Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

Silver zinc zeolite (Zeolite, LTA1 framework type, surface-modified with silver and zinc ions)

EC Number: -

CAS Number: 130328-20-0

CLH-O-0000001412-86-90/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

4 December 2015

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Substance Name:

Silver zinc zeolite (Zeolite, LTA framework type, surface modified with silver and zinc ions)

EC Number: -

CAS Number: 130328-20-0

Index Number: -

Contact details for dossier submitter:

Swedish Chemicals agency

Version number: 4 **Date:** 13th April 2015

CONTENTS

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Silver zinc zeolite*** **(**Zeolite, LTA[1] framework type, surface-modified with silver and zinc ions) This entry covers LTA framework type zeolite which has been surface-modified with both silver and zinc ions at contents Ag 0.5%-6%, Zn 5%-16%, and potentially with phosphorus oxides, NH4+, Mg2+ and/or Ca2+ each at level <3%
EC number:	
CAS number:	***130328-20-0***
Annex VI Index number:	
Degree of purity:	***>99%***
Impurities:	***No impurities present at ≥1% and none of the impurities present at lower levels are considered relevant for the classification of the substance***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	-	-
Current proposal for consideration by RAC	Skin Irrit. 2; H315 (causes skin irritation) Eye Dam. 1; H318 (causes serious eye damage) Repr. 1B; H360D (may damage the unborn child) Carc. 2; H351	

[1] LTA = Linde type A

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
	(suspected of causing cancer) STOT RE 2; H373 (may cause damage to organs through prolonged or repeated exposure.) Aquatic Acute 1; H400; M-factor = 100; (Very toxic to aquatic life) Aquatic Chronic 1; H410; M-factor = 100; (Very toxic to aquatic life with long lasting effects)	
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin Irrit. 2; H315 (causes skin irritation) Eye Dam. 1; H318 (causes serious eye damage) Repr. 1B; H360D (may damage the unborn child) Carc. 2; H351 (suspected of causing cancer) STOT RE 2; H373 (may cause damage to organs through prolonged or repeated exposure.) Aquatic Acute 1; H400; M-factor = 100; (Very toxic to aquatic life) Aquatic Chronic 1; H410; M-factor = 100; (Very toxic to aquatic life with long lasting effects)	

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				Data lacking
2.2.	Flammable gases				Data lacking
2.3.	Flammable aerosols				Data lacking
2.4.	Oxidising gases				Data lacking
2.5.	Gases under pressure				Data lacking
2.6.	Flammable liquids				Data lacking
2.7.	Flammable solids				Data lacking
2.8.	Self-reactive substances and mixtures				Data lacking
2.9.	Pyrophoric liquids				Data lacking
2.10.	Pyrophoric solids				Data lacking
2.11.	Self-heating substances and mixtures				Data lacking
2.12.	Substances and mixtures which in contact with water emit flammable gases				Data lacking
2.13.	Oxidising liquids				Data lacking
2.14.	Oxidising solids				Data lacking
2.15.	Organic peroxides				Data lacking
2.16.	Substance and mixtures corrosive to metals				Data lacking
3.1.	Acute toxicity - oral				Conclusive but not sufficient for classification
	Acute toxicity - dermal				Conclusive but not sufficient for classification
	Acute toxicity - inhalation				Conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Skin Irrit. 2 H315	-	-	

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.3.	Serious eye damage / eye irritation	Eye Dam. 1 H318	-		
3.4.	Respiratory sensitisation				Data lacking
3.4.	Skin sensitisation				Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity				Inconclusive
3.6.	Carcinogenicity	Carc. 2 H351		-	
3.7.	Reproductive toxicity	Repr. 1B H360D		-	
3.8.	Specific target organ toxicity – single exposure				Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	STOT RE 2 H373		-	
3.10.	Aspiration hazard				Data lacking
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1 H400 Aquatic Chronic 1 H410	M-factor = 100 M-factor = 100	-	
5.1.	Hazardous to the ozone layer				Data lacking

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Pictogram: 

Signal word: Danger

Pictogram codes: GHS08, GHS05, GHS09
Hazard statements: H318, H315, H373, H360D, H351, H410
Precautionary statements: Responsibility of the applicant.

Proposed notes assigned to an entry:

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Classification and labelling of silver zinc zeolite has not been previously discussed.

A competent authority report on silver zinc zeolite including a hazard assessment and a classification proposal has been prepared within the review programme for biocides under 98/8/EC (Biocides directive) and this report is currently being reviewed by other EU Member States and the applicant.
The active chemical entity in silver zinc zeolite is the silver ion released during use. Silver ions are effective against a broad spectrum of microorganisms (e.g. Gram-positive and Gram-negative bacteria, moulds/fungi and yeasts). The type of silver zinc zeolites considered in the competent authority report and in this report (i.e. IRGAGUARD B 502i and AgION® Silver Antimicrobial Types AK and AJ) are incorporated into polymers or compounded into coatings which in turn may potentially be used in a vast range of consumer applications.
A first discussion of the toxicological section of the report was held at the Technical Meeting for Biocides in June 2013. During this meeting it was decided that the proposal for classification with respect to irritation and with respect to reproductive toxicity should be forwarded to the Committee for Risk Assessment without further input from the Technical Meeting. It was also agreed that an electronic consultation between EU Member States should be conducted in order to decide if a new carcinogenicity study should be requested considering that the existing study suffered from deficiencies such as lack of GLP compliance and lack of a sufficiently high dose group. SE being the RMS received a response from six Member States. Only one Member State favoured a request for further testing but there was no consensus whether or not to support a proposal for classification with respect to carcinogenicity in category 2.
A majority of Member States supported a request for further *in vivo* mutagenicity data to clarify if the positive results observed *in vitro* would manifest *in vivo*. Such study would replace the existing *in vivo* genotoxicity study which failed to demonstrate exposure of the target system. However, in case the proposal for classification as Reprotoxic in category 1b is accepted and agreed by the European Commission, one of the exclusion criteria in the biocides regulation (BPR) will be fulfilled and the substance will not be approved for use as a biocide on the European market. In that case, the in vivo genotoxicity study would become superfluous. Therefore, for animal welfare reasons, conducting the in vivo genotoxicity study is pending the decision on classification with respect to reprotoxicity.

2.2 Short summary of the scientific justification for the CLH proposal

Physico-chemical hazards

No experimental data are available in relation to physico-chemical hazards except for data on boiling point (>350°C). However based on the composition and nature of the substance (purely inorganic stable complex) it can be concluded that silver zinc zeolite shall not be classified as an explosive under CLP.

Moreover, based on the nature of the substance (purely inorganic stable complex) testing of flammability can be waived under CLP and it shall thus not be classified for this hazard.

In conclusion no classification for physico-chemical hazards are proposed under DSD for silver zinc zeolite based on the nature of the substance and experience in use (i.e. safely handled in various testing in water and air).

Human health hazards

There may be different types of silver zinc zeolites commercially available but the types considered in this report are denoted Irgaguard B 502i, AgION Antimicrobial Type AJ and AgION Antimicrobial Type AK. The differences in chemical composition between these types are not expected to have a significant impact on the toxicological properties and they are thus assumed to be toxicologically equivalent. Irritation studies performed with AgION Antimicrobial Type AK demonstrated irreversible erythema and oedema in rabbits during the 14 day observation period of the skin irritation study and moderate to severe redness of conjunctiva in rabbits that for one rabbit persisted during the entire 21 day observation period of the eye irritation study.

Silver zinc zeolite is thus considered to meet the criteria for classification with respect to irritation:
Skin. Irrit. 2; H315 (causes skin irritation)
Eye Dam. 1; H318 (causes serious eye damage)

Silver zinc zeolite was clastogenic when tested *in vitro* but in the absence of reliable *in vivo* data, it is presently not possible to decide if silver zinc zeolite fulfils the criteria for classification in Category 2 (CLP).

Results of a chronic toxicity/carcinogenicity study in rat indicate that silver zinc zeolite AgION Antimicrobial Type AJ causes statistically significant positive trends for leukaemia in both sexes and pituitary adenomas in females. The tumour types could not be dismissed as it is considered unlikely that positive trends, in which all doses are considered, appear by chance and there was no reason to disregard results in the concurrent controls. If the substance has an ability to promote initiated cells into tumours, an increased frequency of those tumour types common to the strain used seems reasonable.

Silver zinc zeolite is thus proposed to be classified
Carc 2; H351 (suspected of causing cancer)

Silver zinc zeolite treatment during mating, gestation and lactation resulted in a reduced bodyweight gain in F1 pups (and a subsequent delay in day of vaginal opening and preputial separation), a decrease in livebirth index, increase in stillborn index and reduced bodyweights in F2 pups. The results are supported by existing information indicating similar effects, to a varying extent, for example in a developmental toxicity study with silver acetate in rats and a published study with silver chloride. The latter clearly demonstrates developmental toxicity in rats.

Silver zinc zeolite is thus proposed to be classified
Repr.1B H360D (may damage the unborn child)

Discoloration of organs and tissues was observed in all studies with repeated exposure to silver zinc zeolite. In the chronic/carcinogenicity study") in rats it was observed at a dose of 0.1 % (stated to correspond to "at least 30 mg/kg bw) and in the same study in mice it was observed at the lowest dose tested (0.1% corresponding to "at least 67 mg/kg bw"). It was also observed in the two-generation study in rats at the lowest dose in parents as well as offspring (72/87 mg/kg bw (pre-mating)). Despite existing uncertainty whether or not pigmentation of organs and tissues can be associated with major functional changes, an irreversible accumulation of a heavy metal in organs and tissues is considered an undesired effect. Since accumulation of silver in organs and tissues has

been observed at a dose level that lies within the guidance range (adjusted from 10< C≤ 100 mg/kg bw to 5< C≤ 50 mg/kg bw for 6 month exposure) for STOT-RE, category 2.

Silver zinc zeolite is thus proposed to be classified **STOT RE 2; H373 (may cause damage to organs through prolonged or repeated exposure.)**

Environmental hazards

Silver zinc zeolite is in acute tests toxic to fish and inhibits growth of algae. No reliable test results are available for crustaceans and other taxonomic groups. No chronic toxicity data are available for silver zinc zeolite.

Silver zinc zeolite is a metal compound, but T/D protocol results are not available.

For the purpose of classification for environmental hazards, silver zinc zeolite is considered to be readily soluble. Zinc and silver are environmentally relevant, but silver is considered to be the most relevant compound released.

Using available data generated with soluble silver salts, silver zinc zeolite is proposed to be classified as

Acute (short-term) aquatic hazard: category Acute 1, M-factor: 100.

Long-term aquatic hazard: category Chronic 1, M-factor: 100

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

There is currently no classification and labelling for silver zinc zeolite in Annex VI, Table 3.1 in the CLP Regulation.

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

There is currently no classification and labelling for silver zinc zeolite in Annex VI, Table 3.2 in the CLP Regulation.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

There is currently no self-classification and labelling proposed for silver zinc zeolite based on the CLP regulation criteria.

2.4.2 Current self-classification and labelling based on DSD criteria

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Not applicable for biocides.

RAC general comment
The CLH proposal from the Dossier Submitter (DS) covers three Linde Type A (LTA) framework zeolites which have been surface-modified with both silver (Ag) and zinc (Zn) ions with contents of Ag between 0.5% and 6% and of Zn between 5% and16%, and potentially with phosphorus, NH_4^+, Mg^{2+} and/or Ca^{2+}, each at a level < 3%. The data considered by the DS has been generated using different silver zinc zeolites (SZZ), AgION® Silver Antimicrobial Type AK, AgION® Silver Antimicrobial Type AJ and Irgaguard® types B5000 or B 502i. These are denoted AgION Type AK, AgION Type AJ and Irgaguard, respectively. These names are used throughout this opinion. An unspecified SZZ was used in some studies. The DS noted that it is similar to an Irgaguard type. Two other zeolites (HealthShield® and Zeomic®) are referred to in the CLH proposal. These are equivalent to AgION Type AK and AgION Type AJ, respectively. RAC notes that the group so referred to in this opinion could cover additional types of SZZ than the three active substances referred to above. SZZ are active substances with many different uses and applications. Most are incorporated into polymers, compounded into coatings or applied topically onto materials with the purpose of inhibiting growth of a variety of bacterial and fungal species in order to: i) protect humans against pathogens; ii) prevent deterioration of the physical properties or appearance of materials, or; iii) prevent development of undesired odours caused by microbial activity. SZZ belongs to a group of ion-exchange carriers with silver ions as the active chemical entity. Instead of dissolution, silver and zinc ions are released from the zeolite matrix during use. Zeolites are natural or synthetic alumino-silicates, regular in shape and highly porous adsorptive crystals composed of tetrahedrons centered on aluminum and silicon atoms and linked through the oxygen atoms at the apices of the tetrahedra. It is known that the active antimicrobial entity in common between the three SZZ is the silver (Ag^+) cation released during use. The zinc ion (Zn^{2+}) can also be exchanged with other anions but it plays a role as a chemical and colour stabiliser, reducing product discoloration, rather than for antimicrobial efficacy. The zeolites are considered as an inert matrix. The cation Ag^+ is highly effective in halting the growth of bacteria, likely through the binding of silver ions with thiols and disulfide groups in proteins and peptides associated with the permeable and sensistive microbial cell wall. **Justification for applying the strategy for readily soluble metal compounds** SZZ do not dissolve, instead, silver and zinc ions are released from the zeolite matrix during use. As the substance is an inorganic metal compound, Annex IV to the Guidance on the application of the CLP criteria version 4.1, June 2015 (CLP Guidance) applies. The Annex defines the different classification strategies to be applied to either metals or (readily or poorly soluble) metal compounds. It also describes the characteristics of metals as follows (see Annex IV.1): *"Metals, M^0, in their elemental state are not soluble in water but may transform to yield the available form. This means that a metal in the elemental state may react with the media to form soluble cationic or anionic products,*

and in the process the metal will oxidise, or transform from the neutral or zero state to a higher one." With regard to metal compounds it is stated in the guidance that *"in a simple metal compound, such as an oxide or sulphide, the metal already exist in an oxidised state, so that further metal oxidation is unlikely to occur when the compound is introduced into an aqueous medium."*

In the manufacturing process of SZZ, silver and zinc are introduced as metal salts, i.e. silver nitrate ($AgNO_3$) and zinc nitrate ($Zn(NO_3)_2$), respectively with no reduction step to change the oxidation state of the metal ions to elemental metal. As a result, silver and zinc are present in the zeolite matrix in their oxidised state (positively charged ions) and will be released by ion-exchange without further metal oxidation taking place. Indeed, neither silver nor zinc is present as Ag^0 or Zn^0 in the final material SZZ or during its uses.

During the RAC-35 plenary discussion, an industry representative questioned the environmental classification approach taken by RAC and stressed that the strategy for metals should have been followed (according to Annex IV.5.2 of the CLP guidance) given the clear definition of compounds (i.e. either a salt or an oxide) and based on the consideration that silver and zinc are present in SZZ in their metallic form.

It is understood by RAC that the description for metals as stated in Annex IV.1 to the CLP guidance is not met. The environmental classification of SZZ proposed by the DS and adopted in the RAC opinion is based on applying the strategy for readily soluble metal compounds (i.e. the DS considered SZZ as a readily soluble metal compound due to lack of data on water solubility of the compound in accordance with the CLP guidance, Annex IV.5.3) by re-calculating the toxicity of the silver ion derived in tests with soluble silver salts back to the toxicity of the entire compound (using the maximum silver content of 6%). The RAC is of the opinion that it is justified to classify SZZ using the strategy for metal compounds. Despite the fact that silver ions do not dissolve from SZZ but are released by ion-exchange, silver (and zinc) in SZZ is not present in its elemental state and therefore the description of metals as stated in Annex IV.1 of the CLP guidance is not met.

Justification for grouping SZZ

During public consultation, several Member States (MSs) asked to further elaborate the justification for the grouping of hazard data and doubted that this CLH proposal covers a single substance or entry for the purpose of the CLP Regulation, especially taking into consideration differences found for some human health endpoints.

According to the DS, there are many different types of SZZ commercially available. There are also various silver containing active substances (SCAS) notified under Directive 98/8/EC that were not included in the DS proposal and are thus not part of this RAC opinion (e.g. silver phosphate glass, silver copper zeolite, silver nitrate, etc).

Under Directive 98/8/EC, the gouping was considered by Member States during discussion of the SZZ draft Competent Authority Report (CAR) submitted by the evaluating Competent Authority at the Biocides Technical Meeting in June 2013 where it was agreed that read across was acceptable for the three types of SZZ based on the assumption that the common toxic moiety is the silver ion (Ag^+). However, discussion of the possibility to read across between other SCAS was deferred until RAC had provided its opinion on the harmonised classification of the three SZZ proposed by Sweden.

According to confidential reports submitted with the CLH dossier, the release rates of silver and zinc over a fixed period of time under physiological conditions are relatively comparable between the three SZZ. The other data provided in the CLH report and other documents (content of silver, zinc, calcium, magnesium, ammonium ions, particle and pore size) do not suggest that there would be any difference in bioavailability or

toxicokinetics and therefore in systemic toxicity. Although the toxic moiety appears to be the silver ion for systemic endpoints, it is likely that both the zinc ion and the zeolite structure play a role in the bioavailability, toxicokinetics and toxicity of the silver ion.

An additional argument in favour of grouping the three SZZ considered in this opinion is that a pattern of systemic toxic effects consistently appeared in most of the repeated dose toxicity studies (see the Table in the STOT-RE section). RAC noted that the differences among the three SZZ reported for local toxicity might be attributed to differences in experimental conditions (i.e., the vehicle for application of the material, which might determine the silver and zinc release; rinsing or not rinsing the exposed area, etc.) or to different matrix effects.

In conclusion, based on several lines of evidence, **the grouping of SZZ is supported by RAC.** It is unknown whether the zinc and zeolite components might also contribute to the toxicity, in particular after inhalation of the zeolite which is inert and insoluble matrix.

As seen in the overview table below, none of the three types of SZZ is accompanied by a complete set of data. However, it is notable that all human health hazards were assessed on the basis of at least one study with SZZ AgION Type AK (the worst case), except carcinogenicity, which was assessed using SZZ AgION Type AJ. Indeed, several hazard classes lacked experimental studies for one or two of the three silver zinc zeolites. The grouping is further discussed and justified for each hazard class separately.

Table 1 (RAC): Hazard-specific studies using SZZ AgION Type AK and/or SZZ AgION Type AJ

Endpoint/hazard class	AgION Type AJ	AgION Type AK	Irgaguard	Unspecified silver zinc zeolite*	DS proposal for silver zinc zeolites
Phys-chemical properties	x	x	x		No C&L
Acute toxicity (oral, dermal, inhalation)	x	x		x	No C&L
STOT SE					No C&L
Skin irritation	x	x	x		**Skin Irrit. 2**, H315
Eye Irritation		x	x		**Eye Dam. 1**, H318
Skin sensitisation	x	x	x		No C&L
STOT RE		x			**STOT RE 2;** H373
Mutagenicity		x	x		No conclusion
Carcinogenicity	x				**Carc 2;** H351
Reproductive toxicity		x			**Repr. 1B;** H360D
Other endpoints					No conclusion

Note: x, study available for the endpoint and the type/form of SZZ; C&L, classification and labelling
* The unspecified SZZ is assumed to be very similar to Irguaguard B502i

Justification for not applying specific concentration limits (SCLs)

During public consultation, a Member State (MS) proposed to set specific concentration limits (SCLs) for all hazard classes. The rationale was that SZZ, which are part of the SCAS family, may have different silver content and release rates. The MS suggested to take into account such differences in potency and additivity by setting SCLs. The DS responded that according to the CLP guidance on the application of the CLP criteria,

additivity is not applicable to most of the hazard classes. In addition, setting SCLs above the GCL could be considered but the DS doubted that this would be possible from the limited data available for most endpoints. RAC is also of the opinion that SCLs could not be derived due to the lack of adequate data. In addition, the silver (local or systemic) exposure will be dependent upon many variables including silver content and release, which in turn depends on several factors including the zeolite structure, the type of ions present, the pore size, the surface and internal chemical modifications, the physiological environment etc.

RAC concludes that based on the physico-chemical similarity of the SZZ in this opinion and the nature and quality of the available experimental studies, it is considered justified to not set SCLs or to address potency considerations.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	
EC name:	Zeolites, AgZn
CAS number (EC inventory):	130328-20-0
CAS number:	130328-20-0
CAS name:	Zeolites, AgZn
IUPAC name:	Silver zinc zeolite **(**Zeolite, LTA[2] framework type, surface-modified with silver and zinc ions) This entry covers LTA framework type zeolite which has been surface-modified with both silver and zinc ions at contents Ag 0.5%-6%, Zn 5%-16%, and potentially with phosphorus oxides, NH4+, Mg2+ and/or Ca2+ each at level <3%
CLP Annex VI Index number:	
Molecular formula:	Generic (excluding additional ions/elements considered to be confidential business information): Ag_2O ZnO (Na_2O Al_2O_3 SiO_2)
Molecular weight range:	Not applicable to the generic definition (the specific molecular weight of the test material are disclosed in a separate confidential Annex)

Structural formula:

Not applicable

1.2 Composition of the substance

[2] LTA = Linde type A

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Zeolite, LTA[3] framework type, surface-modified with silver and zinc ions	>99%		

Current Annex VI entry:

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
No impurities present at ≥1% and none of the impurities present at lower levels are considered relevant for the classification of the substance (information on impurities are provided in a separate confidential Annex)	-	-	-

Current Annex VI entry:

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None	-	-	-	-

Current Annex VI entry:

1.2.1 Composition of test material

The data considered in this dossier has been generated using different distinct silver zinc zeolites (AgION Antimicrobial Type AJ and Type AK, Irgaguard B8000 (Bactekiller AZ) and Irgaguard B502i). The compositions of these materials are given in a separate confidential Annex to this report (see section 2.3 on page 12).

[3] LTA = Linde type A

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Solid	Doc IIIB 3.1-01 Shepler, (2001) Anonymous, (2006) Fiessler, (2007)	
Melting/freezing point	No melting point <350°C	Doc IIIA 3.1.1-01 Cunningham, (2001) Fiessler, (2007)	
Boiling point	Not relevant due to the high melting point		Valid justification
Relative density	Irgaguard B8000: 2.149	Doc IIIA 3.1.3-01 Fiessler, (2007)	
Vapour pressure	Not volatile due to the inorganic nature of the substance as also indicated by the high melting point (i.e. vapour pressure e $<10^{-5}$ Pa)		Valid justification
Surface tension	Not applicable as silver zinc zeolite is not soluble in water and as the material only releases inorganic ions in water		Valid justification
Water solubility	Not soluble with regards to the whole substance (substance as defined) With regards to silver and zinc dissolution: AgION Antimicrobial Type AJ (loading 10 mg/ml): <u>Silver (max conc.)</u> pH 5: 9.2 mg/l (after 29 days) pH 7: 2.9 mg/l (after 11 days) pH 9: 0.2 mg/l (after 35 days) <u>Zinc (max conc.)</u> pH 5: 467 mg/l (after 37 days) pH 7: 51 mg/l (after 37 days) pH 9: 0.5 mg/l (after 17 days) Irgaguard B8000 (loading 2 mg/ml): <u>Silver (max conc.)</u> pH 5 (phthalate buffer): 23.9 mg/l pH 7 (phosphate buffer): 0.02 mg/l pH 9 (borate buffer): 0.17 mg/l	Doc IIIA 3.5-01 Bussey, (2001) Meinerling and Herrmann, (2007) B3.5-04 (in Document III-A) O'Connor and Woolley, (2010)	

Property	Value	Reference	Comment (e.g. measured or estimated)
	AgION Antimicrobial Type AK (loading corresponding to a max release of 50 mg/L silver) Silver (% of theoretical max) pH 4, 37°C, phosphate buffer: 42.3% (3 h) 39.7% (6 h) 38.1% (12 h) 37.4% (18 h) 36.7% (24 h) 37.0% (72 h) 40.4% (168 h) pH 8, 37°C, phosphate buffer: 18.1% (3 h) 16.0% (6 h) 13.7% (12 h) 12.5% (18 h) 12.2% (24 h) 13.2% (72 h) 13.8% (168 h) Irgaguard B502i (loading corresponding to a max release of 50 mg/L silver) Silver (% of theoretical max) pH 4, 37°C, phosphate buffer: 15.9% (3 h) 22.6% (6 h) 33.9% (12 h) 37.4% (18 h) 37.4% (24 h) 24.1% (72 h) 17.6% (168 h) pH 8, 37°C, phosphate buffer: 0.93% (3 h) 17.8% (6 h) 22.0% (12 h) 22.7% (18 h) 17.1% (24 h) 16.7% (72 h) 14.6% (168 h)		
Partition coefficient n-octanol/water	Not applicable to an inorganic crystalline complex which is neither soluble in water nor in organic solvents		Valid justification
Flash point	Not applicable as the melting point is >40°C		Valid justification
Flammability	Silver zinc zeolite has no capacity to initiate or support combustion; all components are inorganic and non-pyrophoric. Based on the structure and experience in use it can be concluded that silver zinc zeolite is not flammable		Valid justification
Explosive properties	Based on structure and experience in use it can be concluded that silver zinc zeolite is not associated with an		Valid justification

Property	Value	Reference	Comment (e.g. measured or estimated)
	explosive hazard		
Self-ignition temperature	Silver zinc zeolite has no capacity to initiate or support combustion; all components are inorganic and non-pyrophoric. Based on the structure and experience in use it can be concluded that silver zinc zeolite is not flammable		Valid justification
Oxidising properties	Based on structure and experience in use it can be concluded that silver zinc zeolite is not associated with an oxidising hazard.		Valid justification
Granulometry	AgION Antimicrobial Type AJ: Particle size Median 2.5 to 2.8 µm. Min. 0.39 µm. Max. 23 µm. AgION Antimicrobial Type AK: Particle size Median 2.5 to 2.8 µm. Min. 0.39 µm. Max. 23 µm	Doc IIIB 3.11-01 Uchida, (2000)	
Stability in organic solvents and identity of relevant degradation products	No data available		Data not considered relevant as silver zinc zeolite as manufactured does not contain organic solvents and as silver zinc zeolite is not formulated in organic solvents.
Dissociation constant	Not applicable as silver zinc zeolite does not contain any acid or base functionality		Valid justification
Viscosity	Not applicable as silver zinc zeolite as manufactured is a solid with a melting point >40°C		Valid justification

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for hazard classification.

2.2 Identified uses

Silver zinc zeolites are incorporated into polymers, compounded into coatings or applied topically onto materials with the purpose to inhibit growth of a variety of bacterial and fungal species in order to

- protect humans against pathogens,
- prevent deterioration of the physical properties or appearance of materials, or
- prevent development of undesired odours caused by microbial activity.

The level of silver zinc zeolite included in polymers, coatings or applications is <5% by weight.

PT 2

Agion® Silver Antimicrobial Types AK and AJ: Walls, flooring, floor coverings. Car parts, shower curtains, mats, protective covers, tape, waste containers, brush handles, mops, vacuum cleaner bags, plumbing equipment including toilet seats, office equipment, personal care items (hair and tooth brushes, sports and dental mouth-guards), bathroom hardware, footwear, wire and cable insulation, indoor and outdoor furniture, spars, bathtubs, showers and filters.

IRGAGUARD B 5000 formulation is incorporated into plastic items such as door handles, toilet seats, tooth brushes, refuse bags, PVC flooring, PVC wall covers and air conditioning components.

PT 4

Agion® Silver Antimicrobial Types AK and AJ: Packaging, food and drink containers, food trays and covers, sponges, plastic film, food wrap kitchen utensils, cutting boards, cups, food and drink processing equipment. These are articles intended to come into contact with foodstuff.

Gaskets, general purpose containers, tubing, brush bristles, liners, non-woven fabrics, appliances and equipment, counter tops, sinks, tiles, dishes, bottles, conveyer belts, waste bags and bins, plastic film (other than for food packaging) are treated articles that – used in food handling or preparation - may unintentionally come into contact with food (see point suggested for discussion below). These articles - if used in an area where food is prepared – are reasonably being expected to come into contact with food or feed. It is currently not clear whether these uses fall under PT 4.

Agion® Silver Antimicrobial Types AK and AJ: Water filter housing and components, water bottle dispensers and components, water dispensers, ice machine trays, ice machine bins, ice machine water hoses, ice dispensers and other components, water bottles, cups, water storage vessels.

Silver zinc zeolite bonded to granular activated carbon (GAC): The GAC is used in the manufacture of water filters which are used to improve water quality. The antimicrobial effect of silver helps prolong the life of the filter.

PT 7

Agion® Silver Antimicrobial Types AK and AJ : polymer coating, film, laminate and paper.

IRGAGUARD B 5000 formulation is incorporated into laminates or other surface finishes which are applied, for example, to flooring, furniture and artificial leathers.

Polymers include films made of acrylic, latex, nylon, polyester, polyethylene, polypropylene, polystyrene, polyvinylchloride, rubber (natural & synthetic derivatives), urethane, and vinyl, and PMMA laminate /finishes applied to such materials.

PT 9

IRGAGUARD B 5000 formulation is incorporated directly into polymers used, for example, in sanitary applications, waste bins, bath tubs, sealants and fibres. Polymers include moulded parts, pellets or sheets made of polycarbonate, polyester, nylon, polyethylene, polypropylene, polystyrene,

polyvinylchloride, rubber (natural & synthetic derivatives), urethane and vinyl, and PMMA laminate /finishes applied to such materials.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
OECD 102 (melting point)	No melting or decomposition up to 350°C		Doc IIIA 3.1.1-01 Cunningham, (2001) Fiessler, (2007)

3.1 Physico-chemical hazards

3.1.1 Summary and discussion of physico-chemical hazards

Based on the nature of silver zinc zeolite (a purely inorganic complex) experimental data has only been generated for the boiling point. All other relevant physico-chemical parameters have been waived. It could be concluded that silver zinc zeolite complying with the generic definition is not highly flammable or explosive and that it does not possess oxidizing properties.

3.1.2 Comparison with criteria

Explosivity

Silver zinc zeolite complying with the generic definition does not contain any chemical groups associated with explosive properties, which is a sufficient data waiver under CLP. Silver zinc zeolite shall thus not be classified as an explosive under CLP.

Flammability

Under CLP there is a possibility to waive testing for inorganic stable substances. Based on the nature of the substance (a purely inorganic stable complex) it is thus possible to conclude that silver zinc zeolite should not be classified as a flammable solid under CLP (H228).

Moreover, based on the nature of silver zinc zeolite (a purely inorganic stable complex) and experience in use it can be concluded that it should not be classified as a “substance and mixture which, in contact with water, emit flammable gases” (H260 or H261). This is based on the nature of silver zinc zeolite (a purely inorganic stable complex) and the fact that it has been tested for water solubility.

Oxidizing properties

Under CLP it is stated that for inorganic substances testing can be waived if they do not contain oxygen or halogen. As silver zinc zeolite does contain oxygen it appears that waiving of testing does not apply. Nevertheless, based on the nature of silver zinc zeolite (a purely inorganic stable complex) it seems possible to conclude that it should not be classified as an oxidizing solid.

Other physico-chemical hazards

There is not sufficient information available to conclude on the classification of technical silver zinc zeolite under any other physico-chemical hazard classes in CLP. However, based on the nature of the substance (purely inorganic stable complex) and experience in use it seems possible to conclude that silver zinc zeolite shall not be classified as "self-reactive substances and mixtures" (H240, H241 or H242), "self-heating substances and mixtures" (H251 or H252) or "substances and mixtures corrosive to metals" (H290).

3.1.3 Conclusions on classification and labelling

No classification is proposed for technical silver zinc zeolite in relation to its physico-chemical properties based on the available data and information.

RAC evaluation of physical hazards
Summary of the Dossier Submitter's proposal The DS reported that only boiling point data has been generated, but taking into consideration that SZZ is a inorganic complex, all other relevant physico-chemical parameters can be waived. The DS proposed no classification in relation to physico-chemical hazards. **Comments received during public consultation** No comments were received during public consultation on this hazard class. **Assessment and comparison with the classification criteria** SZZ does not contain any chemical groups associated with explosive properties. Under CLP there is a possibility to waive testing for flammability of inorganic stable substances and thus it is can be concluded that SZZ should not be classified as a flammable solid. Moreover, based on the nature of SZZ it can be concluded that it should not be classified as a substance which, in contact with water, emits flammable gases. Also based on the nature of SZZ (a purely inorganic stable complex) and experience in its use, it seems possible to conclude that it should not be classified as an oxidising solid, nor as a self-reactive substance, self-heating substance, or substance corrosive to metals. In conclusion, RAC agrees with the proposal for no classification of SZZ regarding physico-chemical hazards.

4 HUMAN HEALTH HAZARD ASSESSMENT

Description of the data submitted:

The dossier received from the European Silver Task Force (ESTF) for review under the Biocidal Directive 98/8/EC (now replaced by the Biocides Regulation (EU) 528/2012) is a joint dossier including eight different silver containing active substances (SCAS) notified in the

review programme: elemental silver, silver chloride, silver glass, silver sodium hydrogen zirconium phosphate, silver zeolite A, silver zinc zeolite, silver nitrate and disilver oxide. The identity of some of these SCAS was revised during the evaluation process but as this has no impact on the evaluation of silver zinc zeolite it will not be further discussed in this report.

The hazard assessment presented in the biocide dossier submitted by the applicant is shared by all SCAS included. However, due to uncertainties associated with read-across (see below), it is considered scientifically justified and fair to prepare separate hazard assessments for each of the SCAS. These hazard assessments are, as far as possible, based on substance-specific data and read-across to other information available has only been applied in case a data gap for a certain endpoint has been identified. The ESTF has agreed to this proposal and separate biocide competent authority reports and consequently also classification reports are thus prepared for each SCAS.
Doc IIIA of the Competent Authority Report (CAR) which contains summaries of all studies submitted is regarded as a database of experimental studies, literature data, expert statements and published research from which information for a certain SCAS can be obtained.

The biocide CAR forming the basis for this CLH dossier is structured as follows:

- **Doc. I: Evaluation Report (including a proposal for decision on approval)**
- **Doc. II Risk Assessment:**
  - **Doc IIA: Effects assessment of active substance**
  - **Doc IIB: Effects and exposure assessment of biocidal product(s)**
  - **Doc IIC: Risk Characterisation for use of active substance in biocidal product(s)**
- **Doc III: Study Summaries**
  - **Doc IIIA: Active substance**
  - **Doc IIIB: Biocidal product(s)**

When studies in the study summary database are discussed in this CLH report, they are referred to as in Doc IIIA, i.e. with section number (annex point number). If there are several studies available for the same endpoint they are assigned separate numbers in brackets. The different section numbers (annex point numbers) of Doc IIIA are shown in table 11 below. These study summaries are accepted as confidential business information.
As stated above, there may be many different types of silver zinc zeolites commercially available but the hazard assessment in this report includes the types of silver zinc zeolites used in the representative formulations Irgaguard B 5000, AgION Antimicrobial Type AJ, and AgION Antimicrobial Type AK only. These are denoted Irgaguard B 502i, AgION Antimicrobial Type AJ and AgION Antimicrobial Type AK.
As seen in the overview table below, none of the three types of silver zinc zeolites is accompanied by a complete set of data. However, as discussed in the confidential attachment to section 13 of the technical dossier (Technical equivalence, technical specification and read-across), the differences in chemical composition between these types are not expected to significantly impact on the toxicological properties and these three types of silver zinc zeolites are thus considered to be toxicologically equivalent.
The equivalence of other types of silver zinc zeolites must be carefully assessed.
Read-across between SCAS: None of the SCAS was supported by a complete set of toxicological core data but data gaps were claimed to be filled by read-across to data obtained for a different

SCAS or from data available in the open literature. The applicant considered read across justified since the silver ion released from all SCAS should be viewed as the active biocidal substance. However, this read-across is complicated since different SCAS and different sub-types of the SCAS may have different chemical, physical and possibly also toxicological properties, in particular regarding potential effects of the carrier molecule and regarding the release of silver ions (and other metal ions). While it may be possible to identify a "worst case carrier" and use data obtained for this substance as "worst case" data for other SCAS, it is more difficult to manage the problem with not knowing the rates of silver release; the dose of silver that the animals were actually exposed to in the toxicological studies is consequently unknown.
If the studies had been conducted with a highly soluble salt it could have been acceptable to merely adjust effect levels for silver content but in this database, the data available is rather obtained from studies performed with substances of low solubility. Therefore, there is a concern that the true effect level of silver ions is underestimated if a LOAEL/NOAEL for silver equivalents is estimated from the silver content only. Consequently, it would not ensure protection from adverse health effects if applied to another SCAS having a similar silver content but a higher release.
In order to obtain further information regarding the real exposure levels in the toxicological studies, the applicant was asked for data on silver release from the different SCAS during conditions assumed to mimic physiological conditions. In 2010, the final report of a release study performed with different types of SCAS was submitted to the RMS. The results of this study show a variation in silver release between 2 and 42% of the maximum silver content for the different SCAS when tested at pH 4, 37°C (assumed to represent the conditions in the rat stomach and intestine). From this data, the actual exposure to silver ion equivalents has been estimated when read across between silver containing active substances was needed.
Silver zinc zeolite: For silver zinc zeolite, there is data available on almost all the different endpoints, at least for one of the three types of silver zinc zeolites. Read across to results obtained with other SCAS is thus generally not applied. Therefore, in contrast to the report within the review programme for biocides which contains the full data base on silver containing active substances, this report only includes the studies actually used for classification of silver zinc zeolite.

Table 11: Data available for the representative types of silver zinc zeolites

	Irgaguard B 502i	**AgION Antimicrobial Type AJ**	**Ag**ION**Antimicrobial Type AK**
6.1.1. Acute Oral toxicity	Yes*	Yes (in Doc IIIB)	Yes (in Doc IIIB)
6.1.2. Acute Dermal toxicity	Yes*	Yes (in Doc IIIB)	Yes (in Doc IIIB)
6.1.3. Acute Inhalation toxicity	Yes	No	Yes (in Doc IIIB)
6.1.4 Skin and eye irritation	Skin: Yes,Yes* Eye: Yes**	Skin:Yes (in Doc IIIB) Eye: No	Skin: Yes (in Doc IIIB) Eye: Yes (in Doc IIIB)
6.1.5 Skin sensitisation	Yes	Yes (in Doc IIIB)	Yes (in Doc IIIB)
6.2 Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study	No	No	No
6.3.1 Repeated dose toxicity (oral)	No	No	No
6.3.2 Repeated dose toxicity (dermal)	No	No	No
6.3.3 Repeated dose toxicity (inhalation)	No	No	No
6.4.1 Subchronic oral toxicity test	No	No	Yes
6.4.2 Subchronic dermal toxicity test	No	No	No
6.4.3 Subchronic inhalation toxicity test	No	No	No
6.5 Chronic toxicity	No	Yes	No
6.6.1 In-vitro gene mutation study in bacteria	Yes*	No	Yes
6.6.2 In vitro cytogenicity study in mammalian cells	Yes**	No	No
6.6.3 gene mutation assay in mammalian cells	Yes**	No	Yes
6.6.4 If positive in 6.6.1, 6.6.2 or 6.6.3, then an in-vivo mutagenicity study will be required...	Yes (inconclusive)	No	No
6.6.5 If negative in 6.6.4 but positive in-vitro tests then undertake a second in-vivo study…	No	No	No

	Irgaguard B 502i	**AgION Antimicrobial Type AJ**	**Ag**ION**Antimicrobial Type AK**
6.6.6 If positive in 6.6.4 then a test to assess possible germ cell effects may be required	No	No	No
6.6.7 If the results are negative for the three tests … only required if metabolites of concern are formed in mammals	No	No	No
6.7. Carcinogenicity study	No	Yes	No
6.8.1. Teratogenicity test	No	No	No
6.8.2. Two generations reproduction study	No	No	Yes
6.9 Neurotoxicity study	No	No	No
6.10 Mechanistic study - any studies necessary to clarify effects reported in toxicity studies	No	No	No
6.11 Studies on other routes of administration (parenteral routes)	No	No	No
6.12 Medical data in anonymous form	No	No	No
*Test performed with unspecified type of zeolite, assumed to represent Irgaguard B502i (further discussed in confidential appendix "Technical equivalence, technical specification and read-across" attached to section 13 of IUCLID). ** Test performed with Irgaguard B8000 (further discussed in confidential appendix "Technical equivalence, technical specification and read-across" attached to section 13 of IUCLID).			

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

There are no studies available in which the absorption, metabolism, distribution and elimination of orally administered silver zinc zeolite has been investigated. However, some summary reports prepared by experts at the US EPA, the Agency for Toxic Substances and Disease Registry (ATSDR) and the Oak Ridge Reservation Environmental Restoration Program are available in the open literature and these contain information on the toxicokinetics of orally administered silver compounds. These summaries are based on case reports or published research performed with silver nitrate, silver lactate or with metallic silver. Despite the number of summaries, the data is yet limited since most of the documents are based on the same source of information e.g. the ATSDR summary (Doc IIIA, 6.2(08)). The publications referred to in the summaries were, with a few exceptions, not included in the dossier but those considered relevant for the assessment of silver zinc zeolite have been requested from and been provided by the applicant. Although these studies were performed with silver compounds that may not accurately represent silver zinc zeolite, the data is yet considered to provide toxicokinetic information that suffice for a risk assessment of

silver zinc zeolite if applied in a conservative manner.
Toxicokinetic information via the dermal route is limited to a study on the absorption of subcutaneously administered silver zinc zeolite in 1 % carboxymethyl as well as of a percutaneously applied cream containing 10 % silver zinc zeolite. However, the type of silver zinc zeolite used and the composition of the cream tested were not specified in the report thus it is uncertain whether or not the results obtained accurately represent the types of silver zinc zeolites considered in this report. Therefore, the result of this study is given low significance in this assessment.

4.1.1 Non-human information

As stated in the previous section, studies referred to in the summary documents that were considered relevant for the toxicokinetics of silver zinc zeolite were requested from the applicant. The applicant did deliver the original publications for most of these studies (i.e. Furchner et al. (1968), Scott and Hamilton (1950), Phalen and Morrow (1973), Newton and Holmes (1966) and Skog, E. and Wahlberg, J.E)) and they have been evaluated in an addendum to section 6 of the competent authority report (CAR).
It is noted that this information is rather old by now and that most of the studies are poorly reported.

Oral absorption/Excretion: the general view among the different summaries is that only a small amount of silver (<10 %) is absorbed by mammals after oral administration. This view is based primarily on data from Furchner et al who studied the excretion of silver in mice, rats, dogs and monkeys administered silver nitrate by the oral or intravenous route. The research by Furchner et al indicates a biexponentional excretion profile in mice and monkeys and a triexponential excretion profile in dogs and rats upon oral administration. Since only dogs were assayed for a sufficiently long period, it was assumed that the long component would have been detected if excretion had been assayed longer also in the other species. The two-day clearance via urine and faeces ranged between 90 % and 99 % for the different species after an oral dose and between 15 and 82 % after an intravenous dose. Only a minor fraction was excreted in urine. The interspecies differences in clearance rate were explained as differences in the time for passage through the gut.
This study was not performed according to any guideline or GLP and there was no information on the test substance (with respect to purity and other physical data), test animals (housing and feeding conditions) and the residues in bile, tissues and carcass were not measured.
The strength of the study is that the results are based on a large data set with four different species and between 4 and 28 animals in each experiment.
From the Day 2 cumulative excretion data for the four species it is considered appropriate to use 5 % for oral absorption of silver ions in mammals. Since the oral absorption is used to adjust the reference value AEL (acceptable exposure level) it is conservative to use a low value. For silver zinc zeolite, this figure is expected to be conservative since the excretion data may include residues that actually were absorbed and then excreted in bile. Moreover, the absorption could also be higher if silver is absorbed also in the form of silver zinc zeolite.

Dermal absorption: The only study available in which the dermal absorption of silver zinc zeolite was studied is an in vivo study in male rats in which the dermal absorption of an unspecified silver zinc zeolite, either in 1 % solution of carboxymethyl cellulose sodium or as a 10 % cream of unknown composition was investigated. The 1 % solution was administered subcutaneously whereas the 10 % cream was applied percutaneously.
The result from the percutaneous experiment showed a total dermal absorption through intact skin at 168 hours of 2.4% when residues in urine (0.12 %), feces (1.1 %), skin (1 %) and other sites of the body (0.17 %) were taken into account. Since the blood radioactivity after percutaneous

administration was comparable to background levels, the distribution pattern was studied in rats after subcutaneous administration of silver zinc zeolite. Highest levels were observed in the liver at 24 hours but levels declined rapidly after 72 and 168 hours. A similar pattern was seen in blood, plasma and lung. However, the concentration in the Harderian gland, thymus and brain increased progressively and there was no decrease observed after 168 hours. Levels in kidney, spleen and pancreas were also still close to peak levels after 168 hours indicating a tendency for retention in some organs. High levels of radioactivity were observed in the small intestine and cecum after 6 hours and in the large intestine after 24 hours indicating excretion in feces via bile.
In literature, the most common figure reported with respect to dermal absorption of silver is 1%. This figure is also used by the applicant and is based on a study by E. Skog and J.E Wahlberg (1963) in which the uptake of silver nitrate through intact skin of guinea pigs was studied.
This study is relatively old and was not performed according to any guideline or principles of GLP. Moreover, the methodology used and the results obtained were poorly reported.
The dermal absorption was determined as the amount of radioactivity that disappeared from a treated area on living guinea pigs during five hours. For the majority of animals, the dermal absorption was below 1 % but the dermal absorption in one animal was in the range 3.0-3.9.
Due to all uncertainties in the study, it is considered appropriate to conclude a dermal absorption based on the upper-range value (i.e. 4 %) in order to cover all animals in the study. This value is expected to be conservative because it is based on the assumption that all radioactivity that disappeared from the test area entered the systemic circulation through the skin.

