Committee for Risk Assessment

RAC

Annex 1

Background document

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

sodium N-(hydroxymethyl)glycinate; [formaldehyde released from sodium N-(hydroxymethyl)glycinate]

EC Number: 274-357-8
CAS Number: 70161-44-3

CLH-O-0000001412-86-231/F

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

Adopted

14 September 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name:

sodium N-(hydroxymethyl)glycinate;

[formaldehyde released from sodium N-(hydroxymethyl)glycinate]

EC Number: **274-357-8**

CAS Number: **70161-44-3**

Index Number: **not allocated**

Contact details for dossier submitter:

Umweltbundesamt GmbH

on behalf of

AT Competent Authority

Federal Ministry of Agriculture, Forestry, Environment and Water Management

Version number: 3 **Date: 17/07/2017**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON SODIUM N-(HYDROXYMETHYL)GLYCINATE

CONTENTS

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING 5

1.1 SUBSTANCE 5
1.2 HARMONISED CLASSIFICATION AND LABELLING PROPOSAL 5
1.3 PROPOSED HARMONISED CLASSIFICATION AND LABELLING BASED ON CLP REGULATION AND/OR DSD CRITERIA. 8

BACKGROUND TO THE CLH PROPOSAL 12

1.4 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING 12
1.5 SHORT SUMMARY OF THE SCIENTIFIC JUSTIFICATION FOR THE CLH PROPOSAL 12
1.6 CURRENT HARMONISED CLASSIFICATION AND LABELLING 14
1.7 CURRENT SELF-CLASSIFICATION AND LABELLING 14

2 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL 15

Part B

SCIENTIFIC EVALUATION OF THE DATA 16

1 IDENTITY OF THE SUBSTANCE 16

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE 16
1.2 COMPOSITION OF THE SUBSTANCE 17
1.2.1 Composition of test material 18
1.3 PHYSICO-CHEMICAL PROPERTIES 18

2 MANUFACTURE AND USES 22

2.1 MANUFACTURE 22
2.2 IDENTIFIED USES 22

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES 23

3.1 ALL HAZARD CLASSES 24
3.1.1 Summary and discussion of 24
3.1.2 Comparison with criteria 24
3.1.3 Conclusions on classification and labelling 24

4 HUMAN HEALTH HAZARD ASSESSMENT 25

4.1 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION) 25
4.1.1 Non-human information - SHMG 25
4.1.2 Non-human information – Formaldehyde 25
4.1.3 Human information - SHMG 26
4.1.4 Summary and discussion on toxicokinetics 26
4.2 ACUTE TOXICITY 26
4.2.1 Non-human information 26
4.2.1.1 Acute toxicity: oral 26
4.2.1.2 Acute toxicity: inhalation 27
4.2.1.3 Acute toxicity: dermal 28
4.2.1.4 Acute toxicity: other routes 28
4.2.2 Human information 29
4.2.3 Comparison of acute toxicity data of SHMG and the hydrolysis product formaldehyde 29
4.2.4 Summary and discussion of acute toxicity 29
4.2.5 Comparison with criteria 30
4.2.6 Conclusions on classification and labelling 30

4.3 SPECIFIC TARGET ORGAN TOXICITY – SINGLE EXPOSURE (STOT SE) 32
4.4 IRRITATION 33
4.4.1 Skin irritation *33*
4.4.1.1 Non-human information 33
4.4.1.2 Human information 34
4.4.1.3 Comparison of skin and eye irritation data of SHMG and the hydrolysis product Formaldehyde 34
4.4.1.4 Summary and discussion of skin irritation 35
4.4.1.5 Comparison with criteria 36
4.4.1.6 Conclusions on classification and labelling 36
4.4.2 Eye irritation *39*
4.4.2.1 Non-human information 39
4.4.2.2 Human information 40
4.4.2.3 Comparison of skin and eye irritation data of SHMG and the hydrolysis product Formaldehyde 40
4.4.2.4 Summary and discussion of eye irritation 40
4.4.2.5 Comparison with criteria 41
4.4.2.6 Conclusions on classification and labelling 42
4.4.3 Respiratory tract irritation *44*
4.5 CORROSIVITY 44
4.6 SENSITISATION 44
4.6.1 Skin sensititsation *44*
4.6.1.1 Non-human information 44
4.6.1.2 Human information 45
4.6.1.3 Comparison of skin sensitizing data of SHMG and the hydrolysis product formaldehyde 46
4.6.1.4 Summary and discussion of skin sensitisation 46
4.6.1.5 Comparison with criteria 47
4.6.1.6 Conclusions on classification and labelling 47
4.6.2 Respiratory sensitisation *48*
No specific data are available. 48
4.7 REPEATED DOSE TOXICITY 49
4.7.1 Non-human information *49*
4.7.1.1 Repeated dose toxicity: oral 49
4.7.1.2 Repeated dose toxicity: inhalation 49
4.7.1.3 Repeated dose toxicity: dermal 49
4.7.1.4 Repeated dose toxicity: other routes 49
4.7.1.5 Human information 49
4.7.1.6 Other relevant information: Comparison of repeated dose toxicity data for SHMG and the hydrolysis product formaldehyde 50
4.7.1.7 Summary and discussion of repeated dose toxicity 51
4.8 SPECIFIC TARGET ORGAN TOXICITY (CLP REGULATION) – REPEATED EXPOSURE (STOT RE) 51
4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation *51*
4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE *52*
4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE *52*
4.9 GERM CELL MUTAGENICITY (MUTAGENICITY) 52
4.9.1 Non-human information *52*
4.9.1.1 In vitro data 52
4.9.1.2 In vivo data 54
4.9.2 Human information *55*
4.9.4 Summary and discussion of mutagenicity *56*
4.9.5 Comparison with criteria *60*
4.9.6 Conclusions on classification and labelling *61*
4.10 CARCINOGENICITY 63
4.10.1 SHMG *63*
4.10.3 Summary and discussion of carcinogenicity *63*
4.10.4 Comparison with criteria *66*
4.10.5 Conclusions on classification and labelling *66*
4.11 TOXICITY FOR REPRODUCTION 68
4.11.1 Effects on fertility *68*
4.11.1.1 Non-human information 68
4.11.1.2 Human information 68
4.11.2 Developmental toxicity *68*
4.11.2.1 Non-human information 68
4.11.2.2 Human information 68

4.11.3 Other relevant information: Comparison of reproductive toxicity data for SHMG and the hydrolysis product formaldehyde ... *69*
4.11.4 Summary and discussion of reproductive toxicity ... *69*
4.11.5 Comparison with criteria ... *70*
4.11.6 Conclusions on classification and labelling ... *70*
4.12 OTHER EFFECTS ... 71
4.12.1 Non-human information ... *71*
4.12.1.1 Neurotoxicity - SHMG ... 71
4.12.1.2 Other relevant information: Comparison of neurotoxicity information for SHMG and the hydrolysis product formaldehyde ... 72
4.12.1.3 Immunotoxicity ... 72
4.12.1.4 Specific investigations: other studies ... 72
4.12.1.5 Human information ... 73
4.12.2 Summary and discussion ... *73*
4.12.3 Comparison with criteria ... *73*
4.12.4 Conclusions on classification and labelling ... *74*
4.13 OVERVIEW ON AVAILABLE DATA FOR SHMG IN COMPARISON TO DATA FOR FORMALDEHYDE ... 74

5 ENVIRONMENTAL HAZARD ASSESSMENT ... 77

5.1 DEGRADATION ... 77
5.1.1 Stability ... *77*
5.1.2 Biodegradation ... *80*
5.1.2.1 Biodegradation estimation ... 80
5.1.2.2 Screening tests ... 80
5.1.2.3 Simulation tests ... 81
5.1.3 Summary and discussion of degradation ... *82*
5.2 ENVIRONMENTAL DISTRIBUTION ... 82
5.2.1 Adsorption/Desorption ... *82*
5.2.2 Volatilisation ... *83*
5.2.3 Distribution modelling ... *84*
5.3 AQUATIC BIOACCUMULATION ... 84
5.3.1 Aquatic bioaccumulation ... *84*
5.3.1.1 Bioaccumulation estimation ... 84
Products of hydrolysis ... 85
5.3.1.2 Measured bioaccumulation data ... 85
5.3.2 Summary and discussion of aquatic bioaccumulation ... *85*
5.4 AQUATIC TOXICITY ... 85
5.4.1 Fish ... *85*
5.4.1.1 Short-term toxicity to fish ... 85
5.4.1.2 Long-term toxicity to fish ... 87
5.4.2 Aquatic invertebrates ... *87*
5.4.2.1 Short-term toxicity to aquatic invertebrates ... 87
5.4.2.2 Long-term toxicity to aquatic invertebrates ... 88
5.4.3 Algae and aquatic plants ... *88*
5.4.4 Other aquatic organisms (including sediment) ... *90*
5.6 COMPARISON WITH CRITERIA FOR ENVIRONMENTAL HAZARDS (SECTIONS 5.1 – 5.4) ... 92
5.7 CONCLUSIONS ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS (SECTIONS 5.1 – 5.4) ... 94

6 OTHER INFORMATION ... 98

7 REFERENCES ... 99

8 ANNEXES ... 107

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1.1_1: Substance identity

Substance name:	sodium N-(hydroxymethyl)glycinate; [formaldehyde released from sodium N-(hydroxymethyl)glycinate]
EC number:	274-357-8
CAS number:	70161-44-3
Annex VI Index number:	not allocated
Degree of purity:	>= 98.0 % w/w
Impurities:	See DOC IIA confidential, attached to IUCLID section 13

Remark: The active substance as manufactured is an aqueous solution of Sodium N-(hydroxymethyl)glycinate (short: SHMG). The solvent water may be separated without changing the composition of the active substance or affecting its stability. The active substance as manufactured does not contain additives. However, as the active substance is manufactured only as ca. 50% aqueous solution of Sodium N-(hydroxymethyl)glycinate, water is excluded arithmetically. # Detailed information on the chemical composition of the active substance and the a.s. as manufactured is confidential. Thus this information is provided in a separate file. (Please see Doc. II-A, Appendix "Confidential data and information" of the attached CAR). If not stated otherwise the % SHMG solutions are always related to the pure (100%) substance.

1.2 Harmonised classification and labelling proposal

Table 1.2_1: The current Annex VI entry and the proposed harmonised classification for SHMG

	CLP Regulation
Current entry in Annex VI, CLP Regulation	None
Current proposal for consideration by RAC	Acute Tox. 4: H302: Harmful if swallowed Skin Irrit. 2, H315: Causes skin irritation

	Eye Irrit. 2, H319: Causes serious eye irritation Skin Sens. 1, H317: May cause an allergic skin reaction Muta 2, H341: Suspected of causing genetic defects; Note 9: "The classification as a mutagen need not apply if it can be shown that the maximum theoretical concentration of releasable formaldehyde, irrespective of the source, in the mixture as placed on the market is less than 1%." Carc. 1B, H350: May cause cancer; Note 8: "The classification as a carcinogen need not apply if it can be shown that the maximum theoretical concentration of releasable formaldehyde, irrespective of the source, in the mixture as placed on the market is less than 0.1%."
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 4: H302: Harmful if swallowed Skin Irrit. 2, H315: Causes skin irritation Eye Irrit. 2, H319: Causes serious eye irritation Skin Sens. 1, H317: May cause an allergic skin reaction Muta 2, H341: Suspected of causing genetic defects Carc. 1B, H350: May cause cancer

Table 1.2_2: The current Annex VI entry and harmonised classification of formaldehyde

	CLP Regulation (including criteria according to 2nd ATP of CLP)
Formaldehyde	
Current opinion by RAC	Carc. 1B H350 Muta. 2 H341 Acute Tox. 3* H301 Acute Tox. 3* H311 Acute Tox. 3* H331 Skin Corr. 1B H314 Skin Sens. 1 H317 Specific Conc. Limits: * Skin Corr.1B; H314: C ≥ 25 % Skin Irrit. 2; H315: 5 % ≤ C < 25 % Eye Irrit. 2; H319: 5 % ≤ C < 25 % STOT SE 3; H335: C ≥ 5 % Skin Sens. 1; H317: C ≥ 0.2 %

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON SODIUM N-(HYDROXYMETHYL)GLYCINATE

In 2012 RAC adopted its opinion on the proposal submitted by France for a harmonised classification and labelling at EU level of formaldehyde[1]. However, the endpoint and classification as hazardous to the aquatic environment were not part of the dossier and have not been evaluated by RAC.

[1] https://echa.europa.eu/documents/10162/13626/rac_opinion_formaldehyde_en.pdf

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

Table 1.3-1:: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.2.	Flammable gases	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.3.	Flammable aerosols	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.5.	Gases under pressure	n.a.	n.a.	currently not classified	data lacking
2.6.	Flammable liquids	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.7.	Flammable solids	n.a.	n.a.	currently not classified	data lacking
2.8.	Self-reactive substances and mixtures	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	n.a.	n.a.	currently not classified	data lacking
2.10.	Pyrophoric solids	n.a.	n.a.	currently not classified	data lacking
2.11.	Self-heating substances and mixtures	n.a.	n.a.	currently not classified	data lacking
2.12.	Substances and mixtures which in contact with water emit flammable gases	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification

2.15.	Organic peroxides	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Acute Tox. 4: H302: Harmful if swallowed	n.a.	n.a.	n.a.
	Acute toxicity - dermal	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Skin Irrit. 2, H315: Causes skin irritation	n.a.	n.a.	n.a.
3.3.	Serious eye damage / eye irritation	Eye Irrit. 2, H319: Causes serious eye irritation	n.a.	n.a.	n.a.
3.4.	Respiratory sensitisation	n.a.	n.a.	n.a.	data lacking
3.4.	Skin sensitisation	Skin Sens. 1, H317: May cause an allergic skin reaction	n.a.	n.a.	n.a.
3.5.	Germ cell mutagenicity	Muta 2, H341: Suspected of causing genetic defects	Note 9: “The classification as a mutagen need not apply if it can be shown that the maximum theoretical concentration of releasable formaldehyde, irrespective of the source, in the mixture as placed on the market is less than 1%.”	n.a.	n.a.
3.6.	Carcinogenicity	Carc. 1B, H350: May cause cancer	Note 8: “The classification as a carcinogen need not apply if it can be shown that the maximum theoretical concentration of releasable formaldehyde, irrespective of the source, in the mixture as placed on the market is less than 0.1%.”	n.a.	n.a.
3.7.	Reproductive toxicity	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification

3.8.	Specific target organ toxicity –single exposure	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
5.1.	Hazardous to the ozone layer	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification

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

Labelling:

GHS Pictograms	
Signal words	Danger
Hazard statements	H302: Harmful if swallowed
	H315: Causes skin irritation
	H319: Causes serious eye irritation
	H317: May cause an allergic skin reaction
	H341: Suspected of causing genetic defects
	H350: May cause cancer
Precautionary Statements	P202: Do not handle until all safety precautions have been read and understood. P280: Wear protective gloves/protective clothing/eye protection/face protection. P260: Do not breathe mist/vapours/ spray.

	P302 + P352: IF ON SKIN: Wash with plenty of water P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P308 + P313: IF exposed or concerned: Get medical advice/ attention. P362 + P364: Take off contaminated clothing. And wash it before reuse. P405: Store locked up. P501: Dispose of contents/container to …

Proposed notes assigned to an entry:

Note 8: "The classification as a carcinogen need not apply if it can be shown that the maximum theoretical concentration of releasable formaldehyde, irrespective of the source, in the mixture as placed on the market is less than 0.1%."

Note 9: "The classification as a mutagen need not apply if it can be shown that the maximum theoretical concentration of releasable formaldehyde, irrespective of the source, in the mixture as placed on the market is less than 1%."

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON SODIUM N-(HYDROXYMETHYL)GLYCINATE

BACKGROUND TO THE CLH PROPOSAL

1.4 History of the previous classification and labelling

There is no current classification according to Annex I of Council Directive 67/548/EEC.

There is also no current classification according to Table 3.1 of Annex VI of Regulation (EC) No 1272/2008.

1.5 Short summary of the scientific justification for the CLH proposal

The active substance as manufactured represents sodium hydroxymethyl glycinate (SHMG) as a 50% aqueous solution. It represents a reaction product of formaldehyde and glycine. When SHMG is diluted in water SHMG hydrolyses to formaldehyde and glycine. Contact with biological media should lead to a reaction of formaldehyde with protein and shift the equilibrium towards further formaldehyde release. Acidic pH (like in stomach) would further support fast hydrolysis. Therefore we may theoretically assume a rate of 100% final hydrolysis in biological media. A 50% SHMG solution corresponds to less than 12% (w/w) formaldehyde (for more details see section 4.1.1.).

In use concentrations of SHMG are usually very low (0.05% to 0.25%). With such high dilution in water SHMG hydrolyses fully to formaldehyde and glycine. Glycine is an amino acid, a natural cell component, a food ingredient and compared to formaldehyde of low biological reactivity.

Therefore the toxicity of SHMG relates primarily to the toxicity of formaldehyde.

Formaldehyde is corrosive. With SHMG 100% applied as moistened or dry powder, hydrolysis and reaction kinetics may have limited skin irritation effects to levels below the need for classification. SHMG as manufactured (50% w/w solution) was not tested for skin irritation (just at concentrations ≤ 5%, where it does not appear to be skin irritating) and the theoretical considerations given in section 4.1.1. (i.e. up to 12% formaldehyde formed by hydrolysis of SHMG) relate to a formaldehyde concentration within the skin irritation range (see SLCs for formaldehyde for skin irrit. 2: 5 - 25%). The eye irritation studies with SHMG 50% (w/w) and SHMG 100% support eye irritation (but not eye corrosion). Consequently based on a total weight of evidence approach classification of **SHMG is proposed for skin irritation (Category 2) and for eye irritation (category 2)**.

Formaldehyde is a well-known human skin sensitizer. Several skin sensitizing studies are available for SHMG. The most reliable study is from Reagan 1984 where intradermal challenge was carried out with a 5% SHMG solution including also adjuvans, followed by a topical induction with moistened powder and several SHMG topical challenge concentrations. Positive reactions were found with 50% and 5% SHMG solutions. No differentiation according to potency (Category 1A or 1B) is possible, since no lower intradermal induction concentrations than 5% were tested. The study appears valid and appropriate for the **classification for skin sensitization** of SHMG 100%.

Formaldehyde is classified for acute toxicity category 3 for all exposure routes. LD50 values and LC50 values calculated for SHMG as manufactured (50% aqueous solution) are above classification limit values. If calculated for SHMG 100% the oral LD50 values are within the range of **oral acute category 4**, but above the range of acute respiratory or acute dermal category 4. In the absence of further information classification for acute oral toxicity category 4 is proposed.

Formaldehyde is classified as Carcinogen Cat 1B and Mutagenicity Cat 2 on the basis of available animal and human data. No carcinogenicity data are available for SHMG, but mutagenicity data are comparable with formaldehyde. SHMG is **proposed to be classified for carcinogenicity category**

1B and mutagenicity category 2 based on the mechanistic considerations of total releasable amount of formaldehyde upon contact with biological media and read across of the carcinogenic and mutagenic property of formaldehyde. Due to the consideration that formaldehyde release is dominating the toxicity of SHMG and the classification of formaldehyde is read across to SHMG it is suggested that a specific note 8 is included for carcinogenicity (category 1B): "The classification as a carcinogen need not apply if it can be shown that the maximum theoretical concentration of releasable formaldehyde, irrespective of the source, in the mixture as placed on the market is less than 0.1%." Similarly for genotoxicity (category 2) a specific note 9 shall be included: "The classification as a mutagen need not apply if it can be shown that the maximum theoretical concentration of releasable formaldehyde, irrespective of the source, in the mixture as placed on the market is less than 1%." The applicant proposes not to classify SHMG for carcinogenicity and mutagenicity based on considering just the amount of free formaldehyde in SHMG. Supportive arguments for both options are provided in the specific chapter on carcinogenicity.

Environment:

SHMG hydrolyses rapidly (<< 1day) to formaldehyde and sodium glycinate in the aquatic environment. Therefore in addition to the data on SHMG itself also data on the hydrolysis products formaldehyde and sodium glycinate were considered for classification. Glycine is a naturally occurring amino acid, it is not persistent in the environment and its ecotoxicity is of no concern.

Acute Category:

All available acute L(E)C50 values for SHMG as well as for the hydrolysis product formaldehyde are >1 mg/L, therefore no classification is needed for SHMG.

Chronic Categories:

For SHMG one 72hr-NOEC is available for algae, which is >1 mg/L (2.5 mg/L). For fish and crustaceans acute LC50s are >10 mg/L (75 mg/L and 39 mg/L, respectively) and SHMG is rapid degradable (based on ready biodegradability); additionally a measured log Kow of -1.533 is available. On the basis of these data no classification for any of the chronic categories is needed for SHMG.

There is only one reliable chronic NOEC value of >1 mg/L available for formaldehyde from crustacean. For fish and algae EC50 values >1 mg/L are available, which in combination with ready biodegradability, a measured log Kow of 0.35 and a calculated BCF_{fish} of 0.396 L/kg doesn't lead to a classification. However, the NOEC for daphnia is 1.04 mg/L, which is close to the criterion (<1 mg/L) for classification.

Hazards to the ozone layer:

On the basis of low vapour pressure, low Henry´s Law constants and rapid degradation through reaction with hydroxyl radicals for SHMG as well as for its hydrolysis products there are no indications for danger to the ozone layer.

Also SHMG as well as its hydrolysis products are not listed in Annex I and II of Regulation (EC) No 1005/2009 of the European Parliament and of the Council of 16 September 2009 on substances that deplete the ozone layer.

In conclusion no classification for hazards to the aquatic environment and to the ozone layer is proposed for SHMG.

1.6 Current harmonised classification and labelling

No current classification and labelling

1.7 Current self-classification and labelling

Table 2.4_1 Classification and labelling according to Reg. 1272/2008/EC of SHMG **by the participant/manufacturer** for the Biocidal Products Regulation 528/2012/EC

Classification	**By the participant**
Classification	Eye Irrit. 2 Skin Sens. 1
Hazard statements	H319: Causes severe eye irritation
	H317: May cause an allergic skin reaction
Specific classification limits	-
GHS Pictograms	
Signal words	Warning
Precautionary Statements	To be completed after decision for classification

RAC general comment
With regards to substances that act via the released formaldehyde or any other substance acting by similar circumstances, RAC highlights that the classification is based on the (intrinsic) hazardous properties from the substance as such or its hydrolysis product, other cleavage products or any other metabolites and follows the criteria given by the CLP Regulation. For sodium N-(hydroxymethyl)glycinate (SHMG), no read across or risk-based approach is used for classification. The hydrolysis product, formaldehyde, is understood as the active agent (mainly locally active). The considerations summarised by the dossier submitter (DS) in section 4.10.3 (*Summary and discussion of carcinogenicity*) of the CLH report facilitates the general understanding of the classification proposal: "The active substance as manufactured represents sodium hydroxymethyl glycinate (SHMG) as a 50% aqueous solution. It represents a reaction product of formaldehyde and glycine. When SHMG is diluted in water, SHMG hydrolyses to formaldehyde and

glycine. The high pH in the 50% solution (pH=11) or in a more diluted 5% solution slows down hydrolysis and formaldehyde release. However, the hydrolysis study indicates that in unbuffered aqueous solutions of 10%, 1% and 0.25% SHMG the pH is between 10.7 and 11.7, but still about 18%, 40% and 66% were hydrolysed. Contact with biological media should lead to a reaction of formaldehyde with proteins and shift the equilibrium towards further formaldehyde release. Acidic pH (like in stomach) would further support fast hydrolysis, the DT_{50} was smaller than 1.4 hours at pH 4 and 7. Therefore, we may theoretically assume a rate of 100% final hydrolysis in biological media. Given the molecular weight of 127 g/mol for SHMG and 30 g/mol for formaldehyde (factor 4.23) a 50% SHMG solution corresponds to less than 12% (w/w) formaldehyde. In-use concentrations of SHMG are usually very low (0.05% to 0.25%). With such high dilution in water, SHMG hydrolyses fully to formaldehyde and glycine. Glycine is an amino acid, a natural cell component, a food ingredient and compared to formaldehyde of low biological reactivity. Therefore, it is considered that the toxicity of SHMG relates primarily to the toxicity of formaldehyde."

The DS proposed to theoretically assume a rate of 100% final hydrolysis in biological media.

During the public consultation, a number of Industry Associations contested the approach to consider the theoretical maximum release of formaldehyde, and proposed instead to use measured levels of 'free' formaldehyde in the solutions for classification.

The DS responded that measured values of free formaldehyde do not adequately mirror the exposure situation, where contact with biological tissues and fluids would lead to formaldehyde reacting with the biological targets, shifting the equilibrium towards further formaldehyde release. The DS proposed to follow the previous RAC opinions on other formaldehyde releasers and to consider total formaldehyde release upon which to base classification.

2 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The substance is under evaluation as a biocidal active substance for product type 6 under BPR, Regulation (EU) 528/2012 and that as such there is no need for justification for the CLH proposal. As foreseen by Commission Delegated Regulation (EU) No 1062/2014 the competent authority report (CAR) shall be sent to ECHA no later than 31 December 2019. Since we propose classification Canc. 1B for this substance the exclusion criteria according Art. 5(1) of the BPR would be fulfilled. This means that ECHA will not accept the draft CAR for further processing without a RAC opinion. Subsequently the eCA will finalise the draft CAR as soon as possible.

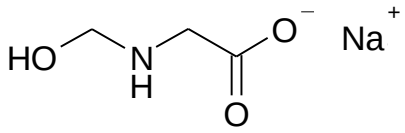
Part B.

SCIENTIFIC EVALUATION OF THE DATA

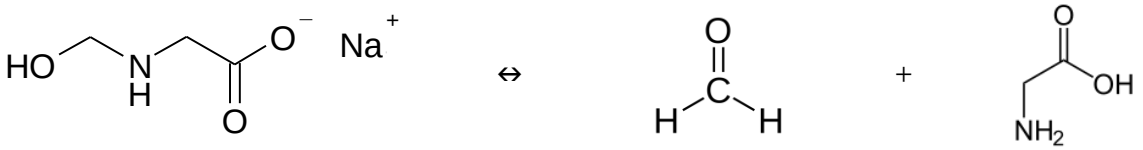
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1.1_1: Substance identity

CAS-No.	70161-44-3
EC-No.	274-357-8
Other No. (CIPAC, ELINCS)	-
IUPAC Name	Sodium N-(hydroxymethyl)glycinate Glycine, N-(hydroxymethyl)-, monosodium salt; Sodium (hydroxymethylamino) acetate
Common name, synonyms	Sodium hydroxymethyl glycinate; abbreviation used in this document: SHMG
Molecular formula	$C_3H_7NO_3.Na$
Structural formula	
Molar mass (g/mol)	127.10

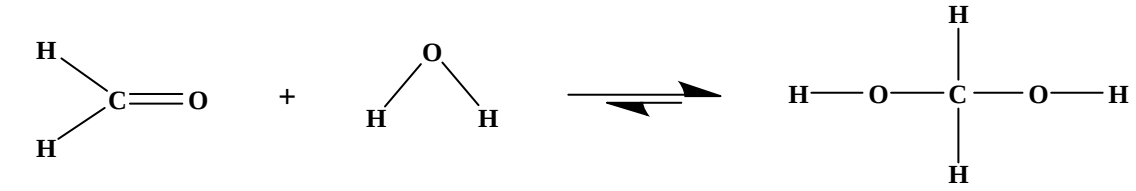
Sodium N-(hydroxymethyl)glycinate is classified as a formaldehyde donor and will hydrolyse in aqueous systems to formaldehyde and sodium glycinate (which further dissociate to glycine); The donor equilibrates with formaldehyde:



DONOR-FORMALDEHYDE (Sodium N-(hydroxymethyl)glycinate) ⇔ FORMALDEHYDE + DONOR (Glycine)

The hydrolysis is pH dependent: at basic pH values sodium hydroxymethyl glycinate reveals partial hydrolytic stability, whilst at acidic pH values the substance is fully degraded to glycine and formaldehyde (See Doc. III-A 7.1.1.1.1.).

The formaldehyde donated by the parent formaldehyde-donor compound is hydrated through a reaction with the solvent (water) forming methylene glycol. The methylene glycol enters into an equilibrium relation with monomeric (gaseous; 'free') formaldehyde. This equilibrium predominantly lies in the direction of the methylene glycol and the concentration of monomeric (gaseous; 'free') formaldehyde is frequently less than 0.1 ppm:



(Free formaldehyde at 0.04%) **(Water)** **(Methylene glycol at 99.96%)**

(Refer to Kirk-Othmer Encyclopedia of Chemical Technology[2] and Walker's frequently cited monograph on formaldehyde[3]. On pages 59-62 of this monograph, the chemistry of formaldehyde dissolved in water is discussed at length. Data from Table 14 (shown below) confirm that formaldehyde dissolved in water equilibrates with a minimal concentration of monomeric gaseous or 'free') formaldehyde.)

Based on the published information, at a typical use-level of 0.10%w/w of a formaldehyde-donor such as Sodium N-(hydroxymethyl)glycinate for the preservation of water-based products, the monomeric (gaseous or 'free') formaldehyde concentration in the liquid is less than 0.1 ppm.

1.2 Composition of the substance

The active substance as manufactured is an aqueous solution of Sodium N-(hydroxymethyl)glycinate (short: SHMG). The solvent water may be separated without changing the composition of the active substance or affecting its stability. The active substance as manufactured does not contain additives. However, as the active substance is manufactured only as

[2] Kirk-Othmer Encyclopedia of Chemical Technology[2]. 2004. 5th ed. Chapter 12. pg. 107

[3] Walker, Joseph Frederic. 1975. Formaldehyde. Robert E. Krieger Publishing Co., Inc. New York

ca. 50% aqueous solution of Sodium N-(hydroxymethyl)glycinate, water is excluded arithmetically. The minimum degree of purity of the active substance excluding water is **min. 98%w/w** SHMG.

The manufacturer has requested that all impurities remain confidential since it may provide an indication on the possible method of manufacturing. Information on impurities is provided in the confidential IUCLID section 1.2 (Composition) and in Doc. II-A confidential of the Competent Authority Report attached to IUCLID section 13.

1.2.1 Composition of test material

The active substance as manufactured is a 50% aqueous solution of Sodium N-(hydroxymethyl) glycinate and used as biocidal product. The respective brand names are Nuosept 44 for technical applications, Integra 44 for HI&I applications and Suttocide A for personal care applications (Suttocide A can describe both: a powder (solid a.s. without water) and the 50% aqueous solution of the active substance). Several studies use these trade names as denomination of the test substance instead of the chemical name.

1.3 Physico-chemical properties

The physico – chemical properties are studied either for the purified active substance of stated specification (98 %w/w SHMG) or for the active substance as manufactured (SHMG as 50% aqueous solution) according to the demands of the data requirements.

