Committee for Risk Assessment

RAC

Annex 1

Background document

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

Chloralose (INN); (R)-1,2-O-(2,2,2-trichloroethylidene)-α-D-gluco- furanose; glucochloralose; anhydroglucochloral

EC number: 240-016-7
CAS number: 15879-93-3

CLH-O-0000004852-71-03/F

Adopted

12 September 2014

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.

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Chloralose

EC Number: 240-016-7

CAS Number: 15879-93-3

Index Number: 605-013-00-0

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Version number: 3 **Date: January 2014**

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Part A.

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 SUBSTANCE

Table 1: Substance identity

Substance name:	IUPAC name: (R)-1,2-O-(2,2,2-trichloroethylidene)-alpha-D-glucofuranose Common name: Chloralose Synonyms: Alphachloralose, α-D-glucochloralose, glucochloral, Anhydroglucochloral
EC number:	240-016-7
CAS number:	15879-93-3
Annex VI Index number:	605-013-00-0
Degree of purity:	≥ 82.5% w/w
Impurities:	≤ 15.0% w/w (S)-1,2-O-(2,2,2-trichloroethylidene)-alpha-D-glucofuranose

1.2 HARMONISED CLASSIFICATION AND LABELLING PROPOSAL

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

	CLP Regulation (according to 2nd ATP of CLP)
Current entry in Annex VI, CLP Regulation	Acute Tox. 4 *; H302 Acute Tox. 4 *; H332
Current proposal for consideration by RAC	Acute Tox. 4; H302 Acute Tox. 4 *; H332 STOT SE 3; H336 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 Acute M-factor = 10 Chronic M-factor = 10
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 4; H302 Acute Tox. 4*; H332 STOT SE 3; H336 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 Acute M-factor = 10 Chronic M-factor = 10

***** - Minimum classification

1.3 PROPOSED HARMONISED CLASSIFICATION AND LABELLING BASED ON CLP REGULATION AND/OR DSD CRITERIA

Table 3: Proposed classification according to the CLP Regulation (including criteria according to 2nd ATP of CLP)

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification	Reason for no classification
2.1.	Explosives	None	-	None	Conclusive but not sufficient for classification
2.2.	Flammable gases	None			Conclusive but not sufficient for classification.
2.3.	Flammable aerosols	None			Conclusive but not sufficient for classification
2.4.	Oxidising gases	None.			Conclusive but not sufficient for classification.
2.5.	Gases under pressure	None			Conclusive but not sufficient for classification
2.6.	Flammable liquids	None.			Conclusive but not sufficient for classification
2.7.	Flammable solids	None	-	None	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	None	-	None	Data lacking
2.9.	Pyrophoric liquids	None.			Conclusive but not sufficient for classification
2.10.	Pyrophoric solids.	None	-	None	Data lacking.
2.11.	Self-heating substances and mixtures	None	-	None	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	None	-	None	Data lacking
2.13.	Oxidising liquids	None.			Conclusive but

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification	Reason for no classification
					not sufficient for classification.
2.14.	Oxidising solids	None	-	None	Conclusive but not sufficient for classification
2.15.	Organic peroxides	None			Conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	None	-	None	Data lacking
3.1.	Acute toxicity - oral	Acute Tox.4; H302	n.a.	Acute Tox.4*; H302	-
	Acute toxicity - dermal	None	-	None	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	Acute Tox.4*; H332	n.a.	Acute Tox.4*; H332	-
3.2.	Skin corrosion / irritation	None	-	None	Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	None	-	None	Conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	None	-	None	Data lacking
3.4.	Skin sensitisation	None	-	None	Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	None	-	None	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	None	-	None	Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	None	-	None	Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	STOT SE 3; H336	-	None	-

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification	Reason for no classification
3.9.	Specific target organ toxicity – repeated exposure	None	-	None	Conclusive but not sufficient for classification
3.10.	Aspiration hazard	None	-	None	Data lacking
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1; H400 Aquatic Chronic 1; H410	Acute M-factor = 10 Chronic M-factor = 10	None	-
5.1.	Hazardous to the ozone layer	None	-	None	Conclusive but not sufficient for classification

*minimal classification

Labelling:

Signal word: Warning

Pictograms: GHS07, GHS09

Hazard statements: H302+H332: Harmful if swallowed or if inhaled.
H336: May cause drowsiness or dizziness.
H410: Very toxic to aquatic life with long lasting effects.

Precautionary statements: P261 Avoid breathing dust/fume/ gas/mist/vapours/spray.
P270: Do not eat, drink or smoke when using this product.
P273: Avoid release to the environment.
P301 + P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P304 + P340: IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P391: Collect spillage.
P403 + P233: Store in a well-ventilated place. Keep container tightly closed.
P501: Dispose of contents/container to … … in accordance with local/regional/national/ international regulation (to be specified).

Proposed notes assigned to an entry:

Note C: The supplier must state on the label that the substance is a mixture of isomers.

