Summary

Silver chloride is used for the treatment of wines to remove fermentation and storage-related abnormal odours (odours caused by reduction reactions, characterised by the presence of hydrogen sulphide and thiols). Silver chloride added to wine must be applied to an inert support, like kieselguhr (diatomaceous earth), bentonite, kaolin, etc. The precipitate must be eliminated by any appropriate physical procedure.

Silver sulphide formed during the treatment remains adsorbed by the inert carrier material and together they can be separated by filtration.

The European Union requested the addition silver chloride to the Annex of the Wine Agreement in late 2015. Provisional approval was granted for the use of this product in European wine exported to Australia under the Wine Agreement. To ensure consistency with the Food Standards Code it is requested to amend the table to clause 14 (Permitted processing aids with miscellaneous functions) of Standard 1.3.3 of the Food Standards Code to include silver chloride as a processing aid. In the revised food Standards Code it would probably fall under Schedule 18-9 (Processing Aids that perform various technological purposes). As the Australian wine industry does not wish to use silver chloride as a processing aid no request is being made to amend Standard 4.5.1.

The inert carrier materials, such as, for instance, kieselguhr (diatomaceous earth), bentonite, kaolin, etc. should comply with the prescriptions of the Food Standards Code. The precipitates silver chloride forms with unwanted components in alcoholic beverages during processing are removed via filtration or similar processes.

Standard 1.3.4 requires that substances added to food, including processing aids, comply with relevant specifications as detailed in the Code. Silver Chloride meets the OIV specification which is one of the secondary references for specifications in Standard 1.3.4 (Identity and Purity). Therefore, no new specification is required for the Code.

The OIV recommended dose is not over 1 g/hl. Although the silver complex will be filtered out, any residual silver must be minimised and meet the Food Standards Code requirements.

Part D: APPLICATION TO AMEND THE AUSTRALIA AND NEW ZEALAND FOOD STANDARD CODE FOR THE INCLUSION OF SILVER CHLORIDE AS A PROCESSING AID FOR WINE

Contents

Summary

Silver chloride is used for the treatment of wines to remove fermentation and storage-related abnormal odours (odours caused by reduction reactions, characterised by the presence of hydrogen sulphide and thiols). Silver chloride added to wine must be applied to an inert support, like kieselguhr (diatomaceous earth), bentonite, kaolin, etc. The precipitate must be eliminated by any appropriate physical procedure. Silver sulphide formed during the treatment remains adsorbed by the inert carrier material and together they can be separated by filtration.

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Standard 1.3.4 requires that substances added to food, including processing aids, comply with relevant specifications as detailed in the Code. Silver Chloride meets the OIV specification which is one of the secondary references for specifications in Standard 1.3.4 (Identity and Purity). Therefore, no new specification is required for the Code.

The OIV recommended dose is not over 1 g/hl. Although the silver complex will be filtered out, any residual silver must be minimised and meet the Food Standards Code requirements.

3.1 GENERAL REQUIREMENTS

3.1.2 Applicant details

Winemakers' Federation Australia

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AUSTRALIA

[redacted]

3.1.3 PURPOSE OF THE APPLICATION

The intention of this application is to allow silver chloride as a processing aid for wine. It is requested to amend the table to clause 14 (Permitted processing aids with miscellaneous functions) of Standard 1.3.3 of the Food Standards Code to include silver chloride as a processing aid. In the revised food Standards Code it would probably fall under Schedule 18-9 (Processing Aids that perform various technological purposes). As the Australian wine industry does not wish to use silver chloride as a processing aid no request is being made to amend Standard 4.5.1 Wine Production requirements (Australia only).

3.1.4 JUSTIFICATION FOR THE APPLICATION

a) Need for the Proposed Change.

Silver chloride is used for the treatment of wines to remove fermentation and storage-related abnormal odours (odours caused by reduction reactions, characterised by the presence of hydrogen sulphide and thiols). Silver chloride added to wine must be applied to an inert support, like kieselguhr (diatomaceous earth), bentonite, kaolin, etc. The precipitate must be eliminated by any appropriate physical procedure(OIV 2012).

Silver sulphide formed during the treatment remains adsorbed by the inert carrier material and together they can be separated by filtration.

The European Union requested the addition of silver chloride to the Annex of the Wine Agreement in late 2015 (Oenoppia, 2015). Provisional approval was granted for the use of these products in European wine exported to Australia under the Wine Agreement. To ensure consistency with the Food Standards Code it is requested to amend the table to clause 14 (Permitted processing aids with miscellaneous functions) of Standard 1.3.3 of the Food Standards Code to include silver chloride as a processing aid. In the revised food Standards Code it would probably fall under Schedule 18-9 (Processing Aids that perform various technological purposes). As the Australian wine industry does not wish to use silver chloride as a processing aid no request is being made to amend Standard 4.5.1.

The inert carrier materials, such as, for instance, kieselguhr (diatomaceous earth), bentonite, kaolin, etc. should comply with the prescriptions of the Food Standards Code. The precipitates silver chloride forms with unwanted components in alcoholic beverages during processing are removed via filtration or similar processes.

Standard 1.3.4 requires that substances added to food, including processing aids, comply with relevant specifications as detailed in the Code. Silver Chloride meets the OIV specification which is one of the secondary references for specifications in Standard 1.3.4 (Identity and Purity). Therefore, no new specification is required for the Code.

The OIV recommended dose is not over 1 g/hl. Although the silver complex will be filtered out, any residual silver must be minimised and meet the Food Standards Code requirements.

b) Advantages of the Proposed Change Over the Status Quo

The change will enable Australia to meet its international obligations under the EU-Australia wine Agreement.

c) Status of Similar Application made in other Countries

No applications are being made by the applicant to other national jurisdictions.

A. REGULATORY IMPACT INFORMATION

1. Costs and benefits

There are no costs to Australian industry or consumers and the sector will befit from improved relations with the European Commission and more broadly the European wine sector.

a) Costs and benefits to the consumers

There are no costs to Australian industry or consumers and the sector will befit from improved relations with the European Commission and more broadly the European wine sector.

b) Costs and Benefits to Industry and Business in General.

There are no costs to Australian industry or consumers and the sector will befit from improved relations with the European Commission and more broadly the European wine sector.

c) Costs and Benefits to Government.

There will be no increased regulatory or enforcement costs for the government.

2. Impact on International Trade

The change will enable Australia to meet its international obligations under the EU-Australia wine Agreement.

3.1.5 INFORMATION TO SUPPORT THE APPLICATION

1. General

There are no negative public health implications. The silver complexes will be filtered out of the wine prior to bottling.

(a) Consumer Choice Issues

There are no consumer issues.

(b) Evidence of General Food Industry or Specific Company Support

The Winemaker's Federation of Australia (WFA) has submitted this application on behalf of the Australian wine sector. WFA is strongly committed to ensuring the integrity of Australia's Food Standards and the international trading system through compliance with World Trade Organisation obligations and international treaty obligations.

A. Technical Information on the Processing Aid

1. Information on the type of processing aid

Silver chloride is a chemical compound with the chemical formula AgCl. This white crystalline solid is well known for its low solubility in water (this behavior being reminiscent of the chlorides of Tl^{+} and Pb^{2+}). Upon illumination or heating, silver chloride converts to silver (and chlorine), which is signaled by greyish or purplish coloration to some samples. AgCl occurs naturally as a mineral chlorargyrite.

Silver chloride is used for the treatment of wines to remove fermentation and storage-related abnormal odours (odours caused by reduction reactions, characterised by the presence of hydrogen sulphide and thiols), thus falling in category (d) – *Permitted decolourants, clarifying, filtration and adsorbent agents* in Standard 1.3.3.

2. Information on the identity of the processing aid

OIV (2014a) prepared a monograph on Silver Chloride - N° C.A.S.: 7783-90-6 . Further information on the chemical structure is available on PuBChem open Chemistry Data Book (attached).

3. Information on the chemical and physical properties of the processing aid

Silver chloride reacts with sulphurous components, for instance, hydrogen sulphide (H2S), disulphides, mercaptans or thioacetates to form silver sulphide during the treatment which remains adsorbed by the inert carrier material and together they can be separated by filtration. Independent of the pH value, silver chloride is very slightly soluble thus the risk of residues in wine is very low, provided the wine is filtered after treatment.

Dosages vary according to the amount of sulphides present. For example, Erbolsch recommend:

- Slight to moderate sulphide off-flavours 20 g/100 L
- Persistent sulphide off-flavours 30 - 50 g/100 L
- Maximally permitted addition (EU legislation) 50 g/100 L

The silver chloride on the adsorbant base is directly added to the pre-filtered wine. Separation is conducted by filtration two days after application at the earliest and no silver residues are expected in the wine due to the poor solubility of silver chloride.

4. Manufacturing process

Silver chloride is synthesized by combining aqueous solutions of silver nitrate and sodium chloride.

$$AgNO_{3(aq)} + NaCl_{(aq)} \rightarrow AgCl_{(s)} + NaNO_{3(aq)}$$

Silver chloride, in its pure state, is a white solid matter.

The silver chloride used should have a minimum purity of 99%. Determination of the silver content is conducted according to the atomic absorption spectrophotometry (AAS) method (7.8).
The silver chloride content in the inert carrier material should be higher than or equal to 2% (OIV, 2014a).

5. Specification for identity and purity

OIV (2014a) has developed a specification for silver chloride. In the European Union, the use of silver chloride in wine requires the purity and identification specifications to be laid down and published in the OIV's International Oenological Codex. Standard 1.3.4 requires that substances added to food, including processing aids, comply with relevant specifications as detailed in the Code. Silver Chloride meets the OIV specification which is one of the secondary references for specifications in Standard 1.3.4 (Identity and Purity). Therefore, no new specification is required for the Code.

6. Analytical method of detection

OIV (2014b) has developed analytical methods of detection for silver (Method OIV-MA-AS322-09 Type IV method , COMPENDIUM OF INTERNATIONAL METHODS OF ANALYSIS**).** Tests for silver chloride are provided in OIV (2014a).

B. Information Related to the Safety of a chemical processing aid

1. General Information on the Industrial use of this chemical

Silver Chloride is used as an Antibacterial agent for concrete and to help prevent bacteria from growing on Glass (when melted into the glass).

Silver substances currently approved for use in Europe in cosmetic production include silver oxide, silver chloride, metallic silver, silver citrate and silver nitrate. Functions of these silver containing substances vary from deodorizing and antimicrobial to preservative and skin conditioning.

2. General information on the use of the chemical as a food processing aid in other countries

<u>Australia</u>

Silver ions are a permitted processing aid used in packaged water and in water used as an ingredient in other foods in Table to Clause 11 of Standard 1.3.3 of the Australia New Zealand Food Standards Code, at a level of 0.01 mg/kg.

<u>European Union</u>

Under EC Regulation 606/2009 (as amended by Commission Delegated Regulation (EU) 2015/1576 of 6 July 2015) the use of silver chloride is permitted to treat wines to remove fermentation and storage-related abnormal odours (caused by reduction reactions characterised by the presence of hydrogen sulphide and thiols. The conditions of use are outlined in Annex 21:

Requirements

(1) *The treatment is to be carried out under the responsibility of an oenologist or qualified technician.*
(2) *The treatment must be recorded in the registers referred to in Article 147(2) of Regulation (EU) No 1308/2013.*
(3) *The silver chloride added to wine must be applied to an inert support, like kieselguhr (diatomaceous earth), bentonite, kaolin, etc. The precipitate must be eliminated by any appropriate physical procedure and must be treated by specialised sector.*
(4) *No more than 1 g/hl, residue in the wine < 0.1 mg/l (silver)'*

United States

In the United States, treatment of wine with silver is not permitted.

3. Data on the toxicokinetics and metabolism of the chemical processing aid, and if necessary its metabolites

Silver (E 174) has been previously evaluated by the EU Scientific Committee for Food (SCF) in 1975, and by the Joint FAO/ WHO Expert Committee on Food Additives (JECFA) in 1977 (JECFA, 1977; 1978). Both committees did not establish an acceptable daily intake (ADI) due to inadequate data. EFSA has also evaluated a number of silver complexes intended for use in food contact materials latest in 2011 and classified silver in the SCF list 3 with a group of specific migration limit of 0.05 mg/kg food (EFSA 2016). More recently, EFSA (2016) re-evaluated silver as an additive (noting that in this application silver chloride is used as a processing aid on an inert carrier.

4. Information on the toxicity of the chemical processing aid and, if necessary, its major metabolites.

Silver is the major potential breakdown product from silver chloride.

5. Safety assessment reports prepared by international agencies or other national government agencies if available

European Union

Silver (E 174) has been previously evaluated by the EU Scientific Committee for Food (SCF) in 1975, and by the Joint FAO/ WHO Expert Committee on Food Additives (JECFA) in 1977 (JECFA, 1977; 1978). Both committees did not establish an acceptable daily intake (ADI) due to inadequate data. EFSA has also evaluated a number of silver complexes intended for use in food contact materials latest in 2011 and classified silver in the SCF list 3 with a group of specific migration limit of 0.05 mg/kg food (EFSA 2016). More recently, EFSA (2016) re-evaluated silver as an additive (noting that in this application silver chloride is used as a processing aid on an inert carrier).

EFSA (2011) also evaluated silver zeolite A for use in food contact materials. EFSa concluded that there was no safety concern for the consumer if migration of the silver ion does not exceed the group specific migration limit of 0.05 mg Ag/kg food.

F. Information Related to the Dietary Exposure to the Processing Aid.

1. **A list of foods or food groups likely to contain the processing aid or its metabolites.**

Silver is used infrequently as an additive (see for example, EFSA attached). Its use in wine will be restricted to use as a processing aid and it will be removed from the final product prior to bottling.

2. **The levels of residues of the processing aid or its metabolites for each food group.**

Silver chloride reacts with sulphurous components, for instance, hydrogen sulphide (H2S), disulphides, mercaptans or thioacetates to form silver sulphide during the treatment which remains adsorbed by the inert carrier material and together they can be separated by filtration. Independent of the pH value, silver chloride is very slightly soluble thus the risk of residues in wine is very low, provided the wine is filtered after treatment.

In Europe, the silver chloride added to wine must be applied to an inert support, like kieselguhr (diatomaceous *earth), bentonite, kaolin, etc. The precipitate must be eliminated by any appropriate physical procedure and must be treated by specialised sector. No more than 1 g/hl, residue in the wine < 0.1 mg/l (silver).*

These prescriptions should also apply in Australia.

3. **For food or food groups not currently listed in the most recent Australian or New Zealand national Nutrition Surveys (NNSs), information on the likely consumption.**

n/a

4. **The percentage of the food group in which the processing aid is likely to be found or the percentage of the market likely to use the processing aid.**

We believe there will be very little use of silver chloride due to the high cost of the processing aid..

5. **Information relating to the levels of residues in foods in other countries.**

Silver is widely distributed in the free state (elemental silver) and in many minerals in which it is found in combination with different elements including sulfur, arsenic, antimony and chlorine: argentite, a sulfide, is the principal ore mineral. It is used in some confectionary. The best reference is probably EFSA (attached).

6. **For foods where consumption has changed in recent years, information on likely food consumption.**

n/a

3.1.6 Assessment Procedure

The appropriate assessment procedure is **General Procedure Level 1.**

3.1.7 CONFIDENTIAL COMMERCIAL INFORMATION

No confidential or commercial information is incorporated in this application.

3.1.8 EXCLUSIVE CAPTURABLE BENEFIT.

There is no exclusive capturable benefit to the applicant.

3.1.9 INTERNATIONAL AND OTHER STANDARDS

A. Codex Alimentarius Commission (Codex) Standards

Silver Chloride is a processing aid.

B. Other National Standards

OIV

The OIV adopted the oenological practice to use silver chloride to remove fermentation and storage-related abnormal odours (caused by reduction reactions characterised by the presence of hydrogen sulphide and thiols at its 12th General assembly in Mendoza on 14 November 2014 (Oenoppia 2015). The prescriptions for its use with musts and wine are attached (OIV, 2012). Following the adoption of a product monograph in 2015 (OIV 2014a), the European Commission amended Regulation 606/2009 on 23 September 2015 to permit the use of silver chloride for musts and wines.

European Union

Following the adoption of a product monograph in 2015 (OIV 2014a), the European Commission amended Regulation 606/2009 on 23 September 2015 to permit the use of silver chloride for musts and wines (EC 2015).

United States

Silver chloride is considered as an Indirect Additive used in Food Contact Substances

3.1.10 STATUTORY DECLARATION

Attached

3.1.11 CHECKLIST

Attached

References

EC (2009) **COMMISSION REGULATION (EC) No 606/2009 of 10 July 2009 laying down certain detailed rules for implementing Council Regulation (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions.**

EC (2015). Commission Delegated Regulation (EU) 2015/1576 of 6 July 2015 amending Regulation (EC) No 606/2009 as regards certain oenological practices and Regulation (EC) No. 436/2009 as regards registering of these practices in the wine sector registers Official Journal European Union 23.9.2015, (L246/1):1-4. Available at http://faolex.fao.org/docs/pdf/eur148537.pdf

EFSA (2011), Scientific opinion on the safety evaluation of the substance silver zeolite, EFSA, EFSA Journal 2011;9(2):1999

EFSA (2016), Scientific opinion on the re-evaluation of silver (E 174) as food additive, EFSA Panel on Food Additives and Nutrient Sources added to Food, EFSA Journal 2016;14(1):4364.

ERBOLSCH, (undated) Ercofid - silver chloride preparation applied on an inert carrier to remove sulphide off-flavours, Data Sheet.

National Center for Biotechnology Information. PubChem Compound Database; CID=24561, https://pubchem.ncbi.nlm.nih.gov/compound/24561 (accessed Jan. 29, 2016).

OENOPPIA (2015), **Information about wine regulation**, Press Release, Paris, 25 August 2015.

OIV, (2012) Treatment with Silver Chloride, International Code of Oenological Practices, OIV Code Sheet – Issue 2012/01 II.3.5-19.

OIV (2014a), **SILVER CHLORIDE,** COEI-1-CHLARG: 2014

OIV (2014b) Silver, Method OIV-MA-AS322-09 Type IV method , COMPENDIUM OF INTERNATIONAL METHODS OF ANALYSIS**.**

WHO (1977) WHO Food Additives Series No.12, Summary of Toxicological Data of Certain Food Additives.

Attachments

Attachment 1: Statutory Declaration
Attachment 2: Checklist
Attachment 3: Summary
Attachment 4: Application
Attachment 5: OIV, (2012) Treatment with Silver Chloride, International Code of Oenological Practices, OIV Code Sheet – Issue 2012/01 II.3.5-19.

Attachment 6: EC (2015), Commission Delegated Regulation (EU) 2015/1576 of 6 July 2015 amending Regulation (EC) No 606/2009 as regards certain oenological practices and Regulation (EC) No. 436/2009 as regards registering of these practices in the wine sector registers Official Journal European Union 23.9.2015, (L246/1):1-4. Available at http://faolex.fao.org/docs/pdf/eur148537.pdf

Attachment 7: OIV (2014a), **SILVER CHLORIDE,** COEI-1-CHLARG: 2014

Attachment 8: National Center for Biotechnology Information. PubChem Compound Database; CID=24561, https://pubchem.ncbi.nlm.nih.gov/compound/24561 (accessed Jan. 29, 2016).

Attachment 9: OIV (2014b) Silver, Method OIV-MA-AS322-09 Type IV method , COMPENDIUM OF INTERNATIONAL METHODS OF ANALYSIS**.**

Attachment 10: OENOPPIA (2015), **Information about wine regulation**, Press Release, Paris, 25 August 2015.

Attachment 11: EFSA (2016), Scientific opinion on the re-evaluation of silver (E 174) as food additive, EFSA Panel on Food Additives and Nutrient Sources added to Food, EFSA Journal 2016;14(1):4364.

Attachment 12: EFSA (2011), Scientific opinion on the safety evaluation of the substance silver zeolite, EFSA, EFSA Journal 2011;9(2):1999.

Attachment 13: ERBOLSCH, (undated) Ercofid - silver chloride preparation applied on an inert carrier to remove sulphide off-flavours, Data Sheet.

Attachment 14: WHO (1977) WHO Food Additives Series No.12, Summary of Toxicological Data of Certain Food Additives.

Abbreviations

ADI	Acceptable Daily Intake
CAS	Chemical Abstracts Serial number
EEC	European Economic Community
EINECS	European Inventory of Existing Chemical Substances
FAO	Food and Agriculture Organization (of the United Nations)
FDA	Food and Drug Administration (of the USA)
GRAS	Generally Recognized As Safe
INS	International Numbering System (Codex Alimentarius numbers for food additives)
IPPA	International Pectin Producers Association
JECFA	Joint Expert Committee for Food Additives (FAO/WHO)
SCF	Scientific Committee for Food (the European Union expert committee)

3. WINES

3.5.15. TREATMENT WITH SILVER CHLORIDE (OIV-OENO 2009-145)

Definition:

Addition of silver chloride to wine

Objective:

To reduce odour defects due to hydrogen sulphide and some mercaptans.

Prescriptions

a) The dose used must not be over 1 g/hl
b) The silver chloride must be previously applied to an inert support, like kieselguhr (diatomaceous earth) or kaolin
c) The main operation must be preceded by trials to determine the amount of product to add.
d) The precipitate must be eliminated by any appropriate physical procedure
e) Residues must be treated by specialised sector
f) Treated wine must be analysed to insure that the maximal residue level do not exceed 0.1 mg/L in silver
g) The treatment must be carried out under the responsibility of an oenologist or a specialised technician
h) Silver chloride must comply with the provisions of the International Oenological Codex

Recommendation of the OIV:

Admitted.

Attachment 8

II

(Non-legislative acts)

REGULATIONS

COMMISSION DELEGATED REGULATION (EU) 2015/1576

of 6 July 2015

amending Regulation (EC) No 606/2009 as regards certain oenological practices and Regulation (EC) No 436/2009 as regards the registering of those practices in the wine sector registers

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EU) No 1308/2013 of the European Parliament and of the Council of 17 December 2013 establishing a common organisation of the markets in agricultural products and repealing Council Regulations (EEC) No 922/72, (EEC) No 234/79, (EC) No 1037/2001 and (EC) No 1234/2007 [1], and in particular Article 75(2) and (3)(g) and Article 147(3)(e) thereof,

Whereas:

(1) In accordance with Article 3 of Commission Regulation (EC) No 606/2009 [2], authorised oenological practices are laid down in Annex I A to that Regulation. The International Organisation of Vine and Wine (OIV) has adopted resolutions allowing three new oenological practices. In order to take account of technical progress and to provide Union producers with the same possibilities as those available to third-country producers, those new oenological practices should be authorised in the Union under the conditions of use defined by the OIV.

(2) Certain oenological practices are particularly exposed to the risk of fraudulent use and must be recorded in the registers pursuant to Article 41 of Commission Regulation (EC) No 436/2009 [3]. For this reason, the three new oenological practices, that is the treatment of wines using a membrane technology coupled with activated carbone, the use of polyvinylimidazole-polyvinylpyrrolidone copolymers and the use of silver chloride, the last two substances being processing aids, should be entered in the registers.

(3) Regulations (EC) No 606/2009 and (EC) No 436/2009 should therefore be amended accordingly,

HAS ADOPTED THIS REGULATION:

Article 1

Amendment of Regulation (EC) No 606/2009

Annex I A to Regulation (EC) No 606/2009 is amended in accordance with the Annex to this Regulation.

---

[1] OJ L 347, 20.12.2013, p. 671.

[2] Commission Regulation (EC) No 606/2009 of 10 July 2009 laying down certain detailed rules for implementing Council Regulation (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions (OJ L 193, 24.7.2009, p. 1).

[3] Commission Regulation (EC) No 436/2009 of 26 May 2009 laying down detailed rules for the application of Council Regulation (EC) No 479/2008 as regards the vineyard register, compulsory declarations and the gathering of information to monitor the wine market, the documents accompanying consignments of wine products and the wine sector registers to be kept (OJ L 128, 27.5.2009, p. 15).

Article 2

Amendment of Regulation (EC) No 436/2009

In the first subparagraph of Article 41(1) of Regulation (EC) No 436/2009, the following points are added:

'(x) treatment using a membrane technology coupled with activated carbon;

(y) use of polyvinylimidazole-polyvinylpyrrolidone copolymers;

(z) use of silver chloride.'

Article 3

Entry into force

This Regulation shall enter into force on the seventh day following that of its publication in the *Official Journal of the European Union*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 6 July 2015.

For the Commission
The President
Jean-Claude JUNCKER

ANNEX

Annex I A to Regulation (EC) No 606/2009 is amended as follows:

(1) in the table, the following rows 53, 54 and 55 are added:

1		2	3
Oenological practice		Conditions of use	Limits on use Applications
'53	Treatment of wines using a membrane technology coupled with activated carbon to reduce excess 4-ethylphenol and 4-ethylguaiacol	For wines and under the conditions laid down in Appendix 19	
54	Use of polyvinylimidazole-polyvinylpyrrolidone copolymers (PVI/PVP)	For musts and wines and under the conditions laid down in Appendix 20	No more than 500 mg/l (where added to both the must and the wine, the total overall quantity must not exceed 500 mg/l)
55	Use of silver chloride	For wines and under the conditions laid down in Appendix 21	No more than 1 g/hl, residue in the wine < 0,1 mg/l (silver)'

(2) the following Appendices 19, 20 and 21 are added:

'Appendix 19

Requirements for the treatment of wines using a membrane technology coupled with activated carbon to reduce excess 4-ethylphenol and 4-ethylguaiacol

The aim of the treatment is to reduce the content of 4-ethylphenol and 4-ethylguaiacol of microbial origin that constitutes organoleptic defects and masks the aromas of the wine.

Requirements:

(1) The treatment is to be carried out under the responsibility of an oenologist or qualified technician.

(2) The treatment must be recorded in the registers referred to in Article 147(2) of Regulation (EU) No 1308/2013.

(3) The membranes used must comply with the requirements of Regulations (EC) No 1935/2004 and (EC) No 10/2011 and with the national provisions adopted for the implementation thereof. They must comply with the requirements of the International Oenological Codex published by the OIV.

Appendix 20

Requirements for polyvinylimidazole-polyvinylpyrrolidone copolymers (PVI/PVP)

The purpose of the use of PVI/PVP is to prevent defects caused by too high metal contents and to reduce undesirable high concentration of metals.

Requirements:

(1) Copolymers must be eliminated by filtration no later than two days after their addition taking into account the precautionary principle.

(2) In the case of cloudy musts, the copolymer must be added no earlier than a maximum of two days before filtration.

(3) The treatment is to be carried out under the responsibility of an oenologist or qualified technician.

(4) The treatment must be recorded in the registers referred to in Article 147(2) of Regulation (EU) No 1308/2013.

Appendix 21

Requirements for silver chloride

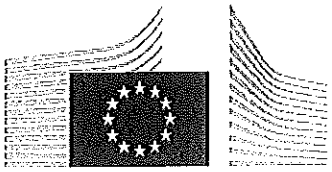
Silver chloride is used for the treatment of wines to remove fermentation and storage-related abnormal odours (caused by reduction reactions characterised by the presence of hydrogen sulphide and thiols).

Requirements:

(1) The treatment is to be carried out under the responsibility of an oenologist or qualified technician.

(2) The treatment must be recorded in the registers referred to in Article 147(2) of Regulation (EU) No 1308/2013.

(3) The silver chloride added to wine must be applied to an inert support, like kieselguhr (diatomaceous earth), bentonite, kaolin, etc. The precipitate must be eliminated by any appropriate physical procedure and must be treated by specialised sector.'



EUROPEAN
COMMISSION

Brussels, 6.7.2015
C(2015) 4510 final

ANNEX 1

ANNEX

to the

COMMISSION DELEGATED REGULATION

amending Regulation (EC) No 606/2009 as regards certain oenological practices and Regulation (EC) No 436/2009 as regards the registering of those practices in the wine sector registers

ANNEX

Annex I A to Regulation (EC) No 606/2009 is amended as follows:

(1) in the table, the following rows 53, 54 and 55 are added:

"

1		2	3
'Oenological practice		Conditions of use	Limits on use Applications
53	Treatment of wines using a membrane technology coupled with activated carbon to reduce excess 4-ethylphenol and 4-ethylguaiacol	For wines and under the conditions laid down in Appendix 19	
54	Use of polyvinylimidazole-polyvinylpyrrolidone copolymers (PVI/PVP)	For musts and wines and under the conditions laid down in Appendix 20	No more than 500 mg/l (where added to both the must and the wine, the total overall quantity must not exceed 500 mg/l)
55	Use of silver chloride	For wines and under the conditions laid down in Appendix 21	No more than 1 g/hl, residue in the wine <0,1 mg/l (silver)'

"

(2) the following Appendices 19, 20 and 21 are added:

"Appendix 19

Requirements for the treatment of wines using a membrane technology coupled with activated carbon to reduce excess 4-ethylphenol and 4-ethylguaiacol

The aim of the treatment is to reduce the content of 4-ethylphenol and 4-ethylguaiacol of microbial origin that constitutes organoleptic defects and masks the aromas of the wine.

Requirements:

(1) The treatment is to be carried out under the responsibility of an oenologist or qualified technician.

(2) The treatment must be recorded in the registers referred to in Article 147(2) of Regulation (EU) No 1308/2013.

(3) The membranes used must comply with the requirements of Regulations (EC) No 1935/2004 and (EC) No 10/2011 and with the national provisions adopted for the implementation thereof. They must comply with the requirements of the International Oenological Codex published by the OIV.

Appendix 20

Requirements for polyvinylimidazole-polyvinylpyrrolidone copolymers (PVI/PVP)

The purpose of the use of PVI/PVP is to prevent defects caused by too high metal contents and to reduce undesirable high concentration of metals.