Table 1.3_1: Summary of physico - chemical properties

Property	Results	Reference	Comment	
			Purity/ Specification	**Method**
Melting point	Sharp endotherm onset ca 192°C, possibly due to melting	**Doc. III-A3; Study IIIA 3.1.1**	98 %w/w	EC method A.1 Differential scanning calorimetry
Boiling point	Decomposition above ca 200°C; No indication of boiling.	**Doc. III-A3; Study IIIA 3.1.2**	98 %w/w	EC method A.2 Differential scanning calorimetry
Relative density	1.653 g/ml (SD of 0.002) at 20°C	**Doc. III-A3; Study IIIA 3.1.3/01**	98 %w/w	EC method A.3 Gas comparison pyknometry
	1.2901 g/ml (SD of 0.0001) at 20°C	**Doc. III-A3; Study IIIA 3.1.3/02**	50% aqueous solution	Gas comparison pyknometry
Vapour pressure	1.42 x 10-5 Pa at 25°C 2.27 x 10-7 Pa at 20°C	**Doc. III-A3; Study IIIA 3.2**	98 %w/w	EC method A.4 Knudsen Effusion

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON SODIUM N-(HYDROXYMETHYL)GLYCINATE

Property	Results	Reference	Comment	
			Purity/ Specification	Method
Henry´s law constant	Result (Bond Method): 1.81E-012 atm-m3/mole corresponding to 1.83E-07 Pa x m3/mole	**Doc. III-A3; Study IIIA 3.2.1/01 Study IIIA 3.2.1/02**	calculation for 100%	HENRYWIN v3.10 HENRYWIN v3.20
	Result VP/WS method using EPI values: 4.063E-018 atm-m3/mole corresponding to 4.117E-13 Pa x m3/mole	**Doc. III-A3; Study IIIA 3.2.1/01 Study IIIA 3.2.1/02**	calculation for 100%	HENRYWIN v3.10 HENRYWIN v3.20
	Result VP/WS method using values of 3.2_01 and 3.5: 1.8E-09 Pa x m3/mole at 25°C	**Doc. III-A3; Study IIIA 3.2.1/02**	calculation for 100%	HENRYWIN v3.20
Physical state	Solid, powder	**Doc. III-A3; Study IIIA 3.3.1/02**	98 %w/w	Visual inspection
	Liquid	**Doc. III-A3; Study IIIA 3.3.1/01**	50% aqueous solution	Visual inspection
Colour	White	**Doc. III-A3; Study IIIA 3.3.2/02**	98 %w/w	Visual inspection
	Very pale to pale yellow	**Doc. III-A3; Study IIIA 3.3.2/01**	50% aqueous solution	Visual inspection
Odour	Mild characteristics odour of aromatic compounds	**Doc. III-A3; Study IIIA 3.3.3**	50% aqueous solution	Sniff test
Absorption spectra: UV/VIS	In unadjusted aqueous solution: No absorption max In acified aqueous solution: 208.6nm, ε = 45 In basified aqueous solution: 220.8nm, ε = 84 The major peaks of the respective spectra were consistent with the accepted structure of the test material	**Doc. III-A3; Study IIIA 3.4**	50% in water	Solutions of test material were prepared by diluting in solvents. The spectra of these were recorded against an appropriate blank using a U3000 spectrophotometer
Absorption spectra: IR	The test substance (Sodium hydroxymethyl glycinate) was supplied as a 50% solution in water. Water absorbs strongly in the infrared region consequently the infrared spectrum could not be recorded.	**Doc. III-A3; Study IIIA 3.4**	--	

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON SODIUM N-(HYDROXYMETHYL)GLYCINATE

Property	Results	Reference	Comment	
			Purity/ Specification	**Method**
Absorption spectra: NMR	Singlet at 3.12 ppm Broad singlet at 3.37 ppm Overlapping singlet at 4.80 ppm Overlapping singlet at 4.81 ppm The 1H NMR spectrum is considered consistent with the accepted structure of the test material	**Doc. III-A3; Study IIIA 3.4**	50% in water	A proton NMR spectrum was recorded for TS as a solution in a deuterated methanol on a Jeol EX 270 NMR Spectrometer
Absorption spectra: MS	The active substance as manufactured is in equilibrium with the starting materials. Because of the dynamic nature of the equilibrium analytical standard methods like LC/MS, GC/MS and photometry are difficult to use for characterisation of the composition and the determination of the active-ingredient-content or the impurities.	**Doc. III-A3; Justification**	--	Justification
Water solubility	The effect of pH on the solubility of the a.s. in water at 20°C: a small amount of precipitation at approx. pHs 5, 6 & 7 which disappeared upon stirring	**Doc. III-A3; Study IIIA 3.5/01**	50% aqueous solution	Non analytical method based on that described in EC directive 92/69 method A6 & OECD Guideline 105 (1995)
	Water solubility at 25°C (mg/L): 1e+006	**Doc. III-A3; Study IIIA 3.5/02**	calculation for 100%	From Log Kow (WSKOW v1.41)
Dissociation constant	pKb: 8.41 carboxylic acid salt ($-COO^-NA^+$) pKb: ≥11.0 secondary amine (-NH-)	**Doc. III-A3; Study IIIA 3.6**	100%	potentiometric titration
Solubility in organic solvents, incl. the effect of temperature on solubility	Solubility range in different organic solvents is below 10g/l at 22 ± 0.5°C	**Doc. III-A3; Study IIIA 3.7**	98%	MT 181
Stability in organic solvents used in b.p. and identity of relevant breakdown products	Sodiumhydroxy methylglycinate contains no organic solvent and is not used in solvent based formulations.	**Doc. III-A3; Justification**	--	Justification
Partition coefficient n-octanol/water	result: log Pow = -1.533 temperature: 26°C	**Doc. III-A3; Study IIIA 3.9**	50% aqueous solution	GlpKa method

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON SODIUM N-(HYDROXYMETHYL)GLYCINATE

Property	Results	Reference	Comment	
			Purity/ Specification	**Method**
	Overall, due to hydrolysis experimental determination is technically not feasible.	**Doc. III-A3; Justification**	100%	Justification
Thermal stability	Decomposition above ca 150 °C Decomposition above ca 200 °C Hydrolysis is a weak endothermic reaction	**Doc. III-A3; Study IIIA 3.10/01** **Doc. III-A3; Study IIIA 3.10/02** **Doc. III-A3; Study IIIA 3.10/03**	50% aqueous solution 98 %w/w 1% aqueous solution	Differential scanning calorimetry (During determination of the melting point) NMR - Temperature dependence of hydrolysis equilibrium
Flammability	In the closed cup equilibrium method (EC A9 test) using a 50% solution no flash was observed up to the maximum temperature tested of 110°C. Testing on contact with water (EC A12) is regarded to be not necessary, as it is handled as aqueous solution, and experience is available that reaction with water is not known. There are no components in SHMG that are expected to react violently or to self ignite.	**Doc. III-A3; Justification**	--	Justification
Flash-point	No flash was observed up to the maximum temperature tested of 110°C	**Doc. III-A3; Study IIIA 3.12**	50% aqueous solution	Closed cup equilibrium method (EC A9 test)
	This endpoint is not applicable for solids.	**Doc. III-A3; Justification**	100%	Justification
Surface tension	temperature: 20ºC ± 0.5 ºC result: 64.8 mN/m Due to the fact that surface tension is higher than 60 mN/m, the test item has no surface-active properties.	**Doc. III-A3; Study IIIA 3.13**	51.1% (by Titration)	OECD 115
Viscosity	results: 24.7 mm²/s at 20ºC 10.1 mm²/s at 40ºC	**Doc. III-A3; Study IIIA 3.14**	51.1% (by Titration)	OECD 114 CIPAC guideline MT22
	This endpoint is not applicable for solids.	**Doc. III-A3; Justification**	100%	Justification
Explosive properties	From the structural formula of Sodium N-(hydroxymethyl)glycinate it can be concluded that the	**Doc. III-A3; Justification**	--	Justification

Property	Results	Reference	Comment	
			Purity/ Specification	Method
	substance does not evolve any explosive properties. Additionally This is a 50% aqueous solution. It does not exhibit a flash point therefore is not expected to be explosive in nature.			
Oxidizing properties	There are no structural indications that this is an oxidiser	**Doc. III-A3; Justification**	--	Justification
Reactivity towards container material	Minimally corrosive to steel. Product will be packaged in polyethylene drums. No interaction between the product and packaging materials is expected.	**Doc. III-A3; Study IIIA 3.17/01**	Powder, dissolved to 1% aqueous solution	ASTM G31
	There is was no deterioration of the concentration of the a.s. when stored over a 3 year period. Observation show no effect on the container material	**Doc. III-A3; Study IIIA 3.17/02**	--	--

2 MANUFACTURE AND USES

2.1 Manufacture

Biocides: Does not need to be specified for the CLH proposal.

2.2 Identified uses

Sodium N-(hydroxymethyl)glycinate is an in-can preservative (PT6), typicalyl used for the preservation of:

Washing and cleaning fluids, household

Detergents, industrial & institutional

Paints & coatings

Textiles

Adhesives

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 3_1: Summary table for relevant physico-chemical studies

Property	Method	Purity/Specification	Results	Reference
Thermal stability, identity of relevant breakdown products	Differential scanning calorimetry (During determination of the melting point)	50% aqueous solution 98 %w/w	Decomposition above ca 150 °C Decomposition above ca 200 °C	J Greenwood (May 2001) J Greenwood (2003)
	NMR - Temperature dependence of hydrolysis equilibrium	1% aqueous solution	Hydrolysis is a weak endothermic reaction	Preiß (2009)
Flammability, including auto-flammability and identity of combustion products	Justification	--	In the closed cup equilibrium method (EC A9 test) using a 50% solution no flash was observed up to the maximum temperature tested of 110°C. Testing on contact with water (EC A12) is regarded to be not necessary, as it is handled as aqueous solution, and experience is available that reaction with water is not known. There are no components in SHMG that are expected to react violently or to self ignite.	See justification Doc IIIA 3.11
Flash point 1	Closed cup equilibrium method (EC A9 test)	50% aqueous solution	No flash was observed up to the maximum temperature tested of 110°C	J Greenwood (May 2001)
Flash point 2	Justification	100%	This endpoint is not applicable for solids.	See justification Doc IIIA 3.12
Explosive properties	Justification	--	From the structural formula of Sodium N-(hydroxymethyl)glycinate it can be concluded that the substance does not evolve any explosive properties. Additionally This is a 50% aqueous solution. It does not exhibit a flash point therefore is not expected to be explosive in nature.	See justification Doc IIIA 3.15

Property	Method	Purity/Specification	Results	Reference
Oxidizing properties	Justification	-	There are no structural indications that this is an oxidiser	See justification Doc IIIA 3.16
Reactivity towards container material	ASTM G31	Powder, dissolved to 1% aqueous solution	Minimally corrosive to steel. Product will be packaged in polyethylene drums. No interaction between the product and packaging materials is expected.	Sutton Laboratories (1991)
	Company Statement	--	There is was no deterioration of the concentration of the a.s. when stored over a 3 year period. Observation show no effect on the container material	Dr. M Funk (2008)

3.1 ALL hazard classes

3.1.1 Summary and discussion of

No classification is proposed based on available data.

3.1.2 Comparison with criteria

No classification is proposed based on available data.

3.1.3 Conclusions on classification and labelling

No classification is proposed based on available data.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information - SHMG

The active substance as manufactured represents sodium hydroxymethyl glycinate (SHMG) as a 50% aqueous solution. It represents a reaction product of formaldehyde and glycine. When SHMG is diluted in water SHMG hydrolyses to formaldehyde and glycine. The high pH in the 50% solution (pH=11) or in a more diluted 5% solution slows down hydrolysis and formaldehyde release. However the hydrolysis study indicates that in unbuffered aqueous solutions of 10%, 1% and 0.25% SHMG the pH is between 10.7 and 11.7, but still about 18%, 40% and 66% were hydrolysed. Contact with biological media should lead to a reaction of formaldehyde with protein and shift the equilibrium towards further formaldehyde release. Acidic pH (like in stomach) would further support fast hydrolysis, the DT50 was smaller than 1.4 hours at pH of 4 and 7. Therefore we may theoretically assume a rate of 100% final hydrolysis in biological media. Given the molecular weight of 127 g/mol for SHMG and 30 g/mol for Formaldehyde (factor 4.23) a 50% SHMG solution corresponds to less than 12% (w/w) formaldehyde.

In use concentrations of SHMG are usually very low (0.05% to 0.25%). With such high dilution in water SHMG hydrolyses fully to formaldehyde and glycine.

ADME and dermal absorption studies have not been performed with sodium hydroxymethyl glycinate.

The toxicokinetic parameters of sodium hydroxymethyl glycinate including absorption in the gut are assumed to be the same as those of formaldehyde.

4.1.2 Non-human information – Formaldehyde

Table 4.1.2_1: Toxicokinetics and metabolism of formaldehyde

Endpoint	**Formaldehyde (for details see Appendix Formaldehyde Core Dossier)**		
	Dermal	**Inhalation**	**Oral**
Absorption	Tier1: 100 % uptake (based on ^{14}C in excreta, organs and carcass, and on in vitro data on human skin), Tier 2: 20 µg/cm^2 h or 300 µg/cm^2 h was estimated for a 3.7% or a 37% formaldehyde solution	100 % uptake (based on ^{14}C) (rodents/primates at rest: ~ 90 and 70 % in nasal passages, man/oronasal breathing: up to ~ 45 % tracheo-bronchially), systemic bioavailability below 10 % (first-pass metabolism)	100 % uptake, rapid (based on ^{14}C in exhaled air, urine and carcass), systemic bioavailability low (first-pass metabolism)
Distribution	systemic bioavailability low ^{14}C label widely distributed (introduction into C1-pool)		
Metabolism	1) Reaction with GSH followed by enzymatic conversion to formate and utilisation for C1-transfer or oxidation to CO2 2) Direct enzymatic conversion to formate and utilisation for C1-transfer or oxidation to CO2 3) Reaction with THF followed by conversion to 5-methyl or 5-formyl THF and utilisation for C1-transfer, or transformation to 10-formyl THF and release of formate or oxidation to CO2		

Endpoint	Formaldehyde (for details see Appendix Formaldehyde Core Dossier)		
	Dermal	**Inhalation**	**Oral**
	4) Adduct formation with cysteine, urea, proteins and nucleic acids Pronounced first-pass metabolism at site of entry		
Toxicologically significant metabolite	Toxicity of metabolites not assessed separately Urine: formate, hydroxymethylurea		
Rate and extent of excretion	Metabolic elimination, high, but variable rate and extent of metabolite excretion (based on ^{14}C) mainly with air and urine (initial plasma t1/2 12 h, terminal t1/2 50 h, 10-40 % ^{14}C residues after 3-4 d)		

4.1.3 Human information - SHMG

No data specific for SHMG are available.

4.1.4 Summary and discussion on toxicokinetics

No data specific for SHMG are available. However it can be considered that SHMG hydrolyze to formaldehyde and glycine with contact to biological tissues and with dilution in aqueus media and with acidic pH (as in stomach).

For formaldehyde 100% absorption via all routes of exposure has to be assumed, though predominantly reaction products and metabolites of formaldehyde will be systemically available.

The oxidation of formaldehyde to formic acid catalysed by formaldehyde dehydrogenase is considered to be the main defence mechanism against the formation of covalent binding of formaldehyde to macromolecules like proteins or DNA. Formaldehyde is eliminated rapidly as formic acid in the urine or as CO2 in the expired air or it enters the carbon anabolism in the body.

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Table 4.2.1.1: Acute toxicity: oral - SHMG

Route	**Method Guideline**	**Species Strain Sex no/group**	**dose levels duration of exposure**	**Value LD50/ LC50**	**Reference**
Oral, gavage	OECD Guidelines for Testing of Chemicals, Number 401	Wistar albino rats (5/sex/group)	Dose rates, 500, 750, 1000 and 1500 mg/kg bw of SHMG powder as 50% aqueous solution. Observed 1,2 and 4 hours post treatment and once daily for 14 days.	1100 mg/kg bw (calculated pure a.s. excluding any water) 2200 mg/kg bw (tested a.s. as manufactured as aqueous	ISP 1997, Single Dose Oral Toxicity in Rats/LD50 in Rats, Report No: MB97-5686.01, Doc IIIA 6.1.1/01

Route	Method Guideline	Species Strain Sex no/group	dose levels duration of exposure	Value LD50/ LC50	Reference
				solution)	
Oral, gavage	similar to OECD 401	BR Sprague-Dawley Albino (5/sex/group)	600, 700, 810, 950, 1100 and 1280 mg/kg bw SHMG powder as 25% w/v aqueous solution. Observed daily for 14 days post treatment.	1070 mg/kg bw (calculated pure a.s. excluding any water) 2140 mg/kg bw (calculated a.s. as manufactured as 50% aqueous solution)	ISP (1979) Approximate Oral Toxicity (LD50) in Rats. Project # 6185a, Doc IIIA 6.1.1/02
Oral	similar to OECD 401	Rat 10/group	1000, 1400, 1600, 1800, 2000 & 2200 mg/kg bw SHMG powder (test-concentration not reported in study report)	1410 mg/kg bw (pure a.s. excluding any water) 2820 mg/kg bw (a.s. as manufactured as aqueous solution)	ISP (1979) Sutocide A - Oral LD50 in Rats; Project ID: H-9304, Doc III A6.1.1/03
Oral gavage	similar to OECD 401	Wistar Albino Rat; 5/sex/group	1000, 2500, 200, 5000 mg/kg bw of SHMG 50% aqueous solution	1050 mg/kg bw (calculated pure a.s. excluding any water) 2100 mg/kg bw (tested a.s. as manufactured as aqueous solution)	ISP (1992) Single Dose Oral Toxicity in Rats / LD50 in Rats; Project # MB92-1554A Research Protocol # 66-03; Doc IIIA, 6.1.1/04

4.2.1.2 Acute toxicity: inhalation

Table 4.2.1.2: Acute toxicity: inhalation - SHMG

Route	Method Guideline	Species Strain Sex no/group	dose levels duration of exposure	Value LD50/ LC50	Reference
Inhalation whole body exposure	FIFRA 40 CFR 158, Guideline Reference #81-3	Ten Wistar albino rats (5/sex)	aerosol of SHMG powder at 2.3 mg/L. MMAD ~ 7 µm, GSD~ 2 µm 4 hours exposure Signs of toxicity and pharmacologic effects	> 2.3 mg/L (tested pure a.s. excluding any water) >4.6 mg/L (calculated a.s. as manufactured	ISP (1997) Acute Inhalation Toxicity in Rats/LC 50 in Rats. Project # MB97-5686.05 Research Protocol # 318-10, Doc IIIA 6.1.3/01

Route	Method Guideline	Species Strain Sex no/group	dose levels duration of exposure	Value LD50/ LC50	Reference
			were observed during the exposure at 1 hour post-treatment and once daily for 14 days	as aqueous solution)	
Inhalation whole body exposure	FIFRA 40 CFR 158, Guideline Reference #81-3	Rats Sprague Dawley 15 rats: 5/sex/dose group 3 dose groups	4.9, 5.92 and 6.91 mg/L aerosol of SHMG 50% aqueous solution MMAD ~ 2.5 µm, GSD 1.7-1.8 µm 4.5 hours exposure; 14 days post exposure observation	3 mg/L (calculated pure a.s. excluding any water) 6 mg/L (tested a.s. as manufactured as aqueous solution)	ISP (1992), EPA Acute Inhalation Toxicity in Rat – Defined LC50 Laboratory Project ID T-1557; Doc IIIA 6.1.3.02

4.2.1.3 Acute toxicity: dermal

Table 4.2.1.3 Acute toxicity: dermal - SHMG

Route	Method Guideline	Species Strain Sex no/group	dose levels duration of exposure	Value LD50/ LC50	Reference
Dermal	similar to OECD 402	New Zealand white rabbits (5/sex/group)	2000 mg/kg bw of moistened SHMG powder for 24 hours under occlusion. The rabbits were observed for signs of toxicity and pharmacologic effects at 1, 2 and 4 hours post-treatment and once daily for 14 days.	>2000 mg/kg (pure a.s. excluding any water) >4000 mg/kg (a.s. as manufactured as aqueous solution)	ISP (1997) Acute Dermal Toxicity in Rabbits / LD50 in Rabbits. Research Project # MB97-5686.02 Research Protocol # 175-04; Doc IIIA 6.1.2/01
Dermal	similar to OECD 402	New Zealand white rabbits (5/sex/group)	2000 mg/kg bw SHMG (powder). 24 hours exposure under occlusion, observed daily for 14 days post treatment	>2000 mg/kg (pure a.s. excluding any water) >4000 mg/kg (a.s. as manufactured as aqueous solution)	ISP (1979) Acute Dermal Toxicity in Rabbits; Food & Drug Research Laboratories, Inc. Lab Project ID: 6185a; Doc IIIA 6.1.2/02

4.2.1.4 Acute toxicity: other routes

No data.

4.2.2 Human information

No data.

4.2.3 Comparison of acute toxicity data of SHMG and the hydrolysis product formaldehyde

Table 4.2.3: Comparison of acute toxicity data of the active substance and its components

Endpoint	**SHMG** (as manufactured, ~50% aqueous solution)	**SHMG** (100%)	**Formaldehyde (FA)**
Acute oral toxicity	Rat LD_{50} = 2200 mg/kg bw (tested as 50% aqueous solution) corresponding to 260 mg formaldehyde/kg bw	Rat LD_{50} = 1100 mg/kg bw (calculated) corresponding to 260 mg formaldehyde/kg bw	category 3: LD50 = 50 - 300 mg/kg bw day
Acute dermal toxicity	Rat LD_{50} > 4000 mg/kg bw (calculated) corresponding to >472 mg formaldehyde/kg bw	Rat LD_{50} > 2000 mg/kg bw (tested as moistened a.s. powder) corresponding to >472 mg formaldehyde/kg bw	category 3: LD50 = 200 - 1000 mg/kg bw day corrosive
Acute inhalation toxicity	>4.6 mg/L (calculated) corresponding to > 0.54 mg formaldehyde/L 6 mg/L (tested as 50% aqueous solution) corresponding to 0.7 mg formaldehyde/L	> 2.3 mg/L corresponding to > 0.54 mg formaldehyde/L (tested as solid aerosol) corresponding to > 0.54 mg formaldehyde/L	category 3: LD50 = 2 - 10 mg/L

Detailed data on formaldehyde are presented in the Formaldehyde Appendix. The LD50 values appear to be in the same (classification) range for formaldehyde and SHMG maximal releasable formaldehyde. However the LD50 values of tests with formaldehyde and SHMG are difficult to compare since tested concentrations were different (oral studies) or no information on applied concentration is available for formaldehyde (dermal).

4.2.4 Summary and discussion of acute toxicity

In acute toxicity studies local irritation at the site of first contact is the main effect for all routes of exposure. The following studies were submitted: 4 studies on acute oral toxicity and 2 studies on acute dermal toxicity and 2 studies for acute inhalation toxicity. LD50 values and LC50 values calculated for SHMG as manufactured (50% aqueous solution) are above classification limit values.

If calculated for SHMG 100% the oral LD50 values are within the range of oral acute category 4. This calculation approach is uncertain, since 100% SHMG is likely to have different irritant or corrosive properties than the 50% solution that was tested, and this difference in local effects may significantly influence LD ranges. However classification based on the calculated LD50 range for

oral category 4 is nevertheless proposed, due to the absence of further information and from a formal point of view and with regard to the harmonised classifications of other formaldehyde releasers that considered acute systemic toxicity relevant for strong irritant to corrosive substances.

The respiratory LC50 for 100% SHMG, tested as solid aerosol, is above 2.3 mg/L. In the absence of further data no acute respiratory toxicity classification is proposed.

The dermal LD50 value for 100% SHMG, tested as moistened powder, is above 2000 mg/kg bw and thus there is clear evidence that classification for acute dermal toxicity is not required.

4.2.5 Comparison with criteria

In rats the acute oral LD50 is 1100 mg/kg bw for the pure a.s. excluding any water which corresponds to 2200 mg/kg bw for the a.s. as manufactured as aqueous solution (ISP 1997, Doc III A6.1.1/01). Considering the oral category 4 classification range of LD50 > 300 and ≤ 2000 mg/kg bw, respective classification for the pure a.s. with H302: Harmful if swallowed appears adequate.

The LC50 (4 h) in inhalation studies is > 2.3 mg/L for the pure SHMG excluding any water (~ 4.6 mg/L SHMG 50% solution) and 6 mg/L for SHMG as manufactured (50% aqueous solution (ISP 1997, Doc III A6.1.3/01, ISP 1992, Doc III A6.1.3/02). Concentrations below 20 mg/L could lead to classification for acute respiratory toxicity. However considering that no lethality was observed at the top concentration level and no further data are available, no classification is proposed.

Dermal exposure LD50 is > 2000 mg/kg for the pure a.s excluding any water corresponds to >4000 mg/kg for the a.s. as manufactured as aqueous solution (ISP 1997, Doc III A6.1.2/01). No classification is proposed considering that no lethality was observed at the top dose level and this level coincides with the upper border of the range leading to dermal category 4 classification.

4.2.6 Conclusions on classification and labelling

Classification for oral acute toxicity category 4, H302 - Harmful if swallowed, is required.

Classification for acute inhalation or dermal toxicity is not necessary.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Based on 4 studies on acute oral toxicity and 2 studies each on acute dermal and inhalation toxicity the DS proposed classification as oral acute toxicity category 4 (H302 - Harmful if swallowed) and no classification for the dermal and inhalation route.

The acute oral LD_{50} is 1100 mg/kg bw in rats for the pure SHMG excluding any water, which corresponds to 2200 mg/kg bw for the substance as manufactured, i.e. as a 50% aqueous solution.

The LC_{50} (4 h) in inhalation studies was > 2.3 mg/L for the pure (solid) SHMG powder excluding any water (~ 4.6 mg/L SHMG 50% solution) and estimated to be 6 mg/L aerosol from SHMG as manufactured (50% aqueous solution). The DS recognised that concentrations below 20 mg/L could lead to classification. In the CLH report the DS argued that no lethality was observed at 6 mg/L, no classification was suggested (however, see section below).

The acute dermal LD_{50} is > 2000 mg/kg, for the pure SHMG was tested as moistened powder, which corresponds to > 4000 mg/kg for SHMG as manufactured as aqueous solution. No classification was proposed by the DS considering that no lethality was observed at the top dose level and this level coincides with the upper value of the acute toxicity estimate for classification as dermal category 4.

Comments received during public consultation

One company/manufacturer commenter proposed classification as acute toxicity category 4 for the oral (H302 - Harmful if swallowed) and also for inhalation route (H332 - Harmful if inhaled). The proposal was mainly based on the studies testing the solid material.

In their response, the DS recommended RAC to consider the proposal by the company/manufacturer for acute inhalation category 4, recognising that the DS´s initial proposal, which relied on data for the dry powder only, may need correction.

Assessment and comparison with the classification criteria

RAC agrees with the DS' proposal that the classification as **oral acute toxicity category 4 (H302 - Harmful if swallowed)** is warranted with an **ATE (oral) value of 1050 mg/kg bw** for the pure SHMG is adequate.

The LC_{50} (4 h) in inhalation studies was > 2.3 mg/L for the pure (solid) SHMG powder excluding any water (corresponding to ~ 4.6 mg/L SHMG 50% solution) and estimated to be 6 mg/L (aerosol) from SHMG as manufactured (50% aqueous solution).

In the inhalation study on SHMG as 50% aqueous solution (Doc IIIA 6.1.3.01), the DS' statement that no lethality was observed contrasts with the study report which says that 10% mortality by day 2 was seen at 4.9 mg/L, 70% mortalities were seen at 5.92 mg/L before day 7 and at 6.91 mg/L within 24 hours.

More weight is given to the aerosol study in comparison to the inhalation study on solid material (Doc IIIA 6.1.3.01) that up to 2.3 mg/L solid material did not show mortalities. Particle size (MMAD) of the solid test substance was 7 µm ± GSD of 2.06 µm. The test material was milled to less than 4 µm. No information, however, on the resulting MMAD and GSD is given in the report. The calculated corresponding concentration of 4.6 mg/L for a 50% aqueous solution (Table 4.2.3 of the CLH report) may be at the edge of lethality as the first mortalities in the aerosol study started to occur at 4.9 mg/L. Thus, the lack of mortality in the study on solid test material could be considered as consistent to the aerosol study.

Therefore, in contrast to the original DS proposal, RAC considers appropriate the classification as acute inhalation toxicity category 4 (LC_{50} > 1 and ≤ 5 mg/L for dust/mist) based on the estimated LC_{50} value of 6 mg/L from SHMG (50% aqueous solution). Exposure duration of 4.5 hours would, after correction to 4 h- values, not change the category, but leads to a corrected LC_{50} value of 6.75 mg/L that corresponds to ~3.3 mg/L for the pure substance. RAC agrees on 3.0 mg/L as ATE value.

In conclusion, RAC considers appropriate to add the classification as **acute inhalation toxicity category 4 (H332 - Harmful if inhaled)** with an **ATE (inhalation) value of 3.0 mg/L**.

RAC agreed with **no classification for dermal acute toxicity**. The LD_{50} > 2000 mg/kg

bw of the moistened solid SHMG is above the limit concentration for category 4 (≤ 2000 mg/kg bw).

Supplemental information - In depth analyses by RAC

For information only: formaldehyde has a minimum classification in CLP, Annex VI for acute oral toxicity in category 3, (H301 – Toxic if swallowed), for acute dermal toxicity category 3 (H311 – Toxic in contact with skin) and for acute inhalation toxicity category 3 (H331 – Toxic if inhaled).

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

No specific STOT SE effects on organs (H370, H371) or with regard to STOT SE 3 respiratory irritation (H335) or drowsiness or dizziness (H336) were observed.

Besides irritant effects at the site of contact no other specific target organ toxicities were observed or expected.

Therefore no classification is required.

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

Summary of the Dossier Submitter's proposal

Respiratory tract irritation from inhalation exposure is to be expected. In the absence of specific data and the DS' proposal to classify for skin and eye irritation, no additional classification for STOT SE 3 was proposed by the DS.

Comments received during public consultation

One MSCA proposed to classify for respiratory tract irritation. In their argumentation, SHMG as manufactured corresponds to 12% maximal releasable formaldehyde which is within the respiratory tract irritation range of SLCs (STOT SE 3; H335: C ≥ 5%).

Assessment and comparison with the classification criteria

During the first 24 h after exposure to 4.9 mg/L SHMG aerosol, irregular respiration, gasping, rales and laboured respiration were observed (acute inhalation study, Doc IIIA 6.1.3.01). Clinical signs of respiratory tract irritation were not interpreted as exclusive indication of (sub-)lethality since the death rate at this dose was 10% and animals recovered by day 10. Based on these observed effects, RAC considers that classification as **STOT SE 3 (H335 - May cause respiratory irritation) is warranted** for SHMG.