Inhalation: Based on the information submitted, the clearance of inhaled silver varies between species. In dogs, up to 90 % of the inhaled amount was transported in the blood to the liver and excreted in bile. The lung clearance was triphasic with half-lives of 1.7, 8.4 and 40 days (accounting for 59, 39 and 2 % of administered dose) in lungs. The half-lives in liver were 9 and 40 days (accounting for 97, and 3 % of excreted dose) (Phalen and Morrow (1973)).
In a rabbit study, 30 % of deposited silver particles were cleared from the lungs within a day and a further 30 % was cleared in the following week.

Distribution: According to information available in the open literature, the silver absorbed from silver nitrate undergoes a first-pass effect in the liver and is excreted into bile. The amount of biliary excretion appears to vary between species. Based on a study in rat, silver is conjugated to gluthathione prior to being excreted in bile (6.2(07)).

The silver absorbed from silver nitrate appears to be widely distributed in the rat. Scott and Hamilton (evaluated in an addendum to Doc IIIA, section 6) observed that the highest amount of silver after an intramuscular dose of silver nitrate was found in the GI tract followed by liver, blood, kidney, skin, muscle, bone, heart, lungs and spleen. The distribution was slightly changed if a higher dose was administered and the highest amount were then observed in liver followed by GI tract, skin, blood, spleen, muscle, bone, kidney, heart and lungs. When the percentage radioactivity per gram tissue is considered rather than the percentage per organ, the highest silver load four days after intravenous administration is noted in adrenals.
The difference observed between the concentration of silver in liver and faeces following a low or a high dose of silver nitrate indicate that the biliary excretion mechanism (at least in the rat) can be saturated.
Microscopic analyses of tissues from rats orally exposed to silver nitrate and silver chloride in sodium thiosulfate showed that silver was regularly found in histiocytes of lymph nodes and liver, in association with the reticulum fibrils of the sinuses of the lymph nodes and the periphery of the malpihian bodies of the spleen and in close approximation to blood vessels (between endothelium and epithelium of thyroid, choroid of the brain and the glomeruli and tubules of the kidney) (Olcott (1948) in an addendum to Doc IIIA, section 6). Silver was also found near or in fine blood vessels

of pancreas, adrenal medulla, pituitary body (in pars nervosa), choroid of the eye and in striated muscle. According to Olcott (1948), a few black granules were observed in the bone marrow but it was not possible to determine whether or not this was silver and the bone marrow of rats exposed to silver or water appeared the same. It is therefore not possible to conclude from this data whether or not the substance is distributed to the bone marrow (further discussed in section 4.9).
Published research referred to in a thesis (evaluated in Doc IIIA, section 6.9(02)) show silver deposition in many structures of the nervous system, primarily in parts that are unprotected by the blood-brain or blood-nerve barriers. Areas such as the red nucleus, brain stem motor nuclei and deep cerebellar nuclei are stated to accumulate the largest amounts of silver but smaller amounts are found dispersed throughout the whole brain. The author states that offspring of pregnant rats exposed to silver during pregnancy show an uptake into neurons, glia and vessel walls with a distribution similar to that seen in the adult.

4.1.2 Human information

Deposition of silver grains has been observed in tissue samples from individuals exposed to silver or different silver compounds via inhalation, oral and dermal routes. This indicates that silver can be absorbed systemically via several routes in humans.
However, there is no information on whether silver or the different SCAS are absorbed in the form of the parent compound, as dissociated constituents (silver ions) or in the form of silver complexes.

Oral absorption: East et al. (1980) reported an oral absorption of 18 % in a 30-week experiment performed with a 47-year old previously healthy woman who had taken excessively large oral doses of anti-smoking lozenges containing silver acetate over a period of 2.5 years (see section 6.12.2(04) and summary reports in the CA report). This retention level is much higher than what has been reported by other investigators and East et al. (1980) cited other studies on this particular anti-smoking formulation demonstrating that "within the limits of experimental error, no silver is retained after oral administration." It should be noted that this result is based on one individual only. East concluded from the results that this may not hold true for excessive intakes like that ingested by this individual. The US EPA has concluded that the study is unsuitable to serve as the basis for a quantitative risk assessment.

Dermal absorption: Silver has been detected in body fluids of humans dermally exposed to silver nitrate formulations during treatment of serious burn damage. Silver deposits in the dermis of a photochemical worker exposed to silver thiosulfate for six months demonstrate that silver also penetrates intact skin (ATSDR (1990)).
The low dermal absorption of silver observed in animals is supported by the result of an in vitro study in which a formulation containing silver chloride adsorbed onto titanium dioxide was applied on human skin. The total penetration of silver from the cream was considered to be less than 0.31 % of the applied dose. However, this dermal absorption refers to a certain formulation and the result may not accurately represent the inherent dermal absorption of silver ions or silver chloride adsorbed onto titanium dioxide since different co-formulants may have influenced the absorption rate.

Inhalation: The clearance observed in humans follows a biphasic profile with half-lives of 1 day and 52 days which are assumed to represent lung and liver clearance respectively (Newton and Holmes (1966) in addendum to Doc IIIA, section 6).

4.1.3 Summary and discussion on toxicokinetics

There is no adequate information to address the toxicokinetics of silver zinc zeolite specifically. The most relevant information available is a published study performed with silver nitrate in four different species. The study is yet relatively old and poorly reported thus confidence in data is rather low. Moreover, it is not known if silver is absorbed in the form of silver ions, silver complexes, silver-protein complexes or in combinations of these and it is thus unclear if the toxicokinetics of silver zinc zeolite is similar to that of silver nitrate or the other silver compounds investigated.
Industry-sponsored published information claims that zeolites may partially decompose during acidic conditions such as in the stomach and that the intact molecule is not bioavailable after oral intake or through the dermal and inhalational routes (Fruijtier-Pölloth 2009).
Assuming this is correct, the absorption of each component could be considered separately and the data available for silver nitrate could thus be considered relevant for the fate of silver ions released from silver zinc zeolite. However, if there is significant absorption also of the parent molecule, further differences in oral absorption may exist. The animal data presented by Furchner et al was obtained with silver nitrate which is a highly soluble salt. It is therefore assumed that total release of silver ions from silver nitrate occurred prior to absorption and that no significant uptake of silver in the form of silver nitrate occurred. The amount of silver excreted after two days in urine and faeces indicated that less than 10 % of the administered dose was absorbed orally by the four species tested. To balance the deficiencies in the data base and the uncertainties associated with read-across, it is assumed that 5 % of silver ions released from silver zinc zeolite in the GI tract will be absorbed. This assumption will result in a more conservative AEL (see section 4.1.1).
According to the industry-sponsored publication, 2-3 % of silicon was absorbed in the gastrointestinal tract of dogs but there appeared to be no significant absorption of aluminium.
Information relevant for the toxicokinetics of zinc is available in the risk assessment report for zinc chloride (http://ecb.jrc.ec.europa.eu/esis/). According to this report, the oral absorption of soluble zinc salts (chloride, sulfate) is 20-30 % in well-nutritioned humans (40-50 % in animals) and 12-18 % of less solule salts (zinc oxide).
This indicates that the oral absorption of zinc is higher than silver and it is thus more conservative to use the oral absorption of silver (ions) also for silver zinc zeolite when correcting the reference value (i.e. AEL).
There is no appropriate information on the dermal absorption of silver zinc zeolite.
A default value of 100 % is often applied in the absence of adequate test data on dermal absorption. The OECD guidance document describes a similar case in which the dermal absorption of an untested substance is considered to be ”no higher than 10 %” if valid experimental data reveals 6% absorption for a ”similar” compound (section 10.1 read-across). If this would be applied to the present case, the dermal absorption of silver zinc zeolite would be 10%.
However, the dermal absorption concluded from experimental data with silver nitrate (4 %) is expected to be conservative since it is based on disappearance kinetics rather than measured uptake values and the majority of samples indicated an uptake of 1% rather than 4 %. Moreover, the dermal absorption of a substance is normally not higher than the oral absorption (IGHCR 2006). Therefore, the dermal absorption and the oral absorption are considered to be the same, e.g. 5 %.

4.2 Acute toxicity

Table 12: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Oral			
OECD TG 401 Rat Albino Sprague-Dawley 5/sex Single oral dose, 5000 mg/kg bw 14 day observation period **Type of silver zinc zeolite not specified**	LD_{50}>5000 mg/kg bw	Observations of peri-nasal staining, diarrhoea, urinary stains. Reliability 2	Doc IIIA 6.1.1(04) (1989)
USA EPA FIFRA Guideline 81-1 Rat Sprague-Dawley derived albino, 5/sex Single oral dose, 2000 mg/kg 14 day observation period Zeomic Type AK10D Silver Zeolite A (Same as AgION® Silver Antimicrobial Type AK)	LD_{50}>2000 mg/kg bw	None Reliability 1	Doc IIIB 6.1.1(01) (2000)
USA EPA OPPTS 870.1100 Rat Slc:SD (SPF), 8/sex Single oral dose, 5000mg/kg 14 day observation period Zeomic Type AJ10D Silver Zeolite A (Same as AgION® Silver Antimicrobial Type AJ)	LD_{50}>5000 mg/kg bw	Lack of detailed information on the test substance. Study performed according to Japanese GLP standards Reliability 2-3	Doc IIIB 6.1.1(02) (1987)
Dermal			
EPA FIFRA 81-2 Rabbit New Zealand White Single dermal dose 5000 mg/kg bw, 24 hour exposure 14 day observation period **Type of silver zinc zeolite not specified**	LD_{50}>5000 mg/kg bw	Slight erythema Reliability 2	Doc IIIA 6.1.2(03) (1989b)
EPA: 81-2; 870.1200 Rat Sprague-Dawley derived, albino, 5/sex Single dermal dose 2000 mg/kg, 24 hour exposure 14 day observation period Zeomic Type AK10D Silver Zeolite A (Same as AgION® Silver Antimicrobial Type AK)	LD_{50}>2000 mg/kg bw	None Reliability 1	Doc IIIB 6.1.2(01) (2000)
USA EPA OPPTS 870.1200 Rat	LD_{50}>2000 mg/kg bw	None	Doc IIIB 6.1.2(02)

Method	Results	Remarks	Reference
Slc:SD (SPF), 8/sex Single dermal dose, 2000 mg/kg, 24 hour exposure 14 day observation period AJ10D Silver Zeolite A (Same as AgION® Silver Antimicrobial Type AJ)		Lack of detailed information on the test substance, study performed according to Japanese GLP standards Reliability 2-3	(1987)
Inhalation			
OECD TG 403 Inhalation whole body Rat Sprague-Dawley, 5/sex 1.43 mg/L, 4 hours 14 day observation period **Type of silver zinc zeolite not specified**	LC_{50}>1.43 mg/L	Observations of discoloration around ears, eyes or mouth, wheezing and nasal discharge (all slight). Reliability 2	Doc IIIA 6.1.3(02) (1989)
OECD TG 403 Inhalation nose only Rat Sprague-Dawley SD, 5/sex 2.28 mg/L, 4 hours 14 day observation period TKA 45039 (IRGAGUARD B 5021)	LC_{50}>2.28 mg/L	Reduced bodyweight gain MMAD above recommendations in Guidance on the Application of the CLP criteria Reliability 2-3	Doc IIIA 6.1.3(04) (2002)
EPA: OPPTS 870.1300 Inhalation, head and nose-only Rat Sprague-Dawley, albino 5/sex 4h, 2.86 mg/L Zeomic Type AK10D Silver Zeolite A (Same as AgION® Silver Antimicrobial Type AK)	LC_{50}>2.86 mg/L	Graying in the upper left lung of 1/10 animals. Reliability 1	IIIB 6.1.3(01) (2000)

4.2.1 Non-human information

The different types of silver zinc zeolites considered are Irgaguard B 502i, AgION Antimicrobial Type AJ and AgION Antimicrobial Type AK.

4.2.1.1 Acute toxicity: oral

Studies on acute oral toxicity are available for an unspecified type of silver zinc zeolite (Doc IIIA, section 6.1.1 (04)) and for AgION Antimicrobial Types AJ and AK (doc IIIB, section 6.1.1 (01, 02)). The type of silver zinc zeolite used in study 6.1.1 (04) is not specified in the original study report but is claimed by the applicant to be a type which differs from Irgaguard B502i but yet can

be used to represent this type. This read-across is considered acceptable (see confidential attachment to section 13 of the technical dossier).

All animals survived treatment in all three studies and there were no major abnormal findings among the animals. Clinical signs were noted in animals treated with the unspecified type of silver zinc zeolite and included peri-nasal staining, diarrhoea, urinary stains. In all three studies, the LD50 values were above the highest doses tested, i.e. 2000 mg/kg bw for AgION Antimicrobial Type AK and 5000 mg/kg bw for the unspecified type of silver zinc zeolite and AgION Antimicrobial Type AJ.
The silver and zinc content of AgION® Silver Antimicrobial Type AJ was not specified in the original report and the batch used for the acute toxicity studies is not recognized from the physico-chemical section thus the silver and zinc content of this substance is unclear. Considering that no mortalities occurred with AgION® Silver Antimicrobial Type AK which is assumed to be equally or more toxic with respect to the chemical composition, further information on AgION® Silver Antimicrobial Type AJ is not considered necessary.

4.2.1.2 Acute toxicity: inhalation

All animals survived the treatment with all three silver zinc zeolites and the clinical signs observed in animals treated with the unspecified silver zinc zeolite included discoloration in the facial area, nasal and ocular discharges. In the nose-only study with Irgaguard B 502i, there were observations of discoloration in the facial area and reduced bodyweight gain and in the study with AgION Antimicrobial Type AK, decreased activity/piloerection occurred during the first four days and grey discoloration of one lung was observed in a single animal.
The result obtained with TKA 45039 (Irgaguard B 502i) is weakened by the fact that the mass median aerodynamic diameter of the particles generated at the maximum attainable concentration (2.28 mg/L) is larger (6.2±3.0 µm with 34% of particles ≤ 4 µm) than the sizes considered relevant in draft OECD TG 403 and in Regulation (EC) No 1272/2008 (CLP) (i.e. MMAD between 1-4 µm). However, it is discussed in the current OECD TG 403 and in the draft report of the expert consultation meeting on acute inhalation toxicity that it may be technically challenging to both achieve a concentration of 5 mg/L and particles of respirable particle size. Moreover, a low acute toxicity via inhalation is supported by the result obtained in the whole body exposure study with the unspecified type of silver zinc zeolite since no mortalities occurred at the highest attainable concentration of 1.43 mg/L.
Therefore, although the respirable fraction was lower than recommended in the study with Irgaguard B502i, the results from the two studies combined indicate that no classification would be necessary for Irgaguard B502i.
There is no experimental data available for Type AJ but due to chemical similarities between Types AK and AJ, data obtained for Type AK is assumed to be relevant also for Type AJ (see confidential appendix " Technical equivalence, technical specification and read-across" attached to section 13 of IUCLID). The maximum dose tested with AgION Antimicrobial Type AK was 2.86 mg/L but it is not stated if this was the maximum attainable dose. Due to the absence of mortality in the study and taking into consideration information from different guidelines stating that it may be difficult to achieve a concentration above 2 mg/L and at the same time obtain particles of respirable size, the RMS finds it realistic to assume that the acute toxicity of AgION Antimicrobial Type AK (and Type AJ) is higher than the maximum attainable concentration.

4.2.1.3 Acute toxicity: dermal

Data on acute dermal toxicity is available for an unspecified type of silver zinc zeolite which is assumed to represent Irgaguard B502i and for the AgION Antimicrobial Types AJ and AK.

All animals survived treatment with all three silver zinc zeolites and the only clinical sign observed was a slight erythema at two sites on day two in rabbits exposed to 5000 mg/kg bw of unspecified silver zinc zeolite.
The LD 50 values were above 2000mg/kg bw for AgION Antimicrobial Types AJ and AK and above 5000 mg/kg bw for the unspecified type of silver zinc zeolite.

4.2.1.4 Acute toxicity: other routes

No substance-specific data available.

4.2.2 Human information

No substance-specific data available.

4.2.3 Summary and discussion of acute toxicity

The acute oral, dermal and inhalation toxicity data available is considered sufficient for a hazard assessment of the types of silver zinc zeolites denoted Irgaguard B502i and AgION Antimicrobial Types J and K. Although specific data is not available for all endpoints, it is considered acceptable to use data obtained with a similar type to fill the data gap (see confidential appendix “Technical equivalence, technical specification and read-across” attached to section 13 of IUCLID).
Data obtained for a similar type of silver zinc zeolite along with scientific arguments are also considered to support the assumption that uncertainties identified in some studies (with respect to MMAD and maximum attainable concentration in acute inhalation toxicity studies) do not invalidate the results of the studies. No further animal testing is thus considered necessary.

4.2.4 Comparison with criteria

The maximum limit for classification with respect to acute oral and dermal toxicity in Regulation EC No 1272/2008 (CLP) is Acute Toxicity Estimate (ATE) of ≥ 2000 mg/kg bw. Since the LD50 values set for Irgaguard B502i and AgION Antimicrobial Types K and J are above this level, no classification for acute oral and dermal toxicity is proposed.
The maximum limit for classification with respect to acute inhalation toxicity in Regulation EC No 1272/2008 is an ATE of ≥ 5 mg/L for substances in dust/mist form. Due to some uncertainties in the data it is difficult to conclude that the acute toxicity via inhalation is above the maximum limit for classification in 1272/2008 however the weight of evidence suggests that it is reasonable to assume that this is the case for these three silver zinc zeolites.
Therefore, Irgaguard B502i and AgION Antimicrobial Types K and J are not considered to fulfil criteria for classification.

4.2.5 Conclusions on classification and labelling

Irgaguard B502i and AgION Antimicrobial Types K and J are not considered to fulfil the criteria for classification with respect to acute toxicity via oral, dermal or inhalation routes.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of SZZ for acute oral toxicity on the basis of the three studies in rats:

i. A study (Klimish score 1) reporting an LD_{50} higher than 2000 mg/kg bw for AgION Type AK;
ii. A second study (Klimish score 2) reporting an LD_{50} higher than 5000 mg/kg bw for AgION Type AJ;
iii. A third study (Klimish score 2) reporting an LD_{50} higher than 5000 mg/kg bw for an unspecified SZZ.

The DS proposed no classification of SZZ for acute dermal toxicity on the basis of the following three studies in rats or rabbits:

i. A rat study (Klimish score 1) reporting an LD_{50} higher than 2000 mg/kg bw for SZZ AgION Type AK;
ii. A rat study (Klimish score 2) reporting an LD_{50} higher than 2000 mg/kg bw for SZZ AgION Type AJ;
iii. A rabbit study (Klimish score 2) reporting an LD_{50} higher than 5000 mg/kg bw for an unspecified SZZ.

The DS proposed no classification of SZZ for acute inhalation toxicity on the basis of the following three studies in rats:

i. A study (Klimish score 1) reporting an LD_{50} higher than 2.86 mg/L for AgION Type AK;
ii. A study of (Klimish score 2-3) reporting an LD_{50} higher than 2.28 mg/L for Irgaguard;
iii. A study (Klimish score 2) reporting an LD_{50} higher than 1.43 mg/L for an unspecified SZZ.

Comments received during public consultation

No comments were received during public consultation on this hazard class.

Assessment and comparison with the classification criteria

The tables below summarise the acute oral, dermal and inhalation toxicity studies (respectively) that were reported by the DS in the CLH report.

Acute oral toxicity

Table 2 (RAC): Summary of acute oral toxicity studies. In all cases the observation period was 14 days.

Method	Result	Remarks and reliability (Klimish score)
USA EPA FIFRA Guideline 81-1	No major abnormal	Doc IIIB 6.1.1(01)

Rat (Sprague-Dawley) 5/sex Single oral dose 2000 mg/kg Zeomic Type AK10D Silver Zeolite A (AgION® Silver Antimicrobial Type AK)	findings No clinical signs No mortalities LD50>2000 mg/kg bw	(Moore, 2000a) Reliability 1
USA EPA OPPTS 870.1100 Rat (Slc:SD (SPF)) 8/sex Single oral dose: 5000mg/kg Zeomic Type AJ10D Silver Zeolite A (same as AgION Type AJ)	No major abnormal findings No clinical signs No mortalities LD50>5000 mg/kg bw	Doc IIIB 6.1.1(02) (Shimizu, 1987a) Silver and zinc content not specified. Study performed according to Japanese GLP standards Reliability 2
OECD TG 401 Rat (Albino Sprague-Dawley) 5/sex Single oral dose: 5000 mg/kg bw Type of SZZ not specified	No major abnormal findings Clinical observations: peri-nasal staining, diarrhoea, urinary stains. No mortalities LD50>5000 mg/kg bw	Doc IIIA 6.1.1(04) (James, 1989a) Reliability 2

Acute dermal toxicity

Table 3 (RAC): Summary of acute dermal toxicity studies. In all cases the observation period was 14 days.

Method	Result	Remarks and reliability (Klimish score)
EPA: 81-2; 870.1200 Rat (Sprague-Dawley) 5/sex Single dose: 2000 mg/kg bw (24 hours) Zeomic Type AK10D Silver Zeolite A (AgION Type AK)	No mortalities LD_{50}>2000 mg/kg bw	Doc IIIB 6.1.2(01) (Moore, 2000b) Reliability 1
USA EPA OPPTS 870.1200 Rat (Slc:SD (SPF)) 8/sex Single dose: 2000 mg/kg bw (24 hours) AJ10D Silver Zeolite A (AgION Type AJ)	No mortalities LD_{50}>2000 mg/kg bw	Doc IIIB 6.1.2(01) (Shimizu, 2000b) Lack of detailed information on the test substance, Performed according to Japanese GLP standards Reliability 2
EPA FIFRA 81-2 Rabbit (New Zealand White) Single dose: 5000 mg/kg bw (24 hours) Type of SZZ not specified	No mortalities Slight erythema on day 2 LD_{50}>5000 mg/kg bw	Doc IIIA 6.1.2(03) (James, 1989b) Reliability 2

Acute inhalation toxicity

Table 4 (RAC): Summary of acute inhalation toxicity studies. In all cases the observation period was 14 days.

Method	Result	Remarks and reliability (Klimish score)
EPA: OPPTS 870.1300	No mortalities	Doc IIIB 6.1.3(01)

Rat (Sprague-Dawley) 5/sex 2.86 mg/L (4 hours) Zeomic Type AK10D Silver Zeolite A (AgION Type AK)	Decreasing activity, piloerection and graying in the upper left lung of 1/10 animals LC_{50}>2.86 mg/L	(Leeper, 2000) Head and nose-only Reliability 1
OECD TG 403 Rat (Sprague-Dawley) 5/sex 2.28 mg/L (4 hours) Irgaguard	No mortalities Discoloration of facilal area and reduced bodyweight gain LC_{50}>2.28 mg/L	Doc IIIA 6.1.3(04) (Wilson, 2002a) Nose only MMAD = 6.2±3.0 µm with 34% particles ≤ 4 µm (above the limit of 4 µm) Reliability 2
OECD TG 403 Rat (Sprague-Dawley) 5/sex 1.43 mg/L (4 hours) Type of SZZ not specified	No mortalities Observations of discoloration around ears, eyes or mouth, wheezing and nasal discharge (all slight). LC_{50}>1.43 mg/L	Doc IIIA 6.1.3(02) (Stuart, 1989) Whole body Reliability 2

The limit concentration for triggering classification for both oral and dermal routes is 2000 mg/kg. In the case of doses of 2000 and 5000 mg/kg did not cause mortalities. Only the unspecified SZZ caused some clinical observations in rats (peri-nasal staining, diarrhoea, urinary stains) and slight transient erythema in rabbits.

The limit concentration for triggering classification by inhalation route is 5 mg/L. None of the available studies reached such high concentration, but the highest tested concentration (2.86 mg/L) did not cause mortalities and thus it is unlikely that the LD_{50} might be lower than 5 mg/L.

Therefore, taking into consideration the above state data **RAC agrees with the DS that silver zinc zeolite does not fulfil the criteria for classification for acute toxicity with respect to the oral, dermal and inhalation routes.**

4.3 Specific target organ toxicity – single exposure (STOT SE)

None of the effects noted among the acute toxicity studies are considered to meet the criteria in Regulation EC No 1272/2008 for classification STOT-SE.

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

Not relevant.

4.3.2 Comparison with criteria

Not relevant.

4.3.3 Conclusions on classification and labelling

None of the effects noted in the acute toxicity studies are considered to fulfil criteria for classification.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS proposed no classification because none of the effects noted among the acute toxicity studies are considered to meet the criteria for classification as STOT-SE.

Comments received during public consultation

No comments were received during public consultation on this hazard class.

Assessment and comparison with the classification criteria

According to the CLP Regulation, classification for STOT SE should be considered where there is clear evidence of toxicity to a specific organ, especially when it is observed in the absence of lethality. Standard acute toxicity studies do not indicate that there is specific organ toxicity following a single exposure. Overall, it is concluded that classification of SZZ for STOT SE is not warranted.

The hazard class STOT-SE 3 should cover 'transient' respiratory tract irritation and narcotic effects that are observed in animal studies. Lethargy, lack of coordination, loss of righting reflex and ataxia when occurring after a single exposure can justify classification of substances for narcotic effects in Category 3. Classification in Category 3 is primarily based on human data, which was not available for SZZ. None of these effects were reported in the available acute toxicity studies.

RAC therefore proposes, in agreement with the DS proposal, to not classify SZZ for STOT-SE.

4.4 Irritation

4.4.1 Skin irritation

Table 13: Summary table of relevant skin irritation studies

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td>OECD TG 404
Rabbit
New Zealand White, 3/sex
0.5 g in 0.5% carboxymethylcellulose
4 hour exposure
Observations at 1, 22, 44 and 68 hours after patch removal
Type of silver zinc zeolite not specified (see confidential annex)</td><td>1h: Very slight erythema in 3/6 animals

Average score at 22, 44, 68 h in individual animals
<table><tr><td></td><td>erythema</td><td>oedema</td></tr><tr><td></td><td>mean</td><td>mean</td></tr><tr><td>1M</td><td>0
(0,0,0)</td><td>0
(0,0,0)</td></tr><tr><td>2M</td><td>0
(0,0,0)</td><td>0
(0,0,0)</td></tr><tr><td>3M</td><td>0
(0,0,0)</td><td>0
(0,0,0)</td></tr><tr><td>4F</td><td>0
(0,0,0)</td><td>0
(0,0,0)</td></tr><tr><td>5F</td><td>0
(0,0,0)</td><td>0
(0,0,0)</td></tr><tr><td>6F</td><td>0
(0,0,0)</td><td>0
(0,0,0))</td></tr></table></td><td>Reliability 2</td><td>Doc IIIA
6.1.4-04
(1989c)</td></tr>
<tr><td>OECD TG 404
Rabbit
New Zealand White, 3 males
0.5 g in 0.3ml deionized water
4 hour exposure,
Observations at 1h, 24h, 48h and 72 h after patch removal
TKA 45039 (Irgaguard B 502i)</td><td>24, 48 hours:
erythema (very slight)

Average score at 24, 48, 72 h in individual animals
<table><tr><td></td><td>erythema</td><td>oedema</td></tr><tr><td></td><td>mean</td><td>mean</td></tr><tr><td>1</td><td>0.33
(1,0,0)</td><td>0
(0,0,0)</td></tr><tr><td>2</td><td>0
(0,0,0)</td><td>0
(0,0,0)</td></tr><tr><td>3</td><td>0.67
(1,1,0)</td><td>0
(0,0,0)</td></tr></table></td><td>Reliability 1</td><td>Doc IIIA
6.1.4-18
(2002)</td></tr>
<tr><td>USA EPA 870.2500
Rabbit
New Zealand White, 3/sex
0.5 g wetted with physiological saline
4 hour exposure,
Observations at 5 h, 24h, 48h, 72h, day 7, day 14 after patch removal
AgION Antimicrobial Type AK</td><td>Average score at 24, 48, 72h
Erythema:1.7, 1.7, 2.2
Oedema:1.2, 0.7, 0.8

Average score at24, 48, 72h and score at termination (day 14) in individual animals:
<table><tr><td></td><td colspan="2">erythema</td><td colspan="2">oedema</td></tr><tr><td></td><td>mean</td><td>14d</td><td>mean</td><td>14d</td></tr><tr><td>1M</td><td>1
(1,1,1)</td><td>-</td><td>0
(0,0,0)</td><td>-</td></tr><tr><td>2M</td><td>2
(2,2,2)</td><td>2</td><td>0
(0,0,0)</td><td>1*</td></tr><tr><td>3M</td><td>2
(2,2,2)</td><td>4</td><td>0.67
(0,1,1)</td><td>4**</td></tr><tr><td>4F</td><td>2.3
(2,2,3)</td><td>4</td><td>1
(0,1,2)</td><td>4**</td></tr><tr><td>5F</td><td>2
(2,2,2)</td><td>1</td><td>0.67
(0,1,1)</td><td>0</td></tr></table></td><td>Not reversible

Reliability 1</td><td>IIIB
6.2(04)
(2000)</td></tr>
</table>

<table>
<tr>
<td></td>
<td>
<table>
<tr><td>6F</td><td>1.7
(1,1,3)</td><td>1</td><td>0.67
(0,1,1)</td><td>1</td></tr>
</table>
*scar
**crust
</td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA EPA 870.2500
Rabbit
New Zealand White, 6M
0.5 g in 0.5mL distilled water
24 hour exposure,
Observations at 0.5h, 48h, 72h, after patch removal
AgION Antimicrobial Type AJ</td>
<td>
Average score at 24.5, 48, 72h
Erythema: 0,0,0
Oedema: 0,0,0

Average score at 24, 48, 72h in individual animals
<table>
<tr><td></td><td>erythema</td><td>oedema</td></tr>
<tr><td></td><td>mean</td><td>mean</td></tr>
<tr><td>1</td><td>0
(0,0,0)</td><td>0
(0,0,0)</td></tr>
<tr><td>2</td><td>0
(0,0,0)</td><td>0
(0,0,0)</td></tr>
<tr><td>3</td><td>0
(0,0,0)</td><td>0
(0,0,0)</td></tr>
<tr><td>4</td><td>0
(0,0,0)</td><td>0
(0,0,0)</td></tr>
<tr><td>5</td><td>0
(0,0,0)</td><td>0
(0,0,0)</td></tr>
<tr><td>6</td><td>0
(0,0,0)</td><td>0
(0,0,0))</td></tr>
</table>
</td>
<td>The study was performed according to Japanese GLP standards

Reliability 2</td>
<td>IIIB 6.2(05) (1987)</td>
</tr>
</table>

4.4.1.1 Non-human information

In the study IIIB 6.2(04), treatment with AgION Antimicrobial Type AK caused irreversible grade 3-4 erythema and grade 2-4 edema in 4/6 rabbits during the 14 day observation period (day 7). The severity of effects increased over time and persisted at day 14 of observation.

Surprisingly, no skin reactions were observed in the study performed with Type AJ (Doc IIIB, 6.2(05), 1987). This study was performed using both abraded and intact skin of male rabbits. The exposure time used (24 hours) was longer than recommended in the current OECD TG 404 (6 hours) and the temperature fluctuated outside protocol limits during the study.
None of these deviations from the current guideline is considered to have affected the outcome of the study in such way that any irritation potential could have been masked. In fact, the conditions of this study, i.e. the use of abraded skin and the use of a longer exposure time could be expected to favour detection of irritation.
TKA 45039 (Irgaguard B502i) and the unspecified type of silver zinc zeolite also caused skin reactions but these were limited to very slight and reversible erythema (Doc IIIA, 6.1.4(18) and Doc IIIA, 6.1.4(04), respectively).
Based on the physico-chemical properties of the substance, there is no apparent explanation why one of the substances meets the criteria for classification while the others do not. The applicant argues that this is due to AgION Antimicrobial Type AK being applied in saline while the others are applied in distilled water. This implicitly means that similar results could be expected for the other silver zinc zeolites if applied in saline, a vehicle which is relevant considering the saline nature of the human skin.

4.4.1.2 Human information

No substance-specific data available.

4.4.1.3 Summary and discussion of skin irritation

Treatment with silver zinc zeolite Type AK resulted in persistent erythema and oedema whereas Type AJ, the unspecified type of silver zinc zeolite and TKA 45039 caused no or very slight erythema.

4.4.1.4 Comparison with criteria

According to Guidance to the Regulation EC No 1272/2008, a substance should be classified for skin corrosion if it produces *"irreversible damage to the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to 4 hours."* The hazard category Skin Corrosion has three subcategories

Corrosive in > 1 of 3 animals
1A ≤ 3 minutes ≤ 1 hour
1B > 3 minutes - ≤ 1 hour ≤ 14 days
1C > 1 hour - ≤ 4 hours ≤ 14 days

Furthermore, a substance should be classified for skin irritation (Category 2) if it fulfils the following criteria:

"(1) Mean value of ≥ 2,3 - ≤ 4,0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or
(2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperplasia, and scaling; or
(3) In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above."

According to the guidance document on the application of CLP criteria, the following criteria applies when 6 rabbits are used in the test:

"a. Classification as skin corrosive – Category 1 if destruction of skin tissue (visible necrosis through the epidermis and into the dermis) occurs in at least one animal after exposure up to 4 hours.

b. Classification as skin irritation – Category 2 if at least 4 out of 6 rabbits show a mean score per animal of ≥ 2.3 ≤ 4.0 for erythema/eschar or for oedema;"

AgION Antimicrobial Type AK produced erythema/eschar in all animals and oedema in 4/6 animals but the individual mean values at 24, 48 and 72 hours were below the range given for the first criterion in category 2 (i.e.≥ 2,3 - ≤ 4,0)." However, the severity of the reactions increased over time and a score of 4 for erythema/eschar as well as a score of 4 for oedema (and formation of crust) was achieved in 2/5 animals (no data was available for one of the rabbits) at day 14 of the observation period. In all remaining animals, a lower grade of erythema/eschar was evident and a lower grade of oedema was observed

in all but one animal. Considering that reactions persisted at the end of the 14 day observation period and that formation of crust occurred in 2/5 (40%) of the animals, a frequency above 1/3 (33%), it could be argued that the criterion for classification Skin Corr 1C is met. However, "crust" is not considered to fully correspond to" visible necrosis through the epidermis and into the dermis" and taking also into consideration the variability in the severity of effects observed between males and females, classification in Category 2 seems more appropriate.

4.4.1.5 Conclusions on classification and labelling

AgION Antimicrobial Type AK is considered to fulfil criteria for classification as Skin Irrit. 2 (H315) based on the erythrema and oedema observed which increased to grade 3-4 for erythrema and grade 2-4 for oedema in 4/6 animals during the 14 day observation period.
Despite that only the results obtained with AgION Antimicrobial Types K fulfils the classification criteria, the RMS proposes that classification as Skin irrit. 2 (H315) should be considered for all three silver zinc zeolites since the lack of irritation in studies with AgION Antimicrobial Type AJ and Irgaguard B502i may be related to the use of a vehicle that is less representative of human skin (see section 4.4.1.1). In the absence of appropriate dose-response data, no specific concentration limits can be set.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed classification of the three SZZ as Skin Irrit. 2 (H315) based on erythema (grade 3-4) and oedema (grade 2-4) observed 14 days after the exposure to SZZ AgION Type AK only. Treatment with SZZ AgION Type AJ and an unspecified type of SZZ caused no or very slight erythema.

The DS argued in favour of classifying all types of zeolites as Skin Irrit. 2 (H315), because the lack of irritation seen with some types of SZZ may be due to the use of distilled water, a vehicle that is less representative to human skin.

Comments received during public consultation

A Member State (MS) asked to consider SZZ for corrosivity. The DS responded that crust formation is not considered to meet the definition of a scar, which was only observed in 1/6 animals (while the CLP criteria for corrosivity refer to $\geq$ 1/3 animals).

The MS also stated that the differences in response between the different zeolites could also be caused by differences in Ag and/or Zn content and their release under aqueous conditions, which would indicate that different classifications would be applicable to the different substances. The DS responded that the type of vehicle used may explain the differences observed in skin irritation studies although it does not explain differences observed in eye irritation studies. The DS concluded that even though the results from skin irritation studies differ between the SZZ tested, a reasonable assumption is that these are due to different conditions of the test system and/or result from biological variation rather than from differences in potency.

Assessment and comparison with the classification criteria

The table below summarises the available skin corrosion/irritation studies.

Table 5 (RAC): Summary of skin corrosion/irritation studies

<table>
<tr><th>Method</th><th>Result</th><th>Remarks and reliability (Klimish score)</th></tr>
<tr><td>USA EPA 870.2500
Rabbit (New Zealand White)
3/sex
0.5 g wetted with physiological saline (4 hour exposure)
AgION Type AK</td><td>Average score at 24, 48, 72h:Erythema:1.7, 1.7, 2.2
Oedema:1.2, 0.7, 0.8

Score at termination in individual animals:

<table><tr><td></td><td>Erythema</td><td>Oedema</td></tr><tr><td>1M</td><td>-</td><td>-</td></tr><tr><td>2M</td><td>2</td><td>1 (scar)</td></tr><tr><td>3M</td><td>4</td><td>4 (crust)</td></tr><tr><td>4F</td><td>4</td><td>4 (crust)</td></tr><tr><td>5F</td><td>1</td><td>0</td></tr><tr><td>6F</td><td>1</td><td>1</td></tr></table></td><td>Doc IIIB 6.2(04) (Nitka, 2000)

Observations at 5h, 24h, 48h, 72h, day 7, day 14 after patch removal

No report for 14 days in 1 male

Non-reversible effects

Reliability 1</td></tr>
<tr><td>OECD TG 404
Rabbit (New Zealand White)
3 males
0.5 g in 0.3mL deionised water (4 hour exposure)

Test material: Irgaguard</td><td><u>24, 48 hours</u>:
Erythema (very slight)
Individual and average scores at 24, 48, 72 h in individual animals:

<table><tr><td></td><td>Erythema</td><td>Oedema</td></tr><tr><td>1</td><td>0.33 (1,0,0)</td><td>0 (0,0,0)</td></tr><tr><td>2</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td></tr><tr><td>3</td><td>0.67 (1,1,0)</td><td>0 (0,0,0)</td></tr></table></td><td>Doc IIIA 6.1.4-18 (Wilson, 2002b)

Reliability 1

Observations at 1h, 24h, 48h and 72 h after patch removal</td></tr>
<tr><td>OECD TG 404
Rabbit (New Zealand White)
3/sex
0.5 g in 0.5% carboxymethylcellulose (4 hour exposure)
<u>Type of SZZ unspecified</u></td><td>1h: Very slight erythema in 3/6 animals
Individual scores at 22, 44, 68 h in all animals: 0</td><td>Doc IIIA 6.1.4-04 (James, 1989c)

Reliability 2

Observations at 1, 22, 44 and <u>68 hours after patch removal</u></td></tr>
<tr><td>USA EPA 870.2500
Rabbit (New Zealand White)
6M
0.5 g in 0.5mL distilled water (24 hour exposure)
AgION Type AJ</td><td>Average score at 24.5, 48, 72h: 0 (for both erythema and oedema)
Individual scores at 22, 44, 68 h in all animals: 0</td><td>Doc IIIB 6.2(05) (Kawasaki, 1987)

The study was performed according to Japanese GLP standards

Observations at 0.5h, 48h, 72h after patch removal

Reliability 2</td></tr>
</table>

Thus, an overall conclusion of the available information shows:

i. One study with AgION Type AJ (reliability 2) yielding no skin reactions.
ii. One study with Irgaguard (reliability 1) and one study with an unspecified type of SZZ (reliability 2) yielding very slight erythema.
iii. One study with AgION Type AK (reliability 1) yielding detectable skin reactions at 24, 48 and 72 hours (not enough for triggering classification) but progressing to severe erythema and oedema which persisted 14 days after exposure.

RAC notes the absence of dermal reaction obtained with the SZZ AgION type AJ. The differences between AgION type AJ and AgION type AK might be related to the vehicle,

because Irgaguard and AgION AJ types were applied in water, while the AgION Type AK was applied in saline.

RAC notes a comment from industry that the elution rate of ions may depend on the content and type of of the water in which the material is immersed. With an ion-exchange carrier such as SZZ, a silver or other cation cannot emerge unless it is exchanged with some other cations. Thus elution into e.g. saline is significantly faster than into pure water. This hypothesis is consistent with a vehicle effect.

According to the CLP Regulation, a substance should be classified as a skin irritant category 2 if it fulfils the following criteria:

(1) Mean value of ≥ 2,3 - ≤ 4,0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or; or

(2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals.

The study with AgION Type AK fulfils the second of these criteria because 2 animals displayed erythema and oedema grade 4 at the end of the observation period (day 14). The study was conducted with physiological saline, which is considered more representative of physiological exposures.

RAC notes that the study with AgION Antimicrobial Type AK yielded one animal with scar formation and it should be considered as skin corrosion. However, grouping all available information it is notable that scarring appeared only in one animal among 21 tested in four different studies and therefore the weight of the evidence is not in favour of classifying SZZ as corrosive to the skin.

In conclusion, RAC supports the proposal issued by DS for classifying SZZ as Skin Irrit. 2; H315 (Causes skin irritation).

4.4.2 Eye irritation

Table 14: Summary table of relevant eye irritation studies

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td>OECD TG 405
Rabbit New Zealand White
(2m/1f)
0.095g (0.1ml) TKA 45039 (IRGAGUARD B 5021)
Observations at 1h, 24h, 48h, 72h, day 7 after instillation</td><td>1 h
Iritis
Conjunctivitis

Average score at 24, 48, 72 h in individual animals
<table>
<tr><td></td><td>cornea</td><td>iris</td><td colspan="3">conjunctiva</td></tr>
<tr><td></td><td>mean</td><td>mean</td><td>redness</td><td>chemosis</td><td>discharge</td></tr>
<tr><td>1M</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>1 (2,1,0)</td><td>0.33 (1,0,0)</td><td>0 (0,0,0)</td></tr>
<tr><td>2F</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>0.67 (1,1,0)</td><td>0.33 (1,0,0)</td><td>0 (0,0,0)</td></tr>
<tr><td>3M</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>1 (1,1,1)</td><td>0.33 (1,0,0)</td><td>0 (0,0,0)</td></tr>
</table></td><td>Classification not required</td><td>IIIA 6.1.4-06 (2002)</td></tr>
<tr><td>OECD TG 405
Rabbit
New Zealand White
(4m/5f)
No-rinse group: 2m/4f
Rinse group:2m/1f

0.058g (0.1ml) Silver zinc zeolite
Observations at 1h, 24h, 48h, 72h, after instillation</td><td>1 h
Iritis
Conjunctival irritation (redness, swelling and production of ocular discharge)
The conjunctival irritation was resolved by day 7.

Average score at 24, 48, 72 h in individual animals
<table>
<tr><td></td><td>cornea</td><td>iris</td><td colspan="3">conjunctiva</td></tr>
<tr><td></td><td>mean</td><td>mean</td><td>redness</td><td>chemosis</td><td>discharge</td></tr>
<tr><td colspan="6">Rinse group</td></tr>
<tr><td>1M</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>1 (1.1.1)</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td></tr>
<tr><td>2F</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>0.67 (1,1,0)</td><td>0.67 (1,1,0)</td><td>0 (0,0,0)</td></tr>
<tr><td>3F</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>0.67 (1,1,0)</td><td>0.33 (1,0,0)</td><td>0 (0,0,0)</td></tr>
<tr><td>4F</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>1.33 (2,1,1)</td><td>0.33 (1,0,0)</td><td>0 (0,0,0)</td></tr>
<tr><td>5F</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>0.33 (1,0,0)</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td></tr>
<tr><td>6M</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>0.67 (1,1,0)</td><td>0.33 (1,0,0)</td><td>0 (0,0,0)</td></tr>
<tr><td colspan="6">Rinse group</td></tr>
<tr><td>1M</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>1 (2,1,0)</td><td>0.33 (1,0,0)</td><td>0 (0,0,0)</td></tr>
<tr><td>2M</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>0.67 (1,1,0)</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td></tr>
<tr><td>3F</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>0.33 (1,0,0)</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td></tr>
</table></td><td>Classification not required</td><td>IIIA 6.1.4-07 (1989)</td></tr>
<tr><td>OECD TG 405
Rabbit New Zealand White
(3 males)
0.06g
AgION Antimicrobial Type AD
Observations at 1h, 24h, 48h, 72h after instillation</td><td>1h, 24h, 48h
opacity, irititis, conjunctivitis
In 2/3 rabbits, the individual scores for conjunctival redness (1.7) are only slightly below the cut-off (2) for classification as category 2 in Regulation EC 1272/2008.