2 BACKGROUND TO THE CLH PROPOSAL

2.1 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Chloralose is listed in Annex VI, under index number 605-013-00-0 "*chloralose (INN); (R)-1,2-O-(2,2,2-trichloroethylidene)-α-D-glucofuranose; glucochloralose; anhydroglucochloral*" as:

- Table 3.1 of Regulation (EC) No. 1272/2008: Acute Tox. 4*; H302; Acute Tox. 4*; H332;
- Table 3.2 of Regulation (EC) No. 1272/2008: Xn; R20/22.

Taking into consideration the additional information obtained under Directive 98/8/EC, it is considered necessary to update this classification.

2.2 SHORT SUMMARY OF THE SCIENTIFIC JUSTIFICATION FOR THE CLH PROPOSAL

Physico-chemical hazards

Based on the information provided under Directive 98/8/EC, chloralose is considered to be thermally stable, non-flammable, non-explosive, non-self ignited and non-oxidising. Therefore no classification is proposed under CLP regulation.

Human health hazards

Based on information submitted under Directive 98/8/EC, chloralose is proposed to be classified under CLP considering the following results:

A LD_{50}=341 mg/kg bw was obtained from an acute oral toxicity study performed with Sprague-Dawley ICO: OFA-SD (IOPS Caw) rats (5M+5F), GLP (EC Method B.1.), doses: 125 mg/kg bw; 200 mg/kg bw; 320 mg/kg bw; 2000 mg/kg bw, single dose, post exposure period of 14 days.

As the LD_{50} value is within the range of $300 < ATE \leq 2000$, the classification as Acute Tox cat 4 under CLP is warranted.

A LC_{50} could only be ascertained as being >1.99 mg/L from an acute inhalation toxicity study (nose only), GLP (EC Method B.2) performed with Spraque-Dawley Crl:CD® (SD) IGS BR rats (male and female), doses: 1.04 mg/L; 1.99 mg/L; 4.55 mg/L, applied for 4 hours and post exposure period of 14 days. 1.99 mg/L was the highest concentration where actual exposure was achieved in stable conditions. The study was considered not valid for classification purposes due to significant variability in the test substance concentration by more than 15% of the mean value at the highest concentration tested (4.55 mg/L). In addition, only 40.9% of particles were less than 4μm (respirable particle size). Therefore, it is considered not possible to withdraw the minimum classification (*) regarding this endpoint, and hence classification as Acute Tox. 4*; H332 under CLP is proposed to be maintained.

Chloralose has a toxicological mode of action based on acute CNS depressant effects causing sedation, ataxia and respiratory depressant effects. It has been used as sedative, hypnotic and anesthetic agents in veterinary and human fields. These types of effects have been also observed in experimental studies submitted in the dossier under Directive 98/8/EC, namely, the acute toxicity

studies performed with rats by the oral and inhalation routes. The following clinical observations were reported:

In the oral study, the onset of sedation, lateral decubitus, coma, and dyspnoea happened within 15 mins following administration at the doses of 125, 200 and 320 mg/kg. Most effects were reversible from 60 mins.

In the inhalation study, the observed effects (ataxia, hunched posture, lethargy, prostration) were observed in all dose groups with different severity according to dose levels applied. All animals that survived in the 3 dose groups showed reversibility in the effects, at the latest from Days 8 to 11 post-exposure.

This mode of action is also supported by human data referring to poisoning cases and medical use as a sedative and anesthetic. As the narcotic effects were demonstrated to be acute and of transient nature, a proposal for classification under CLP for STOT SE 3; H336 seems justified.

Environmental hazards

Taking into consideration the 48h-EC_{50} value from a *Daphnia magna* study (0.027 mg/L) and the 48h-E_bC_{50} from an Algae study (0.02 mg/L) conducted under Directive 98/8/CE, chloralose is considered to be very toxic for aquatic organisms. Chloralose meets the criteria to be classified as Aquatic Acute 1; H400 and since the 48h-EC_{50} value for the most sensitive organism (*Daphnia magna*) is within the range of 0.01-0.1 mg/L, an Acute M-factor of 10 should be assigned.

Chloralose is not readily nor inherent biodegradable and is not expected to undergo abiotic degradation by hydrolysis or by photolysis in water. Due to its low adsorption in soils and being readily soluble in water, chloralose is expected to move from soil to water. The low vapour pressure and low Henry's law constant of chloralose, indicates that it is non volatile and is not expected to move from water and soil to air. If present in the air, chloralose is expected to be quickly degraded by photo-oxidation.

For the long term aquatic hazard the available data is for one trophic level therefore the surrogate approach should be used. Considering that the substance is not rapidly degradable the most stringent outcome is for *Daphnia magna*, 48h-EC_{50} = 0.027 mg/L, hence category Chronic 1. This value is within the range of 0.01-0.1 mg/L and therefore a Chronic M-factor 10 is allocated.