4.4 Irritation

4.4.1 Skin irritation

4.4.1.1 Non-human information

Table 4.4.1.1_1: Skin irritation - SHMG

Species	**Method**	**Average score 24, 48, 72 h**		**Reversibility yes/no**	**Result**	**Reference**
		Erythema	**Edema**			
Skin – rabbit	Similar to OECD 404, but 6 rabbits; Test article: SHMG powder, moistened Klimisch score 2, GLP	24h = 0.33 48h = 0 72h = 0	24h = 0 48h = 0 72h = 0	Yes	**dermal irritation below classification** criteria for the moistened powder	ISP (1997) Primary Dermal Irritation in Rabbits; Research Project # MB97-5686.03 Research Protocol # 182-0; Doc IIIA 6.1.4/01
Skin – rabbit	Probably similar to OECD 404, but very poor method description; Test article: SHMG powder, not moistened Klimisch score 3, non-GLP	24h = 1 72h = 0	24h = 0.5 72h = 0	Yes	**dermal irritation below classification** criteria for the dry powder	ISP (1979) Primary Skin Irritation in Rabbits, H8713; Doc IIIA 6.1.4/02
Skin – rabbit	Similar to OECD 404 with deviations: 24 hour exposure, 6 rabbits; Test article: SHMG powder, not moistened Klimisch score 2, non-GLP	24h = 1. 5 72h = 0.4	24h = 1.2 72h = 0.2	Not fully, but only analysed till 72 hours	**dermal irritation below classification** criteria for the dry powder	ISP (1979) Primary Skin Irritation in Rabbits, 6261a-1; Doc IIIA 6.1.4/03
Skin – rabbit	Probably similar to OECD 404, but very poor method description; Test article: SHMG powder in 5% aqueous solution	24h = 0 72h = 0	24h = 0 72h = 0	-	**no dermal irritation** for the 5% aqueous solution	ISP (1979) Primary Skin Irritation in Rabbits, H8713A; Doc IIIA 6.1.4/04

	Klimisch score 3, non-GLP					
Skin – rabbit	Similar to OECD 404 with deviations: 24 hour exposure, no 48h data, 6 rabbits; Test article: SHMG powder, in 5% aqueous solution Klimisch score 2, non-GLP	24h: 3.2 72h: 1.2	24h: 2.7 72h: 0.6	Not fully, but only analysed till 72 hours	**dermal irritation** for the 5% aqueous solution	ISP (1979) Primary Skin Irritation in Rabbits, 6261a-2; Doc IIIA 6.1.4/06
Skin – rabbit	Similar to OECD 404 with deviations: 24 hour exposure, no 48h data Test article: SHMG powder, in 5% aqueous solution Klimisch score 2, non-GLP	24h: 0 72h: 0	24h: 0 72h: 0	-	**no dermal irritation** for the 5% aqueous solution	ISP (1980) Primary Skin Irritation in Rabbits, 04516; Doc IIIA 6.1.4/07
Skin – rabbit	Similar to OECD 404 with deviations: 24 hour exposure, no 48h data Test article: SHMG powder undiluted, in 0.5% and 5% aqueous solution Klimisch score 2, GLP	Undiluted 24h and 72h: 0 0.5% and 5%: 24h: 1 72h: 0	Undiluted 24h and 72h: 0 0.5% 24h and 72h: 0 5%: 24h: 0.33 72h: 0	Yes	**dermal irritation below classification** criteria for the dry powder, 0.5% and 5% aqueous solution	ISP (1984) Primary Dermal Irritation in Rabbits, 8158; Doc IIIA 6.1.4/05

4.4.1.2 Human information

No information available.

4.4.1.3 Comparison of skin and eye irritation data of SHMG and the hydrolysis product Formaldehyde

Table 4.4.1.3_1 Comparison of the active substance and its components

Endpoint	**SHMG** (100%)	**Formaldehyde**

eye damage/irritation	Serious eye irritation of SHMG tested as ~50% aqueous solution, corresponding to less than 12% formaldehyde)	Severely eye irritating or serious eye damage opacity of the cornea following application of aqueous formaldehyde solutions with concentrations between 7 and 15 %.
skin irritation/corrosion	5% aqueous solution were not irritating in the animal experiment (corresponding to less than 1.2% formaldehyde) WoE conclusion for SHMG as manufactured (50% aqueous solution, 12% maximal releasable formaldehyde) considering available data, hydrolysis and eye irritation data): Skin irritation WoE conclusion for SHMG 100% (calculated without water, 24% maximal releasable formaldehyde) considering maximal releasable formaldehyde and absence of consistent data: Skin irritation (like SHMG as manufactured, 50% aqueous solution)	concentrations of 7-9% caused erosions on the rat skin and a 1% solution still caused irritation in 5% of humans. WoE conclusion for 25-55% formaldehyde in aqueous solution: Causes burns

Detailed data on formaldehyde are presented in the Formaldehyde Appendix. Available skin irritation data for SHMG do not indicate irritation in animal studies with SHMG-solutions up to 5% which corresponds to about 1.2% maximal releasable formaldehyde. This appears to be in line with available data on skin irritation of formaldehyde. For SHMG as manufactured (50% aqueous solution, 12% maximal releasable formaldehyde) no skin but just eye irritation test data are available. The latter indicate serious eye irritation. For formaldehyde serious eye irritation or eye damage is reported in animals at concentrations between 7 and 15 %. The studies for SHMG and formaldehyde in aqueous solution cannot be directly compared since for the free formaldehyde solution reversibility was not tested or tested only till one week. The data are not conclusive with regard to potential quantitative differences in reactivity of free formaldehyde and formaldehyde released from SHMG.

4.4.1.4 Summary and discussion of skin irritation

The skin irritation test data summarized above for the SHMG in dry form as well as in the form of a 0.5% and 5% aqueous solution support that these forms do not qualify for classification for skin irritation EU CLP category 2, or are in the worst case borderline to classification.

The high pH in the 0.5% or 5% solution or resulting from solving SHMG in physiological fluids slows down hydrolysis and formaldehyde release. However the hydrolysis study indicates that in unbuffered aqueous solutions of 10%, 1% and 0.25% the pH is between 10.7 and 11.7, but nevertheless about 18%, 40% and 66% were hydrolysed. Contact with biological media should lead to a reaction of formaldehyde with protein and shift the equilibrium towards further formaldehyde release. Therefore we may theoretically assume a rate of 100% final hydrolysis. Given the molecular weight of 127 g/mol for SHMG 30 g/mol for Formaldehyde (factor 4.23) a 5% SHMG solution corresponds to less than 1.2% (w/w) formaldehyde. Therefore (and furthermore

considering the limited reproducibility of testing methods and the theoretical assumption underlying the calculation) the negative study findings in the dermal irritation studies with up to 5% SHMG are not necessarily in disagreement with the formaldehyde data. Formaldehyde is corrosive, concentrations of 7-9% caused erosions on the rat skin and a 1% solution still caused irritation in 5% of humans. 1% represents also the standard classification limit of skin corrosives for skin irritation.

Within the guinea pig skin sensitization study from 1984 (see section 4.6.1; Doc IIIA 6.1.5/02) a 50% aqueous solution of SHMG did not cause irritation in the negative control animals and this was also observed in the guinea pig skin study from 1997 (IIIA 6.1.5/01) where moistened powder of SHMG was used for challenge. These findings in the skin sensitization study may support that also SHMG 50% and 100% are not skin irritating for guinea pigs.

4.4.1.5 Comparison with criteria

In summary from the data available and the theoretical considerations of hydrolysis and MW ratios of SHMG and formaldehyde it may be concluded that SHMG products with active substance concentrations up to 5% (w/w) do not need to be classified for skin irritation (erythema and oedema average scores below 2.3).

For SHMG 50% and 100% data potentially relevant for estimating skin irritation are available within the skin sensitization studies using guinea pigs. However SHMG as manufactured (50% w/w solution) was not tested for skin irritation in the standard rabbit or standard in vitro tests and the theoretical considerations given in section 4.1.1. (i.e. up to 12% formaldehyde formed by hydrolysis of SHMG) relate to a formaldehyde concentration within the skin irritation range (see SLCs for skin corr. and skin irrit. in CLP Annex VI[4]). The eye irritation study with 50% (w/w) SHMG supports eye irritation but not eye corrosion, which also supports a conclusion of rather skin irritation than skin corrosion.

With moistened or dry powder, hydrolysis and reaction kinetics may have limited skin irritation effects to levels below the need for classification (erythema and oedema average scores below 2.3). This is mechanistically not clear, but since the biocidal active substance as manufactured is 50% (w/w) SHMG, no further investigation of these aspects appears necessary for the context of the biocides regulation. With regard to the CLP regulation it seems adequate to classify SHMG 100% based on a weight of evidence and expert judgment (Annex I, point 1.1.1.) similarly for skin irritation (rather than no classification), since otherwise standard classification rules would not lead to classification of diluted products and this would appear not adequate considering increased formaldehyde release in aqueous solutions and from contact with biological material (for details of hydrolysis, pH and temperature dependence see Doc IIIA 7.1.1.1.1).

4.4.1.6 Conclusions on classification and labelling

Consequently based on a total weight of evidence approach classification for skin irritation is proposed for the SHMG as manufactured (50% w/w solution) as well as for SHMG 100%.

---

[4] Skin Corr. 1B; H314: C ≥ 25 %; Skin Irrit. 2; H315: 5 % ≤ C < 25 %; Eye Irrit. 2; H319: 5 % ≤ C < 25 %; STOT SE 3; H335: C ≥ 5 %; Skin Sens. 1; H317: C ≥ 0,

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

No study on the SHMG as the marketed (50% aqueous solution) is available. There are skin irritation test data in rabbits for the SHMG in dry form as well as in the form of a 0.5% and 5% aqueous solution, which showed weak (below classification) dermal irritation (cf. CLH report, Table 4.4.1.1_1 - Skin irritation).

The DS, in a weight of evidence approach, considered the theoretical hydrolysis rates, pH values of unbuffered aqueous solutions, as well as information from skin sensitisation studies in guinea pigs, and proposed classification for skin irritation, category 2, for SHMG as manufactured (50% w/w aqueous solution) as well as for SHMG 100%.

Comments received during public consultation

One Industry/trade association considered no classification for skin irritation as appropriate. They questioned the relevance of theoretical concentrations of formaldehyde after hydrolysis and relied their argumentation on the lack of significant irritation by SHMG from the available skin irritation tests, as well as the lack of irritation effects in the sensitising studies on guinea pigs. In a pilot study (Doc IIIA 6.1.5.0.2) on sensitisation, skin irritation was analysed after topical application with 25%, 50%, 75% and 100% concentration (in distilled water) (no data on exposure duration and conditions). No erythema and no oedema were observed.

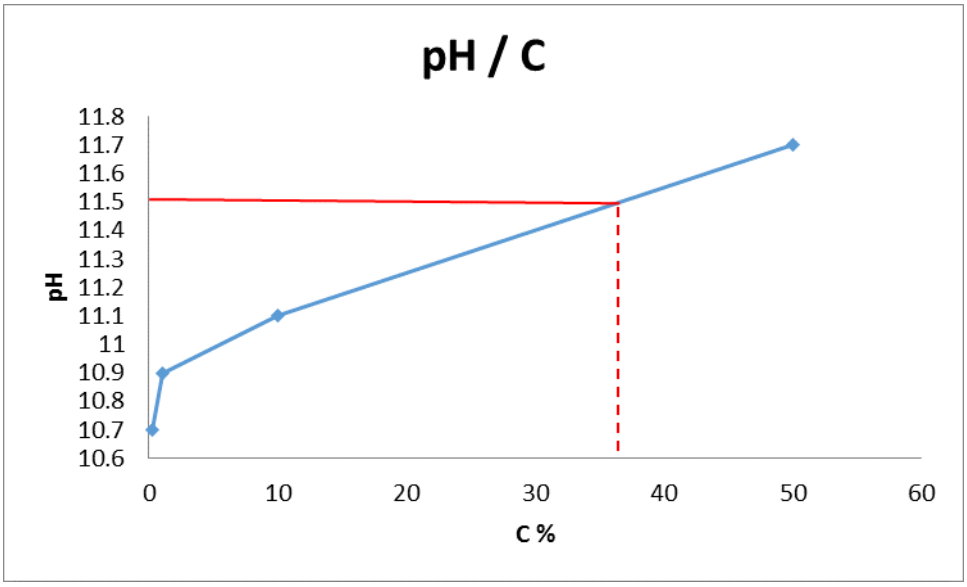
Assessment and comparison with the classification criteria

RAC notes the lack of skin irritation data for the SHMG as marketed (50% solution), and the lack or low-level effects from the dry/moistened powder, 0.5 and 5% solutions, which would not justify classification for skin irritation.

RAC acknowledges the theoretical considerations of the DS. Different lines of evidence need to be considered:

- The SHMG 5% aqueous solution showed weak irritative effects with scores below the mean value of 2.3 for the 24, 48, 72 hours after removal. RAC assumes SHMG as 50% aqueous solution to exert stronger irritative effects.
- Testing of dry material SHMG powder, not moistened or moistened, induced mild irritation (below classification criteria). RAC considers testing of dry material as less predictive for the human situation due to the non-sweating conditions of the rat skin.
- RAC notes the measured data revealing high pH values of ≥ 10.7 in un-buffered SHMG aqueous solutions of 0.25% and above (see hydrolysis study Doc IIIA 7.1.1.1.1., Table 2). The measured pHs were 10.7 (0.25%), 10.9 (1%), 11.1 (10%) and 11.7 (50% SHMG) (see figure below). RAC also notes the CLH report does not refer to the 50% solution.

Figure: pH values at different concentrations of SHMG aqueous solution



According to CLP Guidance 3.2.2.1.2.2., pH extreme values ≥ 11.5 are expected to produce significant effects on the skin and classification as a corrosive should be considered. The observed pH rises with the concentration of SHMG in aqueous solution and theoretically the value of 11.5 is expected to be exceeded at concentrations > 35%.

- The DS considered that 5% SHMG may theoretically hydrolyse at an assumed 100% hydrolysis rate to 1.2% formaldehyde. Formaldehyde is classified in CLP, Annex VI as Skin Corr. 1B, the general concentration limit for classification as skin irritant is ≥ 5% (5 % ≤ C < 25 %).

  The hydrolysis test revealed that SHMG will completely hydrolyse within short time (half-time could not be estimated for the non-buffered solution and was < 1.4 h for 0.25% and 1% buffered solutions at pH 4, 7, and 9). Following the assumption that the hydrolysis product formaldehyde is produced, 50% SHMG could at maximum produce formaldehyde concentrations of 12%. Based on the formaldehyde production, SHMG as a 50% aqueous solution should be classified as skin irritant.

- The DS did also take the information from a maximisation test (GPMT) into account (Doc IIIA 6.1.5.02). The dermally applied challenge dose (topically exposed during 24 hours) of 50% aqueous solution of SHMG produced very slight to grade 4 erythema indicative of the sensitising potential. Erythema is here considered as the response of senstising properties of the previously interdermally applied SHMG. The available information in guinea pigs on the range of grades for the erythema is not robust to assess skin irritation properties, as this study type is not accepted for the endpoint skin corrosivity/irritation. However, it should be noted (as supplementary information) that no corrosive effects were noted at

challenge concentrations up to 50%. Skin irritation was not seen in a pilot study (to estimate the irritative concentration) with 24%, 50%, 75% and 100% aqueous concentrations. However, the study design (no data on exposure duration) and reporting is insufficient compared with standard studies on skin irritation.

Another Guinea pig (Buehler) study (Doc IIIA 6.1.5.01) was considered less informative since the test substance was the moistened powder.

In general, CLP guidance questions the relevance of Guinea pigs to predict the irritative properties due to the low sensitivity of Guinea pigs (Guinea pigs < rats < rabbits).

Considering the available data, the elements of evidence and that no clear indication of corrosivity was observed in the eye irritation tests, RAC regards the pH-values alone insufficiently convincing to conclude on the corrosivity of SHMG. RAC, however, takes into consideration that high pH values may be generated at higher SHMG concentrations and may contribute to the irritating effects of SHMG.

In a weight of evidence approach, taking into consideration the mild irritation observed with a SHMG 5% aqueous solution, the lack of data for SHMG 50% aqueous solution, for which the irritative effect is expected to be stronger and likely to fulfil the classification criteria, the concern from the hydrolysis product formaldehyde (classified as a corrosive) and the high pH values in un-buffered SHMG solutions, RAC agrees with the DS proposal to classify **SHMG** as **skin irritation 2 (H315 - Causes skin irritation)**.

4.4.2 Eye irritation

4.4.2.1 Non-human information

Table 4.4.2.1_1: Eye irritation. Sodium hydroxymethyl glycinate

Species	**Method**	**Average Score:** 24/48/72h				**Reversibility yes/no**	**Result**	**Reference**
		Cornea	**Iris**	**Redness Conjunctiva**	**Chemosis**			
Rabbit	Comparable with OECD TG 405, but 6 animals, eyes not washed Test article: SHMG 50% aqueous solution (pH 11) Klimisch score 1	0	0	1.4 (2 from 6 animals ≥ 2)	1 (all animals < 2)	Yes. All irritations cleared by Day 10.	Eye irritation borderline to classification criteria for EU CLP category 2 (redness score ≥ 2 for 2/6 animals; CLP criteria: ≥ 2/3 animals)	ISP (1990). Rabbit Eye Irritation in Study, PH421-SU-002-90; Doc IIIA 6.1.4/12
Rabbit	Comparable with OECD TG 405, but 6 animals, eyes	2	0.8	2.4	2.4	Yes. All irritations cleared by Day 14.	Eye irritation within classification criteria for EU	ISP (1997) Primary Eye Irritation / Corrosion in

	not washed Test article: SHMG powder Klimisch score 2						CLP category 2 (redness and chemosis score ≥ 2 for ≥ 2/3 animals)	Rabbits MB97-5686.04; Doc IIIA 6.1.4/08
Rabbit	Expectedly comparable with OECD TG 405, but very scarce method descption Test article: SHMG powder in 5% aqueous solution Klimisch score 3	0	0	0	0	-	No eye irritation	ISP (1979) Acute Eye Irritation in Rabbits, H-8712; Doc IIIA 6.1.4/09
Rabbit	Comparable with OECD TG 405, but 6 animals, eyes not washed Test article: SHMG powder in 5% aqueous solution Klimisch score 2, non-GLP	0	0	1 (2 from 6 animals score 2)	0	Yes, till day 7	Eye irritation borderline to classification criteria for EU CLP category 2 (redness score ≥ 2 for 2/6 animals; CLP criteria: ≥ 2/3 animals)	ISP (1979) Acute Eye Irritation in Rabbits; 6261a-2; Doc IIIA 6.1.4/11

4.4.2.2 Human information

No information available.

4.4.2.3 Comparison of skin and eye irritation data of SHMG and the hydrolysis product Formaldehyde

See above, chapter 4.4.1.3.

4.4.2.4 Summary and discussion of eye irritation

The eye irritation test data summarized above for the SHMG in dry form as well as in the form of a 5% and 50% aqueous solution support that all these forms qualify for classification for eye irritation EU CLP category 2, but not category 1.

The high pH in the 50% solution (pH=11) or in the 5% solution or resulting from solving SHMG in physiological fluids slows down hydrolysis and formaldehyde release. However the hydrolysis study indicates that in unbuffered aqueous solutions of 10%, 1% and 0.25% the pH is between 10.7 and 11.7, but still about 18%, 40% and 66% were hydrolysed. Contact with biological media should lead to a reaction of formaldehyde with protein and shift the equilibrium towards further formaldehyde release. Therefore we may theoretically assume a rate of 100% final hydrolysis. Given the molecular weight of 127 g/mol for SHMG and 30 g/mol for Formaldehyde (factor 4.23) a 5% SHMG solution corresponds to less than 1.2% (w/w) formaldehyde. Therefore the fact that with

up to 5% SHMG no irreversible eye effects but only eye irritation was observed is not necessarily in disagreement with the formaldehyde data. Formaldehyde is corrosive and 0.01 mL of 7-9% formaldehyde solution in water resulted in non reversible opaque cornea in rabbits, with a 1% solution about 5% of humans showed a skin irritation.

However the absence of irreversible eye effects for SHMG as manufactured (50% w/w solution) is not necessarily expected from theoretical considerations indicating hydrolysis to formaldehyde concentrations which is in the range from skin irritation to skin corrosion (up to 12% formaldehyde). However since the hydrolysis and reaction kinetics of SHMG are complex and not fully clear and the eye irritation study with SHMG as manufactured was conducted according to GLP and actual testing methods standards the active substance as manufactured can be classified only for serious eye irritation (GHS category 2, H319).

With moistened or dry powder, hydrolysis and reaction kinetics may have limited eye effects to levels below the need for classification for irreversible effects. This is mechanistically not clear, but the eye irritation study carried out with powder indicated eye irritating effects (GHS category 2, H319). For details of hydrolysis, pH and temperature dependence see Doc IIIA 7.1.1.1.1.

4.4.2.5 Comparison with criteria

The classification criteria for eye irritation category 2 is that the mean score for 24, 48 and 72 hours post application is at least in 2 from 3 animals ≥ 2 for conjunctiva redness and/or ≥ 1 for corneal opacity and/or ≥ 1 for iritis and/or ≥ 2 for conjunctival oedema.

One rabbit eye irritation test with a 5% SHMG solution is available where no eye irritation was observed (all scores were 0, Doc IIIA 6.1.4/09), but the test is considered as not reliable.

In another rabbit eye irritation test with a 5% SHMG solution (Doc IIIA 6.1.4/11) conjunctiva redness score was borderline with regard to the above criteria: 2 from 6 animals showed a score of 2. All other endpoints were below the criteria and all irritations cleared by day 7. Considering that the criteria for classification are a conjunctiva redness score of ≥ 2 for at least 2 from 3 animals the conjunctiva score in this test may appear borderline to classification.

In the rabbit eye irritation study with SHMG as manufactured (50% aqueous solution; Doc IIIA 6.1.4/12) the conjunctiva redness score was ≥ 2 in 2 from 6 animals and an average score of 1.4. All other endpoints were below the criteria and all irritations cleared by day 10. Considering that the criteria for classification are a conjunctiva redness score of ≥ 2 for at least 2 from 3 animals the conjunctiva score in this test may also appear borderline to classification.

In the rabbit eye irritation test with SHMG powder (Doc IIIA 6.1.4/08) conjunctiva redness and chemosis average scores for 6 animals were ≥ 2, and all irritations cleared by day 14. Therefore this test supports eye irritation category 2 classification of SHMG in powder form.

However it is considered that

- no criteria are available for extrapolating from a 6 animal experiment to a 3 animal experiment,

- eye irritation experiments with animals are in principle limited in their reproducibility,

- SHMG releases formaldehyde upon contact with biological tissue

4.4.2.6 Conclusions on classification and labelling

Consequently it is concluded that SHMG as manufactured, i.e. the 50% solution and SHMG 100%, should be classified for eye irritation, category 2.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS presented eye irritation data for SHMG in the dry form (powder) and as a 5% and 50% aqueous solution (cf. CLH report, Table 4.4.2.1_1 - Eye irritation) and suggested classification for eye irritation category 2.

While no eye irritation was observed in one rabbit eye irritation test with a 5% SHMG solution (all scores were 0, Doc IIIA 6.1.4/09) that test was considered as not reliable due to a very scarce method description. In another rabbit eye irritation test with a 5% SHMG solution (Doc IIIA 6.1.4/11), conjunctiva redness score of 2 in 2/6 animals were observed, which was considered borderline with regard to the classification criteria (≥2/3 animals) . In the rabbit eye irritation study with SHMG as manufactured (50% aqueous solution, Doc IIIA 6.1.4/12) the conjunctiva redness score was ≥ 2 in 2/6 animals and the redness conjunctiva average (24/48/72 hour) score was 1.4. All other endpoints were below the criteria and all irritations cleared by day 10. Considering that the criteria for classification are a conjunctiva redness score of ≥ 2 for at least 2/3 animals, the results from this test were considered by the DS as indicative of irritating effects which may be sufficient for classification.

In the test with SHMG powder (Doc IIIA 6.1.4/08), the average scores for conjunctive redness and chemosis, for the 6 animals tested, were ≥ 2 and reversible by day 14, which support the eye irritation category 2 classification.

Comments received during public consultation

One company/manufacturer commenter supported eye irritation category 2 based on the available studies. One MSCA also supported this classification.

Assessment and comparison with the classification criteria

For the observed effects (conjunctival erythema (redness) and conjunctival oedema (chemosis) the CLP guidance 3.3.2.3.2.2 indicates in case of 6 rabbits tested, classification for eye irritation category 2 applies if at least 4 out of 6 rabbits show a mean score per animal of ≥ 2. In tests on SHMG 50% aqueous solution mean scores of ≥ 2 for redness were reported in 2 out of 6 animals. These findings alone would not justify classification.

The study report (Doc IIIA 6.1.4/12) indicates that 6 rabbits eyes remained unwashed, 3 rabbits eyes were flushed for one minute with water 20-30 seconds after instillation of test material. No effects in any of the animals were observed on cornea and iris. The sum of the mean scores for conjunctiva effects were 9.7 after 1 hour, 9.0 after 24 h, 5.0 after 48 h, 3.5 after 72 h and effects were cleared by day 10.

The information given in the evaluation by RMS was that the average (24/48/72 hour) scores for redness ≥ 2 was seen in 2 (non-rinsed) animals, all other were < 2, while the average for chemosis for the same time points was 2 in one animal, for all other < 2. In this section, a copy of the original study report indicated the individual results for six (non-rinsed) animals. According to this table (6.1.4.12_1 in Doc IIIA 6.1.4/12), the 24/48/72 hour mean score was ≥ 2 for 2 but not for 4/6 animals as required by the CLP criteria. Solely from the results of this study, there would be no need for classification and DS considered the results as borderline for classification as eye irritant.

The testing of the SHMG powder (Doc IIIA 6.1.4/08) was conducted on 5 male and 4 female rabbits (cf. CLH report, Table 4.4.2.1_1) on 6 animals seems to be incorrect. The study summary in the CAR documents says that six rabbits eyes (out of the total of 9 rabbits) remained unwashed, 3 rabbits eyes washed with 20 mL distilled water 30 seconds post dose (no data on sex distribution).

The results were reported as follow: in unwashed eyes, corneal opacity, noted in 6/6 eyes with mean score 2. However, on day 7, pannus (effect indicating corrosivity) were seen in 3 eyes. Iritis, noted in 6/6 eyes with mean score 1 in 5/6, cleared by day 7. Conjunctival irritation redness and chemosis, each with mean scores ≥ 2 were noted in 6/6 eyes. All effects were cleared by day 14. These observed effects support classification as Eye Irrit. 2, while the pannus lesion in 3 eyes at day 7 indicates a corrosive effect that, however, was reversible at day 14. In washed eyes, corneal capacity and iritis, noted in 2/3 eyes, cleared by day 7. Conjunctival irritation, noted in 3/3 eyes, cleared by day 14. All effects were reported to be cleared by day 14. The mean scores in washed eyes were lower than in unwashed eyes; based on the mean scores of cornea opacity of 2, SHMG powder could be considered irritant.

RAC is aware that testing of a powder may generate particle-related irritative effects that may contribute to the severity of the test substance-related effects. For solid SHMG , this type of contribution may be difficult to assess as hydrolysis after contact with the physiological tear fluid may occur and generate formaldehyde at the site of contact.

Formaldehyde is classified as skin corrosive category 1B (which labelling covers also eye corrosivity), and classification as eye irritant category 2 is required for concentrations ≥ 5% and < 25%.

The pannus effects observed in the study on SHMG powder appeared with delay on day 7 after treatment. Although it indicates abnormal fibrovascular/granulation tissue and is an indicator for corrosivity, it was reversible until day 14 of the observation time and thus was not sufficient for corrosivity classification.

Based on SHMG irritating properties as powder, which tests showed effects consistent with the CLP criteria for classification as eye irritant category 2, the evidence from mild irritation of SHMG 50% aqueous solution and the concern from the hydrolysis product formaldehyde (classified as corrosive) RAC agrees, in a weight of evidence approach, with the DS proposal to classificaty **SHMG as Eye Irrit. 2, (H319 - Causes serious eye irritation)**.

Supplemental information - In depth analyses by RAC

For information: according to the OECD TG 405 the eyes should not be washed for at least 24 hours following instillation of the test substance, except for solids and in case of

immediate corrosive or irritating effects. At 24 hours, a washout may be used if considered appropriate. The earliest information in the available studies was at 1 hour. The results of nonwashed eyes were considered as the most relevant data (in comparison to data from rinsed eyes).

4.4.3 Respiratory tract irritation

No specific information is available for SHMG.

Due to the eye irritation properties and formaldehyde release upon contact with biological tissue in principle also respiratory tract irritation is to be expected from respiratory exposure with SHMG. However in the absence of specific data and considering the classification proposal for skin and eye irritation and carcinogenicity via respiratory exposure no additional classification for respiratory tract irritation (STOT SE 3) is proposed.

4.5 Corrosivity

See chapter 4.4.2. above.