Requirements:

(1) Copolymers must be eliminated by filtration no later than two days after their addition taking into account the precautionary principle.

(2) In the case of cloudy musts, the copolymer must be added no earlier than a maximum of two days before filtration.

(3) The treatment is to be carried out under the responsibility of an oenologist or qualified technician.

(4) The treatment must be recorded in the registers referred to in Article 147(2) of Regulation (EU) No 1308/2013.

Appendix 21

Requirements for silver chloride

Silver chloride is used for the treatment of wines to remove fermentation and storage-related abnormal odours (caused by reduction reactions characterised by the presence of hydrogen sulphide and thiols).

Requirements:

(1) The treatment is to be carried out under the responsibility of an oenologist or qualified technician.

(2) The treatment must be recorded in the registers referred to in Article 147(2) of Regulation (EU) No 1308/2013.

(3) The silver chloride added to wine must be applied to an inert support, like kieselguhr (diatomaceous earth), bentonite, kaolin, etc. The precipitate must be eliminated by any appropriate physical procedure and must be treated by specialised sector."

SILVER$^{(I)}$ CHLORIDE
N° C.A.S.: 7783-90-6
(Oeno (505/2014)

(I) S**ilver chloride used for the treatment of wine should be adsorbed into an inert carrier material**

1. Object, origin and scope of application

This monograph relates to silver chloride used for adsorption into an inert carrier material with a view to its use in wine

Silver chloride is used for the treatment of wines to remove fermentation and storage-related abnormal odours (odours caused by reduction reactions, characterised by the presence of hydrogen sulphide and thiols).
Silver sulphide formed during the treatment remains adsorbed by the inert carrier material and together they can be separated by filtration.
The inert carrier materials, such as, for instance, kieselguhr (diatomaceous earth), bentonite, kaolin, etc. should comply with the prescriptions of the International Oenological Codex.

2. Labelling

The product concentration, batch number, use-by-date, safety warnings and storage conditions should be indicated on the label.

3. Appearance

Silver chloride, in its pure state, is a white solid matter.

4. Composition (test trials)

The silver chloride used should have a minimum purity of 99%. Determination of the silver content is conducted according to the atomic absorption spectrophotometry (AAS) method (7.8).
The silver chloride content in the inert carrier material should be higher than or equal to 2%.

5. Identification of silver chloride

On exposure to light, silver chloride undergoes photolytic decomposition (with darkening).

Silver chloride is partially soluble in a 3% ammoniacal solution (bromide and iodide do not go into solution in the cold) and subsequent addition of potassium iodide solution results in the precipitation of yellow silver iodide (higher sensitivity to light than AgCl). Alternatively, a diluted solution of red potassium hexacyanoferrate(III) can be added instead of iodide. A brown precipitate ($Ag_3[Fe(CN)_6]$) is formed.

6. Solubility of silver chloride

In water at 25 °C: 0.00188 g/L.
Insoluble in alcohol and nitric acid.
Soluble in sulphuric acid, hydrochloric acid, thiosulphate and ammonium solutions upon complex formation.

7. Tests

7.1 Preparation of test solution

Place 0.5 g of sodium chloride and 20 mL of 0.1 mol/L sodium thiosulphate solution in a 50 mL beaker. Mix for 30 minutes. Afterwards, allow to rest/sediment for 5 minutes. Filter the supernatant using a single-use syringe with a filter, pore size 0.45 µm. Transfer 0.5 mL filtrate to a 100 mL volumetric flask and fill up to the calibration mark with distilled water.

7.2 Appearance of test solution

The solution must be colourless, possibly cloudy. The filtrate is colourless.

7.3 Iron

Determine the content according to the atomic absorption spectrophotometry (AAS) method described in Chapter II of the International Oenological Codex; content below 5 mg/kg.

7.4 Nickel

Determine the content according to the atomic absorption spectrophotometry (AAS) method described in Chapter II of the International Oenological Codex; content below 5 mg/kg.

7.5 Lead

Determine the content according to the atomic absorption spectrophotometry (AAS) method described in Chapter II of the International Oenological Codex; content below 5 mg/kg.

7.6 Mercury

Determine the content according to the atomic absorption spectrophotometry (AAS) method described in Chapter II of the International Oenological Codex; content below 1 mg/kg.

7.7 Arsenic

Determine the content according to the atomic absorption spectrophotometry (AAS) method described in Chapter II of the International Oenological Codex; content below 3 mg/kg.

7.8 Silver

Determination by atomic absorption spectrophotometry (AAS), described in the Compendium of International Methods of analysis of wines an musts, after preparation of a test solution (7.1). Calibration with 1 mg/L, 2.5 mg/L and 5 mg/L Ag-reference solutions.

8. Storage

Silver chloride must be stored in a dry place, protected from light in hermetically sealed packaging.

OEnoppia
Oenological Products and Practices
International Association

PRESS RELEASE

For immediate release

INFORMATION ABOUT WINE REGULATION

Paris, 25 August 2015

New oenological practices adopted by the OIV – their authorization in Europe is not automatic!

The General Assembly of the OIV held last July 10 in Mainz voted the adoption of two new oenological practices : the treatment of wines with malolactic fermentation activators and treatment of musts and wines with glutathione. But beware, these practices are not allowed in so far in 2015 for harvest in European countries. If European regulation is based on resolutions of the OIV for any new oenological practice, these products can not be used in the wineries before the insertion procedure in the list of authorized oenological practices (Regulation of the Commission (EC) 606/2009). This procedure takes several months.

In the interests of promoting international standardization and technical consistency, European wine legislation (Council Regulation [EC] 479/2008, replaced by Parliament and Council Regulation (EU) 1308/2013) explicitly stipulates since 2008 that the International Vine and Wine Organization's (OIV) recommendations should be referred to for adopting any new oenological practice. Nevertheless, since the OIV is a 46-country intergovernmental organization enacting international standards, its role as a point of reference for regulations does not lead to automatic authorization of new oenological practices in European Union countries. Any new practice must be incorporated into European regulations. The European Commission consults member countries to submit revisions of enactments and ensure that new oenological practices comply with specific criteria established at European level (food safety, protection of wine's natural characteristics, environmental impact, etc). It usually takes between 6 and 12 months to amend European regulations. This includes 2-4 months needed to consider proposals in the European Parliament and Council. In addition, we

should stress that establishing a new oenological practice (Code of Oenological Practices) always goes hand in hand with a corresponding product monograph written by the OIV (Oenological Codex). A monograph may be adopted after a practice. In such cases, the European Commission waits until the monograph has been adopted before introducing the new practice. The monograph gives product purity specifications and is an essential guarantee of oenological quality and food safety.

The European Parliament and Council are currently considering regulations to introduce oenological practices adopted by the OIV at its 12th General Assembly in Mendoza on 14 November 2014 (adoption of PVI-PVP to reduce an excess of metals, silver chloride to correct the reduction in wines and treatment of wines with a membrane coupling technique and activated carbon to reduce excess 4-ethylphenol and 4-ethylguaiacol). This bill is available in the Commission's document register[1] and is expected to come into force in November 2015 if no objections are raised by the European Parliament or Council.

New oenological practices adopted at the OIV's 13th General Assembly on 10 July 2015

Two new oenological practices – representing major oenological progress – were adopted at the OIV's last General Assembly in Mainz, Germany: treatment of wines with malolactic fermentation activators (OIV-OENO Resolution 531-2015), and treatment of musts and wines with glutathione (OIV-OENO Resolution 445-2015; OIV-OENO Resolution 446-2015).

The option to use malolactic fermentation activators has opened up a new way to improve control of malolactic fermentation, just like alcoholic fermentation. The following products can be used: inactivated yeasts and yeast autolysates for lactic acid bacteria nutrition, yeast hulls for detoxification, and microcrystalline cellulose for support.

Glutathione has long been known to have a role in grape metabolism. Scientific research has shown its protective function (by its quinone-blocking action) in oxidation phenomena. Directly adding pure glutathione will enable this action to be reproduced if wines are exposed to oxygen.

When will these new practices be authorized in Europe?

[1] http://ec.europa.eu/transparency/regdoc/rep/3/2015/FR/3-2015-4510-FR-F1-1.PDF
http://ec.europa.eu/transparency/regdoc/rep/3/2015/FR/3-2015-4510-FR-F1-1-ANNEX-1.PDF

These new practices will be authorized in European countries as soon as the product specifications have been adopted by the OIV and they have been incorporated into Regulation (EC) 606/2009. Although product specifications for malolactic fermentation activators are already in the OIV's International Oenological Codex (monographs on autolysates, inactivated yeasts, yeast hulls and microcrystalline cellulose), glutathione specifications are still in the process of being adopted.

Malolactic fermentation activators are therefore due to be authorized in Europe some time in 2016.

European authorization for glutathione depends on two other conditions, for which it is currently hard to make time-scale commitments (at least 2 years): the adoption of the monograph by the OIV, and a food-safety risk assessment for use as a European food additive (assessment carried out by the EFSA).

How do you know whether or not an oenological practice is authorized?

It is vital to remember that all new oenological practices must be listed in Regulation (EC) 606/2009 to be authorized in European countries. Please consult the consolidated version of Regulation 606/2009[2] directly on the European Commission's Official Journal website (eur-lex.europa.eu) to check if a new practice is officially authorized.

About Oenoppia

Oenoppia is a professional association under French law, bringing together the main designers, producers and distributors of specialist oenological products. Specialist oenological products cover all ingredients, additives and processing aids involving specific oenological expertise and use and that are based on scientific knowledge of grape and wine constituents. Oenoppia's member companies have signed an ethical charter for regulatory compliance and responsible use of oenological products.

Oenoppia. 21-23, Rue Croulebarbe. 75013 Paris. www.oenoppia.com

[2] Latest version consolidated on 27/08/2015: http://eur-lex.europa.eu/legal-content/FR/TXT/PDF/?uri=CELEX:02009R0606-20150419&qid=1440668873225&from=FR

European Food Safety Authority

EFSA Journal 2016;14(1):4364

SCIENTIFIC OPINION

Scientific opinion on the re-evaluation of silver (E 174) as food additive[1]

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The present opinion deals with the re-evaluation of the safety of silver (E 174) when used as a food additive. Silver in food additive E 174 is present in its elemental form. The Panel noted that there are data gaps and concerns to be addressed to conduct a risk assessment with respect to the use of silver (E 174): lack of data on toxicity studies on elemental silver or the food additive (E 174); unknown particle size distribution of the food additive (E 174); evidence of the release of silver ions from elemental silver, which may be of concern. However, the extent of the release of the silver ions is unknown in the case of silver (E 174). The Panel concluded that the information available was insufficient to assess the safety of silver as food additive. The major issues included chemical identification an d characterisation of silver E 174 (e.g. quantity of nanoparticles and release of ionic silver) and similar information on the material used in the available toxicity studies. Therefore, the Panel concluded that the relevance of the available toxicological studies to the safety evaluation of silver as a food additive E 174 could not be established. The Panel recommended that the specifications for E 174 should include the mean particle size and particle size distribution (± SD), as well as the percentage (in number) of particles in the nanoscale (with at least one dimension below 100 nm), present in the powder form of silver (E 174) used as a food additive. The methodology applied should comply with the EFSA Guidance document, e.g. scanning electron microscopy (SEM) or transmission electron microscopy (TEM). The Panel recommended that additional data in line with the current Guidance document on evaluation of food additives would be required.

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KEY WORDS

Silver, E 174, food colour

[1] On request from the European Commission, Question No EFSA-Q-2011-00346, adopted on 10 December 2015.

[2] Panel members: Fernando Aguilar, Riccardo Crebelli, Alessandro Di Domenico, Birgit Dusemund, Maria Jose Frutos, Pierre Galtier, David Gott, Ursula Gundert-Remy, Claude Lambré, Jean-Charles Leblanc, Oliver Lindtner, Peter Moldeus, Alicja Mortensen, Pasquale Mosesso, Agneta Oskarsson, Dominique Parent-Massin, Ivan Stankovic, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen, Matthew Wright and Maged Younes. Correspondence: fip@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Standing Working Group on the re-evaluation of food colours: Fernando Aguilar, Riccardo Crebelli, Alessandro Di Domenico, Maria Jose Frutos, Pierre Galtier, David Gott, Claude Lambré, Jean-Charles Leblanc, Agneta Oskarsson, Jeanne Stadler, Paul Tobback, Ine Waalkens-Berendsen and Rudolf Antonius Woutersen, for the preparatory work on this scientific opinion and EFSA staff members, Federica Lodi, Ana Rincon and Alexandra Tard for the support provided to this scientific opinion. The ANS Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

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Available online: www.efsa.europa.eu/efsajournal

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SUMMARY

Following a request from the European Commission (EC), the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety of silver (E 174) when used as a food additive.

The Panel based its evaluation on previous evaluations and on the additional literature that became available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available.

To assist in identifying any emerging issue or any relevant information for the risk assessment, the European Food Safety Authority (EFSA) has outsourced a contract to deliver an updated literature review on toxicological endpoints, dietary exposure and occurrence levels of silver (E 174) which covered the period up to the end of 2014. Further update has been performed by the Panel.

Silver (E 174) is authorised as a food additive in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008.[4] Silver (E 174) has been previously evaluated by the EU Scientific Committee for Food (SCF) in 1975 (SCF, 1975) and by the Joint FAO/ WHO Expert Committee on Food Additives (JECFA) in 1977 (JECFA, 1977; 1978). Both committees did not establish an acceptable daily intake (ADI) due to inadequate data.

Silver in food additive E 174 is present in its elemental form. Specifications for silver have been defined in the EU in Commission Regulation (EU) No 231/2012. The purity is specified to be not less than 99.5% for silver-coloured powder or tiny sheets. Silver can also occur in crystalline form as a white metal.

During the last call for data, a study on confectionery pearls coated with silver E 174 was performed, finding that a 20% of the mean total silver concentration in the pearls was released as particles after the water treatment of the pearls (Verleysen et al., 2015).

The Panel noted that in Commission Regulation (EU) No 231/2012, no information is included regarding the particle size of silver powder. According to the Panel, the characterisation of the particle size in the powder of E 174 should be included in the specifications. The fully characterisation should include the particles size distribution together with determination and quantification of any nanoparticulate material.

The Panel noted that silver nanoparticles (AgNPs) are released from confectionary pearls (Verleysen et al., 2015) and nanosilver is unstable and releases ions. The Panel was aware of the extensive database on ionic silver or AgNPs, however, the relevance of these data to the evaluation of silver as a food additive (E 174) was not apparent. Therefore, the Panel considered these data could not be directly applied to the evaluation of the food additive.

In this opinion, only data with non-capped nanoparticles are included. However, when corresponding capped nanoparticles have been studied in the same experiments, also those data are included.

Following oral exposure of animals to ionic silver or AgNPs, silver is systemically available. Silver concentrations in the organs were highly correlated to the size of the nanoparticles concentrations being higher in animals exposed to smaller nanoparticles and to the amount of silver ions released from the AgNPs. Bioavailability seems to be in the range of 2–20% depending on many factors including the animal species.

However, the Panel noted that, due to the many variables involved, the conversion rate of metal silver from nanoparticles to silver ions in biological systems is unknown. Moreover, the formation of

[4] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008.

reactive oxygen species (ROS) from the fraction of AgNPs which may be present in the food additive has not been determined. The rate of both processes depends on the size of particles and their relative surface.

Silver distribution has been reported to all organs and tissues in animals. Silver distribution to the brain following oral exposure has been described in several studies, which is in contrast to the conclusions of previous studies with silver nitrate or lactate, that silver would not cross the blood–brain barrier (van Breemen and Clemente, 1955). However, it is also in the recent studies not clear whether silver is present in the brain endothelial cells or in the brain tissue. Silver ions were also detected in the milk of rat dams receiving a daily oral administration of silver chloride, and in the liver and in the brain of the pups. In rodents, silver is primarily excreted via the bile and faeces, but a small amount is also excreted via the urine.

The Panel noted that only one study described the fate of microsized silver particles in animals (Park et al., 2010). In this study, no silver was detected in any of the tissues of mice given an oral administration of microsized silver particles (323 nm), whereas silver was present in tissues of mice receiving a similar administration of nanosized silver particles (21 to 71 nm).

The Panel was aware that there are many data reporting distribution of silver in various human organs following prolonged exposure to very high doses of silver in different forms. The Panel was also aware that there are numerous data reporting adverse effects of silver due to its use in the medical field (Lansdown, 2010; Maillard and Hartemann, 2013) or as a result of occupational exposure (Drake and Hazelwood, 2005). Overall, the Panel noted that in the case of medical and occupational exposure to silver, the doses and/or the route of exposure (inhalation, no inclusion in a food matrix) were usually irrelevant to the exposure resulting from the use of silver as a food additive.

No toxicity studies were reported on elemental silver.

There are no data available to evaluate the *in vivo* genotoxicity of ionic silver. Concerning AgNPs, the available studies provide clear evidence of a genotoxic potential in various *in vitro* test systems. The *in vivo* oral genotoxicity studies performed provide less conclusive evidence, and do not allow a definitive assessment of the possible genotoxic hazard associated with oral exposure to AgNPs. Overall, the Panel concluded that the available data are inadequate to evaluate the genotoxic hazard associated with the use of silver as food additive.

No studies on the carcinogenic potential of either ionic silver compounds or AgNPs have been identified.

In an oral one-generation reproductive toxicity study with silver acetate in drinking water at dose levels of 0, 0.4, 4 or 40 mg silver acetate/kg body weight (bw)/day (0, 0.26, 2.6 or 26 mg ionic silver/kg bw/day) in rats a no-observed-adverse-effect level (NOAEL) for developmental effects (based on an increased number of pups, pup death and decreased weight gain of pups) of 0.4 mg silver acetate/kg bw/day (0.26 mg ionic silver/kg bw/day) was observed (Documentation provided to EFSA No5). The NOAEL for fertility was 4 mg silver acetate/kg bw/day (2.6 mg ionic silver/kg bw/day).

From the maximum level exposure assessment, mean estimates ranged from < 0.01 to 2.6 µg/kg bw/day across all population groups. Estimates based on the high percentile (95th percentile) ranged from 0 to12 µg/kg bw/day across all population groups.

From the refined estimated exposure scenario in the brand-loyal scenario, mean exposure to silver (E 174) from its use as a food additive ranged from < 0.01 µg/kg bw/day for infants to 2.6 µg/kg bw/day in children. The high exposure to silver (E 174) ranged from 0 µg/kg bw/day for infants to 12 µg/kg bw/day in children. In the non-brand-loyal scenario, mean exposure to silver (E 174) ranged from < 0.01 µg/kg bw/day for infants to 1.6 µg/kg bw/day in children. The high exposure ranged from 0 µg/kg bw/day for infants to 3.2 µg/kg bw/day in children.

The exposure from the food additive and the regular diet (ANSES, 2011) could lead to a mean intake for children around 3.5 µg/kg bw/day (non-brand-loyal scenario). On average, exposure from the food additive would represent around 30% of total dietary exposure to silver.

Overall, the Panel noted that there are data gaps and concerns that need to be addressed in order to conduct a risk assessment with respect to the use of silver (E 174) as food additive:

- Data from toxicity studies on elemental silver or the food additive (E 174) are lacking.
- The particle size distribution of the food additive (E 174) is unknown.
- There is evidence of the release of silver ions from elemental silver, which may be of concern. However, the extent of the release of the silver ions, which depends on multiple factors such as pH and particle size, is unknown in the case of silver (E 174) used as food additive.

The Panel concluded that the information available was insufficient to assess the safety of silver as food additive. The major issues included chemical identification and characterisation of silver E 174 (e.g. quantity of nanoparticles and release of ionic silver) and similar information on the material used in the available toxicity studies. Therefore, the Panel concluded that the relevance of the available toxicological studies to the safety evaluation of silver as a food additive E 174 could not be established.

The Panel recommended that the specifications for E 174 should include the mean particle size and particle size distribution (± SD), as well as the percentage (in number) of particles in the nanoscale (with at least one dimension below 100 nm), present in the powder form of silver (E 174) used as a food additive. The methodology applied should comply with the EFSA Guidance document (EFSA Scientific Committee, 2011), e.g. scanning electron microscopy (SEM) or transmission electron microscopy (TEM).

The Panel recommended that additional data in line with the current Guidance document on evaluation of food additives (EFSA, 2012) would be required.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1333/2008[5] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010.[6] This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[7] of 2001. The report 'Food additives in Europe 2000[8]' submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with a highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010 the 2003 Terms of References are replaced by those below.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

[5] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives, OJ L 354, 31.12.2008, p. 16–33.

[6] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.

[7] Report from the Commission on Dietary Food Additive Intake in the European Union, Brussels, 01.10.2001, COM (2001) 542 final.

[8] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002:560.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of silver (E 174) when used as a food additive. Silver (E 174) is authorised as a food additive in the EU in accordance with Annex II to Regulation (EC) No 1333/2008.[9]

Silver (E 174) has been previously evaluated by the EU Scientific Committee for Food (SCF) in 1975 (SCF, 1975), and by the Joint FAO/ WHO Expert Committee on Food Additives (JECFA) in 1977 (JECFA, 1977; 1978). Both committees did not establish an acceptable daily intake (ADI) due to inadequate data. EFSA has also evaluated a number of silver complexes intended for use in food contact materials latest in 2011 (EFSA, 2011) and classified silver in the SCF list 3 with a group of specific migration limit of 0.05 mg/kg food.

The Panel on Food Additives and Nutrient Sources added to Food (ANS) was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following public calls for data.[10,11,12,13] The Panel noted that not all of the original studies on which previous evaluations were based were available for this re-evaluation.

To assist in identifying any emerging issue or any relevant information for the risk assessment, EFSA has outsourced a contract to deliver an updated literature review on toxicological endpoints, dietary exposure and occurrence levels of silver (E 174), which covered the period up to the end of 2014. The Panel has performed further update.

2. Technical data

2.1. Identity of the substance

Silver in food additive E 174 is present in its elemental form. The chemical element has atomic number 47 and symbol Ag: it has an atomic weight of 107.87 g/mol, Chemical Abstract Service (CAS) Registry No 7440-22-4, and EC No (or European Inventory of Existing Commercial chemical Substances (EINECS) number) 231-131-3. According to Commission Regulation (EU) No 231/2012,[14] silver occurs as silver-coloured powder or tiny sheets.

Silver is also described to occur in a crystalline form as a white, lustrous, soft and ductile/malleable metal (Cotton et al., 1999; Holler et al., 2007; Kirk-Othmer, 2006). It has a density of 10.5 g/cm^3 at 20°C and a melting point of 962°C. Pure silver has the highest thermal and electrical conductivities of all metals.

[9] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008.

[10] Call for scientific data on food colours to support re-evaluation of all food colours authorised under the EU legislation. Published: 8 December 2006. Available from: http://www.efsa.europa.eu/en/dataclosed/call/afc061208.htm

[11] Call for scientific data on Silver (E 174) and Gold (E 175), used as food colours. Published: 23 February 2011. Available from: http://www.efsa.europa.eu/en/dataclosed/call/ans110224

[12] Call for food additives usages level and/or concentration data in food and beverages intended for human consumption. Available from: http://www.efsa.europa.eu/en/dataclosed/call/datex140310.htm

[13] Call for scientific data on selected food additives permitted in the EU- Extended deadline: 1 September 2014 (batch A), 1 November 2014 (batch B).http://www.efsa.europa.eu/en/dataclosed/call/140324.htm

[14] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83 of 22.3.2012, p. 1–295.

2.2. Specifications

Commission Regulation (EU) No 231/2012 on specifications for food additives lays down the specifications for silver (E 174) used as a food additive (Table 1). JECFA has not defined specifications for silver.

Table 1: Specifications established for silver (E 174) according to Commission Regulation (EU) No 231/2012.

	Commission Regulation (EU) 231/2012
Synonyms	Argentum
Definition	
Colour Index No	77820
EINECS No	231-131-3
Chemical name	Silver
Chemical formula	Ag
Atomic weight	107.87
Assay	Content not less than 99.5% Ag
Description	Silver-coloured powder or tiny sheets
Identification	—
Purity	—

The Panel noted that, according to the limited information provided by industry, silver used as food colour may have a minimum certified silver content of 99.999% (total impurities, ≤ 10 mg/kg) (see Section 2.3). During the last call for data, a study on confectionery pearls coated with silver E 174 was performed, finding that a 20% of the mean total silver concentration in the pearls was released as particles after the water treatment of the pearls (Verleysen et al., 2015).

In response to a request from EFSA on the silver particles size, an interested party (Documentation provided to EFSA No4) provided information on particle size distribution of the additive gold, in its powdered form and suggested that these data could be also valid for silver. The Panel did not agree with this proposal.

The Panel noted that in Commission Regulation (EU) No 231/2012, no information is included regarding the particle size of silver powder, and therefore the characterisation of the particle size in the powder of E 174 should be included among the specifications. The fully characterisation should include the particles size distribution together with determination and quantification of any nanoparticulate material.

The Panel noted that the manufacturing process of powdered or particulate food additives resulted in material with a range of sizes. Although the mean or median size of the particles is generally significantly greater than 100 nm, a fraction can be present with at least one dimension below 100 nm. The material used for toxicological testing would have contained this nanofraction. The test requirements stipulated in current EFSA guidance documents and EC guidelines for the intended use in the food/feed area apply in principle to unintended nanoforms as well as to engineered nanomaterials (ENM). Therefore, the Panel considers that in principle for a specific food additive containing a fraction of particles with at least one dimension below 100 nm, adequately conducted toxicity tests should be able to detect hazards associated with this food additive including its nanoparticulate fraction. The Panel considers that for the re-evaluation of food additives this procedure would be sufficient for evaluating constituent nanoform fraction in accordance with the recommendation of the EFSA Nano Network in 2014 (EFSA, 2015).

The Panel noted that the coating of AgNPs with different compounds is made with the purpose of improving their stability and dispersability, thus not being relevant for the food additive E 174 where silver is present in its elemental form.

According to product specifications for the commercial products of AgNPs (non-food additive powder), the colours are beige to dark grey or silver < 100nm; grey < 150nm; silver or grey 2–3.5µm; whereas for colloidal suspensions of AgNPs, the colour is pale-yellow (Lok et al., 2007; Liu and Hurt, 2010).

Information on AgNPs has been reported in the Organisation for Economic Co-operation and Development (OECD) report (OECD, 2015). The mean diameters of the AgNPs in the powder were < 55 nm with non-aggregated forms and a size distribution from 6 to 55 nm. The melting point was identified as 961.9°C and a boiling point of 2,212°C. The density at 20°C is ca 10.43–ca 10.49 g/cm3. The tests made with AgNPs coated with different compounds, as citrate or polyvinylpryrolidone (PVP) stabilised AgNPs in colloidal suspensions, demonstrated an excellent stability preventing aggregation of the dispersions. However, these suspensions can be destabilised by changes in the media as low pH and light for the citrate-coated nanoparticles.

Pearlescent pigments surface treatment for confectionery is described in the open patent literature (Myers et al., 2008; Campomanes and Vilches, 2010). They are commercially available under a number of trade name and colours (provide a wide range of colour effects including, but not limited to, silver fine, silver sheen, silver lustre, silver sparkle, gold shimmer, red shimmer, blue shimmer, green shimmer, gold sheen, light gold). A fluid carriers can be used in the surface treatment. The fluid carriers of the invention as described by Myers et al., 2008, 'Pearlescent pigment surface treatment for Confectionery'**,** can include, but are not limited to, a range of different compounds such as acetone, acetylated monoglycerides, different oils or waxes. Because the fluid carrier forms part of the pigmented coating composition which is applied to the surface of a hard candy substrate, it is advantageous to use fluid carriers that contribute little to no moisture to the pigmented coating composition.

2.3. Manufacturing process

Neither the SCF nor JECFA have provided any information concerning the manufacture of silver as food additive. Some data were submitted to EFSA following its public call for data.[15]

Silver is widely distributed in the free state (elemental silver) and in many minerals in which it is found in combination with different elements including sulfur, arsenic, antimony and chlorine: argentite, a sulfide, is the principal ore mineral. These minerals are commonly associated with lead, copper, zinc and gold (Cotton et al., 1999; Kirk-Othmer, 2006). Silver is traditionally extracted by treatment with cyanide solutions in the presence of air; it is also recovered from the work-up of copper and lead ores. The metal obtained is ultimately refined by electro-deposition to a high purity grade: for instance, for the American Society for Testing and Materials International, the minimum standard for commercial silver is 99.90% (ASTM B413). Higher purity grades can be obtained and are readily available from the market. Common impurities are (in descending order) copper, lead, iron and bismuth.

According to the limited information provided by industry as a response to EFSA's call for data (Documentation provided to EFSA, No6), silver used as food colour has a minimum certified silver content of 99.999% (total impurities, ≤ 10 mg/kg). The Panel noted that this certified content is higher than that required by Commission Regulation (EU) No 231/2012. According to the aforementioned reference, production starts from sheets as thin as some tenths of a micron. These sheets are reduced by a mechanical milling process to commercial sizes. Size is controlled by means of different grids

[15] Call for scientific data on Silver (E 174) and Gold (E 175), used as food colours. Published: 23 February 2011. Available from: http://www.efsa.europa.eu/en/dataclosed/call/ans110224

that are mounted on the mill. In case of production of squared leaves, the metal sheets are manually cut by means of a knife. When unsquared leaves are produced, no further cutting operations are performed on the sheets. To eliminate potential microorganisms, silver is heated to not less than 100°C for at least 120 s.

