and sulphur.	RL = 2 reasonably documented publication	Matsumura, T.; et al. (1992): Detection of selenium in generalized and localized argyria: report of four cases with X-ray microanalysis. J. Derm. 19, 87-93	125
4.1.2.06.2	**Clinical study, human, exposure of workers** Test substance: Silver Guideline: Not applicable 5 patients with occupational argyria; employees of a silver manufactory for more than 12 years; light-, electron microscopy and x.ray microanalysis of skin biopsies.	Pub.: Y GLP: N	Small brown-black particle were seem in the dermis by light microscopy. Granules were shown to be electron-dense by electron microscopy. They were most numerous in the basal lamina of sweat glands, but were also present basal lamina of the epidermis and elastic fibres. X-ray analysis confirmed that many granules contained silver and sulphur. However, selenium, mercury, titanium and iron were also identified.	RL = 2 Methods are described in detail and results are presented adequately in the text, tables and figures.	Bleehen, S.S.; et al. (1981): Occupational argyria; light and electron microscopic studies and X-ray microanalysis. Brit. J. Derm. 104, 19-26	162
4.1.2.06.2	**Clinical study, human, exposure of workers** Test substance: Silver salts and metallic silver Guideline: No 50 employees of an industrial silver plant were examined.	Pub.: N Data holder: Umicore GLP: N	8 of 26 individuals exposed showed localised discolorations of uncovered skin and mucosa. There was no evidence of generalised argyria. The concentrations of silver in the skin of workers exposed to silver salts and to metallic silver were significantly higher for the silver workers than for the controls. There was no correlation between workplace exposure concentrations and duration of exposure	RL = 2 Methods are described in detail and results are presented adequately in the text and tables.	Wölbling, R.H.; et al. (1988): Silberablagerung in der Haut von Beschäftigten der silberverarbeitenden Industrie - Dermatologische Untersuchungen und quantitative Messungen mittels Atomabsorptionsspektrometrie. Johann Wolfgang Goethe-Universität Frankfurt am Main	22
4.1.2.06.2	**Clinical field study, human, exposure of workers** Test substance: Silver nitrate or silver oxide Guideline: Not applicable Report on clinical findings of 30 individuals exposed to silver nitrate and silver oxide at the work place.	Pub.: Y GLP: N	20 individuals had argyria (deposits of silver in the eye) with six showing also generalised argyria. No marked influence on the health was detected. Twelve of the 30 workers had measurable silver levels in the range of 1.1-8.4 µg/100 cc.	RL = 2 Methods are described in detail and results are presented adequately in the text and tables	Rosenman, K.D.; et al. (1979): Argyria: Clinical implications of exposure to silver nitrate and silver oxide. J. Occup. Med. 21, 430-435	2

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06.2	**Case report, human, chronic exposure - generalised argyria** Test substance: Silver nitrate Guideline: Not applicable Fourty-six year old white woman; topical silver nitrate for bleeding gingival three times weekly over two and a half years.	Pub.: Y GLP: N	Generalised argyria secondary to topical silver nitrate application on the oral mucosa occurred in a woman which used the silver nitrate applicators uncontrolled.	RL = 2	Marshall, J.P.; Schneider, R.P. (1977): Systemic argyria: secondary to topical silver nitrate. Arch. Dermatol. 113, 1077-1079	35
4.1.2.06.2	**Case report, human, chronic exposure - generalised argyria** Test substance: Neo-Silvol (10% silver nitrate) Guideline: Not applicable Seventy-five year old man; 15 years exposure to Neo-Silvol.	Pub.: Y GLP: N	Several cases of argyria have been described in the literature mainly resulting from extensive and long term use (several years) of disinfectant solutions of silver nitrate for the treatment of gingitivitis or silver nitrate containing eye or nose drops. The patient showed clinical signs of argyria and the diagnosis was confirmed by skin biopsy.	RL = 2 reasonably documented publication	Tanner, L.S.; Gross, D.J. (1990): Generalized argyria. Cutis 45, 237-239	58
4.1.2.06.2	**Case reports human, chronic exposure - fate in the dermis** Test substance: Néocarbagol, Carbagol, Collargent-Acétarsol, Collargol Guideline: Not applicable Results from 4 cases (human females; age: 46, 58, 72, 78 years) are described. The fate of silver in the dermis of chronic argyria was evaluated.	Pub.: Y GLP: N	Silver was easily recognisable in the tissue as irregular aggregates of elementary granules. Microanalysis X showed that the metal was bound with sulphur. In case of recent intoxication, the main location was intracellular (elementary particles in lysosomes). In the case of earlier intoxication, silver was found either in fibrilar components of connective tissue or in the basal material of sweat glands. In summary, silver is first phagocytised by macrophages, but these cells are not able to degenerate silver completely. Then silver is found on connective fibres (remains on sulphated glycoproteins).	RL = 4 not assignable, for language reasons French publication; only English abstract available; information presented here was taken from the abstract.	Reymond, J.L.; et al. (1980): Cutaneous argyria: an electron microscopic study of four eases with microanalysis X study of one case (author's transl) Original Title: Etude en microscopie electronique et en microanalyse X de 4 cas. Ann. Dermatol. Venereol. 107, 251-255	164

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06.2	**Case report, human, chronic exposure - generalised argyria** Test substance: Silver nitrate (3% solution) Guideline: Not applicable 52 year old man; approximately 10 years treatment of gingival erosions from ill fitting dentures with 3 % silver nitrate solution.	Pub.: Y GLP: N	Generalised argyria was diagnosed. High silver concentrations were determined in skin biopsies and silver selenid deposits were identified in the basal membranes of kidney by electron microscopy. Kidney function, however was not impaired and only negligible reactive changes were observed in kidney biopsies. Treatment with D-penicillamine or N-Acetyl-D-penicillamine did not increase urinary silver excretion.	RL = 2 reasonably documented publication	Aaseth, J.; et al. (1981): Argyria-tissue deposition of silver as selenide. Scand. J. Clinic. Lab. Invest. 41, 247-251	161
4.1.2.06.2	**Case report, human, chronic exposure - generalised argyria** Test substance: Silver nitrate Guideline: Not applicable 55-year old woman; a generalised argyria after 9 years of treatment of the oral mucosa with a silver nitrate stick was reported	Pub.: Y GLP: N	Symptoms observed were skin discoloration, progressive taste- and smell disorders, vertigo and hypesthesia. In tissue samples deposits in basal membranes, in macrophages, in the perineurium of peripheral nerves, along elastic and collagenous fibres and in necrotic cells of the oral submucosa. The deposits consisted partly of silver sulphide.	RL = 2 reasonably documented publication	Westhofen, M.; Schafer, H. (1986): Generalized argyrosis in man: neurotological, ultrastructural and X-ray microanalytical findings. Arch. Oto-rhino-laryngol.243, 260-264	149
4.1.2.06.2	**Repeated oral treatment of human - serum concentrations and accumulation in skin** Test substance: Silver acetate Guideline: Not applicable 21 human (11 males/10 females); treatment with anti-smoking chewing gum for 12 weeks; up to 6 pieces per day; chewing for 15-30 min.	Pub.: Y GLP: N	Serum concentrations of silver clearly rose after chewing gum use had started, and concentrations quickly returned to normal after use had ceased. The number of silver granules in skin biopsies increased. No one developed clinical signs of argyria.	RL = 2 Methods are described in detail and results are presented adequately in the text and tables.	Jensen, E.J.; et al. (1988): Serum concentrations and accumulation of silver in skin during three months treatment with an anti-smoking chewing gum containing silver acetate. Human Toxicol. 7, 535-540	139
4.1.2.06.2	**Human case report, localised argyria** Test substance: Silver /photographic fixing/staining solution Guideline: not applicable human case report,	Pub.: Y GLP: N	Localised dermal argyria of the skin (hands) in a female photographic technician is described, with a detailed histopathological localisation analysis in affected skin areas.	RL =2 reasonably well-documented human case report	Buckley, W.R. (1963): Localised Argyria. Arch. Dermatol. 88, 531-539	224