Average score at 24, 48, 72 h in individual animals
<table>
<tr><td></td><td>cornea</td><td>iris</td><td colspan="3">conjunctiva</td></tr>
<tr><td></td><td>mean</td><td>mean</td><td>redness</td><td>chemosis</td><td>discharge</td></tr>
</table></td><td>Classification not required</td><td>IIIA 6.1.4-16 (2006e)</td></tr>
</table>

<table>
<tr><td></td><td><table>
<tr><td>1</td><td>0.67
(1,1,0)</td><td>0.67
(1,1,0))</td><td>1.67
(3,2,0)</td><td>1.33
(2,2,0)</td><td>1
(2,1,0)</td></tr>
<tr><td>2</td><td>0.67
(1,1,0)</td><td>0.67
(1,1,0)</td><td>1.67
(3,2,0)</td><td>0.67
(1,1,0)</td><td>0.33
(1,0,0)</td></tr>
<tr><td>3</td><td>0
(0,0,0)</td><td>0.33
(1,0,0)</td><td>1
(2,1,0)</td><td>0.33
(1,0,0)</td><td>0.33
(1,0,0)</td></tr>
</table></td><td></td><td></td></tr>
<tr><td>EPA: OPPTS 870.2400
(81-4) New Zealand White Rabbit 3M, 3F
0.1 g HealthShield Grade: AK10D
(Same as AgION® Silver Antimicrobial Type AK)</td><td>Average score at 24, 48, 72 h in individual animals<table>
<tr><td></td><td>cornea</td><td>iris</td><td colspan="3">conjunctiva</td></tr>
<tr><td></td><td>mean</td><td>mean</td><td>redness</td><td>chemosis</td><td>discharge</td></tr>
<tr><td>1M</td><td>0
(0,0,0)</td><td>0
(0,0,0)</td><td>3
(3,3,3)</td><td>0.33
(1.0,0)</td><td>0
(0,0,0)</td></tr>
<tr><td>2M</td><td>1
(1,1,1)</td><td>0.67
(2,0,0)</td><td>3
(3,3,3)</td><td>1.33
(4.0,0)</td><td>0.33
(1,0,0)</td></tr>
<tr><td>3M</td><td>0
(0,0,0)</td><td>0.33
(1,0,0)</td><td>3
(3,3,3)</td><td>0.33
(1,0,0)</td><td>0
(0,0,0)</td></tr>
<tr><td>4F</td><td>0
(0,0,0)</td><td>0
(0,0,0)</td><td>3
(3,3,3)</td><td>0.33
(1.0,0)</td><td>0
(0,0,0)</td></tr>
<tr><td>5F</td><td>0.67
(1,1,0)</td><td>0.67
(2,0,0</td><td>3
(3,3,3)</td><td>1
(3,0,0)</td><td>0.67
(2.0,0)</td></tr>
<tr><td>6F</td><td>0
(0,0,0)</td><td>0.33
(1,0,0)</td><td>2.67
(3,3,2)</td><td>1.33
(3,1,0)</td><td>0
(0,0,0)</td></tr>
</table></td><td>CLP:
Eye Dam 1;H318 DSD: Xi;R41</td><td>IIIB 6.2(01) (2000)</td></tr>
</table>

4.4.2.1 Non-human information

The documentation submitted for the evaluation of eye irritation of silver zinc zeolites Irgaguard B502i and AgION Antimicrobial Types AK and AJ includes guideline studies performed with Irgaguard B502i, AgION Antimicrobial Type AD and an unspecified type assumed to represent Irgaguard B502i. Data obtained with AgION Antimicrobial Type AK is available in the database for the formulation (i.e. Doc IIIB, section 6). There is no information available on Type AJ but as discussed in the confidential Appendix 2 - Technical equivalence, technical specification and read-across, use of data for Type AK is considered acceptable.
Instillation of the unspecified silver zinc zeolite or TKA 45039 (Irgaguard B502i) resulted in an initial iritis and conjunctivitis (redness, swelling and discharge) in all test eyes after 1 hour. The conjunctival irritation had resolved in all test eyes by day 7. Overall, the severity of the effects noted in both studies was below the criteria for classification (see table 14).
The effects noted in a study performed with Type AD (which is not used in any of the representative formulations) did not fulfil the criteria for classification but it is noted that the individual scores for conjunctival redness (1.7) in 2/3 rabbits treated with AgION Antimicrobial Type AD are only slightly below the cut-off (2) for classification as Eye irrit. 2 in Regulation EC 1272/2008 (Doc IIIA, 6.1.4(16)).
Instillation of AgION Antimicrobial Type AK in the eye caused a moderate to severe redness of conjunctiva that cleared in all but one of the six animals within 10 to 14 days. In this male, the redness of the conjunctivae persisted during the entire 21 day observation period. Animals also suffered from corneal opacity, chemosis and discharge that generally cleared within 3 days. The average scores for conjunctival redness and chemosis in all six animals for the 24, 48 and 72 hours were 3.0 and 2.0 respectively.
AgION Antimicrobial Type AK is considered to meet the criteria for classification Eye Dam. 1, H318 (causes serious eye damage) based on the conjunctival redness that persisted at the same severity grade during the entire observation period in one animal. In remaining animals a severity grade of 2 persisted at least to day 7.

In the absence of specific information on Type AJ, this silver zinc zeolite is considered to have the same eye irritating potential as Type AK.

4.4.2.2 Human information

No substance-specific data available.

4.4.2.3 Summary and discussion of eye irritation

Based on the physico-chemical properties of the substance, there is no apparent explanation why AgION Antimicrobial Type AK meets the criteria for classification while Irgaguard B502i does not. In contrast to the skin irritation studies, there is no vehicle that possibly influences the results. Since AgION Antimicrobial Type AD which contains a higher amount of silver than Type AK caused less irritation than Type AK, the irritation potential may not primarily be linked to silver.
Since none of the two studies can be dismissed for quality reasons, it is considered appropriate to consider all three silver zinc zeolites as having the potential to cause eye damage.

4.4.2.4 Comparison with criteria

According to Regulation EC No 1272/2008, a substance should be classified Category 1 (serious eye damage) *If, when applied to the eye of an animal, a substance produces: at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or at least in 2 of 3 tested animals, a positive response of:corneal opacity ≥ 3 and/or iritis > 1,5 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material.*

According to the guidance document on the application of the CLP criteria, the following applies if 6 rabbits are used in the test:

a. Classification as serious eye damage – Category 1 if:
i. at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or
(ii) at least 4 out of 6 rabbits show a mean score per animal of ≥ 3 for corneal opacity and/or > 1.5 for iritis
b. Classification as eye irritation – Category 2 if at least 4 out of 6 rabbits show a mean score per animal of:
i. ≥ 1 for corneal opacity and/or
ii. ≥ 1 for iritis and/or
iii. ≥ 2 conjunctival erythema (redness) and/or
iv. ≥ 2 conjunctival oedema (swelling) (chemosis) and which fully reverses within an observation period of normally 21 days."

Since conjunctival redness (grade 3) persisted during the entire observation period in one animal, criterion a (i) is considered fulfilled.

4.4.2.5 Conclusions on classification and labelling

Based on the average scores and the observation of non-fully reversible conjunctival redness in one rabbit within 21 days in the study with AgION Antimicrobial Type AK, silver zinc zeolite is considered to meet the criteria for classification Eye Dam. 1; H318 according to CLP. Despite that only the results obtained with AgION Antimicrobial Types AK fulfils the classification criteria as Eye Dam. 1 (H318), the RMS proposes that classification should be considered for all three silver zinc zeolites. In the absence of appropriate dose-response data, no specific concentration limits can be set.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed classification of the three SZZ as Eye Damage 1 (H318) based on the average scores and the observation of non-fully reversible conjunctival redness in one rabbit within 21 days in the study with ON Type AK only. Treatment with other SZZ caused slight effects (redness, chemosis). The DS argued in favour of classifying all types of zeolites as Eye Damage 1 (H318) on the basis of differences between the available studies.

Comments received during public consultation

One MS stated that the differences in response between the different zeolites could be caused by differences in Ag and/or Zn content and release under watery conditions, which would indicate that different classifications would be applicable to the different substances. The DS disagreed and responded that the significant differences observed in eye irritation studies may be due to other factors like different amounts of zeolites applied in the eyes and that a lower number of animals may also have influenced the outcome of the studies.

Assessment and comparison with the classification criteria

The table below summarises the available eye corrosion/irritation studies.

Table 6 (RAC): Summary of eye corrosion/irritation studies

Method	Result	Classification according to CLP criteria
EPA: OPPTS 870.2400 (81-4) New Zealand (White Rabbit) 3M, 3F 0.1 g HealthShield Grade: AK10D (Same as AgION Type AK)	Average score at 24, 48, 72 h in individual animals:	**Eye Dam 1;H318** Doc IIIB 6.2(01) (Nitka, 2000) Reliability 1

			Conjunctiva		
	cornea	iris	redness	chemosis	Discharge
1M	0 (0,0,0)	0 (0,0,0)	3 (3,3,3)	0.33 (1,0,0)	0 (0,0,0)
2M	1 (1,1,1)	0.67 (2,0,0)	3 (3,3,3)	1.33 (4,0,0)	0.33 (1,0,0)
3M	0 (0,0,0)	0.33 (1,0,0)	3 (3,3,3)	0.33 (1,0,0)	0 (0,0,0)
4F	0 (0,0,0)	0 (0,0,0)	3 (3,3,3)	0.33 (1,0,0)	0 (0,0,0)
5F	0.67 (1,1,0)	0.67 (2,0,0)	3 (3,3,3)	1 (3,0,0)	0.67 (2,0,0)
6F	0 (0,0,0)	0.33 (1,0,0)	2.67 (3,3,2)	1.33 (3,1,0)	0 (0,0,0)
Mean	0.33, 0.33, 0.16	1, 0, 0	3, 3, 2.8	2.2, 0.16, 0	0.5, 0, 0

<table>
<tr>
<td>OECD TG 405

Rabbit (New Zealand White)

2M, 1F

0.095g (0.1 mL) Test material: Irgaguard</td>
<td>1 h: iritis, conjunctivitis

Average score at 24, 48, 72 h in individual animals:

<table>
<tr><td></td><td></td><td></td><td colspan="3">Conjunctiva</td></tr>
<tr><td></td><td>cornea</td><td>iris</td><td>redness</td><td>chemosis</td><td>Discharge</td></tr>
<tr><td>1M</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>1 (2,1,0)</td><td>0.33 (1,0,0)</td><td>0 (0,0,0)</td></tr>
<tr><td>2F</td><td>1 (1,1,1)</td><td>0 (0,0,0)</td><td>0.67 (1,1,0)</td><td>0.33 (1,0,0)</td><td>0.33 (1,0,0)</td></tr>
<tr><td>3M</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>1 (1,1,1)</td><td>0.33 (1,0,0)</td><td>0 (0,0,0)</td></tr>
</table>
</td>
<td>Classification not required

Doc IIIA 6.1.4-06 (Wilson 2002c)

Reliability 1</td>
</tr>
<tr>
<td>OECD TG 405

Rabbit (New Zealand White)

4M, 5F

No-rinse group: 2M/4F
Rinse group:2M/1F

0.058g (0.1 mL) unspecified zinc zeolite</td>
<td>1 h: iritis, conjunctival irritation (redness, swelling and production of ocular discharge)

The conjunctival irritation was resolved by day 7.

Average score at 24, 48, 72 h in individual animals:

<table>
<tr><td></td><td></td><td></td><td colspan="3">Conjunctiva</td></tr>
<tr><td></td><td>cornea</td><td>iris</td><td>redness</td><td>Chemosis</td><td>Discharge</td></tr>
<tr><td>1M</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>1 (1,1,1)</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td></tr>
<tr><td>2F</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>0.67 (1,1,0)</td><td>0.67 (1,1,0)</td><td>0 (0,0,0)</td></tr>
<tr><td>3F</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>0.67 (1,1,0)</td><td>0.33 (1,0,0)</td><td>0 (0,0,0)</td></tr>
<tr><td>4F</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>1.33 (2,1,1)</td><td>0.33 (1,0,0)</td><td>0 (0,0,0)</td></tr>
<tr><td>5F</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>0.33 (1,0,0)</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td></tr>
<tr><td>6M</td><td>0 (0,0,0)</td><td>0 (0,0,0)</td><td>0.67 (1,1,0)</td><td>0.33 (1,0,0)</td><td>0 (0,0,0)</td></tr>
</table>
</td>
<td>Classification not required

Doc IIIA 6.1.4-07 (Rush, 1989)

Reliability 2</td>
</tr>
<tr>
<td>OECD TG 405

Rabbit (New Zealand White)

3 M

0.06g AgION Type AD</td>
<td>1h, 24h, 48h: opacity, irititis, conjunctivitis

In 2/3 rabbits, the individual scores for conjunctival redness (1.7) are only slightly below the cut-off (2) for classification as category 2.

Average score at 24, 48, 72 h in individual animals

<table>
<tr><td></td><td></td><td></td><td colspan="3">Conjunctiva</td></tr>
<tr><td></td><td>cornea</td><td>iris</td><td>redness</td><td>chemosis</td><td>Discharge</td></tr>
<tr><td>1M</td><td>0.67 (1,1,0)</td><td>0.67 (1,1,0)</td><td>1.67 (3,2,0)</td><td>1.33 (2,2,0)</td><td>1 (2,1,0)</td></tr>
<tr><td>2F</td><td>0.67 (1,1,0)</td><td>0.67 (1,1,0)</td><td>1.67 (3,2,0)</td><td>0.67 (1,1,0)</td><td>0.33 (1,0,0)</td></tr>
<tr><td>3M</td><td>0 (0,0,0)</td><td>0.33 (1,0,0)</td><td>1 (2,1,0)</td><td>0.33 (1,0,0)</td><td>0.33 (1,0,0)</td></tr>
</table>
</td>
<td>Classification not required

Doc IIIA 6.1.4-16 (Moore 2006e)

Reliability 1</td>
</tr>
</table>

RAC notes that:

i. Three studies with three different SZZ, including two zeolites that are either not specified or do not belong to the group of zeolites covered by this proposal, showed mean scores for corneal opacity and iritis lower than 1 and conjunctival redness and oedema lower than 2 following grading at 24, 48 and 72 hours after instillation of the test material.

ii. One study with SZZ Type AK showed mean scores for redness of 3 in nearly all

animals following grading at 24, 48 and 72 hours after instillation of the test material (the sixth scored 2.67). In the same study, scores for chemosis varied between 0.33 and 1.33 in all animals but most effects only appeared at 24 hours after instillation. The redness of the conjunctivae persisted during the entire 21 day observation period for one out of 6 animals.

RAC notes a comment from industry that the elution rate of ions may depend on the content and type of of the water in which the material is immersed. In the case of comparable eye irritation studies, a vehicle effect is not possible. RAC agrees with the DS that a number of other factors like different amounts of zeolites applied in the eyes, lower number of animals, rinsing or not rinsing the eyes during the assays or potentiation of silver release through mechanical friction among solid zeolites may have influenced the outcome of the studies.

According to the CLP criteria, serious eye damage means the production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application. The same hazard category is also applied to eye effects in 2 of 3 tested animals (or 4 of 6 tested animals), with corneal opacity scores ≥ 3 and/or iritis scores > 1.5. Instillation of AgION Type AK in the eye caused a moderate to severe redness of conjunctiva that cleared in all but one of the six animals within 10 to 14 days. In one male, the redness of the conjunctivae persisted during the entire 21 day observation period, which fulfils the CLP criteria for classification as Eye Dam. 1.

Thus, according to the CLP criteria, SZZ Type AK (the same type of zeolite used for classifying as skin irritant) fulfils the criteria to be classified as Eye Damage 1 and therefore **RAC, in accordance with DS, supports the classification of all silver zinc zeolite as Eye Damage Category 1; H318 (Causes serious eye damage).**

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

No data available.

4.4.3.2 Human information

No substance-specific data available.
In a study referred to in the review prepared by the Oak Ridge Reservation Environmental Restoration Program (1979)[4], 30 workers were exposed to silver nitrate and silver oxide dusts for periods of less than one year to greater than ten years. Twenty five individuals experienced respiratory irritation (sneezing, stuffiness, running nose or sore throat) at some time during their employment. Twenty of thirty workers reported coughing, wheezing, chest tightness and abdominal pain; the latter finding was closely correlated with blood silver levels. The eight hour time weighted

[4] http://rais.ornl.gov/tox/profiles/silver_f_V1.html

average exposure (determined 4 months prior to the study) was in the range 0.039 to 0.378 mg silver/m^3 for this subpopulation.

4.4.3.3 Summary and discussion of respiratory tract irritation

Respiratory irritation in the form of sneezing, stuffiness, running nose or sore throat coughing, wheezing, chest tightness or abdominal pain has been reported in workers exposed to silver nitrate or silver oxide.There is no robust information on the level and duration of exposure.

4.4.3.4 Comparison with criteria

According to Regulation 1272/2008, a substance causing respiratory tract irritation may be classified in hazard category STOT-SE category 3 (i.e. Specific Target Organ Toxicant-single exposure) based on the following criteria:

(a) respiratory irritant effects (characterised by localised redness, oedema, pruritis and/or pain) that impair function with symptoms such as cough, pain, choking, and breathing difficulties are included. This evaluation will be based primarily on human data;

(b) subjective human observations could be supported by objective measurements of clear respiratory tract irritation (RTI) (such as electrophysiological responses, biomarkers of inflammation in nasal or bronchoalveolar lavage fluids);

(c) the symptoms observed in humans shall also be typical of those that would be produced in the exposed population rather than being an isolated idiosyncratic reaction or response triggered only in individuals with hypersensitive airways. Ambiguous reports simply of 'irritation' shall be excluded as this term is commonly used to describe a wide range of sensations including those such as smell, unpleasant taste, a tickling sensation, and dryness, which are outside the scope of classification for respiratory irritation;

(d) there are currently no validated animal tests that deal specifically with RTI, however, useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of weight of evidence evaluation;

(e) this special classification would occur only when more severe organ effects including in the respiratory system are not observed.

In the absence of substance specific data on silver zinc zeolite or any reliable information on other silver substances, it is not possible to assess if silver zinc zeolite would meet criteria for classification.

4.4.3.5 Conclusions on classification and labelling

There is no substance specific data to assess if silver zinc zeolite has the potential to induce respiratory irritation. Cases of respiratory reactions to silver nitrate or silver oxide has been described in the open literature but data is insufficient to clarify if these should be categorised as sensitisation reactions or as respiratory irritation. Moreover, the exposure level and duration as well

as the clinical history of the subjects is unknown.
Therefore, it is not possible to decide, based on the information available for this endpoint, whether or not silver zinc zeolite would meet criteria for classification.

4.5 Corrosivity

Table 15: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
Please refer to information in section 4.4.1.			

4.5.1 Non-human information

Please refer to information in section 4.4.1.

4.5.2 Human information

No data available.

4.5.3 Summary and discussion of corrosivity

Please refer to information in section 4.4.1.

4.5.4 Comparison with criteria

Please refer to information in section 4.4.1.

4.5.5 Conclusions on classification and labelling

Please refer to information in section 4.4.1.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 16: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Magnusson & Kligman Maximisation Study OPPTS (870.2600), OECD (406) Guinea pigs 10/sex, 5/sex control Induction: Intradermal injection day 0: 5% (TKA 45039 also known as IRGAGUARD B 502l) in propylene glycol Topical Application day 7, exposure 48 hours: 100%w/w TKA 45039 in drops of polypropylene glycol Challenge day 21 exposure 24 hours: 100 %w/w TKA 45039 in drops of polypropylene glycol Evaluation 24, 48 and hours post challenge	Negative	The sensitivity of the system was shown by a study with α-hexylcinnamaldehyde (HCA) conducted during the past 6 months.	IIIA 6.1.5-09 (2002)
Buehler Guinea pigs 5/sex, 5/sex control animals for challenge (naïve group) Induction (1/week) x 3, exposure 6 hours: 60% w/w Silver zinc zeolite test solution in 0.5% CMC Challenge 2 weeks post third application: 60%w/w test solution in 0.5% CMC Evaluation 24, 48 and 72 hours post challenge	Negative	Fewer animals than required were used. The highest dose to cause irritation was not identified in dose-finding study. The sensitivity of the system was shown by 1-chloro-2, 4-dinitrobenzene (DNCB) replacing the test material in each phase.	IIIA 6.1.5-03 (1989d)
Buehler EPA 870.2600 Guinea pig 6/sex (5/sex controls) Induction (1/week) x 3, exposure 6 hours: 0.4 g of test substance moistened with saline Challenge 2 weeks post third application: 0.4 g of test substance moistened with saline Evaluation 24, 48 and 72 hours post challenge HealthShield GradeAK10D (Same as AgION® Silver	Negative	Lack of reliability test The treated area is not reported. 20 test animals is recommended for Buehler test in OECD TG 406	IIIB 6.3(01) (2000)

Method	Results	Remarks	Reference
Antimicrobial Type AK)			
JMHW No. 24 Maximization test Guinea pig 15 animals (f) Positive control: 5 animals (f) Zeomic AJ10N (Same as AgION® Silver Antimicrobial Type AJ) Challenge: 10, 1, or 0.1% Zeomic DMSO suspensions at 24 or 48 hours after patch removal.	Negative	Positive reactions were observed in 100% of animals in a concurrent positive control group exposed to 2,4-dinitrochloro-benzene.	IIIB 6.3(02) (1996)

4.6.1.1 Non-human information

The results obtained in studies with AgION Antimicrobial Types AK (Buehler), Irgaguard B502i (GPMT) and AJ (Buehler) do not indicate that these types of silver zinc zeolites have any sensitizing properties. The result obtained with Type AK should be interpreted with some caution since fewer animals than recommended in Council Regulation 440/2008 was used, the size of the treated area was not reported and the study report does not contain any information on the latest reliability check. However, the presence of faint erythema in a few test animals may be interpreted as an indication that the experimental technique was sensitive and that the results thus are reliable. Considering also that negative results were obtained for the three other silver zinc zeolites, i.e. Type AJ, Irgaguard B502i and the unspecified silver zinc zeolite, it seems realistic to assume that Type AK also lacks sensitizing properties.

4.6.1.2 Human information

There is no human data available for silver zinc zeolite. For elemental silver and silver nitrate, information available in the IUCLID Chemical Data Sheet posted on the website for the European chemical Substances Information System (ESIS) states that mild allergenic responses observed have been attributed to 20 years exposure to silver in dental amalgams. This case report is also described in the report prepared by the Agency for Toxic Substances and Disease Registry (Doc IIIA, section 6.2(09)). According to the ATSDR report, mild allergenic responses have also been observed in a worker dermally exposed to powdered silver cyanide (6 months of exposure) and a worker in contact with radiographic processing solutions (exposure 10 years). Two cases of skin sensitisation following burn treatment with silver sulfadiazine cream have been reported (USEPA 1980).
The applicant argues that other components of amalgam are responsible for the sensitization reactions observed and refers to an article by McCullough, M.J. and Tyas, M.J (2008). These authors state “The allergens thought to be responsible are usually mercury or mercury compounds, and rarely tin, zinc, copper, silver, gold or palladium.” but there are no references given to support the statement. Sensitisation reactions following therapeutic uses of silver nitrate, colloidal silver or silversulfadiazine are described in a textbook by A.B. G Lansdown[5]. The book also states that

[5] Alan BG Lansdown; Silver in Healthcare (2010) ISBN:978-1-84973-006-8

allergic reactions were observed in patch tests with 5 or 10% solutions of silver nitrate when patients were exposed to “aged” (i.e. more ionised) solutions but not to freshly prepared solutions.

4.6.1.3 Summary and discussion of skin sensitisation

The sensitising potential of Irgaguard B502i and AgION Antimicrobial Types AK and AJ has been tested in guinea pigs. Negative results were obtained in all three studies.
Despite the uncertainty regarding the sensitivity of the method in the test with AgION Antimicrobial Type AK, the weight of evidence (i.e. signs of irritation in the study, chemical similarity with Antimicrobial Type AJ) suggests that none of the three types of silver zinc zeolites cause sensitisation.
Based on human cases reported, silver ions do however seem to have an intrinsic potential to induce sensitisation but this potential is apparently not evident at the levels present in the three silver zinc zeolites considered in this report.

4.6.1.4 Comparison with criteria

According to Regulation (EC) No 1272/2008, an incidence of 15 % sensitised guinea pigs in a Buehler test and ≥ 30 % in a Guinea Pig Maximisation Test is considered a positive response triggering classification. The incidences observed with Irgaguard B502i, AgION Antimicrobial Types AJ and AK were 0/20, 0/10 and 0/12 respectively.

4.6.1.5 Conclusions on classification and labelling

The results obtained in the studies performed with Irgaguard B502i, AgION Antimicrobial Types AJ and AK do not meet the criteria for classification with respect to skin sensitisation.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter’s proposal

The DS proposed no classification on the basis of four negative assays with four different SZZ.

Comments received during public consultation

No comments were received during public consultation on this hazard class.

Assessment and comparison with the classification criteria

The table below summarises the available skin sensitisation studies.

Table 7 (RAC): Summary of skin sensitisation studies

Method	Results	Remarks
Magnusson & Kligman Maximisation Study OPPTS (870.2600), OECD (406)	Negative	Doc IIIA 6.1.5-09 (Wilson 2002d) Evaluation 24, 48 and hours post challenge

Guinea pigs: 10/sex, 5/sex control Induction: Intradermal injection day 0: 5% Irgaguard in propylene glycol Topical application day 7, exposure 48 hours: 100% w/w Irgaguard in drops of polypropylene glycol Challenge day 21 exposure 24 hours: **100 %w/w Irgaguard** in drops of polypropylene glycol		The sensitivity of the system was shown by a study with α-hexylcinnamaldehyd e (HCA) conducted during the past 6 months. Reliability 1
Buehler Guinea pigs: 5/sex, 5/sex control animals for challenge (naïve group) Induction: (1/week) x 3, exposure 6 hours: 60% w/w unspecified SZZ test solution in 0.5% CMC Challenge 2 weeks post third application: 60%w/w unspecified SZZ test solution in 0.5% CMC	Negative	Doc IIIA 6.1.5-03 (1989d) Evaluation 24, 48 and 72 hours post challenge Fewer animals than required were used. The highest dose to cause irritation was not identified in dose-finding study. The sensitivity of the system was shown by 1-chloro-2, 4-dinitrobenzene (DNCB) replacing the test material in each phase. Reliability 2
Buehler EPA 870.2600 Guinea pig: 6/sex (5/sex controls) Induction (1/week) x 3, exposure 6 hours: 0.4 g of test substance moistened with saline Challenge 2 weeks post third application: 0.4 g of test substance moistened with saline **HealthShield GradeAK10D (AgION Type AK)**	Negative	Doc IIIB 6.3(01) (Nitka, 2000) Evaluation 24, 48 and 72 hours post challenge Lack of reliability test The treated area is not reported. 20 test animals is recommended for Buehler test in OECD TG 406, while this test used only 12 animals No positive control Reliability 1
JMHW No. 24 Maximization test Guinea pig: 15 F + positive control: 5 F Zeomic AJ10N (Same as AgION® Silver Antimicrobial Type AJ)	Negative	Doc IIIB 6.3(02) (Matsuda, 1996) Positive reactions were observed in 100% of animals in a concurrent positive control group exposed to 2,4-dinitrochloro- benzene.

Challenge: 10, 1, or 0.1% **Zeomic** DMSO suspensions at 24 or 48 hours after patch removal.		Reliability 2

The DS suggested a careful analysis of the results obtained with AgION® Silver Antimicrobial Type AK, because very faint erythema was found in some animals. However, a report submitted by the Industry showed that this faint erythema appeared only during the induction phase in 2 males on days 0 and 14. Thus, the result of this assay, despite other reported deficiencies, can be considered as negative.

In conclusion, **RAC agrees with the DS that no classification of silver zinc zeolite for skin sensitisation is warranted.**

4.6.2 Respiratory sensitisation

Table 17: Summary table of relevant respiratory sensitisation studies

Method	Results	Remarks	Reference
No data available.			

4.6.2.1 Non-human information

No data available.

4.6.2.2 Human information

No data on silver zinc zeolite available.

4.6.2.3 Summary and discussion of respiratory sensitisation

There is no information on respiratory sensitisation reactions to silver zinc zeolite but respiratory irritation following inhalation of silver nitrate or silver oxide have been reported. However, the information is limited and do not include data on exact exposure level, exposure duration or clinical history (smoking history, medical and occupational history) of the subjects.

4.6.2.4 Comparison with criteria

According to Regulation 1272/2008, substances should be classified for respiratory sensitisation if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity; and/or if there are positive results from an appropriate animal test. Substances should be placed in Category 1A if they show *"a high frequency of occurrence in humans; or a probability of occurrence of a high sensitisation rate in humans based on animal or other tests (1)"*. Severity of

reaction may also be considered." Substances should be categorised in 1B if they show a "*low to moderate frequency of occurrence in humans; or a probability of occurrence of a low to moderate sensitisation rate in humans based on animal or other test(1) . Severity of reaction may also be considered."*

Regulation 1272/2008 further states *"In this context, hypersensitivity is normally seen as asthma, but other hypersensitivity reactions such as rhinitis/conjunctivitis and alveolitis are also considered. The condition will have the clinical character of an allergic reaction. However, immunological mechanisms do not have to be demonstrated. "* In the absence of substance specific data on silver zinc zeolite or any reliable information on other silver substances, it is not possible to assess if silver zinc zeolite meets criteria for classification.

4.6.2.5 Conclusions on classification and labelling

There is no substance specific data if silver zinc zeolite has the potential to induce respiratory sensitisation. Cases of respiratory reactions to silver nitrate or silver oxide has been described in the open literature but data is insufficient to clarify if these should be categorised as sensitisation reactions or as respiratory irritation. Moreover, the exposure level and duration as well as the clinical history of the subjects is unknown.

Therefore, it is not possible to decide, based on the information available for this endpoint, whether or not silver zinc zeolite would meet criteria for classification..

RAC evaluation of respiratory sensitisation
Summary of the Dossier Submitter's proposal The DS proposed no classification on the basis of the absence of reliable information in humans and the total absence of information in animals. **Comments received during public consultation** No comments were received during public consultation on this hazard class. **Assessment and comparison with the classification criteria** There are no data available with animals. **RAC agrees with DS that no classification for respiratory sensitisation** is warranted because with the available information it is not possible to assess if SZZ would meet criteria for classification.

4.7 Repeated dose toxicity

Table 18: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
Oral 90 days Rat Sprague-Dawley (Crl:CD (SD)IGS BR) 10/sex 0, 1000, 6250, 12500 ppm Zeomic (stated to be AgION Silver Antimicrobial AK) (~ 64/78, 398/489 and 916/939 mg/kg bw in males and females)	**12500 ppm:** ↓Bodyweight (m, ≤ 8%) ↑Effects on behaviour/activity ↑Erythrocytes (m,10%), platelets (m, 97%) ↓Hb (m/f, 15/10%), HCT (m/f, 9/7%), MCV (m/f 18/11%),MCH (m/f, 23/15%), MCHC (m/f, 6/4%) ↑ALP (m/f, 70/143%) ↑Pigmentation of pancreas, thymus, mandibular lymph node ↑Mild hemorrhage, inflammation in the Harderian gland (M) ↑Chronic nephritis (M) ↑Urinary pH (m, 11%)↑ ↓Urine volume (m/f, n.s.s) **6250 ppm** ↑Effects on behaviour/activity ↑Pigmentation of pancreas, thymus, mandibular lymph node ↑ALP (m/f 44/80%) Other effects noted: 12500 ppm: ↑Eosinophils (f, 85%) ↑Cholesterol (m/f, 59/67%) ↑Rel heart weight (m, 11%) ↓(f) Counts of vertical and stereotypy activity(20-30 min) 6500 ppm: ↓MCV, MCH (m) ↑Cholesterol (m/f, 58/39%) 1000 ppm: ↑Cholesterol (m, 41%) ↓Counts of horizontal, vertical and stereotypy activity during the first ten minutes (m)	NOAEL: 1000 ppm LOAEL: 6250 ppm	IIIA 6.4.1(06) (2001)
Oral 90 days Dog Beagle 4/sex 0, 10, 50 and 250 mg Zeomic AK10D /kg/day	**250 mg/kg bw** ↑Vomiting, head shaking (m,f) ↓Hemoglobin (m, 20%) ↑Increased severity of corticomedullary tubular basophilia and lymphoid infiltration, interstitial fibrosis and hyaline/cellular casts ↑Discoloration of the pancreas and gastrointestinal tract Other effects noted: 250 mg/kg bw ↑APTT (f, 15%), Creatinine (m, 17%) Cholesterol (f, 42%) ALP, (f (week 6), 64%),↑Calcium (f, 3.5%) ↓GLDH (f (week 6), 20%), phospholipids (f, 33%) ↑Urinary volume (f (week 6), 250%) ↓Potassium (63%) ↑Ovaries/uterus enlarged All dose levels:	NOAEL: 50 mg/kg/day LOAEL: 250 mg/kg/day	IIIA 6.4.1(07) (2003)

Method	Results	Remarks	Reference
	↑Vomiting		
Oral Combined chronic and carcinogenicity Mouse B6C3F1 75/sex* 0, 0.1, 0.3 and 0.9% AgION Zeomic AJ 10N "at least" 0, 67, 211 and 617 mg/kg bw/day * Termination: five/sex at 3 months, ten/sex at six months, ten at 22 months and the remaining at 24 months.	**0.9%** ↓RBC (15/14%) HCT (18/22%) MCH (2/5%) MCV (3/8%) Hb (18%,m/f) ↑MCHC (2.5/4.3%) ↑ renal cysts* (m, f) ↑enlargement of Langerhan´s islands (m) ↓kidney (8%), liver (10%), brain, weight (10%) (f) ↑pancreas (19%, m) ↑pigmentation of liver and pancreas **0.3%** ↓HCT, MCV, Hb ↑MCHC (f) ↑ ovarian cysts ↑pigmentation of liver and pancreas **0.1%** ↑ ovarian cysts ↑pigmentation of liver and pancreas Other effects: 0.9% ↓bodyweight gain <10% (m) ↑severity of thrombi (m, f) ↓spleen weight (37%, m) ↓brain (10%, f) 0.3% ↓bodyweight gain <10% (m) ↓spleen weight (31%, m) ↓brain (6%, f) 0.1% ↓spleen weight (31%, m) ↓brain (6%, f) *dose-response	NOAEL: n.d. LOAEL: 0.1%	IIIA 6.5-05 (1992a)
Oral Combined chronic and carcinogenicity Rat F344 70/sex** 0.01, 0.03, 0.1 and 0.3% AgION Zeomic AJ 10N ("at least" 0, 3, 9, 30 and 87 mg /kg bw/day) ** Termination: ten rats/sex at 6 and 12 months and the remaining at 24 months.	**0.1 %**: ↑Pigmentation of liver, kidneys, pancreas, stomach, lymph nodes choroid plexus ↑ALT (m/f 175/58%), AST (f 96%), ALP (m/f 25/39%), LDL-C (m/f 28/19%) ↑endometrial polyps ↑WBC (f 134%) ↓ HCT (10%), MCH (3/3%), MCHC (f, 3%), Hb (f 12%) Other effects: all dose levels ↑Severity of hepatic bile duct proliferation ↓AST (m ≤42%, at 12 months) ↑ALT (m ≤172%, at 24 months) ↓LDH (f≤90%, at 24 months) 0.3% ↓thymus weight n.s.s (38%, f) ALB (m 11%) 0.1, 0.3% ↓TP (m ≤10%),	NOAEL (chronic)*: 0.03 % LOAEL: 0.1% *carcino-genicity is discussed in 4.10	IIIA 6.5-06 (1992b)

Method	Results	Remarks	Reference
	↑endometrial polyps 0.03%: ↑endometrial polyps		

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Data on repeated dose oral toxicity is only available for the type of silver zinc zeolite denoted AgION Antimicrobial Type AK. As discussed in the confidential appendix "Technical equivalence, technical specification and read-across", the toxicological profiles of Irgaguard B502i, AgION Type AJ and AgION Type AK are not expected to differ significantly from each other. Read across between repeated dose toxicity data is therefore considered acceptable in order to avoid further animal testing.

The repeated dose toxicity of AgION Antimicrobial Type AK was investigated in CD rats as well as in Beagle dogs.
All rats survived treatment with 1000, 6250 and 12500 ppm AgION Antimicrobial Type AK (6.4.1(06)) with the exception of a few single rats in each dose group that died during blood sampling. The bodyweights of high dose males were reduced at 5 of the 14 study weeks but the reduction was only 8% or less. The bodyweight gain was reduced by 10% but this parameter was not statistically analysed. The bodyweights and bodyweight gains of high dose females were not affected.
Administration of 6250 ppm (278/366 mg/kg bw) or higher doses resulted in effects on behaviour/activity (hypersensitivity to touch, vocalization, increased activity, aggressive behaviour), pigmentation of pancreas, thymus, the mandibular lymph node and an increase in cholesterol and alkaline phosphatase (ALP).
Increased levels of erythrocytes (10%) and platelets (97%) were observed in high dose males and decreased levels of Hb (m/f, 15/10%), HCT (m/f, 9/7%), MCV (m/f 18/11%), MCH (m/f, 23/15%), MCHC (m/f, 6/4%)) were observed in high dose males and females.
There were no statistically significant differences observed in the neurobehaviour, FOB or motor activity evaluations performed except for increased touch response in high dose animals and a few minor statistically significant effects observed in the neurological examinations performed.
The NOAEL was set at 1000 ppm (64/78 mg/kg bw) based on the effects on behaviour/activity, the increased levels of ALP (m/f 44/80%) and the pigmentation of pancreas, thymus, mandibular lymph node observed in animals administered 6250 ppm (~398/489 mg/kg bw/day).

All dogs survived treatment with 10, 50 and 250 mg/kg bw/day AgION Antimicrobial Type AK. Clinical signs such as head shaking, salivation and vomiting were observed in dogs administered 250 mg/kg bw and the haematological/biochemical analyses showed a decreased level of hemoglobin (m/f 20/8%) and increased levels of cholesterol, phospholipids and ALP. The histopathological examinations showed discoloration of pancreas and gastrointestinal tract and histopathological changes in the kidney (increased severity of corticomedullary tubular basophilia and lymphoid infiltration, interstitial fibrosis and hyaline/cellular casts).
Clinical signs that were observed in all high dose animals throughout the study period were claimed to be related to the administration route (capsules) or taste or irritancy of the test substance. Although this may well be the case, there is no comparative data supporting this and the occurrence

of vomiting yet brings an uncertainty regarding the dose actually achieved.
The level of hemoglobin was 20 % lower in high dose males compared to controls. Some changes in blood parameters were occasionally noted also in high dose females (reduced MCV (3%) and prolonged partial thromboplastin time (10%)) but they were not considered toxicologically significant. Effects on haematological parameters indicative of anemia were also noted in the rat study. According to the study author of the rat study 6.4.1(06), alterations in erythropoietic parameters (haemoglobin, haematocrit, MVC, MCH, MCHC and platelet counts) are suggestive of possible zinc toxicity. Zinc toxicity may result in inhibition of heme synthesis and/or acute erythrocytic destruction.
Enlarged and discoloured ovaries were observed in 3 of 4 high dose female dogs along with enlarged uterus (microscopically: diestrus epithelium). This was not regarded as toxicologically significant by the study author but without similar findings in control animals, the significance of these findings must be considered unclear.
The NOAEL was set at 50 mg/kg bw/day based on the clinical signs, decrease in haemoglobin (20% in males), histopathological changes in kidneys and the discoloration of the pancreas and gastrointestinal tract observed at 250 mg/kg bw/day (LOAEL).

Chronic toxicity (carcinogenicity findings are discussed in section 4.10): AgION Zeomic AJ was administered in the diet to mice in daily doses of 0, 0.1, 0.3 and 0.9% corresponding to an intake of “at least” 0, 67, 211 and 617 mg/kg bw/day (minimum drug intake). Rats received daily doses of 0, 0.01, 0.03, 0.1 and 0.3% corresponding to an intake of “at least” 0, 3, 9, 30 and 87 mg /kg bw/day (minimum drug intake).

The cumulative survival rate and the mean survival time were similar between treated mice and controls. Clinical signs were not tabulated and the information given in the report is limited to a sentence stating that abdominal masses and corneal clouding was reported in all mice (including controls) and that pigmentation of skin was noted in treated animals. The bodyweight gain was reduced in the two highest dose groups but was below 10% at all measurements except for high dose males during weeks 18-65 when it was 18% lower than in controls. Thereafter, the bodyweight gain was higher in high-dose animals compared to controls and the bodyweight gain at terminal sacrifice (24 months) was within 10% of the bodyweight gain in controls for both female and male mice. Effects on hematological parameters (decrease in HCT, Hb, MCV and increase in MCHC) were observed at the two highest dose levels (see table 18).
At termination, decreased weights of spleen, brain and pancreas were observed along with pigmentation of liver and pancreas in all treated mice. Thymus was not weighed at termination. The histopathological examination revealed an increased dose-related frequency of renal cysts in males and females and the high dose group also showed increased kidney weights in females and enlargement of Langerhan´s islands in males. The frequency of renal cysts in males and females and the enlargement of Langerhan´s island in males showed a statistically significant dose-response. Renal effects were observed also in the 90 day studies and the renal cysts observed at 617 mg/kg bw are thus considered toxicologically significant. The total number of cardiac thrombi was identical between control and high dose males but the proportion of severe cardiac thrombi was increased in high dose males. Since it was not statistically significant and since no similar effect was observed in females, the observation is not given further significance here.

A statistically significant increase in the incidence of ovarian cysts was evident although there was no clear dose-response. Since the frequency was increased already in the low dose group, it is not possible to set a NOAEL for this effect.
The chronic LOAEL in mice is thus considered to be below the lowest dose level (i.e. 0.1% which corresponds to 67 mg AgION Type AJ/kg bw/day) based on the increased frequency of ovarian and renal cysts, pigmentation of liver and pancreas and decreased organ weights in all treated mice.

The cumulative survival rate and the mean survival time in rats were similar between treated animals and controls. Clinical signs were not tabulated and the information given in the summary is limited to a sentence stating that abdominal and subcutaneous masses and corneal clouding was reported in all rats (including controls) and that pigmentation of skin was noted in treated animals. All treated rats had changed levels of liver enzymes (AST, ALT and LDH) and hepatic bile duct proliferation indicating that the liver is a target organ. The total count of white blood cells was 2-5 times higher in high dose males and females at 24 months. Effects on hematological parameters (decrease in HCT, Hb (12%), MCH and MCHC) were observed at 24 months in the two highest dose levels in females but there were no effects in males.
There were no effects in the lower dose groups at 24 months or at any dose at 6 and 12 months. Pathological examination revealed pigmentation of liver, kidneys, pancreas, stomach, lymph nodes and the choroid plexus in rats administered the highest dose.
The chronic NOAEL is considered to be 0.03% (~ 9 mg AgION Type AJ/kg bw/day) based on pigmentation observed at the LOAEL 0.1% (~ 30 mg AgION Type AJ/kg bw/day).

4.7.1.2 Repeated dose toxicity: inhalation

There is no inhalation toxicity study with silver zinc zeolite available.

4.7.1.3 Repeated dose toxicity: dermal

There is no dermal toxicity study performed with silver zinc zeolite available. A dermal 90 day study performed with a different silver containing active substance is included in the data submitted by the applicant. Some effects on bodyweight gain were observed at all dose levels in the study but the effects were neither statistically significant nor related to the dose. Histopathological findings in the kidneys (dilated/atrophic ducts) occurred in high dose animals (1000 mg/kg bw) but they were not considered adverse. Since this active substance does not contain zinc, the relevance of this study for the three types of silver zinc zeolites being evaluated is limited.

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.1.5 Human information

There is no human data specific to silver zinc zeolite available.