4.6 Sensitisation

4.6.1 Skin sensititsation

4.6.1.1 Non-human information

Table 4.6.1.1_1: Sensitisation - animals: Sodium hydroxymethyl glycinate

Species	Method	Number of animals sensitized/total number of animals	Result	Reference
Guinea pig. Hartley albino	Comparable to OECD 406, Buehler test, but 10 instead of 20 dosed animals Test article: SHMG powder, moistened Klimisch score 3, GLP	10 guinea pigs Days 1, 8, 15: topical induction with moistened SHMG powder (0.4 g/ 0.1ml) 5 control animals	Day 15: after 6 hours topical induction with moistened SHMG powder (0.4 g/ 0.1ml) 24h post induction: 5/10 positive (score 0.5 each) Day 29: 6 hours challenge with moistened SHMG powder (0.4 g/ 0.1ml) 24, 48, 72 h post challenge: 0/10 positive Positive control: 10/15 positive (score ~1)	ISP (1997), MB97-5686.06; Doc IIIA 6.1.5/01
Guinea pig. Hartley albino	comparable to OECD TG 406 / GPMT Test article: SHMG in solution Klimisch score 2, GLP	10 guinea pigs Day1: Intradermal induction with 0.1ml of 5% SHMG in distilled water & 5% SHMG in FCA Day 8: topical induction with moistened powder	Day 22: 50% challenge concentration: 24h post removal: 5/10 positive (score 1 to 2) 48h post removal: 7/10 positive (score 1 to 2) Positive control DNCP: 6/6 positive Day 29: 5% and 0.5% challenge concentration: 5%, 24h post removal: 4/10 positive (score 1)	ISP (1984), No 8158; Doc IIIA 6.1.5/02

			5%, 48h post removal: 4/10 positive (score 1) 0.5%, 24h post removal: 1/10 positive (score 1) 0.5%, 24h post removal: 2/10 positive (score 1)	
Guinea pig	FIFRA Guideline 81-6 Not comparable to actual OECD TG 406 Test article: SHMG as a 0.1% solution Klimisch score 3, no GLP	8 guinea pigs Day 1 to day 10: daily intracutaneous induction with 0.1% solution in physiological saline without adjuvans	Day 24 intradermal challenge with 0.05ml 0.1% solution: 24h post removal: 0/8 animals positive (average response to the challenge injection was not greater than the average response to the induction injection, for each animal) No positive control tested.	ISP (1980), No. 10864; Doc IIIA 6.1.5/03
Guinea pig	FIFRA Guideline Reference #81-6 Not comparable to actual OECD TG 406 Test article: SHMG as a 0.25% solution Klimisch score 3, GLP	10 guinea pigs 9 topical inductions within 3 weeks with 0.25% solution in distilled water	After 5 weeks topical challenge with 0.25% solution in distilled water: 24h and 48h post challenge: 0/10 animals positive Positive control DNCP: 6/6 positive	ISP (1985), No. 8453A; IIIA 6.1.5/04

4.6.1.2 Human information

Table 4.6.1.2_1: Sensitisation - human: Sodium hydroxymethyl glycinate

Species	**Method**	**Number of animals sensitized/total number of animals**	**Result**	**Reference**
human	CFR 21, Part 50 and 56 Test article: SHMG as a 0.5% (w/v) solution in water, neutralised to pH 7 with lactic acid Klimisch score 2	102 humans 9 topical applications within 3 weeks with 0.5% solution in water, neutralised to pH 7 with lactic acid	After about 2 weeks rest topical challenge with 0.5% to previously untreated sites. 24 & 48 hours after challenge: 1/102 positives, considered as non-specific	Michael Frentzko, BA, Robert W. Shanahan, PhD & Nathan Dorman, MD (1991); Doc IIIA 6.12

A human study, engaging 102 humans with informed consent was carried out with a 0.5% SHMG solution neutralised with lactic acid to pH 7, topically applied 9 times within 3 weeks. After a rest of 2 weeks also challenge was carried out with a 0.5% SHMG solution. This stoechiometrically corresponds to formaldehyde concentrations below 0.12%. No skin irritation and no sensitizing reaction was observed with these concentrations. This observation supports the classification limit of 0.2% for formaldehyde.

4.6.1.3 Comparison of skin sensitizing data of SHMG and the hydrolysis product formaldehyde

Detailed data on formaldehyde are presented in the Formaldehyde Appendix. SHMG and Formaldehyde appear to induce skin sensitization.

Table 3.4.2_1. Comparison of the active substance and its components

Endpoint	**SHMG** (100% and 50% in aqueous solution)	**Formaldehyde**
Sensitization	Sensitizing (animal data) GPMT: 5% intradermal induction, 50% + 5% topical challenge No data to support specific concentration limit; general concentration limit for classification for SHMG 100%: 1% (corresponding to max 0.24% formaldehyde)	Sensitizing (animal and human data) Specific concentration limit for classification 0.2%

4.6.1.4 Summary and discussion of skin sensitisation

The guinea pig study from 1980 (Doc IIIA 6.1.5/03) was conducted with 10 intradermal inductions with 0.1 ml of 0.1% SHMG (without adjuvans), corresponding to a formaldehyde concentration below 0.025%. Also challenge was carried out with intradermal application of 0.1% SHMG, but with reduced volume (0.05 ml). Skin irritation was observed all animals but the average response to the challenge injection was not greater than the average response to the induction injection. Therefore it was concluded that these test conditions do not lead to skin sensitization. However due to the low doses (below the formaldehyde classification limit) the results are not reliable for the evaluation of SHMG as manufactured, which is a 50% (w/w) solution.

The guinea pig study from 1985 (Doc IIIA 6.1.5/04) was carried out with 9 topical inductions with 0.25% SHMG in distilled water, corresponding to a formaldehyde concentration below 0.06% and no irritation was observed at this concentration. Also topical challenge was carried out with this concentration and no reaction was observed in 10 animals. Therefore it was concluded that these test conditions do not lead to skin sensitization. However due to the low and non-irritant doses (below the formaldehyde classification limit) and the low number of animals (10 instead of standard 20 for the Buehler test) the results are not reliable for the evaluation of SHMG as manufactured, which is a 50% (w/w) solution.

The most reliable study is from 1984 (IIIA 6.1.5/02) where intradermal challenge was carried out with a 5% SHMG solution including also adjuvans, followed by a topical induction with moistened powder and 50%, 5% and 0.5% SHMG topical challenge concentrations. These correspond to 12%, 1.2% and 0.12% formaldehyde. Positive reactions were found with 50% and 5% SHMG solutions. No differentiation according to potency (Category 1A or 1B) is possible, since no lower intradermal induction concentrations than 5% were tested. The study appears valid and appropriate for the evaluation of SHMG as manufactured (50% solution).

In summary it may be concluded that SHMG as manufactured (50% solution) is to be classified for skin sensitization category 1.

One guinea pig study is available where moistened powder was used for topical induction (no adjuvans) and for topical challenge (Doc IIIA 6.1.5/01). Slight irritation was observed after induction, but no reaction was observed with challenge. However the positive control was weak (10 from 15 positive with score ~ 1) and 10 instead of 20 animals were used in the dose group. Anyway with moistened powder, hydrolysis and reaction kinetics may have limited the effects expected from the released formaldehyde. This is mechanistically not clear and therefore the results are not reliable for the evaluation of the active substance as manufactured, which is a 50% (w/w) solution of

SHMG. No further investigation of these data appears necessary for assessment within the biocides regulation. For details of hydrolysis, pH and temperature dependence see Doc IIIA 7.1.1.1.1.

The classification limit for formaldehyde (0.2%) stoechiometrically corresponds to a SHMG concentration of 0.85%. However considering the minimal difference to the general concentration limit of 1% and the quantitative uncertainties of the kinetics of hydrolysis and without specific data for SHMG the standard concentration limit of 1% should apply for SHMG.

4.6.1.5 Comparison with criteria

The most reliable study is the guinea pig maximisation test from 1984 (IIIA 6.1.5/02) where intradermal challenge was carried out with a 5% SHMG solution including also adjuvans, followed by a topical induction with moistened powder and 50%, 5% and 0.5% SHMG topical challenge concentrations. These correspond to 12%, 1.2% and 0.12% formaldehyde. 4 from 10 animals showed positive reactions with 5% challenge, 5 (24h post removal) and 7 (48h post removal) from 10 animals showed positive reactions with 50% challenge. This corresponds to the criteria for classification, i.e. more than 30% positive animals.

In contrast the Buehler study carried out with moistened powder in 1997 (IIIA 6.1.5/01) did not indicate a skin sensitization property (0/10 animals positive after challenge), however the study is of limited reliability. With regard to the CLP regulation it is considered adequate to classify SHMG 100% based on a weight of evidence and expert judgment (Annex I, point 1.1.1.) similarly as SHMG as manufactured (50% aqueous solution) for skin sensitization (rather than no classification), since otherwise standard classification rules would not lead to classification of diluted products this would appear not adequate considering increased formaldehyde release in aqueous solutions and from contact with biological material.

4.6.1.6 Conclusions on classification and labelling

Therefore SHMG as manufactured as well as SHMG 100% shall be classified for skin sensitization. No differentiation according to potency (Category 1A or 1B) is possible, since no lower intradermal induction concentrations than 5% were tested. The study appears valid and appropriate for the evaluation of SHMG as manufactured (50% solution) and for SHMG 100%.

RAC evaluation of skin sensitisation
Summary of the Dossier Submitter's proposal Data from a human study with 102 individuals who received 9 topical applications of 0.5% SHMG solution neutralized to pH 7 within 3 weeks did not show any irritation or sensitising reaction at a previously untreated site after another challenge following a rest of 2 weeks. The applied concentration of 0.5% SHMG solution corresponds stoechiometrically to 0.12% formaldehyde which is below the (harmonised classification) concentration limit for formaldehyde of 0.2% (as skin sensitising category 1). The DS presented the available animal (guinea pig) studies for SHMG (cf. CLH report, Table 4.6.1.1_1) and proposed the GPMT from 1984 (CAR IIIA 6.1/02) as the most reliable study. The other studies, including Buehler test from 1997, were considered not reliable due to the low number of animals (10 instead of standard 20 for the Buehler test)

and the low and non-irritant doses employed.

In the 1984 GPMT, positive reactions were observed in animals after intradermal induction with SHMG 5% solution followed by a topical induction with moistened powder at day 8 and topical challenge concentrations of 50%, 5% and 0.5% SHMG followed (corresponding to 12%, 1.2% and 0.12% formaldehyde). Positive reactions were observed at 50% SHMG (first challenge on day 22) in 5/10 animals (24 h post removal) and 7/10 animals (48 h post removal). Potency differentiation to category 1A or 1B was not possible since no induction concentration lower than 5% was tested. SHMG (both pure and as manufactured, 50% solution) are proposed by the DS to be classified as skin sensitizer category 1.

The generic concentration limit of ≥1% for skin sensitizer category 1 corresponds to 0.24 % of formaldehyde as hydrolysis product. This is slightly above the specific concentration limit of 0.2% for formaldehyde. The DS found the difference between 0.85% SHMG (which corresponds to 0.2% formaldehyde) non-significant and proposed to apply the generic concentration limit of 1% for SHMG.

Comments received during public consultation

One company/manufacturer commenter disagreed with the DS' evaluation and found the negative Buehler test with moistened powder more reliable.

Assessment and comparison with the classification criteria

A sensitising potential was identified from the GPMT (CAR IIIA 6.1.5/02) with 70% and 40% positive animals at 50% and 5% challenge concentrations, respectively, following intradermal induction with a 5% concentration. Since no lower induction concentrations were tested, a discrimination between category 1A and 1B is not possible.

Based on the observation of ≥ 30 % positive reactions and the DS' considerations on the SCL, RAC agrees with the DS proposal to classify SHMG as **skin sensitizer category 1 (H317: May cause an allergic skin reaction) with no specific concentration limits**.

4.6.2 Respiratory sensitisation

No specific data are available.

4.7 Repeated dose toxicity

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Table 4.7.1.1_1: Repeated dose toxicity: Sodium hydroxymethyl glycinate

Route	**Duration of study**	**Species Strain Sex No./group**	**Dose levels frequency of application**	**Results**	**LO(A)EL**	**NO(A)EL**	**Reference**
Oral gavage	28 day Similar to OECD 407, GLP	Sprague Dawley rats (10/sex/ group)	0, 40, 160 and 640 mg/kg/day, dosed 5% w/v in water	High dose: 1 female mortality; significantly ↓ total protein (m + f); focal subacute gastritis and focal ulceration of glandular stomach in a few animals (m + f); significantly ↓ body weight on day 14 and 21 (m, ≤ 8%) mid and low dose: No significant adverse effects	640 mg/kg bw day as 5% solution	160 mg/kg bw /day as 5% solution	ISP (1990) 28 Day Oral Toxicity Study – Rat Study No. PH 436-SU-001-90; Doc IIIA 6.3.1
Oral gavage	90 day	Sprague Dawley rats (10/sex/ group)	0, 10, 40 and 160 mg/kg/day dosed 2% w/v in water	No adverse effects were observed	No value	160 mg/kg/day as 2% solution	ISP (1984) Suttacide A – 90 Day Oral (Gavage) Toxicity Study in Rats; Study No. 7824; Doc IIIA 6.4.1/01

4.7.1.2 Repeated dose toxicity: inhalation

No data are available.

4.7.1.3 Repeated dose toxicity: dermal

No data are available.

4.7.1.4 Repeated dose toxicity: other routes

No data are available.

4.7.1.5 Human information

No Information is available.

4.7.1.6 Other relevant information: Comparison of repeated dose toxicity data for SHMG and the hydrolysis product formaldehyde

Table 4.7.1.62_1: Comparison of the active substance and its components

Parameters	**SHMG**	**Formaldehyde**
Oral exposure	Gavage (aqueous solution)	Via drinking water
Study duration Target organs	**28 days** dominant local effects 1 mortality (f); significantly ↓ total protein (m + f), focal subacute gastritis and focal ulceration of glandular stomach in few animals (m+f), significant body weight ↓ (d14, d21, m $\leq$ 8%)	**28, 90 days** limited data
Species LOAEL in mg/kg bw/day NOAEL in mg/kg bw/day	Rat 640 (dosed as 5% solution) 160 (dosed as 5% solution)	
Study duration Target organs Species LOAEL in mg/kg bw/day NOAEL in mg/kg bw/day	**90 days** no adverse effects Rat >160 (dosed as 2% solution) 160 (dosed as 2% solution) 160 mg/kg bw day SHMG corresponds to approx. 38 mg/kg bw day max releasable formaldehyde 2% SHMG corresponds to approx. 0.47% max releasable formaldehyde.	**2 years** dominant local effects Rat 82 (m) or 109 (f) (0.19%) 15 (m) or 21 (f) (0.026%)
Dermal exposure Study duration Species LOAEL (mg/kg bw/day) NOAEL (mg/kg bw/day)	No data Local effects expected	Local effects *, data not sufficient for assessment
Inhalation exposure effects target organs Study duration Species LOAEC (mg/m³) NOAEC (mg/m³)	No data Local effects expected	Local effects - eye irritancy long term (lit. review) human 0.12

*: limited validity

Detailed data on formaldehyde are presented in the Formaldehyde Appendix. The repeated dose toxicity data available for SHMG and formaldehyde are not fully comparable, since for SHMG only 28 day and 90 day gavage studies are available and for formaldehyde the 28 day and 90 day studies were drinking water studies that were considered of limited reliability in the formaldehyde core dossier. However it appears that for both substances local effects were dominant. The 90 day rat study NOAEL of SHMG is in the range between the 2 year rat study NOAEL and LOAEL of formaldehyde, if compared on the basis of molar formaldehyde equivalents (38 mg/kg bw day max releasable formaldehyde is between NOAEL of 15 and LOAEL of 82 mg/kg bw day formaldehyde) and fits well with the 2 year formaldehyde NOAEL after correction for subchronic to chronic exposure time extrapolation (assessment factor 2). If compared on the basis of NOAECs and corrected for sub-chronic to chronic exposure the SHMG-maximal-released formaldehyde NOAEC

was in the magnitude of the free formaldehyde LOAEC, i.e. the free formaldehyde may be considered as slightly more reactive, but this may also fall within the reproducibility, extrapolation and study interpretation uncertainties.

For SHMG no data for repeated dose dermal exposure or repeated dose respiratory exposure are available. However dominant local effects are expected and the available data for formaldehyde (including human data) will be used for AEL derivation and risk assessment.

4.7.1.7 Summary and discussion of repeated dose toxicity

28 day study:

Sprague Dawley rats (10/sex/group) were dosed orally by gavage with 0, 40, 160 and 640 mg/kg/day of the active ingredient SUTTOCIDE® A as 5% (w/v) aqueous solution for 28 consecutive days according to standard OECD testing requirements and GLP. No clinical signs indicative of systemic toxicity were observed in this study. All animals except one female in the high dose group survived the duration of the treatment. The death was considered to be partly the result of a technical dosing error but gross and microscopic pathology revealed damage to the stomach. Therefore, a test material effect could not be ruled out. Statistically significant decreases were observed in the male group mean body weights for the 640 mg/kg/day dose group on Days 14 and 21. Female body weights and bodyweight gains were not affected. At this highest dose also significantly lower total protein values were found in male and female rats as well as non significantly reduced lower mean haemoglobin and haematocrit values in males and focal subacute gastritis and focal ulceration of glandular stomach in a few males and females. The decreased total serum protein and slight decreases in hemograms may reflect the possibility of malabsorption of nutrients and blood loss accompanying gastric mucosal damage at the high dose.

Overall the primary effect appears to be local GI effect with a NOAEL of 160 mg/kg bw day if applied as 5% aqueaous solution. (1990, Doc III A6.3.1).

90 day study:

Sprague Dawley rats (10/sex/group) were dosed orally by gavage with 0, 10, 40 and 160 mg/kg/day of the active ingredient SUTTOCIDE® A as 2% (w/v) aqueous solution for 90 consecutive days according to standard OECD testing requirements and GLP. No treatment related deaths occurred nor were there any daily observations of toxicity. There were no changes in body weights, food consumption, organ weights, or haematological, clinical chemistry or urine parameters which indicated any SUTTOCIDE® A related toxicity. Also, no gross necropsy or histopathological effects were observed in any organ or tissue examined (1984, Doc III A6.4.1/01).

Overall no LOAEL but a NOAEL of 160 mg/kg bw day was observed with the test substance applied as 2% aqueous solution. (1984, Doc III A6.4.1).

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Overall the primary effect appears to be local GI effect with a NOAEL of 160 mg/kg bw day if applied as 5% aqueous solution in the 28 day study. The 90 day study was carried out with a 2% aqueous solution, no LOAEL was observed, there were no adverse effects in the top dose of 160 mg/kg bw day.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

The LOAEL was above 100 mg/kg bw day in the 28 day as well as in the 90 day study, which is above the STOT RE 2 guidance value of 100 mg/kg bw day.

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

No classification for STOT RE is warranted.

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

Summary of the Dossier Submitter's proposal

An oral 28-day study on rats that received SHMG 5% w/v in water at doses of 0, 40, 160 and 640 mg/kg bw/d revealed adverse effects at the high dose level only. One unscheduled death in a female, lower protein levels, non-significantly lower mean haemoglobin and haematocrit values and subacute gastritis and ulceration of the glandular stomach in male and female animals were observed. Significantly lower body weight was seen on day 14 and 21 in male rats (≤ 8%). No adverse effects were observed in an oral 90-day study on rats that received received SHMG 2% w/v in water at doses of 0, 10, 40 and 160 mg/kg bw/d. Since no adverse effects that may justify classification for STOT RE (guidance value ≤100 mg/kg bw/d for category 2) in any of these studies, no classification was proposed.

Comments received during public consultation

No comments received.

Assessment and comparison with the classification criteria

For information, 160 mg/kg bw/d SHMG as 2% aqueous solution corresponds to 38 mg/kg bw/d formaldehyde (no harmonised classification for STOT RE). **RAC concurred with the DS' proposal that no classification is needed**.

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

4.9.1.1 In vitro data

Table 4.9.1.1_1: Genotoxicity in vitro: Sodium hydroxymethyl glycinate

Test system Method Guideline	**Organism/ strain(s)**	**Concentrations tested (give range)**	**Result**		**Remark give information on cytotoxicity and other**	**Reference**
			+ S9	**- S9**		
			+/-/+	+/-/+		
FIFRA Guideline 84-2 (Ames Test)	S. typhimurium: TA 1535, TA 1537, TA 98, TA 100, TA 1538,	Tested at 3.3, 6.7, 10, 33, 67, 100, 333, 667, 1000 & 3333 µg/plate	-?	+/-?	ambiguous results: 1.5 fold induction of revertants with TA100 +S9, not all TG 471 strains tested, higher concentrations could have been tested; no repeat experiment	Steven R. Haworth, Ph.D. (1983) Salmonella / Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) Microbiological Associates Lab, Lab Project ID: T2114.501; Doc IIIA 6.6.1
In vitro Mammalian Cell Gene Mutation Test (tk locus)	Mouse lymphoma L5178Y cells	2.5, 5, 10, 20, 40, 60, 80, 100 and 120 µg/ml) tested in duplicate cultures	+	+	sodium hydroxymethyl glycinate (50% aqueous solution) did induce mutation at the tk locus of L5178Y mouse lymphoma cells in the absence and presence on S-9.	M Lloyd (2002) Sodium hydroxymethyl glycinate: Mutation at the Thymidine Kinase (tk) Locus of Mouse Lymphona L5178Y Cells (MLA) using the MicortitreR Fluctuation Technique Covance Laboratories Ltd, Covance study no. 1184/61, Covance report no. 1184/61-D6173; Doc IIIA 6.6.3
In vitro Mammalian Chromosome Aberration Test	Cultured human peripheral blood lymphocytes	Doses - S9, 28.62, 44.72 and 87.34 µg/ml; +S9, 87.34, 109.2 and 170.6 µg/ml. Treatments were in the absence and presence of S9 for 3 hours followed by a 17 hour recovery prior to harvest.	+	+	Yes, Integra 44 (50% aqueous solution) induced chromosomal aberrations in cultured human peripheral blood lymphocytes in vitro	J Whitwell (2002) Induction of Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes Covance Laboratories Ltd. Covance Study No. 1184/51 Covance report No. 1184/51-D6172; Doc IIIA 6.6.2/02 .
	Chinese Hamster Ovary	Initial Assay 2, 4, 8, 15, 30 and 60 µg/ml (without activation system) 3, 6, 11, 23, 45 and 90 µg/ml (with activation system) Repeat Assay 8, 15, 30, 45 and 90	+	+	SHMG (50% aqueous solution) was clastogenic in the in vitro mammalian cytogenetic assay using Chinese	Donald L Putman, Ph.D / Elizabeth H. Schadly, B.S (1992) In Vitro Mammalian Cytogenetic Test Micobiological Associates, Inc. Lab Study No. TA959.337003 Sponsor project No. SUTA-CHO-2; Doc IIIA 6.6.2/01

		µg/ml (without activation system) 30, 45, 60 and 90 µg/ml			hamster ovary cells.	
Rat Hepatocyte UDS Assay	Primary rat hepatocytes from a single male Fischer 344 rat	Tested at 0.75, 2.5, 5, 7.5, 10, 20, 40, 60, 80 and 100 µg/ml (expressed in terms of active ingredient)	-	-	The active ingredient in SUTTOCIDE ® A (50% aqueous solution) was not genotoxic in the rat hepatocyte/DNA repair assay. Concentrations ≥ 40 µg/ml were severely cytotoxic	Leon F. Stankowski (1995) Revised Rat Hepatocytes Primary Culture/DNA Repair Test on Suttocide A Pharmakon Research international. Study No. PH311-SU-002-90; Doc IIIA 6.6.2/04

* results are given as positive (+), negative (-) or inconclusive (+)

4.9.1.2 In vivo data

Table 4.9.1.2_1: Genotoxicity in vivo:Sodium hydroxymethyl glycinate

Type of test Method/ Guideline	**Species Strain Sex no/group**	**frequency of application**	**sampling times**	**dose levels**	**Results give dose, sampling time and result +/-/+**	**Remarks**	**Reference**
Bone Marrow micronucleus test	out bred Han Wistar Crl:WI (Glx/BRL/Han) BR rats male rats: 12 in negative control; 12 in positive control; 6 in low dose; 6 in mid dose; 15 in high dose	Twice in two consecutive days oral	24 h after second administration	300, 600 and 1200 mg/kg/day	The active ingredient sodium hydroxylmethyl glycinate did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of rats and did not change the PCE/NCE ratio.	Clinical signs: mortality, lethargy, coldness, abnormal gait, abnormal breathing, loose faeces, blue extremities, piloerection; It is likely that neither SHMG nor formaldehyde reached the bone marrow	ISP Induction of micronuclei in the bone marrow of treated rats. Study No.: 1184/74 report No.:1184/74-D6172; Doc III A6.6.4/01
Mouse bone marrow micronucleus test	CD-1 5m / 5f per dose group	Single dose oral	30, 48 and 72 hrs after treatment	750, 1250 and 1750 mg/kg of 50% aqueous solution	The test substance did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of mice and did not	Clinical signs: decreased activity, piloerection. Ptosis, and decreased muscle tone. no mortality (mortality in higher doses	ISP (1987), Micronucleus test (MNT) on Suttocide A ; Study No. PH309-SU-001-87 Doc III A6.6.4/02

					change the PCE/NCE ratio.	tested in range finding study) It is likely that neither SHMG nor formaldehyde reached the bone marrow	
Rat Hepatocyte UDS Assay	rats were dosed once orally 3 animals per dose group at 2 post-exposure time points were analysed	One dose	Hepatocytes were harvested 2-4 and 12-18 hours after treatment	doses of 200 (1/10LD50), 700 (1/3LD50) and 2000 (LD50) mg/kg	SUTTOCIDE® A (50% aqueous solution) did not induce a significant increase in the mean nuclear grain counts in hepatocytes isolated from treated animals.	Clinical signs at the 2-4 hour post treatment harvest, two of the five animals from group 9 (2000 mg/kg dose level) appeared lethargic (mortality in higher doses tested in range finding study) It is likely that neither SHMG nor formaldehyde reached the bone marrow	ISP (1994); In Vivo – In Vitro Rat Hepatocyte Unscheduled DNA Synthesis Assay Laboratory Study No. TD994.381 Study Project No. SUTA-UDSNVIVO-1/93 Doc III A6.6.5/01

4.9.2 Human information

No information available.

4.9.3 Other relevant information: Comparison of genotoxicity data for SHMG and the hydrolysis product formaldehyde

Detailed data on formaldehyde are presented in the Formaldehyde Appendix. The results of the in vitro and in vivo genotoxicity tests for SHMG and free formaldehyde are similar. Within in vitro tests both substances appear mutagenic, within the standard animal tests for systemic genotoxicity both substances appear negative. In vivo data for local genotoxicity are available only for formaldehyde, these are positive. It is considered that the genotoxicity of SHMG is related to the formaldehyde release.

Table 4.9.3_1 Comparison of the active substance and formaldehyde – in vitro genotoxicty results

Parameters	**SHMG**	**Formaldehyde**
Gene mutation in bacteria	Ambiguous results	Mutagenic
Chromosome aberration in eukaryotic cells	Clastogenic	Clastogenic ≥ 7.5 µg/ml
Gene mutation in mammalian cells	Mutagenic	Mutagenic
DNA damage in bacteria and eukaryotic cells	No data	Genotoxic

Overall assessment	Mutagenic activity in vitro	Mutagenic activity in vitro

MA: metabolic activation

Table 4.9.3_2 Comparison of the active substance and formaldehyde – in vitro genotoxicty results

Parameters	**SHMG**	**Formaldehyde**
Systemic genotoxicity	negative in bone marrow micronucleus test in rat and mouse and rat hepatocyte UDS assay; it is likely that neither SHMG nor formaldehyde reached the target tissues	negative (cytogenetic & micronucleus assay) contradictory results in humans
Local genotoxicity	No data (but see positive in vitro data)	Positive (clastogenic in the gastrointestinal tract of rats after oral exposure; clastogenic in the upper respiratory tract of humans after inhalation; DNA-protein cross-links at the site of first contact after inhalation exposure)

4.9.4 Summary and discussion of mutagenicity

Mutagenicity in bacteria

SUTTOCIDE® A (study performed with aqueous solution) was tested in the Salmonella/mammalian microsome Mutagenicity assay using five tester strains, TA98, TA100, TA1535, TA1537 and TA1538, both in the presence and absence of an S9 exogenous metabolic activation system. A range-finding study indicated that treatment with SUTTOCIDE® A (50% aqueous solution) at 3.3-3333 µg/plate with and without S9 resulted in absence of background lawn at 3333 µg/plate and slightly reduced background lawn at 1000 µg/plate in TA100. In the definitive experiment doses of 15, 100, 500, 750, 1000 µg/plate in the presence and absence of S9 were tested in triplicate for the mutagenicity assay. Revertant frequencies in SUTTOCIDE® A (50% aqueous solution) increased with concentration up to 1.5 fold in TA100 +S9. Also in the range finding experiment with TA100 increased revertant frequencies were observed. The study author considered a 1.5 fold increase as not-positive, a 2-fold increase should have been reached for a positive conclusion. However according to TG 471 a concentration related increase of revertants may be considered as positive finding. Moreover in TA100 maximum number of revertants was found in the top dose and the range finding results point to the fact that the substance may also have been tested up to slightly higher concentrations. The results were not confirmed in an independent assay (as recommended in TG 471). It is concluded that the results for SUTTOCIDE® A are ambiguous in this bacterial mutation assay (Steven R Haworth 1983, Doc III A6.6.1).

In vitro Mammalian Cell Gene Mutation Test

The active ingredient (Integra 44 (a.s. as manufactured as aqueous solution)) was mutagenic at the tk locus in mouse lymphoma cells. In the cytotoxicity range-finding experiment, nine doses (2.5, 5, 10, 20, 40, 60, 80, 100 and 120 µg/ml) tested in duplicate cultures were chosen for the main mutation experiment. The highest doses selected to determine viability and 5-trifluorothymidine resistance were 100 µg/ml (-S9) and 120 µg/ml (+S9), which yielded 3% and 8% relative survival, respectively. In the absence of S9, statistically significant ($p<0.05$) increases in mutant frequency

were observed from 20-100 μg/ml (relative survival 90.17-2.68%), while in the presence of S9 statistically significant increases in mutant frequency were observed from 40-120 μg/ml (relative survival 88.43-7.64). A marked increase in mutant frequency were observed with the positive controls 4-nitroquinoline 1-oxide and benzo(a)pyrene. All positive and vehicle control data were within acceptable ranges. Results were not confirmed in an independent assay due to the large increases in mutant frequency observed in this experiment (M Lloyd 2002, Doc III A6.6.3).

In vitro Mammalian Chromosome Aberration Test

There are two separate studies for in vitro mammalian chromosome aberration studies available for sodium hydroxymethyl glycinate.

In the first study: Integra 44 (50% aqueous solution) induced chromosomal aberrations in cultured human peripheral blood lymphocytes in vitro. Integra 44 (in purified water) was tested in an in vitro cytogenetics assay using duplicate human lymphocyte cultures prepared from the pooled blood of three male donors in a single experiment. In the range finding study, treatments covering a broad range of doses (28.62-1271 μg/ml), separated by narrow intervals, were performed both in the absence and presence of S9 metabolic activation. The highest concentrations chosen for the definitive test were based on mitotic index; 87.34 μg/ml (-S9) and 170.6 μg/ml (+S9) induced approximately 59% and 66% mitotic inhibition, respectively. In the chromosomal aberration study, the following doses were selected for analysis: -S9, 28.62, 44.72 and 87.34 μg/ml; +S9, 87.34, 109.2 and 170.6 μg/ml. Treatments were in the absence and presence of S9 for 3 hours followed by a 17 hour recovery prior to harvest. In order to harvest metaphase cells, 2 hours prior to harvest 1 μg/ml colchicine was added to the system. Lymphocytes were then fixed onto slides and scored for chromosomal aberrations; 200 cells were analyzed for each dose level in the absence or presence of S9. Significantly increased frequencies of cells with structural aberrations were observed in cultures without S9 at 44.72 μg/ml ($p \leq 0.05$) and 87.34 μg/ml ($p \leq 0.001$). Significant increases in structural aberrations were also observed in cultures with S9 at all three doses ($p \leq 0.001$). The positive controls 4-nitroquinoline 1- oxide and cyclophosphamide gave resulted in a significant increase in chromosomal aberrations. Although provision was made to perform a second experiment, the clear positive results from the first experiment were such that this was not considered necessary. All positive and vehicle control data were within acceptable ranges (J Whitwell 2002, Doc III A6.6.2/02).