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06.2	**Case report, human, chronic exposure - generalised argyria** Test substance: Silver nitrate Guideline: Not applicable 33-year old female; painting of the tongue with 10% silver nitrate repeatedly over 6 years for treatment of oral ulcers.	Pub.: Y GLP: N	Generalised argyria resulting from deposition of silver in skin, mucosa, tongue, sclera and conjunctiva visible by a blue to slate-gray colour particularly in sun-exposed areas.	RL = 2 reasonably documented publication	Lee, S.M.; Lee, S.H. (1994): Generalized argyria after habitual use of AgNO3. J. Dermatol. 21, 50-53	122
4.1.2.06.2	**Clinical study, human, exposure of workers** Test substance: Silver bromide (90%), silver oxide, silver sulphide and metallic silver Guideline: Not applicable 27 Caucasian males and 27 matched referents; occupational exposure to primarily insoluble silver compounds.	Pub.: Y GLP: N	The authors found that despite the increased presence of silver in the blood, faces, and hair of Caucasian recovery workers versus referents, there was no evidence that chronic silver exposure adversely affected the health of these employees. No cases of generalised argyria. 29% of the silver workers exhibited ocular silver deposition, but did not reveal significant deficits in visual performance.	RL = 2 Methods are described in detail and results are presented adequately in the text and tables.	Pifer, J.W.; et al. (1989): Absence of toxic effects in silver reclamation workers. Scand. J. Work. Environ. Health 15, 210-221	185
4.1.2.06.2	**Clinical study, human, exposure of workers** Test substance: Silver nitrate and silver oxide Guideline: Not applicable 29 active and one retired employee (29 male/! FEMALE9; occupational exposure to silver; ophthalmologic evaluation.	Pub.: Y GLP: N	20 and 15 of 30 employees of an industrial plant involved in the manufacture of silver nitrate and silver oxide showed pigmentation of the conjunctiva and the cornea, respectively. A direct relationship existed between the levels of pigmentation and duration of employment. Ocular pigmentation was seen more frequently than cutaneous pigmentation.	RL = 2 Methods are described in detail and results are presented adequately in the text and tables	Moss, A.P.; Sugar, A.; et al. (1979): The ocular manifestations and functional effects of occupational argyrosis. Arch. Ophthalmol. 97, 906-908	227
4.1.2.06.2	**Case report, human, chronic exposure - Argyria (argyria of the eye)** Test substance: Silver nitrate Guideline: Not applicable	Pub.: Y GLP: N	A person using a silver dye on his eye browns and eye lashes for 25 years developed argyria in the conjunctiva of both eyes.	RL = 4 not assignable this published article is bibliographically not available to EBRC; Information taken from IUCLID data set.	Hill, W.R.; Pillsbury, D.M. (1939): Argyria. The pharmacology of silver. Williams and Wilkins	189

4.1.2.06	Repeated dose toxicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.06.2	**Clinical study, human, exposure of workers** Test substance: Silver and other metal powders Guideline: Not applicable A cross sectional study conducted on 27 workers engaged in manufacturing of precious metal powders.	Pub.: Y GLP: N	Of the 27 workers, 96% had elevated raised urine silver concentrations (0.5-52.0 µg/l) and 92% had raised blood silver concentrations (0.05-6.2 µg/100ml). Most workers had symptoms of respiratory irritation, and nose bleeds were reported in 8/27. Deposition of silver in the cornea of the eye was detected in five of eight of the long term workers. Estimated creatinine clearance was also significantly lower in the group exposed to silver than in the control group. Kidney function appears to have been adversely affected by exposures at work but because of the co-exposure to cadmium the role of silver in causing the decrement in kidney function could not be definitely determined.	RL = 2 Methods are described in detail and results are presented adequately in the text and tables.	Rosenman, K.D.; et al. (1987): Potential nephrotoxic effects of exposure to silver. Brit. J. Ind. Med. 44, 267-272	144

4.1.2.07	Mutagenicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.07	**Mutagenicity - Reviews**					
4.1.2.07	**Review** Test substance: Guideline:	Pub.: Y GLP: N		RL = 4 available literature: #184, #182, #38 missing literature: Goff + Powers 1975, Loeb et al. 1977, Luk et al. 1975, Mauss et al. 1980, Robinson et al. 1982, Scicchitano + Pegg 1987, McCoy + Rosenkranz 1978 ; Nishioka 1975	Anonymous (1990): Toxicological profile for silver. ATSDR - Agency for Toxic Substances and Disease Registry	31

4.1.2.07	Mutagenicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.07	**Review** Test substance: Guideline:	Pub.: Y GLP: N		RL = 4 available literature: #173, #182; missing literature: 2 x Zakour et al. 1981, 2 x Robinson et al. 1982 ; Di Paolo + Casto 1979	Sundermann, F.W. (1984): Recent advances in metal carcinogenesis. Ann. Clin. Lab. Sci. 14, 93-122	40
4.1.2.07	**Review** Test substance: Guideline:	Pub.: Y GLP: N	negative in several bacterial assays Silver ions can interact in vitro with DNA in ways that cause DNA strand breaks and affect the fidelity of DNA replication. The following primary literature on mutagenicity is cited and not available to EBRC at this stage: Tso and Fung, 1981 (bacterial assay) Rossmann and Molina, 1986 (bacterial assay) ATSDR, 1989 (bacterial assay, in vitro DNA effects) Sirover and Loeb, 1976 (in vitro DNA effects) Sundermann, 1984 (in vitro DNA effects) Scicchitano and Pegg, 1987 (in vitro DNA effects) Denizau and Marion, 1989 (genotoxic effects in rat hepatocytes)	RL = 4 Secondary literature	Jongerius, O.; Jongeneelen F.J. (1990): Occupational exposure limits. Criteria Document for metallic silver. Commission of European Communities	222
4.1.2.07.1	**Mutagenicity – Studies in vitro**					
4.1.2.07.1	**Genetic toxicity in vitro – Drosophila melanogaster** Test substance: Silver nitrate Guideline: No Drosophila melanogaster strains flr3 (female) and mwh (male) were mated; concentration: 0.5 mM Drosophila wing somatic mutation and recombination test	Pub.: Y GLP: N	positive	RL = 2 Methods described in detail and results presented adequately in the text, tables and figures.	Yesilada, E. (2001): Genotoxicity testing of some metals in the drosophila wing somatic mutation and recombination test. Bull. Environ. Contam. Toxicol. 66, 464-469	42

4.1.2.07	Mutagenicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.07.1	**Genetic toxicity in vitro - chromosomal aberrations in Root tip cells** Test substance: Guideline: Root tip cells of Crepis capillaris L. Concentration: 1 E-6 - 6 E-6 M	Pub.: Y GLP: N	positive	RL = 4 Russian study; no English abstract; information taken from IUCLID (test system not commonly accepted).	Reutova, N.V.; Shevtchenko, V.A. (1991): Silver as a potential mutagen. Genetika, 27, 1280-1284	203
4.1.2.07.1	Test substance: Guideline:	Pub.: Y GLP: N	Silver-reinforced glass ionomer dental cement was used.	RL = not relevant	Kaplan, C.; et al. (2004): Mutagenic potentials of dental cements as detected by the Salmonella/microsome test. Biomat. 25, 4019-4027	72
4.1.2.07.1	Test substance: Guideline:	Pub.: Y GLP: N	Stainless steel rods coated with silver were used.	RL = not relevant	Bosetti, M.; et al. (2002): Silver coated materials for external fixation devices: in vitro biocompatibility and genotoxicity. Biomaterials 23, 887-892	85
4.1.2.07.1	**Genetic toxicity in vitro - viral transformation assay** Test substance: Silver nitrate Guideline: Not applicable Syrian hamster embryo cells transformed by SA7 virus; concentration: 0.06 mM; treatment period 18 hrs.	Pub.: Y GLP: N	Silver nitrate was reported to increase the viral transformation rate when cells were treated for 18 hours with 0.6 mM.	RL = 3 No validated test protocol; endpoint of questionable significance	Casto, B.C.; et al. (1979): Enhancement of viral transformation for evaluation of the carcinogenic or mutagenic potential of inorganic metal salts. Cancer Res. 39, 193-198	44
4.1.2.07.1	**Genetic toxicity in vitro – chromosomal malsegregation in Saccaromyces cerevisiae** Test substance: Silver nitrite Guideline: Bo S. cerevisiae strain D61M	Pub.: Y GLP: N	Positive Most effective concentration 0.14 ppm; test substance induced mitotic aneuploidy	RL = 3 No standard test protocol; no detailed description of results obtained with the test substance.	Zimmermann, F.K. (1983): Mutagenicity screening with fungal systems. Ann. NY Acad. Sci. 407, 186-196	62