However, pigmentation of tissues and organs which were observed in all repeated dose studies performed (90-day studies, chronic/carcinogenicity study and the two-generation study) is an effect likely due to the deposition of silver and it has been observed also in humans exposed to different silver containing substances. In fact, pigmentation of human skin, denoted argyria, has so far been considered the prominent toxicological effect caused by repeated exposure to silver or silver compounds.
Argyria can be generalized (a blueish-gray discoloration of the skin, hair and internal organs), localized or confined to the structures in the eye (argyrosis) and there seems to be variability in the susceptibility to this effect. The lowest dose to cause argyria is reported in a study from 1935 describing a patient who received approximately 1 g silver (in the form of silver arsphenamine) by

intravenous administration during 2 to 9 years. The discoloration is most prominent in areas exposed to the sun which has been explained (but not proven) to be the result of an increase in melanin production in response to silver deposition. According to the Agency for Toxic Substances and Disease Registry (Doc IIIA, 6.2(08)), the deposition of silver in tissues is due to precipitation of insoluble silver salts like silver chloride and silver phosphate. These salts are then transformed into soluble silver sulfide albuminates that bind or form complexes with amino or carboxylgroups in RNA, DNA and proteins or are reduced by ascorbic acid or catecholamines. Argyria in humans may be caused by photoreduction of silver chloride to metallic silver which in turn may be oxidized by tissue, subsequently forming black silver sulfide. Biopsy samples taken from affected individuals show deposition in tissues such as the kidney, liver and the gastrointestinal tract (6.2(04)). Mineral deposits have been observed in basal membranes for macrophages, in the pericurium of the peripheral nerves, along elastic and collagenous fibres and in the necrotic cells of the oral mucosa using light and electron microscopy (6.12.2(05)). According to published literature, clinical conditions in humans that can be associated with argyria seems restricted to a few isolated cases which include hepatic and renal failure (6.12.2(07)), neurological disorders including taste and smell disorders, vertigo and hypaesthesia (6.12.2(05)) and respiratory irritation along with reduced night vision in workers exposed to dusts of silver compounds (6.12.2(08)).
Although the toxicological significance of tissue pigmentation is somewhat unclear, it is considered appropriate to be precautious and deposition of silver in tissues is therefore regarded as an undesired effect that may lead to adverse effects in humans.

4.7.1.6 Other relevant information

The dossier submitted for review under the Biocides Directive 98/8/EC also contains some data on the repeated dose toxicity of other silver containing active substances. Since these substances do not contain zinc, the results of these studies may not fully represent silver zinc zeolite and this information is thus not included in this report. The data available on silver zinc zeolite specifically, is yet considered sufficient for the purpose of assessing specific target organ toxicity after repeated exposure (see below).

4.7.1.7 Summary and discussion of repeated dose toxicity

Data on short-term repeated dose oral toxicity is only available for the type of silver zinc zeolite denoted AgION Antimicrobial Type AK whereas data on long-term oral toxicity is only available for the type of silver zinc zeolite denoted AgION Antimicrobial Type AJ.
As discussed in the confidential attachment to section 13 of the technical dossier (Technical equivalence, technical specification and read-across), the toxicological profiles of Irgaguard B502i, AgION Type AJ and AgION Type AK are not expected to differ significantly from each other and read across between repeated dose toxicity data is therefore considered acceptable.

The repeated dose toxicity of silver zinc zeolite was investigated in two 90 day studies in CD rats and Beagle dogs respectively and chronic toxicity was investigated in B6C3F1 mice and Fischer 344 rats. Repeated dose toxicity was also investigated in a two-generation study with Sprague-Dawley CD rats (section 4.11).

Repeated intake of silver zinc zeolite for 90 days caused clinical signs such as head shaking, salivation and vomiting in dogs, possibly due to taste or irritancy of the test substance. A decreased level of haemoglobin (m/f 20/8%) and increased levels of cholesterol, phospholipids and ALP were

observed in high dose dogs (250 mg/kg bw). The histopathological examinations showed discoloration of pancreas and gastrointestinal tract and changes in the kidney.

Repeated intake of silver zinc zeolite for 90 days in rats resulted in effects on behaviour/activity (hypersensitivity to touch, vocalization, increased activity, aggressive behaviour) but only single statistically significant differences in the evaluations of neurobehaviour, FOB or motor activity performed. In addition, silver zinc zeolite caused effects on haematological and biochemical parameters (increase in cholesterol and alkaline phosphatase ALP)) and pigmentation of pancreas, thymus and the mandibular lymph node were observed at the histopathological examination.
Chronic intake of silver zinc zeolite also affected haematological and biochemical parameters in rats and mice and caused pigmentation of skin and internal organs. A dose-related increase of renal cysts was observed in both male and female mice and the high dose females also had increased kidney weight. As discussed above, effects on kidneys were also observed in the 90 day studies.
A statistically significant increase in the incidence of ovarian cysts was evident at all dose levels although there was no clear dose-response. Since this could be a hormonally mediated effect, it is unclear if a dose-response would be expected taking into account the complex regulation of hormones (e.g. feed-back mechanisms).
In similarity with the 90 day and chronic toxicity studies, effects on haematological parameters (i.e. increased levels of erythrocytes, platelets and decreased levels of hemoglobin (Hb), hematocrit (HCT), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were observed also in high dose male and female parents of the two-generation study in rats. As in the other repeated dose toxicity studies, pigmentation was observed in several tissues of mid and high dose animals and mild pigmentation of pancreas and thymus was observed also in some females of the low dose group. Besides pigmentation, the pathological examinations revealed histopathological changes in the kidneys including hydronephrosis and decreased size of thymus/thymic athrophy in both parents and pups of the fertility study (see section 4.11).

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to CLP

In summary, five different adverse effects to be considered for classification was identified in the repeated dose toxicity studies; reduced haemoglobin levels, pigmentation of organs/ tissues, nephrotoxicity, reduced thymus weight and increased mortality (table 19).

Table 19:

Study	LOAEL (reduced haemoglobin)	LOAEL (pigmentation)	LOAEL (nephrotoxicity)	LOAEL (reduced thymus)	LOAEL death
90-day rat	916/939 mg/kg bw/day ↓ (m/f, 15/10%)	398/489 mg/kg bw/day	Chronic nephritis 916/939 mg/kg bw	-	No treatment related deaths
90-day dog	250 mg/kg bw/day ↓ (m, 20%) *50 mg/kg bw/day ↓(m, 10%)*	250 mg/kg bw/day	Increased severity of corticomedullary tubular basophilia and lymphoid infiltration, interstitial fibrosis and hyaline/cellular casts 250 mg/kg bw/day	-	No deaths
Chronic rat	30 mg/kg bw/day	30 mg/kg bw/day	-	87 mg/kg	No treatment

Study	LOAEL (reduced haemoglobin)	LOAEL (pigmentation)	LOAEL (nephrotoxicity)	LOAEL (reduced thymus)	LOAEL death
	↓ (f 6%, 24 months)			bw/day (38%, f) n.s.s	related deaths
Chronic mouse	~211 mg/kg bw/day ↓ (m 5%, 12 months) (f 4%, 24 months)	~67 mg/kg bw/day (lowest dose tested)	Renal cysts: 617 mg/kg bw/day		No treatment related deaths
Two-generation rat	984/1109 mg/kg bw/day ↓F0 (m/f 16/12%),	72/87 mg/kg bw/day (F0, F1)	Hydronephrosis: 72/87 mg/kg bw/day (see section 4.11)	≤72/87 mg/kg bw/day (F1,F2)	~472/548 mg/kg bw

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to CLP

According to CLP, *Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure.*
Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Guidance dose/concentration values are provided below (see 3.9.2.9), to be used as part of a weight-of-evidence evaluation.

Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basisof observations from appropriate studies in experimental animals in which significant toxiceffects, of relevance to human health, were produced at generally moderate exposureconcentrations. Guidance dose/concentration values are provided below (see 3.9.2.9) in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2 (see 3.9.2.6).

Reduced haemoglobin levels: In the guidance document on haemaolytic anemia prepared within the European Chemicals Bureau (document ECBI/07/03 Add. 11) and in the Guidance to Regulation (EC) No 1272/2008, a reduction of 20 % or more in Hb concentration is considered to be a sufficient stand-alone criterion for haemolytic anaemia.
However, since the 20% reduction was observed at a dose level of 250 mg/kg bw (10% Hb reduction at 50 mg/kg bw) which is 2.5 times above the upper range (10< C≤ 100 mg/kg bw) for STOT-RE, category 2, it is not considered necessary to classify silver zinc zeolite for this effect.

Pigmentation: discoloration of organs and tissues was observed at a dose of 0.1 % (“at least 30 mg/kg bw”) in the chronic/carcinogenicity study in rats, at the lowest dose tested in the chronic/carcinogenicity study in mice (0.1% corresponding to “at least 67 mg/kg bw”) and in

parents and offspring at the lowest dose tested in the two-generation study in rats (72/87 mg/kg bw (pre-mating)).
Despite pigmentation caused by silver zinc zeolite is an irreversible effect, it is uncertain whether the criterion "serious damage" is fulfilled since the effect has not been clearly associated with major functional changes. Nevertheless, an irreversible accumulation of a heavy metal in organs and tissues is considered an undesired effect.
In the previous legislation on classification (DSD[6]), the risk phrase R33 could be used when accumulation in the human body was expected. The CLP does not contain an equivalent category and hazard statement but criteria for STOT-RE, category 2 is considered fulfilled as silver accumulates in organs and tissues at a dose level within the guidance range ($10 < C \leq 100$ mg/kg bw adjusted to $5 < C \leq 50$ mg/kg bw for 6 month exposure).

Nephrotoxicity: the majority of the adverse kidney effects observed among mice, rats and dogs occurred at dose levels above the guidance value. Hydronephrosis occurred in adult animals of all generations. The effect occurred also in 3 females and one male (compared to none in controls) of the F1 1000 ppm adults (~72/87 mg/kg bw/day) which was the lowest dose tested. Although this value is above the range $4.5 < C \leq 45$ mg/kg bw (adjusted for 202 day exposure during gestation, pre-mating until termination of affected F1 animals), the LOAEL is unknown and it can thus not be excluded that effects do occur also at doses within the guidance value range. According to section 3.9.2.9.8 of the CLP, "The guidance values and ranges mentioned in paragraphs 3.9.2.9.6 and 3.9.2.9.7 are intended only for guidance purposes, i.e. to be used as part of the weight of evidence approach, and to assist with decisions about classification. They are not intended as strict demarcation values."
On the other hand, the frequency observed at the lowest dose level was only slightly increased (3 males and one female compared to none in controls).

Thymus weight: Reduced thymus weight was observed in the two generation study in rat. The thymus weight was not investigated in adult animals but the histopathological examination revealed thymus atrophy in F1 adults administered the highest dose (~984/1109 mg/kg bw/day). Reduced thymus weights were observed in pups of both generations and occurred also at the lowest dose tested (i.e. ~72/87 mg/kg bw/day). Since the exposure period was approximately 43 days, the guidance value range is adjusted to ($21 < C \leq 208$ mg/kg bw) and the effect level is thus within this range. Since the thymus was not weighed in F0 animals it is difficult to assess if the reduced thymus weight observed in pups results from repeated exposure or if it results from developmental toxicity caused by silver zinc zeolite. The fact that there were no observations of thymic atrophy in F0 but in the next generations may reflect a developmental effect but reduced thymus weight was also noted in the chronic toxicity/carcinogenicity study thus the effect could also be due to a long term exposure. However, the thymus weight was only statistically significantly reduced in male rats at 12 months and no difference in weight compared to controls was observed in male rats sacrificed at 24 months. The thymus weight was reduced in high dose females but statistical significance was not achieved. No effects on thymus weight were observed in the 90 day studies. Reduced thymus weights were also noted in F1 and F2 pups in a two-generation study performed with a different silver containing active substance (Doc IIIA, 6.8.2(03)). A reduced absolute weight of thymus was observed in F0 males but there was no statistical significance when related to bodyweight. No effects were seen in F0 females. There were no effects noted in the 90 day studies in dogs or rats performed with this silver containing active substance (in rats the weight was not recorded but there were no histopathological abnormalities detected in thymus).
Since the effect was most pronounced in pups in the two-generation study and the relevance of the

[6] Directive 67/548/EEC (Dangerous Substances Directive)

effect noted in the chronic toxicity/carcinogenicity study was somewhat unclear, the results are considered to indicate a developmental effect rather than an effect due to long-term exposure.

Mortality: the mortality rate was increased in adult high dose animals of both F0 and F1 generations in the two-generation study. In F0, the high dose was 12500 ppm (approximately ~984/1109 mg/kg bw/day) but due to termination of the 12500 ppm dose in F1, the high dose group in the next generation was 6250 ppm (472/548 mg/kg bw/day).
The histopathological examinations of F0 and F1 animals that died did not clarify the cause of death but in F1 animals, there were changes in the urinary tract that may have contributed to mortality. Since the mortality observed in F1 animals (472/548 mg/kg bw/day) occurred at a dose level above the guidance value range, classification is not triggered.

Table 20: Mortality rate observed in the two-generation study

Mortality adults	12500	6250	1000	0
F0	m:10% f:0%	m:3.3% f:0%	m:0% f:0%	m:0% f:3.3%
F1	m:93.3% f:76.7% *the group was terminated prior to mating.*	m:23.3% f:3.3%	m:3.3% f:0%	m:0% f:0%

4.7.2 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Based on the pigmentation and the hydronephrosis observed at the lowest dose level in the two-generation study, classification STOT RE 2; H373 is proposed.

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS summarised and assessed several repeated dose toxicity studies as follows: i) two oral 90 days studies (rats and dogs); ii) two combined chronic carcinogenicity studies (rat and mouse), and; iii) one 2 generation study in rats. The following adverse effects after repeated exposure were considered for classification: reduced haemoglobin levels, pigmentation of organ and tissues, nephrotoxicity, reduced thymus weights and increase in mortality. The DS proposed to classify SZZ as STOT RE 2 on the basis of pigmentation and hydronephrosis observed in the 2-generation study.

Comments received during public consultation

One MS and Industry questioned the proposed classification for STOT-RE because pigmentation does not appear to be an adverse effect and because all other reported effects occurred at above the guidance values for warranting classification.

The DS responded that the LOAEL for nephrotoxicity cannot be set and it is thus not known if such effects would occur within the guidance value range. In addition, the DS noted that from the existing data it was not possible to exclude the possibility that accumulation of a heavy metal in organs and tissues could be related to the systemic effects observed.

Other MS agreed with the proposal to classify SZZ as STOT RE 2.

Assessment and comparison with the classification criteria

The following three tables summarise the main relevant findings in the 90 day repeated toxicity studies, the carcinogenicity studies and the 2 generation study, respectively. The last table (Table 11 (RAC)) presents the main systemic effects described for each type of SZZ belonging to the class analysed in this opinion.

Table 8 (RAC): Summary of 90 days repeated toxicity studies. In both studies the exposure was by the oral route.

Method	Results	Remarks
Rat Sprague-Dawley (Crl:CD (SD)IGS BR) 10/sex 0, 1000, 6250, 12500 ppm Zeomic (stated to be AgION Silver Antimicrobial AK) (~ 0, 64/78, 398/489 and 916/939 mg/kg bw in males and females)	12500 ppm: ↓Bodyweight (m, ≤ 8%); ↑Effects on behaviour/activity; ↑Erythrocytes (m, 10%); ↑platelets (m, 97%); ↓Haemoglobin (m/f, 15/10%); HCT (m/f, 9%/7%); MCV (m/f 18%/11%); MCH (m/f, 23%/15%); MCHC (m/f, 6%/4%); ↑ALP (m/f, 70%/143%); ↑Eosinophils (f, 85%); ↑Cholesterol (m/f, 59%/67%); ↑Relative heart weight (m, 11%); ↓(f) Counts of vertical and stereotypy activity (20-30 min). ↑Effects on behaviour/activity ↑Pigmentation of pancreas, thymus, mandibular lymph node ↑Mild haemorrhage, inflammation in the Harderian gland (M) ↑Chronic nephritis (M) ↑Urinary pH (m, 11%)↑ ↓Urine volume (m/f, n.s.s) 6250 ppm: ↓MCV, MCH (m); ↑Cholesterol (m/f, 58%/39%); ↑ALP (m/f 44%/80%) ↑Effects on behaviour/activity ↑Pigmentation of pancreas, thymus, mandibular lymph node 1000 ppm: ↑Cholesterol (m, 41%); ↓Counts of horizontal, vertical and stereotypy activity during the first ten minutes (m)	Doc IIIA 6.4.1(06) (Serota 2001) LOAEL: 1000 ppm (64/78 mg/kg bw/day) Haematological changes are the only effects appearing at concentrations of concern for warranting classification Reliability 1
Dog Beagle 4/sex 0, 10, 50 and 250 mg Zeomic AK10D /kg/day	250 mg/kg bw: head shaking (m,f); ↓Haemoglobin (m, 20%); ↑APTT (f, 15%), Creatinine (m, 17%); Cholesterol (f, 42%); ALP, (f (week 6), 64%),↑Calcium (f, 3.5%); ↓GLDH (f (week 6), 20%); phospholipids (f, 33%); ↓Potassium (63%) ↑Urinary volume (f (week 6), 250%) ↑Ovaries/uterus enlarged ↑Increased severity of corticomedullary tubular basophilia and lymphoid infiltration, interstitial fibrosis and hyaline/cellular casts ↑Discoloration of the pancreas and gastrointestinal tract All dose levels: ↑Vomiting	Doc IIIA 6.4.1(07) (Teunissen, 2003) NOAEL: 50 mg/kg/day LOAEL: 250 mg/kg/day Reliability 1

Note: n.s.s, no statistically significant; rel, relative; abs, absolute

Table 9 (RAC): Summary of general toxicity reported in the combined chronic and carcinogenicity toxicity studies. In both studies the exposure was by oral route.

Method	Results	Remarks
Mouse B6C3F1 75/sex* 0, 0.1, 0.3 and 0.9% AgION Zeomic AJ 10N ("at least" 0, 67, 211 and 617 mg/kg bw/day) * Termination: five/sex at 3 months, ten/sex at six months, ten at 22 months and the remaining at 24 months.	<u>0.9%</u> ↓in (m/f) RBC (15%/14%), HCT (18%/22%), MCH (2%/5%), MCV (3%/8%) and Haemoglobin (18%, m/f) ↑MCHC (m/f, 2.5%/4.3%) ↓in (m/f) weights of kidney (8%) and liver (10%) ↑pancreas weight (19%, m) ↑pigmentation of liver and pancreas ↓bodyweight gain <10% (m) ↑severity of thrombi (m, f) ↓spleen weight (37%, m) ↓brain weight (10%, f) ↑% Renal cysts (6% f, 8% m) <u>0.3%</u> ↓HCT, MCV, Hb ↑MCHC (f) ↑pigmentation of liver and pancreas ↓bodyweight gain <10% (m) ↓spleen weight (31%, m) ↓brain weight (6%, f) <u>0.1%</u> ↑pigmentation of liver and pancreas ↓spleen weight (31%, m) ↓brain weight (6%, f)	Doc IIIA 6.5-05 (Takizawa, 1992a) LOAEL: 0.1% (67 mg/kg bw/day) Reliability 2
Rat F344 70/sex** 0, 0.01, 0.03, 0.1 and 0.3% AgION Zeomic AJ 10N ("at least" 0, 3, 9, 30 and 87 mg /kg bw/day) ** Termination: ten rats/sex at 6 and 12 months and the remaining at 24 months.	<u>0.1 %:</u> ↑Pigmentation of liver, kidneys, pancreas, stomach, lymph nodes choroid plexus ↑ALT (m/f 175%/58%), AST (f 96%), ALP (m/f 25%/39%), LDL-C (m/f 28%/19%) ↑WBC (f 134%) ↓ HCT (10%), MCH (3%/3%), MCHC (f, 3%), Haemoglobin (f 12%) <u>0.3%</u> ↓thymus weight n.s.s (38%, f) ALB (m 11%) <u>0.1, 0.3%</u> ↓TP (m ≤10%), <u>Other effects all dose levels:</u> ↑Severity of hepatic bile duct proliferation ↓AST (m ≤42%, at 12 months) ↑ALT (m ≤172%, at 24 months) ↓LDH (f ≤90%, at 24 months)	Doc IIIA 6.5-06 (Takizawa 1992b) LOAEL: 0.1% (30 mg/kg bw/day) Reliability 4 (the primary deficiencies in this study are the lack of GLP compliance and the absence of individual animal data)

Note: n.s.s, no statistically significant; rel, relative; abs, absolute

Table 10 (RAC): Summary of general toxicity reported in the 2-generation reproductive toxicity study. Organ pigmentation in F_0 is the only reported effect appearing at concentrations of concern for warranting STOT-RE classification.

Method	Results	Remarks
OECD 416 Maturation, mating, gestation and lactation for two successive generations	**Parental** <u>F_0 12500 ppm:</u> ↑ Mortality (m 10%) ↓ Bodyweight (m ≤10% (pre/post pairing, f 6% gestation day 20, ≤ 11%) ↓ Bodyweight gain: (m ≤17% (pre pairing), f gestation day	Doc IIIA 6.8.2-04 (Schroeder 2002) LOAEL general toxicity = 1000

Oral in diet Rat SpragueDawley Crl: CD® (SD) IGS BR 30/sex 0, 1000, 6250 and 12500 ppm AgION Silver Antimicrobial Type AK /day	14-20:29%, 0-20:16%) ↓ Food consumption (premating m ≤8%, lactation day 0-4:27%, 4-7: 12%, 7-14: 21%, 14-21: 27%) ↑ RBC (m/f 13%/15%), Platelets (m/f 42%/45%) ↓ Hb (m/f 16%/12%), HCT (m 9%), MCH (m/f 25%/23%), MCHC (m/f 7%/6%) ↑ Pigmentation of organs ↑ Histopathological changes in kidneys, including hydronephrosis (8m/2f , 3m in controls) and urinary tract ↓ kidney weight (m abs/rel 14%/3%, f rel brain 7%) ↑ epididymis left/right (rel bw 11%/9%), spleen (m, 7%) testis (rel left/right 12%/10%) <u>F_0 6250 ppm:</u> ↑ Mortality (m, 3.3%) ↑ RBC (f 11%), ↓ MCV (m/f, 6%/9%), MCH (m/f 6%/12%), MCHC (f, 3%) ↑ Pigmentation of organs ↑ Histopathological changes in kidneys (including hydronephrosis 7m/2f, 3m in controls) ↓ kidney weight (m, abs/rel bw 13%/7%), spleen (m, abs/rel bw 14%/21%) <u>F_0 1000 ppm:</u> ↑ Pigmentation of organs <u>F_1 1250 mg/kg bw/day</u> ↑ Mortality (m/f 93.3%/76.7%) ↓ Bodyweight (premating m/f ≤ 56%/46%) ↓ Bodyweight gain (premating m/f ≤ 47%/40%) ↑ Histopathological changes ↑ Thymus atrophy <u>F_1 6250 ppm</u> ↑ Mortality (m/f 23.3%/3.3%) ↓ Bodyweight (during premating period, weeks 1-10: m/f 25-13%/19-2% (n.s.s), post-pairing m ≤12%, gestation n.s.s, lactation ≤ 10%) ↑ Histopathological changes (including hydronephrosis 10 m/4f , 0 in controls) ↑ Kidney weight (m/f, abs 19%/11%, rel bw 9%/8%, rel brain weight 13%/7%) ↓ organ weights: Brain (m/f, 7%/5%), Adrenal (m, abs 18%, rel brain weight 12%), epididymis left/right (abs 14/11%, rel brain weight (left 9%)) Spleen (m, rel bw 11%), Testis (abs left/rel brain weight right 12%/7%), Prostate (rel brain weight 13%), Seminal vesicle (8%), Liver (f, 8%) ↑ Thymus atrophy (thymus not weighed in F1 adults) <u>F_1 1000 ppm</u> ↑ Mortality (m 3.3%) ↑ Pigmentation of organs ↑ Hydronephrosis (3m, 1f, 0 in controls)	ppm (organ pigmentation in F_0 and hydronephrosis and mortality in F_1) Reliability 1
	Offspring	
	<u>F_1 12500 ppm</u> ↑ clinical signs ↓ body weights m+f Day 0: 15%; Day 4:pre/post culling:	

	19% Day 7: 23%; Day 14: 26% Day 21: 36% Day 26: 47% ↓ organ weights: Brain 18% (rel bw ↑ 58%) Spleen 26% (rel bw ↑ 31%), Thymus (m/f abs 74%/70%, rel bw 53%/47%, rel brain 69%/64%) ↓ sex ratio ↑ histopathological changes F_1 6250 ppm ↑ clinical signs ↓ body weights m+f Day 14: 13% Day 21: 25% Day 26: 47% ↓ organ weights: Brain 10%, rel bw ↑ 27%; Thymus (m/f abs 58%/55%, rel bw 39%/39%, rel brain 53%/51%); ↑ Spleen (m/f rel bw 31%/32%) ↑ histopathological changes F_1 1000 ppm ↓ organ weights: Thymus (m abs 13%, m/f rel bw 10%/9%, m rel brain 11%) F_2 6250 ppm ↓ bodyweights Day 0: 5% Day 4: pre/post culling: 12% Day 7: 15% Day 14: 18% Day 21: 20% ↑ histopathological changes ↓ organ weights: Brain (m/f 10/7%, rel bw ↑ 21%/25%), Thymus (m/f abs 50%/54%, rel bw 37%/42%, rel brain 47%/50%), Spleen (m abs 18%) F_2 1000 ppm ↓ Thymus weight (m rel bw 11%)	

Note: n.s.s, no statistically significant; rel, relative; abs, absolute

Table 11 (RAC)): Summary of systemic toxicity reported for each of the SZZ belonging to the class analysed in this opinion

	Type of SZZ		
Studies	**Irgaguard**	**AgION Type AJ**	**AgIONial Type AK**
90 day repeated dose in rat			Haematology alterations Behaviour Organ pigmentation Neprotoxicity ↓ Bodyweigh
90 days repeated dose in dog (highest dose close to 4 times lower than in 90 days study in rat)			Haematology alterations Vomiting
Combined chronic and carcinogenicity in rat		Haematology alterations Organ pigmentation ↓thymus weight	
Combined chronic and carcinogenicity		Haematology alterations Organ pigmentation	

in mouse			
2-generation reproductive study in rat			Haematology alterations in F_0 and F_1 Organ pigmentation in F_0, F_1 and F_1 pups ↓ Bodyweight in F_1 pups ↓ Thymus weight F_1 pups and F_2 pups Nephrotoxicity in F_0 and F_1

Note: n.s.s, no statistically significant; rel, relative; abs, absolute

The table below offers an overview on adverse effects relevant for STOT-RE classification that were consistently observed in available repeated toxicity studies.

Table 12 (RAC): Adverse effects of silver zinc zeolite relevant for STOT-RE classification. Bolded text refers to those effects that appear at doses relevant for classification as STOT RE

Effect	Study	Lowest reported dose (mg/kg bw/day)	Guidance value for STOT-RE classification (mg/kg bw/day)
Haematological changes	**90 days (rat)**	**64/78**	**10 ≤ C ≤ 100**
	90 days (dog)	250	10 ≤ C ≤ 100
	Carcinogenicity (mouse)	211	1.25 ≤ C ≤ 12.5
	Carcinogenicity (rat)	30	1.25 ≤ C ≤ 12.5
Mortality	2-generation study (F_1)	100	4.5 ≤ C ≤ 45 (adjusted for 202 days of exposure)
Nephrotoxicity (chronic nephritis, hydronephrosis, renal cysts)	90 days (rat)	916-939	10 ≤ C ≤ 100
	2-generation study (F_1)	100	4.5 ≤ C ≤ 45 (adjusted for 202 days of exposure)
	90 days (mouse)	617	1.25 ≤ C ≤ 12.54
Thymus weight/atrophy	Carcinogenicity (rat)	87	1.25 ≤ C ≤ 12.5
	2-generation study (F_1-F_2)	100	4.5 ≤ C ≤ 45 (adjusted for 202 days of exposure)
Organ pigmentation	90 days (rat)	398/489	10 ≤ C ≤ 100
	Carcinogenicity (mouse)	67	1.25 ≤ C ≤ 12.5
	Carcinogenicity (rat)	30	1.25 ≤ C ≤ 12.5
	2-generation study (F_0)	**100**	**21 ≤ C ≤ 210 (adjusted for 43 days of exposure)**
	2-generation study (F_1)	100	4.5 ≤ C ≤ 45 (adjusted for 202 days of exposure)

The lowest doses causing the adverse effects stated in the above table were always at least 2 times higher than the cut-off values for warranting classification as STOT-RE 2, except the organ pigmentation in F_0 in the 2-generation study and haematological changes in the 90-days study in rat.

The CLP Guidance states that small changes in clinical biochemistry and haematology are not sufficient to support classification. The most pronounced haematological effect was an increase of 41% in cholesterol concentration. Therefore RAC is of the opinion that haematological effects are not relevant for STOT-RE classification.

Pigmentation of tissues and organs which were observed in all repeated dose studies performed (90-day studies, chronic/carcinogenicity study and the two-generation study)

is an effect likely due to the precipitation of insoluble silver salts. RAC notes that some other reported effects as changes in behaviour (hypersensitivity to touch, vocalisation, increased activity, aggressive behaviour) or enlargements of Islets of Langerhans (see below in the carcinogenicity section), might be related to silver accumulation in the brain or pancreas, respectively.

The precipitation of a heavy metal in organisms is an irreversible bioaccumulative process. Since the human health consequences are not known in the case of silver, it is uncertain whether this effect fulfils the severity criterion described in the CLP Guidance.

RAC is of the opinion that the long-term systemic toxicity of SZZ by the oral route is dependent on a toxic moiety which is common to all SZZ. Although the rate of absorption, distribution and deposition/precipitation of silver ions in tissues and organs may vary between the zeolites, medium- to long-term exposure by the oral route will lead to similar hazardous effects to human health. Therefore **RAC concludes that silver zinc zeolite does not meet the criteria for classification for STOT-RE.**

4.8 Germ cell mutagenicity (Mutagenicity)

Table 21: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Test substance/dose	Results	Remarks	Reference
In vitro				
Ames/Salmonella Mutagenesis Assay S. typhimurium and E. coli	Silver Zinc Zeolite Type AK 0.15, 0.5, 1.5, 5, 15, 50, 150 and 500 µg/plate with and without S9 Positive controls: 2-aminoanthracene (+S9 mix) 9-aminoacridine, sodium azide, 2-nitrofluorene, benzopyrene Negative control: water + 0.15% agar	Negative	Bacterial toxicity evident at dose concentrations of 500 µg/plate and higher. Positive controls increased the mutation frequency indicating a sufficient sensitivity of cultures and the S9 mix.	IIIA 6.6.1-11 2003
Ames/Salmonella Mutagenesis Assay	Silver zinc zeolite Without S9: 0.0005, 0.001, 0.0015, 0.003, 0.005, 0.01 and 0.015 mg/plate. With S9: 0.003, 005, 0.01, 0.015, 0.03, 0.05 and 0.15 mg/plate Positive controls: 2-aminoanthracene (+S9 mix), 9-aminoacridine, sodium azide, 2-nitrofluorene. Negative control: distilled water	Negative	Silver zinc zeolite was tested as a suspension since it was insoluble in usual solvents. –S9: bacterial toxicity was evident at concentrations in excess of 0.015 mg/plate +S9: concentrations greater than 0.15 mg/plate in the activated The ability to detect DNA cross-linking mutagens was not investigated. Positive controls increased the mutation frequency indicating a sufficient sensitivity of cultures and the S9 mix.	IIIA 6.6.1-03 1990a
Mammalian cell mutation – Mouse lymphoma L5278Y cells. Forward mutation at TK locus	Silver Zinc Zeolite Type AK 0 to-25 µg/ml without S-9 and 0 to 175 µg/ml with S-9 Positive controls: methyl methanesulphonate (-S9) 3-methylcholanthrene (+ S9)	Positive response within cytotoxic dose ranges with or without S9	Cytotoxicity at 10 µg/mL and higher without S9. Cytotoxicity at 100 µg/mL and higher with S9 Positive controls increased the mutation frequency indicating a sufficient sensitivity of the assay.	IIIA 6.6.3-03 2003
Mammalian cell mutation – Mouse lymphoma L5278Y cells. Forward mutation at TK locus	Irgaguard B 8000 (assumed to represent Irgaguard B502i) - S9 assay 1 3.1, 6.3, 12.5, 25.0 and 50 µg/mL -S9 assay 2 6.3, 12.5, 25.0 and 50	-S9: Positive +S9: Negative	The applicant´s version is modified by the notions that there was a reproducible increase (3-fold) in the number of mutant colonies in the highest dose in the experiments without metabolic activation and after longer expocure (24h). In addition, there is an increase in the number of	IIIA 6.6.3-05 2002

Method	Test substance/dose	Results	Remarks	Reference
	µg/mL + S9 13.1, 26.3, 52.5, 105.0 and 210.0 µg/mL in assay 1; Positive controls: Methylmethanesulphonate (-S9) 3-methylcholanthrene (+S9)		small colonies, indicating a possible clastogenic activity. Cytotoxicity in Assay 1: ≥50 µg/mL without S9. ≥210 µg/mL with S9 Cytotoxicity in Assay2: ≥50 µg/mL without S9. Positive controls increased the mutation frequency and the amount of small versus large colonies, indicating a sufficient sensitivity of the assay.	
In vitro chromosome aberration test in Chinese Hamster V79 cells with TKA 40265	Irgaguard B 8000 -S9: **0.9, 1.9, 3.8, 7.5,** 15,30 µg/mL +S9: **6.3, 12.5, 25.0, 50.0** 75.0, 100 (in bold: evaluated concentrations) Positive controls: ethylmethane sulphonate (-S9) cyclophosphamide (+S9)	-S9: Positive +S9: Negative	Irgaguard B8000 was considered to be clastogenic without metabolic activation at the highest analysable concentration under the conditions of the study. Cytotoxicity (mitotic index below 50%): ≥7.5 µg/mL without S9. ≥50 µg/mL with S9 Positive controls increased the number of cells with structural chromosomal aberrations, indicating a sufficient sensitivity of the assay.	IIIA 6.6.2-07 2003
In vivo				
In vivo chromosome aberration assay in rats	Assumed to be Irgaguard 8000: 500, 1500 and 5000 mg/kg (gavage, Sprague-Dawley rats, 5/sex) Positive control: cyclophosphamide,	Negative	The test article did not produce any signs of toxicity in the target tissue The sampling time was not optimal. Only 50 metaphase cells were scored per animal. The positive control indicated that the system was capable of detecting chemicals causing chromosomal damage.	IIIA 6.6.4-01 1991

4.8.1 Non-human information

4.8.1.1 In vitro data

The data available with respect to in vitro mutagenicity of silver zinc zeolite include studies performed with two forms of silver zinc zeolite; AgION Antimicrobial Type AK and Irgaguard 8000 as well as an unspecified form assumed to be equivalent to Irgaguard 8000 (based on parameters such as study sponsor etc). Negative results were obtained in two Ames salmonella

mutagenesis assays performed whereas clear positive responses were obtained in the mutation assays performed in mammalian cells (TK-locus tests) for AgION Antimicrobial Type AK (with and without S9) and silver zinc zeolite (assumed to be Irgaguard 8000) (without S9). This was shown as a reproducible increase (3-fold) in the number of mutant colonies at the highest dose in the experiments. In addition, there was an increase in the number of small colonies in the study with Irgaguard 8000, indicating a possible clastogenic activity. A clastogenic response was also observed in the chromosomal aberration assay performed with Irgaguard 8000 in the absence of metabolic activation.

4.8.1.2 In vivo data

The in vivo mutagenicity of silver zinc zeolite (assumed to be Irgaguard 8000) was investigated in a micronucleus assay. No significant increase in chromosomal aberrations was observed but there were no signs of toxicity at the target tissue and no clinical signs of systemic toxicity thus it is uncertain whether the bone marrow actually was exposed to silver zinc zeolite to an extent that is meaningful for genotoxicity testing. Furthermore, the sampling times used in the study (6, 18, and 24 h post exposure) were not optimal. According to OECD guideline, samples should be taken at two separate times following treatment on one day. For rodents, the first sampling interval is 1.5 normal cell cycle length (the latter being normally 12-18 hr) following treatment. Since the time required for uptake and metabolism of the test substance as well as its effect on cell cycle kinetics can affect the optimum time for chromosome aberration detection, a later sample collection 24 hr after the first sample time is recommended. In essence, this implies that only the sampling time after 24 hours would have had the possibility to detect any genotoxic effects (under the prerequisite that a sufficient amount of the test substance reached the target tissue). According to OECD guideline, samples should be taken at two separate times following treatment on one day. Hence, a later sampling time than 24 hours would have been preferable.
Finally, only 50 metaphase cells were scored per animal whereas OECD guideline recommends that at least 100 metaphase cells should be scored.
Data presented in the toxicokinetics section shows the highest concentrations of silver in the reticuloendothelial tissues (liver, spleen, bone, lymph nodes, skin and kidney) of rats orally administered silver nitrate or silver chloride. Even if this may indicate that the target tissue was exposed to the test substances, uncertainty remains if this really was the case in the present study. According to Olcott (1948), a few black granules were observed in the bone marrow of rats exposed to silver nitrate and silver chloride in sodium thioate but it was not possible to determine whether or not this was silver and the bone marrow of rats exposed to silver or water appeared the same. Considering that silver is excreted in bile and the estimated oral absorption is only 5%, the in vivo micronucleus test via the oral route seems to be unsuitable for investigating the genotoxic potential of silver.
In this situation, a comet assay investigating a number of tissues, including the first site-contact tissue and the liver, may be a better assay to study the *in vivo* genotoxicity.

4.8.2 Human information

No data available.

4.8.3 Other relevant information

The technical guidance on the data requirements of Directive 98/8/EC states that when there is positive *in vitro* genotoxicity data available and the result from a first *in vivo* study is negative, it is preferable to have results from an *in vivo* assay investigating possible genotoxicity in other tissues. Such a study is not available for silver zinc zeolite. However, the data submitted also contains four *in vivo* micronucleus studies (Doc IIIA, 6.6.4-03, 6.6.4(04-05), 6.6.4(02) and an *in vivo* liver UDS assay (Doc IIIA, 6.6.3(07)) performed with other silver containing silver substances. Among these assays there were only indications that the test substance reached the target tissue in two of the *in vivo* micronucleus studies and in the UDS assay.
However, the UDS assay is not a fully suitable assay to detect possible clastogenic effects and the results obtained with these silver containing active substances are of limited use only since they do not contain zinc and the genotoxic potential of zinc is not fully established. According to the risk assessment report (RAR) on zinc prepared by the Netherlands, the results of the genotoxicity tests performed with zinc chloride are rather inconclusive:

"Several data were provided on the genotoxicity of zinc chloride. Data on other zinc compounds have also been used, based on the assumption that after intake the biological activities of the zinc compounds are determined by the zinc cation.The available data indicate that the genotoxicity results vary widely. Conflicting results have been found, even in the same test systems. Overall, the results of the in vitro tests indicate that zinc has genotoxic potential in vitro based on positive results in mammalian test systems for gene mutations and chromosomal aberrations and on the positive in vitro UDS test. In vivo, increases in chromosomal aberrations were found in calcium-deficient mice exposed via the diet as well as in mice with normal calcium status when dosed intraperitoneally. In mice also negative results were obtained and even at higher intraperitoneal dose levels. Rats tested negative for chromosomal aberrations after oral dosing, either via gavage or via the diet. The positive result for chromosomal aberrations in vitro is considered over ruled by negative in vivo tests for this endpoint. The positive sperm head abnormality test is considered sufficiently counter-balanced by two negative SLRL tests as well as two negative dominant lethal tests. Moreover, this sperm test is not adequately reported and without details on scoring criteria, interpretation of the observations is rather subjective. In addition, sperm head abnormalities are indicative rather than proof for genotoxicity. Based on the available data there is insufficient ground to classify zinc as genotoxic. It should be noted that the potential to induce gene mutations was not adequately tested in vivo. However, there is no clear evidence from the available data that zinc is genotoxic in vivo and without a clear indication for carcinogenicity a guidance for further testing with respect to target tissue is not available".

4.8.4 Summary and discussion of mutagenicity

The result from the *in vivo* assay is not considered to overrule the positive findings in the three in vitro genotoxicity studies since there were no indications in the study that the target tissue was exposed to the test substance. The *in vivo* study was performed using oral administration and since the oral absorption is approximately 5%, the lack of cytotoxicity in the bone marrow may likely reflect no or very low exposure of the target tissue. The draft guidance on biocide requirements reads: *"Specific considerations for in vivo genotoxicity testing; For substances that are short-lived, reactive, in vitro mutagens, or for which no indications of systemic availability have been presented, an alternative strategy involving studies to focus on tissues at initial sites of contact with the body should be considered (e.g. local genotoxicity, photomutagenicity). Expert judgment should be used on a case-by-case basis to decide which tests are the most appropriate. The main options are the in vivo Comet assay, gene mutation tests with transgenic rodents, and DNA adduct studies.*

For any given substance, expert judgment, based on all the available toxicological information, will indicate which of these tests are the most appropriate."
A comet assay in which genotoxicity in additional tissues (including the first site of contact and the liver) is thus considered the most appropriate way forward to investigate if the effects observed *in vitro* would occur also *in vivo.*

4.8.5 Comparison with criteria

According to 1272/2008 (CLP), *"substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. Substances known to induce heritable mutations in the germ cells of humans"* should be classified as germ cell mutagens in Category1.
Since there is no human data on silver zinc zeolite or other SCAS available, the criteria for classification in this category is not considered fulfilled.
The CLP regulation states further: *"Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans" should be placed in category 2.*
Criteria for classification in Category 2 include:

- *positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:*
- *somatic cell mutagenicity tests in vivo, in mammals; or*
- *other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.*

According to CLP, *substances showing positive results only in one or more in vitro mutagenicity assays should normally not be classified. Their further investigation using in vivo assays, however, is strongly indicated.* Positive *in vitro* data for substances that are structurally similar to known germ cell mutagens should yet be considered for classification in Category 2 (CLP).
Silver zinc zeolite was negative in two bacterial reverse tests but positive in all three *in vitro* cell mutagenicity tests performed. In the absence of reliable *in vivo* data, it is presently not possible to conclude if silver zinc zeolite fulfils the CLP criteria for mutagenicity in Category 2 (Muta. 2, H341).

4.8.6 Conclusions on classification and labelling

Silver zinc zeolite was clastogenic when tested in vitro but in the absence of reliable in vivo data, it is presently not possible to conclude if silver zinc zeolite fulfils the CLP criteria for mutagenicity in Category 2 (Muta. 2, H341).

RAC evaluation of germ cell mutagenicity
Summary of the Dossier Submitter's proposal The available data included five well conducted *in vitro* mutagenicity studies performed with AgION Type AK, Irgaguard as well as an unspecified form of zeolite. An *in vivo* micronucleus assay was performed in SD rats with Irgaguard but this test was considered unreliable by the DS.

According to the DS, negative results were obtained in two Ames *salmonella* mutagenesis assays with AgION Type AK or the unspecified form of zeolite. By contrast, a clear positive response was obtained with AgION Type AK in a well conducted mammalian cell mutation assay (mouse lymphoma L5278Y cells) with or without S9. The second mouse lymphoma assay conducted with Irgaguard was also positive but only in the absence of S9. The number of mutant colonies was increased after 24h (highest concentration). The number of small colonies was also increased, indicating a possible clastogenic effect .

An *in vitro* chromosome aberration test in Chinese Hamster V79 cells was positive with Irgaguard without S9 only. In an *in vivo* study, although the positive control indicated that the system was capable of detecting chemicals causing chromosomal damage, the chromosomal aberration test conducted in rats with Irgaguard was regarded as unreliable. This was due to uncertainties and deviations from the relevant OECD TG, including a possible lack of exposure of the bone marrow, an inapproriate sampling time after administration and a low number of cells in metaphase analysed from each animal.

The DS could not conclude on mutagenicity in the absence of reliable *in vivo* data and as a consequence, did not propose classification for this hazard class.

Comments received during public consultation

Industry questioned the assessment made by the DS on the *in vivo* micronucleus assay and argued that distribution studies confirm that silver (tested with other silver substances) can reach many tissues, including blood and bone marrow. Industry further noted that the demonstrated absence of silver-induced clastogenicity *in vivo* should be recognised as evidence that insufficient silver can be administered *in vivo* to induce a clastogenic effect.

The DS responded that the absorption of orally administered silver is low (below 5%) and thus bone marrow exposure can be expected to be minimal. The absence of mutagenicity due to lack of exposure only means that the test system used is inappropriate for the substance.

One MS also commented that the mutagenicity study was inconclusive. Another MS noted that silver is considered to form discoloration and deposits in tissues following repeated exposure. The *in vivo* micronucleus test, conducted using a single administration, may not have been adequate to reflect the toxicokinetics of silver.

Assessment and comparison with the classification criteria

The table below summarises the available information for mutagenicity studies.

Table 13 (RAC): Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Substance	Results	Remarks
In vitro			
Ames/Salmonella Mutagenesis Assay S. typhimurium and E. coli	AgION Type AK 0.15, 0.5, 1.5, 5, 15, 50, 150 and 500 µg/plate with and without S9 Positive controls: 2-aminoanthracene (+S9 mix) 9-aminoacridine, sodium azide, 2-nitrofluorene, benzopyrene	Negative	Doc IIIA 6.6.1-11, (May 2003) Reliability 2 Bacterial toxicity evident at dose concentrations of 500 µg/plate and higher.