The Panel noted that the response to EFSA's call for data was limited to only one manufacturer of silver, and considered that a manufacturing process concerning a specific case may not be representative of the market situation.

2.4. Methods of analysis in food

A number of methods for analysis of silver in food have been described in the published literature.

One rapid determination method for silver in oysters suing is the so-called 24.6-s neutron activation product ^{110}Ag (Fukushima and Chatt, 2013).

Determination of silver in food (wheat flour or green tea leaf) was described by use of microcolumn high-performance liquid chromatography (Hu et al., 2004).

Flame atomic absorption spectrophotometry has been employed for the determination of silver in foodstuffs (cereals, meat, fish, fats, sugars and preserves, root vegetables, green vegetables, beverages and milk) (Jackson et al., 1980). The level of silver ions released from silver nanocomposites in apple juice has also been measured with atomic absorption spectrometer (Jokar et al., 2014).

The levels of silver in biological samples can also be measured using dispersive liquid-liquid microextraction (DLLME) and graphite furnace atomic absorption spectrometry (GFAAS) (Dittert et al., 2014).

AgNPs detection, characterisation and quantification in pears have been performed by using a combination of techniques as transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy dispersive spectrometer and inductively coupled plasma optical emission spectrometry (ICP-OES) (Zhang et al., 2012). An inductively coupled plasma mass spectrometry (ICP-MS) system with quadrupole mass analyser, multichannel detector was used for the measurement of the silver levels in tomato (Enamorado et al., 2014). The ICP-MS method has been also used for the determination of the levels of silver and other trace elements in muscle tissues of some seafood species as red mullet, grey mullet and tiger prawn (Yarsan et al., 2014).

Detection of AgNPs in aqueous food matrices (e.g. water, coffee or milk) by using particle-induced X-ray emission has also been described (Lozano et al., 2012), as well as by using single particle (SP)-ICP spectrometry for detection in water or migration of silver from nanosilver-polyethylene composite packaging into food simulants (Mitrano et al., 2012; Song et al., 2011). Ramos et al. (2014) have used the asymmetric flow field fractionation coupled with inductively coupled plasma mass spectrometry for the separation, characterisation and quantification of AgNPs in complex nutraceuticals and beverages (Ramos et al., 2014).

A new methodology based on the combination of conventional and advanced TEM methods, ICP-MS and SP-ICP-MS has been applied for the analysis of the AgNPs released from the coating of silver-coloured pearls meant for decoration of pastry (with an average of 8.4 µg Ag/pearl), following a treatment with water. The physico-chemical properties of the particles in the eluted fraction are also characterised by electron diffraction and a combination of high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) imaging with energy dispersive X-ray (EDX) spectroscopy and mapping. For the TEM analysis, the silver pearls were treated with water in a proportion of 1 pearl /25 µL water. For the ICP-MS analysis, a proportion of 1 pearl/2 mL water was used for the pearl dissolution and 0.5 mL of this suspension was further diluted in 50 mL of water. A

latter suspension was further diluted 50 and 100 times for analysis (total dilution factors of 5,000 and 10,000, respectively) (Verleysen et al., 2015).

2.5. Reaction and fate in food

The influence of AgNPs on food components has been studied in wheat grains treated with AgNPs stabilised by sodium citrate to prevent them from infections. Significant differences have appeared after the treatment in the gluten due to changes in the protein secondary structure (Nawrocka and Ciesla, 2013).

Beera et al. (2012) reported the presence of silver ions released from AgNPs (69 nm) aqueous suspensions, that contained 39, 59 or 69% silver ions depending on the batch. The silver ion fraction was much lower for colloidal solutions with citrate-coated protein-encapsulated AgNPs (from 2.6 to 5.9% of total Ag and a mean particle size from 15.9 to 19.8 nm) than for non-coated AgNPs aqueous suspensions. The toxicological implications of this *in vitro* study are described in Section 3.2.6.

Following the last call for data, a published study by Verleysen et al. (2015), on the release of AgNPs (< 100 nm) after a water treatment of confectionery pearls consisting of sugar coated with silver (E 174) intended for decoration of pastry was submitted. In this study, the amount of silver reported was of 8.4 µg Ag/g pearl, with a variation of 38% among pearls, and the number of nanoparticles released was quantified representing an amount of 4.4×10^9 Ag nanoparticles/g of pearl. The mass concentration of the detected particles was 1.8 ± 0.6 µg/g pearl. This number represents 20% of the mean total silver concentration in the pearls. The single, aggregated and/or agglomerated particles were characterised in size, shape, crystal structure and chemical composition through different TEM and SP-ICP-MS methods.

The Panel noted that nanosilver is unstable and releases ions through gradual reaction with oxygen and protons or with pre-existing oxide films in fluid media and that the oxidative dissolution is influenced by pH, coatings and ligands (Liu and Hurt, 2010; Liu et al., 2012). Other studies have related the antibacterial activity of AgNPs to their sensitivity of oxidation, being dependent of optimally displayed oxidised surfaces, presented in well-dispersed suspensions. It has also been found that partially oxidised AgNPs have antibacterial activities (Lok et al., 2007).

In contact with air, silver is not very reactive although sulfur and sulfur compounds (e.g. hydrogen sulfide, sulfur dioxide) blacken its surface as Ag_2S is formed (tarnishing) (Kirk-Othmer, 2006).

The Panel noted that with the exception of complex ions, the only stable cationic species is ionic silver (Ag^+); the other oxidation states (Ag^{2+} and Ag^{3+}) are either unstable in water or exist only in insoluble compounds or complexed species. Therefore, the silver ion released from the oxidation of silver should be Ag^+.

2.6. Case of need and proposed uses

Maximum levels of silver (E 174) have been defined in Annex II to Regulation (EC) No 1333/2008 on food additives. These levels are referred by the Panel as maximum permitted levels (MPLs) in this document.

Currently, silver (E 174) is an authorised food additive in the EU at quantum satis (QS) in three food categories (Table 2).

Table 2: MPLs of silver (E 174) in foods according to the Annex II to Regulation (EC) No 1333/2008.

FCS[(a)] Category No	Foods	Restrictions/exceptions	Maximum permitted level (MPL) (mg/l or mg/kg as appropriate)
05.2	Other confectionery including breath-refreshening microsweets	Only external coating of confectionery	Quantum satis
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Only decoration of chocolates	Quantum satis
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Only liqueurs	Quantum satis

(a): FCS: Food categorisation System (food nomenclature) presented in the Annex II to Regulation (EC) No 1333/2008.

2.7. Reported use levels of silver (E 174) in food

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment, especially for those food additives for which no MPL is set and which are authorised according to QS.

In 2011, EFSA launched a public call[16] for scientific data on silver (E 174) used as a food colour, to support the re-evaluation of silver (E 174) authorised under the EU legislation. Among other information, information on the human exposure to the food additive from the different types of food where it is permitted (e.g. consumption pattern and uses, actual use levels and maximum use levels, frequency of consumption and other factors influencing exposure) was requested. In response to this public call, very few usage data on silver (E 174) in the external coating of confectionery and decoration of chocolates were submitted to EFSA by one data provider, FoodDrinkEurope (FDE, formerly CIAA) (Documentation provided to EFSA, No1) (Appendix A).

In addition, in the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 setting up a programme for the re-evaluation of approved food additives, EFSA launched a public call[17] for food additives usage level and/or concentration data in food and beverages. Data on silver (E 174) were requested from relevant stakeholders. European food manufacturers, national food authorities, research institutions, academics, food business operators and any other interested stakeholders were invited to submit usage and/or concentration data on silver (E 174) in foods. No information concerning actual use levels of silver (E 174) in food were obtained from the industry in response to this call for data.

According to the GNDP database, silver (E 174) was found to be present in one liqueur (grappa-based liqueur) and few foods (for a total of 34 products between 2010 and now). According to the Mintel GNDP database,[18] in Europe, silver (E 174) is used mostly in sugar confectionery (silver-coated sugar pearls used for decoration purposes and sugar-coated almonds) and less often as external coating of chocolates.

2.8. Information on existing authorisations and evaluations

Silver, used as a food additive, has been previously evaluated by the SCF in 1975 (SCF, 1975). In that evaluation, the SCF did not establish an ADI because of the inadequacy of available biological data, but accepted the continued use for only external colouring and decoration. The full SCF statement

[16] http://www.efsa.europa.eu/sites/default/files/consultation/ans110224.pdf
[17] http://www.efsa.europa.eu/sites/default/files/consultation/140310.pdf
[18] Mintel Global New Products Database (http://www.mintel.com/global-new-products-database). Accessed on 21/9/2015.

reads as follows: 'No specification was available to the Committee. The Committee did not establish an ADI because of the inadequacy of the available biological data but felt able to accept the use of this colour for surface colouring and decoration of food only, without further investigations.' No references were given.

Silver was evaluated by JECFA in 1977 (JECFA, 1977, 1978). The Committee concluded (JECFA, 1978): 'In view of the rare use of this metal and in the absence of knowledge of the exact nature of silver used on or in foods, specifications were not prepared. The data available suggest that this substance might accumulate in certain tissues following long-term exposure. There were, however, insufficient data to evaluate this point fully, nor were any adequate long-term studies available. Thus, no evaluation could be made.'

In 2000, the Scientific Committee on medicinal products and medical devices (SC, 2000) delivered an opinion on the use of silver E 174 in which it is proposed that use of this metal as a colourant be prohibited in medicinal products. This committee stated that 'the potential exposure to silver used as a colouring agent in medicinal products by oral route has to be added to that ingested daily with food and water, and both types of exposure are extremely difficult to quantify. Therefore, it is the Committee's opinion that use of this metal as a colourant be prohibited in medicinal products.'

In 2009, the BfR (Bundesinstitut für Risicobewertung) recommended: 'manufacturers to avoid the use of nanoscale silver or nanoscale silver compounds in foods and everyday products until such time that the data are comprehensive enough to allow a conclusive risk assessment which would ensure that products are safe for consumer health' (BfR Opinion, 2009).

A technical report was submitted to EFSA in 2010 on trace elements in animal nutrition and elements for risk assessment that includes a report on silver (Van Paemel et al., 2010). This report stated that AgNPs were beneficial for growth in weaned piglets, mainly due to their antimicrobial properties. However, excessive ingestion of silver is associated with copper and selenium deficiency in poultry.

In 2011, EFSA published a scientific opinion on the safety evaluation of the substance silver zeolite A (silver zinc sodium ammonium alumino silicate), silver content 2–5% for use in food contact materials (EFSA, 2011a). The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) classified silver zeolite in the SCF list 3 with a specific migration limit of 0.05 mg Ag/kg food based on the human no-observed-adverse-effect level (NOAEL) of about 10 g/kg silver for a total lifetime oral intake (WHO, 2008) for drinking water.

In 2014, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) issued a report on the evaluation of environmental risks linked to the exposure to AgNPs (ANSES, 2015). In this document, the presence of AgNPs in consumer products in different fields including the food sector has been reviewed together with the toxicological studies. This document reported that it was not clear whether the observed effects in some tests *in vitro* and *in vivo* are due exclusively to the silver ions or to the AgNPs or to a combined effect of the ions and nanoparticles.

2.9. Exposure assessment

2.9.1. Food consumption data used for exposure assessment

2.9.1.1 EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure

Assessment' (EFSA, 2011b). New consumption surveys recently added in the Comprehensive database were also taken into account in this assessment.[19]

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible underreporting by subjects and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

Food consumption data for the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 3).

Table 3: Population groups considered for the exposure estimates of silver (E 174)

Population	Age range	Countries with food consumption surveys covering more than one day
Infants	From 4 months up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, UK
Children [(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly [(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Sweden, UK

(a): The terms children and the elderly correspond, respectively, to other children and the merge of the elderly and the very elderly in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011c). Nomenclature from the FoodEx classification system has been linked to the Food Classification System (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, FoodEx food codes were matched to the FCS food categories.

2.9.1.2 Food categories selected for the exposure assessment of silver (E 174)

The food categories in which the use of silver (E 174) is authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system food codes), at the most detailed level possible (up to FoodEx level 4) (EFSA, 2011c).

No use levels were reported for liqueurs, therefore this food category was not taken into account in the present estimate. This may have resulted in an underestimation of the exposure.

[19] Available online at: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

Silver (E 174) is authorised in the two other food categories: Other confectionery including breath-freshening microsweets (FCS 05.2) and Decorations, coatings and fillings (FCS 05.4) as coating. The Panel noted that silver (E 174) is probably present on few chocolates or confectionary products only. However, such details on the presence or not of silver (E 174) on chocolates and confectionery are not available in the FoodEx nomenclature. In order to provide a more realistic estimate, some food items were removed from the list of confectionery or chocolates as they usually do not contain silver (E 174) (e.g. liquorice candies, jelly candies, gum drops, nougats, halva, chocolate bars, chocolate cream). The other products may contain silver (E 174) and were taken into account in the present estimate. It has been considered by the Panel that such products with silver are not consumed on a daily basis (special occasion e.g. Christmas, Easter, etc.), then, an assumption of consumption of 10 times per year has been applied.

Added to that, silver (E 174) is only used at low level as coating ingredient in the product (below 1%). This percentage was applied to the consumption of cocoa products and confectionery selected, to retrieve consumption of the food additive.

2.9.2. Exposure to silver (E 174) from its use as a food additive

The Panel estimated chronic exposure to silver (E 174) for the following population groups: infants, toddlers, children, adolescents, adults and the elderly. Dietary exposure was calculated by multiplying silver (E 174) concentrations reported in Appendix A for each food category with their respective consumption amount per kilogram of body weight for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Surveys with only one day per subject were excluded as they are considered not adequate to assess repeated exposure.

This was carried out for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 3). Based on these distributions, the mean and 95th percentile of exposure were calculated per survey for the total population and per population group. High percentile exposure was only calculated for those population groups where the sample size was sufficiently large to allow calculation of the 95th percentile of exposure (EFSA, 2011b). Therefore, in this assessment, high levels of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not included. Thus, for this assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 3).

Exposure assessment of silver (E 174) was carried out by the ANS Panel based on (1) maximum reported use levels (defined as the *maximum level exposure assessment scenario*) and (2) reported use levels (defined as the *refined exposure assessment scenario*) as provided to EFSA by industry. These two scenarios are discussed in detail below.

2.9.2.1 Maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008. As silver (E 174) is authorised according to QS in all food categories, a 'maximum level exposure assessment' scenario was estimated based on the maximum reported use levels provided by industry (Appendix A), as described in the EFSA Conceptual framework (EFSA ANS Panel, 2014).

The Panel considers the exposure estimates derived following this scenario as the most conservative as it is assumed that the consumer will be continuously (over a lifetime) exposed to silver (E 174) present in food at the maximum reported use levels.

2.9.2.2 Refined exposure assessment scenario

The refined exposure assessment scenario is based on use levels reported by industry. This exposure scenario can consider only food categories for which the above data were available to the Panel.

Appendix A summarises the concentration levels of silver (E 174) used in the refined exposure assessment scenario. Based on the available data set, the Panel calculated two refined exposure estimates based on different model populations:

- The brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to silver (E 174) present at the maximum reported use level for one food category. This exposure estimate is calculated as follows:
  - combining food consumption with the maximum reported use level for the main contributing food category at the individual level;
  - using the mean of the typical reported use levels for the remaining food categories.
- The non-brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to silver (E 174) present at the mean reported use levels in food. This exposure estimate is calculated using the mean of the typical reported use levels for all food categories.

The Panel noted that only two food categories out of the three food categories in which the use of silver (E 174) is authorised could be taken into account. If, nevertheless, silver (E 174) is used in the remaining food category of liqueurs for which concentration data were not available, the calculated exposure estimates might result in underestimation of the actual exposure to silver (E 174).

2.9.2.3 Anticipated exposure to silver (E 174)

Table 4 summarises the estimated exposure to silver (E 174) from its use as a food additive in six population groups (Table 3) according to the different exposure scenarios (Sections 2.9.2.1 and 2.9.2.2). Detailed results per population group and survey are presented in Appendix B.

Table 4: Summary of anticipated exposure to silver (E 174) from its use as a food additive in the maximum level exposure assessment scenario and in the refined exposure scenario, in six population groups (minimum–maximum across the dietary surveys in µg/kg body weight (bw)/day)

	Infants (4-11 months)	**Toddlers (12–35 months)**	**Children (3–9 years)**	**Adolescents (10–17 years)**	**Adults (18–64 years)**	**The elderly (≥ 65 years)**
Maximum level exposure assessment scenario						
Mean	< 0.01–0.17	0.07–1.9	0.22–2.6	0.10–2.2	0.03–0.65	0.02–0.18
High level	0.0–0.79	0.32–4.3	1.1–12.0	0.60–8.6	0.20–3.6	0.11–0.70
Refined estimated exposure scenario						
Brand-loyal scenario						
Mean	< 0.01–0.17	0.07–1.7	0.21–2.6	0.09–2.1	0.03–0.64	0.02–0.17
High level	0.0–0.79	0.32–4.1	1.1–12.0	0.60–8.6	0.20–3.5	0.11–0.67
Non-brand-loyal scenario						
Mean	< 0.01–0.17	0.03–1.6	0.18–1.0	0.09–0.77	0.03–0.22	0.02–0.15
High level	0.0–0.69	0.23-2.7	0.87–3.2	0.60–2.3	0.19–0.97	0.09–0.64

2.9.3. Main food categories contributing to exposure to silver (E 174)

Table 5: Main food categories contributing to exposure to silver (E 174) using maximum usage levels (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

FCS category number	FCS Food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[(a)]					
05.2	Other confectionery including breath-freshening microsweets	10.9–37.2 (4)	14.9–56.5 (10)	11.9–86.6 (18)	8.5–91.8 (17)	5.5–80.4 (16)	9.2–76.2 (13)
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4 – only decorations of chocolates	62.8–100 (6)	43.5–100 (10)	13.4–88.1 (18)	8.2–91.5 (17)	19.6–96.2 (17)	23.8–97.0 (14)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific population.

Table 6: Main food categories contributing to exposure to silver (E 174) using the brand-loyal refined exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

FCS category number	FCS Food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[(a)]					
05.2	Other confectionery including breath-freshening microsweets	10.9–37.2 (4)	7.2–56.4 (9)	8.4–86.4 (18)	6.5–91.8 (16)	7.3–80.2 (15)	8.2–75.8 (13)
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4 – only decorations of chocolates	62.8–100 (6)	43.6–100 (10)	13.6–91.6 (18)	8.2–95.1 (17)	19.8–96.2 (17)	24.2–98.1 (14)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific population.

Table 7: Main food categories contributing to exposure to silver (E 174) using the non-brand-loyal refined exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

FCS category number	FCS Food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[(a)]					
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4 – only decorations of chocolates	99.9–100 (6)	99.9–100 (10)	99.3–100 (18)	98.7–100 (17)	99.5–100 (17)	99.6–100 (14)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific population.

2.9.4. Uncertainty analysis

Uncertainties in the exposure assessment of silver (E 174) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and summarised in Table 8.

Table 8: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction[(a)]
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of reported use levels to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties to which types of food the levels refer to	+/–
Food categories included in the exposure assessment: - the most relevant chocolates/confectionary selected assumed to contain the food additive - data not available for certain food categories which were excluded from the exposure estimates (only the liqueurs)	+ –
Concentration data: - levels considered applicable for all items within the entire food category	+
Maximum level exposure assessment scenario: -food categories authorised at the highest level reported	+
Refined exposure assessment scenarios: - exposure calculations based on one maximum and one mean levels (reported use from industries)	+/–
Uncertainty in possible national differences in use levels of food categories	+/–

(a): +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Overall, the Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure to silver (E 174) as a food additive in European countries for the refined scenario.

2.9.5. Dietary occurrence from sources other than the food additive

In a study conducted to determine the presence of trace elements in some seafood species, the levels of Ag detected were 0.030 ± 0.017 mg/kg in red mullet, 0.038 ± 0.024 mg/kg in grey mullet and 0.032 ± 0.029 mg/kg in green tiger prawns (Yarsan et al., 2014).

Following a call for data, the National Institute of Nutrition and Seafood Research (Documentation provided to EFSA No8) has reported silver total concentrations (mg/kg) in seafood and other food matrices. Most samples were from wild fish catches or aquaculture where silver was not present as food additive. The analyses were performed for several years from 2006 to 2011. From the 11,434 samples analysed, 7,842 were below the limit of quantification (LOQ). The mean levels found for some species are presented in Table 9. The Panel noted that the values were very low for some species as trout and rainbow trout, presenting a high standard deviation (data not shown).

Table 9: Silver concentration (mg/kg) in some species analysed during different periods.

SPECIES							
Oysters 2006-2011	1.049	Coalfish	0.024	Mackerel	0.023	Common whelk	0.027
Greenland halibut 2006-2011	0.006	European plaice 2007	0.005	Caplin 2007-2010	0.09	Scallop 2006-2011	0.027
Trout 2007-2009	-	Shrimp 2007-2010	0.185	Ling 2008	0.048	Cusk 2008	0.063
Rosefish 2007	0.074	Rainbow trout 2008	-	Salmon 2005-2011	0.72	Blue mussel 2006-2011	0.013
Cod 2005-2011	0.259	Polar cod 2006-2010	0.022	Halibut 2005-2008	0.045		
Soya	2.27	Spiny dogfish 2007-2008	-	Crab 2007-2010	0.465		

The 2nd French Total Diet Study (ANSES, 2011) estimated the intake of silver. Most of the analysed samples had a silver level below the limit of detection (LOD)/ LOQ (82%). The highest concentration levels were found in molluscs, crustaceans and offal. For adults, the mean exposure ranged from 1.29 µg/kg bw/day (lower bound) to 2.65 µg/kg bw/day (upper bound); at the 95th percentile, exposure levels ranged from 2.82 µg/kg bw/day (lower bound) to 4.78 µg/kg bw/day (upper bound). For children, the mean exposure ranged from 1.60 µg/kg bw/day (lower bound) to 3.47 µg/kg bw/day (upper bound); at the high exposure levels ranged from 3.60 µg/kg bw/day (lower bound) to 6.59 µg/kg bw/day (upper bound). Main contributors were molluscs and crustaceans for adults and milk and water for children.

2.9.6. Dietary exposure from all sources

The exposure from the food additive and the regular diet (ANSES, 2011) could lead to a mean intake for children around 3.5 µg/kg bw/day (non-brand-loyal scenario). On average, exposure from the food additive would represent around 30% of the total dietary exposure to silver.

3. Biological and toxicological data

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations and additional literature that became available since then. No new toxicological or biological information was submitted to the Panel for the re-evaluation of silver following EFSA public calls for data. The Panel noted that not all of the original studies on which previous evaluations were based were available for this re-evaluation.

The present opinion briefly summarises the major studies evaluated previously by the SCF (SCF, 1975) and JECFA (JECFA, 1977, 1978) in these evaluations and describes the additional studies in more detail.

Data on elemental silver are not available.

The Panel noted that AgNPs are released from confectionery pearls (Verleysen et al., 2015) and nanosilver is unstable and releases ions (see Section 2.5). The Panel was aware of the extensive database on ionic silver or AgNPs, however the relevance of these data to the evaluation of silver as a food additive (E 174) was not apparent. Therefore, the Panel considered these data could not be directly applied to the evaluation of the food additive.

In this opinion, only data with non-capped nanoparticles are included. However, when corresponding capped nanoparticles have been studied in the same experiments, also those data are included. References of the studies with capped material are given in Appendix C.

3.1. Absorption, distribution, metabolism and excretion (ADME)

The most important studies evaluated by JECFA (JECFA, 1977), as well as several additional studies identified in the literature search are summarised below.

3.1.1. Ionic silver

Studies evaluated by JECFA

Furchner et al. (1968) investigated the ADME of silver (^{110m} ionic silver, as the nitrate) in mouse, rat, monkey and dog following oral administration. Female RF mice (body weight 27 g, age 3 months, n = 12) were given 0.25 µCi; male Sprague–Dawley rats (body weight 360 g, age 3 months, n = 6) were given 0.5 µCi; male beagle dogs (body weight 13,300 g, age 90 months, n = 4) were given 0.6 µCi, and male *Macaca mulatta* monkeys (body weight 6,700 g, age 48 months, n=4) were given 0.6 µCi. Faeces and urine were collected. Animals were euthanised at various time points to assess the concentration–time profile of radioactivity in tissues. The following tissues were investigated: testis, brain, spleen, kidney, liver, heart, lung, intestine, fur, blood and carcass. Ninety per cent or more of oral applied radioactivity were excreted in the faeces. Cumulative excretion at day 2 was 99.6% for the mouse, 98.3% for the rat, 90.4% for the dog and 94.4% for the monkey. The urinary/faecal excretion ratios for ^{110m}ionic silver were 0.001 for the mouse, 0.001 for the rat, 0.025–0.061 for the dog and 0.019–0.258 for the monkey.

Ham and Tange (1972) found silver granules after given silver nitrate in the drinking water to be deposited in the rat glomerular basement membrane. Albino and hooded female rats (strain not specified; age not specified; body weight of 90–100 g; number of animals not specified exactly, but at least 26 per strain due to the study protocol were given a 0.25% solution of silver nitrate as drinking water (equivalent to 142 mg ionic silver/kg bw/day[20]). A pair of each of the rat strains was euthanised at 1, 2, 3, 4, 8 and 12 weeks. Silver nitrate administration was then withdrawn and all animals were given tap water. In addition, pairs of rats were euthanised at 1, 2, 3, 6, 10 months and four animals per strain were euthanised at 16 months. The kidney was investigated by microscopy and electron

[20] Calculated by the Panel according to EFSA Scientific Committee (2012).

microscopy. Moreover, the silver content in the liver and kidney was determined. Silver granules were found particularly in the glomerular basement membrane. After administration had been discontinued, silver granules in the basal membrane continued to increase in size as renal excretion of silver from the body continued. After some months the silver granules decreased in number and size and eventually disappeared. The silver content of the liver showed gradual decline after intake had ceased over the duration of the experiment.

Additional studies, not evaluated by JECFA

3.1.1.1. Mice

Wang et al. reported radioactivity (orally administered silver ions given as a tracer ^{105}ionic silver) to be located in the liver, heart, spleen, kidney, fur and muscle of mice (Wang et al., 2001).

Pelkonen et al. (2003) investigated the concentration time profile of radioactivity into different tissues when given in the drinking water [^{110m}Ag]Silver nitrate (0.03 mg silver/L, equivalent to 0.005 mg ionic silver/kg bw/day[21]) was given to five male outbred NIH/S mice (10–12 weeks old, body weight 23–26 g) for 1 or 2 weeks. Tissue distribution was analysed by gamma radioactivity. The highest concentrations were found in muscle, followed by cerebellum, spleen, duodenum and myocardial muscle and no accumulation was observed.

3.1.1.2. Rats

Dijkstra et al. (1996) administered silver ions as a water-soluble silver salt (80 nmol silver ions/100 g bw, 8.6 mg ionic silver/kg bw, salt not specified) intravenously to Wistar rats (body weight 260–300 g, age not specified, n=4) and collected the bile for 4 hours via an indwelling catheter in the bile duct in 30 min intervals. Biliary excretion of silver ions occurred; the recovery in bile was 48.5 and 23.2% of the dose in NW rats and in GY rats, respectively.

Distribution in several organs

Olcott (1948) has studied the tissue distribution of orally administered silver nitrate and silver chloride in rats (age starting shortly after weaning, n=2–3) given a 1:1000 dilution of the silver salts in about 1:300 sodium thiosulphate for their lifetime (equivalent to 32 and 38 mg ionic silver/kg bw/day for silver nitrate and silver chloride, respectively[21]). A range of organs were investigated. The eyes became progressively darker. The tongue, teeth and salivary glands were black. The thyroid was regularly grey to black on microscopic examination. The parathyroids contained deposition of moderate numbers of granules. The heart was grey at necropsy. The liver was slightly dark on gross examination. The pancreas was one of the most deeply pigmented parts of the body. Granules of silver were recognised in the brain tissue or in the vessels. The kidney was often very dark on gross examination. Dark spots were found in the glomerulus. Silver was deposited in the basement membrane of the glomerular tuft, lesser amounts of pigment were found in the basement membrane of the collecting tubules or in the wall of the small blood vessels.

Walker (1971) investigated the organ distribution of silver ions given in the drinking water. Male Sprague–Dawley rats (12/group, age 8 weeks, body weight not specified) were given silver nitrate at 6, 12 or 24 mM in the drinking water (equivalent to 59, 118 and 236 mg ionic silver/kg bw/day[21]). The 6 and 24 mM groups were discontinued after 12 and 2 weeks, respectively. The 12 mM group was dosed for 0, 2, 4, 6, 8, 10, 12, 16, 25 and 60 weeks; a series of six rats were given 12 mM for 10 weeks and were then restored to ordinary drinking water and euthanised at 2, 4, 6, 8, 10 and 12 weeks later (lag phase study). Kidney, skin, eye, liver and muscle were taken from some animals for electron microscopy. In addition, 20 organs or tissues were taken from each animal for light microscopy. By the macroscopic pathological investigations, it was observed that the animals had stained muzzle and teeth. Microscopic investigation showed the following: within 6 weeks of commencing

[21] Calculated by the Panel according to EFSA Scientific Committee (2012).

administration, sites where silver deposits were detected included the glomerulus, colon and liver. After a further 6 weeks, silver deposits were detected in choroid plexus, thyroid and skin appendage basement membranes. At 25 weeks of administration, silver was found in skin surface, urinary bladder and prostatic acinar membranes. In the lag phase study, it was found that deposition continued 4 weeks after discontinuation of silver administration.