4.1.2.07	Mutagenicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.07.1	Test substance: Guideline:	Pub.: Y GLP: N	Endpoint cytotoxicity.	RL = not relevant	Mazzotti, F.; et al. (2002): In vitro setting of dose-effect relationships of 32 metal compounds in the Balb/3T3 cell line, as a basis for predicting their carcinogenic potential. Alternatives Lab. Anim. 30, 209-217	52
4.1.2.07.1	**Genetic toxicity in vitro - Salmonella typhimurium reverse mutation assay** Test substance: Silver Guideline: no S. typhimurium strains LT2, TA 100; concentration: 0.0001 - 0.1 M; without metabolic activation Method: Ames Test	Pub.: Y GLP: N	negative	RL = 2 Only 2 S. typhimurium strains tested.	Tso, W.-W.; Fung, W.-P. (1981): Mutagenicity of metallic cations. Tox. Let. 8, 195-200	59
4.1.2.07.1	**Genetic toxicity in vitro - bacterial mutation assay (photogenotoxicity)** Test substance: silver nitrate (Alfa Products) Guideline: No E. coli strains WP2 (trpE), WP2s (trpE, uvrA); concentration: 0.1 uM (highest conc.); without metabolic activation Enhancement of UV mutagenesis in E. coli WP2	Pub.: Y GLP: N	Negative Silver nitrate had no effect on UV mutagenesis in E. Coli WP2	RL = 3 No standard test protocol; methods described briefly; no detailed description of results obtained with the test substance.	Rossman, T.G.; Molina, M. (1986): The genetic toxicology of metal compounds. 2. Enhancement of ultraviolet light-induced mutagenesis in escherichia coli WP2. Environ. Mutagen. 8, 263-271	38

4.1.2.07	Mutagenicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.07.1	**Genetic toxicity in vitro - bacterial mutation assay** Test substance: Silver nitrate Guideline: No E. coli strain K12 PQ37; concentration: 0.001 - 10 nmol/tube; without metabolic activation SOS chromotest	Pub.: Y GLP: N	negative Toxic concentration: 1 nmol/tube	RL = - RL= 3(4) French study; no English abstract; no detailed description of results obtained with the test substance.	Olivier, Ph; Marzin, D. (1986): Etude de l'activite mutagene des metaux a l'aide du sos chromotest. Larc Medical 6, 252-254	215
4.1.2.07.1	**Genetic toxicity in vitro - HGPRT assay** Test substance: Guideline: Chinese hamster ovary (CHO) cells Metabolic activation: with and without S-9 mix Duration of exposure: 5 h Negative control: solvent	Pub.: Y GLP: N	Positive The authors themselves describe the positive outcome of their testing as "preliminary" and subject to confirmation, which is why the result of this test is interpreted here as "ambiguous".	RL = 3 Methods described in detail; no detailed description of results obtained with the test substance.	Hsie, A.W.; et al. (1979): Quantitative mammalian cell mutagenesis and a preliminary study of the mutagenic potential of metallic compounds. Kharasch, N. (Ed.): Trace Metals in Health and Disease, 55-69	173
4.1.2.07.1	**Genetic toxicity in vitro - unscheduled DNA synthesis, in vitro** Test substance: Silver nitrate (A.R. grade) Guideline: No Pollen from Petunia hybrida W166K; concentration: 2 mM; without metabolic activation Labelled DNA was extracted and counted according to the method of Jackson and Linskens (1978, 1979). DNA polymerase activity was determined as described by Takats and Wever (1972).	Pub.: Y GLP: N	No significant unscheduled DNA synthesis was detected with Ag. However Ag has been shown to be inhibitory in this system. So it is difficult to comment on its ability to actually induce UDS.	RL = 2 No standard test protocol; methods described sufficiently; no detailed description of results obtained with the test substance.	Jackson, J.F.; Linskens, H.F. (1982): Metal ion induced unscheduled DNA synthesis in Petunia pollen. Mol. Gen. Genet. 187, 112-115	216

4.1.2.07	Mutagenicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.07.1	**Genetic toxicity in vitro - Bacillus subtilis recombination assay** Test substance: Silver nitrate (Maruchi chemicals) Guideline: No Bacillus subtilis strains H17 and M45; concentration: 0.001 - 10 M; without metabolic activation	Pub.: Y GLP: N	negative	RL = 3 Valid test protocol; no detailed description of results obtained with the test substance.	Kanematsu, N.; et al. (1980): Ret assay and mutagenicity studies on metal compounds. Mutat. Res. 77, 109-116	182
4.1.2.07.1	**Genetic toxicity in vitro - bacterial mutation assay** Test substance: silver nitrate (Alfa products) Guideline: No E. coli strain B/r WP2s (lambda); without metabolic activation Induction of lambda prophage in E. coli	Pub.: Y GLP: N	Negative Growth inhibition at 2.5 E-7 mol/l.	RL = 2 Test system not commonly used; methods described sufficiently; no detailed description of results obtained with the test substance.	Rossman, T.G.; Molina, M.; et al. (1984): The genetic toxicology of metal compunds: I. Induction of lambda prophage in E.coli WP2S lambda. Environ. Mutagen. 6, 59-69	204
4.1.2.07.1	**Genetic toxicity in vitro - bacterial mutation assay** Test substance: Silver nitrate (Merck) Guideline: No E. coli strains PQ37, PQ35; concentration: 0.003 - 3 nM/mL; without metabolic activation SOS chromotest	Pub.: Y GLP: N	Negative Toxic concentration: 3 nM/mL	RL = 2 No standard test protocol; methods described sufficiently; no detailed description of results obtained with the test substance.	Oliver, Ph.; Marzin, D. (1987): Study of the genotoxic potential of 48 inorganic derivatives with the SOS chromotest. Mutat. Res. 189, 263-269	183

4.1.2.07	Mutagenicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.07.1	**Genetic toxicity in vitro - bacterial mutation assay** Test substance: Silver nitrate Guideline: No Micrococcus pyogenies var. aureus strain FDA209, penicilline and streptomycine resistant; Concentration: 0.000001% ; without metabolic activation Induction of antibiotic resistance mutants	Pub.: Y GLP: N	negative	RL = 3 Very old study; no standard test protocol; methods described briefly; no detailed description of results obtained with the test substance.	Clark, J.B. (1953): The mutagenic action of various chemicals on Micrococcus aureus. Proc. Oklahoma Acad. Sci. 34, 114-118	213
4.1.2.07.1	**Genetic toxicity in vitro - bacterial mutation assay** Test substance: Silver nitrate Guideline: No E. coli strains B/Sd-4/1,3,4,5 and B/Sd-4/3,4; concentration: 0.000005% or 0.0001 (24hours), 0.000025, 0.00005% or 0.0001% (3 hrs); without metabolic activation; control Streptomycine method	Pub.: Y GLP: N	Negative Concentrations ranged up to cytotoxic concentrations.	RL = 2 Very old study; no standard test protocol; methods described briefly; relative detailed presentation of the results in tables.	Demerec, M.; et al. (1951): A survey of chemocals for mutagenic action on E. coli.. Am. Nat. 85, 119-136	184
4.1.2.07.1	**Genetic toxicity in vitro – chromosomal malsegregation in Saccaromyces cerevisiae** Test substance: Silver nitrate Guideline: No S. cerevisiae strain DIS13	Pub.: Y GLP: N	Negative (maximum efficient dose 7µg/ml) No diploid or aneuploid cells were observed.	RL = 3 No standard protocol; detailed description of the test system, but not the methods used; limited description of results obtained with the test substance.	Sora, S.; Magni, G.E. (1988): Induction of meiotic chromosomal malsegregation in yeast. Mutat. Res. 201, 375-384	207