	Negative control: water + 0.15% agar		Positive controls validated the assay
Ames/Salmonella Mutagenesis Assay	SZZ, undefined Without S9: 0.0005, 0.001, 0.0015, 0.003, 0.005, 0.01 and 0.015 mg/plate. With S9: 0.003, 005, 0.01, 0.015, 0.03, 0.05 and 0.15 mg/plate Positive controls: 2-aminoanthracene (+S9 mix), 9-aminoacridine, sodium azide, 2-nitrofluorene. Negative control: distilled water	Negative	Doc IIIA 6.6.1-03 (Loveday 1990a) Reliability 1 SZZ was tested as a suspension since it was insoluble in usual solvents. Bacterial toxicity at the highest concentrations Positive controls validated the assay
Mammalian cell mutation – Mouse lymphoma L5278Y cells. Forward mutation at TK locus	AgION Type AK 0 to-25 µg/mL without S-9 and 0 to 175 µg/mL with S-9 Positive controls: methyl methanesulphonate (-S9) 3-methylcholanthrene (+ S9)	Positive response within cytotoxic dose ranges with or without S9	Doc IIIA 6.6.3-03 (Clare 2003) Reliability 1 Cytotoxicity at 10 µg/mL and higher (without S9) and 100 µg/mL and higher (with S9) Positive controls validated the assay
Mammalian cell mutation – Mouse lymphoma L5278Y cells. Forward mutation at TK locus	Irgaguard - S9 assay 1: 3.1, 6.3, 12.5, 25.0 and 50 µg/mL -S9 assay 2: 6.3, 12.5, 25.0 and 50 µg/mL + S9: 13.1, 26.3, 52.5, 105.0 and 210.0 µg/mL in assay 1; Positive controls: Methylmethanesulphonate (-S9), 3-methylcholanthrene (+S9)	-S9: Positive +S9: Negative	Doc IIIA 6.6.3-05 (Wollney 2002) Reliability 2 There was an increase in the number of small colonies in the highest concentration, indicating a possible clastogenic activity. Cytotoxicity in Assay 1: ≥50 µg/mL without S9 and ≥210 µg/mL with S9 Cytotoxicity in Assay 2:≥50 µg/mL without S9.

			Positive controls validated the assay
In vitro chromosome aberration test in Chinese Hamster V79 cells with TKA 40265	Irgaguard -S9:**0.9, 1.9, 3.8, 7.5,** 15,30 µg/mL (in bold: evaluated concentrations) +S9: **6.3, 12.5, 25.0, 50.0** 75.0, 100 µg/mL (in bold: evaluated concentrations) Positive controls: ethylmethane sulphonate (-S9) cyclophosphamide (+S9)	-S9: Positive +S9: Negative	Doc IIIA 6.6.2-07 (Schulz 2003) Reliability 2 Cytotoxicity (mitotic index below 50%): ≥7.5 µg/mL without S9 and ≥50 µg/mL with S9 Positive controls validated the assay
In vivo			
In vivo chromosome aberration assay in rats	Irgaguard: 500, 1500 and 5000 mg/kg (gavage, Sprague-Dawley rats, 5/sex) Positive control: cyclophosphamide,	Negative	Doc IIIA 6.6.4-01 (Loveday 1991) Reliability 2 The test article did not produce any signs of toxicity in the target tissue The sampling time was not optimal. Only 50 metaphase cells were scored per animal. The positive control indicated that the system was capable of detecting chemicals causing chromosomal damage.

Five *in vitro* tests and one *in vivo* tests are presented in the CLH report. Two *in vitro* assays in bacteria were negative, two *in vitro* cell mutation assays in mouse lymphoma L5278Y cells were positive only at cytotoxic concentrations and an *in vitro* chromosome aberration test in Chinese hamster V79 cells which was positive at non-cytotoxic concentrations. *T*he *in vivo* chromosome aberration assay in rats was negative, although several deficiencies were reported by the DS such as: i) non-optimal sampling time (the latest sampling time was 24 h and at least one later sampling time would have been appropriate); and, ii) only 50 metaphase cells were scored per animal whereas the relevant OECD guideline recommends that at least 100 metaphase cells should be analysed.

According to the CLP criteria, classification for germ cell mutagenicity in category 1A is based on positive evidence from human studies. No such evidence exists, therefore classification in that category is not supported. Classification for germ cell mutagenicity in category 1B is also not supported by the data. There is no evidence for positive effects in

the *in vivo* heritable germ cell mutagenicity tests.

Classification into category 2 cannot be concluded on the basis of the available information. Two *in vitro* mammalian cell studies were positive for clastogenicity mainly in presence of S9-mix. In addition, RAC agrees with the DS that the *in vivo* micronucleus assay in rats was not sufficiently reliable for a definitive conclusion on the absence of *in vivo* clastogencity of SZZ. RAC considers that the available data are insufficient to conclude on this hazard class. According to the CLP criteria, classification in Category 2 for mutagenicity is based on positive results obtained in at least one valid *in vivo* mammalian somatic cell mutagenicity test and classification may also be supported by positive *in vitro* mutagenicity results.

RAC notes that the possible active mutagenic moiety *in vitro* is the Ag^+ ion which is, however, not bioactivated by the S9 mix. Therefore results obtained with and without S9 mix should theoretically yield the same qualitative, if not quantitative results. Although speculative, a possible explanation for the differences might be related to a potential complexation of silver ions with thiols and disulfide groups in the S9 mix, reducing its availability to the cells.

RAC also notes that it is unknown whether silver ions are able to reach germinal cells because gonad pigmentation has not been described, although ovarian cysts and endometril polyps were described in the carcinogenicity studies.

In conclusion, **RAC considers that the criteria for classifying SZZ as a germ cell mutagen have not been met and therefore that no classification for this substance is warranted.**

4.9 Carcinogenicity

Table 23: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
Combined chronic and carcinogenicity Oral Mouse B6C3F1 75/sex* AgION Zeomic AJ 10N 0, 0.1, 0.3 and 0.9% "at least" 0, 67, 211 and 617 mg/kg bw/day	No statistically significant increase of tumours in treated animals. <u>0.9%</u> ↓RBC, HCT, MCH, MCV, Hb ↑MCHC ↑ renal cysts* (M, F) ↑enlargement of Langerhan´s islands (M) ↓kidney (8%), liver (10%), brain, weight (10%) (F) ↑pancreas (19%, M) ↑pigmentation of liver and pancreas <u>0.3%</u> ↓HCT, MCV, Hb ↑MCHC (F) ↑ ovarian cysts ↑pigmentation of liver and pancreas <u>0.1%</u> ↑ ovarian cysts ↑pigmentation of liver and pancreas <u>*Other effects;*</u> <u>*0.9%*</u> ↓*bodyweight gain <10% (M)* ↑*severity of thrombi (M, F)* ↓*spleen weight (37%, M)* ↓*brain (10%, F)* <u>*0.3%*</u> ↓*bodyweight gain <10% (M)* ↓*spleen weight (31%, M)* ↓*brain (6%, F)* <u>*0.1%*</u> ↓*spleen weight (31%, M)* ↓*brain (6%, F)* **dose-response*	NOAEL not determined LOAEL: 0.1%	IIIA 6.5-05 (1992a)
Combined chronic and carcinogenicity Oral Rat 70/sex** AgION Zeomic AJ 10N 0.01, 0.03, 0.1 and 0.3% ("at least" 0, 3, 9, 30 and 87 mg /kg bw/day)	Statistically significant positive trends for: Leukemia (m,f) Pituitary adenomas (f) Endometrial polyps <u>0.1 %</u> ↑Pigmentation of liver, kidneys, pancreas, stomach, lymph nodes choroid plexus ↑ALT (M/F 175/58%), AST (F 96%), ALP (M/F 25/39%), LDL-C (M/F 28/19%) ↑endometrial polyps ↑WBC (F 134%) ↓ HCT (10%), MCH (3/3%), MCHC (F 3%), Hb (F 12%) <u>0.03%</u>	NOAEL: 0.03 % (~9 mg/kg bw/day)	IIIA 6.5-06 (1992b)

Method	Results	Remarks	Reference
	↑endometrial polyps *Other effects:* *all dose levels* *↑Severity of hepatic bile duct proliferation* *↓AST* *(M ≤42%, at 12 months)* *↑ALT* *(M ≤172%, at 24 months)* *↓LDH (F≤90%, at 24 months)* *0.3%* *↓thymus weight n.s.s(38%, F)* *0.1, 0.3%* *↓TP (M ≤10%, M ALB ≤10%*		
* Termination: five/sex at 3 months, ten/sex at six months, ten at 22 months and the remaining at 24 months. ** Termination: ten rats/sex at 6 and 12 months and the remaining at 24 months.			

4.9.1 Non-human information

4.9.1.1 Carcinogenicity: oral

AgION Zeomic AJ was administered in the diet to mice in daily doses of 0, 0.1, 0.3 and 0.9% which, according to the applicant, correspond to an intake of "at least" 0, 67, 211 and 617 mg/kg bw/day (minimum drug intake). In the same study, rats received daily doses of 0, 0.01, 0.03, 0.1 and 0.3% which correspond to an intake of "at least" 0, 3, 9, 30 and 87 mg /kg bw/day (minimum drug intake). The findings relevant for the carcinogenicity assessment are discussed in this section whereas other findings in the study are more thoroughly discussed in the section on repeated dose toxicity (section 4.7).

Mice: The cumulative survival rate and the mean survival time were similar between treated mice and controls.
The histopathological examination revealed an increased dose-related frequency of renal cysts in males and enlargement of Langerhan´s islands was observed in males. The frequency of renal cysts was low but followed a statistically significant dose-response.
The total number of tumours per animal at termination was lower in high dose males (1.00) compared to controls (1.26) and was comparable between high dose females and controls. A statistically significant increase in the incidence of ovarian cysts was evident although there was no clear dose-response. Since the frequency was increased already in the low dose group, it is not possible to set a NOAEL for this effect.
The chronic LOAEL in mice is considered to be at or below the lowest dose level, 0.1%, corresponding to 67 mg AgION Type AJ/kg bw/day based on the increased frequency of ovarian cysts, pigmentation of liver and pancreas and decreased organ weights in all treated mice (see section 4.7).
Rats: The cumulative survival rate and the mean survival time in rats were similar between treated animals and controls.
The total number of tumours per animal at termination was lower in high dose males (1.86) compared to controls (1.96) whereas the total number in high dose females was higher (2.11) than

in controls (1.37). The difference was not statistically significant.
The statistical analysis did however reveal a dose-related increase in the frequency of leukemia and infiltration of leukemia cells into different tissues in both male and female rats. Since the tumorous/non-tumorous changes observed were combined for scheduled and intercurrent deaths, it is not clear when in time the leukemia developed. According to the study report, tissues from the right femoral bone were collected. However, it is not clear if the bone marrow was analysed for histopathological changes.
The increased frequency of leukemia was dismissed by the study author since the frequency was within the range observed in historical control data (this data was not submitted but was referred to as Tajima Y, Data of biological characteristics of experimental animals, Soft Science Inc., 1989. Later, the applicant stated (appendix 4) that the historical control range was 23.5-27.9% in males and 17.5-21% in females). While historical control data may be useful for analyses of deviations in single data points it is not considered accurate to disregard a positive trend based on such information. The historical control data is further discussed in appendix 4 (confidential attachment prepared by the applicant) but this historical control data do not meet the general criteria for use of such information (e.g. whether data is comparable with respect to supplier, test facility, housing conditions, diet, group size, administration route, survival rates, assessment criteria etc).

Data on historical control data for leukaemia in F344 rats (table provided by the applicant)[7]

Source	Incidence Mean (range)	Time Period
Ando *et al* (2008)[1]	Male 19.1 (4-30)% Female 21.3 (5-34%)	1990-1999
Haseman *et al* (1998)[2]	Male 50.5 (32-74)% Female 28.1 (14-52)	1990-1997
Miyaishi *et al* (2000)[3]	Male 83.6% Female 57.5%	up to 2000
Solleveld *et al* (1984)[4]	Male 29.7% Female 19.0%	up to 1984
Haseman & Rao (1992)[5]	Male 48.9% Female 25.2%	1980-1984

Moreover, according to the historical control data above, there are large variations observed with respect to background incidence of these tumour types thus this information is considered to be of limited use. Finally, the frequencies observed in controls and in the low dose groups are fairly similar thus there is no apparent reason to question the results in the controls which have been studied during the same conditions as the treated rats.
Consequently, it is not considered appropriate to disregard the concurrent control data.
As stated above, a statistically significant positive trend, in which all doses are considered, is a stronger indication of the biological relevance of an effect than a statistically significant difference

[7] The chronic toxicity/carcinogenicity study was performed in 1989-2002. According to the guidance on the application of CLP criteria, *"The historical data must be from the same animal strain/species, and ideally, be from the same laboratory to minimise any potential confounding due to variations in laboratory conditions, study conditions, animal suppliers, husbandry etc. It is also known that tumour incidences in control animals can change over time, due to factors such as genetic drift, changes in diagnostic criteria for pathological changes/tumour types, and husbandry factors (including the standard diet used), so the historical data should be contemporary to the study being evaluated (e.g. within a period of up to around 5 years of the study). Historical data older than this should be used with caution and acknowledgement of its lower relevance and reliability. (RIVM, 2005; Fung et al, 1996; Greim et al, 2003)."*

at a single dose level. The P values obtained in a Cochran-Armitage trend test were 0.026 and 0.019 (one sided) for females and males, respectively. The positive trend is thus clearly statistically significant and the probability for a statistically significant trend to arise in both sexes merely by chance is considered less likely.
The results of genotoxicity studies indicate that silver zinc zeolite is clastogenic in vitro. Due to uncertainties whether or not the target tissue in the micronucleus test (i.e the bone marrow) was exposed at a sufficient level, it is not possible to conclude if the substance should be considered clastogenic and if so, if there is a threshold level for this effect.
However, the increased incidences of tumours may not necessarily be linked to genotoxicity. If the substance would act as a promoter, initiated cells could progress into tumours and thus increase the frequency of tumours such as those types commonly observed in the rat strain studied. Such mode of action means that the existence of a human counterpart becomes less relevant since the ability to promote initiated cells into tumours may not be linked to certain cell types.
According to the study report, there were dose related increases of pituitary adenomas and endometrial polyps in females and the trends were statistically significant. The findings were dismissed by the study authors since they were irregularly distributed and occurred at a lower incidence than reported in the historical control data referred to (26.9-44% according to the applicant in appendix IV). In similarity with the line of reasoning for leukemia, it is not considered accurate to dismiss a statistically significant trend by historical control data (especially considering the limited information on the historical control data). Moreover, the increased incidence of endometrial polyps could be a hormonally mediated effect and due to the complex regulation of hormones (negative feed-back mechanisms etc) a strict dose-response may not be expected. However, the positive trend for endometrial polyps was dismissed by the Technical Meeting in June 2013 and the effect is thus not considered further.
The NOAEL for carcinogenicity (increased incidence of leukemia in males and females and pituitary adenomas in females) is set at 0.1%, corresponding to 30 mg AgION Type AJ/kg bw/day since the dose-responses is no longer statistically significant when the highest dose group is excluded from the analysis (LOAEL 0.3% corresponding to 87 mg/kg bw/d)[8]. Due to the uncertainties associated with the genotoxic potential of silver zinc zeolite, the NOAEL set is only relevant for the effects in this particular study. The overall NOAEL in the study is set at 0.03% (i.e. 9 mg AgION Type AJ/kg bw/day) based on the pigmentation observed at the LOAEL 0.1% (30 mg/kg bw/day).

Table 24:

MICE	**0**	**0.1**	**0.3**	**0.9**	
Renal cysts*	M:0/49 F: 0/49	M:0/48 F: 0/49	M:0/49 F: 1/50	M:4/50 F: 3/49	
Enlargement of Langerhan´s islands**	M:3/49 F: 0/49	M:7/48 F: 0/549	M:13**/49 F: 0/50	M:11/50** F: 0/49	
Ovarian cysts	6/49	22/49***	19/50***	16/49***	
RATS	**0**	**0.01**	**0.03**	**0.1**	**0.3**
*Endometrial polyps** *Not considered to be a true effect by the Technical Meeting for Biocides, June 2013.*	*0/49*	*2/50*	*5/49*	*9/50***	*7/49***

[8] Setting of a NOAEL based on a step-down procedure is described in paragraph 134 of.OECD guidance "Current approaches in the statistical analysis of ecotoxicological data: A guidance to application".

Pituitary adenomas*	M:1/50 **F:11/49**	M:0/49 **F: 16/50**	M:3/50 **F: 12/49**	M:0/48 **F: 19/50**	M:1/49 **F: 20/49**
Leukemia*	M:7/50 **F: 2/49**	M:7/49 **F: 5/50**	M:7/50 **F: 6/49**	M:11/48 **F: 5/50**	M:14/49 **F: 9/49**
* Statistically significant dose response relation ** Statistically significant p<0.05 *** Statistically significant p<0.01					

4.9.1.2 Carcinogenicity: inhalation

No data available.

4.9.1.3 Carcinogenicity: dermal

No data available.

4.9.2 Human information

According to summary reports available in literature, human exposure to silver has not been associated with cancer but it is also pointed out in summaries that the carcinogenic potential of silver has not been carefully examined. Local sarcomas have been observed after subcutaneous implantation of silver foil (summary document in 6.5 (07)/6.7 (02)) but the author of the original study (Furst (1979)) states that the relevance of such results for exposure via ingestion is difficult to interpret as they may arise due to a phenomenon called solid state carcinogenesis.
The ATSDR report submitted in 6.2 (08) states that subcutaneous imbedding of silver foil seemed to produce fibrosarcomas earlier and more frequently than several other metal foils. However, the results were only preliminary since the analysis of some of the metals was not complete at the time of publication.
Although the quality and reliability of the original test data cannot be assessed from second-hand information, the original publication has not been traced since it was not expected to add useful information (based on the poor quality of other publications reviewed that were published around this time (1956)).

4.9.3 Other relevant information

The information on carcinogenicity included in different summaries (Doc IIIA, sections 6.5(07)/6.7(02) and 6.7 (04-05)) is mainly based on a study by Schmahl and Steinhoff (1960) and a study by Furst, R. and Schlauder, M.C. (1977).
In the study by Schmahl and Steinhoff, subcutaneous injections of colloidal silver resulted in tumours in those rats that survived longer than 14 months. Six of the eight tumours found among the 26 rats (23%) were located the injection site. There were no vehicle controls included in the study but the spontaneous tumour frequency at any site was stated to be 1-3%. Based on this meagre information, it is assumed that the frequency of tumours located at other sites was 2/26 (7.7%) and thus above the spontaneous frequency.
In contrast, no fibrosarcomas developed at the injection sites in Fischer 344 rats intramuscularly injected with silver metal powder (Furst and Schlauder). A few cases of mild local inflammation

were noted at injection sites but only in the latter stages of the study. At necropsy there were several incidences of encapsulation of the vehicle or injected metal powder but none of the injected legs showed muscular atrophy.
The chronic toxicity/carcinogenicity study performed with silver zinc zeolite has also been reviewed by the US EPA (2002)[9]. The US EPA states in the discussion on neoplastic findings in rats "our reviewer believes that these dose-response trends may be linked to treatment and the use of a higher dose may have better linked the treatment to tumour incidence". The US EPA considered the study "unacceptable in that it was not conducted under GLP and that there are no data to support that the top dose represented or even approximated an adequate dose for carcinogenicity testing.
In contrast to the US EPA, the study is considered acceptable despite having deficiencies since tumour findings were noted even though doses did not approximate the maximum tolerated dose.

4.9.4 Summary and discussion of carcinogenicity

Rats receiving silver zinc zeolite developed leukaemia (both sexes) and pituitary adenomas (females) to a higher extent than control animals. The historical control data submitted is not considered to take precedence over concurrent controls as there are no grounds for dismissing the control data. The tumour findings are not considered invalidated by the deficiencies of the study (low dose levels, lack of biochemical analyses etc) since statistically significant trends were observed despite that dose levels were too low to cause generalised toxicity.
Therefore, data is considered to indicate that silver zinc zeolite has carcinogenic properties.

4.9.5 Comparison with criteria

According to the CLP, *"known or presumed human carcinogens"* are classified in Category 1 for carcinogenicity on the basis of epidemiological (Cat 1A) and/or animal data (Cat 1B). If based on animal data, evidence should be *"sufficient" as defined by defined by the International Agency for Research on Cancer (IARC).* A substance may be placed in Category 2 (suspected human carcinogen) *"on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited[10] evidence of carcinogenicity in human studies."* The CLP guidance also states that appearance of only spontaneous tumours, especially if they appear only at high dose levels, may be sufficient to downgrade a classification from Category 1B to Category 2, or even no classification
The information available with respect to carcinogenicity is a chronic toxicity/carcinogenicity study in mice and rats. The study shows a statistically significant positive trend for leukaemia in rats, both males and females and pituitary adenomas in females. These effects occurred despite that the dose levels used in the study were low and only little general toxicity was observed at the highest dose level.

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[9] http://www.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-072503_28-Aug-02_006.pdf

[10] Annex I 3.6.2.2.3 states "limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs."

In this case, the tumour types observed were indeed tumour types that are commonly observed in rats of this strain. However, as pointed out earlier, a statistically significant positive trend, in which all doses are considered, is a strong indication of the biological relevance of the effect. Therefore, the tumours are considered related to treatment. Moreover, in case the substance has an ability to promote initiated cells into tumours, it seems logical that the frequency of the common tumour types increases.

4.9.6 Conclusions on classification and labelling

The positive trend for leukaemia observed is statistically significant and the probability for this to arise in both sexes merely by chance is considered less likely. Likewise, the positive trend for pituitary adenomas in females is statistically significant and thus also considered relevant. If tumour types should be dismissed despite being statistically significant and occurring at doses with low general toxicity only on the basis that they are common in the rat strain used, substances causing certain tumours such as leukaemia would never be detected. The results of the study indicate that the substance has potential to cause tumours in rats and it is thus considered appropriate to communicate this to users through classification and labelling. Since the basis for classification is a rat study rather than epidemiological data, the criteria for Category 1A in CLP are not considered fulfilled. Neither are criteria for Category 1B (CLP) fulfilled since the rat study is not considered as "sufficient" evidence.
Therefore, silver zinc zeolite is proposed to be classified Carc 2; H351 (Suspected of causing cancer).
The applicant disagrees with the proposal for classification. Appendix 4 (confidential attachment) to this document contains a statement in which the RMS has included a response to the arguments put forward by the applicant.

RAC evaluation of carcinogenicity
Summary of the Dossier Submitter's proposal The DS proposed to classify SZZ as Carc. 2 on the basis of an increased incidence of leukaemia in Fisher rats (both sexes) and an increased incidence of pituitary adenomas in females in rats. The incidences of these tumours were statistically significant and dose-related. The DS summarised two combined chronic and carcinogenicity studies with AgION type AJ, one conducted in mice and a second in rats. The zeolite was administered in the diet to mice at concentrations of 0, 0.1%, 0.3% and 0.9% in their feed, corresponding to approximate intakes of 0, 67, 211 and 617 mg/kg bw/day. Rats were given concentrations of of 0, 0.01%, 0.03%, 0.1% and 0.3% in their feed, corresponding to approximate intakes of 0, 3, 9, 30 and 87 mg /kg bw/day (minimum test material intake). In mice, the DS reported that there were no statistically significant increase in any tumour type in treated animals compared to controls. The NOAEL could not be determined due to statistically significant changes occurring at the lowest dose of 0.9%, including pigmentation, increased ovarian cysts in females and decreased spleen and brain weights in males. In rats, the DS reported that the statistical analysis did reveal a dose-related increase in the frequency of leukemia and infiltration of leukemia cells into different tissues in both male and female rats. The DS also considered relevant the positive trend for pituitary adenomas in females.The DS noted that the positive trend observed for leukaemia is statistically significant and the probability for this to arise in both sexes merely by chance

was not considered very likely.

Therefore, the DS proposed to classify SZZ as Carc 2; H351 (Suspected of causing cancer) according to CLP.

Comments received during public consultation

Industry opposed the proposed classification as Carc. 2. Their main arguments were related to the rates of spontaneous (mononuclear cell) leukemia and pituitary adenomas in Fisher rats, which were below the mean historical control incidence reported in the literature. The arguments were:

i. The differences in tumour incidence between controls and different dose levels were not statistically significant in pairwise comparisons.
ii. The tumour types observed had a high background incidence in the strain of rat used and the incidences observed were within the range reported in historical control data.
iii. The type of leukaemia observed was uncommon in other rat strains and has not been observed in humans and therefore is not relevant for humans.
iv. No leukemia has been observed in mice, so greater weight for the classification decision should be placed on the mouse data as the background incidence for these effects was low and varied within a narrow range in this species.
v. The conclusion of the DS was influenced by equivocal results obtained in *in vitro* genotoxicity studies.
vi. The CLP guidance specifically cites the high background incidences of pituitary gland tumours and leukaemia in F344 rats in relation to the use of historical control data.

The DS responded to the comments from industry that statistically significant positive trends for leukaemia and pituitary adenomas are stronger indications of the relevance of an effect and it seems unlikely that specifically leukaemia would appear by chance in both sexes. In addition, the DS argued that classification of a substance for carcinogenicity need not necessarily be linked to mutagenicity and that SZZ could act as a tumour promoter that transforms initiated cells into cells of the tumour types seen in these studies.

One MS commented on the validity of the classification for carcinogenicity in the absence of additional information on the type of leukemia observed as well as on the need for a thorough analysis of the mechanism of action and the human relevance. The DS responded that the type of leukaemia was not specified in the original report, that there were no mechanism of action studies or analysis of the human relevance.

Another MS commented that a statistically positive trend might not be sufficient for classification for carcinogenicity due to the limited reliability of the results from the carcinogenicity study. The MS noted that effects on haematological parameters in repeated dose studies could be taken into account. The DS responded that a statistically significant positive trend for leukaemia is a stronger indication. In addition, haematological effects were observed in repeated dose toxicity studies, including in the two-generation reproductive toxicity study in rats. In that study, haematological parameters were only analysed in the P females and showed some effects in dams given the test material at 12500 and 6250 ppm. Further, mild extramedullary haematopoiesis was observed in a single high dose P dam but these were not observed among the F1 6250 dams.

Assessment and comparison with the classification criteria

Neoplastic and non-neoplastic lesions reported in two combined chronic and carcinogenicity studies with AgION type AJ, one conducted in mice and the second in rats are summarised in the table below.

Table 14 (RAC): Neoplastic and non-neoplastic lesions in the combined chronic toxicity - carcinogenicity studies

MICE	**0%**	**0.1%**	**0.3%**	**0.9%**	
Renal cysts*	M:0/49 F: 0/49	M:0/48 F: 0/49	M:0/49 F: 1/50	M:4/50 F: 3/49	
Enlargement of the islets of Langerhans**	M:3/49 F: 0/49	M:7/48 F: 0/549	M:13**/49 F: 0/50	M:11/50** F: 0/49	
Ovarian cysts	6/49	22/49***	19/50***	16/49***	
RAT	**0%**	**0.01%**	**0.03%**	**0.1%**	**0.3%**
Endometrial polyps*	0/49	2/50	5/49	9/50**	7/49**
Pituitary adenomas*	M:1/50 F:11/49	M:0/49 F: 16/50	M:3/50 F: 12/49	M:0/48 F: 19/50	M:1/49 F: 20/49
Leukaemia*	M:7/50 F: 2/49	M:7/49 F: 5/50	M:7/50 F: 6/49	M:11/48 F: 5/50	M:14/49 F: 9/49

* Statistically significant dose response relationship
** Statistically significant p<0.05
*** Statistically significant p<0.01

Mice

The cumulative survival rate and the mean survival time were similar between treated mice and controls. The total number of tumours per animal at termination was lower in high dose males (1.00) compared to controls (1.26) and was comparable between high dose females and controls.

Renal cysts

The histopathological examination revealed an increased dose-related incidence of renal cysts in males and females. However, the CLH report did not include information about the potential malignancy of these cysts and the number of cases did not seem to be statistically different from controls. Historical control data for renal cysts was also absent. However, nephrotoxicity was also reported in the 90-days study and at the high dose group the cysts were accompanied with increases in kidney weights in females. These data suggest that these cysts might be more relevant for general toxicity than for carcinogenicity (see the STOT-RE section). Thus, RAC does not consider renal cysts as being relevant for carcinogenic classification.

Enlargement of islets of Langerhans

The frequency of the enlargement of the islets of Langerhans in males (no cases were reported in treated or control females) showed a statistically significant dose-response relationship. This lesion was described in the CLH report as an "enlargement" and not as a tumour and no further indication about whether it was potentially malignant was reported. RAC does not consider enlargement of islets of Langerhans in males as being relevant for classification for carcinogenicity.

Ovarian cysts

A statistically significant increase in the incidence of ovarian cysts was evident at all dose

levels, although there was no clear dose-response relationship. Again, the CLH report did not include a histopathological assessment about whether these were potentially malignant or about the incidence of these lesions in historical controls and in this context RAC does not consider ovarian cysts as being relevant for carcinogenic classification.

Rats

The cumulative survival rate and the mean survival time in rats were similar between treated animals and controls. The total number of tumours per animal at termination was lower in high dose males (1.86) compared to controls (1.96) whereas the total number in high dose females was higher (2.11) than in controls (1.37). The difference was not statistically significant.

Endometrial polyps

There were dose related increases in endometrial polyps in females. However, the CLH report dids not contain information about the type of polyps or whether they were potentially malignant. The Industry highlighted in a report submitted at PC that according to the original study, the incidence of endometrial polyps was within the range of the historical control data and therefore the highest incidence reported in this study (18%) has to be considered a consequence of biological variability. In addition, these endometrial polyps were not considered to be a true effect by the Technical Meeting for Biocides (June, 2013). In consequence, RAC does not consider endometrial polyps as relevant for classification for carcinogenicity.

Pituitary adenomas

There was a dose related increase in pituitary adenomas in females only. RAC notes that:

i. Despite the positive trend none of the treated groups showed a statistically significant increase relative to the control group;
ii. The incidence in the group given 0.03% (24%) was essentially the same as the control incidence (22%), and on this basis the slightly larger incidence at 0.01% (32%) may be regarded as being due to biological variability. The incidences at 0.1% and 0.3% (38% and 40%, respectively) were only slightly higher than the incidence at 0.01% and therefore within the expected range of variation;
iii. ECHA Guidance on the Application of the CLP Criteria explicitly mentions pituitary adenomas in F344 rats as an example of animal tissues with a high spontaneous tumour incidence;
iv. ECHA Guidance on the Application of the CLP Criteria states concerning cases such as pituitary adenomas in F344 rats that *"the tumour incidence in the treated group may be significantly above the concurrent control but could still be within the historical incidence range for that tumour type in that species and therefore may not be providing reliable evidence of treatment related carcinogenicity"*;
v. The industry highlighted in a report submitted during PC that according to the original study report the incidence of pituitary adenomas in all (control and treated) groups of the study was within the range observed in historical control data and consequently the incidences of pituitary adenomas was not treatment-related. In addition, the range of historical control data reported by the Industry were very similar to that reported by the National Toxicology Program (Haseman et al., 1984);
vi. The mean survival period in both groups was not significantly different. This suggests that the pituitary adenomas either had a late onset, a slow progression or both and in any case, the contribution of this finding to mortality was not significant;
vii. Pituitary adenomas were not described in the carcinogenicity study in mouse,

where the level of spontaneous incidence was supposedly lower than in F344 rats.

viii. Pituitary adenomas are considered benign tumours and no carcinomas were observed.

The DS considered that a statistically significant positive trend, in which all doses are considered, is a strong indication of the biological relevance of the effect and cannot be due to the chance. The DS also argued against the validity of historical control data because the study was dated in 1992 while the historical control data were based on a publication dated earlier (Tajima, 1989). The CLP Guidance (2014) would tend to support this. However, these were the only historical data available to RAC for this finding to assist with interpreting the study results.

Taken all together, RAC considers that the weight of the above evidence when compared with the CLP criteria suggests that pituitary adenomas are <u>not</u> relevant for classification of SZZ for carcinogenicity.

<u>Leukaemia</u>

A statistical analysis did reveal a dose-related increase in the frequency of leukaemia and infiltration of leukaemia cells into different tissues in both male and female rats. Since the neoplastic/non-neoplastic changes observed were combined for scheduled and intercurrent deaths, it is not clear when in time the leukemia developed. According to the CLH report, tissues from the right femoral bone were collected. However, it is not clear if the bone marrow was analysed for histopathological changes.

RAC notes that:

i. The type of leukaemia is not cited in the CLH report. It seems that the particular type of leukaemia seen in the F344 rat is uncommon in other rat strains and histologically comparable tumour types are not seen in humans;

ii. RIVM concluded that substance induced increases in the incidence of this tumour type are considered not relevant as an indication for carcinogenicity in humans (Muller, 2005);

iii. The mean survival period in both groups was not significantly different. This suggests that the leukaemia either had a late onset, a slow progression or both and in any case, its contribution to mortality was not significant;

iv. According to the original study the incidence of leukaemia in females in all (control and treated) groups of the study was within the range of the historical controls. The incidence of leukaemia in males at the highest concentration (28.6%) was less than 1% higher than the highest incidence reported for historical controls (27.9%).

v. The range of historical control data reported were very similar to those reported from studies conducted under the National Toxicology Program (Haseman *et al*., 1998) for a time period covering the conduct of the carcinogenicity study (1990-1997);

vi. Leukaemia were not described in the carcinogenicity study in mice.

Comparison with the classification criteria:

For this hazard class, one representative SZZ (AgION type AJ) was tested in combined chronic and carcinogenicity studies in both rats and mice. RAC is of the opinion that the long-term systemic toxicity and carcinogenicity of SZZ by the oral route is dependent on a toxic moiety which is common to all SZZ. Although the rate of absorption, distribution and deposition/precipitation of silver ions in tissues and organs may vary between the SZZ, medium- to long-term exposure by the oral route will lead to similar hazardous

effects to human health.

As there is no epidemiological evidence regarding the carcinogenicity of SZZ in humans, a classification in Category 1A is not appropriate. RAC also considers that the animal evidence is insufficient for classification in category 1B.

RAC considers that a classification in category 2 is not appropriate based on the weight of evidence analysis above and the comparison of the findings with the CLP criteria. RAC recognises that the carcinogenicity study in rats demonstrated significant positive trends for leukemia in both males and females. RAC also recognises that there are equivocal results obtained from *in vitro* mutagenicity studies and that the there is a lack of reliable *in vivo* mutagenicity data with SZZ. However, in evaluating the overall weight of evidence, RAC has considered the following:

i. the weak statistical significance of the reported incidences in pituitary adenomas without carcinomas
ii. the weak statistical significance of incidences in leukaemia in a very susceptible strain of rats and the absence of leukemia in mice;
iii. the similar cumulative survival rate and the mean survival time in rats and mice;
iv. the comparable ratio of tumours/animal among control and exposed rats and mice at the termination of the studies;
v. the doubts on the human relevance of the leukaemia reported in rats; and
vi. the apparent sex dependence of the reported tumours.

Thus, **RAC considers that, based on the weight of the evidence analysis of carcinogenicity, SZZ does not warrant classification for carcinogenicity.**

4.10 Toxicity for reproduction

Table 25: Summary table of relevant reproductive toxicity studies

Method	Results	Reference
OECD 416 Maturation, mating, gestation and lactation for two successive generations Rat SpragueDawley Crl: CD® (SD) IGS BR 30/sex AgION Silver Antimicrobial Type AK Oral in diet m/f: 72/87, 472/548, 984/1109 mg/kg bw (premating) This corresponds to approximately 1.5/1.8, 9.8/11.3; and 20.3/22.9 mg silver ion equivalents/kg bw/d in males and females	**Parental:** <u>F0 12500:</u> ↑ Mortality (m 10%) ↓Bodyweight (m≤10% (pre/post pairing, f 6% gestation day 20, ≤ 11%) ↓Bodyweight gain (m≤17% (pre pairing), f gestation 14-20:29% 0-20:16%) ↓Food consumption (premating m ≤8%, lactation 0-4:27%, 4-7: 12%, 7-14: 21%, 14-21: 27%) ↑RBC (m/f 13/15%), platelets (m/f 42/45) ↓Hb (m/f 16/12%), HCT (m 9%) MCH (m/f 25/23%) MCHC (m/f 7/6%), ↑Pigmentation of organs ↑Histopathological changes in kidneys (including hydronephrosis (8m/2f , 3m in controls) , urinary tract ↓ kidney weight (m abs/rel 14/3%, f rel brain 7%) rel brain weight (m, 9%) ↑ epididymis left/right (rel bw 11/9%) Spleen (m, 7%) Testis (rel left/right 12/10%) <u>F0 6250:</u> ↑ Mortality (m, 3.3%) ↑RBC (f 11%), ↓ MCV (m/f, 6/9%), MCH (m/f 6/12%), MCHC (f, 3%) ↑Pigmentation of organs ↑Histopathological changes in kidneys (including hydronephrosis 7m/2f, 3m in controls)) ↓kidney weight (m, abs/rel bw 13/7%) spleen (m, abs/rel bw 14/21%) <u>F0: 1000:</u> ↑Pigmentation of organs <u>F1 12500:</u> ↑Mortality (m/f 93.3/76.7%) ↓Bodyweight (premating m/f ≤ 56/46%) ↓Bodyweight gain (premating m/f ≤ 47/40%) ↑Histopathological changes ↑Thymus atrophy <u>F1:6250:</u> ↑Mortality (m/f 23.3/3.3%) ↓Bodyweight (premating w1-10 m/f 25-13/19-2 (n.s.s)%, post-pairing m ≤12%, gestation n.s.s, lactation≤ 10%) ↑Histopathological changes (including hydronephrosis 10 m/4f , 0 in controls) ↑Kidney weight (m/f, abs 19/11%, rel bw 9/8%, rel brain 13/7%) ↓Brain (m/f, 7/5%) Adrenal (m, abs 18%, rel brain 12%)	IIIA 6.8.2-04 2002

Method	Results	Reference
	epididymis left/right (abs 14/11%, rel brain (left 9%)) Spleen (m, rel bw 11%) Testis (abs left/rel brain right 12/7%) Prostate (rel brain 13%) Seminal vesicle (8%) Liver (f, 8%) ↑Thymus atrophy (thymus not weighed in F1 adults) <u>F1 1000:</u> ↑Mortality (m 3.3%) ↑Pigmentation of organs ↑ Hydronephrosis (3m, 1f, 0 in controls) **Offspring:** <u>F1 12500:</u> ↓total pups born/litter (15%) ↑stillborn index ↓livebirth index ↓liveborn/litter (27%) ↓pup survival indices (Days 0-4 precull 46% (45% day 4 pre-culling then ≤29%)) ↑clinical signs ↓body weights M+f Day 0: 15% Day 4:pre/post culling: 19% Day 7: 23% Day 14: 26% Day 21: 36% Day 26: 47% ↓organ weights Brain 18% (rel bw ↑58%) Spleen 26% (rel bw ↑31%) Thymus (m/f abs 74/70%, rel bw 53/47%, rel brain 69/64%) ↓sex ratio ↑day of vaginal opening (day 59.9, control: 35.1) and preputial separation (day 56.7, control: day 44.5) ↑histopathological changes F1 6250: ↑clinical signs ↓ body weights M+f Day 14: 13% Day 21: 25% Day 26: 47% ↓organ weights Brain 10%, rel bw ↑27% Thymus (m/f abs 58/55%, rel bw 39/39%, rel brain 53/51%) ↑Spleen (m/f rel bw 31/32%) ↑day of vaginal opening (day 39.8) and preputial separation (day 47.4) ↑histopathological changes <u>F1 1000:</u> ↓organ weights Thymus (m abs 13%, m/f rel bw 10/9%, m rel brain 11%) <u>F2 6250:</u> ↑stillborn index ↓livebirth index ↓bodyweights Day 0: 5% Day 4:	

Method	Results	Reference
	pre/post culling: 12% Day 7: 15% Day 14: 18% Day 21: 20% ↑histopathological changes ↓organ weights Brain (m/f 10/7%, rel bw ↑21/25%) Thymus (m/f abs 50/54%, rel bw 37/42%, rel brain 47/50%) Spleen (m abs 18%) <u>F2 1000:</u> ↓Thymus weight (m rel bw 11%) Reproduction: ↑stillborn index (F1, F2) ↓livebirth index (F1, F2) ↑day of vaginal opening and preputial separation	

4.10.1 Effects on fertility

4.10.1.1 Non-human information

In a two-generation reproduction and fertility study in rats, the silver zinc zeolite denoted AgION Silver Antimicrobial Type AK was administered through the maturation, mating, gestation and lactation periods for two successive generations.

Parents

F0: Three males administered the high dose and one male administered the mid dose died during the study. The cause of death could not be established but the deaths were considered related to treatment by the study author. Bodyweight and bodyweight gains were reduced in males during premating by ≤ 10 and 17% respectively. After mating, the male bodyweight gain was comparable for all groups.
One female control animal died during the study but no deaths occurred among the treated F0 females. The bodyweights were reduced in high dose females at day 20 of gestation and at day 7, 14 and 21 of lactation but did not fall below 11% of the bodyweight in controls. The bodyweight gain was reduced during gestation, during days 0-20 by 16% and days 14-20 by 29%. The bodyweight gain during lactation was at some of the measurements significantly increased or decreased compared to controls, but the overall bodyweight gain during lactation (days 0-26) was not statistically significantly different from controls.
Food consumption was reduced between 12 and 27% in the high dose group during lactation and the changes were statistically significant. The reduced bodyweight gain and food intake is further discussed in section 4.11.5.
High dose males and females had increased levels of erythrocytes, platelets and decreased levels of hemoglobin (Hb), hematocrit (HCT), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Some of these parameters were also slightly affected in mid dose males and females. The same effects were seen also in the repeated dose studies performed with silver zinc zeolite Type AK and were considered to be caused by zinc. According to the repeated dose study report, zinc prevents uptake of copper in the GI tract which suppresses

production of ceruloplasmin. This in turn leads to decreased iron transport and decreased synthesis of hemoglobin.
There were no clinical signs observed and no effects on reproductive parameters that were statistically significant.
Pigmentation was observed in several tissues of mid and high dose animals and mild pigmentation of pancreas and thymus was observed also in some females of the low dose group.
Histopathological changes in the kidneys (including hydronephrosis) were noted in high and mid dose animals. Kidney weights were decreased in high dose male and females. The thymus was not weighed.
The gestation length was slightly increased (22.3 compared to 21.9 days in controls) in treated animals and the change was statistically significant for the mid and high dose group. Adverse effects on reproduction was manifested in high dose animals as reduced mean number of live and total pups at birth, reduced live birth index, increased number of stillborn pups and increased stillborn index (see tables 25 and 27). Complete pup mortality was observed in six females of the high dose group. Since the number of corpora lutea was not recorded in the animals, it is not possible to establish if the reduced total number of pups born were due to pre or post-implantation losses.
F1: The mortality in the high dose (12500 ppm) animals was considerable and 28/30 males and 23/30 females died prior to the end of the premating period. The group was therefore terminated after this phase and there were, consequently no pups from this group. The cause of death was not clearly established but discoloration of organs, histopathological changes in the kidneys, decreased size of thymus, enlarged heart and spleen, penile distention/extension and red discoloration were noted among the dead animals.
Body weights of F1 males administered 6250 or 12500 ppm were lower than controls at the start of and throughout the premating, pairing and post-pairing periods and until termination of the high dose group. The body weight gain in males administered 6250 ppm was however comparable to controls over the entire premating period. Bodyweights of mid dose F1 females were statistically lower during the first six weeks of premating and also at one timepoint during lactation but there were no statistically significant effects on body weight gains during overall (week 1-12) premating, gestation or lactation. Food consumption was reduced in high dose animals and in mid dose males during the entire study.
The macroscopic examinations of F1 animals revealed changes in the urinary tract and in the kidneys. Effects on kidneys included mild caliculi, mild to moderate pelvic dilation and an increased incidence of mild to moderate cortical surface irregularity. Most often cortical surface irregularity corresponded to microscopical changes such as chronic interstitial nephritis and/or infarction. In addition, two males administered 6250 ppm had mild calculus formation in the urinary bladder. Low and mid dose animals had an increased frequency of hydronephrosis (increased frequency compared to P0) Tan/brown discoloration of multiple organs were observed in animals (pancreas, thymus, glandular stomach, duodenum, jejunum, mandibular salivary glands, Harderian glands, exorbital lacrimal glands, pineal gland and urinary bladder. A low incidence of thymic athrophy was noted in animals administered 1000 (premating 71/87 mg/kg bw/d in males and females respectively)) or 6250 ppm (m/f: 477/582 mg/kg bw/d).
Organ weight analysis of animals administered 6250 ppm showed an increased relative weight of spleen (only significant in males), reduced absolute brain weight in males and females, reduced absolute/relative weight of prostate, reduced absolute weight of seminal vesicle, reduced absolute/relative weight of both testes and reduced absolute weight of uterus/oviducts/cervix. Reduced kidney weights were observed in males and females administered 1000 or 6250 ppm. Other statistically significant changes observed were not considered related to treatment. Splenomegaly correlated microscopically with increased extramedullary hematopoiesis and is assumed to be related to treatment since anemia was observed in the F0 parents.