2.3 CURRENT HARMONISED CLASSIFICATION AND LABELLING

2.3.1 CURRENT CLASSIFICATION AND LABELLING IN ANNEX VI, TABLE 3.1 IN THE CLP REGULATION

Table 4: Current classification and labelling in Annex VI, Table 3.1

Classification		**Labelling**		**Specific concentration Limits, M-factors**
Hazard Class and Category Codes	**Hazard statement Codes**	**Pictogram, Signal Word Codes**	**Hazard statement Codes**	
Acute Tox. 4 * Acute Tox. 4 *	H332 H302	GHS07 Wng	H332 H302	

* Minimum classification

2.3.2 CURRENT CLASSIFICATION AND LABELLING IN ANNEX VI, TABLE 3.2 IN THE CLP REGULATION

Table 5: Current classification and labelling in Annex VI, Table 3.2

Classification	Labelling	Concentration Limits
Xn, R 20/22	Xn; R: 20/22 S: (2-)16-24/25-28	

2.4 CURRENT SELF-CLASSIFICATION AND LABELLING

2.4.1 CURRENT SELF-CLASSIFICATION AND LABELLING BASED ON THE CLP REGULATION CRITERIA

Table 6: Notified classification and labelling according to CLP criteria in C&L Inventory (version 06/12/2013)

Classification		Labelling			Specific Concentration limits, M-Factors	Notes	Number of Notifiers
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)			
Acute Tox. 4	H302	H302		Wng			35
Acute Tox. 4	H312	H312					
Acute Tox. 4	H302	H302		GHS07 Wng			30
Acute Tox. 4	H332	H332					
		H300		GHS06 Dgr			2
		H332					

2.4.2 CURRENT SELF-CLASSIFICATION AND LABELLING BASED ON DSD CRITERIA

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

According with Article 36(2) of Regulation (EC) No. 1272/2008, chloralose, as an active substance under Directive 98/8/EC, should be subjected to harmonised classification and labelling.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

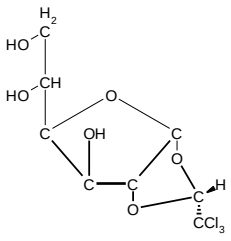
1 IDENTITY OF THE SUBSTANCE

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE

Table 7: Substance identity

EC number:	240-016-7
EC name:	chloralose
CAS number (EC inventory):	
CAS number:	15879-93-3
CAS name:	chloralose
IUPAC name:	(R)-1,2-O-(2,2,2-trichloroethylidene)–alpha–D-glucofuranose
CLP Annex VI Index number:	605-013-00-0
Molecular formula:	$C_8H_{11}Cl_3O_6$
Molecular weight range:	309.54

Structural formula:



1.2 COMPOSITION OF THE SUBSTANCE

Table 8: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Chloralose (α isomer)	≥85% w/w	≥82.5% w/w	

Table 9:Current Annex VI entry

Index number	Substance	EC number	CAS number	Classification
605-013-00-0	Chloralose (INN); (R)-1,2-O-(2,2,2-trichloroethylidene)-α-D-glucofuranose; glucochloralose; anhydroglucochloral	240-016-7	15879-93-3	Acute Tox. 4 *; H332 Acute Tox. 4 *; H302

* Minimum classification

Table 10:Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
(S)-1,2-O-(2,2,2-trichloroethylidene)-α-D-glucofuranose (ß isomer)	≤15% w/w	≤15% w/w	Substance CAS 16376-36-6 (ß isomer) is a non-active ingredient. Chloralose does not contain any other impurities above or equal to the concentration limit of 0.1 % w/w.

Current Annex VI entry: not applicable

Table 11: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks

Current Annex VI entry: not applicable

1.2.1 COMPOSITION OF TEST MATERIAL

The composition of test substance is the same as the substance to be classified and all tests were performed with cloralose which is the sum of both α and β chloralose (α ≥ 85%, β ≤ 15%) with purity ≥ 97%.

1.3 PHYSICO-CHEMICAL PROPERTIES

Table 12: Summary of physico-chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Solid (powder) White to yellowish white Odourless	Not applicable.	Not applicable
Melting/freezing point	176.6°C (97 % purity)	(1)	Measured. (OECD Method 113)
Boiling point	Not achieved	(1)	Chloralose decomposes immediately after melting. Decomposition temperature: 182.0°C. (OECD Method 113)
Relative density	0.7739 ± 0.0007 at 20°C	(2)	Measured. (EC Method A.3)
Vapour pressure	0.00883 Pa at 25°C	(3)	Estimated. (EC Method A.4) Calculated from the regression curve derived by plotting Log P vs. 1/T, at 25ºC.
Surface tension	Mean surface tension = 50.076 ± 0.045 mN/m at 20°C	(4)	Measured. (EC Method A.5)
Water solubility	Mean solubility at 24ºC: pH 5: 4.86g/L pH 7: 4.84 g/L pH 9: 4.73 g/L	(5)	Measured. (EC Method A.6)
Partition coefficient n-octanol/water	Mean log (K_{ow}) at 22-26ºC = 0.85 ± 0.03	(6)	Measured. (EC Method A.8)
Flash point	Not applicable	Not applicable	Chloralose is a solid. Therefore is not possible to determine the flash point.
Flammability	Not flammable: No ignition or combustion of the test powder train was observed.	(7)	Measured. (EC method A.10)
Explosive properties	Chloralose is not considered to be sensitive to impact, friction or heating under confinement.	(8)	Measured. (EC method A.14)

Property	Value	Reference	Comment (e.g. measured or estimated)
Self-ignition temperature	Chloralose was observed to undergo an endothermic event (possible melting) from 166°C. No self heating of the sample was observed during the test.	(9)	Measured. (EC method A.16)
Oxidising properties	The burning time of chloralose is more than the reference sample in the preliminary screening test. Therefore the sample is not expected to be an oxidising solid and completion of the full test is not deemed essential.	(1)	Measured. (EC method A.17)
Granulometry	Data lacking	-	-
Stability in organic solvents and identity of relevant degradation products	Not applicable	Not applicable	Chloralose, as manufactured, does not contain organic solvents.
Dissociation constant	Not applicable	Not applicable	The water solubility of chloralose has been determined therefore it is not scientifically necessary to determine the dissociation constant.
Viscosity	Not applicable	Not applicable	Chloralose is solid.

