Committee for Risk Assessment

RAC

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

Adopted

4 December 2015

4 December 2015

CLH-O-0000001412-86-90/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

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

EC Number: **-**

CAS Number: **130328-20-0**

The proposal was submitted by **Sweden** and received by RAC on **20 April 2015.**

In this opinion all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Sweden has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **2 June 2015**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **3 August 2015**.

ADOPTION OF THE OPINION OF RAC

Rapporteur appointed by RAC: **Miguel Sogorb**

Co-rapporteur appointed by RAC: **Riitta Leinonen**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling of silver zinc zeolite was adopted on **4 December 2015** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

Annex VI	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard Statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Entry	No current entry in Annex VI										
Proposal by the Dossier Submitter	TBD	Silver zinc zeolite (Zeolite, LTA1 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, NH^{4+}, Mg^{2+} and/or Ca^{2+} each at level <3%	-	130328-20-0	Carc. 2 Repr. 1B Skin Irrit. 2 Eye Dam. 1 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H360D H315 H318 H373 H400 H410	GHS08 GHS05 GHS09 Dgr	H351 H360D H315 H318 H373 H410		M=100 M=100	
RAC opinion	TBD		-	130328-20-0	Repr. 2 Skin Irrit. 2 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H361d H315 H318 H400 H410	GHS08 GHS05 GHS09 Dgr	H361d H315 H318 H410		M=100 M=100	
Resulting Entry	TBD				Repr. 2 Skin Irrit. 2 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H361d H315 H318 H400 H410	GHS08 GHS05 GHS09 Dgr	H361d H315 H318 H410		M=100 M=100	

GROUNDS FOR ADOPTION OF THE OPINION

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 (Table 1) 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: 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.

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.

HUMAN HEALTH HAZARD EVALUATION

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: 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 Rat (Sprague-Dawley) 5/sex Single oral dose 2000 mg/kg Zeomic Type AK10D Silver Zeolite A (AgION® Silver Antimicrobial Type AK)	No major abnormal findings No clinical signs No mortalities LD50>2000 mg/kg bw	Doc IIIB 6.1.1(01) (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: 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: 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 Rat (Sprague-Dawley) 5/sex 2.86 mg/L (4 hours) Zeomic Type AK10D Silver Zeolite A (AgION Type AK)	No mortalities Decreasing activity, piloerection and graying in the upper left lung of 1/10 animals LC_{50}>2.86 mg/L	Doc IIIB 6.1.3(01) (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.**

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.

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: Summary of skin corrosion/irritation studies

Method	Result	Remarks and reliability (Klimish score)
USA EPA 870.2500 Rabbit (New Zealand White) 3/sex 0.5 g wetted with physiological saline (4 hour exposure) AgION Type AK	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: 1M: Erythema -, Oedema - 2M: Erythema 2, Oedema 1 (scar) 3M: Erythema 4, Oedema 4 (crust) 4F: Erythema 4, Oedema 4 (crust) 5F: Erythema 1, Oedema 0 6F: Erythema 1, Oedema 1	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
OECD TG 404 Rabbit (New Zealand White) 3 males 0.5 g in 0.3mL deionised water (4 hour exposure) Test material: Irgaguard	24, 48 hours: Erythema (very slight) Individual and average scores at 24, 48, 72 h in individual animals: 1: Erythema 0.33 (1,0,0), Oedema 0 (0,0,0) 2: Erythema 0 (0,0,0), Oedema 0 (0,0,0) 3: Erythema 0.67 (1,1,0), Oedema 0 (0,0,0)	Doc IIIA 6.1.4-18 (Wilson, 2002b) Reliability 1 Observations at 1h, 24h, 48h and 72 h after patch removal
OECD TG 404 Rabbit (New Zealand White) 3/sex 0.5 g in 0.5% carboxymethylcellulose (4 hour exposure) Type of SZZ unspecified	1h: Very slight erythema in 3/6 animals Individual scores at 22, 44, 68 h in all animals: 0	Doc IIIA 6.1.4-04 (James, 1989c) Reliability 2 Observations at 1, 22, 44 and 68 hours after patch removal
USA EPA 870.2500 Rabbit (New Zealand White) 6M 0.5 g in 0.5mL distilled water (24 hour exposure) AgION Type AJ	Average score at 24.5, 48, 72h: 0 (for both erythema and oedema) Individual scores at 22, 44, 68 h in all animals: 0	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

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).