There were no statistically significant or clearly dose-related effects on the fertility parameters. It is noted however that the percentage of abnormal sperm was higher in treated animals compared to controls (0.50 in the mid dose (6250) group, 1.41 in the low dose group and 0.18 in controls). In the absence of statistical significance and effects on fertility, the significance of this finding is unclear. The percentage of females delivering litters with stillborn pups was increased in the 6250 ppm group and this was also reflected as an increased stillborn index and decreased live birth index.

Offspring

F1 pups: Day 0-4 pup survival was low in the high dose group (53.1% compared to 98.9% in controls) and 5/27 females that delivered litters with live pups failed to retain live pups to Day 4. The male/female sex ratio was reduced at day 0, 4 (pre/post culling), day 21 and 26 but the effect was only statistically significant on day 4 (preculling).
Clinical signs in pups pre-weaning included decreased activity in mid and high dose animals and discoloured skin (blue/pale) and difficult breathing in high dose animals. The discoloration was mainly observed at day26 day whereas decreased activity and breathing difficulties were observed at day 0 or 4. There were no abnormalities detected in the clinical observations of dams made during lactation.
Statistically significant reduced bodyweights were observed at all measurements of male and female pups administered 12500 ppm and at day 14, 21 and 26 in male and female pups administered 6250 ppm.
The absolute weights of brain, spleen and thymus was reduced in pups administered 6250 and 12500 ppm. These changes were statistically significant (except for spleen in 6250 pups). The changes remained statistically significant also when these organ weights (except for the spleen) were related to bodyweights.
A dose-related delay in the day of vaginal opening and preputial separation was observed in all treated animals and the delay was significant in the mid and high dose group. Since the bodyweights were comparable between treated females and controls on the day of vaginal opening, the delay seems related to the reduced bodyweights. The bodyweights of 6250 and 12500 ppm males were yet reduced by 12,5 and 38% respectively at the time of preputial separation.
There were no treatment related histopathological findings in the stillborn pups or in day 4 culled pups. Changes in the kidney (pale, dilation, cyst) liver (pale) were observed at day 26 in males and females administered 6250 or 12500 ppm. Moreover, cardiac changes were observed in both sexes of high and mid dose animals; mildly enlarged heart in 6/14 males and 6/18 females in 12500 group and 5/27 males and 4/26 females in 6250 group compared to 0 in controls). Small thymus was observed in 2/14 high dose males and 2/18 females.
F2 pups: The number of live pups/litter was decreased in the low dose group at day 4, 14 and 21 due to the complete loss of pups in two litters but there was no effect in the 6250 ppm animals. Pup body weights were lower in 6250 ppm pups than in controls at birth and were further reduced throughout the pre weaning period.
Organ weight analysis showed reduced absolute/relative thymus and brain weights in males and females administered 6250 ppm. The macroscopic examinations of F2 pups at day 21 (weaning) revealed mild to moderate decreased size of thymus, mild cardiac enlargement, mild renal pallor, mild hepatic pallor and mild pulmonary pallor in animals of the 6250 ppm group.
Analysis of copper, silver and zinc in homogenates of three whole pups from control, 1000 and 6250 pups showed a general decrease of copper in the treated groups whereas the levels of silver and zinc were generally increased (table 25). This analysis does not confirm but supports the mechanism proposed by Shavlovski (see section 4.11.3).

Table 26: Zinc, silver and copper levels (mg/kg bw) of F2 Day 4 culled pups

	control		1000		6250	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
Silver	<1	<1	1.04	1.06	1.68	2.2
	<1	<1	1.06	<1	1.1	<1
	<1	<1	<1	<1	1.07	1.84
Zinc	7.77	10	8.87	8.05	8.65	10.4
	6.44	6.31	11.8	6.88	7.32	7.56
	8.01	7.62	5.57	5.63	8.85	11.9
Copper	2.24	2.18	1.97	1.67	<1.5	1.86
	2.07	2.49	2.19	1.61	<1.5	<1.5
	2.15	2.72	1.61	1.76	1.96	1.52

A NOAEL for parents and offspring could not be set since pigmentation of organs were observed in all adults at all dose levels and reduced thymus weights were observed in F1 adults and in F2 pups administered the lowest dose (i.e. 1000 ppm). F1 animals administered 1000 ppm also had an increased incidence of hydronephrosis (see tables 25 and 28).
The LOAEL was at or below 1000 ppm which corresponds to 72/87 mg Type AK/kg bw/d (based on pre mating values).
The NOAEL for reproduction was below 1000 ppm (approximately 70 mg Type AK/mg kg bw) based on a decrease in livebirth index, increase in stillborn index, reduced bodyweights in F2 pups administered 6250 ppm (approximately 470 mg Type AK/kg bw/d) and reduced bodyweight gain in F1 pups with a subsequent delay in day of vaginal opening and preputial separation.
The same effects although more severe (and accompanied by a reduced pup survival) were observed in F1 pups of dams administered 12500 ppm.

Table 27: Overview of findings in the two-generation study with silver zinc zeolite

	Mortality	**Bodyweight**	**Bodyweight gain**	**Sexual maturation**	**Haematology**
12500					
P m P f	10% (3/30) 0%	**Premating (end):** ↓11% n.s.s in females **Gest:** ↓6% (only sign day 20) **Lact**:≤11%	**Premating** (1-11): ↓17% n.s.s in females* **Gest**: 14-20:↓29% 0-20: ↓16%** **Lact**: No consistent pattern**	-	**m/f** Hb: ↓16/12 RBC: ↑13/15 MCV: 20/19 MCH:↓25/23 MCHC:↓7/6 Plat:↑42/45
*stat sign increase certain weeks **see text for a discussion on adjusted maternal weight					

	Mortality	Bodyweight	Bodyweight gain	Sexual maturation	Haematology
F1 (p)m F1 (p) f	93.3% (28/30) 76.7% (23/30) **Found dead** **Days m f** 0-10 3 3 10-20 6 3 20-30 10 6 30-40 4 5 40-50 3 3 50-60 3 2 60-70 0 1 70-80 1 -	**Premating (start):** ↓55% (m) ↓45% (f) **Premating (end):** ↓56% (m) ↓44% (f) **Gest:** n.s.s **Lact:** see text	**Premating (1-12):** ↓47% (m) ↓40% (f) **Gest:**n.s.s **Lact:** see text	Day 59.9 Day 56.7 (F1 Control: 35.1/44.5)	No data
F1 pups (P dams)	**Total pups born/litter:** 12.1(↓15%) **Liveborn/litter:** 10.3 (↓27%) **Stillborn/litter:** 1.5 (↑750%) **Live birth index:**85.5% **Stillborn index:** 12.2% **Pup survival indices:** 0-4: 53.1% 4*-21:n.s.s 4*-26:n.s.s	**M+f** Day 0: ↓15 Day 4: pre/post culling: ↓19 Day 7: ↓23 Day 14:↓26 Day21: ↓36 Day 26:↓47	Not determined	-	No data
F2 pups (F1 dams)	No data F1 terminated prior to mating	No data F1 terminated prior to mating	No data F1 terminated prior to mating	- F1 terminated prior to mating	No data F1 terminated prior to mating
6250					
P males P females	3.3% (1/30) 0%	**Premating (end):** ↓7% ↓19-9% on single occasions week 1-6 in females **Gest:** not stat sign **Lact:** ↓7% (day 14 only)	**Pre (1-11):** ↓12% n.s.s in females **Gest:** 14-20:n.s.s 0-20: n.s.s **Lact:** No consistent pattern	-	Hb: n.s.s RBC: n.s.s/↑11 MCV: 6/9 MCH:↓6/12 MCHC:↓7/3 Plat:n.s.s
F1 (m) p F1 (f) p	23.3% (7/30) 3.3% (1/30) **Found dead** **Days m f** 10-20 - 1 20-30 2 - 30-40 1 - 50-60 1 - 110-120 1 - 120-130 -2 -	**Premating (start):** ↓25% (m) ↓19% (f) **Premating (end, week 12):** ↓13% (m) n.s.s in females* **Gest:** not stat sign **Lact:** ≤10%	**Pre (1-12):** n.s.s	Day 39.8 Day 47.4 (F1 Control: 35.1/44.5)	No data

	Mortality	Bodyweight	Bodyweight gain	Sexual maturation	Haematology
			Gest: n.s.s **Lact:** (↓65% day 4, see report)		
*bw stat sign reduced weeks 1-6 only.					
F1 pups	**Total pups born/litter:** n.s.s (13.1, ↓8%)) **Liveborn/litter:** n.s.s (12.8↓9%)) **Stillborn/litter:** n.s.s (0.4, ↑400%) **Live birth index:** n.s.s (97.4%) **Stillborn index:** n.s.s (2.6%) **Pup survival indices:** n.s.s (day 0-4: 96%)	**M+f** Day 0: n.s.s Day 4: pre/post culling: n.s.s Day 7: n.s.s Day 14: ↓13 Day21: ↓25 Day 26: ↓29	Not determined	-	No data
F2 pups	**Total pups born/litter:** n.s.s (13, ↓1%) **Liveborn/litter:** **n.s.s** (12.2, 5%) **Stillborn/litter:** n.s.s (0.7, ↑350%) **Live birth index:**93.1 % **Stillborn index:** 5.4 % **Pup survival indices:** n.s.s (day 0-4. 93.2%)	**M+f** Day 0: ↓5 Day 4: pre/post culling: ↓12 Day 7: ↓15 Day 14: ↓18 Day21: ↓20 Day 26: n.d	Not determined	-	No data
1000					
P males P females	0% 0%	**Pre (end):** n.s.s M/F **Gest:** not stat sign **Lact:** not stat sign	**Pre (1-11):** ↓6% n.s.s in females **Gest:** 14-20:n.s.s 0-20: n.s.s **Lact:** n.s.s	-	
F1 (m) p F1 (f) p	3.3% (1/30) 0%	**Pre (start/end):** n.s.s in m/f **Gest:** not stat sign **Lact:** ↓7% (day 4 only)	**Pre (1-12):** **n.s.s** **Gest:** 14-20:n.s.s 0-20: n.s.s **Lact: n.s.s** (see text)	n.s.s	No data
F1 pups	**Total pups born/litter:** n.s.s (13.2, ↓7%) **Liveborn/litter:** n.s.s (↓9%) **Stillborn/litter:** n.s.s (0.3, ↑300%) **Live birth index:** n.s.s (97.6%)	**m+f** Day 0, 4: pre/post culling, 7, 14, 21, 26: n.s.s	Not determined	-	No data

	Mortality	Bodyweight	Bodyweight gain	Sexual maturation	Haematology
	Stillborn index: n.s.s (2.0%) **Pup survival indices:** n.s.s (day 0-4: 98.8%)				
F2 pups	**Total pups born/litter:** n.s.s (11.3, ↓14%) **Liveborn/litter:** n.s.s (10.9, ↓16%) **Stillborn/litter:** n.s.s (0.3, ↑150%) **Live birth index:** n.s.s (96%) **Stillborn index:** n.s.s (2.6%) **Pup survival indices**: n.s.s (day 0-4. 83.4%)	**m+f** Day 0, 4: pre/post culling, 7, 14, 21, 26: n.s.s	Not determined	-	
0					
P males P females	0% 3.3% (1/30)	- -	- -	-	
F1 (m) p F1 (f) p	0% 0%	- -	- -	35.1 44.5	
F1 pups	**Total pups born/litter:** 14.2 **Liveborn/litter:** 14.1 **Stillborn/litter:** 0.1 **Live birth index:** 99.2% **Stillborn index:** 0.8% **Pup survival indices:** 0-4: 98.9% 4*-21:100% 4*-26:100%	- -	n.d	-	
F2 pups	**Total pups born/litter:** 13.1 **Liveborn/litter:** 12.9 **Stillborn/litter:**0.2 **Live birth index:**98.3% **Stillborn index:** 1.1% **Pup survival indices:** 0-4: 95% 4*-21:99.5%	- -	n.d	-	
*post culling					

Table 28: Pathological findings in several generations

	12500	6250	1000	Control
Incidences of hydronephrosis				

F0	8m, 2f	7m, 2f	2m,1f	3m
F1	terminated	10m, 4f	3m, 1f	-
Reduced thymus weight (% lower than controls)				
F0	not weighed; no histopathological findings			
F1 pups	(m/f) abs 74/70%, rel bw 53/47% rel brain 69/64%	(m/f) abs 58/55% rel bw 39/39% rel brain 53/51%	m, abs 13%, m/f rel bw 10/9% m, rel brain 11%	-
F1 adults	not weighed thymus atrophy noted in males/females	not weighed	not weighed	not weighed
F2 pups	Not available due to termination of F1.	(m/f) abs 50/54%, rel bw 37/42%, rel brain 47/50%	m, rel bw 11%	-

4.10.1.2 Human information

No information specific to silver zinc zeolite is available. The human data on silver substances available is discussed in section 4.11.3.

4.10.2 Developmental toxicity

4.10.2.1 Non-human information

There were no studies in the dossier addressing the teratogenicity of silver zinc zeolite. The studies submitted for the biocides review were performed with four other silver containing substances (see section 4.11.3). All of the studies are performed in rats.

4.10.2.2 Human information

No silver zinc zeolite specific information is available. The only human data found is discussed in section 4.11.3.

4.10.3 Other relevant information

Developmental toxicity

Silver containing active substance 1*(doc IIIA, 6.8.1(02)):* The substance was administered in daily dietary doses of 200, 700, 2000 mg/kg bw to rats during days 6-15 of gestation. Two animals in the mid dose group and two animals in the high dose groups were found dead prior to termination. Three of these deaths were attributed to dosing accidents but the death of one high dose dam was considered related to treatment. This female showed hemorrhage from the urogenital tract, dark red

kidneys and the stomach was distended with gas and test substance.The maternal bodyweight and bodyweight gain was approximately 13 and 24% lower at termination in high dose animals compared to controls. Clinical observations that were considered related to treatment included incidences of wheezing (0/30, 2/30, 6/30 and 8/30 in control, low, mid and high dose groups respectively) and incidences of sedation (11/30), voiding watery faeces (3/30), urogenital discharge (3/30) and thinness (2/30) in the high dose group only.
No treatment-related effects in litter parameters were observed except for a different sex ratio in treated groups (M/F 49.4/50.6, 53.0/47.0 and 54.0/46.1 in low, mid and high dose respectively) compared to controls (M/F 40.8/59.2). This change was not statistically significant thus the toxicological significance is unclear. A few abnormalities were noted in single low and mid dose animals but these were considered to be incidental. There were no statistically significant differences in the incidence of delayed ossification effects but statistical analyses could not be made for the phalanges of bones due to processing accidents and incomplete staining. According to the study report, skeletal abnormalities such as wavy ribs, misshapen radii, ulnae and femurs were observed in three foetuses from the same litter (3/223 foetuses examined) in a high dose female. Since there is no individual data on the different types of delayed ossification included, this information cannot be confirmed. The individual bodyweight data shows that the parent of this litter lost 19g during the treatment period (day 6-17) and the overall weight gain at termination was only 2 g (mean bodyweight gain in controls was 109g) thus the effects may be secondary to maternal toxicity. Besides observations of pale liver and kidney in two high dose females and enlarged spleen in one female in mid and high dose group, there were no other gross abnormalities reported.
The NOAEL for maternal toxicity was 700 mg kg bw based on a reduced bodyweight gain and an increased incidence of clinical signs at 2000 mg/kg bw (LOAEL). The NOAEL for pup/embryotoxicity/teratogenicity was higher than 2000 mg/kg bw based on the absence of toxicity at the highest dose tested. Based on data obtained in the release study these doses correspond to a maternal NOAEL/LOAEL of 10/29 mg silver ion equivalents/kg bw and a NOAEL for pup/embryotoxicity/teratogenicity above 29 mg silver ion equivalents/kg bw.

Silver containing active substance 2 *(doc IIIA, 6.8,1(06)):* The developmental toxicity of the substance was tested first in a preliminary oral gavage study in eight rats and then in a standard developmental toxicity test with 25 Sprague-Dawley rats. In both studies animals were administered 0, 100, 300 and 1000 mg/kg bw during days 6-15 of gestation. All animals survived through the main study except for a mid-dose dam who was killed in extremis with signs of respiratory distress that were considered to be the result of a dosing trauma. There were no clinical signs observed in the studies and no significant effects on food consumption or bodyweights. The pregnancy index, implantation data and live litter size parameters were similar between treated animals and controls and the only difference was a dose related increased of the percentage males per litter that was statistically significant in the high dose group (56.8% compared to 43% in controls). The significance of this finding is unclear since the opposite pattern was observed in the preliminary study (40.3% in high dose and 50.6% in controls) but it is noted that an increased percentage of male foetuses was also observed in the study with silver containing active substance 1. There were no differences among foetal parameters such as litter weight data, visceral/skeletal malformations or variations. The NOAEL and LOAEL for maternal/pup embryotoxicity/teratogenicity was higher than 1000 mg/kg bw based on the absence of toxicity at the highest dose tested. Based on data obtained in the release study, this corresponds to a NOAEL above 25 mg silver ion equivalents/kg bw.

Literature data; silver chloride *(Doc IIIA, 6.8.1(03)***:** In a published study by Shavlovski et al., silver chloride 50 mg/animal (less than approximately 250 mg/kg bw/day) was administered in diet to 20 inbred albino female rats from the first day of the study to day 20 when the rats were sacrificed. A group of five rats were also used to study the effect of silver during the period of

organogenesis (days 7-15 only). The study also investigated effects in untreated control rats, in rats administered injections of human ceruloplasmin and rats administered bipyridyl or penicillin (Cu/Fe chelators).
The results showed that if dams were exposed between days 1-20, the incidence of post-implantation deaths (36%) increased compared to control (9.6%) and historical controls (8.7%) and all newborn animals died within 24 hours. Moreover, the incidences of hydronephrosis (31%) and cryptorchidism (35%) increased substantially compared to controls (5.3 and 1.3% hydronephrosis and cryptorchidism respectively) and historical controls (1.2 and 0.8% respectively).
The survival of newborns was improved if injections of human ceruloplasmin were received during days 2-14 and survival was almost comparable to controls if CP injections were received during days 8-21. The deaths of embryos and newborns were explained as a consequence of copper deficiency caused by silver inhibiting copper from binding to the transportprotein ceruloplasmin. This theory was supported by the increased survival (and reduced frequency of teratogenic effects) in AgCl treated rats who received injections of human ceruloplasmin as well as by the lack of copper in placenta, embryos and blood serum of adult rats treated with AgCl. In addition, malformations were exacerbated when chelator bipyridyl was co-administered. There were no effects in rats treated with AgCl during organogenesis only and this was considered to be due to active ceruloplasmin gradually decreasing from blood.
Although the study was not performed according to GLP or a recognised guideline, the result is considered reliable since the publication has been peer-reviewed and the experiment seems to be well conducted. Several parameters requested in OECD TG 414 were not investigated but the study yet raise serious concern for developmental toxicity of silver, especially since the author states that the treatment did not alter the physiological functions of the dams. Since effects were noted at the only dose level tested, no NOAEL for teratogenic effects can be set in this study.

Literature data; silver acetate *(Doc IIIA, 6.8.1(07)***:** In a different published study the effects of silver acetate on CD albino rats during days 6-19 of gestation was investigated at doses of 10, 30, or 100 mg/kg/day. All animals survived treatment except for a high dose dam exhibiting signs of morbidity and a high dose dam excluded due to a misdirected dose. Clinical signs such as piloerection and minor bodyweight changes were noted in all animals and other signs indicative of toxicity such as alopecia and rooting after dosing were observed in high dose animals. There were no significant effects on maternal body weight gain, food or water consumption during pre-treatment, treatment and gestation period. The number of pregnant dams was reduced in high dose dams (87.5% compared to 96%) but the difference was not statistically significant and did not show a dose-response. Other reproductive parameters did not differ from controls. The percentage litters with late foetal deaths was increased in the high dose group (incidences: 0/24, 0/23, 0/25 and 2/20) resulting in a statistically significant positive trend in the Cochran-Armitage test. The incidence was above historical control data (0-4.35%) but the study authors did not regard the result of this study as clear evidence of prenatal mortality since the number of late fetal deaths/litter was not affected by treatment (it is noted though, that although not statistically significant, the percentage late fetal deaths /litter was 1.22 in high dose group compared to none in control and the lower dose groups). A negative trend that was statistically significant was observed for average male foetal bodyweight/litter and percent litters with late foetal deaths (Cochran-Armitage test) in test for linear trend. The incidence of malformations (external, visceral, skeletal) were lower in the high dose group compared to the control. The number of skeletal variations/litter and the percentage of litters with any variation were increased in high dose animals compared to controls. The skeletal variations included unossified sternebrae, rudimentary rib, short rib, bipartite ossification center. Considering that there were no dose-response and that the difference was not statistically significant, the observation is not given further toxicological significance.
The NOAEL set for maternal toxicity was 30 mg/kg bw based on clinical signs of toxicity and the NOAEL for pups was 30 mg/kg bw based on the decreased average male foetal bodyweight/litter

and average total foetal bodyweight/litter at 100 mg/kg bw (LOAEL). The NOAEL for embryotoxicity/teratogenicity is 30 mg/kg bw based on the increased incidence of the percent litters with late foetal deaths in the high dose group. Based on a silver content of 64.6% and the assumption that silver acetate is completely dissolved in the stomach, this would correspond to a NOAEL of 19.4 mg silver ion equivalents/kg bw.

Literature data; silver lactate: Rungby and Danscher (1983) have demonstrated silver in the brains of neonatal rats exposed in utero when dams received intraperitoneal injections of silver lactate on days 18 and 19 of gestation. This observation shows that silver has an intrinsic ability to pass the blood brain barrier (6.8.1(04)).

Fertility

Silver containing active substance 2 *(doc IIIA, 6.8.2(03)):* The test material was administered in dietary doses of 1000, 5000 and 20000 ppm (72.5/78.2, 363/400 and 1465/1612 mg/kg bw/d and 1.9, 9.9 and 40 mg silver ion equivalents/kg bw/d) to two generations of rats throughout maturation, mating, gestation and lactation.
Parents F0: There were no treatment related deaths in the F0 generation and no effects on bodyweights, food consumption, reproductive parameters or litter parameters (litter size and viability). Increased relative weight of spleen and decreased absolute weight of seminal vesicles/coagulating gland was observed in high and mid dose males whereas a decreased absolute weight of thymus was observed in high dose males only. The pathological examinations showed pigmentation of pancreas in high and mid dose males and females.
Parents F1: Four high dose males and two high dose females died in the FI generation whereas all F1 control animals survived. The bodyweights of male rats were reduced the entire period before pairing and the bodyweights of female rats were reduced during the first three weeks before pairing and during the entire gestation and lactation periods. Food consumption was reduced in males during the last weeks of maturation and during the first days of gestation and lactation in females ($\leq$ 10%). There were no effects on reproductive parameters with the exception of the pre-coital interval which was extended in high dose females compared to controls. This did not affect fertility but it is noted as a biologically significant effect that may indicate endocrine effects. The parturition index was lower in high dose females (90.9%) than in controls (95.4%) but the change was neither dose-related nor statistically significant in chi square analysis. There were no effects on live birth index or the viability index but the number born and the litter size at day 1 was reduced in high dose females compared to controls.
The absolute weights of adrenals, kidneys, seminal vesicles/coagulating gland and right testis were reduced in high dose males and the relative brain weight, epididymides was increased in this group. The absolute and relative prostate weight was reduced more than 25% in high dose males. A dose-related decrease in prostate weight was also observed in F0 males but statistical significance was not achieved. The only statistically significant change observed among organ weights in females was a reduced absolute/relative weight of uterus (28/13%) in the high dose group.
Pigmentation of pancreas, lymph nodes and thymus was observed in high and mid dose animals. According to the study author, there were no significant differences in the proportions of each of the follicle however the total number of follicles (small, medium and large) was lower in high dose animals (7.7/7.5/5.6 in (ovary 1/ ovary 2/ overall respectively) compared to controls (10.4/10.1/10.2 respectively). Since there were no effects on reproductive performance, this observation is not given further significance.
F1 pups: The litter weights and the mean individual weights were reduced by 8 and 9% at the end of lactation (day 21). There were no effects on landmarks of development (pinna unfolding, tooth eruption and eye opening) or on reflexological responses (surface righting reflex, mid-air righting reflex, startle reflex, pupillary reflex).

The weight of thymus was reduced in both male and female mid and high dose pups.
The pathological examination showed pigmentation of pancreas and the mesenteric lymph nodes in high and mid dose males and females.
F2 pups: The litter weights were reduced by 13% at day 1 of lactation and the mean individual weights were reduced by 13% at the end of lactation (day 21). There were no effects on landmarks of development (pinna unfolding, tooth eruption and eye opening) or on reflexological responses (surface righting reflex, mid-air righting reflex, startle reflex, pupillary reflex).
The weight of thymus was reduced in both male and female mid and high dose pups.
The pathological examination showed pigmentation of pancreas and the mesenteric lymph nodes in high and mid dose males and females. The frequency of increased renal pelvic cavitation seemed to be slightly higher in high dose males (6) than in controls (1).
The NOAEL for parents was considered to be 1000 ppm based on organ pigmentation (pancreas, mesenteric lymph nodes in both sexes and generations) and organ weight changes in F0, F1 parents. Based on the lowest reported test substance intake during premating, this NOAEL corresponds to 72.5 mg silver containing active substance/kg bw and approximately 2 mg silver ion equivalents/kg bw/d (F0 males). The corresponding LOAEL is 363 mg silver containing active substance/kg bw and approximately 10 mg silver ion equivalents/kg bw/d (F0 males).
The NOAEL for offspring was 1000 ppm based on the reduced thymus weight in high dose F1 and F2 pups and in male mid dose F1 pups. Based on the lowest reported test substance intake in females during premating, this corresponds to 78 mg silver containing active substance/kg bw (1.9 mg silver ion equivalents/kg bw/d) (F0).
The NOAEL for reproduction was 5000 ppm based on a reduced number born in high dose F1 animals and reduced live litter size (day 1) in high dose F2 animals. Based on the lowest reported test substance intake in females during premating (test substance intake is only available for premating period), this NOAEL corresponds to 400 mg/kg bw (9.9 mg silver ion equivalents/kg bw/d) (F0). The LOAEL was 1612 mg/kg bw (40 mg silver ion equivalents/kg bw/d) (F0)

Other: Silver in the form of an aqueous salt was injected into the testis of rats either as a single dose or as daily doses. Animals were sacrificed and examined histopathologically at different days after the injection. The metal produced histopathological changes in the testis and increased the frequency of pycnotic spermatzoa. Considering that there were no control animals included, that the dose was unknown and that there were no effects on male fertility in the other studies available, this study is not given further significance.

Human information:

According to the summary prepared by the Agency for Toxic Substances and Disease Registry (6.2(08)) it is not known whether silver causes developmental toxicity in humans. There were no studies found regarding developmental effects in humans after exposure to silver but the document refers to a study by Robkin et al. (1973) in which the possibility of a relationship between the concentration of silver in foetal tissues and the occurrence of developmental abnormalities was investigated. The authors reported that the concentration of silver in the foetal liver of 12 anencephalic human foetuses was higher (0.75±0.15 mg/kg) than the values from 12 foetuses obtained either through therapeutic abortions (0.23±0.05 mg/kg), or in 14 spontaneously aborted foetuses (0.21±0.05 mg/kg).The concentration in 9 premature infants was 0.68±0.22 mg/kg. The authors could not determine if the higher concentration of silver in anencephalic foetuses were associated with the malformation, or with foetal age.

4.10.4 Summary and discussion of reproductive toxicity

More than one of the studies on developmental toxicity and reproduction indicates that silver has an embryotoxic potential at doses where the mothers are not severely affected by treatment. This is mainly expressed as decreased viability in foetuses/pups and was seen in varying degrees in the developmental toxicity studies performed with silver chloride (severe effects with late post-implantation deaths, complete pup mortality, increased frequencies of hydronephrosis and cryptorchidism) and silver acetate (slightly increased percentage of litters with late foetal deaths) and in the two-generation study with silver zinc zeolite (reduced number born (15%, F1), increased stillbirth index, reduced liveborn index, reduced pup weight/pup weight gain, small/reduced weight of thymus, increased frequency of hydronephrosis). It is also indicated (reduced number born (11%, F1), reduced live litter size day 1(F2), reduced thymus weight) in a two generation study performed with a different silver containing active substance (Doc IIIA, 6.8.2(03) but was not observed in developmental toxicity studies performed with two other silver containing active substances (6.8.1 (02, 06).
According to the study by Shavlovski et al. (6.8.1 (03)), silver ions can displace copper ions in ceruloplasmin which transports copper to the foetus. In the study, a level of approximately 250 mg/kg bw, led to a copper deficiency that ultimately caused death of the foetuses or newborn when exposure was continuous during the entire gestation period. If exposure was restricted to the period of organogenesis (day 7-15), there were no effects observed. Shavlovski et al. explained this as likely due to a gradual decrease of active ceruloplasmin content in the blood.
Ceruloplasmin is the main copper transporter in the blood and it seems to play a role in cellular uptake of iron[11]. The concentration is usually elevated during preganancy and ceruloplasmin and copper are present in the amniotic fluid and in milk[12]. The information available is not sufficient to elucidate if the effects observed in pups are due to a deficiency of copper, iron or both. Shavlovski et al speculates that the increased mortality could be due to an impaired enzymatic protection (e.g. superoxide dismutase) against oxidative stress.
The competitive binding observed in the studies seems to be an intrinsic property of the silver ion and the severity of effect by different silver containing active substances (SCAS) thus seems to depend on the amount and release of silver and possibly other metal ions with a similar ability to compete for binding. A reason why there were no effects in the developmental toxicity studies with the other two silver containing active substances could be that the amounts of silver ions released from these SCAS at the doses tested were below the LOAEL for embryo/foetal toxicity. While the presence of zinc in silver zinc zeolite may exacerbate the effects of silver in preventing copper from binding to ceruloplasmin, the presence of copper in one of the other substances may be sufficient to keep copper in excess over silver and thus prevent silver from binding. Moreover, since the exposure period was limited to days 6-15 of gestation it is also possible that the lack of effects could be due to active ceruloplasmin still being available in the blood, as discussed by Shavlovski et al.

There are no developmental toxicity studies available for silver zinc zeolite, neither in rats nor in rabbits. The only specific data on silver zinc zeolite available with respect to reproduction is the two-generation study performed in rats. Data is only available for the type of silver zinc zeolite denoted AgION Antimicrobial Type AK however read across between AgION Antimicrobial Type AK, Type AJ and Irgaguard B502i is considered justified (confidential appendix on Technical equivalence, technical specification and read-across). In the absence of developmental toxicity studies, it is not safe to exclude that silver zinc zeolite could induce malformations or cause other

[11] Attieh et al (1999), The Journal of Biological Chemistry.

[12] Linder, M. C et al (1998) American Journal of Clinical Nutrition, vol 67, No 5 (9655-9715) and references therein.

embryotoxic effects than those observed in the two-generation study.
However, taking into account that any observations of malformations would still result in the same classification as proposed based on the foetal/pup mortality observed, it is not considered ethically or scientifically justified to require further testing in rats and rabbits.
The remaining uncertainty is thus whether a study in rabbits would have indicated a lower NOAEL for the increased pup mortality observed. This needs to be considered in risk assessments but is of less relevance for classification.
There were no studies in the dossier addressing transfer of silver to milk.

It is noted that weight changes of sex organs occurred in both generations with both silver zinc zeolite and with a different silver containing active substance. There was no clear pattern as organ weights could be increased in the first generation but decreased in the second. The changes may be the result of technical difficulties during the dissection process however since an increased frequency of pituitary adenomas and endometrial polyps (an effect disregarded by the Technical Meeting for biocides) was observed in the combined chronic/carcinogenicity study in rats and a longer pre-coital interval was observed in F1 (two-generation study with silver containing active substance 2), endocrine effects cannot be safely excluded.

4.10.5 Comparison with criteria

The information available that specifically address the reproductive toxicity of silver zinc zeolite is limited to a two-generation study in rats. No developmental toxicity study is available for this substance.
However, there is no requirement, neither in section 1.1.6 (what data are needed for classification" nor in section 3.7 (reproductive toxicity) of the document "Guidance on the Application of the CLP Criteria", hereafter referred to as CLP guidance, on a certain number of studies for a decision on classification to be taken.
The CLP guidance is rather flexible regarding what data are needed for classification; .."This information can include experimental data generated in tests for physical hazards, toxicological and ecotoxicological tests, historical human data such as accident records or epidemiological studies, or information generated in in vitro tests, (Quantitative) Structure Activity Relationships ((Q)SAR), "read across", or category approaches...Testing on animals must be avoided wherever possible and alternative methods (including in vitro testing, the use of (Q)SARs, read-across and/or category approaches) must always be considered first provided they provide adequate reliability and quality of data."
Considering that the plausible mechanism of toxicity presented is a gradual decrease of active ceruloplasmin from blood, it can be questioned if the standard developmental toxicity studies would have been able to detect silver toxicity (see 3.9.4) unless treatment was continuous during the entire gestation period. Therefore, the two-generation study with exposure during pre-mating, gestation and lactation seems to be a more appropriate study to detect this type of toxicity in the foetus/pup.
The effects observed in the study with silver zinc zeolite, i.e. increased foetal/pup mortality, decreased pup bodyweights/delayed sexual maturation and reduced thymus weights are severe and have been assessed according to the Guidance on the Application of the CLP Criteria, in particular with respect to maternal toxicity:

The CLP guidance states in section 3.7.2.4.2 that "Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal

lethality, significant post-natal functional deficiencies."
The CLP guidance further states that "All types of reproductive toxic effects may be considered as secondary to parental toxicity. With current knowledge it is not possible to identify specific effects indicating toxicity in parental animals which do not have any relevance to reproductive toxicity (e.g. peroxisome proliferation). However parental toxicity that is less than marked should not influence the classification for reproductive toxicity independent of the specific parental effects observed." The criteria for category 1B also indicate that there is a difference between effects being a secondary non-specific consequence of other toxic effects and hence effects being a specific consequence.
The CLP guidance lists some endpoints important for the assessment of maternal influence on developmental effects and these are discussed below:
Mortality: The mortality rate in the study was remarkable, however, for parents it was more or less restricted to the P males of the high dose group (10%) and F1 males of the 6250 ppm group (23%). According to the CLP guidance, maternal mortality greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation. The mortality rate in P0 females was 0% and the rate in F1 females of the 6250 ppm group was not higher than observed in P0 controls (3.3% or 1/30) thus the data cannot be dismissed based on maternal mortality. Considering the higher frequency of histopathological changes in kidneys and the urinary tract, it may be speculated that anatomical and/or biochemical differences make the males more sensitive to the substance and ultimately results in organ failure and death. The mortality in F1 (pre-mating) was considerable (28/30 males and 23/30 females died) indicating a higher sensitivity of the F1 generation compared to the P generation.

Bodyweight/bodyweight change: The bodyweight of P dams in the 12500 group was reduced by 6% on day 20 of gestation and the bodyweight gain was reduced by 16% and 29% during days 0-20 and 14-20 of gestation respectively. The adjusted mean maternal bodyweight change was not calculated but considering that the mean bodyweights of males and females pups were 15% lower compared to controls day 0 and that the number of pups born/litter was 15% lower than controls, the reduced bodyweight gain may have been an intrauterine rather than a maternal effect. This can also be illustrated by roughly adjusting the mean maternal body weight for foetal weights[13]. The results indicate that the terminal bodyweights of high dose dams were actually higher in high dose dams compared to control dams when the total litter weight was subtracted. Therefore, the reduced bodyweight gain observed during gestation in 12500 ppm dams seems due to effects on foetal weight rather than maternal weight. The reduced body weight gain is thus not considered to indicate severe maternal toxicity.
Moreover, reduced bodyweights and subsequent delayed day of vaginal opening and preputial separation was observed in F1 offspring of 6250 ppm P0 females who did not differ significantly from controls with respect to mortality, bodyweight and bodyweight gain during gestation (see table 27).
There were no statistically significant changes of bodyweights/bodyweight gains in F1 6250 (the highest dose in the F1 generation) dams during gestation yet a statistically significant increase/decrease in stillborn and livebirth index (5.4/93.1% compared to 1.1/98.3% in F2 control pups) was observed also in the offspring of this generation (F2 pups). This is a further indication that effects in pups were not due to bodyweight changes during gestation.
According to OECD guidance document on mammalian reproductive toxicity testing and assessment (number 43), a feed restriction study clearly showed that severe weight loss or decrease

[13] Calculated as the (terminal body weight -total pup weights (number x mean bw). The adjusted final weight of control dams (weight day 0: 269.3g) was 215.1g and the corresponding weight in 12500 ppm dams was 223.2g (weight day 0: 265.7g)).

in body weight gain per see induced minor changes in skeleton development but no effects on viability or malformations in the rat (Fleeman, 2005).
Clinical evaluations: None of the clinical signs of maternal intoxication listed in the CLP guidance (i.e. coma, prostration, hyperactivity, loss of righting reflex, ataxia or laboured breathing) were observed among P or F1 dams during the gestation or lactation periods. Haematological parameters were only analysed in the P females and showed some effects in 12500 and 6250 ppm dams (see table 25). Mild extramedullary haematopoiesis was observed in a single high dose P dam but there were no such observation made among the F1 6250 dams.
A reduced food intake was observed in high dose females compared to controls during lactation. However, there were no abnormalities detected in any of the high dose dams during the clinical observations made during lactation. Considering that many of the dams lost some of their pups during the first days, the reduced food intake could solely illustrate the food demand being lower due to less lactating pups.
The effects seen in pups (i.e. reduced number of pups, reduced livebirth/increased stillborn index, reduced bodyweight gain, reduced pup survival indices, clinical signs (pale), histopathological changes in kidneys, heart, liver and reduced thymus) can thus not be considered being due to maternal neglect.
Post-mortem data: Histopathological changes of kidneys and urinary tract were observed in all treated animals. The effects appear to be more severe in males based on higher incidences/severity of chronic interstitial nephritis, calculi and hydronephrosis. The frequencies were higher in F1 animals compared to P animals thus effects appear to increase over generations. A reduced weight of thymus or thymus atrophy was observed in both adult animals and pups. This was also observed in the two-generation study with silver containing active substance 2.
The effects on foetal/pup survival or reduced pup weights (and delayed sexual maturation) observed in offspring is not considered to be explained by any of the post-mortem observations made in the dams.

Overall, the above assessment of maternal effects is not considered to unequivocally demonstrate that the developmental effects are secondary to unspecific maternal toxicity.
The plausible mechanism for silver reproductive toxicity, (i.e. silver interfering with copper binding to ceruloplasmin and thereby reducing the availability of copper, iron or perhaps both metals to the foetus) is supported by the copper analysis of F2 pups in the silver zinc zeolite study. This information support that effects in pups are caused by a specific mechanism rather than being secondary to a non-specific toxicity in the mother. The copper status in the dams has not been investigated but based on the effects observed, pups seems to be more sensitive than dams.
The classification criteria for category 1B in CLP guidance reads *"The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."*
The data on silver zinc zeolite is considered to provide clear evidence of an adverse effect on development, i.e. mortality. The effects on pup survival do not seem due to a "secondary non-specific toxicity in the mother" but rather to a specific mechanism that involves inhibition of copper binding to ceruloplasmin and consequently a reduced availability of copper, iron or both metals in the foetus/pup. Since ceruloplasmin has the same function in humans, the mechanism cannot be considered irrelevant for humans.

4.10.6 Conclusions on classification and labelling

The results of the two-generation study are considered as sufficient stand-alone data for classification with respect to developmental toxicity. However, the results are also supported by other data indicating similar effects, to varying extent (i.e. the two-generation study with silver containing active substance 2, a developmental toxicity study with silver acetate in rats and a published study with silver chloride, the latter clearly demonstrating developmental toxicity in rats). The severity of effects seems to depend on the dose of available silver and possibly zinc ions. The mortality, the reduced pup weights and the reduced thymus weight observed cannot be explained by any unspecific effects in the mother.
Consequently, based on the data above and the considerations in the CLP guidance, silver zinc zeolite is proposed to be classified for developmental toxicity as Repr. 1B, H360D (May damage the unborn child).
The applicant disagrees with the proposal. The confidential appendix 4 to this document contains a statement in which the RMS has included a response to the arguments put forward by the applicant.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed to classify SZZ as toxic to reproduction, category 1B for developmental toxicity, mainly on the basis of a single 2-generation reproductive toxicity study with AgION Type AK which was administered to SD rats through the maturation, mating, gestation and lactation periods for two successive generations. The study was conducted in compliance with OECD TG 416. Prenatal developmental toxicity studies have not been conducted with SZZ.

According to the DS, the effects relevant for classification as Repr. 1B (H360D) were primarily based on foetal/pup mortality, reduced pup weights and reduced thymus weight, that were not considered secondary non-specific consequences of marked toxicity in the dams. Effects were primarily noted in F_1 high dose pups (12500 ppm) and F_2 mid dose pups (6250 ppm). The mortality rate in P males of the high dose group (10%) and F_1 males of the 6250 ppm group (23%) was notable. However, the mortality rate in P_0 females was 0% and the rate in F_1 females of the 6250 ppm group was not higher than that observed in P_0 controls (3.3% or 1/30), thus the data could not be dismissed based on maternal mortality. Considering the higher frequency of histopathological changes in the kidneys and the urinary tract, the DS speculated that anatomical and/or biochemical differences make the males more sensitive to the substance and ultimately result in organ failure and death. The mortality in F_1 (pre-mating) was considerable (28/30 males and 23/30 females died), indicating a higher sensitivity of the F_1 generation compared to the P generation. According to the DS, the reduced bodyweight gain observed during gestation in 12500 ppm dams seemed due to effects on foetal weight rather than maternal weight. The reduced body weight gain was thus not considered to indicate severe maternal toxicity. Similarly, the DS concluded that the relevant effects seen in pups (i.e. reduced number of pups, reduced livebirth/increased stillborn index, reduced bodyweight gain, reduced pup survival indices, clinical signs (pale), histopathological changes in kidneys, heart, liver and reduced thymus weight) were not considered to be due to maternal neglect.

Other parameters that were affected in the 2-generation reproductive toxicity study with AgION Type AK included increased stillbirth index, reduced liveborn index and increased frequency of hydronephrosis. The DS also reported delayed day of vaginal opening and preputial separation observed in F_1 offspring of 6250 ppm P_0 females, but this group did not differ significantly from controls with respect to mortality, bodyweight or bodyweight

gain during gestation.

The DS also indicated additional effects in a two generation study performed with a different silver containing active substance (reduced number born (11%, F_1)), reduced live litter size day 1 (F_2), reduced thymus weight) but these effects contradict other studies with silver containing active substances. The DS also noted that weight changes of sex organs occurred in both generations with both SZZ type AK and with a different silver containing active substance.

The DS also considered that the proposed mechanism (silver ions displacing copper ions from ceruloplasmin, causing adverse effects on the foetus due to reduced copper bioavailability) plausible but also relevant for humans. The DS noted that silver and perhaps zinc displacing copper in ceruloplasmin and thus causing a copper deficiency in pups is plausible. However, the DS argued that it is not known whether this is the only mechanism for the developmental toxicity of SZZ. Apart from fairly crude measurements of F_2 pup homogenates, there were no data on the levels of copper, silver, zinc or iron in parental animals or pups. Therefore, according to the DS, it is not possible to assess if there is also a copper deficiency in the parents and/or if the copper deficiency is more pronounced in the pups. Nevertheless, since dams showed no treatment-related clinical signs whereas pups clearly failed to survive, the sensitivity of pups indeed seems much higher. Likewise, if effects in the pups are due to silver and/or zinc also causing an iron deficiency in the dams, pups obviously cope less well with this deficiency. Therefore, the DS proposed that this intrinsic property of the substance should be communicated to the user by classification and labelling.

Consequently, based on the data summarised above and in the absence of a prenatal developmental toxicity study, the DS proposed SZZ to be classified as Repr. 1B (H360D, May damage the unborn child) under CLP criteria.

Comments received during public consultation

Several comments from Industry opposed the proposed classification as category 1B (H360D) and submitted several reports. These reports covered mechanism of action of maternal toxicity and studies with a silver copper zeolite (not included among those considered in this proposal). A summary of these reports are provided below.

1 Differences with the DS in the interpretation of results

Industry considered that the adverse effects on development were due to maternal toxicity because the F_0 showed a reduced weight gain in late gestation at the high dose level and an abnormal pattern of weight gain during lactation at the mid and high dose levels. Additionally, three high dose and one mid dose males died during the study. Industry argued that the increased mortality of high dose pups was not surprising in view of the maternal toxicity observed at an even lower dose and should be considered secondary to maternal effects: the effects on pup weight were also considered secondary to maternal findings.

In addition, the occurrence of stillborn F_2 pups did correlate with low body weight gains during gestation (indicating maternal toxicity) or low body weight gain during the pre-pairing period in five cases. Therefore, these observations were also not considered to be specific reproductive effects of the test compound. All effects in the offspring were observed at dose levels causing significant parental toxicity.