Distribution in the kidney

Walker (1972) studied the renal content of silver given as silver nitrate in the drinking water. Sixteen male Sprague–Dawley rats (8 weeks of age, body weight not specified) were given silver nitrate for 0, 2, 4, 6, 8, 10, 12, 16, 25, 60 and 81 weeks at a concentration of 12 mM (equivalent to 118 mg ionic silver/kg bw/day[22]). Parts of renal cortex were processed for electron microscopy. Silver was found in the glomerular basement membrane as already described by Ham and Tange (1972).

Creasey and Moffat (1973) investigated the distribution of ingested silver in the rat kidney following administration of 0.15% silver nitrate in drinking water to weanling rats (strain and body weight not specified, n=26) for 4–15 weeks (equivalent to 85 mg ionic silver/kg bw/day[22]). Kidneys were subjected to light and electron microscopy. Granules containing silver that never exceeded 30 nm was found in the glomeruli, around the vascular bundles and capillaries of the outer medulla. In the inner medulla granules containing silver were found in the vasa recta, loops of Henle and in the interstitial cells and matrix.

Distribution in the spleen

Pereira (1977) investigated the localisation of silver in the rat spleen. Young male albino rats (strain, age, number of animals and body weight not specified) were given for many months (time period not specified further) drinking water with 1.5 g silver nitrate per litre (equivalent to 85 mg ionic silver/kg bw/day[22]). Following euthanisation, specimens were prepared for electron microscopy. Silver was found in several structures in the spleen, e.g. dense granules were found in the elastic membranes of the splenic capsule and trabeculae, and in discrete locations throughout the red pulp and marginal zone. In the red pulp, extremely dense granular deposits occurred in the basal laminae. Dense granules were also deposited in the macrophages, reticular fibres and marginal sinus basal laminae of the splenic marginal zone.

Distribution in the brain

Van Breemen and Clemente (1955) investigated the ability of silver ions to cross the blood–brain barrier. Rats (strain, age, number of animals and body weight not specified) were administered 0.5% silver nitrate in their drinking water for 6–8 months (equivalent to 158 mg ionic silver/kg bw/day[22]). The rats were euthanised and sections of the brain were examined by electron microscopy. Precipitated silver was found in the perivascular spaces of the choroid plexus and in the area postrema. Silver was specifically deposited on the outer surfaces of endothelial cells, on collagen fibrils of the stroma and on the vessel side of the cell membranes adjacent to perivascular spaces. Very little silver was found around the capillaries in the cerebrum, cerebellum and most of the medulla.

Rungby and Danscher (1983) investigated the distribution of silver in the brain by electron microscopy. Wistar and Sprague–Dawley rats of both sexes (10 animals/group, weighing at least 120 g) were administered silver nitrate and silver lactate at a concentration of 0.01% dissolved in the drinking water for 4 months (equivalent to 6 mg ionic silver/kg bw/day[22]). Silver nitrate in the drinking water resulted in silver deposition in several brain regions. Regarding cell types, a relatively high content of silver was found in glia with silver nitrate, whereas silver lactate resulted in deposition

[22] Calculated by the Panel according to EFSA Scientific Committee (2012).

preferentially in neurons. Silver was located intracellularly in the lysosomes and extra-cellularly in basement membranes and elastic fibres of the vessels.

Distribution in the eye

Rungby (1986) investigated the ultrastructural localisation of silver in the eyes. Male Wistar rats (body weight 120 g, 3 animals/group) were administered silver nitrate or silver lactate at a concentration of 0.02 % dissolved in the drinking water for 45 days (equivalent to 12 mg ionic silver/kg bw/day[22]). Silver was found in lysosomes of most cell types, with the exception being the neural retina. Extracellularly, silver was present in vascular basal laminae and in connection with connective tissue fibres.

Olcott (1947) also found pigmentation of the eyes following ingestion of silver nitrate. A total number of 159 rats (strain only specified as animals coming from Rockland Farms, New York City, N.Y.; 82 males, 55 females that had no litters and 22 females that had at least one litter; age at least 1 month; body weight not specified) had their eyes examined; 143 of the rats received a 1:1000 solution of silver nitrate whereas 16 rats received a 1:1000 solution of silver chloride held in solution by about 3.5 times as much sodium thiosulphate as the silver salt (equivalent to 32 mg ionic silver /kg bw/day[23]). Silver was given from the age of 1 month until death. Silver was found during life and at necropsy as pigmentation of the eyes. The amount of pigmentation was directly related to the duration of treatment.

Matuk et al. (1981) investigated the distribution of silver in rat eyes. Forty weanling male Wistar rats (age and body weight not specified) were administered a 0.25% silver nitrate solution via the drinking water for up to 8.5 months (equivalent to 81 mg ionic silver/kg bw/day[23]). After 10 weeks, two rats were euthanised and the eyes were processed for electron microscopy. The remaining rats were divided into two groups: 1) A group that continued with the same solution for the next 6 months and 2) a group that was shifted to water for 6 months. At monthly intervals, one rat from each group was euthanised and the eyes were examined for silver deposits by electron microscopy. Particles containing silver were found in the eyes of the rats. The number and size of these particles increased with continued ingestion of silver nitrate, but decreased when silver nitrate was withdrawn. However, fine particles of silver were still present 12 months after the end of silver nitrate ingestion.

The Panel noted that none of the reports indicated whether the granules/deposits found by electron microscopy represented silver in its inorganic form or silver bound to organic compounds, e.g. silver-glutathione.

Occurrence in milk

Ilyechova et al. (2012) reported that silver ions were present in the breast milk of rat dams receiving a dose of 50 mg silver chloride/kg bw/day from the diet, starting on the first day of lactation. This was indicated by a higher silver concentration in the breast tissue of silver-treated dams (4.7 µg/g tissue) than of controls (0.15 µg/g tissue). Also, the stomach of 10-day-old rats breastfed by silver-treated dams contained much more silver (35 µg/g tissue) than stomach from rats receiving milk from control dams (1 µg/g tissue). In this study, the authors also reported that silver ions were transported and accumulated in the liver of the pups and to a much less extent in their brain.

3.1.1.3. Human

The Panel was aware that there are many data reporting distribution of silver in various human organs following prolonged exposure to very high doses of silver in different forms (see Section 3.2.7.2).

[23] Calculated by the Panel according to EFSA Scientific Committee (2012).

3.1.2. AgNPs

Additional studies, not evaluated by JECFA

3.1.2.1 Silver ions from AgNPs in laboratory synthetic set-ups

Several studies have been reported in the literature on the behaviour of AgNPs in different environments. These studies have frequently been performed with citrate-stabilised or other forms of stabilised colloidal AgNPs in aqueous systems. Although considering that stabilised AgNPs are not representative of the food additive E 174, the Panel considered that these studies provide valuable information that can be useful as evidence of the release of silver ions from elemental AgNPs even if the rate of release could be presumably different considering the possible effect of the capping agent used. Some of these studies are reviewed below.

Liu and co-workers (Liu and Hurt, 2010; Liu et al., 2010, 2012) extensively investigated the behaviour of colloidal AgNPs in aqueous systems and in simulated biological environments. In the presence of oxygen, AgNPs were seen to undergo chemical conversion that can affect silver bioavailability and toxicity. Conversions were simulated and included accelerated oxidative dissolution in the simulated GI (GI) tract, thiol binding and exchange, photo-reduction in the near-skin regions of thiol- or protein-bound silver to secondary zerovalent AgNPs, and rapid reactions between silver surfaces and reduced selenium species (Liu et al., 2012). Some biological environments have low pH (e.g. gastric fluid) which facilitates silver dissolution as ionic silver. High concentrations of organic ligands (thiols) and relevant concentrations of selenium in addition to sulfur: both selenium and sulfur can yield silver precipitates, with Ag_2Se being more insoluble than Ag_2S. Similarly, under certain conditions the presence of the chloride ion Cl^- may determine ionic silver to precipitate as AgCl. Silver nanoparticulate surfaces can adsorb ionic silver, so that even simple colloids can be thought to contain three forms of silver: solid elemental silver, free ionic silver or its complexes, and surface-adsorbed ionic silver (Liu and Hurt, 2010).

The same authors developed a kinetic model to describe the observed release of ionic silver from AgNPs surfaces in the aqueous systems and biological environments utilised for the experimental observations (Liu et al., 2012). According to the model, the oxidative dissolution of AgNPs is strongly dependent on pH and particle size, in that dissolution rate was shown to increase with lowering pH and could be much higher for nanoparticles than for microparticles. The authors observed that although gastric fluids should lead to an accelerated dissolution of AgNPs, dissolution can be incomplete for most particles due to the limited residence time in stomach (10–240 min). The dissolution kinetics in laboratory aqueous media was modelled at different pH (ranging from 1.5 to 7.4) and with various particle sizes (Ø = 2–500 nm). The Panel noted that the study included microparticles and that at pH 1.5 (resembling the pH of the stomach), for short incubation times (< 3 h), ionic silver formation rate from 5-nm AgNPs was more than fivefold higher than that at pH 7.4 (resembling the pH of the blood). Small AgNPs (Ø < 20 nm) appeared to dissolve much quicker than larger nanoparticles (Ø > 20 nm). By comparison, 500 nm microparticles seemed to be almost unreactive. Surface area normalisation proved that the high dispersion of nanoparticles had a very strong impact on the formation rate of ionic silver.

Loza et al. (2014) investigated PVP-coated AgNPs dissolution in biological media and related-biological effects. It was observed that AgNPs (in the form of 70-nm-Ø spherical particles) released silver ions if oxidising species like molecular oxygen or hydrogen peroxide were present. The presence of a reducing sugar (glucose) had only a small effect on dissolution rate. In the presence of chloride ions, precipitation of silver chloride nanoparticles occurred (apparently, not on the surface of the initial AgNPs); at physiological salt concentrations, precipitation of silver chloride inhibited the precipitation of silver phosphate. When the AgNPs surface was passivated by cysteine, the dissolution was quantitatively inhibited. AgNPs were subject to an 8-month-long immersion in pure water at a neutral pH: a dissolution of only about 50% was observed (for which no sound explanation was found), and no surface changes were detected in the unreacted AgNPs by TEM. The authors also

carried out a literature survey on the dissolution of AgNPs: it ultimately showed that only qualitative trends could be identified from the available studies as the nature of the nanoparticles and of the immersion media were in general not comparable. Dissolution effects were confirmed by cell culture experiments (human mesenchymal stem cells and neutrophil granulocytes), where AgNPs that were stored under argon had a clearly lower cytotoxicity than those stored under air; they also led to a diminished formation of reactive oxygen species (ROS). This highlighted that silver ions can be released from AgNPs.

3.1.2.2 *In vitro* investigations of the absorption of AgNPs.

Several authors (Bouwmeester et al., 2011; Rogers et al., 2012; Walczak et al., 2012) explored the absorption of AgNPs using *in vitro* systems.

Bouwmeester and co-workers (Bouwmeester et al., 2011) used Caco-2 and M cells as an *in vitro* intestine model to study the passage of AgNPs and their ionic forms, and to assess their effects on whole-genome mRNA expression in the cells. The cells were exposed to AgNPs in four sizes (producer's TEM assessment: Ø = 20, 34, 61, and 113 nm) for 4 h. Exposure to silver ions was included as a control as 6–17% of the AgNPs silver content were found to be transformed into silver ions, the ion levels increasing with decreasing size of nanoparticles. The amount of silver ions that passed the Caco-2 cell barrier was equal after exposure to silver ions and for exposures with nanoparticles. AgNPs induced clear changes in gene expression in a range of stress responses including oxidative stress, endoplasmatic stress response and apoptosis. However, the gene expression response to AgNPs was very similar to that of $AgNO_3$. Translocation of nanoparticles through the epithelium depended on their physico-chemical properties such as size, surface charge, lipophilicity/hydrophilicity and presence/absence of a ligand. The study, carried out with nanoparticles selected in the range of 20–30 nm, indicated that the translocation of silver across the cell membrane in the model utilised was likely to occur as silver ions released from the nanoparticles and not as AgNPs as such. Similarly, the observed effects of the AgNPs were likely exerted by the silver ions that were released from the nanoparticles.

The absorption of silver from ingested AgNPs largely depends on initial particle size, shape and surface coating, properties which will influence particulate aggregation, solubility and chemical composition during transit in the GI tract. Rogers and co-workers (Rogers et al., 2012) used an *in vitro* model to expose citrate-stabilised AgNPs (Ø = 40 nm, nominal) to synthetic human stomach fluid (SSF) at pH 1.5; changes in size, shape, zeta potential, hydrodynamic diameter and chemical composition were determined during a 1-h exposure period by various analytical techniques. According to this experiment, ingested AgNPs may be converted to a variety of aggregated and chemically modified particles in the stomach. The authors acknowledged that, given the wide range of coating compounds that vary in chemical properties or surface charge, the reported results may not be representative of AgNPs preparations in general. Moreover, absorption of ionic silver from these Ag-containing materials will also depend on the interactions between this mixture of Ag-containing species and the absorptive surfaces of the GI tract.

Walczak et al. (2012) investigated the fate of AgNPs in a model mirroring GI digestion following oral ingestion. The study utilised 60-nm AgNPs and silver ions from $AgNO_3$. After exposure to saliva, gastric and intestinal fluid, samples were analysed with various analytical techniques. In the presence of proteins, after exposure to gastric fluid the number of particles dropped significantly, to rise back to original values. A reduction in number of particles was caused by clustering of particles in bigger particles, as revealed by analysis with SEM/EDX spectroscopy: some of the clusters contained AgNPs and chlorine. During exposure to intestinal fluid, these clusters broke back into single 60-nm AgNPs. The authors concluded that, under physiological conditions (i.e. in the presence of proteins), these AgNPs can reach the intestinal wall in their initial size and composition. It was also observed that exposure to intestinal fluid of $AgNO_3$ in the presence of proteins resulted in particle formation. These nanoparticles (Ø in the range of 20–30 nm) were composed of silver, sulfur and chlorine. On the

whole, ingestion of both AgNPs and silver ions ultimately appeared to lead to intestinal exposure to particles, although with a different chemical composition.

The Panel noted that the findings from the above *in vitro* studies allow to draw the conclusion that the transepithelial transport occurs with a similar efficiency for AgNPs as for silver ions suggesting that silver ions are absorbed. This in accordance with the report of the Danish Environmental Protection Agency (DTU, 2013), dealing with the systemic absorption of ingested nanomaterials, which pointed out that the results suggest that AgNPs dissolve in the GI tract prior to intestinal absorption, to enter circulation and subsequently reach primarily the liver and spleen and to a lesser degree other organs.

3.1.2.3 Mice

Park et al. (2010) investigated the tissue distribution of silver in mice following oral administration of a suspension of AgNPs (22, 42 and 71 nm in diameter) and silver from microsized particles (323 nm in diameter). ICR mice (5/group – both sexes but the number of animals per sex not specified, 6 weeks of age, weight not specified) were administered the AgNPs by gavage (vehicle: deionised water) in a dose of 1 mg/kg bw/day for 14 days. The control group received deionised water prepared by the same process to prepare the AgNPs suspension. The silver ion concentration in brain, lung, liver, kidney and testes was measured using ICP-MS after tissue digestion. Silver, measured by ICP-MS after solving the tissues in 70% HNO_3 and treatment with 30% H_2O_2, was found in all the tissues of animals dosed with nanoparticles, except in testes of animals which had received the 71 nm AgNPs. The concentrations were dependent on the particle diameter with lower concentrations in the tissues of animals treated with NP of higher diameter. In contrast to the findings after administration of AgNPs (22–71 nm), there was no silver found in the tissues following administration of microsized silver particles (323 nm).

3.1.2.4 Rats

Van der Zande et al. (2012) investigated the distribution and elimination of AgNPs and silver ions in rats. Male Sprague–Dawley rats (5/group, 6 weeks old, body weight about 245 g) were exposed daily by oral gavage for 28 days to 90 mg/kg bw of AgNPs (18 nm, non-coated or 12 nm, PVP-coated, in diameter) or 9 mg/kg bw of silver nitrate (corresponding to 6 mg ionic silver /kg bw/day). Included were also wash-out groups identically exposed to silver but not sacrificed until day 36 or day 84. At the end of the 4-week treatment, total silver contents were determined with atomic absorption spectrophotometry in a broad range of organs, blood and intestinal contents. Furthermore, SP-ICP-MS was applied to detect silver containing nanoparticles in a selection of these organs and in intestinal contents. Silver was found in all examined organs (liver, spleen, testis, kidney, brain, lung, blood, bladder and heart) with the highest levels in the liver and spleen for all silver treatments. Silver concentrations in the organs were highly correlated with the amount of silver ions in the silver nanoparticle suspensions, indicating, according to the authors, that mainly silver ions passed the intestines in the silver nanoparticle exposed rats. In all groups (the two nanoparticle groups, as well as the silver nitrate group), silver was cleared from most organs after 8 weeks of wash out. However, silver content persisted for the observation period in the brain and in the testis. There were no significant differences in distribution profiles between silver nitrate and the two types of AgNPs.

In a 28-day study by Kim et al. (2008) (performed according to the OECD TG 407) on AgNPs in Sprague–Dawley rats (10/sex/group, 6 weeks old, mean body weight 283 g for males and 192 g for females) were administered 0, 30, 300 and 1,000 mg/kg bw/day AgNPs (diameter of 60 nm, coating not specified) by gavage (vehicle: 0.5% carboxy methyl cellulose) for 28 days. At the end of the 4-week treatment, tissue silver was determined by atomic absorption spectrophotometry. A dose-dependent content of silver was found in all examined tissues ($p < 0.05$ or < 0.01) including the testis, kidney, liver, brain, lung, stomach and blood.

Kim et al. (2010) also performed a 13-week study (according to the OECD TG 408) on AgNPs in Fisher 344 rats (10/sex/group, 5 weeks of age, mean body weight of males and females were

approximately 100 and 90 g, respectively). Rats were administered 0, 30, 125 and 500 mg/kg bw/day AgNPs (diameter of 56 nm, coating not specified) by gavage (vehicle: 0.5% carboxy methyl cellulose). At the end of the 90-day treatment, tissue silver was determined by atomic absorption spectrophotometry. A dose-dependent content of silver was found in all tissues examined including testis, liver, kidney, brain, lung and blood. A gender difference was observed for kidneys where a twofold higher concentration of silver was observed in the kidneys of females as compared to males.

Summary

Upon oral exposure of animals to ionic silver or AgNPs, silver is systemically available. Silver concentrations in the organs were highly correlated to the size of the nanoparticles showing higher concentrations in animals treated with nanoparticles with a smaller diameter and to the amount of silver ions in the suspension of AgNPs. Bioavailability seems to be in the range of 2–20% depending on a range of factors including the animal species.

However, the Panel noted that, due to the many variables involved, the conversion rate of metal silver from nanoparticles to silver ions in biological systems is unknown. Moreover, the formation of ROS from the fraction of AgNPs which may be present in the food additive has not been determined. The rate of both processes depends on the size of particles and their relative surface.

Silver is distributed into all organs and tissues (mainly in the liver). Silver is also distributed into the brain following oral exposure, which is in contrast to the conclusions from the authors of studies from the 50s with silver nitrate or lactate, that silver would not cross the blood–brain barrier (van Breemen and Clemente,1955). However, in the more recent studies, it is also unclear whether silver is present in the brain endothelial cells or in the brain tissue. Silver ions were also detected in the milk of rat dams receiving a daily oral administration of silver chloride, and in the liver and in the brain of the pups. In rodents, silver is primarily excreted via the bile and faeces, but a small amount is also excreted via the urine.

The Panel noted that only one study described the fate of microsized silver particles in animals (Park et al., 2010). In this study, no silver was detected in any of the tissues of mice given an oral administration of microsized silver particles (323 nm), whereas silver was present in tissues of mice receiving a similar administration of nanosized silver particles (21 to 71 nm), indicating the impact of particle size on the conversion into silver ions of metallic silver, given in particulate form.

3.2. Toxicological data

No studies were reported on elemental silver.

3.2.1. Acute oral toxicity

No data were submitted to EFSA following a public call for data. The only oral acute toxicity study evaluated by JECFA (JECFA, 1977), as well as additional studies identified in the literature search are summarised below.

Studies evaluated by JECFA

Ionic silver

In mice, an oral dose of silver nitrate of 50 mg/kg bw (corresponding to 32 mg ionic silver/kg bw) caused death in 50% of the animals within the 14-day observation period (JECFA, 1977).

Additional studies, not evaluated by JECFA

3.2.1.1 Mice

Ionic silver / AgNPs

Cha et al. (2008) compared the acute response of mice livers to nano- or microsized silver particles. A silver nanoparticle solution was prepared based on the reduction in $AgNO_3$ with $NaBH_4$. Male balb/c mice (7-week old) were given a single dose of 2.5 g nanosized silver particles (13 nm; or microsized silver particles (2–3.5 µm) by gavage. Three days later animals were euthanised and livers were processed for microscopy. Both groups exhibited lymphocyte infiltration.

AgNPs

Maneewattanapinyo et al. (2011) showed that spherical AgNPs (with a particle diameter of 10–20 nm) at a limited dose of 5,000 mg/kg bw led neither to mortality nor acute toxic signs in ICR mice in an acute oral toxicity study performed according to the OECD TG 425 (Acute oral toxicity test: the up and down procedure). The AgNPs were synthesised preparing an aqueous solution of AgNO3 with a reducing agent (NaBH4), the AgNPs were purified by centrifugation, washed and adjusted to the initial volume with water. The solutions were diluted with distilled water to obtain different concentrations of AgNPs prior to use in the experiments.

3.2.1.2 Rats

Ionic silver

Tamimi et al. (1998) investigated the acute toxicity of an antismoking mouthwash with the active ingredient being 0.5% silver nitrate. Fischer 344 rats (10/sex, 10–12 months old, body weight 200–250 g) received 1 ml by gavage of either 200, 300 or 400 mg silver nitrate/kg bw of the mouthwash (corresponding to 126, 189 and 256 mg ionic silver/kg bw); the control group received the placebo (not further specified). Animals were observed for 2 weeks, dead animals were subjected to post-mortem examinations immediately after death. The oral LD_{50} values were 428 and 433 mg silver nitrate/kg bw for male and female rats, respectively (corresponding to 280 mg ionic silver/kg bw).

3.2.1.3 Rabbits

Ionic silver

Tamimi et al. (1998) investigated the acute toxicity of an antismoking mouthwash with the active ingredient being 0.5% silver nitrate. Californian rabbits (10/sex, 8–10 months old, body weight 1–1.2 kg) received 10 ml orally by gavage of either 200, 800, 1,000, 1,800 or 4,000 mg silver nitrate/ kg bw of the mouthwash (corresponding to 126, 504, 630, 1,134 or 2,520 mg ionic silver/kg bw); the control group received the placebo (not further specified). The animals were observed for 2 weeks and deceased animals were subjected to post-mortem examinations. The oral LD_{50} values were 1,261 and 1,320 mg/kg bw for male and female rabbits, respectively (corresponding to 794 and 832 mg ionic silver/kg bw).

Overall, oral LD_{50} values of approximately 32, 280 and 800 mg ionic silver/kg bw have been reported for silver nitrate in mice, rats and rabbits, respectively (Tamimi et al., 1998). For AgNPs (10–20 nm), a dose of 5,000 mg/kg bw did not lead to mortality or acute toxic signs in mice (Maneewattanapinyo et al., 2011).

3.2.2. Short-term and subchronic toxicity

No data were submitted to EFSA following a public call for data. In general, the studies in rats evaluated by JECFA (JECFA, 1977) are special purpose studies investigating, for example, the effect of silver acetate in vitamin E-deficient rats or supplementation with essential vitamins and/or minerals; these studies are not considered of relevance for the evaluation of silver as a food additive. Two studies evaluated by JECFA (JECFA, 1977) have been performed in poultry (chicks and turkey poults); these studies are not considered of relevance for the evaluation of silver as a food additive. The remaining two studies evaluated by JECFA (JECFA, 1977) were described in Section 3.2.7 Other studies.

Additional studies, not evaluated by JECFA

3.2.2.1 Mice

AgNPs

Park et al. (2010) investigated the inflammatory response in mice following oral administration of AgNPs (22, 42 and 71 nm in diameter) and silver from microsized particles (323 nm in diameter). The commercial AgNPs were suspended with sonication in tetrahydrofuran (THF) that was evaporated by adding deionised water to the same volume as THF. After the AgNPs, suspension was filtered through different pore sizes and the particle size analysed finding the following average diameters 22, 42, 71 and 323 nm, respectively. THF was completely absent in the final suspension of nanoparticles.

The study consisted of two parts. In the first part, ICR mice (5/group – both sexes but the number of animals per sex not specified, 6 weeks of age, weight not specified) were orally administered the AgNPs by gavage (vehicle: deionised water) at 1 mg/kg bw/day for 14 days. In the second part, ICR mice (6/group – both sexes but the number of animals per sex not specified, 6 weeks of age, weight not specified) were orally administered the AgNPs (42 nm in diameter) by gavage (vehicle: deionised water) at 0.25, 0.5 or 1 mg/kg bw/day for 28 days. The control group in both parts received deionised water prepared by the same process to prepare the AgNPs suspension. In the 14-day study, no changes were observed in body weights, relative organ weights (liver, kidneys, testis, brain and lung) or histopathology (liver, kidney and intestines) in all groups of mice treated with AgNPs (1 mg/kg bw/day). In the 28-day study, the serum levels of alkaline phosphatase (ALP) and aspartate transaminase (AST) were significantly increased ($p < 0.01$) in both sexes administered 1 mg/kg bw/day, and the level of alanine transaminase (ALT) was significantly increased ($p < 0.01$) in females administered 1 mg/kg bw/day. The histopathological examination revealed a slight inflammatory cell infiltration in the kidney cortex in both male and female mice (incidences not reported). According to the authors, the results of the two studies indicated that repeated oral administration of AgNPs may cause organ toxicity in mice and that the AgNPs (22, 42, 71 nm in diameter) are more active than the silver particles of 323 nm in diameter. As the histopathological kidney changes are minimal and the increase in the levels of ALP and AST are not accompanied by histopathological changes in the liver, the Panel considered these lesions of doubtful, if any, toxicological relevance.

3.2.2.2 Rats

Ionic silver

In rats (sex and number not further specified) given silver nitrate or silver chloride in suspension by sodium thiosulfate at a concentration of 1:1000 in the drinking water over long periods (equivalent to 57 and 68 mg ionic silver/kg bw/day for silver nitrate and silver chloride, respectively[24]) hypertrophy of the left ventricle was reported, which, according to the author, is presumed to indicate vascular hypertension that may have been due to the deposition of silver in the basement membranes of the renal glomeruli (Olcott, 1950). The study could not be used for risk assessment as the reporting was limited.

Walker (1971) investigated the effects of silver given in the drinking water. Male Sprague–Dawley rats (12/group, body weight not specified) were given silver nitrate at 6, 12 or 24 mM in the drinking water (equivalent to 59, 118 and 236 mg ionic silver/kg bw/day[24]). The 6 and 24 mM groups were discontinued after 12 and 2 weeks, respectively. The 12 mM group was dosed for 0, 2, 4, 6, 8, 10, 12, 16, 25 and 60 weeks; a series of six rats were given 12 mM for 10 weeks and were then restored to ordinary drinking water and euthanised at 2, 4, 6, 8, 10 and 12 weeks later (lag phase study). In addition, six rats were kept continuously at 12 mM to observe long-term toxicity. The kidney, skin, eye, liver and muscle were taken from some animals for electron microscopy. In addition, 20 organs or tissues were taken from each animal for light microscopy. Rats given 6 mM silver nitrate rapidly

[24] Calculated by the Panel according to EFSA Scientific Committee (2012).

developed brown-stained muzzles and teeth, but otherwise did not display any effects and were therefore only exposed for 12 weeks. Rats given 24 mM silver nitrate had an initial precipitous decrease in water intake, which rose slightly over the next 5 days and 3 out of 12 rats died. The rest of the animals were poorly groomed, listless and still drinking little, and therefore the study was discontinued in week 2. Animals given 12 mM silver nitrate drank less than controls at the beginning of the study, but returned to the control levels by 5 days. These animals had stained muzzles and teeth, and a slight depression in body weight. No other signs of toxicity were observed for up to 60 weeks. The long-term toxicity group showed a rapid deterioration in their clinical appearance at weeks 76–81; five of these rats recovered slowly upon return to normal drinking water.

AgNPs

In a 28-day study (performed according to the OECD TG 407) on colloidal AgNPs in Sprague–Dawley rats (10/sex/group, 6 weeks old, body weight not properly specified) were administered 0, 30, 300 and 1,000 mg/kg bw/day AgNPs (diameter of 60 nm) by gavage (vehicle: 0.5% carboxy methyl cellulose) (Kim et al., 2008). ALP was increased for male rats in the 300 and 1,000 mg/kg bw/day groups, for female rats only in the high-dose group ($p < 0.01$). Cholesterol was increased in male and female rats in the 1,000 mg/kg bw/day group ($p < 0.01$). Red blood cell count, haemoglobin and haematocrit were increased in female rats in the 300 and 1,000 mg/kg bw/day groups ($p < 0.05$ or < 0.01). Mean corpuscular volume was increased in males at 1,000 mg/kg bw/day ($p < 0.05$). The histopathological examination of the livers showed increased incidences of bile duct hyperplasia around the central vein to the hepatic lobule (dose dependently according to the authors) with infiltration of inflammatory cells, including eosinophils, in the hepatic lobule and in the portal tract. In addition, dilated central veins with infiltration of inflammatory cells were reported in and beneath the central veins (no details or incidences presented in the publication). The authors concluded that exposure to 300 mg AgNPs/kg bw/day and higher may result in slight liver damage. The Panel agreed with that conclusion.