2 MANUFACTURE AND USES

2.1 MANUFACTURE

2.2 IDENTIFIED USES

Chloralose is used as a rodenticide substance in a slurry formulation and is presented as ready-to-use bait, at a concentration of 4 % w/w. Chloralose is placed on the market as a mixture of α and β isomers. β isomer is considered as non-active component.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 13: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Explosive Properties EC method A14	The Chloralose sample gave a negative result to all three explosivity tests, and is therefore not considered to be sensitive to impact, friction or heating under confinement according to EC method A14.	None	(8)
Flammability (Solids) EC Method A10	No ignition or combustion of the test powder train was observed after applying the hot flame from the gas burner for a period of two minutes (the maximum period specified in EC method A10). Given this result, chloralose is not classified as flammable.	None	(7)
Self-ignition Temperature EC Method A16	The sample was observed to undergo an endothermic event (possible melting) from 166°C. No self heating of the sample was observed during the test	None	(9)
Oxidising Solids (Screening test) EC Method A17	The burning time of chloralose is more than the reference sample in the preliminary screening test. Therefore the sample is not expected to be an oxidising solid and completion of the full test is not deemed essential	None	(1)
Melting/ freezing temperature Differential Scanning Calorimetry (DSC) analysis according to OECD method 113	176.6 ^{o}C	None	(1)

3.1 PHYSICO-CHEMICAL HAZARDS

3.1.1 SUMMARY AND DISCUSSION OF PHYSICO-CHEMICAL HAZARDS

3.1.2 COMPARISON WITH CRITERIA

According to the information presented in the CLH report, no classification according to the CLP criteria for physico-chemical hazardous properties is warranted.

3.1.3 CONCLUSIONS ON CLASSIFICATION AND LABELLING

Based on data mentioned above no classification is proposed for chloralose regarding physico-chemical hazards according to CLP criteria.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION):

4.1.1 NON-HUMAN INFORMATION

4.1.2 HUMAN INFORMATION

4.1.3 SUMMARY AND DISCUSSION ON TOXICOKINETICS

4.2 ACUTE TOXICITY

Table 14: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Oral – GLP (EC Method B.1.) Sprague-Dawley ICO: OFA-SD (IOPS Caw) rats (5M+5F) Doses: 125 mg/kg bw 200 mg/kg bw 320 mg/kg bw 2000 mg/kg bw single dose Post exposure period: 14 days Purity: 98,5% w/w (86.8% alpha and 13.2% beta isomers)	Rat LD_{50}: M: 611 mg/kg F: 212 mg/kg M+F: 341 mg/kg	None	(10)
Inhalation (nose only) – GLP (EC Method B.2.) Spraque-Dawley Crl:CD® (SD) IGS BR rats (male and female) Doses: 1.04 mg/L 1.99 mg/L 4.55 mg/L Applied for 4 hours Post exposure period of 14 days Purity: ≥ 97% w/w	Rat LC_{50} is > 1.99 mg/L (the highest concentration at which the exposure towards the test substance was stable).	Study not conclusive for classification purposes due to the variability in the test substance concentration; it varied by more than 15% of the mean value at the highest concentration tested (4.55 mg/L). In addition, only 40.9% of particles were less than 4µm (respirable particle size)	(11)

4.2.1 NON-HUMAN INFORMATION

4.2.1.1 ACUTE TOXICITY: ORAL

Four groups of 10 Sprague-Dawley ICO: OFA-SD (IOPS Caw) rats (5 males and 5 females each) were used in the main test. Doses administered by gavage were 125 mg/kg bw, 200 mg/kg bw, 320 mg/kg bw, 2000 mg/kg bw being the concentration in vehicle (water) of 10 ml/kg. The volume administered to each animal was adjusted according to body weight determined on day of treatment. Post exposure period was of 14 days.

In performed study, sedation, lateral decubitus, dyspnoea, and coma were the clinical adverse effects within 15 minutes following administration of the test substance at the doses of 125, 200 and 320 mg/kg. Most effects were reversible from 60 minutes. At 2000 mg/kg tonic and clonic convulsions were noted before death of the animals. The rate of death was 10%, 10%, 50% and 100% at 125, 200, 320 and 2000 mg/kg respectively. Deaths were noted within 4 hours post-treatment.

The body weight gain of the animals was not affected by the treatment with the test substance. Between days 1 and 5, a decrease in body weight was noted for one male of the 125 mg/kg dose group. The body weight of this animal showed an improvement during the remainder of the observation period. As this decrease in body weight was not observed in the other animals treated at 125 mg/kg and in the animals treated with the highest doses, it was probably not attributable to the test substance.