2 Two generation study with silver-copper zeolite

In this study, 4 groups of rats were treated with silver copper zeolite at dietary

concentrations of 1000, 5000 and 20000 ppm. Animals of both generations were treated for approximately 10 weeks prior to pairing, then throughout mating, gestation and lactation. F_1 and F_2 pups were weaned from their mother at 21 days of age. This study followed the current US EPA guideline for this type of study.

Among F_0 parents, key findings included:

i. Mean body weight performance and food consumption were not significantly different from controls in all groups, but slightly lower weights in mid and high dose males had become apparent by the time of sacrifice.
ii. At necropsy, darkening (pigmentation) of pancreas and lymph nodes was observed in mid and high dose animals.
iii. Histopathology findings were limited to pigment in the pancreas and mesenteric lymph nodes in the mid and high dose groups.
iv. The absolute, but not relative, weights of the seminal vesicles and spleen in the mid and high dose groups were lower than control, probably reflecting slightly lower mean body weights. For high dose males, mean absolute, but not relative, thymus weights were slightly lower.
v. Mating performance, including semen values and oocyte counts, was similar in all groups.

Among F_1 offspring, key findings were:

i. Mean litter sizes and mortality were similar in all groups.
ii. Mean high dose pup and litter weights were lower than in controls on Day 21 of lactation; values at lower doses were similar to controls.
iii. Reflexes, sex ratios and the times of vaginal opening and preputial separation were similar in all groups.
iv. Mean thymus weight was lower in high dose males and females. At the mid dose, the findings were statistically significant in males, but the author considered the values to be within the normal range for the laboratory.

Among F_1 parents, key findings were:

i. High dose males were slightly lighter at weaning, and gained less weight after weaning. High dose females were also lighter, but gains were similar to controls, although absolute weights remained slightly lower at pairing. High dose females gained less weight during gestation, especially in the first week: during lactation, body weights were *ca* 30 g less than in controls, but body weight gains were similar.
ii. Food consumption was slightly lower for high dose males and pre-mating females; consumption was also lower in gestation, especially over gestation days 0-14, and in the first week of lactation. Food consumption at lower doses was similar to that in controls.
iii. During the pre-mating period, the test substance intake was approximately 33% higher in F1 animals compared with F0 animals.
iv. At necropsy, darkening (pigmentation) of pancreas and lymph nodes was observed in mid and high dose animals.
v. Histopathology findings were limited to pigment in the pancreas and mesenteric lymph nodes in the mid and high dose groups.
vi. High dose uterus weight was lower than in controls. To some extent this represented the absence of high dose females with physiological dilatation of the uterus; this would be expected to occur in 0-2 females per group and these females have a higher than typical uterus weight; this finding was considered by Industry to be incidental.
vii. Mating performance, including semen values and oocyte counts, was generally

similar in all groups, although the number of high dose females with a pre-coital period longer than 5 days was slightly higher than expected.

Among F_2 offspring, key findings were:

i. The number of implantation sites and therefore of F2 pups born to high dose females was slightly lower than control, possibly reflecting the slightly lighter body weights of the parent females. Pup mortality was similar in all groups.
ii. Mean high dose pup and litter weights were lower to controls on Day 21 of lactation; values at lower doses were essentially similar to controls.
iii. Mean thymus weight was lower in high dose males and females. At the mid dose, the findings were statistically significant in females, but author considers values to be within the normal range for the laboratory.

In terms of effects on sexual function and fertility, there were no adverse effects at any dose level tested.

3 Mechanistic considerations

There was evidence in the literature (Shavlovski *et al*., 1995) that silver toxicity is associated with depletion of copper levels, and that this toxicity can be reduced by the administration of ceruloplasmin (CP). Shavlovski *et al*. (1995) reported that it is not the depletion of CP but rather the absence of copper from CP that has reduced its oxidase activity. Indeed, CP was still present in the blood but lacked its oxidase activity. The effects of low copper levels were improved by injection of CP, but this commercial CP preparation unfortunately contained copper. It was therefore concluded that it is the supplementation with copper bound to CP that reduced the effect of silver toxicity.

Thus Shavlovski *et al*. (1995) have shown that the developmental effects of silver toxicity were secondary to the depletion of copper from CP. Keen *et al.* (1998) have also presented evidence that low copper intake was associated with developmental effects. This was originally noted as enzootic ataxia (swayback) in lambs; Keen *et al*. (1998) also have noted, among other findings, brain defects and severe connective tissue abnormalities.

Zatulovskiy (2012) has shown that when mice were treated with silver chloride in the diet, at a concentration in the feed intended to achieve 50 mg/kg/day, there was a marked depletion in the serum copper concentration. This would be consistent with the findings of Shavlovski *et al*. (1995) that the toxicity of silver chloride is related to depletion of copper and/or CP. Also, Hirasawa *et al.* (1994) have indicated that administration of silver lowers the serum copper levels.

Regardless of the exact copper levels, it is apparent (Zatulovskiy, 2012; Shavlovski *et al*., 1995) that the developmental toxicity of SZZ was attributable to depletion of serum copper concentration. When Shavlovski *et al*. (1995) used a shorter dosing period (Days 7-15 of gestation) there were no developmental effects (in contrast to the findings with dosing over Days 1-20). This absence of effect with the shorter dosing period was probably associated with a lesser degree of lowering of maternal concentration of copper in the serum, based on the findings in mice. Thus it was argued that the developmental toxicity of silver ions can be attributed to lower serum copper.

For SZZ, the toxicity included the effect of the zinc content as well as the silver content. The effect of zinc was studied by Khan *et al*. (2007) in a 2-generation study with zinc chloride. As is standard for a published paper (as opposed to a formal, GLP-compliant report) there were no individual animal data and limited summary tables. However, the reproductive effects were confined to reduced fertility, pup survival and pup weight at 30

mg/kg/day; the NOAEL for reproductive effects was 15 mg/kg/day. These findings were similar to those observed with SCAS's. The level of 15 mg/kg/day equivalent to approximately 7 mg/kg/day of zinc ions.

The effect of silver and/or zinc administration on serum levels of copper were studied by Hirasawa *et al.* (1994). They administered silver (as silver nitrate) at 9.3 µmol/kg/day and/or zinc (as zinc sulphate) at 46.5 µmol/kg/day for 6 days by intraperitoneal injection. These doses equate to approximately 1 mg/kg/day and 3 mg/kg/day of silver and zinc ions, respectively. Administration of silver alone caused a reduction of the serum copper level and the CP oxidase level. When zinc was administered alone, there was an elevation, both of serum copper and of CP oxidase level. However, when both metals were administered, there was a reduction of both serum copper level and the CP oxidase level. This suggests that zinc may not offer a protection from the copper-lowering effect of silver, although it did not produce an exacerbation of the effects of silver. However, although Hirasawa *et al.* (1994) noted that zinc administration produced a slight increase in serum copper levels, this is in contrast to other researchers (Reinstein *et al*., 1984) who cited papers that indicated a decrease in serum copper after zinc administration. Other papers (including Keen *et al*., 1984) indicated that copper and zinc can affect the homeostasis of the other ion.

It has been noted in all GLP studies that developmental toxicity of silver containing materials always occurred in the presence of maternal toxicity. It was also considered relevant that studies in which plasma levels of copper were established (mainly research papers) that silver ions led to lower serum copper levels. It was also noted that the degree of lowering of copper levels in serum increased with increasing duration of dosing, and that with the shorter dosing period, Shavlovski *et al.* (1995) did not observe developmental toxicity.

It is thus plausible that developmental toxicity of SZZ is a secondary consequence of lower serum copper levels. Therefore they concluded that it is only a developmental hazard when a silver containing compound such as SZZ lowers serum copper.

Human patients with hereditary hypoceruloplasminaemia had serum CP concentrations that are 50% of the normal value. These patients did not display clinical abnormalities. This suggests that at least 50% of Cp can be inhibited, destroyed or otherwise rendered inactive without adverse outcomes in humans. In human pregnancy, serum Cp concentration increases three- to four- fold. This suggests that significant levels of Ag^+ are needed to achieve copper displacement in Cp to an extent that would cause toxic effects, especially during pregnancy.

The DS responded to all these considerations but maintained their position that classification as Repr. 1B (H360D) is warranted for SZZ.

In addition to the Industry comments one MS supported the proposed classification, while other disagreed.

Other MSs also commented that discussion in the RAC Plenary was needed on the appropriate classification.

Assessment and comparison with the classification criteria

The table below provides an overview of the reproductive toxicity -related findings and the pathological findings in all generations.

Table 15 (RAC): Overview of reproductive findings in the two-generation study with silver zinc zeolite. n.s.s = non statistically significant.

Group	Mortality	Body-weight	Body-weight gain	Sexual maturation
CONTROL				
P males P females	0% 3.3% (1/30)	- -	- -	-
F1 (m) p F1 (f) p	0% 0%	- -	- -	35.1 44.5
F1 pups	Total pups born/litter: 14.2 Liveborn/litter: 14.1 Stillborn/litter: 0.1 Live birth index: 99.2% Stillborn index: 0.8% Pup survival indices: 0-4: 98.9% 4*-21:100% 4*-26:100%	- -	n.d	-
F2 pups	Total pups born/litter: 13.1 Liveborn/litter: 12.9 Stillborn/litter:0.2 Live birth index:98.3% Stillborn index: 1.1% Pup survival indices: 0-4: 95%, 4:21:99.5%	- -	n.d	-
1000 ppm				
P males P females	0% 0%	Pre (end): n.s.s M/F Gestation: n.s.s Lactation: n.s.s	Pre (1-11): ↓6% (n.s.s in females) Gestation: 14-20:n.s.s 0-20: n.s.s Lactation: n.s.s	-
F1 (m) p F1 (f) p	3.3% (1/30) 0%	Pre (start/end): n.s.s in m/f Gestation: not stat sign Lactation: ↓7% (day 4 only)	Pre (1-12): n.s.s Gestation: 14-20:n.s.s 0-20: n.s.s Lactation: n.s.s (see text)	n.s.s
F1 pups	Total pups born/litter: n.s.s (13.2, ↓7%) Liveborn/litter: n.s.s (↓9%) Stillborn/litter: n.s.s (0.3, ↑300%) Live birth index: n.s.s (97.6%) Stillborn index: n.s.s (2.0%) Pup survival indices: n.s.s (day 0-4: 98.8%)	m+f Day 0, 4: pre/post culling, 7, 14, 21, 26: n.s.s	Not determined	-
F2 pups	Total pups born/litter: n.s.s (11.3, ↓14%) Liveborn/litter: n.s.s (10.9, ↓16%)	m+f Day 0, 4: pre/post culling, 7, 14,	Not determined	-

	Stillborn/litter: n.s.s (0.3, ↑150%) Live birth index: n.s.s (96%) Stillborn index: n.s.s (2.6%) Pup survival indices: n.s.s (day 0-4. 83.4%)	21, 26: n.s.s		
6250 ppm				
P males P females	3.3% (1/30) 0%	Premating (end): ↓7% ↓19-9% on single occasions week 1-6 in females Gestation: not stat sign Lactation: ↓7% (day 14 only)	Pre (1-11): ↓12% n.s.s in females Gestation: 14-20:n.s.s 0-20: n.s.s Lactation: No consistent pattern	-
F1 (m) p F1 (f) p	23.3% (7/30) 3.3% (1/30)	Premating (start): ↓25% (m) ↓19% (f) Premating (end, week 12): ↓13% (m) n.s.s in females* Gestation: not stat sign Lactation: ≤10%	Pre (1-12): n.s.s Gestation: n.s.s Lactation: (↓65% day 4)	Day 39.8 Day 47.4 (F1 Control: 35.1/44.5)
*bw statistically significantly reduced weeks 1-6 only.				
F1 pups	Total pups born/litter: n.s.s (13.1, ↓8%) Liveborn/litter: n.s.s (12.8, ↓9%) Stillborn/litter: n.s.s (0.4, ↑400%) Live birth index: n.s.s (97.4%) Stillborn index: n.s.s (2.6%) Pup survival indices: n.s.s (day 0-4: 96%)	M+f Day 0: n.s.s Day 4 (pre/post culling): n.s.s Day 7: n.s.s Day 14: ↓13 Day21: ↓25 Day 26: ↓29	Not determined	-
F2 pups	Total pups born/litter: n.s.s (13, ↓1%) Liveborn/litter: n.s.s (12.2, 5%) Stillborn/litter: n.s.s (0.7, ↑350%) Live birth index:93.1 % Stillborn index: 5.4 % Pup survival indices: n.s.s (day 0-4. 93.2%)	M+f Day 0: ↓5 Day 4 (pre/post culling): ↓12 Day 7: ↓15 Day 14: ↓18 Day 21: ↓20 Day 26: n.d	Not determined	-
12500 ppm				
P males P females	10% (3/30) 0%	Premating (end): ↓11% n.s.s in females Gestation: ↓6% (only sign day 20) Lactation: ≤ 11%	Premating (1-11): ↓17% n.s.s in females* Gestation: 14-20:↓29% 0-20: ↓16%**	

			Lactation: No consistent pattern**	
*stat sign increase certain weeks **see text for a discussion on adjusted maternal weight				
F1 (p)m F1 (p) f	93.3% (28/30) 76.7% (23/30)	Premating (start): ↓55% (m) ↓45% (f) Premating (end): ↓56% (m) ↓44% (f) Gestation: n.s.s	Premating (1-12): ↓47% (m) ↓40% (f) Gestation:n.s.s	Day 59.9 Day 56.7 (F1 Control: 35.1/44.5)
F1 pups (P dams)	Total pups born/litter: 12.1(↓15%) Liveborn/litter: 10.3 (↓27%) Stillborn/litter: 1.5 (↑750%) Live birth index:85.5% Stillborn index: 12.2% Pup survival indices: 0-4: 53.1% 4*-21:n.s.s 4*-26:n.s.s	M+f Day 0: ↓15 Day 4 (pre/post culling): ↓19 Day 7: ↓23 Day 14:↓26 Day21: ↓36 Day 26:↓47	Not determined	-
F2 pups (F1 dams)	No data F1 terminated prior to mating	No data F1 terminated prior to mating	No data F1 terminated prior to mating	- F1 terminated prior to mating

Note: n.s.s, no statistically significant; n.d., not disponible.

The gestation period was slightly increased (22.3 days compared to 21.9 days in controls) in treated animals and the change was statistically significant for the mid and high dose groups. Adverse effects on reproduction were manifested in high dose animals as reduced mean number of live and total pups at birth, reduced live birth index, increased number of stillborn pups and increased stillborn index. Complete pup mortality was observed in six females of the high dose group. Since the number of corpora lutea was not recorded in the animals, it is not possible to establish if the reduced total number of pups born were due to pre or post-implantation losses.

Fertility

There were no statistically significant or clearly dose-related effects on the fertility parameters. It is noted however that the percentage of abnormal sperm was higher in treated animals compared to controls but the significance of this finding is unclear. **The DS did not propose or conclude on classification for fertility**.

Development

A dose-related delay in vaginal opening and preputial separation was observed in all treated animals and the delay was statistically significant in the mid and high dose groups.

There were no treatment related histopathological findings in the stillborn pups or in day 4 culled pups. Changes in the kidney (pale, dilation, cyst) liver (pale) were observed at day 26 in males and females administered 6250 or 12500 ppm. Moreover, cardiac enlargement was observed in both sexes of high and mid dose animals; mildly enlarged

heart in 6/14 males and 6/18 females in the 12500 group and 5/27 males and 4/26 females in the 6250 group (compared to 0 in controls). Small thymus was observed in 2/14 high dose males and 2/18 females.

The number of live pups/litter was decreased in the low dose group at day 4, 14 and 21 due to the complete loss of pups in two litters but there was no effect in the 6250 ppm animals. Pup body weights were lower in 6250 ppm pups than in controls at birth and were further reduced throughout the pre-weaning period.

Organ weight analysis showed reduced absolute/relative thymus and brain weights in males and females administered 6250 ppm (see table below). The macroscopic examinations of F_2 pups at day 21 (weaning) revealed mild to moderate decreased size of thymus, mild cardiac enlargement, mild renal pallor, mild hepatic pallor and mild pulmonary pallor in animals of the 6250 ppm group.

Table 16 (RAC): Pathological findings in several generations

	DOSE (ppm)			
	12500	6250	1000	0
Incidences of hydronephrosis				
F_0	8m, 2f	7m, 2f	2m,1f	3m
F_1	Terminated	10m, 4f	3m, 1f	-
Reduced thymus weight (% lower than controls)				
F_0	not weighed; no histopathological findings			
F_1 pups	(m/f) abs 74/70%, rel bw 53/47% rel brain 69/64%	(m/f) abs 58/55% rel bw 39/39% rel brain 53/51%	m, abs 13%, m/f rel bw 10/9% m, rel brain 11%	-
F_1 adults	not weighed thymus atrophy noted in males/females	not weighed	not weighed	not weighed
F_2 pups	Not available due to termination of F_1	(m/f) abs 50/54%, rel bw 37/42%, rel brain 47/50%	m, rel bw 11%	-

It was proposed as an explanation for the foetal toxicity of silver ions that they can displace copper ions in ceruloplasmin which transports copper to the foetus. Ceruloplasmin is the main copper transporter in the blood and it seems to play a role in the cellular uptake of iron. The concentration is usually elevated during pregnancy and ceruloplasmin and copper are present in the amniotic fluid and in milk. Analysis of copper, silver and zinc in homogenates of three whole pups from control, 1000 and 6250 ppm groups showed a general decrease of copper in the treated groups whereas the levels of silver and zinc were generally increased, which suggest that effects observed in pups are due to a deficiency of copper, iron or both (see table below).

Table 17 (RAC): Zinc, silver and copper levels (mg/kg bw) of F_2 Day 4 culled pups

	Control		1000 ppm		6250 ppm	
	males	females	males	females	males	females
Silver	<1	<1	1.04	1.06	1.68	2.2
	<1	<1	1.06	<1	1.1	<1
	<1	<1	<1	<1	1.07	1.84
Zinc	7.77	10	8.87	8.05	8.65	10.4
	6.44	6.31	11.8	6.88	7.32	7.56
	8.01	7.62	5.57	5.63	8.8/5	11.9
Copper	2.24	2.18	1.97	1.67	<1.5	1.86
	2.07	2.49	2.19	1.61	<1.5	<1.5
	2.15	2.72	1.61	1.76	1.96	1.52

Industry has presented several reports arguing that the reported developmental effects

were indeed secondary to maternal effects related to reduction in copper bioavailability due to displacement of copper bound to ceruloplasmine.

Comparison with the criteria

The CLH report is succinct in the description of effects of SZZ on fertility paratmeters. However, RAC agrees with the evaluation of the DS that there were no statistically significant or clearly dose-related effects on the fertility outcomes.

Regarding adverse effects on or via lactation, results from the two generation studies in animals provided no clear evidence of adverse effects in the offspring due to transfer of the substance in the milk or adverse effect on the quality of the milk. Absorption, metabolism, distribution and excretion studies do not indicate the likelihood that SZZ is present in potentially toxic levels in breast milk. Therefore, RAC concludes that no classification for adverse effects effects on or via lactation is warranted.

Regarding adverse effects on development, the classification of a substance as known human reproductive toxicant in Category 1A is largely based on evidence from humans. As there is no epidemiological evidence regarding the developmental toxicity of SZZ in humans, a classification in Category 1A is not appropriate.

RAC is of the opinion that the two-generation study with SZZ provided evidence of adverse effects. These effects included increased mortality parameters in F_1 pups at 12500 ppm and 6250 ppm. In addition, reduced pup weight/pup weight gain, significant dose-related small/reduced weight of the thymus and increased frequency of hydronephrosis, primarily in treated males and females (with no such finding in female controls) are considered by RAC to be non-relevant for classification because it is considered that such effects appeared as a consequence of the repeated dose on pups and therefore should be addressed as general systemic toxicity in pups rather than as developmental toxicity. This opinion is supported by the observations that nephrotoxicity and reduction in thymus weight were also reported in 90-day repeated dose and combined chronic-carcinogenicity studies. Macroscopic findings included enlarged hearts in F_1 and F_2 pups. Cardiac changes were observed in both sexes of high and mid dose F_2 pups; mildly enlarged heart in 6/14 males and 6/18 females in 12500 group and 5/27 males and 4/26 females in 6250 group (compared to 0 in controls).

The decreased growth rate of pups was accompanied by developmental delays (time of preputial separation and vaginal opening). A dose-related delay in the day of vaginal opening and preputial separation was observed in all treated animals and the delay was significant in the mid and high dose group. Since the bodyweights were comparable between treated females and controls on the day of vaginal opening, the delay seems related to the reduced bodyweights. The bodyweights of 6250 and 12500 ppm males were reduced by 12,5 and 38% respectively at the time of preputial separation.

According to the CLP guidance, several factors need to be assessed for determining if adverse effects on reproduction have to be considered (or not) as secondary of maternal toxicity. Significant and biologically relevant adverse affects are reviewed below:

Mortality: The mortality rate in the study was remarkable. However, for parents it was more or less restricted to the P males of the high dose group (10%) and F_1 males of the 6250 ppm group (23%). According to the CLP guidance, maternal mortality greater than 10% is considered excessive and the data for that dose level shall normally not be considered for further evaluation. The mortality rate in P_0 females was 0% and the rate in F_1 females of the 6250 ppm group was not higher than observed in P_0 controls (3.3% or 1/30).

Bodyweight/bodyweight change: The bodyweight of P dams in the 12500 ppm group was reduced by 6% on day 20 of gestation and the bodyweight gain was reduced by 16% and 29% during days 0-20 and 14-20 of gestation, respectively. However, adjusting the mean maternal body weight for foetal weights (calculated as the terminal body weight - total pup weights (number x mean bw)) as the terminal bodyweights were higher in high dose dams compared to control dams when the total litter weight was subtracted. Therefore, the reduced bodyweight gain observed during gestation in 12500 ppm dams might be due to effects on foetal weight rather than maternal weight.

Clinical evaluations: None of the clinical signs of maternal intoxication listed in the CLP guidance (i.e. coma, prostration, hyperactivity, loss of righting reflex, ataxia or laboured breathing) were observed among P or F_1 dams during the gestation or lactation periods.

Post-mortem data: Histopathological changes of kidneys and urinary tract were observed in all treated animals. The effects appeared to be more severe in males based on higher incidences/severity of chronic interstitial nephritis, calculi and hydronephrosis. The frequencies were higher in F_1 animals compared to P animals, thus the effects appeared to increase over the generations. A reduced weight of thymus or thymus atrophy was observed in both adult animals and pups.

A specific mechanism that involves inhibition of copper binding to ceruloplasmin and consequently a reduced availability of copper, iron or both metals in the foetus/pup is considered plausible by RAC. Since ceruloplasmin has the same function in humans as in rodents, the mechanism is considered relevant for humans. RAC concludes that the mechanistic information does not raise doubts about the relevance of the effect for humans.

RAC considers the toxicity at the highest concentration too high to be used for establishing classification in Categoty 1B. However, the enlargement of hearts reported at the mid dose in F_2 pups (5/27 males and 4/26 females) appeared in the presence of mild maternal toxicity (mainly hydronephrosis and haematological alterations) and cannot be totally disregarded for classification. This same effect also appeared in F_1 pups at the highest dose (6/14 males and 6/18 females), albeit in the presence of excesive maternal toxicity. RAC considers these effects on the heart as relevant for classification of SZZ as toxic to development Cat 2.

In conclusion, **RAC considers that SZZ meets the criteria to be classified as Repr. 2; H361d (Suspected of damaging the unborn child) but that classification for fertility is not warranted.**

4.11 Other effects

4.11.1 Non-human information

4.11.1.1 Neurotoxicity

There are no robust studies on neurotoxicity available, neither for silver zinc zeolite nor for any other silver containing active substances in the data base. Information on potential neurotoxicity is restricted to published case reports (see section 4.12.1.4) and published research.

Rats: clinical signs indicating effects on behaviour/activity (hypersensitivity to touch, vocalization, increased activity and aggressive behaviour) were noted in the 90 day study among rats administered 6250 ppm (278/366 mg/kg bw) or a higher dose of AgION Antimicrobial Type AK. However, there were no statistically significant differences observed in the neurobehavioral, FOB or motor activity evaluations performed except for increased touch response in high dose animals and a few minor statistically significant effects in the neurological examinations performed.
Dogs: Head shaking were noted in dogs administered 250 mg/kg bw Antimicrobial Type AK for 90 days but it is not clear if this should be regarded as a true neurological effect or if this effect and the vomiting observed rather is a symptom of a general discomfort of treatment.

In the developmental toxicity study with silver containing active substance 1, there were incidences of sedation in 37% of high dose rats whereas there were no effects in controls. In some of these animals, effects such as thinness, watery feces or urogenital discharge were also observed thus it is not clear if this effect should be considered as a true neurotoxic effect or as a secondary effect.
In the two-generation study performed with silver containing active substance 2, the reflexological response to stimuli (surface righting reflex, mid-air righting reflex, startle reflex, pupillary reflex) was examined and no treatment related effects were noted. However, since learning and memory tests were not included, it is not safe to exclude that deposition of silver ions in nervous tissues could adversely affect the nervous system in fetuses/children during development.
Overall, there are no clear indications that silver zinc zeolite is a neurotoxic substance. However, considering that data on neurotoxicity is rather poor, in particular with respect to learning and memory, effects on the nervous system cannot be excluded based on the information available.

4.11.1.2 Immunotoxicity

No data available.

4.11.1.3 Specific investigations: other studies

The dossier contains two different studies to address the data requirement on mechanistic studies. The aim of the first study was to give a better understanding of the effects of copper and silver on bacteria and viruses at the molecular level. While this study provides some information regarding the mode of action, the relevance of this information for an understanding of the effects observed in toxicological studies is considered to be low. The second study is an in vitro experiment performed to determine the role of thiol modification in silver-induced toxicity of freshly isolated hepatocytes. The authors demonstrated that a time and concentration dependent cell damage occurred along with a decrease in intracellular soluble thiols and lipid peroxidation in hepatocytes isolated from male Wistar rats exposed to silver nitrate and silver lactate. Since treatment with radical scavengers delayed but did not protect from cytotoxicity, silver cytotoxicity does not seem to be mediated by lipid peroxidation. The thiol reducing agent dithiothritol had protective effects whereas the glutathione depleting agent diethylmaleate potentiated silver toxicity. Based on these findings, silver was considered to cause toxic effects in rat heptocytes by disturbing the cellular thiol homeostasis. A reduced thiol pool could reduce the ability to cope with oxidative stress.
Nevertheless, none of these studies were performed with silver zinc zeolite and they do not address the major adverse effects (i.e., pigmentation of organs, histopathological changes in the kidneys, reducedthymus, anemia, late foetal deaths, reduced live birth index and pup survival) observed in the toxicological studies with silver zinc zeolite:

4.11.1.4 Human information

There are no case reports on silver zinc zeolite included in the dossier. However, there are several case reports describing effects in humans exposed to other silver substances. Since at least some of the effects observed (e.g. argyria, neurotoxicity, kidney effects etc) are observed also in animal studies with silver zinc zeolite, there is reason to assume that effects could be linked to the silver ion and thus be of relevance for silver zinc zeolite.

According to a pesticide re-registration document for silver prepared by US EPA (1992), excessive industrial and/or medicinal exposures to silver have been associated with arteriosclerosis and lesions of the lungs and kidneys. Exposure to industrial dusts containing high levels of silver nitrate and/or silver oxide may cause breathing problems, lung and throat infections and abdominal pain. Skin contact with certain silver compounds may cause mild allergic reactions such as rash, swelling and inflammation in sensitive people (6.12.2(02)).
A document on silver prepared by US EPA Integrated Risk Information System (IRIS) (6.12.2(03) refers to a publication by Gaul and Staud (1935) reporting 70 cases of generalized argyria following organic and colloidal silver medication, including 13 cases of generalized argyria receiving intravenous silver arsphenamine injection therapy. The authors concluded that argyria may become clinically apparent after a total accumulated i.v. dose of approximately 8 g of silver arsphenamine. The document states that the authors of a book entitled "Argyria, The Pharmacology of Silver" reached the conclusion that a total accumulative i.v. dose of 8 g silver arsphenamine is the limit beyond which argyria may develop (Hill and Pillsbury, 1939). However, since the body accumulates silver throughout life, they considered it theoretically possible that amounts less than this (for example, 4 g silver arsphenamine) can result in argyria. Therefore, based on cases presented in the study, the lowest i.v. dose resulting in argyria in one patient, 1 g metallic silver (calculated as 4 g silver arsphenamine x 0.23 (the fraction of silver in silver arsphenamine)) was considered to be a minimal effect level.
Another reference included is Blumberg and Carey (1934) who reported argyria in an emaciated chronically ill (more than 15 years) 33-year-old female (32.7 kg) who had ingested capsules containing 16 mg silver nitrate three times a day over a period of 1 year (about 30 mg silver/day) for alternate periods of 2 weeks. The authors noted that this marked argyremia was striking because even in cases of documented argyria, blood silver levels are not generally elevated to the extent observed (0.5 mg/L). Normal levels for argyremic patients were reported to range from not detected to 0.005 mg Ag/l blood. Heavy traces of silver in the skin, moderate amounts in the urine and feces, and trace amounts in the saliva were reported in samples tested 3 months after ingestion of the capsules was stopped. However, despite the marked argyremia and detection of silver in the skin, the argyria at 3 months was quite mild. No obvious dark pigmentation was seen other than gingival lines which are considered to be characteristic of the first signs of argyria. The authors suggested that this may have been the case because the woman was not exposed to strong light during the period of silver treatment. The US EPA concludes that this study is not suitable to serve as the basis for a quantitative risk assessment of silver because it is a clinical report for a single patient with compromised health. Furthermore, the actual amount of silver ingested is based on the patient's recollection and cannot be accurately determined.
The last case referred to in the IRIS document was reported by East et al. (1980) and is also presented in 6.12.2(04). The article describes argyria diagnosed in a 47-year previously healthy woman (58.6 kg) who had taken excessively large oral doses of anti-smoking lozenges containing silver acetate over a period of 2.5 years. No information was provided as to the actual amount of silver ingested. Symptoms of argyria appeared after the first 6 months of exposure. Based on whole body neutron activation analysis, the total body burden of silver in this female was estimated to be 6.4 (plus or minus 2) g. Both the total body burden and concentration of silver in the skin were estimated to be 8000 times higher than normal. In a separate 30-week experiment, the same subject

retained 18% of a single dose of orally-administered silver, a retention level much higher than that reported by other investigators. East et al. (1980) cited other studies on this particular anti-smoking formulation (on the market since 1973) which demonstrated that "within the limits of experimental error, no silver is retained after oral administration." The authors conclude that this may not hold true for excessive intakes like that ingested by this individual.
The US EPA concludes that the study is not suitable to serve as the basis for a quantitative risk assessment.
The article presented in 6.12.2(05) describes a case with clinical signs such as taste and smell disorders, vertigo and hypaesthesia in a patient that used a stick of silver nitrate (containing 0.53 g AgNO3) daily over a nine year period in order to treat the oral mucosa. The authors concluded that the affinity of silver for membrane and neuronal structures and the deposition of insoluble silver following extended high exposure on a daily basis had induced progression of the clinical condition of this patient.
Two other case reports describing neurotoxic effects are discussed in the document submitted for 6.8.1(07). According to this document, Sudmann (1994) has reported a case where a patient with silver-impregnated bone cement developed serious neurological deficits five years after implantation. Two years after removal of the bone cement, the patient partially recovered from grave muscle paralysis.
The second case report states that convulsive seizures occurred in a woman ingesting 20 mg daily for 40 years. These seizures abated when silver intake was stopped. With only limited information available, it is difficult to assess the relevance of these case reports for the endpoint neurotoxicity.
A different case report described blue-gray discoloration of skin in a 58 year old man who had treated himself with a colloidal silver solution that was made at home using a 38000Volt generator, 100% pure silver coins and distilled water (6.12.2(06)). The man drank 8 fluid ounces (~2.4 dl) every hour from 8 AM to 8 PM for four days without any intake of any other food or beverages. Four weeks after self-treatment, a bluish appearance to the oral mucosa that progressed to involve the face, trunk and extremities appeared. Examination of the patient revealed a diffuse blue-grey coloration of the skin which was most pronounced in the sun-exposed areas of forearm, hands, face, neck and the “V” of the chest. Discoloration was also noted in the lunulae, sclera, and conjunctivae of the eyes and spotty blue macules were evident on the oral mucosa of the soft palate. Histopathological examinations of biopsies from the forearm revealed fine, minute, round, brown/black granules deposited primarily in the basement membrane around the eccrine glands and to a lesser extent in the fibrous sheath of the pilo-sebaceous units, pilo-erector muscles, dermal elastic fibres and arteriolar walls.
The increased discoloration in the sun exposed tract is explained by the combined effect of sun-induced reduction of colorless silver compounds to elemental silver and an increased melanin production due to silver stimulated melanocyte tyrosinase activity.
A case of fatal renal and hepatic failure is described in 6.12.2(07). The article describes the course of disease in a patient that underwent silver nitrate instillation in the renal pelvis for treatment of chyluria. Since the instillation was completed at a separate hospital, the authors could not confirm the dose administered to this patient. Within 24 hours of dosing the patient developed severe renal and hepatic failure despite given N-acetyl cysteine in view of acute toxic hepatitis and placed on haemodialysis for renal failure. The case was further complicated by development of epistaxis that required post-operative ventilation support. Although the patients’ general condition and liver function tests improved by the type of dialys used, the patient died from cardiorespiratory arrest (probably caused by pulmonary embolism or aspiration pneumonia) approximately 48 hours after extubation and beginning oral feeding.
A summary of the toxicity of silver has been prepared for the Oak Ridge Reservation Environmental Restoration Program and this document has been submitted for several sections of the dossier. It is stated in the document that besides cases of localised or generalised forms of

argyria, accidental or intentional ingestion of large doses of silver nitrate caused corrosive damage to the gastrointestinal tract, abdominal pain, diarrhea, vomiting, shock, convulsions and death. The estimated fatal dose of silver nitrate is ≥ 10g, but recoveries have been reported following ingestion of larger doses. Acute irritation of the respiratory tract can occur from inhalation of silver nitrate dust, but generally only at concentrations that produce argyria. One case report described severe respiratory effects in a worker who had become ill 14 hours after working with molten silver ingots. In a study referred to (Rosenman 1979), 30 workers were exposed to silver nitrate and silver oxide dusts for periods of less than one year to greater than ten years. Twenty five individuals experienced respiratory irritation (sneezing, stuffiness, running nose or sore throat) at some time during their employment. Twenty of thirty workers reported coughing, wheezing, chest tightness and abdominal pain; the latter finding was closely correlated with blood silver levels. Granular silver-containing deposits, observed in the conjunctiva and cornea of 20/30 workers, correlated with duration of employment. Some of the workers reported decreased night vision. The eight hour time weighted average exposure (determined 4 months prior to the study) was in the range 0.039 to 0.378 mg silver/m3 for this subpopulation. Decreased night vision was also reported in a group of workers manufacturing metal silver powder (Rosenman et al. 1987). Increased excretion of the renal enzyme N-acetyl-β-D-glucosaminidase and decreased creatinine clearance seen in these workers may indicate an impaired kidney function however since the same workers were exposed to cadmium which is a known nephrotoxin, the effect cannot with certainty be ascribed to silver.
Chronic exposure to silver for reclamation workers exposed to silver and insoluble silver compounds, revealed conjunctival and corneal argyria in 21 and 25% of the workers respectively. Many also exhibited internal nasal-septal pigmentation. Examination of liver enzyme levels for silver-exposed and non-exposed workers revealed no significant differences.
Ocular damage has been reported from application of solutions containing >2% silver nitrate. Corneal opacification may be so severe as to cause blindness. Application of silver nitrate to gingival may result in necrotizing ulcerative gingivitis. The document further states that case histories indicate that dermal exposure to silver or silver compounds for extended periods can lead to generalised skin discoloration and that mild allergic responses attributed to dermal contact with silver or silver compounds have been reported (6.12.2(08)).

A risk benefit assessment of silver products for medical indications was performed by the US Food and Drug Administration (6.12.5(01)). It is stated in the article that burn treatment with silver nitrate can cause methemoglobinemia, hydrochloridemia, hyponatremia and eschars that adhere to dressings. Silver suladiazine used to replace silver nitrate in this type of treatment may cause leucopenia and nephrotic syndrome rarely. It also states that there is a potential risk for the developing fetus when pregnant women use silver products. The results of a case-control epidemiology study suggested (after adjustment for confounding factors) some association between maternal exposures to 0.001 mg/L of silver in drinking water and some increase in fetal developmental anomalies (ear, face and neck). However, the authors of the epidemiologic study recognized that there are inferential limitations to epidemiologic studies and that further research is needed to explore these findings.

The authors of the risk-benefit assessment concluded that the lack of established effectiveness and potential toxicity of these products should be emphasized. The risk was considered to exceed the unsubstantiated benefit for over the counter silver-containing products.
Argyria is a permanent discoloration of skin and so far, antidote treatment (such as depigmentation creams, hydroquinone, dermal abrasion or chelation therapy with British antilewisite or D-penicillamnine) appears to be without effect (6.12.2(06).

4.11.2 Summary and discussion

Clinical signs of neurotoxicity were observed in some animal studies with silver zinc zeolite and have been reported in humans exposed to different silver substances. However, the animals exhibiting such symptoms also suffered from other effects thus it is difficult to conclude that symptoms do result from primary effects on the nervous system rather than from general toxicity. Likewise, it is not possible to conclude on the neurotoxic potential of silver zinc zeolite based on human case reports since these are poorly described and usually involve high exposure levels. There were no data available indicating that other effects in studies with silver zinc zeolite (pigmentation, kidney effects, developmental effects etc) result from species specific mechanisms. It must therefore be assumed that similar effects could occur also in humans.

4.11.3 Comparison with criteria

Not relevant

4.11.4 Conclusions on classification and labelling

Not relevant

RAC evaluation of aspiration toxicity
Summary of the Dossier Submitter's proposal The DS proposes no classification on the basis of the absence of reliable information in humans and the total absence of information in animals. **Comments received during public consultation** No comments were received during public consultation on this hazard class. **Assessment and comparison with the classification criteria** **RAC agrees with DS and do not support classification for aspiration toxicity** because with the available information it is not possible to assess if SZZ would meet the criteria for classification.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Silver zinc zeolite is an inorganic substance containing the metals silver and zinc, and as such it could be seen as falling into the category of metals and metal compounds, for which a specific classification scheme is available for environmental hazards according to the CLP guidance chapter IV.5. However, the results from a T/D protocol in order to classify the substance according to the CLP strategy are not available. The zeolite part will remain insoluble, whereas metal ions are released more or less, highly depending on the composition of the medium. Silver zinc zeolite could be regarded as poorly or readily soluble, depending on the availability of counter ions for ion exchange or organic matter, which will capture released metal ions (see chapter 5.1.3).

5.1 Degradation

Silver zinc zeolite is a purely inorganic complex and as stated in CLP Annex I section 4.1.2.10.1, for metals and inorganic compounds, the concept of degradability as applied to organic compounds has limited or no meaning.

However, silver zinc zeolite will dissociate in the water compartment to release its constituents in form of silver-ions, zinc-ions and other non-hazardous positively charged ions and the insoluble aluminium silicate (zeolite) complex. The rate of dissolution of silver and zinc is dependent on the conditions (pH, temperature, ion-strength, redox potential and organic matter content) of the receiving water and also on the loading of the silver zinc zeolite to the water. Available data on silver and zinc release from silver zinc zeolite under environmentally relevant conditions are summarized in Table 29 below.

The environmentally relevant silver and zinc ions will then undergo speciation in the water compartment. A lot of information is available from the public domain on the speciation of silver and zinc in the water compartment but this is not considered relevant for the environmental hazard assessment (see chapter 5.4).

Table 29: Summary of relevant information on degradation

Method	Results	Remarks	Reference
OPPTS 860.7840 (flask method testing up to 37 days)	Silver and zinc dissolution (filtered solutions) of AgION Antimicrobial Type AJ at a loading of 10 mg/mL in distilled water (pH adjusted with nitric acid or sodium hydroxide): Silver (max conc., AAS) pH 5: 9.2 mg/l (after 29 days) pH 7: 2.9 mg/l (after 11 days) pH 9: 0.2 mg/l (after 35 days) Zinc (max conc., AAS) pH 5: 467 mg/l (after 37 days) pH 7: 51 mg/l (after 37 days) pH 9: 0.5 mg/l (after 17 days)	The dissolution is higher at lower pH due to the ion-exchange with H^+. The zinc dissolution is higher than that for silver	Doc IIIA 3.5-01 Bussey, (2001)
OECD 105 (flask method, 3 days)	Silver dissolution (filtered solutions) of Irgaguard B8000 at a loading of 2 mg/mL in buffered solutions: Silver (max conc., AAS) pH 5 (phthalate buffer): 23.9 mg/l pH 7 (phosphate buffer): 0.02 mg/l pH 9 (borate buffer): 0.17 mg/l		Doc IIIA 3.5-01 Meinerling and Herrmann, (2007)
In-house method (silver release)	AgION Antimicrobial Type AJ and Irgaguard B502i (loading corresponding to a max release of 50 mg/L silver). Silver determination by ICP-OES (filtered solutions). Testing in acidic (nominal pH 6; poor buffering) and alkaline (nominal pH 9, poor buffering) hard synthetic water (100 mg/L $CaSO_4$ and $MgSO_4$) AgION Antimicrobial Type AK Silver (% of theoretical max)	Due to the poor buffering capacity of the solutions there was no significant difference of the measured pH of the pH 6 and pH 9 solutions (it could be seen as one data set) The silver release is consistently low for both materials under the conditions used.	B3.5-04 (in Document III-A) O'Connor and Woolley, (2010)

Method	Results	Remarks	Reference
	Nominal pH 6, 20°C: 1.60% (3 h) 1.67% (6 h) 1.84% (12 h) 1.86% (18 h) 1.92% (24 h) 1.96% (72 h) 2.29% (168 h) Nominal pH 9, 20°C: 1.48% (3 h) 1.50% (6 h) 1.56% (12 h) 1.65% (18 h) 1.69% (24 h) 1.87% (72 h) 2.11% (168 h) <u>Irgaguard B502i</u> Silver (% of theoretical max) Nominal pH 6, 20°C: <0.02% (3 h) 0.04% (6 h) 0.03% (12 h) 0.05% (18 h) 0.04% (24 h) 0.06% (72 h) 0.13% (168 h) Nominal pH 9, 20°C: 0.03% (3 h) 0.03% (6 h) 0.01% (12 h) 0.02% (18 h) 0.07% (24 h) 0.06% (72 h) 0.12% (168 h)	The data is complemented with that presented for physiological conditions (phosphate buffer, 37°C, pH 4 and pH 8) in Table 9 (phys.chem). Even though not directly relevant to environmental conditions it indicates a significantly higher silver release at low pH (max ~40% at pH 4; max 20% at pH 8). This means that for environmental conditions a higher silver release may occur for acidic conditions than shown by the data presented here. Under environmental conditions and in biological test media, the release of silver ions will be increased by the presence of organic matter, to which the silver ions will adsorb (see chapter 5.2.1.)	

5.1.1 Stability

Hydrolysis

Silver zinc zeolite is an inorganic compound that dissociates in water as outlined in Section 5.1 above, but it does not have any chemical bonds prone to hydrolysis. Hence, hydrolysis is not considered a relevant pathway.

Photolysis

There is no quantitative data available for the effects of photolysis processes on silver zinc zeolite in water. However, photo-reduction and photo-oxidation may affect the rate at which silver and zinc-ions (and other non-hazardous ions) are released from the complex and the speciation of these ions in the water compartment. However, photolysis processes are not considered relevant for the environmental hazard assessment of silver zinc zeolite as such.

5.1.2 Biodegradation

Silver zinc zeolite is an inorganic substance. According to CLP Annex I section 4.1.2.10.1, for metals and inorganic compounds, the concept of degradability as applied to organic compounds has limited or no meaning. Methods for the determination of biodegradability are not applicable to inorganic substances. Therefore biodegradation is not considered a relevant pathway.

5.1.3 Summary and discussion of degradation

Silver zinc zeolite is an inorganic substance that will undergo dissociation in the water compartment to release its constituents in form of silver-ions, zinc-ions and other non-hazardous positively charged ions and the insoluble aluminium silicate (zeolite) complex. The rate of dissolution of silver and zinc is dependent on the conditions (pH, temperature, ion-strength, redox potential and organic matter content) of the receiving water and also on the loading of the silver zinc zeolite to the water. The environmentally relevant silver- and zinc-ions will undergo further speciation in the water compartment but as elements they will not degrade. Photolysis processes may affect the rate of release of silver and zinc in water and the subsequent speciation of these elements in the water compartment but these processes are not considered relevant for the environmental hazard assessment of silver zinc zeolite as such.

As silver zinc zeolite is an inorganic compound, the term biodegradation has no meaning.

In conclusion, since the environmentally relevant constituents of silver zinc zeolite (i.e. silver and zinc-ions) are elements and cannot degrade, silver zinc zeolite must be considered not readily or rapidly degradable.