Jeong et al. conducted a histochemical study of intestinal mucins of the rats of the study of Kim et al. 2008 described above. A dose dependent increase in silver nanoparticle accumulation was found in the small and large intestine lamina propria. Silver nanoparticle treated rats displayed increased higher numbers of goblet cells that had released their mucus granules as compared to the controls. The authors suggested that AgNPs induce discharge of mucus granules and abnormal mucus composition in goblet cells (Jeong et al., 2010).

Hadrup et al. (2012a) investigated the toxic potential of AgNPs and ionic silver in rats. Female Wistar rats (4 weeks old) were administered vehicle control, silver acetate (9 mg ionic silver/kg bw/day), or AgNPs (14 nm in diameter; PVP-coated) at 2.25, 4.5 or 9 mg/kg bw/day by gavage for 28 days; males were only given the vehicle control and 9 mg/kg bw/day AgNPs for 28 days. Body weight, macroscopic and microscopic pathology and a range of biochemical and haematological parameters were investigated. In addition, ionic silver led to decreased body weight gain ($p < 0.01$), decreased relative thymus weight ($p < 0.05$), increased plasma ALP ($p < 0.05$) and decreased plasma urea ($p < 0.05$). AgNPs at 9 mg/kg bw/day increased the haematocrit. Both ionic and nanoparticulate silver increased urine uric acid (only statistically significantly for AgNPs $p < 0.001$) and allantoin urine concentration ($p < 0.01$ and 0.001). In an accompanying *in vitro* investigation, AgNPs, ionic silver (silver acetate) and a 12-kDa-filtered subnano silver particle fraction were used to investigate cell death mechanisms in neuronal-like cells; the effect of subnano silver in the silver nanoparticle preparations strongly suggested that the toxic effects of AgNPs were mediated by free ions as toxic effects *in vitro* on viability (including apoptosis) could be explained by the subnano fraction and ionic silver.

Kim et al. (2010) performed a 13-week study (according to the OECD TG 408) on AgNPs in Fischer 344 rats (10/sex/group, 5 weeks of age, mean body weight of males was approximately 100 g, mean body weight of females was approximately 90 g). Rats were administered AgNPs (diameter of 56 nm)

at doses of 0, 30, 125 and 500 mg/kg bw/day by gavage (vehicle: 0.5% carboxy methyl cellulose). Body weight was decreased in high-dose male rats. ALP was increased in females at 500 mg/kg bw/day ($p < 0.01$). A decrease in serum magnesium was found for females at 125 and 500 mg/kg bw/day ($p < 0.01$). A decrease in serum inorganic phosphorus was found for females at 125 and 500 mg/kg bw/day (both with $p < 0.05$). Increased cholesterol was observed for both sexes (for males from 125 mg/kg bw/day, for females only at 500 mg/kg bw/day ($p < 0.01$)). No significant changes in the haematological parameters were noted except for a decreased reticulocyte counts for female rats at 30 mg/ kg bw/day ($p < 0.05$). The histopathological examination of the liver revealed minimal bile duct hyperplasia in 0/10; 4/10; 5/10 and 4/10 of the control, low, mid, and high-dose male rats, respectively and 0/10; 2/10; 2/10 and 2/10 of the control, low, mid and high-dose female rats, respectively. According to the authors, the higher incidence of minimal bile duct hyperplasia, with or without minimal necrosis or fibrosis suggests a treatment-related effect. As these histopathological liver changes are minimal and do not demonstrate a dose-effect relationship, the Panel considered these lesions of doubtful, if any, toxicological relevance.

Summary

Rats given 12 mM silver nitrate in drinking water (118 mg ionic silver/kg bw/day) for 0, 2, 4, 6, 8, 10, 12, 16, 25 and 60 weeks drank less than controls at the beginning of the study, but returned to the control levels by 5 days (Walker 1971). These animals had stained muzzles and teeth and a slight depression in body weight. Hadrup et al. (2012a) observed in rats after oral administration by gavage of silver acetate (9 mg ionic silver/kg bw/day) for 28 days, a decreased body weight gain, decreased thymus weight and increased liver enzymes and decreased plasma urea and allantoin urine concentration. In mice, repeated oral administration of AgNPs (22, 42, 71 nm in diameter, at 1 mg/kg bw/day for 14 days; or 42 nm in diameter, from 0.25 mg/kg bw/day for 28 days) induced effects on liver enzymes. However, no lesions in the liver were observed. Larger silver particles (323 nm in diameter, at 1 mg/kg bw/day for 14 days) did not induce any changes (Park et al., 2010).

In rats, colloidal AgNPs (diameter of 55–60 nm) resulted in slight liver damage (affected enzymes after 28 days at a dose of 300 mg/kg bw/day (Kim et al., 2008) and after 90 days at a dose of 125 mg/kg bw/day (Kim et al., 2010)). No effects were observed at 30 mg/kg bw/day. According to Kim et al. (2008, 2010), the bile duct hyperplasia observed in the liver in the 90-day study may point to a treatment-related effect of AgNPs. The Panel did not agree with this preliminary conclusion, and considered further research needed.

3.2.3. Genotoxicity

No data were submitted to EFSA following public calls for data. The only study evaluated by JECFA (JECFA, 1977), as well as additional studies identified in the literature search are summarised below.

3.2.3.1 *In vitro* studies

Study evaluated by JECFA

Ionic silver

No genotoxic activity of silver chloride was observed in a rec assay using *Bacillus subtilis* strains H17 and M45 (Nishioka, 1975). The Panel noted that this test system has not been validated and considered this study not relevant for risk assessment.

Additional studies, not evaluated by JECFA

Ionic silver

Eliopoulos and Mourelatos (1998) evaluated a suspension of silver iodide (AgI) in polyacrylamide in the Ames test at concentrations from 10 to 150 µg/mL using *Salmonella* Typhimurium strains TA1535, TA102, TA97 and TA98 with and without metabolic activation. No dose-related increase in

revertants was induced by treatment with AgI. A doubling effect on revertants was only observed with 30 µg/mL in TA102 without metabolic activation and at 150 µg/mL in TA97 with metabolic activation, doses which, according to the authors, appear to be nearly toxic for bacteria. Overall, the results of this study are considered negative.

Eliopoulos and Mourelatos (1998) also evaluated AgI, either dissolved in acetone or suspended in polyacrylamide, for the ability to induce sister chromatid exchanges (SCEs) in human cultured lymphocytes *in vitro* at concentrations of 2.3–1,000 ng/mL (acetone solution) or 5–10,000 ng/mL (suspension in polyacrylamide). AgI induced a doubling of SCEs at and above 100 ng/mL when dissolved in acetone, and at and above 1,000 ng/mL when suspended in polyacrilamide.

Foldbjerg et al. (2011) investigated the effects of $AgNO_3$ in the human alveolar cell line A549. Dose-dependent cellular toxicity caused by ionic silver (0.25–10 µg/mL) was demonstrated by the methyltetrazolium (MTT) and annexin V/propidium iodide assays. Treatment with $AgNO_3$ also induced dose-related mitochondrial damage, intracellular ROS and genotoxicity detected as an increase in bulky DNA adducts by ^{32}P postlabelling. Both cytotoxicity and genotoxicity of ionic silver were greatly decreased by pretreatment with the antioxidant *N*-acetyl-cysteine. The Panel noted that the bulky adducts detected showed a similar migration pattern in treated and untreated cells and accumulated in age-dependent way, and that according to the authors such adducts (I-compounds) 'appear to arise via the interaction of DNA with endogenous reactants formed in the course of metabolism, e.g. ROS'.

AgNPs

Ahamed et al. (2008) examined the DNA damage response to AgNPs (diameter 25 nm) in mouse embryonic stem (mES) cells and mouse embryonic fibroblasts (MEF). Exposure of cells to AgNPs (at final concentration of 50 µg/mL) for 4–72 h upregulated p53, the DNA damage repair proteins Rad51 and induced phosphorylation of the histone H2AX and cell death as measured by the annexin V and MTT assays.

Kawata et al. (2009) evaluated the *in vitro* toxicity of AgNPs (7–10 nm) at non-cytotoxic doses (0.1–3.0 mg/L, for 24 h) in human hepatoma cell line, HepG2, based on cell viability assay, micronucleus test and DNA microarray analysis. Silver carbonate (Ag_2CO_3) was also tested to compare the toxic effects of ionic silver and AgNPs. The cell viability assay demonstrated that AgNPs saccelerated cell proliferation at low doses (< 0.5 mg/L), which was supported by the DNA microarray analysis showing significant induction of genes associated with cell cycle progression. At higher doses (> 1.0 mg/L), only AgNPs induced abnormal cellular morphology and increased the frequency of micronucleus formation (up to 47.9 ± 3.2% of binucleated cells), indicating that AgNPs can elicit a much stronger chromosome damage than ionic silver. Cysteine, a strong ionic silver ligand, only partially abolished the formation of micronuclei (MN) mediated by AgNPs, indicating that ionic silver derived from AgNPs could not fully explain the genotoxic activity of AgNPs.

Kim et al. (2010) evaluated the *in vitro* cytotoxicity and genotoxicity of AgNPs (≤ 100 nm) using the trypan blue exclusion assay, the mouse lymphoma thymidine kinase (tk+/-) gene mutation assay (MLA) and the alkaline comet assay in L5178Y mouse lymphoma and BEAS-2B cells. In both cell types, AgNPs were weakly cytotoxic, with IC20 (20% inhibitory concentration) values > 3.7 and 1.7 mg/mL, respectively. Mutant frequencies in nanosilver-treated L5178Y cells (313–2,500 µg/mL) were slightly but not significantly increased compared to the vehicle controls, with and without S-9. In the comet assay (190–3770 µg(mL), significantly increased tail moment were observed in both L5178Y BEAS-2B cells after treatment with AgNPs, with and without S9, indicating that AgNPs can cause primary DNA damage and cytotoxicity, but not mutagenicity, in cultured mammalian cells.

Hackenberg et al. (2011) evaluated AgNPs (< 50 nm) induced DNA damage, cell death and functional impairment in human mesenchymal stem cells (hMSCs). hMSCs were exposed to AgNPs (0.01, 0.1, 1

and 10 µg/mL) for 1, 3 and 24 h. Cytotoxicity was measured by the trypan blue exclusion test and the fluorescein-diacetate test, DNA damage was evaluated by the alkaline comet assay and chromosomal aberration test. Cytokine release of IL-6, IL-8 and vascular endothelial growth factor (VEGF) was detected by ELISA. TEM revealed AgNPs distribution to cytoplasm and nucleus. Cytotoxic effects were seen at concentrations of 10 µg/mL for all test exposure periods. Both comet assay and chromosomal aberration test showed DNA damage after treatment with AgNPs at 0.1 µg/mL and above. A significant increase in IL-6, IL-8 and VEGF release indicated hMSC activation.

Park et al. (2011) investigated potential genotoxicity of AgNPs in a mouse embryonic fibroblasts cell line harbouring a plasmid containing the bacterial *lacZ* reporter gene (MEF-*lacZ*). AgNPs of average nominal diameters of 20, 80 or 113 nm were characterised by TEM analysis and dynamic light scattering (DLS) analysis. However, the results of TEM and DLS analysis were not reported and therefore the actual particle sizes used in the assay is unknown. Particles of the three nominal diameters were mixed with mouse embryonic fibroblast-lacZ cells at concentrations ranging from 0.1 to 50 µg/mL, after treatment DNA extracts, plasmid rescued and the mutation frequency was determined by transfecting plasmids in a competent lacZ deficient *Escherichia coli* strain. No induction in mutation frequency was observed. The Panel noted that this test system has not been validated for hazard identification and that no positive control was included in the study. Consequently, it is not possible to evaluate the sensitivity of the test method applied and the result reported in this study cannot be considered for risk assessment.

Asare et al. (2012) examined the cytotoxic and genotoxic effects of silver particles (12.5, 50 and 100 µg/mL) of nano- (20 nm) and submicron- (200 nm) sized in human testicular embryonic carcinoma cell line (NT2), and primary testicular cells from C57BL6 mice of wild type (WT) and 8-oxoguanine DNA glycosylase knock-out (mOgg1−/−) genotype. The results indicate that both silver nano- and submicron-particles are cytotoxic and cytostatic, causing apoptosis, necrosis and decreased proliferation in a concentration- and time-dependent manner. The 200 nm silver particles, and to a lower extent the 20 nm AgNPs, appeared to cause a concentration-dependent increase in DNA-strand breaks in NT2 cells, whereas this response did not seem to occur in mouse primary testicular cells.

Flower et al. (2012) evaluated the genotoxicity of spherical AgNPs (40–60 nm) in human peripheral blood cells using the alkaline comet assay. Results indicated that AgNPs (50 and 100 µg/mL) caused DNA damage following a 3 h treatment. A significant positive response was also elicited by short-time (5 min) treatment.

Li et al. (2012) investigated the mutagenicity of AgNPs (5 nm in diameter) in the Ames assay at concentrations from 0.15–76.8 µg/plate using *Salmonella* Typhimurium strains TA98, TA100, TA102, TA1535 and TA1537 without metabolic activation. The test was performed according to OECD TG 471 using the pre-incubation method. No increases in mutant frequency over the vehicle control were found in the range of concentrations that could be assayed (2.4–38.4 µg/plate) due to toxicity.

Li et al. (2012) also investigated the genotoxicity of AgNPs (5 nm in diameter) in the *in vitro* micronucleus assay using human lymphoblastoid TK6 cells at concentrations from 10–30 µg/mL. The micronucleus frequency was increased in a dose-dependent manner. At the highest concentration (30 µg/mL the AgNPs induced a significant 3.17-fold increase (with a net increase in 1.60% in micronucleus frequency over the vehicle control), which, according to the criteria of the authors, was a weak positive response.

Ghosh et al. (2012) investigated the genotoxicity of AgNPs in human lymphocytes. AgNPs were characterised by TEM and SEM analysis and the particles in suspension was measured by DLS analysis. The TEM and SEM images revealed average sizes of 125 and 120 nm, respectively. The DLS analysis showed a maximum peak between 420 and 440 nm. The particles were tested in lymphocytes isolated from human blood at concentrations ranging from 25 to 200 µg/mL. DNA

damage was evaluated by comet assay. The results did not show a concentration-related increase. The highest increase was observed at the lowest concentration ($p < 0.05$) and significant increases were also observed at 50 and 200 µg/mL ($p < 0.05$). However, at 100 and 150 µg/mL significant increases were not observed.

Mei et al. (2012) investigated the effect of AgNPs on the mutation rate in mouse lymphoma cells. AgNPs were characterised by TEM analysis and DLS analysis. TEM analysis showed that 66% of the nanoparticles had diameters in the range of 4–8 nm, 24% in the range of 8–12 nm and 6% were above 12 nm. DLS analysis showed agglomerates sizes of particles ranging from 61 nm in water to 1,609 nm in Fischer's cell culture medium. The nanoparticles were added to the L5178Y/$Tk^{+/-}$ mouse lymphoma cell line in concentrations ranging from 3 to 6 µg/mL. Cytotoxicity investigations in a range finding experiment showed that at 3 µg/mL minor cytotoxicity occurred, whereas concentrations higher than 6 µg/mL induced moderate cytotoxicity. To investigate the genotoxicity, the mouse lymphoma forward mutation $Tk^{+/-}$ assay and the comet assay (both with and without the lesion-specific endonucleases) were performed. Treatments of L5178Y/$Tk^{+/-}$ mouse lymphoma cells resulted in a significant yield of mutants at concentrations between 3 and 6 µg/mL. Molecular analysis of induced mutants displayed both in small and large mutant colonies that mutant phenotype was associated with partial loss of heterozygosity of chromosome 11, suggestive of induced structural chromosome damage. In the comet assay, statistically effects ($p < 0.01$) were only observed in the presence of the lesion-specific endonucleases (at 4.5 µg/mL and above). Treatment with AgNPs also proved to modify the expression of genes involved in the production of ROS, oxidative stress, antioxidants and DNA repair, suggesting that the observed genotoxic effects were due to AgNPs-induced oxidative stress.

Li et al. (2013) investigated the genotoxic effect of AgNPs in primary Syrian hamster embryo cells. AgNPs dissolved in cell culture media determined by DLS analysis showed a hydrodynamic size distribution with a peak at 100 nm and 69% of the number concentration was below 100 nm. Cytotoxicity was determined by the MTT assay. A reduction in cell viability was observed between 25 and 67% in the concentration range 2.5–40 µg/mL. The genotoxic potential was investigated using a cytokinesis-block micronucleus assay. Syrian Hamster Embryo cells were tested in two concentrations of 20 or 40 µg/mL. Statistically significant ($p < 0.001$) increases in MN were recorded for both concentrations.

Kim et al. (2013) investigated the genotoxic effect of AgNPs in Ames test, by comet assay and by the micronucleus assay in Chinese hamster ovary cells. The particles were characterised by SEM, TEM and the hydrodynamic size distribution of the particles in aqueous suspension was determined with DLS analysis. In scanning electron microscopy (SEM) and TEM, the single particle size was 100 nm or less. The DLS analysis showed that about 50% of the particles were in the range of 40–59 nm. In the *Salmonella* Typhimurium assay the strains TA98, TA100, TA1535 and TA1537 were used at concentrations ranging from 100 to 500 µg/plate, with or without rat S9 liver fraction. No effects were observed in the Ames test. Comet assay and micronucleus assay were conducted in Chinese hamster ovary cells with concentrations ranging from 0.01 to 10 µg/mL. Cytotoxicity measurements were not conducted in connection with the comet assay. Therefore, the cytotoxicity is unknown of the concentrations applied in the comet assay. AgNPs induced (statistical significant, $p < 0.01$) DNA damage at all concentrations tested. Micronucleus formation was increased (statistically significant, $p < 0.05$) at doses ranging from 0.1 to 10 µg/mL.

Kruszewski et al. (2013) investigated the effects of AgNPs (20 or 200 nm in diameter) on DNA damage in human cell lines. After 2 hours incubation, 20 nm particles were agglomerated to 87–135 nm agglomerates. The 200 nm particles were agglomerated to 212–271 nm. The two sizes of particles were added to HEPG2, HT29 or A549 human cell lines in concentrations ranging from 10 to 100 µg/mL. DNA damage was measured with comet assay, oxidative base damage was recognised by formamido-pyrimidine glycosylase (FPG) and estimated by use of the FPG + comet assay and frequencies of histone H2AX foci and MN. No effects were observed on the frequency of histone

H2AX foci and induction of MN. Effects were observed in the comet assay both with and without FPG enzyme at concentrations of 10 µg/mL and above.

Karlsson et al. (2014) used a recently developed reporter assay based on mouse embryonic stem (mES) cells that uses GFP (green fluorescent protein)-tagged biomarkers (ToxTracker) for detection of DNA damage, oxidative stress and general cellular stress upon exposure to AgNPs (10 and 40 nm average size). In addition, the conventional alkaline comet assay (with and without FPG glycosylase for oxidative DNA lesions) was carried out. In the experimental condition of this study, AgNPs (5–50 µg/mL) were negative in comet assays and did not elicit neither DNA replication stress nor oxidative stress of p53-associated cellular stress.

Sahu et al. (2014) evaluated the genotoxicity of AgNPs in the human hepatoma HepG2 and human colon carcinoma Caco2 cells using the cytokinesis block-micronucleus assay with acridine orange staining and fluorescence microscopy. Cells were treated for 4 and 24 h with aliquots of a standard solution of citrate AgNPs (0.962 mg Ag/mL) at the final concentrations of 0.5–15 µg/mL. Average diameter of nanoparticles, determined by TEM, was 20.4 nm. A statistically significant increase in MN was observed in both cell types after 4 h exposure to 10 and 15 µg AgNPs/mL; after 24 h exposure significantly increased frequencies of cells with MN were observed at 0.5 µg/mL and above in HepG2 cells, and at the top dose of 15 µg/mL in Caco2 cells. In the range of doses applied, treatments did not elicit any significant cytotoxic/cytostatic effect, as measured by the cytokinesis-block proliferation index.

Vecchio et al. (2014) used a high-throughput screening platform based on the cytokinesis-block micronucleus assay, on-chip cell sorting, and automated image analysis to evaluate the cytotoxic and genotoxic effects of AgNPs of different size (10 and 70 nm) in primary human lymphocytes. Data show a significant genotoxic activity (induction of MN) with all AgNPs at the highest tested dose of 10 µg/mL, while the lower doses of 0.1 and 1 µg/mL were ineffective. AgNPs-induced genotoxicity was in part lymphocyte subtype dependent, with most pronounced response in CD2+ and CD4+ cells.

Butler et al. (2015) investigated how physico-chemical properties of AgNPs affect their cellular uptake and genotoxicity. To this aim, AgNPs of different size (10, 20, 50 and 100 nm) and silver nitrate (AgNO3) were tested for mutagenicity (Ames test), clastogenicity and primary DNA damage (in flow cytometry-based micronucleus test and comet assay in human monocyte and T cell lines). Cellular uptake concurrently evaluated by TEM. AgNPs of all tested sizes, as well as silver nitrate, were negative for mutagenicity in bacteria, which included strains sensitive to oxidative DNA damage (*E. coli* WP2 and *S.* Typhimurium TA102). No bacterial uptake of AgNPs could be identified by TEM. However, as AgNO3 either was not mutagenic in the Ames test, the lack of bacterial uptake of the AgNPs may not be the major reason for the lack of genotoxicity observed. On the other hand, in tests in mammalian cells, micronucleus and comet assay end points were inversely correlated with AgNPs size, with smaller NPs inducing a more distinct genotoxic response. The same genotoxic effects were also induced, with relatively higher efficiency, by silver nitrate. TEM results indicated that AgNPs were confined within intracellular vesicles of mammalian cells and did not penetrate the nucleus. These results suggest that silver ions may be the primary, and perhaps only, cause of genotoxicity elicited by AgNPs in mammalian cells.

3.2.3.2 *In vivo* studies

Additional studies, not evaluated by JECFA

Ionic silver

Eliopoulos and Mourelatos (1998) evaluated AgI for the ability to induce SCEs in P388 lymphocytic leukaemia cells cultured in the mouse peritoneal cavity at doses up to 100 mg AgI/kg bw. No induction of SCEs was observed. The Panel noted that this methodology, which recalls a host-

mediated assay, has not been validated nor further used to genotoxicity assessment, and considered the results of this study of limited or no relevance.

AgNPs

Kim et al (2008) tested AgNPs (average diameter 60 nm) within a 28-day oral toxicity study in rats. Sprague–Dawley rats (10/sex per group) were treated by gavage with daily doses of 30, 300 or 1,000 mg/kg AgNPs suspended in 0.5% carboxymethylcellulose. No increase in micronucleated polychromatic erythrocytes (PCEs), and no deviation of the poly/normochromatic erythrocyte ratio (PCE/NCE) was observed at sacrifice in treated rats compared to controls receiving the vehicle alone. According to the authors, the results suggested that the AgNPs did not induce genetic toxicity in male and female rat bone marrow *in vivo*. The Panel noted that no indication of exposure of bone marrow to the test material, as shown by the altered PCE/NCE ratio, is provided in this study. The Panel also noted that data on tissue distribution generated within the same study indicate a dose-dependent accumulation of silver in several tissues (kidney, liver, lungs, brain, stomach), whereas blood concentration of silver was only minimally elevated. Overall, the Panel concluded that the negative results obtained in this study are insufficient to rule out a genotoxic concern.

Ordzhonikidze et al. (2009) evaluated the toxic and genotoxic effects of AgNPs (size 9±6 nm) in BALB/c mice injected intraperitoneally (i.p.). The effect of the AgNPs was compared to those of the anionic surfactant (AOT), used as AgNPs stabiliser and silver nitrates. Acute toxicity of tested material decreases in the sequence AgNPs>AOT>>AgNO3. Genotoxic effects were assessed by the abnormal sperm heads test and neutral comet assay in splenocytes. The frequencies of abnormal sperm heads, evaluated 21 days after treatment, was similar in mice injected with AgNPs (1.6 mg ionic silver /L) and AOT (5mM), but higher (about 1.5-fold) than in control mice. At the same doses, corresponding to ½ LD50, comet assay showed an increase in the DNA percentage in the comet tail in spleen cells of mice injected with both AgNPs and AOT. However, the Panel noted that the sperm head abnormality test is not a genotoxicity end-point, as sperm morphology can also be affected by cytotoxicity. The Panel also noted a number of inconsistencies and shortcomings in the comet assay, performed with an inadequate (neutral) protocol, without a positive control and with inappropriate study design, averaging data from 14 mice sacrificed at seven different time points (two per point). Overall, the Panel concluded that this study cannot be considered for risk assessment.

Ghosh et al. (2012) investigated the genotoxicity of AgNPs in bone marrow cells of mice. AgNPs of 120 nm in diameter were administered intraperitoneally to Swiss albino male mice (8–12 weeks old, weight 25–30 g). There were six groups of five male mice each. The following groups were investigated: 1) negative control, 2) positive control (i.p. injection of mitomycin), 3) positive control (i.p. cyclophosphamide), groups 4) to 7) were single i.p. injection of AgNPs at 10, 20, 40 and 80 mg/kg bw, respectively. The animals were euthanised after 18 h of exposure. Then the chromosome aberration test and the comet assay were performed. Results were as follows: A statistically significant increase in chromosomal aberrations (mainly chromatid breaks) in bone marrow cells were found with all doses of AgNPs ($p < 0.05$). DNA damage, as measured by the comet assay, was statistically significantly ($p < 0.05$) increased but only in the two lowest doses of 10 and 20 mg AgNP/kg bw groups and not in the 40 and 80 mg AgNP/kg bw groups. ROS generation in bone marrow was also quantified by flow cytometry: according to the authors the results obtained indicate significant ROS generation following treatment with 10 and 20 mg AgNP/kg bw, whereas ROS generation at the subsequent concentrations was negligible and comparable to control (data not shown). The Panel noted that as described the particles appeared to be out the size range defined as nanoparticles in the EFSA Guidance (EFSA, 2011), and furthermore the route of exposure used in this study may have limited relevance for the assessment of the *in vivo* genotoxic hazard *in vivo* associated with oral intake of AgNPs.

Gromadzka-Ostrowska et al. (2012) injected male Wistar rats, 24 animals/group, (age 14 weeks, body weight 308.1 $\pm$ 22.4 g) via the tail vein with a single dose (5 mg/kg bw or 10 mg/kg bw) of 20 nm AgNPs (Groups AG I and AG II) or with 5 mg/kg bw of 200 nm silver particles (group Ag III). A

control group was injected with 0.9% NaCl solution. Animals were sacrificed 24 h, 7 days and 28 days after injection. Epididymal sperm count, sperm morphology, sperm cell DNA damage (using the comet assay) and histopathological examination of the testis were performed. No differences in body weight, food and water consumption were observed. Epididymis weight and testis weight were comparable among the groups. Epididymal sperm count was decreased in the AG I group after 24 h and 28 days when compare to the control. The frequency of abnormal sperm was comparable in the treated and the control groups. The comet assay showed that DNA damage (% DNA in the tail in the germ cells was increased at 24 hours in the AG I and Ag II groups, then decreased after 7 and 28 days. No difference was found for the AG III group. Histopathological examination showed effects (differences of the seminiferous tubule morphology, wider intercellular spaces and higher vacuolisation of the germinal epithelium) in the testis of the animals of the AG III group. The Panel noted that the application of the comet assay to germ cells is complicated by a number of technical and theoretical considerations, and that its use for regulatory purposes has been not recommended (MacGregor, 2015).

Dobrzynska et al. (2014) injected intravenously male Wistar rats (7 per group) with 5 or 10 mg/kg bw spherical AgNPs (average diameter 20 nm) or 5 mg/kg bw Ag spherical microparticles (average diameter 200 nm). Animals were sacrificed 24 hrs, 1 week and 4 weeks later, and genotoxicity evaluated in bone marrow cells by comet and micronucleus assays. No genotoxicity was detected in bone marrow cells by comet assays at any sampling time. A significant (two/three-fold) increase in micronucleated polychromatic erythrocytes (PCE), stained with the conventional May-Gruenwald and Giemsa stains, was reported in all treated groups sacrificed 24 h and 1 week after treatment, and also after 4 weeks for the high-dose nanoparticles group. In the same treated animals, no increase in the number of MN in bone marrow reticulocytes stained with acridine orange was observed. The Panel noted that polychromatic erythrocytes and reticulocytes represent the same cell type, i.e. immature erythrocytes detected with different staining procedures, and thus, there is no reason for the divergent results reported. Moreover the Panel noted that the approach followed up for the statistical analysis of results was incorrect, considering cells rather than animals as statistical units, while data show a large inter-animal variability (CV of 1.7 among solvent controls). This raises doubts on the biological significance of the positive result reported. Overall, the Panel concluded that the results of this study should not be considered for risk assessment.

El Mahdy et al. (2014) injected i.p. mature female albino rats (5 per groups) with 1, 2 and 4 mg/kg bw AgNPs (8.7 nm) daily for 28 days. At the end of treatment chromosomal aberrations were scored in 50 bone marrow cells per animal. A statistically significant increase in structural chromosomal aberrations was reported in treated animals compared to controls receiving the vehicle (distilled water) alone. The Panel noted that 'centromeric attenuations' were the most frequent alteration observed and included in the computations of structural chromosomal aberration, whereas 'centromeric attenuation' consists in a discolouration of the centromeric region with unknown biological significance. The only other aberrations recorded were chromatid deletions which increased in treated animals with no clear relation with dose (1, 3, 7 and 4 deletions in control, low, middle and high dose, respectively). The Panel also noted that such findings were provided by the scoring of just 50 cells per animal, whereas OECD Guideline 475 recommends to score at least 200 metaphases per animal. Based on these concerns, the Panel considered the results of this study as inconclusive.