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: Summary of eye corrosion/irritation studies

<table>
<tr><th>Method</th><th>Result</th><th>Classification according to CLP criteria</th></tr>
<tr><td>EPA: OPPTS 870.2400 (81-4)

New Zealand (White Rabbit)

3M, 3F

0.1 g HealthShield Grade: AK10D (Same as AgION Type AK)</td><td>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>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>
<tr><td>Mean</td><td>0.33, 0.33, 0.16</td><td>1, 0, 0</td><td>3, 3, 2.8</td><td>2.2, 0.16, 0</td><td>0.5, 0, 0</td></tr>
</table></td><td>Eye Dam 1;H318

Doc IIIB 6.2(01) (Nitka, 2000)

Reliability 1</td></tr>
<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)</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.</td><td>Classification not required

Doc IIIA 6.1.4-16</td></tr>
</table>

3 M 0.06g AgION Type AD	Average score at 24, 48, 72 h in individual animals					(Moore 2006e) Reliability 1
			Conjunctiva			
	cornea	iris	redness	chemosis	Discharge	
1M	0.67 (1,1,0)	0.67 (1,1,0)	1.67 (3,2,0)	1.33 (2,2,0)	1 (2,1,0)	
2F	0.67 (1,1,0)	0.67 (1,1,0)	1.67 (3,2,0)	0.67 (1,1,0)	0.33 (1,0,0)	
3M	0 (0,0,0)	0.33 (1,0,0)	1 (2,1,0)	0.33 (1,0,0)	0.33 (1,0,0)	

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 $\geqslant$ 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).**

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.

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: Summary of skin sensitisation studies

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

0.5% CMC		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) Challenge: 10, 1, or 0.1% **Zeomic** DMSO suspensions at 24 or 48 hours after patch removal.	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. 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.**

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) presents the main systemic effects described for each type of SZZ belonging to the class analysed in this opinion.

Table 8: 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	Doc IIIA 6.4.1(07) (Teunissen, 2003) NOAEL: 50 mg/kg/day LOAEL: 250 mg/kg/day Reliability 1

	lymphoid infiltration, interstitial fibrosis and hyaline/cellular casts ↑ Discoloration of the pancreas and gastrointestinal tract All dose levels: ↑ Vomiting	

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

Table 9: 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: 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 Oral in diet Rat SpragueDawley Crl: CD® (SD) IGS BR 30/sex 0, 1000, 6250 and 12500 ppm AgION Silver Antimicrobial Type AK /day	**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 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)	Doc IIIA 6.8.2-04 (Schroeder 2002) LOAEL general toxicity = 1000 ppm (organ pigmentation in F_0 and hydronephrosis and mortality in F_1) Reliability 1

	F_1 1000 ppm ↑ Mortality (m 3.3%) ↑ Pigmentation of organs ↑ Hydronephrosis (3m, 1f, 0 in controls)	
	Offspring	
	F_1 12500 ppm ↑ 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: 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 in mouse		Haematology alterations Organ pigmentation	
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: 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)** 90 days (dog) Carcinogenicity (mouse) Carcinogenicity (rat)	**64/78** 250 211 30	**10 ≤ C ≤ 100** 10 ≤ C ≤ 100 1.25 ≤ C ≤ 12.5 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) 2-generation study (F_1) 90 days (mouse)	916-939 100 617	10 ≤ C ≤ 100 4.5 ≤ C ≤ 45 (adjusted for 202 days of exposure) 1.25 ≤ C ≤ 12.54
Thymus weight/atrophy	Carcinogenicity (rat) 2-generation study (F_1-F_2)	87 100	1.25 ≤ C ≤ 12.5 4.5 ≤ C ≤ 45 (adjusted for 202 days of exposure)
Organ pigmentation	90 days (rat) Carcinogenicity (mouse) Carcinogenicity (rat) **2-generation study (F_0)** 2-generation study (F_1)	398/489 67 30 **100** 100	10 ≤ C ≤ 100 1.25 ≤ C ≤ 12.5 1.25 ≤ C ≤ 12.5 **21 ≤ C ≤ 210 (adjusted for 43 days of exposure)** 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.**

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: 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 control: water + 0.15% agar	Negative	Doc IIIA 6.6.1-11, (May 2003) Reliability 2 Bacterial toxicity evident at dose concentrations of 500 µg/plate and higher. 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.**

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: 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.

Leukaemia

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.**

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: 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	m+f Day 0, 4: pre/post culling, 7, 14, 21, 26: n.s.s	Not determined	-

	(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	-
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:	Premating (1-11): ↓17% n.s.s in females* Gestation:	

		↓6% (only sign day 20) Lactation: ≤ 11%	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: 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: 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.**

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.

ENVIRONMENTAL HAZARD EVALUATION

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. 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. 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. 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. 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 ERVcompound 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 (ERVcompound) 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 ERVcompound of 0.0037 mg/L (EC50, 48h, Daphnia magna) and to the chronic ERVcompound 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 ERVcompound = 0.500 mg/L and a chronic ERVcompound = 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 (M0) 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 ERVcompound is ≤ 1 mg/L (ERVcompound = 0.0037 mg SZZ/L) and as Aquatic Chronic 1; H410 since there is no evidence of rapid environmental transformation and the chronic ERVcompound is ≤ 0.1 mg/L (ERVcompound = 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.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and by RAC (excluding confidential information).