In a second study on chromosomal aberrations .SUTTOCIDE® A (50% aqueous solution) was tested at doses of 1.25, 2.5, 5, 10, 15, 20, 40, 60, and 80 μg/ml in Chinese hamster ovary cells both conditions both in the absence and presence of S9. Cells were exposed for 6 hours followed by an 18 hour recovery period. Cell growth inhibition was found to be 59% (+S9) and 23% (-S9) at 80 μg/ml. In the initial chromosomal aberration test, SUTTOCIDE® A (50% aqueous solution) was tested in the absence of S9 at dose levels of 2, 4, 8, 15, 30 and 60 μg/ml, and in the presence of S9 at dose levels of 3, 6, 11, 23, 45 and 90 μg/ml for 20 hours. Toxicity, as measured by mitotic inhibition, was 71% (-S9) at the 60 μg/ml dose level; in the conditions with S9, no toxicity was observed. Two hours prior to harvest, metaphase cells were collected by addition of 0.1 μg/ml Colcemid. Cells were fixed onto slides, and whenever possible, a minimum of 200 metaphase spreads (100 per duplicate flask) were scored. Statistically significant ($p \leq 0.01$) increases in chromosomal aberrations were observed at 30 and 60 μg/ml (-S9) and 45 and 90 μg/ml (+S9) at harvest time. An independent repeat assay was conducted at dose levels of 8, 15, 30, 45 and 60 μg/ml (-S9) and 30, 45, 60 and 90 μg/ml (+S9) for 20 and 44 hours. Toxicity, as measured by mitotic inhibition, was 57% (20 hr) and 59% (44 hr) in the absence of S9 at 60 μg/ml. In the S9 activated studies, toxicity was 28% (20 hr) and 20% (44 hr) at 90 μg/ml. Cells were harvested and

scored using the same procedures as the initial test. Statistically significant ($p \leq 0.01$) increases in structural chromosome aberrations were observed at 45 and 60 µg/ml (-S9) at both harvest times. Statistically significant ($p \leq 0.01$) increases in the number of numerical aberrations were found at the 44 hour harvest time (-S9) at 45 and 60 µg/ml. In the S9 activated system, significant ($p \leq 0.05$) increases in structural chromosome aberrations were found at the dose level of 90 µg/ml at both harvest times. The Cochran-Armitage trend test for dose-responsiveness was positive only at the 20 hour harvest time. No significant increase in numerical aberrations was observed at the 44 hour harvest time (-S9). The positive controls, triethylenemelamine and cyclophosphamide gave significant increases ($p \leq 0.05$) in chromosomal aberrations when compared to vehicle (water) control). All positive and vehicle control data were within acceptable ranges. (Donald L Putman / Elizabeth Schnadly 1992, Doc III A6.6.2/01).

UDS Assay (in vitro.)

The active ingredient in SUTTOCIDE® A was not genotoxic in the rat hepatocyte/DNA repair assay.

SUTTOCIDE® A (50% aqueous solution) was tested at 0.75, 2.5, 5, 7.5, 10, 20, 40, 60, 80 and 100 µg/ml (expressed in terms of active ingredient) in primary rat hepatocytes from a single male Fischer 344 rat. Cells, cultured in triplicate, were incubated with SUTTOCIDE® A and 10 µCi/ml 3H-thymidine for 18-20 hours. Subsequently, cultures were washed and autoradiograms prepared. None of the ai concentrations induced a mean net nuclear grain (NNG) count greater than 5. The positive control, 2-acetamido fluorine, induced a mean NNG of 9.8 and had 95% of cells in repair. All positive and negative controls were within acceptable historical negative control values (Leon F. Stankowski Jr. 1995, Doc III A6.6.2/04).

Bone Marrow Micronucleus Test in vivo

Two studies were performed to test the mutagenicity in vivo in a bone marrow micronucleus test.

In the first study SUTTOCIDE® A (50 % aqueous solution) was applied to out bred Han Wistar Crl:WI (Glx/BRL/Han) BR rats (3/sex).: In a toxicity range-finding study, SUTTOCIDE® A (50 % aqueous solution) was dosed at 1000, 1400 or 2000 mg/kg/day (expressed in terms of active ingredient) by oral gavage for two consecutive days. No substantial difference in toxicity was observed between males and females therefore, the main study was conducted using male rats only. Due to the number and severity of clinical signs observed at the 1400 and 2000 mg/kg/day dose levels, another intermediate dose level of 1200 mg/kg/day was tested as a range finder in male animals. 1200 mg/kg/day was selected as the maximum dose level for the main study. In the micronucleus test, SUTTOCIDE® A was administered once daily for two consecutive days, at 300, 600 and 1200 mg/kg/day to groups of six male rats, euthanized 24 hours after the second treatment. Following treatment, bone marrow cells were flushed and fixed onto slides for scoring. Clinical signs were observed at the 1200 mg/kg/day dose, and included mortality, lethargy, coldness, abnormal gait, abnormal breathing, loose faeces, blue extremities, piloerection and dilated pupils. 12 rats were tested in each of the negative and positive control groups, 6 rats were tested in each of the low dose and mid dose group and 15 rats in the high dose group, three rats of the high dose group died, likely due to local GI effects. Rats treated with the test article exhibited group mean ratios of PCE/NCE for the vehicle control group and which also fell within normal ranges. This indicates that neither SHMG nor formaldehyde reached the bone marrow inspite of mortality and severe clinical signs in the high dose group. There were no instances of statistically significant increases in micronucleus frequency for any of the groups receiving the test article. The positive control, cyclophosphamide, induced a significant increase in the number of micronucleated PCE

cells relative to vehicle control. All positive and vehicle control data were within acceptable ranges (2002, Doc III A6.6.4/01).

In a second study 875 mg/kg was selected as an estimate of the maximum tolerated dose for the micronucleus test on the basis of a pre-test indicating to the pharmacotoxic signs and mortality at higher doses (1225, 2500 mg/kg bw). In the micronucleus test, ninety mice (5/sex/group) were dosed once with 375, 625 and 875 mg/kg of SUTTOCIDE® A (50% aqueous solution) and analysed after 30, 48 and 72 hours. In all three doses, animals exhibited decreases activity, piloerection, and decreased muscle tone. Following treatment, bone marrow cells were flushed and fixed onto slides for scoring. SUTTOCIDE® A did not induce a statistically significant increase in micronucleated polychromatic erythrocytes, nor did not cause a statistically significant shift in the PCE/NCE ratio. The positive control, cyclophosphamide, gave a significant increase in the incidence of micronucleated PCE, as well as a depression of the PCE/NCE ratio. All positive and vehicle control data were within acceptable ranges (1987, Doc III A6.6.4/02). In conclusion, SUTTOCIDE® A did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of mice. Most likely neither SHMG nor the hydrolysis product formaldehyde has reached the bone marrow.

UDS Assay (in vivo-in vitro)

The active ingredient in SUTTOCIDE® A was not genotoxic in the in vivo rat hepatocyte unscheduled DNA synthesis assay.

Within 2 dose range-finding assays using 5 male Fisher 344 rats per dose group in summary doses of 50, 150, 500, 1500, 2000, 3000, 4000, 5000 mg/kg bw were tested. Based on the probit analysis of the results, an LD50 of 2080 mg/kg was estimated. Accordingly, the following doses of 200 (1/10LD50), 700 (1/3LD50) and 2000 (LD50) mg/kg were selected for the definitive assay. Fifty rats (5/group) were dosed once orally with SUTTOCIDE® A (50% aqueous solution) and the hepatocytes were harvested 2-4 and 12-18 hours after treatment. Thirteen animals were given the 2000 mg/kg dose. At the 2-4 hour timepoint, two out of five animals appeared lethargic; at the 12-18 hour timepoint, six out of eight were found dead while the surviving two animals appeared lethargic and ungroomed. However finally 3 animals per dose groups and per control groups were analysed. Following harvest, hepatocytes were treated with 3H-thymidine, fixed onto 3 slides per animal, and autoradiograms prepared. 50 cells per slide were evaluated, i.e. 150 cells per animal. SUTTOCIDE® A did not induce a significant increase in the mean nuclear grain counts in hepatocytes isolated from treated animals. The positive controls methyl methanesulfonate (2-4 hour group) and 2- acetylamidofluorine (12-18 hour group) gave mean NNG counts of 10.4 and 9.9 respectively. All positive and vehicle control data were within acceptable ranges (1994, Doc III A6.6.2/01).

4.9.5 Comparison with criteria

The in vitro tests for SHMG were positive, with the exception of the AMES test that was ambiguous. The in vivo tests for SHMG were negative in bone marrow micronucleus test in rat and mouse and in the rat hepatocyte UDS assay. The genotoxicity profile as far as available for SHMG correlates with the genotoxicty profile of formaldehyde. It is likely that neither SHMG, nor the hydrolysis product formaldehyde reached the target tissues in the in vivo genotoxicity studies.

Based on the available data and mechanistic considerations of formaldehyde release local genotoxic effects are to be expected from SHMG. The presently available data for SHMG and FA support the conclusion that germ cells are not affected and according to CLP Regulation 1272/2008/EC, Annex 1, paragraph 3.5.2.1 the germ cell mutagenicity “hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny.” However according to the ECHA CLP guidance 2012, chapter 3.5.1 “genotoxicants which are incapable of causing heritable mutations because they cannot reach the germ cells (e.g. genotoxicants only acting locally, "site of contact” genotoxicants)” may be classified as category 2 mutagen in order to provide an indication that the substance could be carcinogenic. Nevertheless, since the substance is already proposed for classification as carcinogenic Cat 1B, there is no need for this further information. Therefore, labelling for mutagenicity according EU Regulation 1272/2008/EC is not required.

However during RAC meetings for the classification of formaldehyde (2012), the hazard classes on mutagenicity and their interpretation with regard to the classification of somatic cell mutagenicity were discussed on a very fundamental level. RAC agreed that “due to the induction of genotoxic effects in vivo on somatic cells at site of contact, which are supported by positive findings from mutagenicity and genotoxicity tests in vitro, classification of formaldehyde for mutagenicity category 2 in accordance with the CLP Regulation, with the hazard statement H341 (Suspected of causing genetic defects) is therefore warranted. The route(s) of exposure should not be stated in the hazard statement as it is not proven that other routes than inhalation can be excluded.”

It is proposed to base classification of SHMG on the data of the hydrolysis product formaldehyde. Arguments for and against reading across the carcinogenicity data and C&L conclusion from formaldehyde to SHMG are listed in chapter 4.9.4. The same arguments are valid for the read across of mutagenicity category 2. A consistent approach for the read across for these 2 endpoints is necessary.

Due to the consideration that formaldehyde release is dominating the toxicity of SHMG and the classification of formaldehyde is read across to SHMG it is suggested to include a note 9 indicating: "The classification as a mutagen need not apply if it can be shown that the maximum theoretical concentration of releasable formaldehyde, irrespective of the source, in the mixture as placed on the market is less than 1%."

4.9.6 Conclusions on classification and labelling

Classification for mutagenicity category 2 is required. A specific note 9 shall be included: "The classification as a mutagen need not apply if it can be shown that the maximum theoretical concentration of releasable formaldehyde, irrespective of the source, in the mixture as placed on the market is less than 1%."

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS proposed to classify SHMG as mutagen category 2 based on the available positive *in vitro* genotoxic data and taking into account the classification of its hydrolysis product formaldehyde (mutagen category 2).

There are several positive *in vitro* tests for SHMG; a mouse lymphoma assay, chromosomal aberration tests with CHO cells and human lymphocytes. A bacterial gene mutation assay (Ames test) is ambiguous, and a UDS test is negative. These data lead to the conclusion that the substance induces mutagenic effects *in vitro*.

The available *in vivo* tests (bone marrow micronucleus in rat and mouse; UDS test) are negative. Regarding the relevance of the negative *in vivo* results, the DS argued that it seems to be likely that neither SHMG, nor its hydrolysis product formaldehyde reached the target tissues. Based on the available data and mechanistic consideration of formaldehyde release, local genotoxic effects are to be expected from SHMG.

The results of the *in vitro* and *in vivo* genotoxicity tests for SHMG and free formaldehyde are similar. Both substances are *in vitro* mutagens; within the standard animal tests for systemic genotoxicity both substances are negative. *In vivo* data for local genotoxicity are available only for formaldehyde, and are positive.

The DS proposed to base the classification of SHMG on the data of the hydrolysis product formaldehyde (classified as Category 2 mutagen) due to the consideration that formaldehyde release is dominating the toxicity of SHMG.

Comments received during public consultation

One MSCA supported the proposed classification as mutagen category 2 based on the

hydrolysis product formaldehyde.

One industry representative is of the opinion that classification for mutagenicity is not justified.

Assessment and comparison with the classification criteria

The evaluation of the genotoxic data of SHMG by the DS and RAC does not differ. SHMG is an *in vitro* mutagen but induces no genotoxic effects *in vivo* in different target organs.

In vitro data

SHMG induced with and without S9 mix gene mutations (mouse lymphoma assay: Lloyd, 2002) and clastogenic effects (chromosomal aberration test with CHO cells (Putman and Schnadly, 1992) and with human lymphocytes (Whitwell, 2002)) in mammalian cell cultures. A bacterial gene mutation test is equivocal (Haworth, 1983), while an UDS test with rat hepatocytes is negative(Stankowski, 1995).

In vivo data

A rat bone marrow micronucleus test (ISP, 2002) and a mouse bone marrow micronucleus test (ISP, 1987) as well as a UDS test (ISP, 1994) were negative.

Due to its reactivity (hydrolysis to formaldehyde), a low systemic availability is expected for SHMG. Therefore, the induction of systemic genotoxic effects in standard animal tests is unlikely. However, a local genotoxic effect produced by the hydrolysis product formaldehyde is expected. Therefore, the read-across to formaldehyde, which has a harmonised classification as mutagen in category 2 due to the induction of local genotoxic effects, is justified.

It is assumed that SHMG has a low systemic availability due to its reactivity. Accordingly, the available *in vivo* results are of low relevance and do not allow the conclusion that the substance is not genotoxic in the whole animal. There is no test with SHMG which assessed whether genotoxic effects will be induced in cells at site of first contact. For the evaluation of toxicological properties of SHMG, the fact that its hydrolysis product formaldehyde is inducing local genotoxic effects and is already classified as Category 2 mutagen is taken into account. Based on positive *in vitro* data of SHMG and on read-across to formaldehyde, RAC agrees with the DS proposal to **classify SHMG as a Germ Cell Mutagen Category 2 (H341 – Suspected of causing genetic defects)**.

The classification proposal for SHMG is in line with previous decisions on formaldehyde and other formaldehyde releasers.

Some RAC members expressed their discomfort with the classification as in their view the criteria should only be interpreted as they specifically relate to germ cell mutagens. They expressed their disagreement on the classification as Muta Cat. 2 based on the local genotoxic effects in a minority position (see associated documents to this opinion).

RAC agreed with the DS proposal to apply Note 9 in line with the previous formaldehyde releaser opinions. **Note 9**: "*The classification as a mutagen need not apply if it can be shown that the maximum theoretical concentration of releasable formaldehyde, irrespective of the source, in the mixture as placed on the market is less than 1%.*"

4.10 Carcinogenicity

4.10.1 SHMG

No long-term carcinogenicity study on experimental animals is available.

It is considered that SHMG hydrolyses to formaldehyde by dilution and by the reaction of formaldehyde with biological media. This assumption is –in qualitative terms- supported by the hydrolysis study and by the intended efficacy mode of action. The available repeated dose studies with SHMG indicate predominantly local effects. Furthermore the tests for systemic genotoxicity were negative for both SHMG and formaldehyde. The hydrolysis products formaldehyde and glycine are unlikely to induce systemic genotoxicity as demonstrated by respective negative genotoxicity tests. Also the systemic carcinogenicity studies for formaldehyde are negative.

Consequently it is to be expected that SHMG shows the same local carcinogenic hazard as Formaldehyde.

In any case for risk assessment the threshold values for formaldehyde need to be taken into account in parallel with the threshold values derived for SHMG.

4.10.2 Other relevant information: Comparison of carcinogenicity data for SHMG and the hydrolysis product formaldehyde

Detailed data on formaldehyde are presented in the Formaldehyde Appendix. No data are available for SHMG. Potential carcinogenicity of SHMG is considered to be related to the carcinogenicity of the released formaldehyde (see above, chapter 3.6.4).

Table 4.10.2_1 Comparison of the active substance and its components

Parameters	**SHMG**	**Formaldehyde**
Systemic carcinogenicity in experimental animals	No data	No carcinogenic activity
Local carcinogenicity in experimental animals	No data	Carcinogenic activity after inhalation at > 7.4 mg/m³
Systemic carcinogenicity in humans	No data	Conflicting results
Local carcinogenicity in humans	No data	Conclusion from not unequivocal epidemiological studies: increased tumour risk after inhalation exposure

4.10.3 Summary and discussion of carcinogenicity

The active substance as manufactured represents sodium hydroxymethyl glycinate (SHMG) as a 50% aqueous solution. It represents a reaction product of formaldehyde and glycine. When SHMG is diluted in water SHMG hydrolyses to formaldehyde and glycine. The high pH in the 50% solution (pH=11) or in a more diluted 5% solution slows down hydrolysis and formaldehyde release. However the hydrolysis study indicates that in unbuffered aqueous solutions of 10%, 1% and 0.25% SHMG the pH is between 10.7 and 11.7, but still about 18%, 40% and 66% were hydrolysed. Contact with biological media should lead to a reaction of formaldehyde with protein

and shift the equilibrium towards further formaldehyde release. Acidic pH (like in stomach) would further support fast hydrolysis, the DT50 was smaller than 1.4 hours at pH of 4 and 7. Therefore we may theoretically assume a rate of 100% final hydrolysis in biological media. Given the molecular weight of 127 g/mol for SHMG and 30 g/mol for Formaldehyde (factor 4.23) a 50% SHMG solution corresponds to less than 12% (w/w) formaldehyde.

In use concentrations of SHMG are usually very low (0.05% to 0.25%). With such high dilution in water SHMG hydrolyses fully to formaldehyde and glycine. Glycine is an amino acid, a natural cell component, a food ingredient and compared to formaldehyde of low biological reactivity.

Therefore it is considered that the toxicity of SHMG relates primarily to the toxicity of formaldehyde.

The available repeated dose studies with SHMG indicate predominantly local effects. Furthermore the tests for systemic genotoxicity were negative for SHMG. Also hydrolysis product formaldehyde is unlikely to induce systemic genotoxicity as demonstrated by respective negative genotoxicity tests. Also the formaldehyde studies for systemic carcinogenicity are negative. Consequently it is to be expected that SHMG shows the same local carcinogenic hazard as Formaldehyde.

The following options are considered for decision on classification and labelling: In the situation when the concentration of formaldehyde in the formaldehyde releasing substance is equal or higher than the general classification limit (0.1% in case of GHS class 1) the classification should be the same as the classification established for formaldehyde. However, when the concentration will be lower than the general classification limit in principle two options may be followed:

(I) Proposal supported by the eMS: The formaldehyde releasing substance should be classified like formaldehyde (category 1B) - based on the considerations of total releasable formaldehyde, intended use, category of users and exposure taking into account the precautionary principles, in this case of difficulties with the risk assessment of substances that are instable with half lives depending on dilution, temperature and pH.

(II) Proposal supported by the applicant in the context of the European Biocidal Products Regulation: The formaldehyde releasing substance should not be classified based on the formal consideration as constituent of a product at the time being “supplied to the user”.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON SODIUM N-(HYDROXYMETHYL)GLYCINATE

Proposal 1 is from the evaluating Member State. Proposal 2 is from the applicant. Below arguments are listed for either proposals:

supportive arguments for proposal 1:	**supportive arguments for proposal 2:**
Classification according to releasable Formaldehyde, i.e. Skin Irrit. 2, Eye Irrit. 2. Skin Sens 1, Muta 2, Carc. 1B	Classification according to “free Formaldehyde”, i.e. Eye Irrit.2, Skin Sens 1
This conclusion was taken by RAC for the formaldehyde releasers evaluated for the biocides regulation (RMS AT; “reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 3:2)” and “reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 1:1)” and “4-(morpholin-4-ylmethyl)morpholine”).	
Risk through formaldehyde-release in water is covered	Classification usually relates to the substance itself and not to potential release or degradation products which occur during different use scenarios
The formaldehyde releaser is difficult to characterise since it is instable with half-lives depending on dilution, temperature and pH.	Analogue to the evaluation of other “substances of concern” or impurities the cut-off values from the GHS system should be considered for the real amount of free formaldehyde
The high pH in the 50% solution (pH=11) or in a more diluted 5% solution slows down hydrolysis and formaldehyde release. However the hydrolysis study indicates that in unbuffered aqueous solutions of 10%, 1% and 0.25% SHMG the pH is between 10.7 and 11.7, but still about 18%, 40% and 66% were hydrolysed.	Formaldehyde -releasers are designed as transport forms and depot compounds and these benefits of slow continuous formaldehyde release should be considered. Formaldehyde releasers should not be equalized with a pure formalin-solution. SHMG is relatively stable at pH above 7.
Contact with biological media should lead to a reaction of formaldehyde with protein and shift the equilibrium towards further formaldehyde release. Acidic pH (like in stomach) would further support fast hydrolysis, the DT50 was smaller than 1.4 hours at pH of 4 and 7. Therefore we may theoretically assume a rate of 100% final hydrolysis in biological media.	
1 g SHMG releases 0.24 g Formaldehyde (factor 4.23)	
Solutions of formaldehyde releasers only need to be classified if maximal releasable formaldehyde content is above 0.1%	Formaldehyde release is a hydrolysis and occurs in dilutions with water → depending on the releaser type this needs dilutions between 1:10 and 1:1000
In vitro genotoxicity data for SHMG support the assumption of <u>local</u> genotoxicity and consequent <u>local</u> carcinogenicity	Other examples for substances (oligomers) that contain formaldehyde and are classified according to free formaldeyhde: ● Polyoxymethylen (CAS formaldehyde-polymer = technical plastic) has different properties compared to FA and is classified differently ● Paraformaldehyde itself (degree of polymerization of 8–10 units) is only classified as toxic (T) and corrosive (C) so far Instead of full classification and labelling a warning label could be applied „can release FA with water

	contact“
	A classification of formaldehyde-releasers on the basis of maximal releasable formaldehyde could be considered as an unusual mixture between the classification process and risk assessment which does not justify either of the both procedures

The applicant summarized the following consequences of classification according to maximal releasable formaldehyde (proposal 1):

- Classification and labelling implies a lot additional requirements for storage and transport
- High protection measures need to be implemented (e.g. respiratory protection at refilling) also in cases where only a low risk is existent (no water contact)
- Possible products and uses will be impossible on the market due missing users acceptance (panics); as a last consequence a whole group of substances showing a high and broad efficacy could disappear from the market and will be replaced by other products showing other problems which presumably do not have a comparable efficacy

4.10.4 Comparison with criteria

Genotoxiciy data for SHMG support local genotoxicity, but no systemic genotoxicity. No carcinogenicity studies are available for SHMG. However carcinogenicity data available for the hydrolysis product formaldehyde support classification for category 1B on the basis of human and animal data. Formally “information on substances or mixtures related to the substance or mixture being classified” should be used within a WoE evaluation for classification and labelling. Arguments for classification in Category 1B and arguments from the applicant supporting for non-classification are listed above. Following a WoE evaluation and considering the conclusion of RAC on other formaldehyde releasers (“reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 3:2)” and “reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 1:1)” and “4-(morpholin-4-ylmethyl)morpholine”) it is proposed to base classification of SHMG on the data of the hydrolysis product formaldehyde.

Due to the consideration that formaldehyde release is dominating the toxicity of SHMG and the classification of formaldehyde is read across to SHMG it is suggested to include a specific note 8 : “The classification as a carcinogen need not apply if it can be shown that the maximum theoretical concentration of releasable formaldehyde, irrespective of the source, in the mixture as placed on the market is less than 0.1%.”

4.10.5 Conclusions on classification and labelling

Classification for carcinogenicity, category 1B is proposed. A specific note 8 shall be included: “The classification as a carcinogen need not apply if it can be shown that the maximum theoretical concentration of releasable formaldehyde, irrespective of the source, in the mixture as placed on the market is less than 0.1%.”

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

There are no carcinogenicity studies on SHMG and the DS proposed to classify SHMB as a carcinogen 1B based on the hydrolysis product formaldehyde.

SHMG as manufactured is a 50% aqueous solution that hydrolyses to formaldehyde and glycine. The DS considered the toxicity of SHMG as related to the toxicity of formaldehyde; complete hydrolysis of a 50% aqueous solution of SHMG would correspond to 12% (w/w) formaldehyde. The DS noted that the formaldehyde releaser is difficult to characterize since it is instable with half-lives depending on dilution, temperature and pH.

The high pH, in the 50% solution (pH=11) or in a more diluted 5% solution, slows down the hydrolysis and the formaldehyde release. However, the hydrolysis study indicated that in unbuffered aqueous solutions of 10%, 1% and 0.25% the pH is between 10.7 and 11.7, but still about 18%, 40% and 66% were hydrolysed.

Contact with biological media should lead to a reaction of formaldehyde with proteins and shift the equilibrium towards further formaldehyde release.

In vitro genotoxicity data for SHMG was considered as supporting the assumption of local genotoxicity and consequently a local carcinogenicity.

The DS suggested adding note 8 (as agreed for other formaldehyde releasers) that solutions of formaldehyde releasers only need to be classified if the maximal releasable formaldehyde content is above 0.1%.

Following a wight of evidence evaluation and considering the conclusion of RAC on other formaldehyde releasers ("reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 3:2)" and "reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 1:1)" and "4-(morpholin-4-ylmethyl)morpholine"), is the DS proposed to base classification of SHMG on the data of the hydrolysis product formaldehyde. For the same reason, the DS suggested to include a specific note 8: "The classification as a carcinogen need not apply if it can be shown that the maximum theoretical concentration of releasable formaldehyde, irrespective of the source, in the mixture as placed on the market is less than 0.1%."

Comments received during public consultation

Company/manufacturer and industry association commenters disagreed with the assessment of SHMB based on equivalency and read across to formaldehyde and argued that repeat dose toxicity study demonstrate the lack of neoplastic growth or aberrant tissue at the site of gavage.

One MACA supported the proposed classification as carcinogen category 1B.

Assessment and comparison with the classification criteria

Consistent with the classification of other formaldehyde releasers and in agreement with

the DS proposal, RAC considers the classification of **SHMG as carcinogen category 1B (H350 - May cause cancer)** to be warranted.

RAC agreed with the DS proposal to apply Note 8 in line with the previous formaldehyde releaser opinions. **Note 8:** "*The classification as a carcinogen need not apply if it can be shown that the maximum theoretical concentration of releasable formaldehyde, irrespective of the source, in the mixture as placed on the market is less than 0.1%.*"

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

4.11.1.1 Non-human information

In long-term studies no adverse effects on the reproductive organs were recorded at the high dose level inducing local effects. This provides a good indication that it is unlikely there will be an effect on fertility following repeated administration of sodium hydroxymethyl glycinate. Further more it is not to be expected, that the breakdown product, formaldehyde, will reach the reproductive organs. The performance of further reproductive animal tests on sodium hydroxymethyl glycinate would not add to the available information and would be in contravention of the biocidal products regulation concerning the performance of unnecessary animal tests.

4.11.1.2 Human information

No information available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Table 4.11.2_1: Teratogenicity Sodium hydroxymethyl glycinate

Route of exposure	**Test type Method Guideline**	**Species Strain Sex no/group**	**Exposure Period**	**Doses (mg/kg/ day)**	**NO(A)EL maternal toxicity**	**NO(A)EL Teratogenicity Embryotoxicity**	**Reference**
Oral gavage	comparable to OECD 414 (except for exposure)	Rat↓ 27 females per dose group	Day 6 through Day 15 of gestation	75 150 225 as 5% aqueous solution	150 mg/kg/day as 5% solution	225 mg/kg/day as 5% solution	ISP (1990), Developmental Toxicity study in Rats; Study No. PH328-SU-002-90 Doc IIIA6.8.1

4.11.2.2 Human information

No information available.

4.11.3 Other relevant information: Comparison of reproductive toxicity data for SHMG and the hydrolysis product formaldehyde

Detailed data on formaldehyde are presented in the Formaldehyde Appendix. Dominant maternal effects likely due to local effects were observed in the developmental toxicity study with SHMG as well as with formaldehyde. The toxicity of SHMG is considered to be related to the toxicity of the released formaldehyde.

Table 4.11.3_1 Comparison of SHMG and formaldehyde – developmental toxicity studies

Exposure route	**SHMG**	**Formaldehyde**
Dermal exposure	No data	No data but corrosive properties
Inhalation	No data	maternal effects in rats (bw ↓) LOAEL 39 ppm (47 mg/m³) NOAEL 20 ppm (24 mg/m³) developmental effects (bw ↓, skeletal ossification ↓) LOAEL 39 ppm (47 mg/m³) NOAEL 20 ppm (24 mg/m³)
Oral gavage exposure	maternal effects in rats (bw and food consumption) LOAEL 225 mg/kg bw day NOAEL 150 mg/kg bw day (tested as 5% aqueous solution) developmental effects LOAEL > 225 mg/kg bw day (tested as 5% aqueous solution)	maternal effects in mice (mortality) LOAEL 185 mg/kg bw/day NOAEL 148 mg/kg bw (tested as 1% aqueous solution) developmental effects (embryo mortality, bw ↓) LOAEL 185 mg/kg bw NOAEL 148 mg/kg bw/day (tested as 1% aqueous solution)

Table 4.11.3_2 Comparison of SHMG and formaldehyde – fertility studies

Type of study	**SHMG**	**Formaldehyde**
Repeated dose toxicity (28, 90 days)	Rat, oral No effects on reproductive organs (mainly local effects)	Different species, oral or inhalation: dominant local effects.
Special studies on fertility	No data	No data

4.11.4 Summary and discussion of reproductive toxicity

Developmental toxicity

Female Sprague Dawley rats were dosed by oral gavage with the active ingredient at doses 0, 75, 150 and 225 mg/kg/day (calculated without water) from Day 6 through Day 15 of gestation. The solution was administered to the animals as an aqueous dilution of SUTTOCIDE® A (50% aqueous

solution) which contained 5% w/v of the active ingredient. Cesarean section was performed on each dam on Day 20 and the uterus of each dam excised and weighed. Numbers of corpora lutea, viable and non-viable fetuses, early and late resorptions, total number of implantations, and fetal and uterine weights were recorded. There were no significant differences observed in any of the end points examined except for maternal toxicity noted in the high dose group as evidenced by suppressed body weight gain and reduced food consumption. Fetuses were examined for evidence of variations and malformations. A significant increase of skeletal malformations was observed in the low dose group only: 4.4% vs. 0% in control, shaped scapula (broad and flat) and short appendicular bones (humerus, radius, ulna, femur, tibia and fibula). However only one litter was affected and no dose dependency was observed. Therefore this was considered as spontaneous and not related to treatment.