4.1.2.07	Mutagenicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.07.1	**Genetic toxicity in vitro – bacterial bioluminiscence test** Test substance: Silver nitrate (highest grade) Guideline: No Photobacterium fischeri strain Pf-3; without metabolic activation; 24 hours of incubation at 28° Bioluminescence test according to Ulitzur (1986)	Pub.: Y GLP: N	Positive: MIC = 0.005 mmol/l; luminescence increased approximately 2 to 10 times in comparison with the control. (MIC = Minimal inhibitory concentration)	RL = 3 No standard test protocol; methods described briefly; no detailed description of results obtained with the test substance.	Ulizur, S.; Barak, M. (1988): Detection of genotoxicity of metallic compounds by the bacterial bioluminescence test. J. Biolum. Chemilum. 2, 95-99	206
4.1.2.07.1	**Genetic toxicity in vitro – HGPRT assay** Test substance: Silver diaminotetraborat Guideline: No System of testing: V79 hamster cells Test concentration: 0.2, 0.5, 1.0, 2.0 µg/ml Metabolic activation: with and without S-9 mix Solvent: phosphate buffered saline Incubation: 24 hrs Treatment: 60 min Negative control: solvent HGPRT assay in V79 cells.	Pub.: Y GLP: N	Negative Concentrations of 0.2-2 µg/ml were chosen, which in the presence of S-9 mix had a cytotoxic effect of about 17-29% and without S-9 mix 19-92%.	RL = 2 Methods described in detail and results presented adequately in the text, tables and figures.	Dusinska, M.; Slamenova, D. (1990): Effects of silver compounds on in vitro cultured mammalian cells: II. Study of genotoxicity and the effect of diamminesilver tetraborate on macromolecular synthesis of V79 cells. Biologia 45, 211-218	192
4.1.2.07.1	**Genetic toxicity in vitro - unscheduled DNA synthesis** Test substance: Silver nitrate (analytical grad J.T. Baker Chem. Co) Guideline: (similar to OECD 482 (1989)) Cultured rat hepatocytes; 3(H)-thymidine incorporation; concentration: 4.6 to 46 µM of silver ions; 2hrs treatment period; 20 hrs incubation period. Determination of UDS and binding to DNA	Pub.: Y GLP: N	A small but significant increase in UDS was only seen at the highest concentration (18.5 µM without hydroxyurea) which was moderately cytotoxic. Silver was accumulated in the nucleus, but to a smaller extend than other metal ions (Cu, Cd, Pb).	RL = 2 Conducted according to a recent test protocol; sufficient information on test system; results are presented briefly in the text and figures	Denizeau, F.; Marion, M. (1989): Genotoxic effects of heavy metals in rat hepatocytes. Cell Bio. Toxicol. 5, 15-25	32

4.1.2.08	Carcinogenicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.08	**Carcinogenicity - Reviews**					
4.1.2.08	Test substance: Guideline:	Pub.: Y GLP: N	No info related to carcinogenicity and silver.	RL = not relevant	Sundermann, F.W. (1984): Recent advances in metal carcinogenesis. Ann. Clin. Lab. Sci. 14, 93-122	40
4.1.2.08	Test substance: Guideline:	Pub.: N Data holder: Umicore GLP: N		RL = 4 Data set: 4.7 Carcinogenicity: 2 entries about s.c. implantation of silver foil (#223, #214) and 1 about cutted discs (not available); one each about s.c. administration (#228), i.v. (#228) and i.m. (#168).	Anonymus (1993): Silver (metallic). AIDA Grunddatensatz, pp.33	28
4.1.2.08.1	**Carcinogenicity – Studies in animals**					
4.1.2.08.1	**Carcinogenicity, rat, s.c. implantation** Test substance: Silver metall foil Guideline: No Strain: Wistar Sex: male Route of administration: implantation Exposure period: 714 days Frequency of treatment: implanted throughout the test Post. obs. Period: no data Doses: Two pieces of foil, 1.5 cm wide e	Pub.: Y GLP: N	Silver metal foil embedded subcutaneously in 25 rats induced fibrosarcomas earlier (275 days compared to 350-714 days) and more frequently (32% (14 rats) at implantation sites compared to 0-5 %) than other metal foil (steel, tantalum, tin and vitallium). Several materials produce such tumours when implanted subcutaneously in animals and the relevance of these tests to carcinogenesis in humans is uncertain.	RL = 4 Review; primary literature available (#214, #223, #228, #168) Route of administration not relevant.	Anonymous (1990): Toxicological profile for silver. ATSDR - Agency for Toxic Substances and Disease Registry	31

4.1.2.08	Carcinogenicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.08.1	**Carcinogenicity, rat, s.c. implantation** Test substance: Silver metall foil Guideline: No Strain: Wistar Sex: male Route of administration: implantation Exposure period: 714 days Frequency of treatment: implanted throughout the test Post. obs. period: no data Doses: Two pieces of foil, 1.5 cm wide	Pub.: Y GLP: N	Silver metal foil embedded subcutaneously in 25 rats induced fibrosarcomas earlier (275 days compared to 350-714 days) and more frequently (32% (14 rats) at implantation sites compared to 0-5 %) than other metal foil (steel, tantalum, tin and vitallium).	RL = 3 Route of administration not relevant.	Coffin, D.L.; Palekar, L.D. (1985): Bioassays for asbestos and other solid materials. Handbook of Carcinogen Testing, Park Ridge NJ Noyes Publications, 384-419	223
4.1.2.08.1	**Carcinogenicity, rat, s.c. implantation** Test substance: Silver metall foil Guideline: No Strain: Wistar Sex: male Route of administration: implantation Exposure period: 714 days Frequency of treatment: implanted throughout the test Post. obs. period: no data Doses: Two pieces of foil, 1.5 cm wide	Pub.: Y GLP: N	Silver metal foil embedded subcutaneously in 25 rats induced fibrosarcomas earlier (275 days compared to 350-714 days) and more frequently (32% (14 rats) at implantation sites compared to 0-5 %) than other metal foil (steel, tantalum, tin and vitallium).	RL = 3 Route of administration not relevant.	Oppenheimer, B.S.; Oppenheimer, E.T.; et al. (1956): Carcinogenic effect of metals in rodents. Cancer Res. 16, 439-441	214
4.1.2.08.1	Test substance: Guideline:	Pub.: N Data holder: Umicore GLP: N	In-vivo fibrogenic potential of HEC waste (granular silver) following single intraabdominal administration.	RL = not relevant	Greenspan, B.J. (1983): HEC-waste (silver) six-month intraabdominal fibrosis study in rats. Bushy Run Research Center, Export, Pennsylvania, USA	18