Macroscopic examination of the main organs of the animals found dead during the study or sacrificed at the end of the study revealed no apparent abnormalities.

The oral LD_{50} value in rats is estimated at about 341 mg/kg.

4.2.1.2 ACUTE TOXICITY: INHALATION

A rat study was performed according to GLP using three groups of 10 Spraque-Dawley Crl:CD® (SD) IGS BR rats (male and female). Each group was submitted to a inhalation test for 4 hours (nose only) been the nominal concentrations of Group 1: 3.00 mg/L, Group 2: 13.0 mg/L and Group 3: 16.7 mg/L with a post exposure period of 14 days.

In this study extensive work was performed (alternative grinding and generation techniques and varying the air flow settings and test material input) in an attempt to achieve a sustainable atmosphere at the maximal concentration tested, but no significant improvements could be made to achieve atmospheric concentrations or particle size distributions. Thus the concentration used in Group 3 was considered to be the maximal attainable.

The actual exposure of the rats during the highest concentration tested is uncertain since the exposure concentration varied by more than 15% of the mean value (4.55 mg/L), only 40.9% of particles were less than 4µm (respirable particle size) and the relative humidity was very low, ranging between 25-35%.

In Group 1 (mean achieved atmosphere concentration of 1.04 mg/L) no deaths occurred, in Group 2 (1.99 mg/L) 1 animal died during the day of exposure and in Group 3 (4.55 mg/L) 1 animal died during exposure.

Hunched posture, pilo-erection and red/brown staining around eyes and/or snout were seen in animals for short periods on removal from the chamber following a 4-hour inhalation study. Wet fur

is commonly recorded during and for a short period after exposure. These signs are considered to be associated with the restraint procedure and, in isolation, are not indicative of toxicity.

One day after exposure, all animals showed increased respiratory rate and pilo-erection. There were frequent instances of lethargy and isolated occurrences of laboured respiration, noisy respiration, prostration and red/brown staining around the eyes and snout. Animals recovered slowly to appear normal from Days 8 to 11 post-exposure.

Normal bodyweight development was noted during the study.

At necropsy no macroscopic abnormalities were detected amongst Group 1 and Group 2 animals necropsied at terminal kill.

Three animals from Group 3 showed the following macroscopic abnormalities at terminal kill:

Lungs – pale, dark patches, pale patches;

Liver – dark or pale.

Amongst animals that died during the course of the study macroscopic abnormalities were detected in lungs (abnormally red, abnormally dark) and liver (dark).

According to results the estimated LC_{50} is >1.99 mg/L (the highest concentration at which the actual exposure is certain).

4.2.1.3 ACUTE TOXICITY: DERMAL

4.2.1.4 ACUTE TOXICITY: OTHER ROUTES

4.2.2 HUMAN INFORMATION

Available human data is mostly on acute intoxications with chloralose in suicidal, homicidal and accidental cases. There are over 20 acute poisonings by chloralose reported in the literature, but fatal cases are rare (12-14). The highest ingested dose reported was 30g (14). The oral toxic dose is reported to be about 1g for adults and 20mg/kg for children (15).

4.2.3 SUMMARY AND DISCUSSION OF ACUTE TOXICITY

In an oral acute toxicity study performed according to EC Method B.1 using Sprague-Dawley ICO: OFA-SD (IOPS Caw) rats (5M+5F), and doses: 125 mg/kg bw; 200 mg/kg bw; 320 mg/kg bw; 2000 mg/kg bw, single dose, post exposure period of 14 days, submitted under Directive 98/8/EC, the value of LD_{50} = 341 mg/kg was obtained which therefore imply a modification of the existing classification for this hazard class from Acute Tox Category 4* to Acute Tox Category 4 in Annex VI of Regulation 1272/2008.

An inhalation acute toxicity study on rat was performed according to GLP. However the value of LC_{50} obtained could only be ascertain as being above 1.99 mg/L, the highest concentration at which the exposure towards the test substance was stable. Therefore this study was deemed to be inconclusive regarding this hazard class. Based on this no modification on the existing classification for this hazard class is proposed and so the minimal classification is maintained.

4.2.4 COMPARISON WITH CRITERIA

The LD_{50} of 341 mg/kg obtained from one rat oral acute toxicity study is within the range (300-2000 mg/kg bw/d) which corresponds to a classification as Acute Tox Category 4, H302, according to Regulation (EC) 1272/2008 criteria.

The obtained result from one rat inhalation acute toxicity of LC_{50} >1.99 mg/L is inconclusive so minimal classification as Acute Tox Category 4*, H332 according to Regulation (EC) 1272/2008 is maintained.

4.2.5 CONCLUSIONS ON CLASSIFICATION AND LABELLING

Based on data mentioned above it is suggested that chloralose is classified in relation to oral acute toxicity in the Annex VI of Regulation (EC) 1272/2008 as Acute Tox. 4, H302 and labeled with Pictogram GHS07 and signal word Warning.

Based on results of the study performed no modification of the existing classification as Acute Tox. 4*; H332, regarding inhalation acute toxicity entry is proposed.