Overall a maternal NOAEL of 150 mg/kg bw day was observed with the test substance applied as 5% aqueous solution. The developmental NOAEL was higher (225 mg/kg bw day; Dennis J Margitich 1990, Doc III A6.8.1).

Fertility

No studies are available for SHMG. The toxicity of SHMG is considered to be related to the hydrolysis product formaldehyde. Dominant local effects are observed for formaldehyde and are assumed for SHMG.

4.11.5 Comparison with criteria

The available data on potential adverse fertility effects or adverse developmental effects are conclusive and do not indicate evidence for classification.

4.11.6 Conclusions on classification and labelling

No classification for reproductive toxicity is necessary.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

No studies allowing to evaluate the SHMG toxicity toward fertility and sexual function are available. The toxicity of SHMG is considered to be related to the hydrolysis product formaldehyde.

In repeated dose studies, no adverse effects on the reproductive organs were recorded at the high dose levels. According to the DS, this provided a good indication that it is unlikely there will be an effect on fertility or sexual function following repeated administration of SHMG. Furthermore, it is not to be expected, that the breakdown product, formaldehyde, will reach the reproductive organs.

Developmental toxicity

The DS presented a developmental study (similar to OECD TG 414) on SHMG.

Female Sprague Dawley rats were dosed by gavage with the active ingredient at doses 0, 75, 150 and 225 mg/kg bw/d from day 6 through day 15 of gestation.SHMG was administered to the animals as a 5% w/v solution. Caesarean section was performed on each dam on day 20 and the uterus of each dam excised and weighed. No effects on numbers of corpora lutea, viable and non-viable foetuses, early and late resorptions, total number of implantations, and foetal and uterine weights were recorded. There were no significant differences observed in any of the end points examined except for maternal toxicity noted in the high dose group as evidenced by suppressed body weight gain and reduced food consumption. Foetuses were examined for evidence of variations and malformations. A significant increase of skeletal malformations was observed in the low dose group only: 4.4% vs. 0% in control. However, the malformation shaped scapula (broad and flat) and short appendicular bones (humerus, radius, ulna, femur, tibia and fibula), were present only in one litter and no dose response was observed.

Overall, a maternal NOAEL of 150 mg/kg bw/d was observed with the test substance applied as 5% aqueous solution. The developmental NOAEL was higher (225 mg/kg bw/d; Doc III A6.8.1).

In conclusion, the DS did not see evidence to classify for reproductive effects.

Comments received during public consultation

No comments received.

Assessment and comparison with the classification criteria

RAC notes the lack of dose-related effects on the reproductive organs from repeated dose studies with SHMG (consistent with the results of the formaldehyde studies) and the lack of adverse effects on the development of offsprings from the available developmental toxicity study with SHMG.

No specific studies on fertility are available.

Based on the lack of data and the lack of indications from the available developmental study and repeated dose studies, RAC agrees with the DS that **no classification for reproductive toxicity for SHMG is warranted**.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity - SHMG

There are no chemical structural alerts and there were no clinical signs that would indicate a potential for neurological effects in the repeat dose studies.

4.12.1.2 Other relevant information: Comparison of neurotoxicity information for SHMG and the hydrolysis product formaldehyde

Detailed data on formaldehyde are presented in the Formaldehyde Appendix. There is no indication for a concern with regard to neurotoxicity for SHMG or for formaldehyde.

Table 4.12.1_2 Comparison of SHMG and its hydrolysis product formaldehyde

	SHMG	**Formaldehyde**
Effects	90 day, gavage rat No neurotoxic effects detected	Rat, inhalation exploratory behaviour and learning affected with LOAEL = 0.12 mg/m³, but considered to be related to an unspecific irritation of the nasal/olfactory mucosa and their relevance to human health is unlikely

4.12.1.3 Immunotoxicity

No specific information available

4.12.1.4 Specific investigations: other studies

SHMG hydrolyses to formaldehyde and glycine. Therefore a summary of potential neurotoxicity due to glycine is provided, supporting that there is no specific concern related to this hydrolysis product:

The non-essential amino-acid Glycine is physiologically acting as inhibitory neurotransmitter in the spinal cord and brainstem via glycine receptor (GlyR)-chloride channels (Xu, 2010; Zafra, 1997). Under certain conditions Glycine is also reported to be a co-agonist of excitatory N-methyl-D-aspartate receptors (NMDARs). Dysregulation of glycine signalling can be associated with some neural diseases (e.g. hyperexcitability disorders).

Endogenous glycine levels in glycinergic neurons are regulated by several mechanisms (Zafra, 1997). High levels can be achieved by a high rate of synthesis, slow degradation, an efficient accumulative reuptake process, or by a combination of these mechanisms. Studies using radioactive precursors suggest that much of the glycine synthesis in the CNS is derived from de novo synthesis from glucose through serine. Lower levels are supposed to be achieved mainly by degradation via the glycine cleavage system (GCS) or by conversion to L-serine. Furthermore uptake of extracellular glycine in different brain tissues is strongly regulated by the carriers GLYT1 and GLYT2, which also contribute to buffer glycine levels within the nervous system (Danysz and Parsons, 1998). A toxicologically relevant impact of exogenous glycine on endogenous glycine levels within the nervous system is thus rather unlikely.

Kawai et al. (2012) analysed the pharmacokinetics and cerebral distribution of glycine for 24 hours following oral administration of 2 g/kg to rats (after fasting). Glycine in plasma reached its maximum concentration of 5.3 mmol/L after 0.5 h (13-fold increase compared to vehicle control). In cerebrospinal fluid (CSF) Tmax was again 0.5 h, but the maximum concentration reached only 53 µmol/L (6-fold increase compared to vehicle control). In the cerebral cortex Tmax was 4 h and the maximum concentration was 1.4 pmol/mg wet tissue (2-fold increase compared to vehicle control). This shows that glycine levels increased more slowly in cortex than in plasma or CSF. Furthermore glycine levels are considerably lower in CSF and cortex compared to plasma levels indicating that distinct delivery mechanisms are present at the blood-CSF-barrier and blood-brain-

barrier. The ED50 of glycine for the glycine receptor and the NMDA receptor is 90-100 µM and 100-300 nM, respectively. Thus, in CSF the glycine levels after oral administration were higher than the ED50 of NMDA receptors but lower than the ED50 for glycine receptors and a modulating effect of exogenous glycine on NMDA receptors after high dose diet cannot be excluded. However, also the baseline level of approximately 10 µm is clearly above the ED50 for the NMDA receptor indicating that NMDA receptors are activated by endogenous glycine as well. A neurotoxic effect following oral administration of glycine is thus not expected. Furthermore, continuous administration of a large amount of glycine (>= 0.4 g/kg/d) has been reported to improve negative symptoms in patients with schizophrenia (Tuominen, 2005).

A detailed in vivo study on neurotoxic effects according to OECD guidelines after exogenous administration of glycine is not available. However, data from in vitro and repeated dose toxicity studies gave no concern for a neurotoxic effect. Glycine is normally present in the brain interstitial space at a concentration of approximately 10 µM. In vitro experiments with hippocampal cultures showed neuronal damage, but only after exposure to Fraunhofer ITEM very high concentrations of glycine (10 mM glycine for 30 min or longer or 4 mM glycine for 24 h). Concentrations up to 3 mM did not increase excitability or produce neurotoxicity (Newell, 1997).

In repeated dose toxicity studies no treatment-related clinical signs were observed after oral administration up to doses of 2000 mg/kg bw/day (28-day study; Shibui, 2013) and 3181 mg/kg bw/day (108 week study; Kitahori, 1994) in rats. Furthermore, no treatment-related organ weight changes or histopathological findings in brain tissue were observed. Thus, there is no concern for a neurotoxic effect derived from repeated dose toxicity studies.

Furthermore, Shoham et al. (2001) analysed the effect of chronic high-dose glycine nutrition on brain cell morphology in rats. Adult rats were randomized to one of three nutritional regimens (no glycine supplementation, 1 g/kg/day, or 5 g/kg/day glycine supplementation) and to one of three treatment durations (1, 3, or 5 months). Serum glycine levels were analysed at sacrifice and brain sections were examined for neurodegeneration. To explore additional neural adaptations to high-dose glycine treatment, the densitiy of class B, N-type Ca2+ channels was analysed. The results showed a dose-dependent increase of serum glycine levels. However, no evidence of neuronal or glial cell excitotoxic damage or degeneration was observed at either of the treatment intervals studied. At 3 and 5 months of glycine treatment, the density of class B, N-type Ca2+ channels was reduced in parietal cortex and hippocampus. The findings indicate that in vivo administration of high-dose glycine did not cause neurodegenerative effects and adaptive processes are present in the brain which may ensure physiological signalling.

4.12.1.5 Human information

No human information available.

4.12.2 Summary and discussion

Please see summary in chapter 4.12.1 above.

4.12.3 Comparison with criteria

No relevant neurotoxicological effects are evident for SHMG or the hydrolysis product formaldehyde. Further data for immunotoxicity or other endpoints are not available. Dominant local effects are expected.

4.12.4 Conclusions on classification and labelling

No classification for STOT SE or RE is necessary.

4.13 Overview on available data for SHMG in comparison to data for formaldehyde

Table 4.13_1 Overview table of animal data available for the assessment of human hazard

Endpoint	Sodium hydroxymethyl glycinate	Formaldehyde
Toxicokinetics		
Absorption	No data[1]	Oral, inhalation: 100% Dermal: 20 µg/cm^2 h or 300 µg/cm^2 h was estimated for a 3.7% or a 37% formaldehyde solution
Distribution	No data[1]	Reactivity at the site of first entry and rapid oxidation; systemic bioavailibilty low ^{14}C label widely distributed into C1-pool
Excretion	No data[1]	Metabolic elimination Exhaled CO_2 Urine: sodium formate
Acute dermal toxicity	Rabbit: > 2000 mg/kg bw (tested as moistened a.s. powder, corresponding to 472 mg formaldehyde/kg bw) >4000 mg/kg bw (calculated for SHMG as manufactured, 50% aqueous solution)	Rabbit LD50 = 270 mg/kg bw
Acute inhalation toxicity	Rat: >2.3 mg/L (tested as solid aerosol, corresponding to 0.54 mg formaldehyde/L >4.6 mg/L (calculated for SHMG as manufactured, 50% aqueous solution)	Rat LC_{50} (4h) = 600 mg/m³
Acute oral toxicity	Rat: 1100 mg/kg bw (calculated for SHMG 100%) 2200 (tested as 50% aqueous solution, corresponding to 260 mg formaldehyde/kg bw)	Rat LD_{50} = 640 mg/kg bw (tested as ~4% aqueous solution)
Eye irritation	Causes serious eye irritation tested as 50% aqueous solution: conjunctiva redness score ≥ 2 in 2/ 6 animals; average score of 1.4, fully reversible till day 10. tested as powder (100%): redness and chemosis score ≥ 2 for ≥ 2/3 animals	Severely eye irritating or serious eye damage opacity of the cornea following application of aqueous formaldehyde solutions with concentrations between 7 and 15 % (but reversibility tested only till 1 week, therefore formaldehyde data not fully comparable with SHMG)
Skin irritation	Causes skin irritation 5% aqueous solution were not irritating in the animal experiment (corresponding to 1.2% maximal releasable formaldehyde); no data for 50% solution; below classification criteria for dry or moistened powder (~SHMG 100%) WoE considering available data, hydrolysis and eye irritation data: Skin irritation	Causes burns concentrations of 7-9% caused erosions on the rat skin and a 1% solution still caused irritation in 5% of humans. WoE conclusion for 25-55% formaldehyde in aqueous solution: Causes burns
Skin and eye irritation in humans	No data	AEC human eye = 0.12 µg/L (gas)
Skin sensitization	Skin sensitizing GPMT: 5% intradermal induction, 50% + 5%	Sensitizing (animal and human data)

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON SODIUM N-(HYDROXYMETHYL)GLYCINATE

Endpoint	Sodium hydroxymethyl glycinate	Formaldehyde
	topical challenge	
Repeated dermal dose toxicity	No data local effects expected	Local effects, data not sufficient for assessment
Repeated inhalation toxicity	No data local effects expected	Local effects – eye irritancy long term (lit. review) human NOAEC 0.12 mg/m^3
Repeated oral toxicity **Study duration** Target organs Species LOAEL in mg/kg bw/day NOAEL in mg/kg bw/day **Study duration** Target organs Species LOAEL in mg/kg bw/day NOAEL in mg/kg bw/day	Gavage (aqueous solution) **28 days** dominant local effects 1 mortality (f); significantly ↓ total protein (m + f), focal subacute gastritis and focal ulceration of glandular stomach in few animals (m+f), significant body weight ↓ (d14, d21, m ≤ 8%) Rat 640 (tested as 5% solution) 160 (tested as 5% solution) **90 days** no adverse effects Rat >160 (tested as 2% solution) 160 (tested as 2% solution) 160 mg/kg bw day SHMG corresponds to approx. 38 mg/kg bw day max releasable formaldehyde 2% SHMG corresponds to approx. 0.47% max releasable formaldehyde.	Via drinking water **28, 90 days** limited data **2 years** dominant local effects Rat 82 (m) or 109 (f) (0.19%) 15 (m) or 21 (f) (0.026%)
Genotoxicity in vitro	Mutagenic activity in vitro	Mutagenic activity in vitro
Systemic genotoxicity in vivo	Negative in rat and mouse micronucleus tests and rat hepatocyte UDS test it is likely that neither SHMG nor formaldehyde reached the target tissues	Negative in cytogenetic & micronucleus assay; contradictory results in humans it is likely that formaldehyde did not reach the target tissues
Local genotoxicity in vivo	No data (but see positive in vitro data)	positive
Systemic carcinogenicity in experimental animals	No data[1]	No carcinogenic activity
Local carcinogenicity in experimental animals	No data	Carcinogenic activity after inhalation at > 7.4 mg/m³
Systemic carcinogenicity in humans	No data	Conflicting results
Local carcinogenicity in humans	No data	Conclusion from not unequivocal epidemiological studies: increased tumour risk after inhalation exposure
Developmental toxicity	No effects without maternal toxicity Oral developmental toxicity study in rats: maternal effects in rats (bw and food consumption): LOAEL 225 mg/kg bw day/ NOAEL 150 mg/kg bw day (tested as 5% aqueous solution)	No effects without maternal toxicity Inhalation developmental toxicity study in rats: NOAEL 24 mg/m³ Oral developmental toxicity study in mice: NOAEL 148 mg/kg bw (tested as 1% aqueous solution)
Fertility	No effects on reproductive organs in 28 and 90 day rat studies	Dominant local effects in repeated dose studies;

Endpoint	**Sodium hydroxymethyl glycinate**	**Formaldehyde**
	No specific study for fertility available[1]	No specific study for fertility available.
Neurotoxicity	no indication for neurotoxicity from clinical signs in the repeated dose studies	Rat, inhalation exploratory behaviour and learning affected with LOAEL = 0.12 mg/m³, but considered to be related to an unspecific irritation of the nasal/olfactory mucosa and their relevance to human health is unlikely

Waiving arguments:

[1)] Scientifically dispensable: The equilibrium of SHMG and its hydrolysis products formaldehyde and glycine shifts to the hydrolysis products by dilution and by the reaction of formaldehyde with biological media as well as with acidic pH (like in stomach). This supports reading across toxicological data for formaldehyde to SHMG on a molar basis. For formaldehyde a large toxicological data basis is available and summarized in the Appendix Formaldehyde. The other hydrolysis product glycine is an amino acid, a natural cell component, a food ingredient and compared to formaldehyde of low biological reactivity. Therefore –compared to formaldehyde- glycine was considered of very low concern.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

5.1.1 Stability

Sodium N-(hydroxymethyl)glycinate

Hydrolysis in water

The hydrolysis of sodium hydroxymethylglycinate (SHMG) was studied according to OECD Guideline 111 "Hydrolysis as a Function of pH", April 13, 2004 and US EPA OPPTS 835.2130. (see **Doc. II-A 7.1.1.1.1, Study A 7.1.1.1.1**). The study is rated Klimisch 2 and was conducted according to GLP. Measurements were performed to investigate the rate and the formation of hydrolysis products as a function of concentration, pH and temperature by ^{13}C NMR. The identified hydrolysis products of SHMG formaldehyde and sodium glycinate were quantified via external calibration. The investigation of hydrolysis as a function of pH were performed for buffered 1% and 0.25% solutions of SHMG at pH 4, 7 and 9. (1% and 0.25% solutions are related to the solid substance). The impact of temperature was measured for the 1% solution of SHMG adjusted to pH 7 at 10°C, 25°C and 40°C. Concentration-dependent hydrolysis of aqueous, non-buffered SHMG solutions (50%, 10%, 1% and 0.25%) were also investigated, although not required by OECD Guideline 111. 50% SHMG corresponds to biocidal products Nuosept 44 as manufactured and 0.25% to the maximum concentration of the end products. Environmentally relevant concentrations are expected to be lower than 0.25%.

Referring to this study, SHMG revealed higher hydrolytic stability at pH 9, than at 4 and 7. Stability decreased slightly with higher temperatures. Referring to measurements of buffered solutions containing 0.25% and 1% SHMG, equilibrium of hydrolysis was achieved within 1.4h at all measured pH levels (4, 7 and 9) and temperatures (10°C, 25°C and 40°C).

Table 5.1.1_1: pH-dependent formation of sodium glycinate (SG) and formaldehyde (FA) of a 0.25%SHMG solution

Component	0.25 % SHMG solution (equally to 0.0195 mol/l)			
	3.9	7.0	9.1	10.9 [a)]
SG [% w/w]	0.206	0.200	0.096	0.061
FA [% w/w]	0.055	0.055	0.030	0.016
SG [mol/l]	0.0212	0.0206	0.0099	0.0063
FA [mol/l]	0.0183	0.0183	0.0100	0.0053

[a)] unbuffered solution

Whereas, SHMG was already fully hydrolysed at pH 4 and 7 (25°C) after 1.4h, less than 50% SHMG were hydrolysed at pH 9 (1.3h, equilibrium at 25°C) (cf. Table 4.1.1.2-1). It is worth noting that the concentrations of the products of hydrolysis in the unbuffered diluted solutions were consistently less than the expected based on the chemical structure of SHMG. This is especially true for sodium glycinate, the signals of which show a strong broadening. Apart from inaccuracies in peak integration, it is assumed that the hydrolysis products are involved in dynamic processes which are responsible for the loss of signal intensity. Based on these findings, the hydrolytically half-life of SHMG is considered to be below or significantly below 1.4h at pH 4 and 7. As more

than 50% SHMG remained stable at pH 9 after 1.3h (equilibrium), no half-life could be derived/estimated for this pH. Nevertheless, it is concluded that significantly more diluted solutions than 0.25% SHMG (expected to be more relevant for environmental conditions) are hydrolysed fully at alkaline pH levels. Referring to the outcome of this study, the active substance is expected to hydrolyse completely and fast in the range of a few hours or less than one hour under environmentally relevant conditions for acidic or neutral pH values. A half-life of less than one day is used and considered for this assessment including alkaline pH based on the expected dilution.

Table 5.1.1_2: Hydrolysis of sodium hydroxymethylglycinate (SHMG)

Guideline / Test method	**pH**	**Temperature [°C]**	**Initial TS concentration, C_0 [% w/w]**	**Reaction rate constant, K_h [1/s x 10^5]**	**Half-life, DT_{50} [h]**	**Coefficient of correlation, r_2**	**Reference**
OECD 111	4, 7	25°C	0.25	not applicable	**< 1.4h**	not applicable	**Doc. III-A 7.1.1.1.1**

Conclusion

SHMG reveals higher hydrolytic stability at higher pH levels and at higher concentrations. Referring to measurements of buffered solutions containing 0.25% and 1% SHMG, equilibrium of hydrolysis was achieved within 1.4h at all measured pH levels (4, 7 and 9) and temperatures (10°C, 25°C and 40°C). Whereas, SHMG was fully hydrolysed at pH 4 and 7 (25°C), less than 50% SHMG were hydrolysed at pH 9 (25°C). Based on these findings, hydrolytically half-life of SHMG is considered to be below or significantly below 1.4h at pH 4 and 7. As more than 50% SHMG remained at pH 9 at equilibrium (achieved after < 1.3h), no half-life could be derived/estimated for this pH. Nevertheless, it is concluded that significantly more diluted solutions than 0.25% SHMG (expected to be more relevant for environmental conditions) "reveal a hydrolytic half-life" and full hydrolysis at alkaline pH respectively. Referring to the outcome of this study, the active substance is expected to hydrolyse completely and fast in the range of a few hours (pH 4, 7) or less than one day (pH 9) under environmentally relevant conditions.

Phototransformation in water

No study on photolysis of SHMG in aqueous solution was submitted (Doc. III-A 7.1.1.1.2, Justification for non-submission). The UV spectrum indicates no absorption of light at wave-lengths >290 nm (see Doc III-A 3, Section 3.4). The US EPA method OPPTS 835.2210 states that the test method is applicable to all chemicals which have a UV-absorption maximum in the range of 290-800 nm. Chemicals with UV absorption maximum of <290 cannot undergo direct photolysis in sunlight. Therefore, the active substance is no candidate for noteworthy photolysis in sunlight and the performance of a test is not necessary. The available information is assumed to be sufficient.

Phototransformation in air

The reaction rate of SHMG with OH-radicals in the atmosphere was calculated using AopWin v1.92 (see **Doc. III-A 7.3.1**). The calculated half-life was 4h corresponding to an OH-radical concentration of $5x10^5$ radicals per cm^3 (cf. Table 4.1.1.2-3; recommended default value according to EC 2003, part II, chapter 3, 2.3.6.3, p.51).

In the gas phase, SHMG is fast degraded in air via reaction with OH radicals; degradation by nitrate and ozone was not estimated Degradation rates for the reaction with ozone and NO_3 radicals were not estimated by the model. The ozone rate constant estimations produced by AOPWIN are

generally important when one or more functional group is attached to any olefinic or acetylenic unit. The AOPWIN program does not estimate reaction rates for nitrate radicals (NO_3). Reaction with NO_3 radicals is expected to be negligible, compared to reaction with OH radicals. Also direct photolysis is not expected, because the UV spectrum of the reaction product (active substance) indicates no absorption of light at wave-lengths > 290 nm (cf. Doc III-A 3.4).

Due the low volatility SHMG (cf. Chapter 1.3, Table 1.3-1), the degradation pathway in air is assumed to be of minor importance. The calculated Henry´s law constant is 1.8E-09 Pa m^3 $mole^{-1}$ (cf. Doc III-A 3) indicates that volatilization from aqueous solutions can be assumed to be also negligible.

Table 5.1.1_3: Phototransformation in air for SHMG

Guideline / Test method	**Molecule / radical**	**Rate constant**	**Molecule/Radical concentration**	**Half-life (τ1/2)**	**Reference**
Estimation direct photolysis	**h υ**	**0 (expected)**	**-**	**-**	**Doc. III-A 7.1.1.1.2 Justification for non-submission**
Estimation indirect photolysis (Calculation AopWin v1.92)	**OH**	$9.6 \cdot 10^{-11}$ cm^3/molecule s	$0.5 \bullet 10^6$ molecule/ cm^3 (24 h-day)	4.01 h	**Doc III-A 7.3.1**

Products of hydrolysis

Formaldehyde

Hydrolysis of formaldehyde can be excluded because of the absence of a hydrolysable group in the molecule (cf. Doc III-A7.1.1.1.1). However, at room temperature formaldehyde undergoes essentially complete hydration in water forming the formaldehyde hydrate “methylene glycol” ($CH_2(OH)_2$) and its oligomers, namely the low molecular mass poly(oxymethylene)glycols with the following structure $HO(CH_2O)nH$ (n = 8). At environmentally relevant concentrations, formaldehyde is expected to exist predominantly as hydrate.

There are no tests on photolysis in water of formaldehyde in aqueous solutions available. The UV spectrum of formaldehyde indicates a weak absorption of light at wavelengths between 240 and 360 nm assuming possible direct photolysis of formaldehyde in water and air. However, in aqueous solutions formaldehyde hydrate is formed (cf. Doc III-A7.1.1.1.1 and Chapter 4.1.1.2.1) having no chromophore that is capable of absorbing sunlight and thus should not decompose by direct photolysis in water. Because of the ready biodegradability, photolysis in surface waters is expected to be of minor importance.

In the air compartment, formaldehyde is susceptible to direct photolysis and in addition, formaldehyde is degraded in air via reaction with OH radicals; the half-life was estimated to be 1.97 days. Degradation by nitrate and ozone is negligible.

Based on the half-life constants of formaldehyde, accumulation in the atmosphere is not to be expected. Furthermore, the Henry's law constant is relatively low. Therefore, Formaldehyde is not expected to volatilise to air from water surfaces in significant quantities and the amount which reaches the air compartment will be washed out by rain (cf. Appendix “Formaldehyde Core Dossier”).

Sodium glycinate

Glycine is a naturally occurring amino acid and readily biodegradable. Abiotic degradation is considered to play a minor role in the degradation of glycine in the environment. Photooxidation in air using AOPWIN v1.92 resulted in a calculated half-life of 13.7 hours (calculated according to TGD, reaction rate constant of 2.8035E-11 cm^3/molec/s).

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No data available.

5.1.2.2 Screening tests

Sodium hydroxymethyl glycinate

The ready biodegradability of the active ingredient in sodium hydroxymethyl glycinate manufactured as aqueous solution was assessed by measurement of carbon dioxide evolution according to OECD Guideline 301B (**Doc III A 7.1.1.2.1_01, Study A 7.1.1.2.1**). In the study a predominantly domestic activated sludge served as inoculum (cf. Table 5.1.2.2-1). Mean carbon dioxide evolution exceeded 60% of the theoretical CO_2 yield over the course of the 28 day incubation, and within 10 days. Mineralization of the test substance reached a maximum of 99% in this study, indicating that the active ingredient in sodium hydroxymethyl glycinate manufactured as aqueous solution is readily biodegradable. The validity criteria of the guideline were met; however the reporting was rather poor. In general the documentation of the study was not complete (e.g. number and volumes of test vessels missing, descriptions of controls). Therefore this study is rated as Klimisch 2.

Table 5.1.2.2_1: Biodegradation of SHMG

Test method	**Test type**	**Test para-meter**	**Inoculum**		**Test substance conc.**	**Degradation**		**Reference**
			Type	**Concen-tration**		**Incubation period**	**Degree [%]**	
OECD Guideline 301B GLP Klimisch 2	Ready	CO_2 evolution	Activated sludge (domestic)	SSP 30 mg/L	35.3 mg/L (=10 mg DOC/L)	28 day	99 after 28 days (~87% within 10d window)	**Doc III A 7.1.1.2.1_01, Study A 7.1.1.2.1**

SSP …suspended solids

Products of hydrolysis

Formaldehyde

4 studies for ready biodegradation of formaldehyde are summarized in Appendix “Formaldehyde Core Dossier”. In addition, there are numerous other studies available, mainly from review articles and current publications. On the basis of results from a study according to OECD 301A

formaldehyde is expected to be readily biodegradable fulfilling the 10-d-window. Additional information on elimination of formaldehyde in STPs and freshwater is available (Appendix "Formaldehyde Core Dossier").

Sodium glycinate

Sodium glycinate (CAS No. 6000-44-8) is considered to be readily biodegradable by QSAR estimations (BIOWIN, EPISUITE v4.1) indicating fast biodegradation (Doc III-A 7.1.1.2.1_02). Glycine (CAS 56-40-6) is a natural occurring amino acid. Glycine is degraded via three pathways according to Freemann, 2005[5]. The glycine cleavage system is widely distributed in animals, plants and bacteria according to Kikuchi et al. 2008[6].

Therefore the dominant degradation process for glycine in the environment is expected to be biodegradation.

Also data from a registration dossier submitted to ECHA indicates readily biodegradability based on an OECD 301 C test proposal[7].

Based on the available evidence sodium glycinate is not expected to be persistent in the environment. Moreover glycine (synthetic or natural) is already permitted in the EU for use in foods under Directive 2001/15/EC. Glycine and its salts including sodium glycinate (E640) are permitted as food additives in the EU under Directive 95/2/EC on food additives other than colours and sweeteners (EFSA, 2008)[8].

Conclusion:

A GLP conform test on ready biodegradability (OECD guideline 301B) of SHMG was performed. According to the study result SHMG is readily biodegradable (99% degradation after 28 days).

The ready biodegradability of formaldehyde was investigated in four tests. Due to the results of a test according to OECD guideline 301A formaldehyde is expected to be readily biodegradable. Provided evidence suggests that also the second hydrolysis product sodium glycinate is readily biodegradable. Glycine is a natural occurring amino acid and the glycine cleavage system is widely distributed in animals, plants and bacteria. Glycine and its salts including sodium glycinate (E640) are permitted as food additives in the EU under Directive 95/2/EC on food additives other than colours and sweeteners.

5.1.2.3 Simulation tests

No data available.

---

[5] W. H. Freeman, publ. (2005): Lehninger Principles of Biochemistry, Fourth Edition, ISBN-10: 071676265X

[6] Kikuchi G1, Motokawa Y, Yoshida T, Hiraga K. (2008): Glycine cleavage system: reaction mechanism, physiological significance, and hyperglycinemia. Proc Jpn Acad Ser B Phys Biol Sci. 2008;84(7):246-63.

[7] http://apps.echa.europa.eu/registered/data/dossiers/DISS-abd5e2a9-6007-3dba-e044-00144f67d249/DISS-abd5e2a9-6007-3dba-e044-00144f67d249_DISS-abd5e2a9-6007-3dba-e044-00144f67d249.html

[8] http://www.efsa.europa.eu/de/efsajournal/doc/718.pdf

5.1.3 Summary and discussion of degradation

In a guideline and GLP conform hydrolysis study SHMG reveals pH and concentration dependant degradation with higher hydrolytic stability at higher pH levels and at higher concentrations. Whereas SHMG was fully hydrolysed at lower and neutral pH values, less than 50% SHMG were hydrolysed at pH 9 after 1.4 hours. Based on these findings, hydrolytically half-life of SHMG is considered to be below or significantly below 1.4h at pH 4 and 7. As more than 50% SHMG remained at pH 9 at equilibrium, no half-life could be derived/estimated for this pH. Nevertheless, it is concluded that significantly more diluted solutions than 0.25% SHMG (expected to be more relevant for environmental conditions) "reveal a hydrolytic half-life" and full hydrolysis at alkaline pH respectively. Photolysis and photodegradation are not expected to be relevant degradation pathways for SHMG in the environment.