4.1.2.08	Carcinogenicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.08.1	Test substance: Guideline:	Pub.: N Data holder: Umicore GLP: N	In-vivo fibrogenic potential of silver oxide following single intraabdominal administration.	RL = not relevant	Greenspan, B.J. (1983): Silver oxide six-month intraabdominal fibrosis study in rats. Bushy Run Research Center, Export, Pennsylvania, USA	17
4.1.2.08.1	Test substance: Guideline:	Pub.: N Data holder: Umicore GLP: N	In-vitro cytogenicity study in rabbit alveolar macrophages with silver oxide.	RL = not relevant	Hengler, W.C. (1979): Silver oxide - macrophage assay. Chemical Hygiene Fellowship, Pittsburgh, Pennsylvania, USA	10
4.1.2.08.1	Test substance: Guideline:	Pub.: N Data holder: Umicore GLP: N	In-vitro cytogenicity study in rabbit alveolar macrophages with silver oxide.	RL = not relevant	Hengler, W.C.; Slesinksi, R.S. (1982): Silver oxide - rabbit alveolar macrophage (RAM) assay. Bushy Run Research Center, Export, Pennsylvania, USA	9
4.1.2.08.1	Test substance: Guideline:	Pub.: N Data holder: Umicore GLP: N	In-vitro cytogenicity study in rabbit alveolar macrophages with HEC waste (granular silver).	RL = not relevant	Hengler, W.C.; Slesinski, R.S. (1982): HEC waste (silver) - rabbit alveolar macrophage (RAM) assay. Bushy Run Research Center, Export, Pennsylvania, USA	8
4.1.2.08.1	**Carcinogenicity, rat** Test substance: Guideline:	Pub.: Y GLP: N	No specific info for silver.	RL = not relevant	Furst, A. (1981): Bioassay of metals for carcinogenesis: Whole animals. Environ. Health Perspect. 40, 83-91	226

4.1.2.08	Carcinogenicity					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.08.1	**Carcinogenicity, rat, i.m.** Test substance: Metall powder Guideline: No Strain: Fischer 344 Route of administration: i.m. Exposure period: 150 days Frequency of treatment: 30 days (5 monthly doses) Post. obs. period: weekly for 4 weeks, then monthly Doses: 0.2 ml metal powder in trioctinoin Control Group: yes Animals were palpated for tumours and killed when moribund or when a growth was noted. Suspected tissues were analysed pathologically.	Pub.: Y GLP: N	No signs of toxicity in animals treated with silver. Autopsy showed some rats had encapsulated vehicle and injected metal at injection site. No muscular atrophy was noted. The authors concluded that silver was not tumorigenic when administered as a finely divided powder.	RL = 3 Route of administration not relevant.	Furst, A.; Schlauder, M.C. (1978): Inactivity of two noble metals as carcinogens. J. Environ. Path. Tox. 1, 51-57	168
4.1.2.08.1	**Carcinogenicity, rat** Test substance: Colloidal silver Guideline: No Strain: DB rats (male/female) Route of administration: s.c. and i.v. Exposure period: 10 month (s.c.), 7 month (i.v.) Frequency of treatment: weekly Post. obs. period: life-time observation Doses: 1.75/2.45 mg/animal (s.c.) 2.5 mg (i.v.) Animals were treated by s.c. injections for 10 months and received i.v. injections in addition for 7 months	Pub.: Y GLP: N	Silver apparently produced fibrosarcoma earlier and more frequently than several other metal foils. The authors reported that colloidal silver injected subcutaneously into rats resulted in tumours in 8/26 rats that survived longer than 14 months. In six of the eight rats, the tumours were at the injection site.	RL = 3 Route of administration and study design not relevant.	Schmähl, D.; Steinhoff, D. (1960): Experimental carcinogenesis in rats with colloidal silver and gold solutions. Z. Krebsforsch. 63, 586-591	228

4.1.2.09	Toxicity for Reproduction					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.09	**Toxicity for reproduction - Reviews**					
4.1.2.09	**Review** Test substance: Guideline:	Pub.: Y GLP: N	see following article are cited EBRC#48 and #163	RL = 4 Review only	Jongerius, O.; Jongeneelen F.J. (1990): Occupational exposure limits. Criteria Document for metallic silver. Commission of European Communities	222
4.1.2.09.1	**Toxicity for reproduction – Effects on fertility and reproduction**					
4.1.2.09.1	**Toxicity to reproduction - fertility male/female rat, oral** Test substance: Silver nitrate or silver chloride Guideline: Not applicable Species: rats Sex: male/female Route of administration: drinking water Exposure period: life time Frequency of treatment: continuously Doses: 1, 0.4 or 0.1% silver salt (solution of silver nitrate or silver chloride dissolved in 0.3% sodium thiosulphate) Route of administration: drinking water Control group: yes, water Macroscopic and light microscopy examination of various tissues including testes, uterus and ovaries.	Pub.: Y GLP: N	Testes were unchanged. Appearance of spermatozoa was normal and no silver deposits were found in the testes. Fertility of male rats was not impaired (no further data). Uterus and ovary: Silver deposition was very low in the uterus. No loss of fertility was reported in female rats (no further data).	RL = 3 Very old study; highly deficient in description of methodology, but results well documented in the text and figures. The study was not intended to investigate repeated dose toxicity.	Olcott, C.T. (1948): Experimental argyrosis. IV. Morphologic changes in the experimental animal. Am. J. Path. 24, 813-833	36
4.1.2.09.1	**Industion of testicular atrophy** Test substance: Silver nitrate Guideline: not applicable study design: direct testicular injection Species: rat and mice, male Frequency of treatment: single injection	Pub.: Y GLP: N	Decreases in testicular weight seen after 7 days, with focal or total testicular necrosis after 2 and 7 days; spermatozoa destroyed after 7 d. Study not considered relevant for risk assessment purposes.	RL = 3 invalid study design, method and results insufficiently described	Kamboj, V.P.; Kar, A.B. (1964): Antitesticular effect of metallic and rare earth salts. J. Reprod. Fertil. 7, 21-28	50

4.1.2.09	Toxicity for Reproduction					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.09.1	**Toxicity to reproduction - fertility male rat, oral** Test substance: Silver nitrate Guideline: no Species: Albino rats Sex: male Route of admin.: s.c. Doses: 0.04 mM/kg No of animals: 3-5 rats per group Five different experiments were conducted: a) single injection, sacrifice after 18 hrs b) repeated injection, sacrifice after 2, 10, 21 or 30d c) single injection, sacrifice after 4, 8 or 12 d d) repeated injections (10 days), sacrifice 1, 4 or 8 d after termination of treatment c) single injection, sacrifice after 24 hrs Testes were removed and investigated by light microscopy (a-d) and x-ray analysis ©	Pub.: Y GLP: N	Silver nitrate produced acute and chronic changes in the histology of testis and interfered to some degree with spermatogenesis. All tissues showed improvement following the initial injection, even in spite of continuous daily injections for up to 30 days.	RL = 2 Very old study; not according to standard protocol; methods described briefly; results presented in detail in the text.	Hoey, M.J. (1966): The effects of metallic salts on the histology and functioning of the rat testis. J. Reprod. Fertil. 12, 461-471	48
4.1.2.09.1	**Developmental toxicity - mechanistic study, rat, oral** Test substance: Silver chloride Guideline: No Strain: Inbred Albino rats Sex : females (pregnant) Route of administration: oral via the diet Exposure period: throughout pregnancy Frequency of treatment: continuously Doses: 50 mg / animal / day Control Group: yes Pregnant rats received an embryotoxic dose of silver chloride in the diet; the effect of ceruloplasmin (CP) on the embryotoxicity/visceral malformations was evaluated.	Pub.: Y GLP: N	Silver chloride did not affect the physiological function of dams. Enzymatically active Cu containing CP was eliminated from the blood. Embryos showed developmental abnormalities and prenatal death or 100% mortality was seen in the first 24 hours. Cu in the placenta and foetal tissue was strongly diminished. Cu and Zn-dismutase activity decreased in embryonic cells and to a lesser degree in tissues of pregnant females. Embryotoxicity was severely diminished by treatment with CP.	RL = 2 No standard protocol; methods adequately described; results presented detailed in the text, table and figures. The study was not intended to evaluate the developmental toxicity; mechanistic study.	Shavlovski, M.M.; et al. (1995): Embryotoxicity of silver ions is diminished by ceruloplasmin--further evidence for its role in the transport of copper. Biometals 8, 122-128	89