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

Currently, chloralose has a minimum classification for acute oral and inhalation toxicity as Acute Tox. 4 * – H302 and Acute Tox. 4 * – H332, respectively. For acute oral toxicity, the dossier submitter confirmed the current classification (and thus proposed removal of the asterisk) based on an oral LD_{50} value of 341 mg/kg bw in rats, as this LD_{50} is within the range of $300 < ATE \leq 2000$ mg/kg bw.

For acute inhalation toxicity, the dossier submitter did not consider it appropriate to withdraw the minimum classification (and thus proposed to maintain the asterisk in the Annex VI entry) because the results of the available study in rats were considered inconclusive for classification purposes. In this study, rats were exposed for 4 hours (nose-only) to mean achieved concentrations of 1.04, 1.99, or 4.55 mg/L, and mortality was 0/10, 1/10 and 1/10 animals, respectively. Whereas the study author concluded the LC_{50} to be >4.55 mg/L, the dossier submitter concluded that the LC_{50} value can only be ascertained as being >1.99 mg/L, as this is the highest concentration at which there were not significant fluctuations in the actual exposure. At the maximum attainable concentration of 4.55 mg/L on the other hand, the actual exposure was considered to be uncertain because the exposure concentration varied by more than 15% of the mean value and only 40.9% of particles were of respirable size (<4 μm).

Comments received during public consultation

One Member State competent authority (MSCA) supported the classification proposal for both acute oral and acute inhalation toxicity. One MSCA supported the classification proposal for acute inhalation toxicity, but suggested that for the oral route Acute Tox. 3 – H301 should be considered in view of the high variability observed between males and females, and that therefore classification should be based on the LD_{50} of 212 mg/kg bw for female rats rather than on the LD_{50} of 341 mg/kg bw for males and females combined. The same comment on the acute oral toxicity was made by a third MSCA.

Assessment and comparison with the classification criteria

There is one acute **oral** toxicity study available. In this study, groups of 5 male and 5 female rats were given a single dose of 125, 200, 320 or 2000 mg/kg bw chloralose by gavage. Mortality was 1/10, 1/10, 5/10 and 10/10 animals, respectively, resulting in LD_{50} values of 212 mg/kg bw for females, 611 mg/kg bw for males and 341 mg/kg bw for males and females combined. Based on the combined value, the dossier submitter proposed chloralose to be classified as Acute Tox. 4 – H302. However, in general, classification is based on the lowest LD_{50} value available. Hence, RAC concluded that chloralose should be classified as **Acute Tox. 3 – H301**, given that the lowest LD_{50} value of 212 mg/kg bw for female rats falls within the range for category 3 ($50 < LD_{50} \leq 300$ mg/kg bw).

As to the only acute **inhalation** study available for chloralose, RAC supported the conclusion of the dossier submitter that its results were inconclusive for classification purposes. There was no information on whether this study was the basis for classifying chloralose originally with Xn; R20 under DSD, or whether it was based on other data. In the absence of adequate information it was not possible for RAC to determine whether this classification, which was translated into Acute Tox. 4* – H332 under CLP, is justified or not. Hence, a recommendation for keeping Acute Tox. 4* – H332 or not cannot be made from a scientific point of view.

4.3 SPECIFIC TARGET ORGAN TOXICITY – SINGLE EXPOSURE (STOT SE)

4.3.1 SUMMARY AND DISCUSSION OF SPECIFIC TARGET ORGAN TOXICITY – SINGLE EXPOSURE

Chloralose has been used as sedative, hypnotic and anesthetic agents in veterinary and human fields. The toxicological mode of action, typically an acute central nervous system (CNS) depressant, causing sedation, respiratory depressant has been also identified in the experimental studies submitted in the dossier under Directive 98/8/EC, namely, the acute toxicity study performed with rats by oral route (10) and inhalation routes (11). The following clinical observations were reported:

In the oral study, the onset of sedation, lateral decubitus, and dyspnoea was within 15 minutes following administration at the doses of 125, 200 and 320 mg/kg. Most effects were reversible from 60 minutes.

In the inhalation study, the observed effects (ataxia, hunched posture, lethargy, prostration) were observed in all dose groups with different severity according to dose levels applied. All animals that survived in the 3 dose groups showed reversibility in the effects, at the latest from Days 8 to 11 post-exposure.

Human information

Chloralose has been used as sedative, hypnotic, anesthetic agent and management of alcohol withdrawal symptoms in humans (16). Chloralose is no longer used in medical practice, but it has been available until recently as 75–300 mg oral and rectal dosage forms as anesthetic for human administration (17).

Available human data is mostly on acute intoxications with chloralose in suicidal, homicidal and accidental cases. There are over 20 acute poisonings by chloralose reported in the literature, but fatal cases are rare (12-14). The highest ingested dose reported was 30g (14). The oral toxic dose is reported to be about 1g for adults and 20mg/kg for children (15).

Medical and acute poisoning cases confirm that, chloralose is a CNS depressant, causing sedation, respiratory depression, and anaesthesia.