A guideline and GLP conform screening test on biodegradation revealed that SHMG is readily biodegradable (99% degradation after 28 days). Concerning the hydrolysis product formaldehyde the ready biodegradability of formaldehyde was investigated in four tests. Due to the results of a test according to OECD guideline 301A formaldehyde is expected to be readily biodegradable. Provided evidence suggests that also the second hydrolysis product sodium glycinate is readily biodegradable. Glycine is a natural occurring amino acid and the glycine cleavage system is widely distributed in animals, plants and bacteria.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Sodium N-(hydroxymethyl)glycinate

Because no experimental Koc was determined by the participant a QSAR estimate was calculated using the Soil Adsorption Coefficient Program (KOCWIN v2.0, EPI Suite v4.11) that estimates the soil adsorption coefficient (K_{OC}) of organic compounds. Corrected Koc values for SHMG were ≤1 L/kg with the MCI Method and the logKow method, respectively, both indicating very low adsorption (cf. **Doc. III-A 7.1.3 - Justification**).

Also the K_{OC} was estimated according to a QSAR model described in TGD (EC 2003, part III, chapter 4.3.2, p. 24). If the measured $\log K_{OW}$ value of -1.533 (cf. Doc III-A 3.9) is used and the QSAR for Nonhydrophobics ($\log Koc = 0.52 \log K_{OW} + 1.02$) the log Koc is calculated with 0.22 (=1.67 L/kg).

However the QSAR estimate for Nonhydrophobics is very uncertain given the range of overestimation for organic acids of 0.55 log units and the underestimation of aliphatic amines of 1-2 log units.

The low adsorption coefficient indicates high mobility in soils and poor adsorption to sewage sludge and sediment solids.

Also SHMG has a pK_b value of 8.41 and >11 and is available under environmental relevant conditions mostly in its charged form. Also the hydrolytic property of the releasing compounds adds to the uncertainties to determine an adequate adsorption constant. Therefore the calculated values are only reliable with restrictions.

Products of hydrolysis

Formaldehyde

There is no study available on adsorption of formaldehyde in soils and sediments. Therefore, the K_{OC} was estimated according a QSAR model described in EU Technical Guidance Document on Risk Assessment (EC 2003). Based on a log K_{OW} of 0.35 and the QSAR for non-hydrophobics, the K_{OC} is calculated to be 15.9 L/kg. Therefore, formaldehyde is expected to exhibit only a very weak adsorption in soils and sediments.

The HPLC-screening test according to OECD Test Guideline (TG) 121 is not feasible as it is outside the scope of the method. A request for a test according to OECD TG 106 will not improve the information on the distribution behaviour of Formaldehyde in terms of overall mobility. A current literature research revealed that no information is available on the adsorption behaviour of low-molecular aldehydes (cf. Appendix "Formaldehyde Core Dossier").

Sodium glycinate

For sodium glycinate (CAS No. 6000-44-8) and glycine (CAS 56-40-6) no experimental evidence on adsorption is available. A QSAR estimate was calculated using the Soil Adsorption Coefficient Program (KOCWIN v2.0, EPI Suite v4.11) that estimates the soil adsorption coefficient (K_{OC}) of organic compounds. Results suggest low soil adsorption based on a calculated Koc value of ≤1L/kg and pH dependency.

Conclusion:

Adsorption of SHMG was determined by QSAR estimates. Corrected estimated Koc values were around 1 L/kg indicating low adsorption to solid particles in soil and sediment systems. However the estimates are compromised by the hydrolytic property of the releasing compound (e.g. determination of logK_{ow}), and the charged state at environmental relevant pH values. Therefore the estimated values have a higher degree of uncertainties.

Formaldehyde is expected to exhibit only a very weak adsorption in soils and sediments based on a log K_{OW} of 0.35 and a QSAR calculated K_{OC} of 15.9 L/kg.

5.2.2 Volatilisation

Table 5.2.2-1: Vapour pressure and Henry´s law constant

Property	Method	Purity/Specification	Results	**Reference**
Vapour pressure	1.42 x 10-5 Pa at 25°C 2.27 x 10-7 Pa at 20°C	**Doc. III-A3;** **Study IIIA 3.2**	98 %w/w	EC method A.4 Knudsen Effusion
Henry´s law constant	Result (Bond Method): 1.81E-012 atm-m3/mole corresponding to 1.83E-07 Pa x m3/mole	**Doc. III-A3;** **Study IIIA 3.2.1/01** **Study IIIA 3.2.1/02**	calculation for 100%	HENRYWIN v3.10 HENRYWIN v3.20

	Result VP/WS method using EPI values: 4.063E-018 atm-m3/mole corresponding to 4.117E-13 Pa x m3/mole	**Doc. III-A3;** **Study IIIA 3.2.1/01** **Study IIIA 3.2.1/02**	calculation for 100%	HENRYWIN v3.10 HENRYWIN v3.20
	Result VP/WS method using values of 3.2_01 and 3.5: 1.8E-09 Pa x m3/mole at 25°C	**Doc. III-A3;** **Study IIIA 3.2.1/02**	calculation for 100%	HENRYWIN v3.20

Due to the low vapour pressure of SHMG (cf. Table 5.2.2_1), volatilisation is assumed to be of minor importance. The calculated Henry's law constant is 1.8E-09 Pa m^3 $mole^{-1}$ (cf. Doc III-A 3) indicates that volatilization from aqueous solutions can be assumed to be also negligible.

5.2.3 Distribution modelling

No data available.

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Sodium N-(hydroxymethyl)glycinate

According to the TGD (EC 2003, part II, chapter 3, p. 126) a BCF_{fish} for substances with a log K_{OW} of 2 - 6 can be calculated using the QSAR developed by Veith et al. (1979). However, the log K_{OW} value for SHMG was determined to be at least -1.533. This value is outside of the domain of the QSAR.

According to ECHA (2012)[9] the effect of hydrolysis may be a significant factor for substances discharged mainly to the aquatic environment: the concentration of a substance in water is reduced by hydrolysis so the extent of bioconcentration in aquatic organisms would also be reduced. Where the half-life, at environmentally relevant pH values (4-9) and temperature, is less than 12 hours, it can be assumed that the rate of hydrolysis is greater than that for uptake by the exposed organisms. The DT50 for SHMG was determined to be less than 1.4 hour at pH 4 and 7, more stability was observed at pH 9 (cf. Chapter 5.1.1). Therefore the likelihood of bioaccumulation is greatly reduced and the determination of a BCF value is not necessary in this specific case. So it is more appropriate to consider the identified hydrolysis products.

[9] ECHA (2012): Guidance on information requirements and chemical safety assessment Chapter R.7c: Endpoint specific guidance, http://echa.europa.eu/documents/10162/13632 /information_requirements_r7c_en.pdf , 2013-10-24

The QSAR model for the estimation of a terrestrial bioconcentration factor is applicable to a logK_{OW} range of 1 to 6 (EC, 2003, part III, p. 41) and 1 to 8 (EC, 2003, part II, p. 141). The BCF - logKow relationship applies generally to neutral organic substances which are not easily biotransformed. The relationship is not valid for ionised substances (EC, 2003, part III, p. 41). Therefore no valid QSAR calculation for terrestrial bioconcentration can be made for SHMG (cf. Doc III-A 7.5.5 - Justification) either.

Products of hydrolysis

Formaldehyde

In experimental studies on bioaccumulation no elevated formaldehyde levels were found. Additional information on log K_{OW} (0.35) as well as the estimated BCF_{fish} (0.396 L/kg_{ww}) and biomagnification factor for fish-eating predators support the experimental findings that Formaldehyde does not accumulate in aquatic biota (cf. Appendix "Formaldehyde Core Dossier").

Sodium glycinate/glycine

Glycine is the smallest amino acid and has an experimental log Kow of -3.21 (cf. Doc. IIIA 7.4.2 – Justification). A BCF calculation for sodium glycinate was performed with BCFBAF™ model v3.01 of the Episuite ™ v4.10. The result showed no bioaccumulation potential.

5.3.1.2 Measured bioaccumulation data

There are no experimental data about bioaccumulation available. Because of the hydrolysis properties of SHMG (**cf. Doc III-A 7.1.1.1.1**) experimental determination of the BCF is not possible (Doc III A7.4.2 – Justification).

5.3.2 Summary and discussion of aquatic bioaccumulation

In view of the rapid hydrolysis, a test on aquatic or terrestrial bioconcentration of SHMG seems scientifically not justified. Also the use of a QSAR estimation for aquatic bioconcentration based on a log Kow <1 that is outside the applicability domain is not scientifically sound. The likelihood of bioaccumulation is greatly reduced and the determination of a BCF value is not necessary in this specific case.

Also no bioaccumulation potential for the hydrolysis products formaldehyde and sodium glycinate was identified based on modelled low Kow values. In addition glycine is a naturally occurring amino acid and the glycine cleavage system is widely distributed in animals, plants and bacteria.

5.4 Aquatic toxicity

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

The acute toxicity of SUTTOCIDE® A (powder, 97% SHMG) in Bluegill (*Lepomis macrochirus*) was determined in a 96-hour flow-through test (Doc III A7.4.1.1/01) conducted according to US

EPA series 72 of Pesticide Assessment Guidelines, FIFRA Subdivision E Hazard Evaluation: Wildlife and Aquatic Organisms (1). Nominal test concentrations of SUTTOCIDE® A used were 13.6, 22.7, 37.8, 63 and 105 mg a.i./L. Observations of mortality and other clinical signs were made at 24, 48, 72 and 96 hours. The 96h-LC_{50} of SUTTOCIDE® A in Bluegill was 100 mg a.i./L for the pure a.s. excluding any water. The no mortality concentration after 96 hours exposure was 37.8 mg a.i./L. As the test concentrations were not measured, this study was rated as Klimisch 3 and is acceptable only as supporting information.

The acute toxicity of SUTTOCIDE® A (powder, 97% SHMG) to Rainbow trout (*Oncorhynchus mykiss*) was determined in a 96-hour flow-through test (Doc III A7.4.1.1/02) conducted according to US EPA Series 72 of Pesticide Assessment Guidelines, FIFRA Subdivision E Hazard Evaluation: Wildlife & Aquatic Organisms (1). The nominal test concentrations of SUTTOCIDE® A used were 13, 21.6, 36, 60 and 100 mg a.i./L. Observations of mortality, stress and unusual behaviour were made at 22, 24, 48, 72 and 96 hours. The 96h-LC_{50} of SUTTOCIDE® A in Rainbow trout was approx. 93.8 mg ai/L for the pure a.s. excluding any water. The no mortality concentration after 96 hours exposure was 60 mg a.i./L. As the test concentrations were not measured, this study was rated as Klimisch 3 and is acceptable only as supporting information.

The acute toxicity of SUTTOCIDE® A (50 % aqueous solution = Integra 44, 49.53% SHMG) to Bluegill (*Lepomis macrochirus*) was determined in a 96-hour flow-through test (**Doc III A7.4.1.1/03**) conducted according to US EPA Series 72 of Pesticide Assessment Guidelines, FIFRA Subdivision E Hazard Evaluation: Wildlife & Aquatic Organisms (1). The nominal test concentrations of SUTTOCIDE® A used were 16, 26, 43, 72 and 120 mg a.i./L corresponding to mean measured concentrations of 5.8, 16, 40, 59 and 109 mg a.i./L. Observations of mortality, stress and unusual behaviour were made at 4, 24, 48, 72 and 96 hours. The results were based on mean measured concentrations. The **96h-LC_{50}** of SUTTOCIDE® A in Bluegill was **75 mg a.i./L for the pure SHMG** excluding any water (corresponding to 150 mg a.i./L for the a.s. as manufactured as aqueous solution). The no mortality concentration after 96 hours exposure was 40 mg ai/L (corresponding to 80 mg a.i./L for the a.s. as manufactured as aqueous solution).

The acute toxicity of SUTTOCIDE® A (50 % aqueous solution = Integra 44, 49.53% SHMG) to Rainbow trout (*Oncorhynchus mykiss*) was determined in a 96-hour flow-through test (Doc III A7.4.1.1/04) conducted according to US EPA Series 72 of Pesticide Assessment Guidelines, FIFRA Subdivision E Hazard Evaluation: Wildlife & Aquatic Organisms (1). Nominal test concentrations of SUTTOCIDE® A used were 16, 26, 43, 72 and 120 mg a.i./L corresponding to mean measured concentrations of 11, 21, 35, 62 and 120 mg a.i./L. Observations of mortality, stress and unusual behaviour were made at 4, 24, 48, 72 and 96 hours. Based on measured test concentrations, the 96h-LC_{50} of SUTTOCIDE® A in Rainbow trout was 106 mg a.i./L for the pure a.s. excluding any water (corresponding to 212 mg a.i./L for the a.s. as manufactured as aqueous solution). The no mortality concentration after 96 hours exposure was 35 mg a.i./L (corresponding to 70 mg a.i./L for the a.s. as manufactured as aqueous solution).

Table 5.4.1.1_1 Acute toxicity to fish

Guideline / Test method	**Species/ Test material**	**Endpoint Type of test**	**Exposure**		**Results [mg a.i./L]**		**Remarks**	**Reference**
			Design	**Duration**	**LC_0**	**LC_{50}**		
FIFRA Subdivision E, Series 72-1 GLP Klimisch 3	*Lepomis macrochirus* SUTTOCIDE®A (powder)	Mortality	Flow-through test	96 hours	37.8[1]	100[1]	-	Doc III A7.4.1.1/01

Guideline / Test method	**Species/ Test material**	**Endpoint Type of test**	**Exposure**		**Results [mg a.i./L]**		**Remarks**	**Reference**
			Design	**Duration**	**LC_0**	**LC_{50}**		
FIFRA Subdivision E, Series 72-1 GLP Klimisch 3	*Oncorhynchus mykiss* SUTTOCIDE®A (powder)	Mortality	Flow-through test	96 hours	60[1]	93.8[1]	-	Doc III A7.4.1.1/02
FIFRA Subdivision E, Series 72-1 **GLP** **Klimisch 1**	***Lepomis macrochirus*** **SUTTOCIDE®A (50 % aqueous solution = Integra 44)**	**Mortality**	**Flow-through test**	**96 hours**	**40**	**75**	**Results are calculated for the pure a.s.**	**Doc III A7.4.1.1/03**
FIFRA Subdivision E, Series 72-1 GLP Klimisch 1	*Oncorhynchus mykiss* SUTTOCIDE®A (50 % aqueous solution = Integra 44)	Mortality	Flow-through test	96 hours	35	106	Results are calculated for the pure a.s.	Doc III A7.4.1.1/04

[1] results based on nominal concentrations

5.4.1.2 Long-term toxicity to fish

No data are available.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

The acute toxicity of SUTTOCIDE® A (powder, 97% SHMG) in *Daphnia magna* was determined in a 48 hour flow-through test (Doc III A7.4.1.2/01) conducted according to US EPA Series 72 of Pesticide Assessment Guidelines, FIFRA Subdivision E Hazard Evaluation: Wildlife & Aquatic Organisms (2). The test was carried out using five concentrations of SUTTOCIDE® A at 13, 21.6, 36, 60 and 100 mg a.i./L. Observations of mortality and other clinical signs were made at approximately 24 and 48 hours. Based on nominal concentrations, the 48h-EC_{50} of SUTTOCIDE® A in *Daphnia magna* was 46.5 mg a.i./L for the pure a.s. excluding any water. The NOEC was 36 mg a.i./L. As the test concentrations were not measured, this study was rated as Klimisch 3 and is acceptable only as supporting information.

The acute toxicity of SUTTOCIDE® A (50% aqueous solution = Integra 44, 49.53% SHMG) in *Daphnia magna* was determined in a 48 hour flow-through test (**Doc III A7.4.1.2/02**) conducted according to US EPA Series 72 of Pesticide Assessment Guidelines, FIFRA Subdivision E Hazard Evaluation: Wildlife & Aquatic Organisms (2). The test was carried out using five concentrations of SUTTOCIDE® A at 16, 26, 43, 71 and 120 mg a.i./L corresponding to mean measured concentrations of 7.3, 15, 29, 53 and 116 mg a.i./L. Observations of mortality and other clinical

signs were made at approximately 24 and 48 hours. Based on measured concentrations, the **48h-EC_{50}** of Suttocide A in *Daphnia magna* was **39 mg a.i./L for the pure SHMG** excluding any water (corresponding to 78 mg a.i./L for the a.s. as manufactured as aqueous solution). The NOEC was 15 mg a.i./L (corresponding to 30 mg a.i./L for the a.s. as manufactured as aqueous solution).

Table 5.4.2.1_1: Acute toxicity to aquatic invertebrates

Guideline / Test method	**Species/ Test material**	**Endpoint Type of test**	**Exposure**		**Results [mg a.i./L]**		**Remarks**	**Reference**
			Design	**Duration**	**EC_0**	**EC_{50}**		
FIFRA Subdivision E, Series 72-2 GLP Klimisch 3	*Daphnia magna* SUTTOCIDE®A (powder)	Mobility	Flow-through test	48 hours	36[1]	46.5[1]	-	Doc III A7.4.1.2/01
FIFRA Subdivision E, Series 72-2 **GLP** **Klimisch 1**	***Daphnia magna*** **SUTTOCIDE®A (50 % aqueous solution = Integra 44)**	**Mobility**	**Flow-through test**	**48 hours**	**15**	**39**	**-**	**Doc III A7.4.1.2/02**

5.4.2.2 Long-term toxicity to aquatic invertebrates

No data available.

5.4.3 Algae and aquatic plants

The effects of SUTTOCIDE® A (50% aqueous solution, Integra 44, 51.12% SHMG) on the growth of the alga *Selenastrum capricornutum* (Doc III A7.4.1.3/01) was determined in accordance with OECD Guideline 201 (following Annex 5 (92/69/EEC) to Commission Directive 92/32/EEC: C.3. Algal Inhibition Test). The test was carried out using six concentrations of SUTTOCIDE® A as 50% aqueous solution at 1.6, 3.125, 6.25, 12.5, 25 and 50 mg/L corresponding to 0.8, 1.6, 3.2, 6.4, 12.8 and 25.6 mg a.i./L for the pure a.s. excluding any water. The 72h-EC_{50} of SUTTOCIDE® A for growth inhibition in *Selenastrum capricornutum* was calculated to be 6.3 mg a.i./L for the pure a.s. excluding any water (corresponding to 12.3 mg a.i./L for the a.s. as manufactured as aqueous solution) based upon area under the growth curves and 8.65 mg a.i./L for the pure a.s. excluding any water (corresponding to 16.9 mg a.i./L for the a.s. as manufactured as aqueous solution) based upon average specific growth rate. Based on areas under the growth curves the NOEC was calculated to be 1.6 mg a.i./L for the pure a.s. excluding any water (corresponding to 3.125 mg a.i./L for the a.s. as manufactured as aqueous solution) and 3.2 mg a.i./L for the pure a.s. excluding any water (corresponding to 6.25 mg a.i./L for the a.s. as manufactured as aqueous solution) based upon average specific growth rate. Because of unexplained inconsistencies between the draft report and the final report of this study, and the missing analysis of the test substance concentration, this study is considered as Klimisch 3 and can only be used as supporting information.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON SODIUM N-(HYDROXYMETHYL)GLYCINATE

The effects of Nuosept™ 44 Microbiocide (50% aqueous solution, 50.09% SHMG) on the growth of the alga *Desmodesmus subspicatus* (**Doc III A7.4.1.3/02**) was determined in accordance with OECD Guideline 201. The test was carried out using six concentrations of Nuosept™ 44 Microbiocide at 1.23, 2.46, 4.91, 9.82, 19.6 and 39.3 mg/L corresponding to 0.625, 1.25, 2.5, 5, 10 and 20 mg a.i./L for the pure a.s. excluding any water. In this study the actual concentrations for the pure a.s. excluding any water were indirectly determined by HPLC analysis of the formaldehyde concentrations at the beginning of the test and after 24, 48 h and 72 h of exposure. The single concentrations of the replicates varied between 64% and 132% of the expected formaldehyde concentration over the 72 h test period. These analyses confirmed that the test item was correctly dosed, since the geometric mean of the measured concentrations of formaldehyde by HPLC ranged between 82 and 100% of the nominal concentration of (theoretically) releasable formaldehyde. The following results were based on the calculated actual concentrations of the a.s. (excluding any water) transformed from mean measured formaldehyde concentrations. The **72h-EC_{50}** of Nuosept™ 44 Microbiocide for growth inhibition in *Selenastrum capricornutum* was calculated to be **5.37 mg /L for the pure SHMG** excluding any water (corresponding to 10.6 mg a.i./L for the a.s. as manufactured as aqueous solution) **based upon area under the growth curves** and **10.1 mg a.i./L for the pure SHMG** excluding any water (corresponding to 19.9 mg a.i./L for the a.s. as manufactured as aqueous solution) **based upon average specific growth rate.** The **NOEC** was calculated to be **2.1 mg/L for the pure SHMG** excluding any water (corresponding to 4.13 mg a.i./L for the a.s. as manufactured as aqueous solution) based upon average specific growth rate.

Table 5.4.3-1: Growth inhibition on algae

Guideline / Test method	**Species/ Test material**	**Endpoint / Type of test**	**Exposure**		**Results [mg a.i./L]**			**Reference**
			Design	**Duration**	**NOE_rC**	**E_bC_{50}**	**E_rC_{50}**	
Annex 5 (92/69/EEC) to Commission Directive 92/32/EEC: C.3. Algal Inhibition Test GLP Klimisch 3	*Selenastrum capricornutum*	Cell multiplication inhibition	Static	72 hours	3.2[1]	6.3[1]	8.65[1]	Doc III A7.4.1.3/01
OECD 201 **GLP** **Klimisch 1**	*Desmodesmus subspicatus*	**Cell multiplication inhibition**	**Static**	**72 hours**	**2.1[2]**	**5.37[2]**	**10.1[2]**	**Doc III A7.4.1.3/02**

[1] results based on nominal concentrations, [2] indirect determined actual concentrations calculated from mean measured formaldehyde concentrations

5.4.4 Other aquatic organisms (including sediment)

Inhibition of microbial activity (aquatic)

Sodium N-(hydroxymethyl)glycinate

The effects of sodium N-(hydroxymethyl)glycinate (50% aqueous solution, 50.06% SHMG) on the respiration of activated sludge (**Doc III A7.4.1.4**) were assessed according to EC Commission Directive 87/302/EEC: Part C, Biodegradation: Activated Sludge Respiration Inhibition Test, C.11. Formaldehyde released from sodium N-(hydroxymethyl)glycinate was tested at nominal concentrations of 100, 200, 400, 600 and 800 mg/L. After a 3 hour contact time the respiration rates of activated sludge were measured. The **EC_{50}** on the respiration of activated sludge was **279 mg /L for the pure SHMG** excluding any water (corresponding to 558 mg a.i./L for the a.s. as manufactured as aqueous solution).

Table 5.4.4_1: Inhibition of microbial activity (aquatic)

Guideline/ Test method	Species / Inoculum	Endpoint /Type of test	Exposure		Results [mg a.i./L]			Remarks	Reference
			Design	Duration	EC_{20}	EC_{50}	EC_{80}		
EC Commission Directive 87/302/EEC: Part C, Biodegradation: Activated Sludge Respiration Inhibition Test **GLP** **Klimisch 1**	**Activated sludge**	**Respiration inhibition**	**Static**	**3 hours**	**166[1]**	**279[1]**	**469[1]**	**-**	**Doc III A7.4.1.4**

[1] results based on nominal concentrations

5.5 Toxicity of hydrolysis products to aquatic organisms

Formaldehyde

The toxicity of formaldehyde to aquatic organisms was tested in several studies covering different trophic levels. The submitted effect values range from 4.7 to 69 mg/L. For comparison, the acute toxicity of formaldehyde to fish ranges from LC_{50} (96 h) = 5.7 - 1020 mg/L (OECD 2002). The lowest reliable effect value of 5.7 mg/L was obtained with the striped bass (*Morone saxatilis*) (Formaldehyde Core Dossier, Doc. III-A 7.4.1.1/05_HCHO).

Acute toxicity towards invertebrates was tested with the cladocerans *Daphnia magna* and *Daphnia pulex*. Further studies using a number of invertebrate species from a wide array of taxa are reported. The lowest reliable 48h-EC_{50} for invertebrates is 5.8 mg/L (*D. pulex*). The test on *Daphnia magna* revealed a 48h-EC50 of 29 mg/L (Formaldehyde Core Dossier, Doc. III-A 7.4.1.2/03_HCHO).

Two algal toxicity studies with *Desmodesmus subspicatus* produced consistent results on growth inhibition with a geometric mean 72h-E_rC_{50} of 5.7 mg/L (Formaldehyde Core Dossier, Doc. III-A 7.4.1.3_HCHO).

Chronic toxicity towards fish (*Danio rerio*) was investigated in a study comparable to OECD Guideline 212. A 6d- EC50 of 6.9 mg/L was obtained from this study. However since no NOEC was reported and the test duration (6 days) was shorter than recommended by the guideline the information can only be used as additional information.

One chronic toxicity study according to OECD guideline 211 with *Daphnia magna* is available. In this study a 21 days NOEC of 1.04 mg/L, based on the age of the first reproduction was found. This study is considered as key study.

It has to be considered, that the applicant has not provided the long-term *Daphnia* study for the approval of SHMG, therefore a new long-term Daphnia study or a letter of access to the already available study needs to be provided by the applicant at product authorisation stage.

The acute toxicity of formaldehyde towards bacteria was investigated in two studies. The test according to OECD Guideline 209, determining the inhibition of respiration in a sewage sludge sample, resulted in an EC_{50} of 20.4 mg/L.

Please see Appendix “Formaldehyde Core Dossier” for detailed information.

Glycine/sodium glycinate

is naturally occurring in diets, humans and animals. Glycine and its salts including sodium glycinate (E640) are permitted as food additives in the EU under Directive 95/2/EC on food additives other than colours and sweeteners. Its ecotoxicity is considered of no concern. This is confirmed by QSAR estimates with the ECOSAR V1.11 model (cf. Table 5.5_1). For neuroendocrine effects please see chapter 4.12.1.4.

Table 5.5_1: Results of ECOSAR v1.11 QSAR estimates of sodium glycinate and glycine

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
--> Acid moeity found: Predicted values multiplied by 10				
Aliphatic Amines-acid	: Fish	96-hr	LC50	5.15e+005 *
Aliphatic Amines-acid	: Daphnid	48-hr	LC50	32749.543
Aliphatic Amines-acid	: Green Algae	96-hr	EC50	93748.484
Aliphatic Amines-acid	: Fish		ChV	2.04e+005
Aliphatic Amines-acid	: Daphnid		ChV	1459.729
Aliphatic Amines-acid	: Green Algae		ChV	19779.402
Neutral Organic SAR	: Fish	96-hr	LC50	4.44e+006 *
(Baseline Toxicity)	: Daphnid	48-hr	LC50	1.53e+006 *
	: Green Algae	96-hr	EC50	1.44e+005
	: Fish		ChV	2.41e+005
	: Daphnid		ChV	37143.629
	: Green Algae		ChV	12443.324

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

5.6 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Aquatic Acute 1:

All available acute L(E)C_{50} values for SHMG and formaldehyde for all three trophic levels are >1 mg/L. The lowest L(E)C_{50} value available is the E_rC_{50} (algae) for SHMG with 11.76 mg/L. Therefore no classification with Aquatic Acute 1 is necessary.

➔ **No classification**

Studies used:

SHMG:

- Doc III A7.4.1.1/03: Wildlife International Ltd (1996), FIFRA Subdivision E, Series 72-1 -> **96h-LC_{50} (fish, calculated, based on measured concentrations of sodium glycinate) =75 mg /L for the pure SHMG excluding water**
- Doc III A7.4.1.2/02: Wildlife International Ltd (1996), FIFRA Subdivision E, Series 72-2 and ASTM Standard E729-88a -> **48h-EC_{50} (crustacean, calculated, based on measured concentrations of sodium glycinate) =39 mg/L for the pure SHMG excluding water**
- Doc III A7.4.1.3/02: BMG Engineering Ltd (2015), OECD 201: Algal Inhibition Test -> **72h-E_rC_{50} (algae, calculated, based on measured concentrations of formaldehyde) =11.76 mg/L for the pure SHMG excluding water**

Formaldehyde: (for details and references see "Formaldehyde Core Dossier")

- FA Doc. III-A 7.4.1.1/05_HCHO -> **(96h) LC_{50} (fish) =5.7 mg/L**
- FA Doc. III-A 7.4.1.2/03_HCHO -> **(48h) EC_{50} (crustacean) = 5.8 mg/L**
- FA Doc. III-A 7.4.1.3_HCHO -> **(72h) E_rC_{50} (algae) = 5.7 mg/L (mean)**

Aquatic Chronic Categories:

For SHMG one 72hr-NOECs are available for algae, which is >1 mg/L (2.5 mg/L). For fish and crustaceans acute LC_{50s} are >10 mg/L (75 mg/L and 39 mg/L, respectively) and SHMG is rapid degradable (based on ready biodegradability); additionally a measured log K_{ow} of -1.533 is available. On the basis of these data no classification for any of the chronic categories is needed for SHMG.

There is only one reliable chronic NOEC value available (>1 mg/L) for formaldehyde from crustacean. For fish and algae EC_{50} values >1 mg/L are available, which in combination with ready biodegradability, a measured log K_{OW} of 0.35 and a calculated BCF_{fish} of 0.396 L/kg doesn't lead to any classification.

Sodium glycinate/glycine is a naturally occurring amino acid and its ecotoxicity is of no concern. This is further substantiated by QSAR estimations.

Therefore no classification for hazards to the aquatic environment is proposed for SHMG, since neither the available data on SHMG itself, nor the data on its hydrolysis products fulfill the criteria. However, for Formaldehyde a NOEC of 1.04 mg/L was derived for daphnia, which is close to the criterion (<1 mg/L) for classification.

Aquatic Chronic 1, 2, 3 and 4:

➔ **No classification**

Studies used:

SHMG:

- Doc. III-A 7.1.1.2.1**:** Covance Laboratories Ltd (2002), OECD 301 B: Assessment of ready biodegradability; CO_2 Evolution Test -> **99% degradation in 28 days, 10-d window fullfilled**
- Doc. III-A 3: Partition coefficient of SHMG, (measured) -> **log K_{ow} =-1.533**
- Doc III A7.4.1.1/03: Wildlife International Ltd (1996), FIFRA Subdivision E, Series 72-1 -> **96h-LC_{50} (fish, calculated, based on measured concentrations of glycine) =75 mg /L for the pure SHMG excluding water**
- Doc III A7.4.1.2/02: Wildlife International Ltd (1996), FIFRA Subdivision E, Series 72-2 and ASTM Standard E729-88a -> **48h- EC_{50} (crustacean, calculated, based on measured concentrations of glycine) =39 mg/L for the pure SHMG excluding water**
- Doc III A7.4.1.3/02: BMG Engineering Ltd (2015), OECD 201: Algal Inhibition Test -> **72h-NOEC (algae, calculated, based on measured concentrations of formaldehyde) =2.5 mg/L for the pure SHMG excluding water**

Formaldehyde: (for details and references see "Formaldehyde Core Dossier")

- FA Doc. III-A 7.1.1.2/04_HCHO**:** OECD 301 A -> **readily biodegradable**
- FA Doc. III-A 3_HCHO: Hansch et al. (1995), Sangaster (1989), in accordance with 92/69/EEC A.9, Shake-Flask Method, Partition coefficient of Formaldehyde -> measured **log K_{ow} =0.35**
- Calculation according to TGD on Risk Assessment -> **BCF $_{fish.\ calculated}$ =0.396**
- FA Doc. III-A 7.4.1.1/05_HCHO -> **96h- LC_{50} (fish) =5.7 mg/L**
- FA Doc. III-A 7.4.1.2/03_HCHO -> **21 days- NOEC (crustacean) = 1.04 mg/L**
- FA Doc. III-A 7.4.1.3_HCHO -> **72h- E_rC_{50} (algae) = 5.7 mg/L (mean)**

Hazards to the ozone layer:

On the basis of low vapour pressure, low Henry´s Law constants and rapid degradation through reaction with hydroxyl radicals for SHMG as well as for its hydrolysis products there are no indications for danger to the ozone layer.