4.1.2.09	Toxicity for Reproduction					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.09.1	**Toxicity to reproduction - in-vitro study with human spermatozoa** Test substance: Silver Guideline: Not applicable Human semen was collected, spermatozoa prepared and treated with different metals; evaluation of motility, metabolism, glucose utilisation and oxidation and lactate accumulation.	Pub.: Y GLP: N	Silver reduced the motility of unwashed spermatozoa to two thirds of the control (after 3 hours, while motility was less pronounced affected in washed spermatozoa. This was accompanied by reduced oxygen consumption and glucose utilisation and oxidation, but there were no changes in lactate accumulation.	RL = 2 The methods are described sufficiently; results are presented adequately in the text and tables.	Holland, M.K.; White, I.G. (1980): Heavy metals and spermatozoa. 1. Inhibition of the motility and metabolism of spermatozoa by metals related to copper. Fertil. Steril. 34, 483-489	163
4.1.2.09.2	**Toxicity for reproduction – Developmental toxicity**					
4.1.2.09.2	**Developmental toxicity - single administration in monkey, intrauterine** Test substance: Silver nitrate Guideline: Strain: 15 Cynomolgus monkeys (Macaca fascicularis) Sex: female Route of administration: intrauterine injection Exposure period: between days 27 and 43 of pregnancy Frequency of treatment: single dose Doses: 1 ml of silver nitrate 1 % in water Control Group: other: 0.9 % saline Monkeys received single intravenous injections and were allowed to deliver; 2 additional monkeys were subjected to hysterectomy 7 days after injection.	Pub.: Y GLP: N	Silver nitrate treatment resulted in vaginal bleeding 1 or 2 days after treatment and lasted for an average of 5.3 days. In all 7 silver treated monkeys, pregnancy was terminated. Four of six control monkeys delivered healthy offspring. All silver treated monkeys recovered and two of seven became pregnant after subsequent mating and delivered healthy infants.	RL = 3 No standard protocol; methods and results described briefly. Route of administration not relevant for human health risk assessment.	Dubin, N.H.; et al. (1981): Effect of silver nitrate on pregnancy termination in cynomologous monkeys. Fertil. Steril. 36, 106-109	46

4.1.2.10	Other toxicity data					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.10	**Other toxicity data**					
4.1.2.10	**Case report on Argyria** Test substance: Silver nitrate Guideline: Fourty-six year old woman; 5 years exposure to silver nitrate application used in oral mucosa.	Pub.: Y GLP: N		RL = not rated	Marshall, J.P.; Schneider, R.P. (1977): Systemic argyria: secondary to topical silver nitrate. Arch. Dermatol. 113, 1077-1079	35
4.1.2.10	**Biochemical or cellular interactions** Test substance: Silver nitrate Guideline: Type: Biochemical or cellular interactions Species: Spraque Dawley Sex: male Route of administration: i.p. The effects on hepatic hemoxygenase activity (HO), cytochrome P-450, GSH, metallothionein (MT) and renal MT and GSH were determined.	Pub.: Y GLP: N	Male Spraque Dawley rats received i.p. injections of the maximum tolerated dose (MTD) (65 umol/kg/day) of silver nitrate or a fraction thereof 36 and 12 hr before sacrifice. At the MTD silver nitrate increased HO and depressed cytochrome P-450 liver and kidney GSH. MT levels were not affected by the treatment. At lower doses silver nitrate did not show any effects on the parameters studied.	RL = not rated	Eaton, D.L.; et al. (1980): Dose–response effects of various metal ions on rat liver metallothionein, glutathione, heme oxygenase, and cytochrome P–450. Toxicol. Appl. Pharmacol. 55, 393-402	47
4.1.2.10	**Cellular interactions** Test substance: silver sulphate Guideline:	Pub.: Y GLP: N	Silver induces degranulation and LTC4 secretion in mast cells. Silver induces not only intracellular production but also extracellular release of ROS, including O2-(radical) and H2O2. The silver induced ROS production is mediated by a FAD-dependent oxidase, which is activated in a PI3K independent manner.	RL = not rated special mechanistic investigation	Yoshimaru, T.; et al. (2006): Silver activates mast cells through reactive oxygen species production and a thiol-sensitive store-independent Ca2+ influx. Free Radical Bio. & Med. 40, 1949-1959	65

4.1.2.10	Other toxicity data					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.10	**Cytotoxicit- in vitro,** Test substance: Titanium-silver alloys Guideline:	Pub.: Y GLP: N		RL = not rated dental applications are not covered under reach	Oh, K.-T.; et al. (2005): Properties of titanium-silver alloys for dental application. J. Biomed. Mat. Res. Part B, Appl. Biomat. 74, 649-658	69
4.1.2.10	**Cellular interactions** Test substance: silver nitrate, silver sulphate Guideline:	Pub.: Y GLP: N	Silver specifically induces degranulation and LTC4 production in RBL-2H3 mast cells. Silver does not induce the production of IL-4 and TNF-a. Silver has no effect on tyrosine phosphorylation of FceRIb, Lyn, Syk and LAT. Silver induces calcium signals that are involved in degranulation but not in LTC4 production.	RL = not rated	Suzuki, Y.; et al. (1950): Silver activates calcium signals in rat basophilic leukemia-2H3 mast cells by a mechanism that differs from the Fc epsilon RI-activated response. J. Immunol. 169, 3954-3962	82
4.1.2.10	**Review** Test substance: Guideline:	Pub.: Y GLP: N	Toxicity of silver and silver products in wound repair	RL = not rated	Lansdown, A.B.G. (2002): Silver. 2: Toxicity in mammals and how its products aid wound repair. J. Wound Care 11, 173-177	88
4.1.2.10	Test substance: silver amalgam Guideline:	Pub.: Y GLP: N		RL = not rated route of administration not relevant for HH rsik assessment; futhermore dental applications are not covered under reach	Hultman, P.; et al. (1998): Activation of the immune system and systemic immune-complex deposits in Brown Norway rats with dental amalgam restorations. J. Dental Res. 77, 1415-1425	101
4.1.2.10	Test substance: Silver nitrate Guideline:	Pub.: Y GLP: N	Secondary amyloidosis	RL = not rated	Grehan, S.; et al. (1997): Down-regulation of the major circulating precursors of proteins deposited in secondary amyloidosis by a recombinant mouse interleukin-1 receptor antagonist. Eur. J. Immunol. 27, 2593-2599	105
4.1.2.10	**Cellular interactions** Test substance: Guideline:	Pub.: Y GLP: N	Interaction of silver and other metals with thiol groups.	RL = not rated	Hultberg, B.; et al. (1997): Copper ions differ from other thiol reactive metal ions in their effects on the concentration and redox status of thiols in HeLa cell cultures. Toxicology 117, 89-97	109