4.3.2 COMPARISON WITH CRITERIA

Two animal acute toxicity studies performed according to GLP, by oral and inhalation route showed reversible CNS depressant effects including sedation, lateral decubitus, hunched posture, ataxia, lethargy, and prostration. This mode of action is also supported by human data referring to poisoning cases and medical use as a sedative and anesthetic. As the narcotic effects were demonstrated to be acute and of transient nature, a proposal for classification under CLP for chloralose as STOT SE 3; H336 seems justified.

4.3.3 CONCLUSIONS ON CLASSIFICATION AND LABELLING

According to CLP Regulation criteria, it is suggested that chloralose is classified as STOT SE 3; H336 and labeled with Pictogram GHS07 and signal word Warning.

4.4 IRRITATION

4.4.1 SKIN IRRITATION

Table 15: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference

4.4.1.1 NON-HUMAN INFORMATION

4.4.1.2 HUMAN INFORMATION

4.4.1.3 SUMMARY AND DISCUSSION OF SKIN IRRITATION

4.4.1.4 COMPARISON WITH CRITERIA

4.4.1.5 CONCLUSIONS ON CLASSIFICATION AND LABELLING

4.4.2 EYE IRRITATION

Table 16: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference

4.4.2.1 NON-HUMAN INFORMATION

4.4.2.2 HUMAN INFORMATION

4.4.2.3 SUMMARY AND DISCUSSION OF EYE IRRITATION

4.4.2.4 COMPARISON WITH CRITERIA

4.4.2.5 CONCLUSIONS ON CLASSIFICATION AND LABELLING

4.4.3 RESPIRATORY TRACT IRRITATION

4.4.3.1 NON-HUMAN INFORMATION

4.4.3.2 HUMAN INFORMATION

4.4.3.3 SUMMARY AND DISCUSSION OF RESPIRATORY TRACT IRRITATION

4.4.3.4 COMPARISON WITH CRITERIA

4.4.3.5 CONCLUSIONS ON CLASSIFICATION AND LABELLING

4.5 CORROSIVITY

4.5.1 NON-HUMAN INFORMATION

4.5.2 HUMAN INFORMATION

4.5.3 SUMMARY AND DISCUSSION OF CORROSIVITY

4.5.4 COMPARISON WITH CRITERIA

4.5.5 CONCLUSIONS ON CLASSIFICATION AND LABELLING

4.6 SENSITISATION

4.6.1 SKIN SENSITISATION

Table 17: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference

4.6.1.1 NON-HUMAN INFORMATION

4.6.1.2 HUMAN INFORMATION

4.6.1.3 SUMMARY AND DISCUSSION OF SKIN SENSITISATION

4.6.1.4 COMPARISON WITH CRITERIA

4.6.1.5 CONCLUSIONS ON CLASSIFICATION AND LABELLING

4.6.2 RESPIRATORY SENSITISATION

4.6.2.1 NON-HUMAN INFORMATION

4.6.2.2 HUMAN INFORMATION

4.6.2.3 SUMMARY AND DISCUSSION OF RESPIRATORY SENSITISATION

4.6.2.4 COMPARISON WITH CRITERIA

4.6.2.5 CONCLUSIONS ON CLASSIFICATION AND LABELLING

4.7 REPEATED DOSE TOXICITY

Table 18: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference

4.7.1 NON-HUMAN INFORMATION

4.7.1.1 REPEATED DOSE TOXICITY: ORAL

4.7.1.2 REPEATED DOSE TOXICITY: INHALATION

4.7.1.3 REPEATED DOSE TOXICITY: DERMAL

4.7.1.4 REPEATED DOSE TOXICITY: OTHER ROUTES

4.7.1.5 SUMMARY AND DISCUSSION OF REPEATED DOSE TOXICITY

4.7.1.6 HUMAN INFORMATION

4.7.1.7 OTHER RELEVANT INFORMATION

4.7.1.8 SUMMARY AND DISCUSSION OF REPEATED DOSE TOXICITY FINDINGS RELEVANT FOR CLASSIFICATION ACCORDING TO DSD

4.7.1.9 COMPARISON WITH CRITERIA OF REPEATED DOSE TOXICITY FINDINGS RELEVANT FOR CLASSIFICATION ACCORDING TO DSD

4.7.1.10 CONCLUSIONS ON CLASSIFICATION AND LABELLING OF REPEATED DOSE TOXICITY FINDINGS RELEVANT FOR CLASSIFICATION ACCORDING TO DSD

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

4.8.2 COMPARISON WITH CRITERIA OF REPEATED DOSE TOXICITY FINDINGS RELEVANT FOR CLASSIFICATION AS STOT RE

4.8.3 CONCLUSIONS ON CLASSIFICATION AND LABELLING OF REPEATED DOSE TOXICITY FINDINGS RELEVANT FOR CLASSIFICATION AS STOT RE