Also SHMG as well as its hydrolysis products are not listed in Annex I and II of Regulation (EC) No 1005/2009 of the European Parliament and of the Council of 16 September 2009 on substances that deplete the ozone layer.

5.7 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

No classification for hazards to the aquatic environment and to the ozone layer is proposed for SHMG, since neither the available data on SHMG itself, nor the data on its hydrolysis products fulfill the criteria.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's (DS) proposal

Sodium N-(hydroxymethyl)glycinate (SHMG) is a reaction product of formaldehyde and glycine. When SHMG is diluted in water, it hydrolyses to formaldehyde and glycine. Glycine is an amino acid, a naturally occurring biological molecule, a food ingredient and compared to formaldehyde of low concern as a toxin. Therefore, the toxicity of SHMG relates primarily to the toxicity of SHMG and formaldehyde.

Degradation

The dossier submitter proposed to consider SHMG as rapidly degradable. The basis for this proposal is an OECD TG 301B test result (Doc III A 7.1.1.2.1_01, Study A 7.1.1.2.1). In the study, a predominantly domestic activated sludge served as inoculum (cf. Table 5.1.2.2-1) was used. Mean carbon dioxide evolution exceeded 60% of the theoretical CO_2 yield over the course of the 28 day incubation, and the 10-day window was fulfilled.

Mineralization of the test substance reached a maximum of 99% in this study, indicating that the active ingredient in sodium hydroxymethyl glycinate manufactured as aqueous solution was readily biodegradable. The validity criteria of the guideline were met; however the reporting was rather poor. In general, the documentation of the study was not complete (e.g. number and volumes of test vessels missing, descriptions of controls). Therefore, this study was rated as Klimisch 2. SHMG is also unstable to hydrolysis with hydrolysis values being below 1.4h at pHs 4, 7, and 9 (at 10, 25, and 40 °C).

Aquatic Bioaccumulation

The DS proposed that SHMG does not meet the CLP criteria for bioaccumulation. The basis for this proposal is a measured log K_{OW} value of -1.533 (cf. Doc III-A 3.9). However, the test substance will hydrolyse under the test conditions, especially at the applied concentration (10 µL in 10 mL ISA water), and thus the partition coefficient of the hydrolysis products has actually been measured. Calculations (KOWWIN v1.67) show a logP_{ow} of -6.19 for the sodium salt and -3.41 for the non-ionized form.

There are no experimental BCF data available. Due to the hydrolysis properties of SHMG (cf. Doc III-A 7.1.1.1.1), experimental determination of the BCF is not possible (Doc III A7.4.2 –Justification). Overall, a low bioaccumulation potential is expected for SHMG.

Acute Toxicity

The dossier submitter proposed to not classify SHMG as acutely hazardous to the aquatic environment. The basis for this proposal was that from relevant and reliable tests for all three trophic levels, the lowest available acute L(E)C_{50} values for SHMG are above 1 mg/L.

Fish	*Lepomis macrochirus*
Doc III A7.4.1.1/03: Wildlife International Ltd (1996), FIFRA Subdivision E, Series 72-1	96h-LC_{50} 75 mg/L for pure SHMG excluding water (calculated, based on mean measured concentrations of sodium glycinate)
Invertebrates	*Dapnia magna*
Doc III A7.4.1.2/02: Wildlife International Ltd (1996), FIFRA Subdivision E, Series 72-2 and ASTM Standard E729-88a	48h-EC_{50} 39 mg/L for pure SHMG excluding water (calculated, based on measured concentrations of sodium glycinate)
Algae	*Desmodesmus subspicatus*
Doc III A7.4.1.3/02: BMG Engineering Ltd (2015), OECD 201: Algal Inhibition Test	72h-ErC_{50} 11.76 mg/L for pure SHMG excluding water (calculated, based on measured concentrations of formaldehyde)

Hydrolysis products

Acute formaldehyde toxicity data are available in the REACH registration dossier, which is disseminated on ECHA's website. Summaries of these data are also presented in the background document (BD). Glycinate and its salts such as sodium glycinate are

naturally occurring substances present in all life, they are therefore unlikely to be of concern. This is confirmed by the QSAR data for glycine and sodium glycinate (summarised in BD). The data for formaldehyde and glycinate/sodium glycinate indicate acute toxicity values above 1 mg/L for all trophic levels, further indicating that no classification is warranted.

Chronic Toxicity

The dossier submitter proposed to not classify SHMG as chronically hazardous to the aquatic environment. The basis for this proposal is that for algae a reliable 72hr-NOE_rC is available which is above 1 mg/L. For fish and crustaceans, no chronic aquatic toxicity data are available. However, as SHMG is rapidly degradable and has a low potential for bioaccumulation, a conclusion of no classification is derived via the surrogate approach.

fish	no chronic aquatic toxicity data available
crustacean	no chronic aquatic toxicity data available
algae	*Desmodesmus subspicatus*
Doc III A7.4.1.3/02: BMG Engineering Ltd (2015), OECD 201: Algal Inhibition Test	72h-NOE_rC 2.5 mg/L for pure SHMG excluding water (calculated, based on mean measured concentrations of formaldehyde)

Hydrolysis Products

Chronic formaldehyde toxicity data are available in the REACH registration dossier, which is disseminated on ECHA's website. Summaries of these data are also presented in the background document (BD). The available chronic toxixity data for formaldehyde is a *Daphnia* 21 d NOEC of 1.04 mg/L, indicating no classification. No chronic data toxicity were available for formaldehyde in fish or algae. However, as formaldehyde appears to be both rapidly degradable (based on ready biodegradation of 99% after 28 d) and has a low potential for bioaccumulation (measured Log k_{ow} 0.35 and calculated BCF 0.396 L/kg), a conclusion of no classification is derived via the surrogate approach. As the QSAR derived chronic toxicity values for glycinate/sodium glycinate indicates toxicity above 1 mg/L for all trophic levels (summarised in BD), this further indicated that no classification for SHMG is warranted.

Comments received during public consultation

Two MSs commented and both agreed with the proposal to not classify SHMG as hazardous to the aquatic environment.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the dossier submitter to assess SHMG as being rapidly degradable,

based on being readily biodegradable under OECD TG 301 B and unstable to hydrolysis.

Aquatic Bioaccumulation

RAC agrees with the dossier submitter that SHMG does not fulfil the criteria on aquatic bioaccumulation, based on a measured Log K_{ow} of -1.533 and being rapidly hydrolysed at environmentally relevant pHs and temperatures.

Acute Toxicity

RAC agrees with the dossier submitter to not classify SHMG as acutely hazardous to the aquatic environment, based on measured acute toxicity values above 1 mg/L for SHMG and formaldehyde, as well as QSAR data for glycine/sodium glycinate at all trophic levels.

Chronic Toxicity

One available 72 h NOE_rC for algae is above 1 mg/L. Long-term toxicity data for fish and invertebrates not available. However, as SHMG is rapidly degradable and has a low potential for bioaccumulation, the conclusion via the surrogate approach is no classification. Furthermore, a *Daphnia* NOEC for formaldehyde was above 1 mg/L. No chronic data toxicity are available for formaldehyde in fish or algae. However, as formaldehyde appears to be both rapidly degradable and has a low potential for bioaccumulation, no classification is derived via the surrogate approach. Furthermore, QSAR data for glycine and sodium glycinate show results considerably above 1 mg/L for all trophic levels. Overall, RAC agrees with the dossier submitter that SHMG does not warrant classification for chronic hazards to the aquatic environment.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

The dossier submitter proposed to <u>not</u> classify SHMG as hazardous to the to the ozone layer. The basis for this proposal is a low vapour pressure, a low Henry´s Law constant and rapid degradation through reaction with hydroxyl radicals for SHMG. Also SHMG is not listed in Annex I and II of Regulation (EC) No 1005/2009 of the European Parliament and of the Council of 16 September 2009 on substances that deplete the ozone layer.

Comments received during public consultation

Two MS commented and both agreed with the proposal to <u>not</u> classify SHMG as hazardous to the ozone layer.

Assessment and comparison with the classification criteria

RAC agrees with the dossier submitter to not classify SHMG as hazardous to the ozone layer.

6 OTHER INFORMATION

Not available

7 REFERENCES

Section No / Reference No	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
IIIA 3.1.1	2003	Sodium hydroxyymethyl glycinate: Determination of the Physico-Chemical Properties (EC Tests A1, A2, A3, A4) Report No: 1184/53-D2149 GLP, Unpublished	Y	ISP
IIIA 3.1.2	2003	Sodium hydroxyymethyl glycinate: Determination of the Physico-Chemical Properties (EC Tests A1, A2, A3, A4) Report No: 1184/53-D2149 GLP, Unpublished	Y	ISP
IIIA 3.1.3/01	2003	Sodium hydroxyymethyl glycinate: Determination of the Physico-Chemical Properties (EC Tests A1, A2, A3, A4) Report No: 1184/53-D2149 GLP, Unpublished	Y	ISP
IIIA 3.1.3/02	2001	Sodium hydroxyymethyl glycinate: Determination of the Physico-Chemical Properties (EC Tests A1, A2, A3, A6, A9) Report No: 1184/53-D2141 GLP, Unpublished	Y	ISP
IIIA 3.2	2003	Sodium hydroxyymethyl glycinate: Determination of the Physico-Chemical Properties (EC Tests A1, A2, A3, A4) Report No: 1184/53-D2149 GLP, Unpublished	Y	ISP
IIIA 3.2.1/01	2007	EPIWIN 3.20 Estimation for Sodium hydroxymethyl glycinate	Y	ISP
IIIA 3.2.1/02	2009	HENRYWIN v3.20 and EPI Summary v4.00 estimations for Sodium hydroxylmethyl glycinate (CAS 70161-44-3)	Y	ISP
IIIA 3.3.1/01	1991	Confidentiality attachment to: Product Chemistry: Physical and chemical characteristics of Suttocide A 50% Solution Report No: SUTTON-1991-9 GLP, Unpublished	Y	ISP
IIIA 3.3.1/02	2003	Sodium hydroxyymethyl glycinate: Determination of the Physico-Chemical Properties (EC Tests A1, A2, A3, A4) Report No: 1184/53-D2149 GLP, Unpublished	Y	ISP
IIIA 3.3.2/01	1991	Confidentiality attachment to: Product Chemistry: Physical and chemical characteristics of Suttocide A 50% Solution Report No: SUTTON-1991-9 GLP, Unpublished	Y	ISP
IIIA 3.3.2/02	2003	Sodium hydroxyymethyl glycinate: Determination of the Physico-Chemical Properties (EC Tests A1, A2, A3, A4) Report No: 1184/53-D2149 GLP, Unpublished	Y	ISP
IIIA 3.3.3	1991	Confidentiality attachment to: Product Chemistry: Physical and chemical characteristics of Suttocide A 50% Solution Report No: SUTTON-1991-9 GLP, Unpublished	Y	ISP

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON SODIUM N-(HYDROXYMETHYL)GLYCINATE

Section No / Reference No	**Year**	**Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
IIIA 3.4	2001	Sodium hydroxyymethyl glycinate: Evaluation of the Spectroscopic Properties Report No: 1184/52-D2141 GLP, Unpublished	Y	ISP
IIIA 3.5/01	2001	Sodium hydroxyymethyl glycinate: Determination of the Physico-Chemical Properties (EC Tests A1, A2, A3, A6, A9) Report No: 1184/53-D2141 GLP, Unpublished	Y	ISP
IIIA 3.5/02	2007	EPIWIN 3.20 Estimation for Sodium hydroxymethyl glycinate	Y	ISP
IIIA 3.6	1991	Confidentiality attachment to: Product Chemistry: Physical and chemical characteristics of Suttocide A 50% Solution Report No: SUTTON-1991-12 GLP, Unpublished	Y	ISP
IIIA 3.7	2009	Determination of the solubility range of Glycine, N-(hydroxymethyl)-, monosodium salt (CAS 70161-44-3) in different organic solvents	Y	ISP
IIIA 3.9	2001	Sodium hydroxyymethyl glycinate: Determination of the Physico-Chemical Properties (EC Tests A1, A2, A3, A6, A9) Report No: 1184/53-D2141 GLP, Unpublished	Y	ISP
IIIA 3.10/01	2001	Sodium hydroxyymethyl glycinate: Determination of the Physico-Chemical Properties (EC Tests A1, A2, A3, A6, A9) Report No: 1184/53-D2141 GLP, Unpublished	Y	ISP
IIIA 3.10/02	2003	Sodium hydroxyymethyl glycinate: Determination of the Physico-Chemical Properties (EC Tests A1, A2, A3, A4) Report No: 1184/53-D2149 GLP, Unpublished	Y	ISP
IIIA 3.10/03	2009	Nuosept 44 (50% aqueous solution of sodium hydroxymethyl glycinate) hydrolysis as a function of pH Fraunhofer ITEM study No. 15G08015	Y	ISP
IIIA 3.12	2001	Sodium hydroxyymethyl glycinate: Determination of the Physico-Chemical Properties (EC Tests A1, A2, A3, A6, A9) Report No: 1184/53-D2141 GLP, Unpublished	Y	ISP
IIIA 3.13	2008	Final Report Nuosept 44 Surface Tension A.5. (OECD 115), Kinematic Viscosity (OECD 114) SIEMENS Report-No.: 20080771.01	Y	ISP
IIIA 3.14	2008	Final Report Nuosept 44 Surface Tension A.5. (OECD 115), Kinematic Viscosity (OECD 114) SIEMENS Report-No.: 20080771.01	Y	ISP
IIIA 3.17	1991	Confidentiality attachment to: Product Chemistry: Physical and chemical characteristics of Suttocide A 50% Solution Report No: SUTTON-1991-9 GLP, Unpublished	Y	ISP
IIIA 3.17	2008	Study on storage stability of Nuosept 44	Y	ISP

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON SODIUM N-(HYDROXYMETHYL)GLYCINATE

Section No / Reference No	**Year**	**Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
1st add info glycine	2005	Material Safety Data Sheet; Glycine (CAS-No. 56-40-6), 10.10.2005 by Sciencelab.com, Inc., 14025 Smith Rd. Houston, Texas 77396	No	-
2ed add info glycine	2005	Material Safety Data Sheet according to 91/115ECC; Glycine (CAS-No. 56-40-6), 06.06.2005 by Tintometer GmbH, Lovibond Water Testing, Schleefstr. 8a-12, DE-44287 Dortmund	No	-
3rd add info glycine	2000	IUCLID Dataset glycine—iron sulphate (1:1) (CAS-No. 56-40-6). IUCLID CD-ROM, Year 2000 Edition, ed. by European Commission	No	-
IIIA 6.1.1/01	1997	Single Dose Oral Toxicity in Rats/LD50 in Rats Report No: MB97-5686.01 GLP, Unpublished	Y	ISP
IIIA 6.1.1/02	1979	Suttocide A - Approximate Acute Oral Toxicity (LD50) in Rats Report No: 6185a GLP, Unpublished	Y	ISP
IIIA 6.1.1/03	1979	Suttocide A - Oral LD50 in Rats Report No: H-9304 GLP, Unpublished	Y	ISP
IIIA 6.1.1/04	1992	Single Dose Oral Toxicity in Rats/LD50 in Rats Report No: MB92-1554 GLP, Unpublished	Y	ISP
IIIA 6.1.2/01	1997	Acute Dermal Toxicity in Rabbits / LD50 Report No: MB97-5686.02 GLP, Unpublished	Y	ISP
IIIA 6.1.2/02	1979	Suttocide A - Acute Dermal Toxicity in Rabbits Report No: 6185a GLP, Unpublished	Y	ISP
IIIA 6.1.3.01	1997	Acute Inhalation Toxicity in Rats/LC 50 in Rats Report No: MB97-5686.025 GLP, Unpublished	Y	ISP
IIIA 6.1.3.02	1992	EPA Acute Inhalation Toxicity - Defined LC 50 Report No: T-1557 GLP, Unpublished	Y	ISP
IIIA 6.1.4/01	1997	Primary Dermal Irritation in Rabbits Report No: MB97-5686.03 GLP, Unpublished	Y	ISP
IIIA 6.1.4/02	1979	Suttocide A- Primary Skin Irritation in Rabbits Report No: H-8713 GLP, Unpublished	Y	ISP
IIIA 6.1.4/03	1979	Primary Skin Irritation in Rabbits Report No: 6261a-1 GLP, Unpublished	Y	ISP
IIIA 6.1.4/04	1979	Primary Skin Irritation in Rabbits Report No: H-8713A GLP, Unpublished	Y	ISP

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON SODIUM N-(HYDROXYMETHYL)GLYCINATE

Section No / Reference No	**Year**	**Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
IIIA 6.1.4/05	1984	Primary Dermal Irritation in Rabbits Food & Drug Research Laboratories, Inc. FDRL Study No. 8158	Y	ISP
IIIA 6.1.4/06	1979	Suttocide A – Primary Skin Irritation in Rabbits Report No: 6261a-2 GLP, Unpublished	Y	ISP
IIIA 6.1.4/07	1980	Suttocide A - Primary Skin Irritation in Rabbits Report No: 04516 GLP, Unpublished	Y	ISP
IIIA 6.1.4/08	1997	Primary Eye Irritation/Corrosion in Rabbits Report No: MB97-5686.04 GLP, Unpublished	Y	ISP
IIIA 6.1.4/09	1979	Suttocide A – Acute Eye Irritation in Rabbits Report No: H-8712 GLP, Unpublished	Y	ISP
IIIA 6.1.4/10	1979	Suttocide A – Acute Eye Irritation in Rabbits Report No: 6261a-1 GLP, Unpublished	Y	ISP
IIIA 6.1.4/11	1979	Suttocide A – Acute Eye Irritation in Rabbits Report No: 6261a-2 GLP, Unpublished	Y	ISP
IIIA 6.1.4/12	1990	Rabbit Eye Irritation Study - With and Without Wash. Report No: PH421-SU-002-90 GLP, Unpublished	Y	ISP
IIIA 6.1.5/01	1997	Delayed Contact Dermal Sensitization Test - Buehler Method Report No: MB97-5686.06 GLP, Unpublished	Y	ISP
IIIA 6.1.5/02	1984	Suttocide A – Dermal Sensitisation Study: Maximisation Test (GPMT) in Guinea Pigs Report No: 8158 GLP, Unpublished	Y	ISP
IIIA 6.1.5/03	1990	Suttocide A – Guinea Pig Sensitisation Study Report No: 10864 GLP, Unpublished	Y	ISP
IIIA 6.1.5/04	1985	Dermal Senstisation Study: Modified Buehler Test in Guinea pigs Report No: 8453A GLP, Unpublished	Y	ISP
IIIA 6.3.1	1990	28 day Oral Toxicity Study – Rat Report No: PH436-SU-001-90 GLP, Unpublished	Y	ISP
IIIA 6.4.1/01	1984	Suttocide A – 90 Day Oral (Gavage) Toxicity Study in Rats Report No: 7824 GLP, Unpublished	Y	ISP
IIIA 6.6.1	1983	Suttocide A - Samonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay Report No:T2114.501 GLP, Unpublished	Y	ISP

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON SODIUM N-(HYDROXYMETHYL)GLYCINATE

Section No / Reference No	**Year**	**Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
IIIA 6.6.2/01	1992	In Vitro Mammalian Cytogenetic Test Report No: TA959.337003 GLP, Unpublished	Y	ISP
IIIA 6.6.2/02	2002	Sodium Hydroxymethylglycinate: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes Report No: 1184/51-D6172 GLP, Unpublished	Y	ISP
IIIA 6.6.2/03	1992	Cell Growth Inhibition of Chinese Hamster Ovary (CHO) Cells Report No: TA959.337039 GLP, Unpublished	Y	ISP
IIIA 6.6.2/04	1995	Revised Rat Hepatocyte Primary Culture/DNA Repair Test on Suttocide A Report No:PH311-SU-002-90 GLP, Unpublished	Y	ISP
IIIA 6.6.3	2002	Sodium hydroxymethyl glycinate: Mutation at the Thymidine Kinase (tk) Locus of Mouse Lymphoma L5178Y cells (MLA) using the Microtitre R Fluctuation Technique Report No: 1184/61-D6173 GLP, Unpublished	Y	ISP
IIIA 6.6.4/01	2002	Sodium hydroxymethyl glycinate: Induction of micronuclei in the bone marrow of treated rats. Report No: 1184/74-D6172 GLP, Unpublished	Y	ISP
IIIA 6.6.4/02	1987	Mirconucleus Test on Suttocide A Report No: PH309-SU-001-87 GLP, Unpublished	Y	ISP
IIIA 6.6.5/01	1994	In Vivo- In Vitro Rat Hepatocyte Unscheduled DNA Synthesis Assay Report No: TD 994.381 GLP, Unpublished	Y	ISP
IIIA 6.6.5/02	1993	Mutagenicity: Unscheduled DNA synthesis (UDS) Assay in Primary Rat Hepatocytes Report No: PH311-SU-002-90 GLP, Unpublished	Y	ISP
IIIA 6.8.1	1990	Developmental Toxicity Study in Rats Report No: PH328-SU-002-90 GLP, Unpublished	Y	ISP
IIIA 6.12	1991	Clinical Safety Evaluation: Suttocide A -Repeated Insult Patch Test (RIPT) with Humans Essex Testing Clinic, Inc. Panel Nos. 90122/90123 ETC Entry Nos. 3106.01-02 Michael Frentzko, BA, Robert W. Shanahan, PhD & Nathan Dorman, MD (1991)	Y	ISP
IIIA 7.1.1.1.1	2009	Nuosept 44 (50% aqueous solution of sodium hydroxymethyl glycinate) hydrolysis as a function of pH Fraunhofer ITEM study No. 15G08015	Y	ISP
IIIA	2002	Sodium Hydroxymethyl glycinate: Assessment of ready	Y	ISP

Section No / Reference No	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
7.1.1.2.1		biodegradability by measurement of carbon dioxide evolution Report No:1184/72-D2149, GLP, Unpublished		
IIIA 7.3.1	2010	AOPWIN v1.92 estimations for Sodium hydroxymethyl glycinate (CAS 70161-44-3) and Hydroxymethylglycine (CAS 15874-34-7), Dr S Hahn, Fraunhofer ITEM, 05 August 2010 (unpublished)	Y	ISP
IIIA 7.4.1.1/01	1991	A 96-hour Flow-Through Acute Toxicity Test with the Bluegill (Lepomis Macrochirus) Wildlife International Ltd, WLI Project No. 300A-103B	Y	ISP
IIIA 7.4.1.1/02	1991	A 96-Hour Flow-Through Acute Toxicity Test with the Rainbow Trout (Oncorhynchus mykiss Report No: 300A-104 GLP, Unpublished	Y	ISP
IIIA 7.4.1.1/03	1996	Suttocide A (Integra 44): A 96 hour flow-through acute toxicity test with the bluegill (Lepomis macrochirus) Wildlife International LTD. Project No.: 300A-108	Y	ISP
IIIA 7.4.1.1/04	1996	Suttocide A (Integra 44): A 96 hour flow-through acute toxicity test with the rainbow trout (Oncorhynchus mykiss) Wildlife International LTD. Project No.: 300A-107	Y	ISP
IIIA 7.4.1.2/01	1991	Suttocide A (Integra 44): A 48-Hour Flow-through Acute Toxicity Test with the Cladoceran (Daphnia Magna). Report No: 300A-101A GLP, Unpublished	Y	ISP
IIIA 7.4.1.2/02	1996	Suttocide A (Integra 44): A 48-Hour Flow-through Acute Toxicity Test with the Cladoceran (Daphnia Magna). Report No: 300A-109	Y	ISP
IIIA 7.4.1.3/01	2002	Suttocide: Inhibition of Growth to the Alga selenastrum capricornutum Report-Nö.: 1184/57-D2149 GLP, Unpublished	Y	ISP
IIIA 7.4.1.3/02	2015	Fresh water algal growth inhibition test with Desmodesmus subspicatus. BMG study no. A15-00577. (July 2015), GLP, unpublished	Y	Ashland Industries Europe GmbH
IIIA 7.4.1.4	2001	Sodium hydroxymethylglycinate: determination of inhibition of respiration of activated sludge Report No: 1184/59-D2145 GLP, Unpublished	Y	ISP
IIIA 7.5.3.1.1	1991	Suttocide A: An Acute Oral Toxicity Study with the Northern Bobwhite Report No: 300-103 GLP, Unpublished	Y	ISP

Section No / Reference No	**Year**	**Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
IIIA 7.5.3.1.2/01	1991	A Dietary LC50 Study with the Northern Bobwhite Report No: 300-101 GLP, Unpublished	Y	ISP
IIIA 7.5.3.1.2/02	1991	Suttocide A: A Dietary LC50 Study with the Mallard Report No: 300-102 GLP, Unpublished	Y	ISP

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON SODIUM N-(HYDROXYMETHYL)GLYCINATE

Section No / Reference No	Author(s)	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
Additional references					
Chapter 1	Kirk-Othmer	2004	Encyclopedia of Chemical Technology . 2004. 5th ed. Chapter 12. pg. 107	N	-
Chapter 1	Walker, Joseph Frederic	1975	Formaldehyde. Robert E. Krieger Publishing Co., Inc. New York	N	-
Chapter 5	ECHA	2012	Guidance on information requirements and chemical safety assessment Chapter R.7c: Endpoint specific guidance, http://echa.europa.eu/documents/10162/13632 /information_requirements_r7c_en.pdf, 2013-03-14	N	-
Chapter 5	EC	2003	Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market Part II, Part III	N	-
Chapter 5	Freeman**,** WH	2005	Lehninger Principles of Biochemistry, Fourth Edition, ISBN-10: 071676265X	N	-
Chapter 5	Kikuchi G1, Motokawa Y, Yoshida T, Hiraga K.	2008	Glycine cleavage system: reaction mechanism, physiological significance, and hyperglycinemia. Proc Jpn Acad Ser B Phys Biol Sci. 2008;84(7):246-63.	N	-
Chapter 4	Danysz W, Parsons CG.	1998	Glycine and N-methyl-D-aspartate receptors: physiological significance and possible therapeutic applications. Pharmacol Rev. 1998 Dec;50(4):597-664.	N	-
Chapter 4	ECHA	2015	ECHA database for glycine (Data retrieved on 5th April 2015). http://apps.echa.europa.eu/registered/data/dossiers/DISS-abd5e2a9-6007-3dba-e044-00144f67d249/DISS-abd5e2a9-6007-3dba-e044-00144f67d249_DISS-abd5e2a9-6007-3dba-e044-00144f67d249.html	N	-
Chapter 4, 5	EFSA	2008	Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from Commission on Flavouring Group Evaluation 79, (FGE.79) Consideration of amino acids and related substances evaluated by JECFA (63rd meeting). EFSA Journal 870, 1 – 46	N	-
Chapter 4	HMDB	2015	The Human Metabolome Database. http://www.hmdb.ca/metabolites/HMDB00123	N	-
Chapter 4	Kawai N, Bannai M, Seki S, Koizumi T, Shinkai K, Nagao K, Matsuzawa D, Takahashi M,	2012	Pharmacokinetics and cerebral distribution of glycine administered to rats. Amino Acids. 2012 Jun;42(6):2129-37.	N	-

Section No / Reference No	Author(s)	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
	Shimizu E.				
Chapter 4	Kitahori Y, Konishi N, Hayashi I, Nakahashi K, Kitamura M, Nakamura Y, Matsuda H, Fukushima Y, Yoshioka N, Hiasa Y	1994	Carcinogenicity study of glycine in Fisher 344 rats. Journal of Toxicologic Pathology, 7 (1994), pp. 471–480	N	-
Chapter 4	Newell DW, Barth A, Ricciardi TN, Malouf AT.	1997	Glycine causes increased excitability and neurotoxicity by activation of NMDA receptors in the hippocampus. Exp Neurol. 1997 May;145(1):235-44.	N	-
Chapter 4	Shibui Y, Miwa T, Yamashita M, Chin K, Kodama T.	2013	A 4-week Repeated Dose Toxicity Study of Glycine in Rats by Gavage Administration. J Toxicol Pathol. 2013 Dec;26(4):405-12.	N	-
Chapter 4	Shoham S, Javitt DC, Heresco-Levy U.	2001	Chronic high-dose glycine nutrition: effects on rat brain cell morphology. Biol Psychiatry. 2001 May 15;49(10):876-85.	N	-
Chapter 4	Tuominen HJ, Tiihonen J, Wahlbeck K.	2005	Glutamatergic drugs for schizophrenia: a systematic review and meta-analysis. Schizophr Res. 2005 Jan 1;72(2-3):225-34. Fraunhofer ITEM	N	-
Chapter 4	WHO	2005	Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series 928. WHO, Geneva	N	-
Chapter 4	Xu TL, Gong N.	2010	Glycine and glycine receptor signaling in hippocampal neurons: diversity, function and regulation. Prog Neurobiol. 2010 Aug;91(4):349-61	N	-
Chapter 4	Zafra F, Aragón C, Giménez C.	1997	Molecular biology of glycinergic neurotransmission. Mol Neurobiol. 1997 Jun;14(3):117-42.	N	-

8 ANNEXES

Throughout the CLH-Report references are made to the first draft of Competent Authority Report (CAR) on Sodium N-(hydoxymethyl)glycinate. Attached to IUCLID section 13 you will find the following parts of the first draft CAR

Doc II-A (first Draft CAR, SHMG, RMS AT, 2015)

Doc II-A confidential (first Draft CAR, SHMG RMS AT, 2015)

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON SODIUM N-(HYDROXYMETHYL)GLYCINATE

Doc III-A (first Draft CAR, SHMG , RMS AT, 2015)
(Please note that this document is still the 1st draft CAR and the applicant had no opportunity to re-check the content for potential confidential information. Therefore it has been decided to claim this document as confidential too)

Doc III-A_confidential
(This documents are definitively confidential since they hold detailed information on the active substance specification and manufacturing process)

HCHO Doc II-A (Formaldehyde core dossier, RMS DE, 2012)

HCHO Doc III-A (Formaldehyde core dossier, RMS DE, 2012)