Patlolla et al. (2015) evaluated the hepatotoxic and genotoxic effects elicited by oral administration of AgNPs to rats. AgNPs (10 nm diameter), suspended in deionised water, were given by gavage to groups of five adult male Sprague–Dawley rats at 5, 25, 50 or 100 mg/kg bw once a day for 5 days. Animals were sacrificed 24 h after last treatment, blood collected and liver excised for the analysis of the following biomarkers: i) liver function enzymes (ALT/glutamic-pyruvate transaminase (GPT), AST/glutamic oxaloacetic transaminase (GOT), ALP) in serum; ii) reactive oxygen species (ROS) and lipid hydroperoxide (LHP) in liver homogenate; iii) DNA damage in liver by alkaline comet assay; iv) liver histopathology. The results obtained show a dose-related increase in serum markers of altered liver function, as well as ROS and LHP generation in liver homogenate. The increases reached

statistical significance at the two highest dose levels (50 and 100 mg/kg bw per day). Histopathological examination of liver tissue highlighted a dose-related increase in frequency and severity of morphological alterations, which were severe at the highest dose displaying central vein damage, hepatocellular vacuolisation, necrosis and pycknosis. A dose-related increase in percentage tail DNA, which attained statistical significance at 50 and 100 mg/kg bw per day, and was also observed in comet assays with liver homogenates. The authors concluded that oral administration of high doses of AgNPs caused oxidative stress, DNA damage and hepatotoxicity in rats. The Panel agreed with this conclusion. However, concerning genotoxicity, the Panel noted that no concurrent evaluation of cell survival was performed in comet assay, and that heavily damaged cells indicative of cell toxicity, the so-called hedgehogs, were not recorded separately as recommended. Therefore, according to the OECD TG 489 recommendation, the Panel concluded that the positive results reported in this study in association with overt organ toxicity should be evaluated with caution for genotoxic risk assessment. The Panel also noted that, according to the results reported, an extensive release of Ag ions (35–70%) occurred when AgNPs were disperse in deionised water, and thus considered that ionic silver may have contributed to the adverse effects reported in this study.

Summary

The limited information available, indicate that ionic silver is non-mutagenic in bacteria but genotoxic and clastogenic in mammalian cells *in vitro* (Butler et al., 2015). No information is available on the genotoxic potential of ionic silver *in vivo*.

Concerning AgNPs, negative results were obtained in mutation tests in bacteria (Li et al., 2012; Kim et al., 2013; Butler et al., 2015), but positive results have been reported in the majority of *in vitro* studies performed in mammalian cells. In these studies, AgNPs induced MN in human (Li et al., 2012; Vecchio et al., 2014; Sahu et al., 2014; Butler et al., 2015) and rodent cells (Kawata, 2009; Li et al., 2013; Kim et al., 2013), DNA lesions detectable by comet assays, optimised for the detection of oxidative damage, in a variety of human (Kawata et al., 2009; Hackenberg et al., 2011; Asare et al., 2012; Flower et al., 2012; Kruszewski et al., 2013; Butler et al., 2015) and rodent (Ahamed et al., 2008; Kim et al., 2010; 2013; Mei et al., 2012) cell lines and gene mutations in mouse lymphoma cells (Mei et al., 2012). Data suggest that the release of silver ions from nanoparticles can contribute or even entirely account for the *in vitro* genotoxicity of AgNPs (Kawata et al., 2009; Butler et al., 2015).

Fewer studies have investigated the *in vivo* genotoxic potential of AgNPs. No induction of MN was observed in rat bone marrow after oral exposure of 30 to 1,000 mg/kg bw for 28 days, with no proof of bone marrow exposure (Kim et al., 2008) whereas oxidative stress and DNA damage was observed in another oral study in rats (Patlolla et al., 2015), but the Panel considered these findings not conclusive. Chromosomal aberrations were induced in bone marrow cells of mice after intraperitoneal exposure (Ghosh et al., 2012), but the Panel noted that the i.p. route of administration is not relevant for the evaluation of risk from oral exposure. Other *in vivo* studies provided inconclusive or unreliable results.

In conclusion, there are no data available to evaluate the *in vivo* genotoxicity of ionic silver. Concerning AgNPs, the available studies provide clear evidence of a genotoxic potential in various *in vitro* test systems. The *in vivo* oral genotoxicity studies performed provide less conclusive evidence, and do not allow a definitive assessment of the possible genotoxic hazard associated with oral exposure to AgNPs.

Overall, the Panel concluded that the available data are inadequate to evaluate the genotoxic hazard associated with the use of silver as food additive.

3.2.4. Chronic toxicity and carcinogenicity

No data were submitted to EFSA following a public call for data. The two studies evaluated by JECFA (JECFA, 1977) investigated the occurrence of tumours following implantation of foil, platelets and pellets of silver or dental alloy under the skin of mice and rats; these studies are not considered of relevance for the evaluation of silver as a food additive. The studies identified in the literature search are summarised below.

Additional studies, not evaluated by JECFA

Ionic silver

Olcott (1948) has studied the effects of orally administered silver nitrate and silver chloride in rats (number and sex not further specified). Various concentrations of the silver salts were assessed. When rats were given a concentration of 1% of the silver salts in the drinking water, they did not survive (equivalent to 317 and 375 mg ionic silver/kg bw/day for silver nitrate and silver chloride, respectively[25]). With a concentration of 0.4% of the silver salts in the drinking water two rats were kept alive for 500 days (equivalent to 127 and 150 mg ionic silver/kg bw/day for silver nitrate and silver chloride, respectively[25]). When given a 1:1000 dilution of the silver salts in about 1:300 sodium thiosulphate for their lifetime (equivalent to 32 and 38 mg ionic silver/kg bw/day for silver nitrate and silver chloride, respectively[25]) the life span of the rats was not shortened. A range of organs was investigated; left heart ventricle hypertrophy was the only finding reported. The study could not be used for risk assessment as the reporting was limited.

Forty weanling male Wistar rats (age and body weight not specified further) were administered a 0.25 % silver nitrate solution via the drinking water for up to 8.5 months (equivalent to 79 mg ionic silver/kg bw/day[25]). After 10 weeks, two rats were euthanised and the eyes were processed for electron microscopy. The remaining rats were divided into two groups: 1) A group that continued with the same solution for the next 6 months and 2) a group that was shifted to water for 6 months. A slightly lower rate of body weight gain was reported until about 23 weeks after the start and at this point some animals began to loose weight and eventually died. The group of rats that had withdrawal of silver nitrate regained their body weight (Matuk et al., 1981).

Overall, no studies on the carcinogenic potential of either ionic silver compounds or AgNPs have been identified. In rats, retarded growth and stained muzzles were the only effects reported following long-term exposure to ionic silver (up to 8.5 months approximately 81 mg ionic silver/kg bw/day, (Matuk et al., 1981) and 60 weeks, approximately 118 mg ionic silver/kg bw/day (Walker 1971)).

3.2.5. Reproductive and developmental toxicity

No data were submitted to EFSA following a public call for data. No studies were evaluated by JECFA (JECFA, 1977). Additional studies identified in the literature search are summarised below.

Additional studies, not evaluated by JECFA

Ionic silver

Reproductive toxicity

The toxicity of silver acetate (purity 99%) was studied in Sprague–Dawley rats (n=20/sex per group) when administered in drinking water in an one-generation reproduction and fertility test (Documentation provided to EFSA No5). Silver acetate was given in the drinking water at dose levels resulting in administration of 0, 0.4, 4 or 40 mg silver acetate/kg bw/day (0, 0.26, 2.6 or 26 mg ionic silver/kg bw/day). Parental male animals were exposed 10 weeks prior to mating and parental female

[25] Calculated by the Panel according to EFSA Scientific Committee (2012).

animals for 2 weeks prior to mating. The F1-pups were sacrificed on postnatal day (PND) 26. Special attention was given to the thymic development.

No clinical signs, changes in body weight and food intake were observed during the study. However, a reduction was observed in fluid consumption of the male and female rats of the high-dose group during the premating period. The author considered this as a consequence of taste aversion. Fluid consumption was also decreased in the high-dose group during gestation and in all silver acetate groups during lactation.

The fertility was decreased in the high-dose group compared to the control and the low- and mid-dose group. The number of pups born alive was decreased in the high-dose group. Reduced pup survival observed in the mid-dose group compared to the control group was not observed in the other dose groups. Data suggested that in the high-dose group pups loss occurred during gestation and early lactation and in the mid-dose group at a later time point. Pup loss (PND 4–21) was decreased in the high-dose group compared to the control, low- and mid-dose groups.

Pup weights of the mid-dose group were decreased on PND 0, 4, 7 and 21 (on PND 21 not statistically significantly) and the number of runts in this group was increased on PND 4, 7 and 21. The author stated that it appeared that the F1-pups from the high-dose group that were sensitive to silver acetate exposure died early but that those that survived recovered by PND 21. Furthermore, they stated that data suggest that the growth rate in F1-female pups of this group that were sensitive to silver acetate did not return to control values by PND 26.

At necropsy of the parental animals and F1 pups on PND 26, no treatment-related effects were observed on organ weight (relative to brain weight) of the thymus, spleen, heart, kidney, liver, ovaries, testes, epididymides, apart from the decreased stomach weight in the parental females and the male and female F1 pups of the high-dose group and the kidney weight of the F1 pups of the mid-dose group.

The only effect found in the clinical chemistry of the parental animals was an elevated serum glucose level in the high-dose group. No adverse effects were observed in the female F1 pups.

In male F1 pups, the following effects were observed on clinical chemistry: a decrease in blood urea nitrogen (BUN) in the all silver acetate treated groups; increase in serum glucose in the high-dose group; increase in serum calcium in the low- and mid-dose group but not in the high-dose group and a decrease in the BUN/creatinine ratio in the mid- and high-dose group.

Exposure to silver in drinking water caused deposition in a number of tissues of the parental (F0) animals but did not cause any significant histopathological changes. No gross effects or histopathological changes were observed in the F1 pups (exposed *in utero* and during lactation) on PND 26.

Based on the presence of runts, pup death and delayed pup growth the authors considered that 0.4 mg silver acetate/kg bw/day (0.26 mg ionic silver/kg bw/day) was the NOAEL for this study. The Panel agreed with this conclusion.

Developmental toxicity

Rats

Shavlovski et al. (1995) investigated the role of ceruloplasmin in the transport of copper when embryotoxicity of silver chloride was induced in rats. Inbred albino female rats (body weight 180–200 g, age and strain not specified further) were given 50 mg silver chloride/animal per day in the feed (with a body weight of 200 g this corresponds to 250 mg silver chloride/kg bw/day corresponding to 188 mg ionic silver/kg bw/day). The rats were exposed from gestation day (GD) 7–15 (five pregnant animals/group) or GD 1–20 (20 pregnant animals/group). On GD 20, animals were euthanised. The

number of live and dead fetuses, as well as malformations was recorded and corpora lutea were counted. Fetuses were weighed and the conditions of their organs were assessed. A range of biochemical parameters were measured in the tissues. For dams exposed from GD 1–20, the postimplantation loss was 36% ($p < 0.001$), fetal weight was decreased ($p < 0.001$) and the number of fetuses having visceral aberrations was considerably higher than in the control group. In addition, the new-born animals all died within the first 24 h after birth. The Panel noted that Shalovski designed this study to investigate the role of ceruloplasmin but standard parameters (body weight, feed consumption) and dose-response assessments were not included. Only one high dose was tested for which no maternal effects were observed after exposure from GD 6 to 15 and exposure from GD 1 to 20. However, it may be assumed that the absence of any detectable copper carrying, enzymatic active ceruloplasmin in the blood and an absence of detectable copper in the serum in the dams and in the fetuses and placenta is the cause of the observed developmental effects.

Silver acetate was administered daily to mated Sprague–Dawley rats (25 animals/group) by gavage from GD 6 to 19 at doses of 0, 10, 30 or 100 mg/kg per day (vehicle 1% aqueous methylcellulose) (NTP, 2002). Silver administration was equivalent to 6.5, 19.4 or 65 mg ionic silver/kg bw/day. Females were sacrificed on GD 20 followed by a full fetal pathological examination. One animal was removed from the high-dose group due to a misdosing and one confirmed pregnant female in the high-dose group was euthanised on GD12 due to morbidity. Treatment-related clinical signs were noted primarily in the mid- and high-dose groups and consisted of weight loss, rooting after dosing and piloerection. A significant ($p < 0.05$) decreasing linear trend was noted for maternal body weight on GD 12, but there were no statistically significant differences between the control group and any treated group. Feed and water consumption did not exhibit dose-related differences between the control group and silver acetate-treated group. There were no differences in the number of corpora lutea and number of implantations. Postimplantation loss, number of live and dead fetuses, and the sex ratio did not differ among groups. An increasing trend was observed for the percent litters with late fetal deaths. Average fetal body weight/litter (sexes combined) and average male fetal body weight/litter exhibited a significant decreasing trend, but no significant pairwise differences between treated groups and the control group. No statistically significant effects were noted for average female body weight. No toxicologically relevant differences were observed in the incidences of fetal malformations or variations. The authors noted that the maternal NOAEL was 10 mg silver acetate/kg bw/day (equivalent to 6.5 mg ionic silver/kg bw/day) based on the clinical signs including weight loss and considered the NOAEL for developmental toxicity to be 100 mg silver acetate (equivalent to 65 mg ionic silver/kg bw/day). The Panel agreed with this conclusion.

Summary

Overall, in an oral one-generation reproductive toxicity study with silver acetate in drinking water at dose levels of 0, 0.4, 4 or 40 mg silver acetate/kg bw/day (0, 0.26, 2.6 or 26 mg ionic silver/kg bw/day) in rats, a NOAEL for developmental effects (increased number of pups, pup death and decreased weight gain of pups) of 0.4 mg silver acetate/kg bw/day (0.26 mg ionic silver/kg bw/day) was observed (Documentation provided to EFSA No5). The NOAEL for fertility was 4 mg silver acetate/kg bw/day (2.6 mg ionic silver/kg bw/day).

In a prenatal developmental toxicity study, developmental toxicity of ionic silver was observed when rats were dosed with silver chloride (188 mg ionic silver/kg bw/day) on GD 1–20 (Shavlovski et al., 1995). No developmental effect was observed by the same authors when rats were only dosed with silver chloride from GD 7–15. This study was only conducted at one-dose level in a low number of animals and maternal toxicity was not described properly. The effects on ceruloplasmin after longer administration were emphasised by the authors. The Panel noted that the study was performed with a high dose.

In another prenatal developmental study (NTP, 2002) with silver acetate performed according the current guidelines at dose levels of up to 100 mg/kg bw/day (65 mg ionic silver/kg bw/day)

administered from GD 6–19, a NAOEL for developmental toxicity was observed at 65 mg ionic silver/kg bw/day as the NOAEL for maternal toxicity was 6.5 mg ionic silver/kg bw/day.

The Panel noted that silver ions affected developmental toxicity at a much lower level (NOAEL 0.26 mg ionic silver/kg bw/day) in the one-generation reproductive toxicity study.

3.2.6. Hypersensitivity, allergenicity, intolerance

3.2.6.1 Allergy

The Panel noted that reports of people suffering from silver allergy after exposure to silver (mostly in jewels or dental amalgams) are usually confounded by the simultaneous presence of nickel, a known sensitising metal. These observations are not relevant to the safety assessment of silver as a food additive.

3.2.6.2 Immunotoxicity

Several recent studies *in vitro* and *in vivo* in mice and rats, have reported that administration of AgNPs induces various immunotoxic effects (Lappas, 2015).

In mice treated orally Park et al. (2010) (study design described in Section 3.2.2.1) for 14 days with AgNPs (22 nm, 42 nm and 71 nm; suspended in 0.5% carboxy methyl cellulose), several alterations in immunological parameters were reported. Cytokines including IL-1, IL-6, IL-4, IL-10, IL-12 and TGF-α were increased in a dose-dependent manner by repeated oral administration. In addition, B cell distribution in lymphocyte and IgE production were increased. Based on these results, the authors suggested that repeated oral administration of AgNPs may cause organ toxicity and inflammatory responses in mice.

Van der Zande et al. (2012) investigated the immunotoxicity of AgNPs and silver ions in rats. Male Sprague–Dawley rats (5/group, 6 weeks old, start body weight about 245 g) were exposed daily by oral gavage for 28 days to 90 mg/kg bw of AgNPs (18 nm, non-coated or 12 nm, PVP-coated, in diameter) or 9 mg/kg bw of silver nitrate (corresponding to 6 mg ionic silver/kg bw). Included were also wash-out groups identically exposed to silver but not euthanised until day 36 or day 84. Immunotoxicity was evaluated by testing the proliferation of T- and B-cells isolated from spleen and mesenteric lymph nodes in response to lipopolysaccharides (LPS) or Concanavalin A. Cytokine levels in culture media from these proliferating T- and B-cells, and the activity of natural killer (NK)-cells isolated from the spleen were also measured. Finally, antibody levels in blood were evaluated. No immunotoxicity was detected.

After oral exposure in drinking water of rats to silver acetate (Ag-Ac) 0, 0.4, 4 and 40 mg/kg bw/day (0, 0.26, 2.6 or 26 mg ionic silver/kg bw/day) as described in Section 3.2.5 (Documentation provided to EFSA No5), splenic and thymic lymphocyte subsets from postnatal (PN) 4- and 26-day-old pups were assessed by flow cytometry for changes in phenotypic markers. Functional indices included natural killer (NK) activity and mitogen-induced lymphocyte proliferation. Spleens from PN 4-day pups had lower percentages of CD8+ lymphocytes in 4 and 40 Ag-Ac groups and reduced Concanavalin A response in all three Ag-Ac groups. Changes in phenotypic markers in splenocytes from PN 26-day pups included significantly lower TCR+ cells in rats fed 4 and 40 mg Ag-Ac and higher B cell population in those that were fed 40 mg Ag-Ac. In conclusion, maternal exposure to Ag-Ac had a significant impact on rat splenic development especially in the early lactation period, but there was no impact on thymic development. The Panel noted that the immunotoxic effects reported mostly pointed to an effect of silver on the phenotype and maturation of the developmental splenic T cell population. The Panel also noted that at the lowest dose administered of 0.4 mg silver acetate, a reduced Concanavalin A response was observed. This dose corresponds to a lowest-observed-adverse-effect level (LOAEL) of 0.26 mg ionic silver/kg bw/day.

Małaczewska (2014) reported that 28-day oral administration to mice of different doses (0.25, 2.5, 25 mg/kg diet equivalent to 0.05, 0.5 and 5 mg/kg bw/day[26]) of silver nanocolloid (10–20 nm) decreased the counts of monocytes in the animals' blood and induced an increase in CD4+/CD8+ T cell distribution, a decrease in NK and NKT cell distribution (doses of 0.5 and 5 mg/kg bw/day) and an increased CD4+:CD8+ ratio (5 mg/kg bw/day). Silver nanocolloid also affected the activity of cells, depressing the proliferation of lymphocytes at the lowest dose tested (0.05 mg/kg bw/day diet) and stimulating phagocytosis as well as the respiratory burst of granulocytes and monocytes (all doses).

Hamilton et al. (2014) studied the sensitivity of a variety of macrophage and epithelial cell lines to 20 nm and 110 nm AgNPs. They reported that 20 nm nanoparticles were more toxic to macrophages and epithelial cells than were 110 nm nanoparticles. According to the authors, this could be due to the more rapid dissolution of the smaller particles in acidic phagolysosomes, which is consistent with silver ion mediated toxicity.

Haase et al. (2014) has reported that the effects of AgNPs and ionic silver on neutrophils and macrophages were similar; both triggered the release of neutrophil extracellular traps and inhibited the formation of nitric monoxide and protein phosphatase activity, and induced increased intracellular levels of ROS.

The Panel noted that the outcomes of all these studies were inconsistent. This could be due to different material, doses, duration of exposure, and animal models used but overall the Panel considered that ionic silver and AgNPs may have an effect on the immune system.

3.2.7. Other studies

3.2.7.1 Animals

Ionic silver

In rats (6/group) given drinking water containing 0.5, 2 or 20 mg Ag/L (No further details regarding the silver compound, except that the description in the JECFA evaluation indicate that silver was administered as a soluble salt, i.e. ionic silver) for 6–12 months, the nucleic acid level in brain and liver was decreased after 1 year at 2 mg Ag/L. At 20 mg Ag/L, the RNA and DNA contents of the brain were increased after 6 months and dystrophic changes in the brain accompanied by a decrease in nucleic acid level were observed after 12 months. The liver was less sensitive towards silver than the brain (Kharchenko et al., 1973, cited in JECFA, 1977).

In rabbits (8/group) administered 0, 0.00025, 0.0023, 0.025 or 0.25 mg Ag/kg (No further details regarding the silver compound; the unit is probably mg Ag/kg bw) via their drinking water for 11 months *marked effects on immunological capacity (measured as phagocytosis)* and histopathological changes of nervous, vascular and glial tissue of the encephalon and medulla were observed at the two highest dose levels (0.025 and 0.25 mg Ag/kg bw). No effects on the haemoglobin concentration, red blood cell count, differential white blood cell count, *proteinogenic function of the liver* and serum sulfhydryl (SH) were noted. *Rats treated with same amounts of silver showed affected conditioned reflexes* (Barkov and El piner, 1968, cited in JECFA, 1977).

Rungby and Danscher (1984) investigated the potential effect of silver on behaviour of animals. Female NMRI mice (20 animals, 60 days old at the beginning of the experiment, body weight not reported) had their drinking water replaced by a 0.015% silver nitrate solution for 125 days (equivalent to 14 mg ionic silver/kg bw/day[26]). Thereafter, the mice were given normal drinking water again. Twenty non-exposed females served as controls. Ten days after termination of silver administration, the mice were tested with regard to activity levels. The silver exposed mice were found to be hypoactive as measured by open field behaviour in the cage.

[26] Calculated by the Panel according to EFSA Scientific Committee (2012).

Ionic silver/ AgNPs

Mice

AgNPs (3–20 nm, not further specified) were dosed to Swiss albino mice by gavage at doses of 0, 5, 10, 15 and 20 mg/kg bw/day for 21 days (Shahare and Yashpal, 2013). Body weight was decreased in all dose groups. The weight loss was the highest in the 10 mg/kg bw group. The authors further only described in this dose group. Damaged intestinal epithelium was found in mice at 10 mg/kg bw/day for 21 days. The authors assumed that loss of microvilli reduced absorptive capacity of the intestinal epithelium and hence weight loss.

Rats

Hadrup et al. (2012 a,c) investigated the toxic and neurotoxic potential of AgNPs and ionic silver in rats. Female Wistar rats (4 weeks old) were administered vehicle control, silver acetate (9 mg ionic ionic silver/kg bw/day), or AgNPs (14 nm in diameter; PVP-coated) at 2.25, 4.5 or 9 mg/kg bw/day by oral gavage for 28 days; males were only given the vehicle control and 9 mg AgNP/kg bw/day for 28 days. Body weight, macroscopic and microscopic pathology and a range of biochemical and haematological parameters, as well as brain neurotransmitters were measured. Perturbation in brain dopamine ($p < 0.01$ and 0.001), noradrenaline ($p < 0.05$) and serotonine ($p < 0.01$) were observed following both ionic silver and AgNPs. In addition ionic silver led to decreased body weight gain ($p < 0.01$), decreased relative thymus weight ($p<0.05$) and increased plasma ALP ($p < 0.05$) and decreased plasma urea ($p < 0.05$). AgNPs at 9 mg/kg bw/day increased the haematocrit. Both ionic and AgNPs increased urine uric acid (only statistically significantly for AgNPs $p < 0.001$) and allantoin urine concentration ($p < 0.01$ and 0.001). In an accompanying *in vitro* investigation, AgNPs, ionic silver (silver acetate) and a 12 kDa filtered subnano silver particle fraction were used to investigate cell death mechanisms in neuronal-like cells; the effect of subnano silver in the silver nanoparticle preparations strongly suggested that the toxic effects of AgNPs were mediated by free ions as toxic effects *in vitro* on viability (including apoptosis) could be explained by the subnano fraction and ionic silver.

Van der Zande et al. (2012) investigated the hepatotoxicity of AgNPs and silver ions in rats. Male Sprague–Dawley rats (5/group, 6 weeks old, start body weight about 245 g) were exposed daily by oral gavage for 28 days to 90 mg/kg bw of AgNPs (18 nm, non-coated or 12 nm, PVP-coated, in diameter) or 9 mg/kg bw of silver nitrate (corresponding to 6 mg ionic silver/kg bw). Included were also wash-out groups identically exposed to silver but not euthanised until day 36 or day 84. Hepatotoxicity was evaluated by analysis of alanine aminotransferase and aspartate aminotransferase levels in plasma. No hepatotoxicity was detected.

Williams et al (2015) described studies on the GI tract of male and female Sprague–Dawley rats using ileal samples from a good laboratory practice (GLP)-compliant NTP study (details of which (biodistribution, bioaccumulation and histopathological examinations) are due to be reported separately and were not available to the Panel). Three sizes of citrate-stabilised AgNPs (10, 75 and 110 nm) and silver acetate were used. The Panel noted that a full interpretation of the published findings cannot be made in the absence of the data on biodistribution, bioaccumulation and histopathological examinations which are not included in the paper but will be published elsewhere. The Panel also noted that the reported effects were only determined in one part of the small intestine (ileum) and may not be representative of the whole GI tract such as the jejunum or colon where there are different microbial populations. Furthermore reported details of the bacterial isolate preparation methods are limited and it was not possible to ascertain whether this occurred under aerobic or anaerobic conditions and the effectiveness of these conditions.

Effect of silver on copper metabolism.

In the study by Ilyechova et al. (2014), two groups of animals received 50 mg silver chloride/kg bw/day: one group of adult rats received the silver-diet for 1 month (Ag-A1) and another group received the silver-diet for 6 months from birth (Ag-N6). The animals in the Ag-N6 group were first fed by females, which received the Ag-diet from the first day of lactation. In Ag-A1 rats, a dramatic decrease in copper status indexes manifested as ceruloplasmin-associated copper deficiency. In Ag-N6 rats, copper status indexes decreased only twofold as compared to control rats. In rats of both groups, silver entered the bloodstream and accumulated in the liver. Silver was incorporated into ceruloplasmin (Cp). In the liver, a prolonged Ag-diet caused a decrease in the expression level of genes associated with copper metabolism.

3.2.7.2 Humans

The Panel was aware that there are numerous data reporting adverse effects of silver due to its use in the medical field (Lansdown, 2010; Maillard, 2013) or as a result of occupational exposure (Drake and Hazelwood, 2005). If these observations confirm that prolonged exposure to very high doses of elemental silver can be responsible for the development of toxic effects, these are mostly due to the release of biologically active silver ions, and they are consecutive to exposure, which is very high and/or not comparable to the exposure resulting from the use of silver as a food additive. Therefore, these data were not directly considered in the risk assessment of silver as a food additive but they reported some useful information about the possible human effects resulting from silver toxicity.

The data indicated that the main reported effect after exposure to high doses of elemental silver in an occupational setting was argyria, which was not associated with pathological damage in a specific target organ. On the contrary, exposure to soluble silver compounds present in drugs may produce toxic effects, including liver and kidney damage, irritation of the eyes, skin, respiratory and intestinal tract, and changes in blood cells. No carcinogenic effects were reported and silver allergy was extremely rare.

Overall, the Panel noted that in the case of medical and occupational exposure to silver, the doses and/or the route of exposure (inhalation, no inclusion in a food matrix) were usually irrelevant to the exposure resulting from the use of silver as a food additive. The Panel also noted that the health risks associated with systemic absorption of ionic silver were low. Argyria and argyrosis are the principle observable changes associated with long-term exposure to ingestion or inhalation of high doses of metallic silver or ionisable silver compounds. The Panel noted that, in these contexts, the possible effects resulting from oral exposure to AgNPs were poorly documented.

4. Discussion

Following a request from the EC, the ANS Panel was asked to deliver a scientific opinion re-evaluating the safety of silver (E 174) when used as a food additive. The Panel based its evaluation on previous evaluations and on the additional literature that became available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available.

To assist in identifying any emerging issue or any relevant information for the risk assessment, EFSA has outsourced a contract to deliver an updated literature review on toxicological endpoints, dietary exposure, and occurrence levels of silver (E 174) which covered the period up to the end of 2014. Further update has been performed by the Panel.

Silver (E 174) is a food colouring authorised as a food additive in the EU that was previously evaluated by the SCF in 1975 (SCF, 1975) and by JECFA in 1977 (JECFA, 1977, 1978) and. None of the committees established an ADI. The previous evaluation by JECFA has been summarised and new available literature has been evaluated and incorporated. EFSA have also evaluated a number of silver

complexes intended for use in food contact materials latest in 2011 (EFSA, 2011) and classified silver in the SCF list 3 with a group of specific migration limit of 0.05 mg/kg food.

The Panel noted that the manufacturing process of powdered or particulate food additives resulted in material with a range of sizes. Although the mean or median size of the particles is generally significantly greater than 100 nm, a fraction can be present with at least one dimension below 100 nm. The material used for toxicological testing would have contained this nanofraction. The test requirements stipulated in current EFSA guidance documents and EC guidelines for the intended use in the food/feed area apply in principle to unintended nanoforms as well as to ENM. Therefore, the Panel considered that in principle for a specific food additive containing a fraction of particles with at least one dimension below 100 nm, adequately conducted toxicity tests should be able to detect hazards associated with this food additive including its nanoparticulate fraction. The Panel considered that for the re-evaluation of food additives this procedure would be sufficient for evaluating constituent nanoform fraction in accordance with the recommendation of the EFSA Nano Network in 2014 (EFSA, 2015).