5.1.4 Adsorption/Desorption

No data are available for the adsorption of silver zinc zeolite as such. As stated in Section 5.1 above, silver zinc zeolite will dissociate into its constituents in water to release the environmentally relevant silver- and zinc-ions. A lot of information is available from the public domain on the adsorption/desorption of silver and zinc. Data on partition coefficients of silver and zinc used in the Competent Authority Report (CAR) for silver zinc zeolite under the Biocide directive and the European Union Risk Assessment Report (RAR) on zinc metal respectively indicate that these elements readily adsorb to particulate matter (see table 30).

Table 30: Summary of partition coefficients for silver and zinc used in EU risk assessments

Element	Compartment	Mean K_d (L/kg) used in risk assessment	Reference
Silver	Soil/soil water	398.11	Doc IIIA A7.1.2.3-01 Allison and Allison, (2005)
	Suspended matter/water	158500	
Zinc	Suspended matter/water	110000	EU RAR on zinc JRC (2010)
	Sediment/water	73000	

5.1.5 Volatilisation

Silver zinc zeolite is an inorganic compound with a high melting point (>350°C) and volatilisation is therefore not relevant.

5.2 Environmental distribution

No data are available for silver zinc zeolite as such. The main route to the water compartment for the usage envisaged is via STP. As outlined in 5.1 silver zinc zeolite will dissociate into its constituents in water to release the environmentally relevant silver- and zinc-ions. As indicated by the partition coefficients in Section 5.2.1 above, silver and zinc readily adsorb to particulate matter and the efficiencies of the STPs for removing silver and zinc from the water fraction through precipitation are thus very high.

In the Competent Authority Report (CAR) for silver zinc zeolite under the Biocide directive, data from the public domain (Doc III-A7.1.2.2.1-06, Shafer *et al*, 1998) indicates removal coefficients of 94-99% for silver with a mean of 97.5%. For the risk assessment in the CAR for silver zinc zeolite a removal coefficient of 91%, based on Swedish monitoring data, was used for silver as a worst case input for the predicted environmental concentrations in the receiving water compartment.

In the European Union Risk Assessment Report (RAR) on zinc metal (JRC, 2010) removal coefficients for the STPs in the range of 90-99.9% are reported.

5.3 Aquatic Bioaccumulation

Bioconcentration: For the inorganic compound silver zinc zeolite, the BCF concept is not applicable. Furthermore, it is unlikely that this insoluble high molecular weight compound is passing biological membranes. However, silver or zinc may be released into the water and taken up by organisms through ion transport channels.

Bioaccumulation: It is possible that suspended particles of the compound are taken up via ingestion, especially by particle filtrating organisms. In the gastrointestinal tract, the compound itself is likely not passing into the body, but counter ions such as zinc or silver ions may be released and enter the organisms. Especially in the case of particle-reactive silver ions, the major route of uptake is via ingestion of silver associated to organic particles.

Standard tests such as OECD 305 are not applicable for metals or other inorganics compounds. A large body of published literature exists concerning bioaccumulation of metals. Uptake is species specific and controlled by physiological mechanisms. Therefore, a generalised conclusion about the

bioaccumulation potential of silver is not possible. Some groups like algae or small crustaceans can accumulate silver to very high levels.

As far as it can be generalised, it appears that fish have physiological mechanisms to keep silver levels low, whereas in invertebrates storage of silver in metabolically unavailable forms is a means of detoxification. Therefore, even if taken up, there is no general correlation between the body burden and the toxicity of silver.

In the course of the risk evaluation under Biocide directive and –regulation literature surveys were carried out for bioaccumulation and biomagnification of silver, which are attached to this CLP report instead of studies or test reports.

Annex I: Literature reviews regarding aquatic bioaccumulation and –magnification of silver

a) Review prepared by RMS Sweden under the Review Programme of the Biocide Directive 98/8/EC, Draft May 2012

b) Position paper submitted by the applicant for silver zinc zeolite (European Silver Task Force via TSGE) under the Review Programme of the Biocide Directive 98/8/EC, Draft May 2012

5.4 Aquatic toxicity and classification of silver zinc zeolite

Silver zinc zeolites are metal compounds, but they are not silver salts. Instead of dissolution, ions are released in aqueous media from an insoluble zeolite matrix. The ecotoxicity tests measure the toxicity of the ions released. The released amount is related to the loading rate, and hence toxicity of the entire compound.

The released amount of silver and zinc ions, which are considered to be the ecotoxicologically relevant constituents of silver zinc zeolite, depends on the composition and pH of the test medium and the presence of organic material (see chapter 5.5).

The reasonably expected uses of silver zinc zeolite are related to incorporation of the compound into coatings and polymers that are formed into solid articles. These articles are used in situations where contact with water may result in release to the drain. Therefore the likely route of entry of silver zinc zeolites into the aquatic environment is via the sewage system. Silver zinc zeolite, thus, is unlikely to enter water bodies in its original composition (i.e. silver and zinc adsorbed to zeolite). The different components will have different fates.

The zeolite part is likely to remain in the polymer matrix, whereas the counter ions to the negatively charged zeolite are released into water. Once released into the sewage system, if so in small amounts, the zeolite may adsorb other metal ions available in the sewage treatment process. Therefore, fate and ecotoxicological effect studies conducted on the original silver zinc zeolite are of low relevance. In the course of the risk evaluation under Biocide directive and -regulation the components were evaluated separately (zeolite, silver ion and zinc ion). Environmental hazards of zeolites were not addressed in detail, due to the negligible contribution from biocidal zeolites as compared to the overall release of zeolites into the aquatic environment, which is dominated by laundry detergents. Information about environmental hazard of zeolites is available in two reports prepared by the HERA project, sponsored by industry associations. The HERA report from 2004[14]

[14] HERA 2004. Human & Environmental Risk Assessment on ingredients of European household cleaning products. Zeolite A, Version 3.0, January, 2004,

and 2005[15] did not identify environmental risks from the use of zeolites in household detergents. The most sensitive aquatic toxicity endpoint is the algae (NOEC = 15.5 mg/l derived for zeolite X), which is above the solubility limit. Considering the much higher toxicity of dissolved silver, further information on the environmental hazards of zeolites is not relevant.

The environmentally relevant released ions are zinc and silver. Silver is considered the most relevant, because organisms are considerably more sensitive to dissolved silver than to dissolved zinc throughout all studied taxonomic groups (see table 32). Only environmental risk connected with dissolved silver was assessed in the CAR. For values for zinc it is referred to the RAR for zinc metal (European Union Risk Assessment Report 2010; CAS: 7440-66-6; EINECS No: 231-175-3; ZINC METAL).

Table 32: Comparison of toxicity values for silver and zinc

Taxonomic group	**Zinc** European Union Risk Assessment	**Silver** This CLH report
acute		
Fish	LC_{50} = 140 µg Zn/L *Thymallus arcticus*	LC_{50} = 2.3 µg Ag/L *Pimephales promelas*
Invertebrates	LC_{50} = 80 µg Zn/L *Ceriodaphnia dubia*	LC_{50} = 0.22 µg Ag/L *Daphnia magna*
Algea	EC_{50} = 136 µg Zn/L *Pseudokirchneriella subcapitata*	EC_{50} = 4 µg/Ag/L *Pseudokirchneriella subcapitata*
chronic		
Fish	NOEC = 44 µg Zn/L *Jordanella floridae*	NOEC = 0.02 µg Ag/L *Oncorhynchus mykiss*
Invertebrates	NOEC = 37 µg Zn/L *Ceriodaphnia dubia*	NOEC = 0.53 µg Ag/L *Ceriodaphnia dubia*
Algea	NOEC = 17 µg Zn/L *Pseudokirchneriella subcapitata*	NOEC = 0.75 µg Ag/L *Pseudokirchneriella subcapitata*

For the purpose of this classification, it is assumed that the silver ion is the ecotoxicologically most relevant ion released from the zeolite. Thus, the aquatic hazard is assessed based on results from ecotoxicity test with soluble silver salts.

5.4.1 Aquatic toxicity of silver zinc zeolite

Remark: The results from toxicity tests with the whole compound will not be used for classification. They are presented here for information only.

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http://www.heraproject.com/files/8-F-BE8D7CFF-A805-0020-23F16E4B786891E8.pdf

[15] HERA 2995. Zeolites A, P and X. Supplement to the HERA report on the Environmental Risk Assessment of Zeolite A. Edition 1.0, September 2005, http://www.heraproject.com/files/25-E-ZeoliteAPX_Sept%202005.pdf

Table 31: Summary of relevant information on aquatic toxicity of silver zinc zeolite. Only studies that are sufficiently reliable are presented here. More studies are presented in the Competent Authority report.

Method	Results	Remarks	Reference
Fish			
Brachydanio rerio US EPA Pesticide Assessment Guideline Subdivision E, 72-1 Zeolite type: IRGAGUARD 8000 (3.8% silver, 6.6%.zinc)	**96h LC_{50} = 0.5 mg/L (loading rate)**		IIIB 7.7.1.1-01 (Ciba/BASF)
Oncorhynchus mykiss Zeolite type: AK10D (4.9% silver, 13.0% zinc (w/w))	96h LC_{50} = 16.9 mg/L NOEC = 5.6 mg/L LOEC = 10 mg/L (loading rate)		IIIB 7.7.1.1-01 (Sciessent/AgION)
Crustacea: no reliable data available			
Algae			
Pseudokirchneriella subcapitata OECD 201 (1984) Zeolite type: IRGAGUARD B 8000 (3.8% silver, 6.6%.zinc)	48h EbC_{50} = 39 mg/L NOEC = 10 mg/L LOEC = 20 mg/L (loading rate)		IIIB 7.7.1.1-03 (Ciba/BASF)

5.4.1.1 Short-term toxicity to fish

CIB/BASF IIIB 7.7.1.1-01 (OECD 203 guideline study)

The applicant submitted an acute toxicity test with zebra fish using zeolite type IRGAGUARD B8000. Based on the loading rate of the substance, a 96h LC_{50} = 0.5 mg/L was determined. Silver concentrations in the test medium could not be determined with sufficient precision. The test medium contained high amounts (up to 88 µg/L) of zinc, most probably contributing to the toxicity. The zinc was apparently not released from the zeolite but present in the medium itself, because it was found in approximately equal amounts in the control and all test media.

AgION/Sciessent IIIB 7.7.1.1-01 (OECD 203 guideline study)

The applicant submitted an acute toxicity test with rainbow trout using zeolite type AK10D. Based on the loading rate of the substance, 96h LC_{50} = 16.9 mg/L, NOEC = 5.6 mg/L, LOEC = 10 mg/L.

Silver concentrations in the test medium were measured. There was no dose-response relation between dissolved silver and mortality. The highest dissolved silver level was 18 µg/L measured at the LOEC level. Zinc levels, both total and dissolved, were proportional to zeolite concentrations. The dissolved zinc concentration at the LC_{50} level was estimated to be 213 µg/L, and most probably contributing to the toxicity.

5.4.1.2 Long-term toxicity to fish

No data available

5.4.1.3 Short-term toxicity to aquatic invertebrates

No reliable data available

5.4.1.4 Long-term toxicity to aquatic invertebrates

No data available

5.4.1.5 Algae and aquatic plants

Ciba/BASF IIIB 7.7.1.1-03

The lowest 72-h E_bC_{50} for toxicity on *Desmodesmus subspicatus* was calculated to be 39 mg/L based on loading rate. The NOEC value was 10 mg/L and LOEC value was 20 mg/L.

The lowest – more relevant - 72-h E_rC_{50} for toxicity on *Desmodesmus subspicatus* cannot be determined because concentrations on the upper end of the range were not tested (i.e. not concentration showing close to 100% inhibition). The NOEC value was 20 mg/L and LOEC value was 40 mg/L.

5.4.1.6 Other aquatic organisms (including sediment)

No data available

5.5 Aquatic toxicity and classification based on dissolved silver ions

The CLP classification strategy IV.5.3.1 for readily soluble compounds proposes: “Where the acute $ERV_{compound}$ is less than or equal to 1 mg/l, consideration must be given to the data available on the rate and extent to which these ions can be generated from the metal compound. Such data, to be valid and useable should have been generated using the T/D protocol.” T/D protocol data are not available for silver zinc zeolite. With regard to released silver ions, silver zinc zeolite could be regarded as being readily soluble, which is in line with CLP guidance chapter IV.5, i.e. there are no clear data of sufficient validity to show that the transformation to metal ions will not occur.

In the release study (see chapter 1.3) up to 23% of silver was released during 168h (pH 8, 37°C, phosphate buffer). In a long-term biological test it can reasonably be assumed that a large part of the silver ions have been released during the test (Only theoretically; long-term tests with silver-zinc zeolites are not available). The release is enhanced due to the presence of organic matter in the test system. In an acute toxicity test with zebra fish using zeolite type IRGAGUARD B8000, silver in solution amounts to around 70 % of the nominal silver concentration at test start, and after 96h to around 24% (IIIB silver zinc zeolite CIBA 7.7.1.1-01).

The maximum content of silver in zeolite is 6% (see identity in chapter 1.1), which is the value used to re-calculate ecotoxicity results to silver zinc zeolite.

Table 33: Acute toxicity of silver to aquatic organisms.
Data normalised to silver and re-calculated for silver zinc zeolite (SSZ).

Species	Substance tested	Exposure time [days]	Effect	LC/EC_{50} [µgAg/L]	Recalculated value [mg/L]	Reference
Fish **Oncorhynchus mykiss**	**$AgNO_3$**	**96h**	**Mortality**	**3.5 dissolved**	**0.058**	**IIIA 7.4.1.1-01 Nebeker 1983**
Fish *Pimephales promelas*	**$AgNO_3$**	**96h**	**Mortality**	**2.3 dissolved**	**0.038**	**IIIA 7.4.1.1-01 Nebeker 1983**
Fish *Pimephales promelas*	**$AgNO_3$**	**96h**	**Mortality**	**2.3 dissolved**	**0.038**	**IIIA 7.4.1.1-02 Erickson 1998**
Crustacea ***Daphnia magna***	**$AgNO_3$**	**48h**	**Mortality**	**0.22 dissolved**	**0.0037**	**IIIA 7.4.1.2-03 Bianchini et al. 2002**
Crustacea ***Daphnia magna***	**$AgNO_3$**	**48h**	**Mortality**	**0.25 dissolved**	**0.0042**	**IIIA 7.4.1.2-01, Nebeker 1983**
Crustacea ***Daphnia magna***	**$AgNO_3$**	**48h**	**Mortality**	**0.52 dissolved**	**0.0087**	**IIIA 7.4.1.2-02, Erickson 1998**
Algae ***Pseudokirchneriella subcapitata***	**AgCl on titanium dioxide**	**72h**	**Growth rate**	**4.0 total**	**0.067**	**IIIA 7.4.1.3-02**

Table 34: Chronic toxicity of silver to aquatic organisms.
Data normalised to silver and re-calculated for silver zinc zeolite (SSZ).

Species	**Substance tested**	Exposure time **[days]**	Effect	NOEC [µgAg/L]	Recalculated value **[mg/L]**	Reference
Fish ***Oncorhynchus mykiss*** ***Embryo and larvae***	**$AgNO_3$**	**60d**	**Mortality**	**0.15 dissolved**	**0.00225**	**IIIA 7.4.3.2-01 Nebeker 1983**
Fish ***Oncorhynchus mykiss*** **Embryo and larvae**	**$AgNO_3$**	**60d**	**Growth**	**0.02 dissolved**	**0.00033**	**IIIA 7.4.3.2-01 Nebeker 1983**
Crustacea ***Daphnia magna***	**$AgNO_3$**	**21d**	**Survival and reproduction**	**0.7 dissolved**	**0.012**	**IIIA 7.4.3.4 Nebeker 1983**
Crustacea ***Daphnia magna***	**$AgNO_3$**	**10d**	**Survival and reproduction**	**0.8 dissolved**	**0.013**	**Rodgers et al. 1997**
Crustacea ***Ceriodaphnia dubia***	**$AgNO_3$**	**10d**	**Survival and reproduction**	**0.53 dissolved**	**0.0088**	**Rodgers et al. 1997**
Crustacea ***Hyalella azteca***	**$AgNO_3$**	**10d**	**Survival and reproduction**	**4.0 dissolved**	**0.067**	**Rodgers et al. 1997**
Insecta ***Chironomus tentans***	**$AgNO_3$**	**10d**	**Survival and reproduction**	**125 dissolved**	**2.08**	**Rodgers et al. 1997**
Algae ***Pseudokirchneriella subcapitata***	**AgCl on titanium dioxide (Ag 15%)**	**72h**	**Growth rate**	**0.75 total**	**0.0125**	**IIIA 7.4.1.3-02**

5.5.1.1 Short-term toxicity to fish

IIIA 7.4.1.1-01 Nebeker 1983 (Published peer-reviewed research)

A series of acute toxicity studies, both flow-through and static were conducted using silver nitrate employing fathead minnow and steelhead and rainbow trout as test organisms. Toxicity results are based on the measured total silver concentrations of the test media. Dissolved silver concentration (<0.45mm) has been determined at least once in the mentioned study. The article states that 59% of the silver was lost after well water was filtered. Using this information, a NOEC of 0.058 Ag/L can be estimated for fathead minnow, and a NOEC of 0.038 µg/L can be estimated for steelhead /rainbow trout (re-calculated to silver zinc zeolite).

IIIA 7.4.1.1-02 Erickson 1998 (Published peer-reviewed research)

A series of studies conducted with fathead minnows investigated the effect of manipulating water hardness, pH and alkalinity, and organic carbon. The effects of adding sodium sulfate and sodium chloride were determined. Finally, the effect of ageing the test media and use of natural versus laboratory test media were investigated. The acute toxicity of silver to juvenile fathead minnows was substantially reduced by increasing hardness with the addition of calcium and magnesium

sulfate, and by increasing dissolved organic carbon with the addition of humic acid. Toxicity was also inversely related with pH and alkalinity when these were jointly altered by the addition of a strong base or acid. Silver was much less toxic in natural river water (106 µg total Ag/L) compared to laboratory water (10.4 µg total Ag/L), probably due to the higher organic carbon content of the river water. The LC_{50}s for flow-through exposure with fathead minnows were approximately two-fold lower than for static exposure with fresh test solutions, but not significantly lower than for static exposure with aged solutions. The lowest endpoint was obtained from the unfed flow-through study and was a 96-hour LC_{50} of 2.3 µg dissolved Ag/L (recalculated to 0.038 µg/L silver zinc zeolite). The decrease in LC_{50} for aged test solutions was unexpected according to the authors of the study, since ageing was supposed to result in formation of complexes with low bioavailability. This finding is not further discussed by the authors. It could indicate that complexed silver may, indeed, be bioavailable, and/or ionic and complexed silver have different toxicologically relevant targets within the organism.

5.5.1.2 Long-term toxicity to fish

IIIA 7.4.3.2-01; Nebeker 1983 (Published peer-reviewed research)

A 60-day embryo-larval study was conducted with steelhead trout. For the endpoint of growth (fish length) the LOEC value is 0.1µg Ag/L. At LOEC the length reduction is 12% and the curve does not show clear dose-response relation. According to TGD 3.2.2. table 16, NOEC can be calculated from LOEC. If LOEC >10% and <20% effect, NOEC can be calculated as LOEC/2. That makes in this case a NOEC of 0.05 µg Ag/L (total silver). Toxicity results are based on the measured total silver concentrations of the test media, leading to an underestimation of silver toxicity. Dissolved silver concentration (<0.45mm) has been determined at least once in the mentioned study. The article states that 59% of the silver was lost after well water was filtered. Using this information, RMS has estimated a NOEC of 0.02 µg/L for inhibition of growth, based on dissolved silver concentrations, or 0.00033 mg/L re-calculated to silver zinc zeolite.

Remark: During the peer review under the Biocides Directive, the reliability of this study was questioned. At the technical meeting in June 2013 it was agreed that this study is sufficiently reliable, if supported by further published studies that are available. In a RIVM report summarises most of, if not all, the available literature ("Environmental risk limits for silver: A proposal for water quality standards in accordance with the Water Framework Directive"[16]) further ecotoxicological studies have been evaluated, and should be used for this purpose. The applicant is currently conducting this update of the available literature, which means that the numerical value of the NOEC might change. However, this change is unlikely to influence the classification proposal, except for the M-factor.

5.5.1.3 Short-term toxicity to aquatic invertebrates

IIIA 7.4.1.2-03 Bianchini et al. 2002 (Published peer-reviewed research)

The aim of the study was to investigate the influence of sulfide (as ZnS) on the toxicity of silver to Daphnia magna. LC_{50} for sulfide-free exposure was 0.22 µg/L, recalculated to silver zinc zeolite 0.0037 mg/L. Mortality to *Daphnia magna* was reduced in the presence of sulfide only when results

[16] http://www.rivm.nl/bibliotheek/rapporten/601714023.pdf.

are based on total measured silver concentrations. This might include particulate silver and it cannot be excluded that the daphnids took up silver by ingestion of particles. However, when measured filtered silver was considered, the toxicity curves were virtually identical, indicating that the dissolved fraction was the source of available silver.

IIIA 7.4.1.2-01, Nebeker 1983 (Published peer-reviewed research)

Several acute tests were conducted with *Daphnia magna*. One test was carried out with addition of food and the remaining three without food. Toxicity results are based on measured total silver concentrations of the test media, leading to an underestimation of silver toxicity. Dissolved silver concentration (<0.45mm) has been determined at least once in the mentioned study. The article states that 59% of the silver was lost after filtration. Using this information, a LC_{50} of 0.25 dissolved µg/Ag/L can be estimated, recalculated to 0.0042 mg/L silver zinc zeolite. Addition of food decreased toxicity to LC_{50} = 12.5µg/L based on measured total silver. Considering that 89% of the silver was lost by filtration as stated, the estimated LC_{50} based on dissolved fraction is 1.4 µgAg/L.

IIIA 7.4.1.2-02, Erickson 1998 (Published peer-reviewed research)

This study concerns the effects of static versus flow-through test regimes as well as feeding and ageing of test solutions on the acute toxicity of silver (as silver nitrate) to *Daphnia magna*. The lowest 48-hour LC_{50} value for *Daphnia magna* was obtained in a static test in non-aged laboratory water without feeding and was 0.52 µg Ag/L, recalculated to 0.0087 mg/L silver zinc zeolite. In addition, the toxicity of silver in natural water was found to be much lower than in laboratory water, by a factor of 60. The major difference between the two waters is the concentration of organic matter, the organic content of the river water being more than an order of magnitude higher.

The geometric mean of the recalculated values for Daphnia (0.0037, 0.0042, 0.0087 mg SSZ/L) – used for classification for acute aquatic hazard $ERV_{compound}$ = 0.0051 mg SSZ/L.

5.5.1.4 Long-term toxicity of silver to aquatic invertebrates

IIIA 7.4.3.4, Nebeker 1983 (Published peer-reviewed research)

One published peer-reviewed scientific article has been provided by the applicant, describing three *Daphnia magna* reproduction studies conducted with silver nitrate in which water hardness was varied. Survival and reproductive success (as young/survived adult) were equally sensitive endpoints. The statement made that water hardness did not affect the survival or reproduction of *D. magna* has not been statistically verified in this study. Dissolved silver concentration (<0.45mm) has been determined at least once in the mentioned study. The article states that 59% of the silver was lost after filtration. Using this information, values can be estimated Survival and reproduction to 0.7 µgAg/L, and recalculated to silver zinc zeolite 0.012 mg/L. The study is afflicted with some short-comings like high mortality in controls as well as lacking or inconclusive information about silver concentrations, purity of the test substance.

IIIA 7.4.1.2-03 Rodgers et al. 1997 (Published peer-reviewed research, added by RMS)

Static toxicity tests over 10d were conducted with several invertebrate species of which *Ceriodaphnia dubia* was the most sensitive with the lowest NOEC 0.53 µg/Ag/L (dissolved), and recalculates to silver zinc zeolite 0.0088 mg/L. Test species and further results are presented in table 34.

5.5.1.5 Toxicity of silver to algae and aquatic plants

Ciba/BASF IIIB 7.7.1.1-03

The lowest 72-h E_bC_{50} for toxicity on *Desmodesmus subspicatus* was calculated to be 39 mg/L based on loading rate. The NOEC value was 10 mg/L and LOEC value was 20 mg/L.

The lowest – more relevant - 72-h E_rC_{50} for toxicity on *Desmodesmus subspicatus* cannot be determined because concentrations on the upper end of the range were not tested (i.e. not concentration showing close to 100% inhibition). The NOEC value was 20 mg/L and LOEC value was 40 mg/L.

5.5.1.6 Toxicity of silver to other aquatic organisms (including sediment)

Table 35: Toxicity of silver to sediment-living organism

Species	**Substance tested**	**Exposure time [days]**	**Effect**	**LC/EC$_{50}$ [µgAg/L]**	**Recalculated value [mg/L]**	**Reference**
Chironomus tentans	**$AgNO_3$**	10d	Survival and growth	BL: **152** WB: 1294 Nominal total silver	**2.53**	IIIA 7.4.3.5.1-01 Call 1999

More studies are presented in the Competent Authority report, but the study by is considered as the only sufficiently reliable, since it was carried out according to a recognised EPA guideline, sediments and test conditions are described in detail and original data are presented. A very important factor is also that the tested sediments were allowed to equilibrate prior to testing. It was confirmed by additional measurements that 22d was sufficient time to establish equilibrium between added test substance and sediment, by showing that porewater silver concentrations remained constant after 15d and beyond. The choice of silver nitrate as test compound can be considered as a worst case.

Remark: This study is a short-term study. For the purpose of refinement of the sediment risk assessment for silver, the applicant is currently conducting sediment test using OECD guidelines 218 and 225.

5.6 Comparison with criteria for environmental hazards (sections 5.1 – 5.3 and 5.5)

Approach based on available toxicity reference data (CLP guidance IV.5.3.2.1)

Physico-chemical properties: The substance is not soluble. Log Kow or BCF not applicable. The substance releases toxic silver and zinc ions.

According to IV.5.3 ”Classification strategies for metal compounds”, a metal compound will be considered as readily soluble if:

- the water solubility (measured through a 24-hour Dissolution Screening test or estimated e.g. from the solubility product) is greater or equal to the acute ERV of the dissolved metal ion concentration; or

- If such data are unavailable, i.e. there are no clear data of sufficient validity to show that the transformation to metal ions will not occur; CLP guidance chapter IV.5.

In line with the latter condition, silver zinc zeolite is considered as readily soluble for the purpose of this classification.

Degradation (evidence of rapid degradation): The substance is not degradable.

Acute aquatic toxicity: For readily soluble metal compounds the CLP classification strategy IV.5.3.1 proposes: "Classify the metal compound as Category Acute 1 if the acute $ERV_{compound} \leq 1$ mg/l. An M-factor is also established as part of this classification."

The lowest value from three studies with *Daphnia magna* (0.0037 mgSSZ/L) is used to derive the $ERV_{compound}$ = 0.0037 mg SSZ/L. which is below 1 mg/l.

Chronic aquatic toxicity: The key study IIIA 7.4.3.2-01 (Nebeker 1983) revealed the lowest chronic aquatic toxicity, being 0.00033 mg/L (recalculated). Irrespective whether there is evidence of rapid environmental transformation or not, the SSZ is classified as Category Chronic 1, since the chronic $ERV_{compound} < 0.01$ mg/l. An M-factor is also established as part of this classification (see chapter 5.6.1).

5.6.1 M-factor (CLP guidance IV.5.4)

For soluble metal compounds M-factors are applied as for organic substances (CLP Guidance Table IV.5.4.1).

Acute: $ERV_{compound}$ = 0.0037 mg SSZ/L, **M-factor = 100**

Long-term: $ERV_{compound}$ = 0.0003 mg SSZ/L, **M-factor = 100** (no rapid environmental transformation assumed)

5.7 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.3 and 5.5 – 5.6)

The approach based on available toxicity reference data (CLP guidance IV.5.3.2.1) is applied for classification

For the purpose of this classification, silver zinc zeolite is considered as readily soluble. According to CLP guidance chapter IV.5.3.1, the metal compound is classified as Category Acute 1 if the acute ERVcompound ≤ 1 mg/l. An M-factor must is established as part of this classification.

Acute $ERV_{compound}$ = 0.0037 mg SSZ/L -> short-term aquatic hazard: **Category Acute 1, M-factor: 100.**

According to CLP guidance chapter IV.5.3.2.1, the metal compound is classified as Chronic 1 if the chronic ERVcompound ≤ 0.1 mg/l and there is no evidence of rapid environmental transformation. Alternatively, the metal compound is classified as Category Chronic 1 if the chronic ERVcompound

≤ 0.01 mg/l and there is evidence of rapid environmental transformation. An M-factor must be established as part of this classification.

Chronic $ERV_{compound}$ = 0.0003 mg SSZ/L -> long-term aquatic hazard: **Category Chronic 1, M-factor: 100** (no rapid environmental transformation assumed).

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Silver zinc zeolite (SZZ) is an inorganic compound containing silver and zinc ions, and as such it falls into the category of metals and metal compounds, for which a specific classification scheme is available in the CLP Guidance.

The DS proposed to classify SZZ as Aquatic Acute 1; H400, M=100 and Aquatic Chronic 1; H410, M=100, following the classification approach in Annex IV.5 to the CLP Guidance on classification strategies for metals and metal compounds. The classification for the environment is based on the comparison of the overall levels of silver and zinc released (based on the silver zinc zeolites with the highest levels of silver and zinc) with the effect levels for silver and zinc ions. The zeolite structure is considered insoluble. The effect levels for silver and zinc ions are based on published literature and derived from various silver salts, not the specific silver zeolites. Thus read-across from one form of silver zinc zeolite to the other forms is not applied. From an environmental hazard perspective it can thus be concluded that the substances are comparable and it is thus justified to classify them as a group. SZZ is an inorganic substance and since the environmentally relevant constituents cannot degrade, the compound must be considered not readily or rapidly degradable. Furthermore, no evidence of rapid environmental transformation is assumed as remobilisation of silver ions cannot be excluded. SZZ is considered as readily soluble for the purpose of classification. The acute $ERV_{compound}$ (Ecotoxicity Reference Value) for SZZ is 0.0037 mg SZZ/L for *Daphnia magna* and the chronic $ERV_{compound}$ is 0.0003 mg SZZ/L for fish (*Onchorhynchus mykiss*).

Degradation

For a purely inorganic compound such as SZZ, the concept of degradability as applied to organic compounds has limited or no meaning. SZZ will dissociate in the water compartment to release its constituents in form of silver ions, zinc ions and other positively and negatively charged ions and the insoluble aluminium silicate (zeolite) complex. The rate of dissolution of silver and zinc is dependent on the water conditions (pH, temperature, ion-strength, redox potential and organic matter content) and also on the loading of the SZZ to the water. The environmentally relevant silver and zinc ions will undergo further speciation in water but as elements they will not degrade.

A test performed with AgION® Antimicrobial Type AJ according to OPPTS 860.7840 using nitric acid and sodium hydroxide for pH adjustment at a loading of 10 mg/mL in distilled water showed that dissolution of both silver and zinc was higher at lower pH due to the ion-exchange with H^+. The zinc dissolution was higher than that for silver. Another test using AgION® Antimicrobial Type AJ and Irgaguard B502i at a loading corresponding to a maximum release of 50 mg Ag/L was performed according to an in-house method (silver determination by ICP-OES (filtered solutions), testing in acidic (nominal pH 6, poor buffering) and alkaline (nominal pH 9, poor buffering) hard synthetic water (100 mg/L $CaSO_4$ and $MgSO_4$). The results showed that due to the poor buffering capacity of the solution there was no significant difference in the silver release between the acidic and alkaline solutions. The silver release was consistently low for both materials.

SZZ does not have any chemical bonds prone to hydrolysis. Photolysis processes are not considered relevant for environmental hazard classification.

Bioaccumulation

A bioconcentration factor (BCF) is not relevant for an inorganic compound like SZZ. It is also unlikely that an insoluble high molecular weight compound would pass biological membranes. However, ions may be taken up by organisms through ion transport channels. It is also possible that suspended particles of the compound are taken up via ingestion, especially for particle filtrating organisms. A generalised conclusion about the bioaccumulation potential of silver is not possible due to the fact that uptake of metals is species specific and controlled by physiological mechanisms.

Aquatic toxicity

In water the zeolite part of SZZ is likely to remain in its original inert form, whereas the silver and zinc ions will be released. The components (zeolite, silver ions and zinc ions) are therefore evaluated separately. Information on the environmental hazard of zeolites was collected from two HERA reports from 2004 and 2005 where the most sensitive aquatic toxicity endpoint was identified for algae with a NOEC of 15.5 mg/L which was above the solubility limit. Due to the considerably higher toxicity of silver and zinc ions than the counter ions (NO_3^-) and the zeolite matrix, further information on these was not considered relevant. Silver is considered the most relevant ion being more toxic to all studied taxonomic groups than zinc as presented in the following two tables.

Table 18 (RAC): Aquatic acute toxicity values for silver and zinc

	Zinc (European Union Risk Assessment)	**Silver (CLH Report)**
Fish	LC_{50} = 140 µg Zn/L *Thymallus arcticus*	LC_{50} = 2.3 µg Ag/L *Pimephales promelas*
Invertebrates	LC_{50} = 80 µg Zn/L *Ceriodaphnia dubia*	LC_{50} = 0.22 µg Ag/L *Daphnia magna*
Algae	EC_{50} = 136 µg Zn/L *Pseudokirchneriella subcapitata*	EC_{50} = 4 µg Ag/L *Pseudokirchneriella subcapitata*

Table 19 (RAC). Aquatic chronic toxicity values for silver and zinc

	Zinc (European Union Risk Assessment)	**Silver (CLH Report)**
Fish	NOEC = 44 µg Zn/L *Jordanella floridae*	NOEC = 0.02 µg Ag/L *Oncorhynchus mykiss*
Invertebrates	NOEC = 37 µg Zn/L *Ceriodaphnia dubia*	NOEC = 0.53 µg Ag/L *Ceriodaphnia dubia*
Algae	NOEC = 17 µg Zn/L *Pseudokirchneriella subcapitata*	NOEC = 0.75 µg Ag/L *Pseudokirchneriella subcapitata*

The toxicity values from tests with soluble silver compounds reviewed by the DS are presented in the following two tables. The maximum content of silver in SZZ is 6 % which is the percentage used to re-calculate the ecotoxicity results derived from tests with $AgNO_3$ and AgCl (on titanium dioxide) to SZZ.

Table 20 (RAC): Aquatic acute toxicity of silver to aquatic organisms and re-calculated acute ERVs for SZZ

Species	**Substance tested**	**Exposure time**	**Effect**	**LC/EC_{50} (mg Ag/L)**	**Re-calculated ERV for SZZ (mg/L)***
Fish *(Oncorhynchus mykiss)*	$AgNO_3$	96h	Mortality	0.0035 dissolved	0.058
Fish *(Pimephales promelas)*	$AgNO_3$	96h	Mortality	0.0023 dissolved	0.038
Fish *(Pimephales promelas)*	$AgNO_3$	96h	Mortality	0.0023 dissolved	0.038

Crustacea *(Daphnia magna)*	**$AgNO_3$**	**48h**	**Mortality**	**0.00022 dissolved**	**0.0037**
Crustacea *(Daphnia magna)*	$AgNO_3$	48h	Mortality	0.00025 dissolved	0.0042
Crustacea *(Daphnia magna)*	$AgNO_3$	48h	Mortality	0.00052 dissolved	0.0087
Algae *(Pseudokirchneriella subcapitata*)	AgCl on titanium dioxide	72h	Growth rate	0.004 total	0.067

* the acute ERVcompound = acute ERV of the metal ion x (molecular weight of the metal compound/atomic weight of the metal). In the case of SZZ percentages are used: acute ERVcompound = ERV of silver ion x (100/6).

Table 21 (RAC): Chronic toxicity of silver to aquatic organisms and re-calculated chronic ERVs for SZZ

Species	**Substance tested**	**Exposure time**	**Effect**	**NOEC (mg Ag/L)**	**Re-calculated value for SZZ (mg/L)***
Fish *(Oncorhynchus mykiss*, embryo and larvae*)*	$AgNO_3$	60d	Mortality	0.00015 Dissolved	0.00225
Fish *(Oncorhynchus mykiss*, embryo and larvae*)*	**$AgNO_3$**	**60d**	**Growth**	**0.00002 dissolved**	**0.00033**
Crustacea (*Daphnia magna*)	$AgNO_3$	21d	Survival and reproduction	0.0007 dissolved	0.012
Crustacea *(Daphnia magna)*	$AgNO_3$	10d	Survival and reproduction	0.0008 dissolved	0.013
Crustacea *(Ceriodaphnia dubia)*	$AgNO_3$	10d	Survival and reproduction	0.00053 dissolved	0.0088
Crustacea (*Hyalella azteca*)	$AgNO_3$	10d	Survival and reproduction	0.004 dissolved	0.067
Insecta (*Chironomus tentans*)	$AgNO_3$	10d	Survival and reproduction	0.125 dissolved	2.08
Algae *(Pseudokirchneriella subcapitata*)	AgCl on titanium dioxide (Ag 15%)	72h	Growth rate	0.00075 total	0.0125

* The Chronic ERVcompound = chronic ERV of the metal ion x (molecular weight of the metal compound/atomic weight of the metal). In the case of SZZ percentages are used: acute ERVcompound = ERV of silver ion x (100/6).

According to Annex IV.5.3 of the CLP Guidance a metal compound is considered as readily soluble if:

- the water solubility (measured through a 24-hour Dissolution Screening Test or estimated e.g. from the solubility product) is greater or equal to the acute ERV of the dissolved metal ion concentration: or

- if such data are unavailable, i.e. there are no clear data of sufficient validity to show that the transformation to metal ions will not occur.

In the case of SZZ a transformation/dissolution (T/D) test is not available. In line with the latter condition the DS considered SZZ as readily soluble for the purpose of classification. No evidence of rapid environmental transformation was assumed for silver ions and consequently for SZZ. The lowest acute aquatic toxicity data for silver from a study with *Daphnia magna* was used to derive the acute $ERV_{compound}$ = 0.0037 mg SZZ/L

which led to a proposed classification as Aquatic Acute 1, M=100. The lowest chronic aquatic toxicity value for silver from a study with *Oncorhynchus mykiss* was used to derive the $ERV_{compound}$ = 0.00033 mg SZZ/L which led to a proposed classification as Aquatic Chronic 1, M=100.

Comments received during public consultation

Two Member States Competent Authorities (MSCA) agreed to the classification proposal while two other MSCAs pointed out that there are more data available on aquatic ecotoxicity including additional species and taxonomic groups e.g. in the 2012 RIVM Report on silver and in the public REACH database for registered silver and argued that these should be assessed. The use of geometric mean according to the CLP guidance was proposed once all relevant acute and chronic endpoints are considered. In addition further consideration of possible removal mechanisms of silver in natural waters was emphasised.

Industry provided additional information on the structure and general physico-chemical properties of SZZ. An industry consortium also stressed the need to base the classification of SZZ tested following the Transformation/Dissolution protocol which, however, are not available. They also commented that SZZ could be rapidly removed directly from the water column due to the strong binding of silver ions to the inorganic and organic reduced sulphur which is present in oxic waters as demonstrated by TICKET-Unit World Model calculations. Industry considered the toxic effects of SZZ to be to a large extent caused by zinc ions which should be included in the classification by using a toxic unit approach. The use of Biotic Ligand Models (BLMs) is recommended to normalise the zinc and silver toxicity data to take account of the different abiotic conditions in the actual tests. Industry consequently proposed that classification as Aquatic Chronic 4 would be appropriate due to the missing T/D data. They also criticised the use of the $ERV_{compound}$ for the derivation of the M-factor because all toxicity is related to the silver ion.

The DS responded that the environmental hazard classification is proposed for the compound silver zinc zeolite, in line with the CLP Guidance, Annex IV.5 for metals and inorganic metal compounds. They agreed that SZZ could also be seen as a zinc compound which in their opinion would not change the proposed classification, hence using a toxic unit approach would not alter the classification and/or the M-factor. The DS further pointed out that the CLH report does not attempt to classify silver. A T/D test was not required because it was not needed in the biocide risk assessment. They agreed that silver ions react quickly with the sulfidic compound and adsorb to particulate organic matter. However, particle feeders may take up the particle bound silver by ingestion and silver sulphide can be redissolved in the stomach. The CLH report was based on studies made available by the applicant in the biocide assessment. Other studies conducted using the most sensitive species *Oncorhynchus mykiss* were reviewed during the process. If the geometric mean of these studies (0.08 µg/L) were used, the classification and the M-factor would not change.

Assessment and comparison with the classification criteria

RAC agrees to base the classification of SZZ on silver since organisms are considerably more sensitive to dissolved silver than to dissolved zinc. RAC also agrees that SZZ is considered readily soluble for classification purposes due to lack of validated water solubility data (cf. ECHA Guidance on the Application of the CLP criteria, Annex IV.5.3). Data from a T/D screening test would have been preferred. In the absence of full T/D test data the use of BLMs is not considered useful.

There are acute and chronic data available on fish, crustacea and algae and in addition on the sediment dwelling organism *Chironomus tentans*. Ecotoxicity reference values for SZZ ($ERV_{compound}$) are calculated from the lowest acute and chronic ecotoxicity reference

values (ERV) derived for silver by correcting the values for the molecular weight of the compound. In the case of SZZ the calculation is based on the 6% maximum silver content of SZZ. RAC agrees to the acute $ERV_{compound}$ of 0.0037 mg/L (EC_{50}, 48h, *Daphnia magna*) and to the chronic $ERV_{compound}$ of 0.00033 mg/L (NOEC, 60d, *Oncorhynchus mykiss*). In comparison the ERV values for SZZ calculated with 16% maximum concentration of zinc in SZZ would be an acute $ERV_{compound}$ = 0.500 mg/L and a chronic $ERV_{compound}$ = 0.106 mg/L which would lead to a classification as Acute 1 and Chronic 2 assuming that zinc is readily soluble.

RAC is of the opinion that currently there is not enough evidence of rapid environmental transformation of SZZ. Chemical speciation of silver is governed by complexation with both inorganic ligands and natural organic matter. As for all equilibrium, there is a concentration- dependent binding constant between silver and the available ligands. It is recognised that sulphide, normally present at low concentrations in natural waters, forms a strong complex with silver ions. However, depending on the levels of sulphide and silver ions present in water, other speciation reactions with varying binding constants with e.g. chloride and natural organic matter may occur (Paquin and Di Toro, 2008). Therefore it is not possible to conclude that silver ions would completely speciate to non-available forms. Also the potential for the reverse change to occur cannot be ruled out. Finally, particle feeders may ingest particle bound silver and sulfidic silver can be redissolved in the stomach.

Although SZZ is not a simple metal compound the classification strategy for metal compounds is used for classification instead of the classification strategy for metals. In Annex IV.1 of the CLP Guidance, metals and metal compounds are characterised as:

a. Metals (M^0) in their elemental state are not soluble in water but may transform to yield the available form. This means that a metal in the elemental state may react with water or dilute aqueous electrolyte to form soluble cationic or anionic products, and in the process the metal will oxidise, or transform, from the neutral or zero oxidation state to a higher one;

b. In a simple metal compound, such as oxide and sulphide, the metal already exists in the oxidised state, so that further metal oxidation is unlikely to occur when the compound is introduced into an aqueous medium.

In light of the description of the structure and functioning of SZZ, RAC concludes that even if not fulfilling the characterisation for metal compounds, SZZ is acting in the same way as metal compounds, i.e. releasing positively charged ions, thus the classification strategy for metal compounds can be used.

In conclusion, and following the classification strategy for metal compounds (Annex IV.5.3), RAC concludes that SZZ should be classified as **Aquatic Acute 1; H400** since the acute $ERV_{compound}$ is ≤ 1 mg/L ($ERV_{compound}$ = 0.0037 mg SZZ/L) and as **Aquatic Chronic 1; H410** since there is no evidence of rapid environmental transformation and the chronic $ERV_{compound}$ is ≤ 0.1 mg/L ($ERV_{compound}$ = 0.00033 mg SZZ/L). The M-factors are **M=100** (0.001 < Acute ERV < 0.01 mg/L) for acute and **M=100** (0.0001 < Chronic ERV < 0.001 mg/L) for long-term aquatic hazard classification, respectively.

5.8 Labelling elements based on the classification

Element	Code
GHS Pictogram	 GHS09
Signal Word	WARNING
Hazard Statement	H410 Very toxic to aquatic life with long lasting effects
Precautionary statement(s)	P273, P391, P501

6 OTHER INFORMATION

7 REFERENCES

Additional references

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8 ANNEXES

Annex I: Literature reviews regarding aquatic bioaccumulation and –magnification of silver

a) Review prepared by RMS Sweden under the Review Programme of the Biocide Directive 98/8/EC, Draft May 2012

b) Position paper submitted by the applicant for silver zinc zeolite (European Silver Task Force via TSGE) under the Review Programme of the Biocide Directive 98/8/EC, Draft May 2012