4.1.2.10	Other toxicity data					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.10	**Cellular interactions** Test substance: Silver nitrate Guideline:	Pub.: Y GLP: N		RL = not rated	Hultman, P.; et al. (1995): Genetic susceptibility to silver-induced anti-fibrillarin autoantibodies in mice. Clin. Immunol. Immunopat. 77, 291-297	115
4.1.2.10	**Cytotoxicity** Test substance: silver- based dental casting alloys Guideline: Balb/c 3T3 mouse fibroblasts	Pub.: Y GLP: N	metal release from alloys and cytotoxicity	RL = not rated dental applications are not covered under reach	Wataha, J.C.; et al. (1995): Correlation between cytotoxicity and the elements released by dental casting alloys. Int. J. Prost. 8, 9-14	119
4.1.2.10	**Cellular interactions** Test substance: Silver nitrate Guideline:	Pub.: Y GLP: N	In this paper silver, amongst other was examined to determine ist influence on the reactive oxygen species (ROS) generating capacity of human neutrophils and on a serum opsonic activity.	RL = 4 Japanese publication; only English abstract available	Ono, Y.; et al. (1994): Effects of metals (silver, nickel, cobalt and chromium) on the reactive oxygen species generating capacity of human neutrophils and on the serum opsonic activity. Nippon Eiseigaku Zasshi. Japan. J. Hyg. 49, 645-653	121
4.1.2.10	**Cellular interactions** Test substance: Silver nitrate Guideline:	Pub.: Y GLP: N	Silver ions in micromolar concentration significantly increase the production of superoxide anions in cells, initiated by formyl-methionyl-leucylphenylalanine.	RL = not rated	Jansson, G.; Harms-Ringdahl, M. (1993): Stimulating effects of mercuric- and silver ions on the superoxide anion production in human polymorphonuclear leukocytes. Free Rad. Res. Comms. 18, 87-98	124
4.1.2.10	Test substance: Silver nitrate/lactate Guideline: not applicable Species: Wistar rats (m/f) administration: i.p. or via drinking water autometallography and effects on nervous system	Pub.: Y GLP: N	The localisation in the nervous system after silver administration is investigated, together with an attempt to evaluate of neurotoxic effects by volumetric analysis oif hippocampi of postnatally developing animals. Plausible and transparent results were not obtained in this study.	RL = 3 inadequate sample sizes, incomplete documentation dosing regimen and results	Rungby, J. (1990): An experimental study on silver in the nervous system and on aspects of its general cellular toxicity. Danish Med. Bull. 37, 442-449	132

4.1.2.10	Other toxicity data					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.10	**Histopathological observation of periodontal tissue of rats** Test substance: silver alloy powder Guideline:	Pub.: Y GLP: N		RL = 4 Japanese publication; only English abstract available	Watanabe, K. (1989): Histopathological study of damage to periodontal tissues by silver alloy metals in rats. Nippon Shishubyo Gakkai Kaishi 31, 1021-1046	135
4.1.2.10	**Histopathological observation of periodontal tissue of rats** Test substance: Silver Guideline:	Pub.: Y GLP: N	Clumps and granules of silver powder were not associated with chronic inflammatory changes. Multinucleate giant cells were shown surrounding silver powder. Silver powders were observed to have been taken into the multinucleate giant cells. Many of these cells also contained fine dark granules in their cytoplasm, and a few contained small pieces of silver. The tissue reaction to silver showed little cytotoxicity.	RL = 4 Japanese publication; only English abstract available	Iijima, S. (1989): Histopathological study of the effect of pure metals to the periodontal tissues. Nippon Shishubyo Gakkai kaishi 31, 997-1020	136
4.1.2.10	**Cellular interactions** Test substance: Silver lactate Guideline: cultured mouse peritonesl macrophages	Pub.: Y GLP: N	It was demonstrated that silver affects viability and structure of cultured macrophages, possibly due to induction of lipid peroxidation, as demonstrated to occur in the liver of silver-exposed mice.	RL = not rated	Rungby, J.; et al. (1987): Silver affects viability and structure of cultured mouse peritoneal macrophages and peroxidative capacity of whole mouse liver. Arch. Toxicol. 59, 408-412	143

4.1.2.10	Other toxicity data					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.10	**Neurotoxic effects on the developing hypocampus** Test substance: Silver lactate monohydrate (silver content 54.78%) Guideline: No Sex: male and female pups (2 litters of 4) Strain: Wistar rats Route of admin.: intraperitoneal Exposure period: 4 postnatal weeks Frequency of treatment: daily Doses: 0.1 mg in week 1, 0.2 mg in week 2 and 0.35 mg in week 3 and 4 Control group: yes, sodium lactate Hippocampus preparations were examined for neuropathological changes by comparison of the volumes of the different (14) components.	Pub.: Y GLP: N	The body weight of experimental animals was significantly smaller than for control animals. No pathological cellular changes were seen. While there was a tendency for the volumes of the hippocampal components of the treated animals to be smaller than those of controls, this effect did not reveal stat. significance. The pyramidal cell layer of the region superior, and as a consequence the whole pyramidal cell layer, was significantly smaller in the silver treated animals. In conclusion, these findings indicate that the perikaria of the pyramidal cells are either the first elements to show silver toxicity or that they are selective for silver neurotoxicity.	RL = 2 Methods are described in detail and results are presented appropriately in the text and table.	Rungby, J.; et al. (1987): A quantitative evaluation of the neurotoxic effect of silver on the volumes of the components of the developing rat hippocampus. Toxicology 43, 261-268	145
4.1.2.10	**Case report on argyria** Test substance: Argyrol (silver containing eye-drops) Guideline: Eighty year old woman	Pub.: Y GLP: N		RL = not rated	Loeffler, K.U.; Lee, W .R. (1987): Argyrosis of the lacrimal sac. Graefe's Arch. Clin. Exp. Ophthalm. 225, 146-150	146
4.1.2.10	**Cellular interactions** Test substance: Silver lactate Guideline:	Pub.: Y GLP: N	After a single intraperitoneal injection of 20mg/kg silver lactate, lipid peroxidation was significantly increased in the liver 3, 12 and 48h after exposure whereas MDA levels in kidney and brain were not significantly affected.	RL = not rated	Rungby, J. (1987): Silver-induced lipid peroxidation in mice: interactions with selenium and nickel. Toxicology 45, 135-142	147
4.1.2.10	**Selenium- effects on silver cellular toxicity and distribution** Test substance: Silver lactate Guideline:	Pub.: Y GLP: N		RL = not rated	Rungby, J.; et al. (1987): Effects of selenium on toxicity and ultrastructural localization of silver in cultured macrophages. Arch. Toxicol. 61, 40-45	148

4.1.2.10	Other toxicity data					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.10	**Case report, human** Test substance: Silver nitrate Guideline: Autopsy of a seventy-eight year old woman, chronic heavy user of over-the-counter nasal drops containing silver nitrate.	Pub.: Y GLP: N	Silver nitrate deposition was observed in circumventricular organs (CVO) and in the paraventricular and supraoptic nuclei of the hypothalamus.	RL = not rated	Landas, S.; et al. (1985): Demonstration of regional blood-brain barrier permeability in human brain. Neurosci. Letters 57, 251-256	152
4.1.2.10	**Cellular interaction** Test substance: Silver nitrate Guideline: synovial cells 3 + 2 day exposure 3 flasks served as control 1 flask per concentration silver concentration : 10µM Cell proliferation, collagen synthesis and non-collagen protein synthesis were studied.	Pub.: Y GLP: N	Silver resulted in decreased DNA content, which was attributed to cytotoxicity. A dose-dependent inhibition of [3H]proline incorporation into bacterial collagenase resistant (non-collagen) protein was observed. Silver inhibited collagen accumulation to a greater extent than non-collagen protein accumulation. The rate of collagen accumulation in medium decreased during incubation.	RL = not rated	Goldberg, R.L.; et al. (1983): Effect of heavy metals on human rheumatoid synovial cell proliferation and collagen synthesis. Biochem. Pharm. 32, 2763-2766	158
4.1.2.10	**Biochemical or cellular interactions** Test substance: Silver nitrate Guideline:	Pub.: Y GLP: N	Silver nitrate increased the rate of hemoglobin catalysed peroxidation of phospholipid liposomes in vitro. The effect could be inhibited by addition of superoxide dismutase and catalase, suggesting an involvement of superoxide radicals and H2O2. Silver ions potentiated hemoglobin autooxidation and increased the binding of hemoglobin to the erythrocyte membrane.	RL = not rated	Ribarov, S.; Brnov, L.; et al. (1986): On the mechanism of AgNO3-induced lipid peroxidation in erythrocytes. Biomed. Acta 45, 321-330	193
4.1.2.10	**Cellular interactions** Test substance: Silver nitrate Guideline:	Pub.: Y GLP: N	The hepatic level of GSH was depleted after single-dose treatment and percentage of GSH depletion was greater still after the repeated-dose treatment. Glutathione reductase activity was increased 24 hours after treatment Glutathione-S-transferase activity was significantly decreased after 24 hours after a single-dose treatment.	RL = not rated	Sheweita, S.A. (1998): Heavy metal-induced changes in the glutathione levels and glutathione reductase/glutathione s-transferase activities in the liver of male mice. Int. J. Toxicol. 17, 383-392	195