Silver in food additive E174 is present in its elemental form. Specifications for silver have been defined in the EU in Commission Regulation (EU) No 231/2012. The purity is specified to be not less than 99.5% for silver-coloured powder or tiny sheets. Silver can also occur in crystalline form as a white metal.

The Panel noted that silver is used in foods as powder, crumbs or flakes of different sizes above 1 mm, but limited information has been provided by industry on distribution of particle sizes in powdered silver. However, there is some evidence that AgNPs could be released from the current application forms, as it has been observed by the data provided after the analysis of the AgNPs released from the coating of silver-coloured pearls for decoration of pastry, being reported an amount of 4.4×10^9 Ag nanoparticles/g of pearl. The mass concentration of the detected particles was 1.8 ± 0.6 µg/g pearl, representing the 20% of the mean concentration of silver in the pearls.

The Panel noted that in Commission Regulation (EU) No 231/2012, no information is included regarding the particle size of silver powder and therefore the characterisation of the particle size in the powder of E 174 should be included among the specifications. The fully characterisation should include the particles size distribution together with determination and quantification of any nanoparticulate material.

The Panel noted that AgNPs are released from confectionary pearls (Verleysen et al., 2015) and nanosilver is unstable and releases ions (see Section 2.5). The Panel was aware of the extensive database on ionic silver or AgNPs, however the relevance of these data to the evaluation of silver as a food additive (E 174) was not apparent. Therefore, the Panel considered these data could not be directly applied to the evaluation of the food additive.

Following oral exposure of animals to ionic silver or AgNPs, silver is systemically available. Silver concentrations in the organs were highly correlated to the size of the nanoparticles concentrations being higher in animals exposed to smaller nanoparticles and to the amount of silver ions released from the AgNPs. Bioavailability seems to be in the range of 2–20% depending on many factors including the animal species.

However, the Panel noted that, due to the many variables involved, the conversion rate of metal silver from nanoparticles to silver ions in biological systems is unknown. Moreover, the formation of ROS from the fraction of AgNPs which may be present in the food additive has not been determined. The rate of both processes depends on the size of particles and their relative surface.

Silver distribution has been reported to all organs and tissues of animals. Silver distribution to the brain following oral exposure has been described in several studies, which is in contrast to the conclusions of previous studies with silver nitrate or lactate, that silver would not cross the blood–

brain barrier (van Breemen and Clemente,1955;). However, it is also in the recent studies not clear whether silver is present in the brain endothelial cells or in the brain tissue. Silver ions were also detected in the milk of rat dams receiving a daily oral administration of silver chloride and in the liver and the brain of the pups. In rodents, silver is primarily excreted via the bile and faeces, but a small amount is also excreted via the urine.

The Panel noted that only one study described the fate of microsized silver particles in animals (Park et al., 2010). In this study, no silver was detected in any of the tissues of mice given an oral administration of microsized silver particles (323 nm), whereas silver was present in tissues of mice receiving a similar administration of nanosized silver particles (21 to 71 nm).

The Panel was aware that there are many data reporting distribution of silver in various human organs following prolonged exposure to very high doses of silver in different forms. The Panel was also aware that there are numerous data reporting adverse effects of silver due to its use in the medical field (Lansdown, 2010; Maillard, 2013) or as a result of occupational exposure (Drake and Hazelwood, 2005). Overall, the Panel noted that in the case of medical and occupational exposure to silver, the doses and/or the route of exposure (inhalation, no inclusion in a food matrix) were usually irrelevant to the exposure resulting from the use of silver as a food additive. The Panel also noted that the health risks associated with systemic absorption of ionic silver were low. Argyria and argyrosis are the principle observable changes associated with long-term exposure to ingestion or inhalation of high doses of metallic silver or ionisable silver compounds. The Panel noted that, in these contexts, the possible effects resulting from oral exposure to AgNPs were poorly documented.

No toxicity studies were reported on elemental silver.

The toxicity of AgNPs, mostly capped with modifying agents, is extensively studied.

Oral LD_{50} values of approximately 32, 280 and 800 mg Ag/kg bw have been reported for ionic silver (silver nitrate) in mice, rats and rabbits, respectively (Tamimi et al., 1998). For AgNPs, a dose of 5,000 mg/kg bw did not lead to mortality or acute toxic signs in mice (Maneewattanapinyo et al., 2011).

In mice, no short-term or subchronic studies on ionic silver were available. Shahare and Yaspal (2013) studied the effects of 10 mg/kg bw/day AgNPs (3–20 nm) after dosing to Swiss albino mice by gavage for 21 days and observed a decreased body weight and intestinal damage.

In rats, colloidal AgNPs (diameter of 55–60 nm) resulted in slight liver damage (affected enzymes after 28 days at a dose of 300 mg/kg bw/day (Kim et al., 2008) and after 90 days at a dose of 125 mg/kg bw/day (Kim et al., 2010). No effects were observed at 30 mg/kg bw/day. According to Kim et al. (2008, 2010), the bile duct hyperplasia observed in the liver in the 90-day study may point to a treatment-related effect of AgNPs. The Panel did not agree with this preliminary conclusion, and considered further research needed. Hadrup et al. (2012a,b,c) observed the following changes in rats after oral administration by gavage of silver acetate (9 mg ionic silver/kg bw/day) for 28 days: a decreased body weight gain, decreased thymus weight and increased liver enzymes and decreased plasma urea allantoin urine concentration and changes in the neurotransmittors. However, Van der Zande et al. (2012) observed no hepatotoxicity after daily exposure by gavage for 28 days to 90 mg/kg bw of AgNPs (18 nm, non-coated or 12 nm, PVP-coated, in diameter) or 9 mg/kg bw of silver nitrate (corresponding to 6 mg ionic silver/kg bw).

There are no data available to evaluate the *in vivo* genotoxicity of ionic silver. Concerning AgNPs, the available studies provide clear evidence of a genotoxic potential in various *in vitro* test systems. The *in vivo* oral genotoxicity studies performed provide less conclusive evidence, and do not allow a definitive assessment of the possible genotoxic hazard associated with oral exposure to AgNPs.

Overall, the Panel concluded that the available data are inadequate to evaluate the genotoxic hazard associated with the use of silver as food additive.

No studies on the carcinogenic potential of either ionic silver compounds or AgNPs have been identified. In rats, retarded growth and stained muzzles were the only effects reported following long-term exposure to ionic silver (up to 8.5 months, approximately 81 mg ionic silver/kg bw/day (Matuk et al., 1981) and 60 weeks, approximately 118 mg ionic silver/kg bw/day (Walker 1971)).

In an oral one-generation reproductive toxicity study with silver acetate in drinking water at dose levels of 0, 0.4, 4 or 40 mg silver acetate/kg bw/day (0, 0.26, 2.6 or 26 mg ionic silver/kg bw/day) in rats, a NOAEL for developmental effects (based on an increased number of pups, pup death and decreased weight gain of pups) of 0.4 mg silver acetate/kg bw/day (0.26 mg ionic silver/kg bw/day) was observed (Documentation provided to EFSA No5). The NOAEL for fertility was 4 mg silver acetate/kg bw/day (2.6 mg ionic silver/kg bw/day).

In a prenatal developmental toxicity study, developmental toxicity of ionic silver was observed when rats were dosed with silver chloride (188 mg ionic silver/kg bw/day) on GD 1–20 (Shavlovski et al., 1995). No developmental effect was observed by the same authors when rats were only dosed with silver chloride from GD 7–15. This study was only conducted at one dose level in a low number of animals and maternal toxicity was not described properly. The effects on ceruloplasmin after longer administration were emphasised by the authors.

In another prenatal developmental study (NTP, 2002) with silver acetate performed according the current guidelines at dose levels of up to 100 mg/kg bw/day (65 mg ionic silver/kg bw/day) administered from GD 6–19, a NAOEL for developmental toxicity was observed at 65 mg ionic silver/kg bw/day as the NOAEL for maternal toxicity was 6.5 mg ionic silver/kg bw/day.

The Panel noted that silver ions affected developmental toxicity at a much lower level (NOAEL 0.26 mg ionic silver/kg bw/day) in the one-generation reproductive toxicity study (Documentation provided to EFSA No5).

The Panel considered some immunotoxicity studies performed following intravenous administration but they were not evaluated because the route of exposure was considered not directly relevant to the exposure resulting from the use of silver as a food additive. The Panel noted that the outcomes of immunotoxicity studies performed with AgNPs *in vitro* and *in vivo* after oral administration were variable but always suggestive of an effect of the treatment with silver on the immune system. Inconsistencies in the outcomes (immune-stimulation or suppression) might be due to different material, doses, duration of exposure and animal or cell models used. Overall, they indicate that silver particles cytotoxicity and immunomodulatory activities are influenced by both their size and the rate of surface dissolution, leading to the release of silver ions, which seemed to be the most active form. Owing to the possibility that silver ions can be released from silver use as a food additive even if not under a nanoparticulate form, the Panel considered that the immunomodulation effects observed in studies using AgNPs are relevant for silver used as a food additive and that further investigation is warranted.

Exposure assessments of food additives under re-evaluation are carried out by the ANS Panel based on (1) MPLs set down in the EU legislation (defined as the regulatory maximum level exposure assessment scenario) and (2) usage or analytical data (defined as the refined exposure assessment scenario). It was not possible to carry out a scenario based on the MPLs set out in EU legislation, as, for all food categories, silver (E 174) is authorised according to QS. However, maximum levels of the available data were used to provide a conservative estimate scenario (noted as the *maximum level exposure assessment scenario*). With regard to the refined exposure assessment scenario, reported use levels were made available by industry only for two food categories. The Panel considers that the refined exposure assessment approach results in more realistic long-term exposure estimates because

of the underlying assumptions and the concentration data used. The Panel noted that the refined exposure estimates will not cover future changes in the level of use of silver (E 174).

From the maximum level exposure assessment, mean estimates ranged from < 0.01 to 2.6 µg/kg bw/day across all population groups. Estimates based on the high percentile (95th percentile) ranged from 0 to12 µg/kg bw/day across all population groups.

From the refined estimated exposure scenario in the brand-loyal scenario, mean exposure to silver (E 174) from its use as a food additive ranged from < 0.01 µg/kg bw/day for infants to 2.6 µg/kg bw/day in children. The high exposure to silver (E 174) ranged from 0 µg/kg bw/day for infants to 12 µg/kg bw/day in children. In the non-brand-loyal scenario, mean exposure to silver (E 174) ranged from <0.01 µg/kg bw/day for infants to 1.6 µg/kg bw/day in children. The high exposure ranged from 0 µg/kg bw/day for infants to 3.2 µg/kg bw/day in children.

The exposure from the food additive and the regular diet (ANSES, 2011) could lead to a mean intake for children around 3.5 µg/kg bw/day (non-brand-loyal scenario). On average, exposure from the food additive would represent around 30% of total dietary exposure to silver (see Table 4).

Overall, the Panel noted that there are data gaps and concerns that need to be addressed in order to conduct a risk assessment with respect to the use of silver (E 174) as food additive:

- Data from toxicity studies on elemental silver or the food additive (E 174) are lacking.
- The particle size distribution of the food additive (E 174) is unknown.
- There is evidence of the release of silver ions from elemental silver, which may be of concern. However, the extent of the release of the silver ions, which depends on multiple factors such as pH and particle size, is unknown in the case of silver (E 174) used as food additive.

5. Conclusions

The Panel concluded that the information available was insufficient to assess the safety of silver as food additive. The major issues included chemical identification and characterisation of silver E 174 (e.g. quantity of nanoparticles and release of ionic silver) and similar information on the material used in the available toxicity studies. Therefore, the Panel concluded that the relevance of the available toxicological studies to the safety evaluation of silver as a food additive E 174 could not be established.

6. Recommendation

The Panel recommended that the specifications for E 174 should include the mean particle size and particle size distribution (± SD), as well as the percentage (in number) of particles in the nanoscale (with at least one dimension below 100 nm), present in the powder form of silver (E 174) used as a food additive. The methodology applied should comply with the EFSA Guidance document (EFSA Scientific Committee, 2011), e.g. SEM or TEM.

The Panel recommended that additional data in line with the current Guidance document on evaluation of food additives (EFSA, 2012) would be required.

DOCUMENTATION PROVIDED TO EFSA

1. CIAA (Confederation of the Food and Drink Industries of the EU). Exercise on occurrence data – EFSA re-evaluation of some food colours. CIAA submission: December 2009.

2. Coda-Cerva, 2014. Reply to EFSA: Call for food additives usages level and/or concentration data in food and beverages intended for human consumption. Submitted on 30.09.2014; Updates on 15.01.2015; 16.03.2015.

3. Eytzinger GmbH, 2011. Reply to EFSA: Call for scientific data on Silver (E 174) and Gold (E 175), used as food colours. Submitted on 27 July 2011.

4. Eytzinger GmbH, 2015. Personal communication from Eytzinger GmbH on the particle size distribution on edible Gold (E 175), 25 November 2015.

5. FDA (Food and Drug Administration), 2012. Effect of maternal exposure to silver ions (silver acetate) on thymic development in F1-generation offspring. Submitted on 18 December 2014.

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7. Memorandum, 2000. Use of silver zeolite as a component of articles intended for food contact applications. Department of Health and Human Services. Unpublished Report sumbitted by FDA (Food and Drug Administration) in 2010.

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Appendices

Appendix A – Summary of the reported use levels and concentration levels (mg/kg or mg/L as appropriate) of silver (E 174) provided by industry

FCS Category number	FCS food category	MPL	Restrictions	n	Reported use levels		Data sources/comments
					Typical mean	Highest maximum level	
05.2	Other confectionery including breath-freshening microsweets	QS	Only external coating of confectionery[(a)]	1	8	7,000	FoodDrinkEurope (representative of 2 EU countries)
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4	QS	Only decoration of chocolates[(a)]	1	5,000	5,000	FoodDrinkEurope
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	QS	Only liqueurs	1	-	-	Not taken into account (no concentration data available)

(a): With the assumption that coating and decorations represent 1% of the products (for both confectionary and chocolates).

Appendix B – Summary of total estimated exposure of silver (E 174) from their use as food additives for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and high level (mg/kg bw/day)

	Number of subjects	Maximum level scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
Infants							
Bulgaria (NUTRICHILD)	659	5.9E-04	0.0E+00	5.9E-04	0.0E+00	2.9E-04	0.0E+00
Germany (VELS)	159	3.1E-03	2.5E-02	2.9E-03	2.5E-02	2.4E-03	2.5E-02
Denmark (IAT 2006_07)	826	2.4E-03	1.0E-02	2.2E-03	8.4E-03	1.3E-03	6.9E-03
Finland (DIPP_2001_2009)	500	9.8E-06	0.0E+00	9.8E-06	0.0E+00	8.8E-06	0.0E+00
United Kingdom (DNSIYC_2011)	1366	4.0E-03	2.9E-02	4.0E-03	2.9E-02	3.8E-03	2.5E-02
Italy (INRAN_SCAI_2005_06)	12	6.1E-03		6.1E-03		6.1E-03	
Toddlers							
Belgium (Regional_Flanders)	36	6.8E-02		6.2E-02		5.8E-02	
Bulgaria (NUTRICHILD)	428	1.8E-02	1.2E-01	1.7E-02	1.0E-01	7.4E-03	5.2E-02
Germany (VELS)	348	4.4E-02	1.1E-01	3.9E-02	1.1E-01	2.4E-02	7.0E-02
Denmark (IAT 2006_07)	917	3.8E-02	1.3E-01	3.5E-02	1.1E-01	1.7E-02	6.5E-02
Spain (enKid)	17	1.4E-02		1.0E-02		1.0E-02	
Finland (DIPP_2001_2009)	500	3.1E-03	1.4E-02	3.1E-03	1.4E-02	1.0E-03	8.3E-03
United Kingdom (NDNS-RollingProgrammeYears1-3)	185	3.2E-02	1.1E-01	3.0E-02	1.1E-01	1.7E-02	6.1E-02
United Kingdom (DNSIYC_2011)	1314	1.6E-02	7.5E-02	1.5E-02	7.3E-02	1.3E-02	6.5E-02
Italy (INRAN_SCAI_2005_06)	36	1.0E-02		9.9E-03		9.2E-03	
Netherlands (VCP_kids)	322	7.5E-02	2.1E-01	6.7E-02	1.8E-01	4.1E-02	1.4E-01
Children							
Austria (ASNS_Children)	128	3.7E-02	1.1E-01	3.4E-02	1.1E-01	2.5E-02	8.6E-02
Belgium (Regional_Flanders)	625	6.0E-02	1.6E-01	5.6E-02	1.5E-01	3.8E-02	1.2E-01
Bulgaria (NUTRICHILD)	433	2.6E-02	1.3E-01	2.5E-02	1.3E-01	1.4E-02	8.9E-02
Czech Republic (SISP04)	389	6.0E-02	2.5E-01	5.9E-02	2.5E-01	2.3E-02	1.2E-01
Germany (EsKiMo)	835	5.9E-02	1.6E-01	5.2E-02	1.5E-01	3.6E-02	1.1E-01
Germany (VELS)	293	5.4E-02	1.4E-01	4.7E-02	1.3E-01	3.2E-02	8.3E-02
Denmark (DANSDA 2005-08)	298	6.0E-02	1.4E-01	5.3E-02	1.3E-01	2.7E-02	7.3E-02
Spain (enKid)	156	2.9E-02	1.2E-01	2.8E-02	1.2E-01	2.0E-02	6.9E-02
Spain (NUT_INK05)	399	2.4E-02	8.1E-02	2.3E-02	7.7E-02	1.6E-02	6.6E-02
Finland (DIPP_2001_2009)	750	1.1E-01	4.6E-01	1.1E-01	4.6E-01	1.3E-02	5.8E-02
France (INCA2)	482	3.1E-02	9.3E-02	2.8E-02	8.6E-02	2.2E-02	8.1E-02
United Kingdom (NDNS-RollingProgrammeYears1-3)	651	3.5E-02	1.3E-01	3.3E-02	1.2E-01	2.0E-02	6.5E-02
Greece (Regional_Crete)	838	1.0E-02	4.7E-02	1.0E-02	4.7E-02	6.7E-03	3.2E-02
Italy	193	1.6E-02	7.9E-02	1.6E-02	7.9E-02	1.5E-02	6.7E-02

	Number of subjects	Maximum level scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
(INRAN_SCAI_2005_06)							
Latvia (EFSA_TEST)	187	3.3E-02	1.2E-01	3.3E-02	1.2E-01	1.9E-02	8.3E-02
Netherlands (VCP_kids)	957	7.9E-02	1.9E-01	7.0E-02	1.8E-01	4.1E-02	1.2E-01
Netherlands (VCPBasis_AVL2007_2010)	447	6.5E-02	1.7E-01	5.8E-02	1.5E-01	3.3E-02	1.0E-01
Sweden (NFA)	1473	5.1E-02	1.6E-01	5.0E-02	1.6E-01	1.1E-02	5.5E-02
Adolescents							
Austria (ASNS_Children)	237	1.3E-02	5.7E-02	1.3E-02	5.2E-02	1.0E-02	4.4E-02
Belgium (Diet_National_2004)	576	2.4E-02	8.5E-02	2.3E-02	7.7E-02	1.9E-02	7.0E-02
Cyprus (Childhealth)	303	1.2E-02	4.6E-02	1.1E-02	4.4E-02	9.9E-03	3.8E-02
Czech Republic (SISP04)	298	2.9E-02	1.6E-01	2.8E-02	1.5E-01	1.1E-02	6.6E-02
Germany (National_ Nutrition_Survey_II)	1011	2.0E-02	1.1E-01	1.9E-02	1.0E-01	7.6E-03	4.2E-02
Germany (EsKiMo)	393	4.4E-02	1.4E-01	3.9E-02	1.1E-01	2.8E-02	8.3E-02
Denmark (DANSDA 2005-08)	377	3.4E-02	9.1E-02	3.1E-02	8.8E-02	1.6E-02	5.2E-02
Spain (AESAN_FIAB)	86	2.7E-02	1.1E-01	2.7E-02	1.1E-01	7.2E-03	3.8E-02
Spain (enKid)	209	1.5E-02	7.6E-02	1.5E-02	6.4E-02	7.0E-03	3.5E-02
Spain (NUT_INK05)	651	1.3E-02	5.8E-02	1.3E-02	5.7E-02	8.2E-03	4.1E-02
Finland (NWSSP07_08)	306	8.1E-02	3.3E-01	8.1E-02	3.3E-01	6.5E-03	3.2E-02
France (INCA2)	973	1.8E-02	6.0E-02	1.7E-02	5.6E-02	1.3E-02	4.8E-02
United Kingdom (NDNS-RollingProgrammeYears1-3)	666	2.1E-02	7.6E-02	2.0E-02	7.0E-02	1.4E-02	4.7E-02
Italy (INRAN_SCAI_2005_06)	247	7.5E-03	3.1E-02	7.3E-03	3.1E-02	7.0E-03	3.0E-02
Latvia (EFSA_TEST)	453	1.4E-02	7.4E-02	1.4E-02	7.4E-02	1.3E-02	6.6E-02
Netherlands (VCPBasis_AVL2007_2010)	1142	3.5E-02	1.1E-01	3.2E-02	1.0E-01	1.8E-02	5.9E-02
Sweden (NFA)	1018	3.8E-02	1.4E-01	3.7E-02	1.4E-01	9.6E-03	5.0E-02
Adults							
Austria (ASNS_Adults)	308	8.5E-03	3.8E-02	8.3E-03	3.8E-02	7.0E-03	3.3E-02
Belgium (Diet_National_2004)	1292	1.2E-02	4.5E-02	1.2E-02	4.4E-02	9.9E-03	4.1E-02
Czech Republic (SISP04)	1666	3.6E-03	2.2E-02	3.6E-03	2.2E-02	2.5E-03	1.8E-02
Germany (National_ Nutrition_Survey_II)	10419	1.6E-02	7.4E-02	1.6E-02	7.1E-02	5.7E-03	3.2E-02
Denmark (DANSDA 2005-08)	1739	1.6E-02	5.2E-02	1.5E-02	4.9E-02	6.9E-03	2.5E-02
Spain (AESAN)	410	5.6E-03	2.8E-02	5.5E-03	2.8E-02	3.8E-03	2.3E-02
Spain (AESAN_FIAB)	981	1.3E-02	4.0E-02	1.3E-02	4.0E-02	3.3E-03	1.9E-02
Finland (FINDIET2012)	1295	2.9E-02	1.7E-01	2.9E-02	1.7E-01	4.7E-03	2.5E-02
France (INCA2)	2276	6.1E-03	2.9E-02	5.8E-03	2.5E-02	4.3E-03	2.1E-02
United Kingdom (NDNS-RollingProgrammeYears1-3)	1266	8.2E-03	3.3E-02	7.9E-03	3.2E-02	5.9E-03	2.7E-02
Hungary (National_Repr_Surv)	1074	3.4E-03	2.0E-02	3.4E-03	2.0E-02	3.3E-03	2.0E-02
Ireland (NANS_2012)	1274	7.9E-03	3.4E-02	7.5E-03	3.2E-02	6.0E-03	2.6E-02

	Number of subjects	Maximum level scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
Italy (INRAN_SCAI_2005_06)	2313	1.9E-03	1.1E-02	1.9E-03	1.1E-02	1.6E-03	9.9E-03
Latvia (EFSA_TEST)	1271	6.3E-03	3.3E-02	6.3E-03	3.2E-02	4.9E-03	3.1E-02
Netherlands (VCPBasis_AVL2007_2010)	2057	1.3E-02	4.8E-02	1.2E-02	4.5E-02	7.7E-03	3.4E-02
Romania (Dieta_Pilot_Adults)	1254	3.8E-03	1.9E-02	3.7E-03	1.9E-02	2.5E-03	1.4E-02
Sweden (Riksmaten 2010)	1430	1.1E-02	5.0E-02	1.1E-02	4.8E-02	4.9E-03	2.4E-02
The elderly							
Austria (ASNS_Adults)	92	3.0E-03	1.2E-02	2.9E-03	1.2E-02	2.9E-03	1.2E-02
Belgium (Diet_National_2004)	1215	5.9E-03	2.8E-02	5.8E-03	2.7E-02	4.6E-03	2.4E-02
Germany (National_ Nutrition_Survey_II)	2496	4.8E-03	2.4E-02	4.8E-03	2.2E-02	2.6E-03	1.7E-02
Denmark (DANSDA 2005-08)	286	7.9E-03	3.0E-02	7.1E-03	2.5E-02	4.0E-03	1.6E-02
Finland (FINDIET2012)	413	8.0E-03	4.5E-02	7.9E-03	3.5E-02	1.3E-03	7.8E-03
France (INCA2)	348	2.9E-03	1.1E-02	2.9E-03	1.1E-02	1.3E-03	8.0E-03
United Kingdom (NDNS-RollingProgrammeYears1-3)	305	6.0E-03	2.1E-02	5.9E-03	2.1E-02	3.1E-03	1.5E-02
Hungary (National_Repr_Surv)	286	1.6E-03	7.4E-03	1.6E-03	7.4E-03	1.1E-03	6.4E-03
Ireland (NANS_2012)	226	1.9E-03	1.2E-02	1.8E-03	1.2E-02	1.6E-03	1.1E-02
Italy (INRAN_SCAI_2005_06)	518	9.1E-04	4.4E-03	8.8E-04	4.3E-03	6.9E-04	3.7E-03
Netherlands (VCPBasis_AVL2007_2010)	173	6.4E-03	2.6E-02	5.9E-03	2.5E-02	4.2E-03	2.3E-02
Netherlands (VCP-Elderly)	739	7.5E-03	2.7E-02	6.9E-03	2.6E-02	5.3E-03	2.3E-02
Romania (Dieta_Pilot_Adults)	128	2.4E-03	1.3E-02	2.3E-03	1.1E-02	8.6E-04	5.0E-03
Sweden (Riksmaten 2010)	367	4.7E-03	2.4E-02	4.5E-03	2.3E-02	3.0E-03	1.7E-02

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ABBREVIATIONS

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
ALP	alkaline phosphatase
ALT	alkaline transaminase
AST	aspartate transaminase
AgNPs	silver nanoparticles
BUN	blood urea nitrogen
Bw	body weight
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
DLLME	dispersive liquid-liquid microextraction
DLS	dynamic light scattering
DNA	deoxyribonucleic acid
EC	European Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
EDX	energy dispersive X-ray
ENM	engineered nanomaterials
FAO	Food and Agriculture Organization of the United Nations
FCS	Food Categorisation System
FDA	US Food and Drug Administration
FDE	Food Drink Europe
GI	gastrointestinal
GLP	good laboratory practice
GFAAS	graphite furnace atomic absorption spectrometry
HAADF-STEM	high-angle annular dark-field scanning transmission electron microscopy
ICP	inductively coupled mass
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	median lethal dose
MN	micronuclei
MPL	maximum permitted level
MS	mass spectrometry
MTT	methyltetrazolium
NCE	normochromatic erythrocyte
NOAEL	no-observed-adverse-effect Level
OECD	Organisation for Economic Co-operation and Development

OES	optical emission spectrometry
PCE	polychromatic erythrocyte
PVP	Polyvinylpyrrolidone
QS	quantum satis
ROS	reactive oxygen species
SCE	sister chromatid exchange
SCF	Scientific Committee for Food
SEM	scanning electron microscopy
SP	single particle
TEM	transmission electron microscopy
WHO	World Health Organization

SCIENTIFIC OPINION

Scientific Opinion on the safety evaluation of the substance, silver zeolite A (silver zinc sodium ammonium alumino silicate), silver content 2 – 5 %, for use in food contact materials[1]

EFSA Panel on food contact materials, enzymes, flavourings and processing aids (CEF)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output replaces the earlier version published 21 February 2011[4]

ABSTRACT

This scientific opinion of EFSA deals with the risk assessment of the substance silver zeolite A (silver zinc sodium ammonium alumino silicate), silver content 2-5 %, REF. No. 86437 for which the CEF Panel concluded that there is no safety concern for the consumer if migration of silver ion does not exceed the group specific migration limit of 0.05 mg Ag/kg food. The CEF Panel noted that the use of the substance may also result in migration of aluminium into food. The potential exposure to Al for an adult weighing 60 kg can be estimated to be at the range of 4.4% of the TWI of 1 mg/kg bw/week set by the AFC Panel (EFSA, 2008).

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KEY WORDS

Silver zeolite A (silver zinc sodium ammonium alumino silicate), silver content 2-5 %; Ref. No. 86437; Aluminium, Food contact materials; Safety assessment; Evaluation.

1 On request from the Ministère de l'Économie de l'Industrie et de l'Emploi, France, Question No EFSA-Q-2009-00708, adopted on 4 February 2011.

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4 The changes do not affect nor alter this scientific output. The adoption date has been updated.

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SUMMARY

Within the general task of evaluating substances intended for use in materials in contact with food according to the Regulation (EC) No.1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with foodstuffs, the CEF Panel received a request from a competent Member State Authority for safety evaluation of a substance following a corresponding application from the industry.

The request received and the outcome of the safety evaluation is summarised below:

The Ministère de l'Économie de l'Industrie et de l'Emploi, France, requested for evaluation of the substance silver zeolite A (silver zinc sodium ammonium alumino silicate), silver content 2-5 % with the European Commission reference number (REF. No.) 86437, for use to control microorganism growth on the article in polyolefins, poly(ethylene terephthalate) (PET) and polycarbonate (PC) made with up to 3% w/w of silver zeolite A containing around 2.5% silver. Finished articles are intended to be used for single contact with all types of foodstuffs at room temperature for a long period. The dossier was submitted on behalf of AgION Technologies Ing., USA.