4.1.2.10	Other toxicity data					
Section	**Description**	**Pub/GLP**	**Key findings**	**Rating/Comments**	**Source**	**EBRC#**
4.1.2.10	**Immunological study** Test substance: silver nitrate analytical grade (Merck) Guideline: Not applicable Sex: female Strain: SJL (H-2s) Exposure period: 5 to 10 weeks Frequency of treatment: continuously Doses: 0.002 %, 0.01 %, 0.05 % Route of administration: drinking water Control Group: yes, tap water Investigation of the influence of silver on the production of IgG anti-nucleolar antibodies and immune complexes (mechanistic study).	Pub.: Y GLP: N	No clinical signs of illness were observed in the treated animals. Serum immunglobulin concentrations: IgM levels in treated mice were not significantly different from controls. IgG1, IgG2b and IG3 concentrations in the 0.05 % group showed a slight but significant decrease after 10 weeks. Serum anti-nuclear antibodies: all mice of the 0.05 and 0.01 % groups developed anti-nuclear antibodies after 5 weeks and the titter was increased after 10 weeks of treatment. No organ specific antibodies were found. Findings in kidneys: No histological damage or signs of vasculitis, no deposits of IgG or Ig3C. In the 0.05 % group, a small but significant increase in mesanglial IgM titer compared to controls was observed. Silver deposition was observed in the kidneys, spleen and livers of the 0.01 % and 0.05 % groups. No effects were observed at 0.002 %.	RL = 2 Methods are described in detail and results are presented appropriately in the text, tables and figures. Special mechanistic study.	Hultman, P.; Eneström, S.; et al. (1994): Selective inducation of anti-fibrillarin autoantibodies by silver nitrate in mice. Clin. Exp. Immunol. 96, 285-291	201

Annex 2

Documentation of literature search on STN

Compound: Silver (and compounds)
Topics: - toxicity data obtained from animal testing
- human clinical epidemiological data
Date of search: 30./31.08.2006

Selection of database(s):

STN provides access to the database TOXCENTER (Toxicology Center), which is a bibliographic database that covers the pharmacological, biochemical, physiological, and toxicological effects of drugs and other chemicals.

TOXCENTER is composed of data relevant for all aspects of toxicity from the following databases:

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ANEUPL   - Aneuploidy File
BIOSIS   - 1969 to the present
CAplus   - 1907 to the present
CIS      - CIS Abstracts
CRISP    - Toxicology Research Projects
DART     - Development and Reproductive Toxicology File
EMIC     - Environmental Mutagen Information Center File
EPIDEM   - Epidemiology Information System,
ETIC     - Environmental Teratology Information Center File
FEDRIP   - Federal Research in Progress
HAPAB    - Health Aspects of Pesticides Abstract Bulletin
HMTC     - Hazardous Materials Technical Center File
IPA      - 1970 to the present
MEDLINE  - 1950 to the present
PESTAB   - Pesticides Abstracts
PPBIB    - Poisonous Plants Bibliography
RISKLINE - Swedish National Chemicals Inspectorate
TSCATS   - Toxic Substances Control Act Test Submissions
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Given the amount of sources for TOXCENTER, it is assumed that the field of toxicology is comprehensively covered by a search in this database.

Search strategy:

TOXCENTER contains - amongst others - online thesauri for Chemical Name (/CN) and Controlled Terms (/CT). In addition, CAS registry numbers can be searched for in the Basic Index field (/BI). Controlled Terms (CT) are key words that describe the relevance of a literature item for a certain topic, issue or scientific problem. Controlled Terms are organised in a hierarchical structure and so called Narrow Terms (NT) are subordinated. For example, the CT "Toxicity Tests" is associated which several NTs, amongst them "acute toxicity tests", "lethal dose 50", "mutagenicity tests", etc... Prior to the actual search, the database TOXCENTER was accessed to obtain lists of available controlled terms, together with their narrow terms, which could then be used for the search.

The following Controlled Terms, together with their associated narrow terms (+NT), where applicable, where selected for this search.

Metabolism
Absorption
Biological Transport
Pharmacokinetics
Toxicity
Toxicity Tests
Epidemiological Studies

Concerning the compounds to be searched for, the CAS Numbers for silver metal (7440-22-4), silver nitrate (7761-88-8) and silver(I)oxide (20667-12-3) were searched for in the Basic Index field (/BI). In addition the word "silver" was searched in the Chemical Name field (/CN), in which all chemicals addressed e.g. in an article, are listed. In doing so, all compounds containing "silver" in their name were covered. However, a preview search had indicated, that an unmanageable amount of results would be returned without further narrowing this search. Therefore, the search was limited to those database entries, which in addition to the above, contained one of the words "silver" or "silber" (german) or "argentum" in the abstract. This decision was taken to keep the dataset manageable and to obtain an overview of existing data. Should data-gaps be observed during the evaluation of the obtained data, more specific searched should be conducted to fill these gaps.

The search was prepared offline in the format of a search script, which was then executed online. The possibility to interact with the process is given. Using this search strategy, and after automatic removal of 3 duplicates, 336 results were obtained. Deciding that this appeared to be a reasonably number of relevant results, the bibliographic information and the abstracts were obtained from the database for 336 results.

Annex 3 – List of literature identified during screening of secondary review articles

cited in EBRC # 24

Durbin (1960)	# 245
Kehoe et al. (1940)	# 242
Kent & McCance (1941)	# 243
Tipton et al. (1966)	# 241
McKeen & Wolf (1963)	# 246
Natusch et al. (1974)	# 247
Standler & Vonnegut (1972)	# 248

cited in EBRC # 28

Goldberg et al. 1950	#231
Venugopal, B; Luckey, T.D. 1978	#232
Konradova, V; Folia Morphol. 1968	#233
Heyl et al. 1979	#239
Marks 1966	#240
Bunyan J. et al. 1968	

cited in EBRC # 31

East et al. (1980)	#234
McIntyre (1978)	#235
Snyder et al. (1975)	#237
Olcott 1947	
Olcott 1950	

cited in EBRC #41:

Skog & Wahlberg, 1964	#230

cited in EBRC # 111

Gamelli R:L. 1993

cited in EBRC # 222

Stokinger 1981 (acute toxicity)
Sollmann, 1957 (acute toxicity in human)
Forycki et al., 1983 (acute toxicity in human)
Shelley et al., 1987 (Agyrosis)
Smith and Carson, 1977 (chronic toxicity in man)
Buckley et al., 1965 (Argyria)
Bell et al., 1952 (Argyria)
Lehnert et al., 1973 (treatment of Argyria)
Jindrichova , 1963 (Argyria in workers)
Perrone et al., 1977 (4 cases of argyria)
Brooks, 1981 (lung toxicity)
Barrie and Harding, 1947 (case reports on workers, lung toxicity)
McLaughlin et al., 1945 (case reports on workers, lung toxicity)
La Torraca, 1962 (Nephrotoxicity)
Tso and Fung, 1981 (bacterial assay)
Rossmann and Molina, 1986 (bacterial assay)
ATSDR, 1989 (bacterial assay, in vitro DNA effects)
Sirover and Loeb, 1976 (in vitro DNA effects)
Sundermann, 1984 (in vitro DNA effects)
Scicchitano and Pegg, 1987 (in vitro DNA effects)
Denizau and Marion, 1989 (genotoxic effects in rat hepatocytes)
Furst and Haro, 1969 (carcinogenicity)

Note: Literature characterised above with an EBRC reference no (i.e., #230-248) were already acquired and screened for relevance; the other are listed here for completeness and should be considered in a more in-depth evaluation.