The CEF Panel concluded that there is no safety concern for the consumer if migration of silver ion does not exceed the group specific migration limit of 0.05 mg Ag/kg food. The CEF Panel noted that the use of the substance may also result in migration of aluminium into food. The potential exposure to Al for an adult weighing 60 kg can be estimated to be at the range of 4.4% of the TWI of 1 mg/kg bw/week set by the AFC Panel (EFSA, 2008).

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BACKGROUND AS PROVIDED BY THE LEGISLATION

Before a substance is authorised to be used in food contact materials and is included in a positive list EFSA´s opinion on its safety is required. This procedure has been established in Articles 8 and 9 of the Regulation (EC) No. 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food[5].

According to this procedure the industry submits applications to the Member States competent Authorities which in their turn transmit the applications to the EFSA for their evaluation. The application is supported by a technical dossier submitted by the industry following the SCF guidelines for the "presentation of an application for safety assessment of a substance to be used in food contact materials prior to its authorisation" (EC, 2001).

In this case, EFSA received an application from the Ministère de l'Économie, de l'Industrie et de l'Emploi, France, requesting the evaluation of the substance silver zeolite A (silver zinc sodium ammonium alumino silicate), silver content 2-5 % with the European Commission reference number (REF. No.) 86437.

TERMS OF REFERENCE AS PROVIDED BY THE LEGISLATION

The EFSA is required by Article 10 of Regulation (EC) No. 1935/2004 of the European Parliament and of the Council on materials and articles intended to come into contact with food to carry out risk assessments on the risks originating from the migration of substances from food contact materials into food and deliver a scientific opinion on:

1. new substances intended to be used in food contact materials before their authorisation and inclusion in a positive list;
2. substances which are already authorised in the framework of Regulation (EC) No. 1935/2004 but need to be re-evaluated.

[5] This Regulation replaces Directive 89/109/EEC of 21 December 1988, OJ L 40, 11.2.1989, P.38.

ASSESSMENT

1. Introduction

The European Food Safety Authority was asked by the "Ministère de l'Économie de l'Industrie et de l'Emploi", France, to evaluate the safety of the silver zeolite A (silver zinc sodium ammonium alumino silicate), silver content 2-5 % and a REF. No. 86437. The request has been registered in the EFSA's register of received questions under the number EFSA-Q-2010-00813. The dossier was submitted on behalf of AgION Technologies Inc.

Since in the past the evaluation of substances used in food contact materials was undertaken by the Scientific Committee on Food (SCF), the same system of classification into a "SCF list" is retained for uniformity purposes. The definitions of the various SCF lists and the abbreviations used are given in the APPENDIX A.

2. General information

According to the applicant, the compound silver zeolite A is a defined mixture $M_{12}(2AlO_2\ 2SiO_2)_6\ 27H_2O$ (with $M = Na^+, Ag^+, Zn^{++}, NH4^+$). The detailed composition corresponds to the raw formulas ranging from $Na_{1.3}$, Ag, $Zn_{9.7}$, $(NH_4)_{5.8}*(AlO_2*SiO_2)_{23}*\ 36H_2O$ to $Na_{1.5}$, Ag, $Zn_{9.4}$, $(NH_4)_{6.8}*(AlO_2*SiO_2)_{27.4}*\ 2.2H_2O$, depending on the grade. Grades used in food contact applications may contain up to 5% (w/w) silver. The function of the substance is to control microorganism growth on the article and to thereby preserve the article. According to the petitioner, silver zeolite A is not intended to have a technical effect on food.

Silver zeolite A was previously evaluated by the EFSA (EFSA, 2005) on the basis of non toxicity data (data on structure and identity, physical and chemical properties, intended use, global migration data, specific migration from low density polyethylene (LDPE), oriented polypropylene, polystyrene, polyvinylchloride and polybutyleneterephthalate into simulants including 3% acetic acid, and sodium-containing test media), toxicity data (gene mutation tests in bacteria, *in vitro* gene mutation assay in mammalian cells, *in-vitro* and *in-vivo* chromosomal aberration test, 90-day dietary toxicity study in rats and dogs, dietary two-generation reproduction and fertility study in rats) and microbiological data (intended microbiological function and applications, spectrum of microbial activity, level of activity, efficacy, lack of antimicrobial activity against microbes in the food).
It was classified in SCF_List 3, with a group restriction of 0.05 mg Ag/kg food, based on the human NOAEL of about 10 g of silver for a total lifetime oral intake allocated by WHO (WHO, 2004) for drinking water. The following restrictions were also allocated:

- Maximum content in polymer: 10% (w/w) of silver zeolite A containing ≤ 5% silver.
- Only for articles intended for repeated use made from polyolefins (up to 40°C for contact times below 1 day) and for poly(alkylene terephthalate) based polymers (up to 99°C for contact times below 2 hours).

The current evaluation deals with a new dossier submitted by the applicant to request the extension of the use in polyolefins, poly(ethylene terephthalate) (PET) and polycarbonate (PC) single use articles (maximum content in plastics: 3% w/w of silver zeolite A containing around 2.5% silver).

The plastics containing the silver zeolite A are intended to be used with all types of foodstuffs (acidic, aqueous, alcoholic, and fatty).

3. Data available in the dossier used for this evaluation

The studies submitted for evaluation followed the SCF guidelines for the presentation of an application for safety assessment of a substance to be used in food contact materials prior to its authorisation (EC, 2001).

Non-toxicity data:

- Data on structure
- Data on intended use
- Data on specific migration from LDPE, PET and PC into 3% acetic acid, 40 mM sodium acetate buffer at pH 5 and 50 mM sodium phosphate buffer at pH 7

Microbiological data:

- None, this aspect was evaluated by the EFSA in 2005 (EFSA, 2005).

Toxicity data:

- None, this aspect was evaluated by the EFSA in 2005 (EFSA, 2005).

4. Evaluation

4.1. Non-toxicological data

Based on the earlier evaluation, the Panel noted that migration would be expected to be highest into acidic simulant and into simulants containing sodium counter-ions.

The migration of silver ion after 10 days at 40°C in 3% acetic acid, in sodium acetate buffer (40 mM, pH 5) and in sodium phosphate buffer (50 mM, pH 7) was investigated in a study on LDPE, PET and PC containing around 3% silver zeolite A. The LDPE sample was made with a silver zeolite A containing 2.5% Ag and 14% Zn while PET and PC were made with a silver zeolite A containing 2.7% Ag and 14.7% Zn. The migration of silver ion was:

a) from LDPE, 25 µg/kg in 3% acetic acid, 16 µg/kg in sodium acetate buffer and 9 µg/kg in sodium phosphate buffer.
b) from PET, 31 µg/kg in 3% acetic acid, below 3 µg/kg in sodium acetate buffer and 7 µg/kg in sodium phosphate buffer.
c) from PC, 17 µg/kg in 3% acetic acid, 4 µg/kg in sodium acetate buffer and 14 µg/kg in sodium phosphate buffer.

In another study, the migration of silver ion after 10 days at 40°C in 3% acetic acid, from LDPE samples containing 1, 2, 3, 4 and 5% silver zeolite A (with a content of 2.5% Ag and 14% Zn), was 21, 25, 40, 54 and 51 µg/kg, respectively.

The results demonstrate that articles containing the substance can be formulated according to the intended use so that the migration of silver does not exceed 50 µg/kg food.

Based on the elemental composition of the substance and assuming that the ratio of the migration of aluminium and silver are in proportion to this composition, a maximum migration of silver of 50 µg/kg would restrict the migration of aluminium to 375 µg/kg food/simulant. This is a conservative assumption considering that silver zeolites are designed to exchange silver ions while aluminium and other metal ions form part of the matrix of the substance.

a. Microbiological data

The microbiological aspects were evaluated by the EFSA in 2005 (EFSA, 2005). Based on the data provided the Panel had concluded that:
The efficacy of the biocide in a wide range of polymers was demonstrated against a wide range of microorganisms exposed in aqueous solutions to the food contact material surface. In all cases there was a three to four log reduction in viability of the test organism after 24 hours exposure.
No evidence was presented that demonstrated the efficacy of the biocide under in-use conditions i.e. that the use of the biocide makes a contribution to food safety and hygiene over and above that resulting from normal hygiene regime employed in food preparation areas.

b. Toxicological data

The toxicological aspects regarding silver were evaluated by the EFSA in 2005 (EFSA, 2005). The Panel also took note of the WHO "Guidelines for drinking-water quality". According to these Guidelines a total lifetime oral intake of about 10 g of silver (equal to 0.39 mg/day/person) can be considered on the basis of epidemiological and pharmacokinetic knowledge as the human NOAEL.

Based on the data above, a restriction of 0.05 mg/kg of food (as silver) for the substance would limit intake to less than 13% of the human NOAEL, under the assumption that each day a kg of food is consumed containing silver at the restriction limit.

The Panel took also note of the opinion of the AFC Panel (EFSA, 2008) setting a TWI of 1 mg/kg bw/week for aluminium. Based on a conservative scenario of consumption of 1 kg of food per day containing aluminium migrated at the level of 375µg/kg, an adult of 60 kg bodyweight in one week time would be exposed to 0.044 mg Al/kg bw/week, corresponding to 4.4% of the TWI set in 2008.

CONCLUSIONS

The CEF Panel after having considered the data provided for the use of the substance in single use articles, as well as the previous evaluation of the substance (EFSA, 2005) for repeated use articles, proposes that the substance silver zeolite A (silver zinc sodium ammonium alumino silicate), silver content 2-5 % be classified in the SCF_List 3, with a group specific migration limit of 0.05 mg Ag/kg food, based on the human NOAEL of about 10 g of silver for a total lifetime oral intake allocated by WHO (WHO, 2004) for drinking water.

The use of the substance may also result in migration of aluminium into food. The potential exposure to Al for an adult weighing 60 kg can be estimated to be at the range of 4.4% of the TWI of 1 mg/kg bw/week set by the AFC Panel (EFSA, 2008).

DOCUMENTATION PROVIDED TO EFSA

Dossier referenced: AgION/efsa/100520. Dated: April 2010. Submitted on behalf of AgION Technologies Inc., USA.

Unpublished data submitted by the petitioner (May 2002 and December 2003 and September 2004).

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APPENDICES

APPENDIX A

DEFINITION OF THE SCF LISTS

The classification into a SCF_List is a tool used for tackling authorisation dossiers and do not prejudice the management decisions that will be taken on the basis of the scientific opinions of the CEF Panel and in the framework of the applicable legislation

List 0	Substances, e.g. foods, which may be used in the production of plastic materials and articles, e.g. food ingredients and certain substances known from the intermediate metabolism in man and for which an ADI need not be established for this purpose.
List 1	Substances, e.g. food additives, for which an ADI (=Acceptable Daily Intake), a t-ADI (=temporary ADI), a MTDI (=Maximum Tolerable Daily Intake), a PMTDI (=Provisional Maximum Tolerable Daily Intake), a PTWI (=Provisional Tolerable Weekly Intake) or the classification "acceptable" has been established by this Committee or by JECFA.
List 2	Substances for which this Committee has established a TDI or a t-TDI.
List 3	Substances for which an ADI or a TDI could not be established, but where the present use could be accepted. Some of these substances are self-limiting because of their organoleptic properties or are volatile and therefore unlikely to be present in the finished product. For other substances with very low migration, a TDI has not been set but the maximum level to be used in any packaging material or a specific limit of migration is stated. This is because the available toxicological data would give a TDI, which allows that a specific limit of migration or a composition limit could be fixed at levels very much higher than the maximum likely intakes arising from present uses of the additive. Depending on the available toxicological studies a restriction of migration into food of 0.05 mg/kg of food (3 mutagenicity studies only) or 5 mg/kg of food (3 mutagenicity studies plus 90-day oral toxicity study and data to demonstrate the absence of potential for bio-accumulation in man) may be allocated.
List 4	**(for monomers)**
4A	Substances for which an ADI or TDI could not be established, but which could be used if the substance migrating into foods or in food simulants is not detectable by an agreed sensitive method.

4B	Substances for which an ADI or TDI could not be established, but which could be used if the levels of monomer residues in materials and articles intended to come into contact with foodstuffs are reduced as much as possible.
List 4	**(for additives)** Substances for which an ADI or TDI could not be established, but which could be used if the substance migrating into foods or in food simulants is not detectable by an agreed sensitive method.
List 5	Substances that should not be used.
List 6	Substances for which there exist suspicions about their toxicity and for which data are lacking or are insufficient. The allocation of substances to this list is mainly based upon similarity of structure with that of chemical substances already evaluated or known to have functional groups that indicate carcinogenic or other severe toxic properties.
6A	Substances suspected to have carcinogenic properties. These substances should not be detectable in foods or in food simulants by an appropriate sensitive method for each substance.
6B	Substances suspected to have toxic properties (other than carcinogenic). Restrictions may be indicated.
List 7	Substances for which some toxicological data exist, but for which an ADI or a TDI could not be established. The required additional information should be furnished.
List 8	Substances for which no or only scanty and inadequate data were available.
List 9	Substances and groups of substances which could not be evaluated due to lack of specifications (substances) or to lack of adequate description (groups of substances). Groups of substances should be replaced, where possible, by individual substances actually in use. Polymers for which the data on identity specified in "SCF Guidelines" are not available.
List W	"Waiting list". Substances not yet included in the Community lists, as they should be considered "new" substances, i.e. substances never approved at national level. These substances cannot be included in the Community lists, lacking the data requested by the Committee.

APPENDIX B

TERMS USED RELEVANT TO MIGRATION:

Overall migration: The sum of the amounts of volatile and non volatile substances, except water, released from a food contact material or article into food or food simulant

Specific migration: The amount of a specific substance released from a food contact material or article into food or food stimulant

ABBREVIATIONS

AFC	Scientific Panel on additives, flavourings, processing aids and materials in contact with food
bw	Body weight
CAS	Chemical abstracts service
CEF	Scientific Panel on food contact materials, enzymes, flavourings and processing aids
EC	European Commission
EFSA	European food safety authority
FCM	Food Contact Material(s)
LDPE	Low density polyethylene
NOAEL	No observed adverse effect level
PET	Poly(ethylene terephthalate)
REF No	Reference Number
SCF	Scientific Committee on food
TWI	Tolerable weekly intake
WHO	World health organisation
w/w	Weight by weight

Ercofid

Silver chloride preparation for the removal of persistent sulphide off-flavours

Product Description

Ercofid is a silver chloride preparation applied on an inert carrier.

Aim of treatment is the removal of any kind of sulphide off-flavours or similar disagreeably smelling off-odours in wine caused by sulphurous components, as for instance, hydrogen sulphide (H_2S), disulphides, mercaptans or thioacetates. Particularly with the treatment of persistent sulphide off-flavours Ercofid has proved to be highly efficient.

Ercofid is very well distributed in the wine which assures intensive contact and a short reaction time.
Due to the high selectivity of silver chloride modifications of the wine aroma are largely excluded. Independent of the pH-value, silver chloride is very slightly soluble thus the risk of residues in wine is very low, even when overdosed. Provided the wine is filtrated after treatment.

Ercofid complies with the current purity requirements of laws and regulations relevant for the treatment of wine. Parameters of importance are proved by laboratory quality control.

Dosage

Pretests must absolutely be conducted to determine the correct amounts to be used.

Application	Dosage
Slight to moderate sulphide off-flavours	20 g/100 L
Persistent sulphide off-flavours	30 - 50 g/100 L
Maximally permitted addition (EU legislation)	50 g/100 L

Ercofid is directly added to the prefiltrated wine. Mix thoroughly to provide for uniform distribution. Stir the wine again after a few hours. Separation is conducted by filtration two days after application at the earliest. If Ercofid is applied properly and pretests are carried through correctly, no silver residues of relevance are to be expected in the wine due to the poor solubility of silver chloride. Under these conditions a blue fining is not required.

Storage

Protect from foreign odours and moisture. Since Ercofid is sensitive to light, store in a dark place. Reseal opened packagings immediately and tightly.

ERBSLÖH Geisenheim AG, Erbslöhstraße 1, 65366 Geisenheim, Germany, Tel: +49 6722 708-0
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INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

WORLD HEALTH ORGANIZATION

SUMMARY OF TOXICOLOGICAL DATA OF CERTAIN FOOD ADDITIVES

WHO FOOD ADDITIVES SERIES NO. 12

The data contained in this document were examined by the
Joint FAO/WHO Expert Committee on Food Additives*
Geneva, 18-27 April 1977

Food and Agriculture Organization of the United Nations
World Health Organization

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SILVER

EVALUATION FOR ACCEPTABLE DAILY INTAKE

BIOLOGICAL DATA

BIOCHEMICAL ASPECTS

Silver does not occur regularly in animal and human tissues but is present in man's environment in air, water, soils and food as well as in specific products. In some marine species silver tends to accumulate in soft tissue. The shells and soft tissues of approximately 50 oysters (Crassostrea virginica Gmelin) analysed were for silver and other elements. The oysters were collected from 10 stations of various salinity ranges along the Georgia coast. Analysis was carried out by atomic absorption spectrophotometrically. The precision of the analysis was about ±5. Silver was below detectability in the shells (i.e. below 1 ppm) while the soft tissues was 28-82 (±10-20) ppm (Casarett and Doull, 1975; Windom and Smith, 1972).

Silver can be absorbed by the gastrointestinal tract. Retention is apparently greatest in the reticulo-endothelial organs. After intravenous injection the concentrations were present in decreasing order in spleen, liver, bone marrow, lungs, muscle and skin (Browning, 1969).

Various studies and clinical observations indicate that silver salts can be absorbed from the lungs, gastrointestinal tract and such insured epithelia as nasal mucosa, conjunctiva, and skin. Absorbed silver is then stored in the reticulo-endothelial cells of the skin, mucous membranes, liver, spleen, possibly bone marrow, in basement membranes, especially those of the renal glomerulus, and presumably in muscles (Ham and Tangue, 1972; Kanai et al., 1976; Bader, 1966; Anderson, 1966; Voldrich et al., 1975).

Radiosilver (^{110m}Ag) administration to mice, rats, monkeys and dogs by oral intravenous and intraperitoneal routes was excreted for more than 90% in the faeces, 90% or more of oral doses were not absorbed. Whole body retention in mice, rats and monkeys was less than 1% of the initial dose after one week. In the same period less than 10% was retained in dogs (Fnrchner et al., 1968).

The major route of excretion is via the gastrointestinal tract, predominantly through desquamation of silver containing cells of the alimentary tract. Urinary excretion has not been reported to occur even after intravenous injection (Casarett and Doull, 1975; Kent and Mc Cance, 1941). It seems that even mild degrees of liver damage considerably impair the ability of the liver to excrete quite small doses of silver (Petering, 1976). Unlike lead or mercury there is no evidence that silver is a cumulative poison (Petering, 1976).

No information was obtained on the biotransformation of silver in the animal body except that absorbed ionic silver is transformed into metallic while being deposited in tissues (Petering, 1976).

Numerous enzymes were inhibited in vitro by silver ions. High affinity to sulfhydryl and histidine imidazole groups was observed. Silver ions compete with molecular oxygen as hydrogen acceptor, resulting in inhibition of glucose oxydase (Nakamura and Ogura, 1968).

Protargol, a silver-protein complex containing 8% silver inhibited the in vitro prostaglandin E_2 synthesis by bull geminal vesicles even at concentrations of $10^{-7}M$ (Deby et al., 1973).

Glutathione peroxydase activity in the liver of rats treated with 76 and 751 ppm silver (as silver acetate) for seven weeks was respectively 30% and 4% of the control values (Swanson et al., 1974).

After a single s.c. injection (3 mg silver/kg bw) $AgNO_3$ induced the synthesis of a low molecular weight protein in the liver of rats, with the characteristics of metallothionein induced by cadmium, zinc or mercury salts (Winge et al., 1975).

Silver ion is a very toxic substance when viewed from the standpoint of its action of an inhibitor of enzymes and as a metabolic inhibitor of lower forms of life. Biochemically, the silver ion (Ag+) can act as potent enzyme inhibitor (Chambers et al., 1974). It has been reported (Wagner et al., 1975) that in vitro administration of silver dramatically decreased liver glutathione peroxidase in rats fed Se-supplemented diets with or without vitamin E. It seems therefore that silver acetate exerts its antagonistic effects on Se (silver induces Se deficiency signs) through an effect on the activity of biosynthesis of glutathione peroxidase.

Much of the biologic action of silver can be attributed to the reaction of silver ion with sulfhydryl groups to produce stable silver mercaptide (Petering, 1976).

Cooper and Jolly (1970) in a review of the ecologic effects of

silver have pointed out that the current experimental practice of seeding clouds with silver iodide to promote rainfall may lead to new hazards for both man and natural biologic systems if the practice is extended (Petering, 1976).

TOXICOLOGICAL STUDIES

Special studies on carcinogenicity

Sarcomas, malignant fibrosarcomas, fibromas, fibro-adenomas and invasions of muscle with corrective tissue were observed after implantation of foil, platelets and pellets made of silver or dental alloy under the skin of mice and rats (Oppenheimer et al., 1956; Shubik and Hartwell, 1969).

Special studies on mutagenicity

No DNA damaging capacity was observed in a recombination-assay with AgCl in a Bacillus Subtilis strain (Nishioka, 1975).

Acute toxicity studies

Oral administration of 50 mg $AgNO_3$/kg bw to mice caused death in 50% of the animals in a 14 day observation period (Goldberg et al., 1949).

Intraperitoneal administration of 2 ml of an aqueous solution containing 0.239 M $AgNO_3$ to guinea pigs (0.216 g $AgNo_3$/kg bw) was fatal in 6/10 animals after seven days (Wahlberg, 1965).

Intraperitoneal injection of 20 mg $AgNo_3$/kg bw in rabbits caused death accompanied by degeneration of liver parenchyma and kidney tubules. Silver granules were observed in these organs (La Torraca, 1962).

Subcutaneous injection of 7 mg $AgNO_3$/kg bw to rats affected testis histology and spermatogenesis. After 18 hours the peripheral tubules were affected and some central tubules were completely degenerated. Some tubules recovered but not the duct system (Hoey, 1966).

A single dose of 500 mg of colloidal silver was lethal to dogs in 12 hours (Shouse and Whipple, 1931). Prior to death there was anorexia, weakness, loss of weight, and anaemia. Death was due to pulmonary congestion and oedema,

Short-term studies

Rats

Rats (90-100 g) were given a 0.25% solution of $AgNO_3$ in distilled water as drinking water for a period ranging from 1 to 12 weeks. Rats were killed at 1, 2, 3, 4, 8 and 12 weeks and at 1, 2, 3, 6, 10 months and also 16 months after silver administration had

stopped. Deposition of silver in the glomerular basement membrane was noticed one week after the initiation of treatment electron microscopically (Ham and Tange, 1972).

1500 ppm Ag^1 (as acetate) in drinking water for two to four weeks caused liver necrosis and death in vitamin E deficient rats. The effect was prevented by 120 ppm D- -tocophirylacetate and partially by 1 ppm Se (Diplock et al., 1967).

Addition of silver acetate to the diet (130-1000 ppm) or drinking water (1500 ppm) of weaning rats fed a vitamin E deficient diet, precipitated a rapidly fatal hepatocellular necrosis and muscular dystrophy on day 14 of the treatment or subsequently. No changes were observed in liver of rats given silver acetate and vitamin E supplements. The mitochondrial changes possessed some of the features seen in rats fed a diet deficient in vitamin E and selenium. A reduced availability of selenium by silver in vitamin E deficient rats is postulated (Grasso et al., 1969).

Rats fed a casein-based diet were given 0.76 and 751 ppm silver (as acetate) in drinking water for a period of seven weeks. Dietary Se (0.5 ppm as Na_2SeO_3) prevented growth depression observed in rats receiving 76 ppm silver and markedly improved growth and survival of those given 751 ppm, but increased liver and kidney silver levels. Liver glutathione peroxidase activity of the treated groups supplemented with selenium was respectively 30% and 4% of the controls. Glutathione peroxidase of erythrocytes was not affected (Swanson et al., 1974).

Cyanocabalamine (3 ppm), vitamin E and selenium (0.05 and 1 ppm) were found to antagonize silver-induced liver necrosis in rats (Bunyan et al., 1968).

Rats (six per group) were treated with drinking water containing 0.5, 2 and 20 mg Ag/l for 6-12 months. 2 mg Ag^+/l decreased the nucleic acid level in brain and liver after one year and 20 mg Ag^+/l increased RNA and DNA contents of the brain after six months and caused dystrophic changes in the brain accompanied by a decrease in nucleic acid level after 12 months. The liver was less sensitive towards silver than the brain (Kharchenko et al., 1973).

Groups of eight rabbits received 0, 0.00025, 0.0023, 0.025 and 0.25 mg Ag/kg via their drinking water during 11 months. Marked effects on immunological capacity (measured as phagocytosis) and histopathological changes of nervous, vascular and glial tissue of the encephalon and medulla were observed in the groups receiving 0.025 and 0.25 mg Ag/kg bw. Treatment had no effects on haemoglobin, R.B.C., differential W.B.C., proteinogenic function of the liver and serum SH groups. Rats treated with same amounts of silver showed affected conditioned reflexes (Barkov and El piner, 1968).

Groups of 20 chicks received 0, 10, 25, 50, 100 and 200 ppm silver during four weeks in combination with 0, 10 or 25 ppm copper in the diet. Silver at 100 ppm reduced growth in the copper deficient but not in the control chicks. At 50 ppm mortality was increased in the copper deficient group, but not in those receiving copper. 10 ppm silver reduced the haemoglobin concentration and the elastin content in the aorta in deficient chicks. These effects were completely overcome by the addition of copper to the diet (Hill and Matrone, 1970),

Turkey poults given dietary silver (900 ppm of added silver nitrate) exhibited reduced body weight gain, haemoglobin, packed cell volume, and aortic elastin content, as well as significantly increased ratio of wet heart weight to body weight. The enlarged hearts were attributed to a copper deficiency induced by the dietary silver. Adding extra copper offset the silver-induced condition (Peterson et al., 1973; Jensen et al., 1974),

OBSERVATION IN MAN

Absorption of silver resembles whole body retention. It is retained in all body tissues (Hamilton et al., 1972a; Tripton et al.,

1966). The silver content of the miocardium, aorta and pancreas tends to decrease with age (Bala et al., 1969) although the amount of silver in the body increases with age (Hill and Pillsbury, 1939). The concentration of silver in healthy human tissues from the United Kingdom was 1-9 µg/kg ash was found. The average silver contents in wet tissue of normal Americans was about 0.05 µg/kg (Tripton, 1963).

The intake from the diet is estimated at 27 µg/day (Hamilton and Minski, 1972) up to 88 µg/day (Kehoe et al., 1940).

Silver toxicity is manifested in a variety of forms, some proven others suspected. Proven forms include: argyria, gastrointestinal irritation, renal and pulmonary lesions. Suspected forms include, among others (ill-defined) arteriosclerosis (Casarett and Doull, 1975).

Argyria denotes the slate blue colour observed in parts of the body of persons exposed chronically to silver (Anderson, 1966). Epidemiologically, two types of argyria are recognized: industrial argyria and iatrogenic argyria.

Regardless of type there are two forms of argyria, local and generalized. The local form involves the formation of grey blue patches on the skin or may manifest itself in the conjunctiva of the eye. In generalized argyria the skin shows widespread pigmentation, often spreading from the face to most uncovered parts of the body. In some cases the skin may become black with a metallic lustre. Heavy

pigmentation of the eye structures can interfere with vision (Casarett and Doull, 1975). Except for this adverse effect argyria is solely a cosmetic problem. The slate blue colour of argyria is not entirely due as one might suspect, to the deposition of metallic silver (Petering, 1976), but largely to an increased deposition of melanin. Silver has a melanocyte-stimulating property (Rich et al., 1972). Cases of generalized argyria have occurred after ingestion or chronic medicinal application of gram quantities of silver. Silver was absorbed during prolonged (nine months) nasal application of Targesine (silver solution). It was calculated that during this time 7000 ml of solution containing 210 g silver had been used (Voldrich et al., 1975).

After chronic medical and occupational exposure to silver, argyria and argyrosis are the most common findings. Although intravenous administration of a total of 0.91-7.6 g (average 2.39) silver as silver arsphenamine in a period of two to nine years has caused argyria, hundreds of patients have received up to 1.7 g Ag (as arsphenamine) without developing argyria.

In argyria silver is regularly deposited in blood vessels, connective tissue, skin, glomeruli of the kidney, choroid plexus, mesenteric glands and thyroid. Adrenals, lungs, dura mater, bones, cartilage muscle and nervous tissue are minimally involved as deposition sites for silver.

In workers argyrosis of the cornea may be accompanied by turbidity of the anterior lens capsule and disturbance of the dark adaptation, usually not resulting in loss of vision.

Argyria is observed only in connexion with occupational medical exposure or after cosmetic application of silver (Hill and Pillsbury, 1939).

The systemic effects of silver are not extensive because of the poor absorption of silver compounds from the intestinal tract (Petering, 1976). It is considered that 10 g of silver nitrate taken orally is a lethal dose of man, although recovery from smaller doses

has been reported (Cooper and Jolly, 1970). The systemic effects of a lethal dose are preceded by severe haemorrhagic gastroenteritis and shock. According to Goodman and Gilman (1965) the silver ion seems first to stimulate and then depress structures in the brain stem. Central vasomotor stimulation results in a rise in blood pressure. At the same time there is bradycardia due to central vagal stimulation. Death eventually results from respiratory depression.

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See Also:
Toxicological Abbreviations
Silver (ICSC)
SILVER (JECFA Evaluation)