4.9 GERM CELL MUTAGENICITY (MUTAGENICITY)

Table 19: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference

4.9.1 NON-HUMAN INFORMATION

4.9.1.1 IN VITRO DATA

4.9.1.2 IN VIVO DATA

4.9.2 SUMMARY AND DISCUSSION OF MUTAGENICITY

4.9.3 CONCLUSIONS ON CLASSIFICATION AND LABELLING

4.10 CARCINOGENICITY

4.10.1 NON-HUMAN INFORMATION – ANIMAL STUDIES

4.10.2 HUMAN INFORMATION

4.10.3 SUMMARY AND DISCUSSION OF CARCINOGENICITY

4.10.4 CONCLUSIONS ON CLASSIFICATION AND LABELLING

4.11 TOXICITY FOR REPRODUCTION

Table 20: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference

4.11.1 REPRODUCTIVE TOXICITY

4.11.1.1 NON-HUMAN INFORMATION – ANIMAL STUDIES

4.11.1.2 HUMAN INFORMATION

4.11.2 SUMMARY AND DISCUSSION OF REPRODUCTIVE TOXICITY

4.11.3 CONCLUSIONS ON CLASSIFICATION AND LABELLING

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 DEGRADATION

Table 21: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Biodegradability (Ready) Closed bottle test (Ready) EC Method C.4-E	Day 7 – 7.88% Day 14 – 9.45% Day 21 – 10.91% Day 28 – 16.97%	River water and garden soil	(18)
Biodegradability (Inherent) Modified Zahn-Wellens/EMPA (Inherent) OECD Guideline 302B	Day 1 – 5% Day 2 – 7% Day 3 – 7% Day 6 – 8% Day 8 – 11% Day 10 – 19% Day 16 – 15% Day 21 – 17% Day 27 – 18% Day 28 – 19%	Sewage sludge from a plant which treats domestic sewage.	(19)
Hydrolysis as a function of pH and identification of breakdown products OECD Test Guideline 111	Half-life, DT_{50} [h] not determined as further testing was not carried out, due to the degradation of chloralose being <10% at any pH	The hydrolysis data revealed that degradation of chloralose was 3.3% at pH 9 in the preliminary test (5 days). At pH 4 and 7 no degradation was verified. No further test was performed.	(20)

5.1.1 STABILITY

Abiotic degradation of chloralose, by hydrolysis, was studied according to OECD test guideline 111, at three different pH values (4, 5 and 9). Only the preliminary test was performed by incubating a solution of the active substance in buffered solutions, in the dark, at 50^{o}C for 5 days, as the observed final degradation was less than 10%. The results indicate that chloralose is hydrolytically stable and the half-life can be expected to exceed one year at 25^{o}C.

The US EPA method entitled Fate, Transport and Transformation Test Guidelines OPPTS 835.2210 Direct Photolysis Rate in Water by Sunlight states that the test method is applicable to all chemicals which have a UV/absorption maxima in the range of 290-800nm. Chloralose has an UV absorption maximum of 194.5 nm (no UV absorbance in the sun light region). So chloralose is not degradable by direct phototransformation and is assumed to be stable against photolysis in water.

5.1.2 BIODEGRADATION

5.1.2.1 BIODEGRADATION ESTIMATION

5.1.2.2 SCREENING TESTS

The biodegradation behaviour of chloralose was studied in laboratory conditions. Two biodegradation studies were performed with chloralose: Closed bottle test (Ready) and Modified Zahn-Wellens/EMPA (Inherent).

The closed bottle ready biodegradation study in compliance with EC Method C.4-E was performed at 20 ± 2 ºC with sampling on days 0, 7, 14, 21 and 28. This test revealed that the substance was not readily biodegradable (16.97% after 28 days). The reference substance exhibited a normal pattern of degradation (up to 94.04% within 28 days).

The Modified Zahn-Wellens/EMPA study in compliance with OECD Guideline 302B was performed at 20-25°C and pH 7.4. The sampling was performed at 0 and 3 hours and on days 1, 2, 3, 6, 8 10, 14, 16, 21, 23, 27 and 28. The test material attained 19% degradation after 28 days.

The results obtained from the abiotic test vessel showed 14% loss of DOC occurred over the study period. Correction of the DOC degradation rate for abiotic loss showed that the test material achieved 5% biodegradation after 28 days.

The reference substance exhibited a normal pattern of degradation (101% within 28 days).

Therefore, chloralose is not readily nor inherent biodegradable.

5.1.2.3 SIMULATION TESTS

Not performed.

5.1.3 SUMMARY AND DISCUSSION OF DEGRADATION

Chloralose is not expected to undergo abiotic degradation by hydrolysis or by photolysis in water, is not rapidly degradable and is not readily nor inherent biodegradable.

5.2 ENVIRONMENTAL DISTRIBUTION

Table 22: Summary of relevant information on distribution

Method	Results			Remarks	Reference
Adsorption/Desorption screening test	Adsorbed a.s. [%]	Ka [1]	KaOC [2]	[1] Ka = Adsorption coefficient [2] KaOC = Adsorption coefficient based on organic carbon content. No data available for Kd (desorption coefficient). KdOC (desorption coefficient based on organic carbon content) and Ka/Kd (Adsorption/desorption	(21)
OECD Guideline 106 EC test method C18 Sandy clay loam (pH 4.81; 3.2%oc) Clay (pH 7.19; 0.98%oc) Clay loam/clay (pH 7.23; 0.70%oc) Sand/loamy sand (pH	31.04 26.23 31.78 26.57 21.43	0.80 0.60 0.84 0.62 0.44	25.00 61.22 120.00 5.49 62.86		

Method	Results			Remarks	Reference
4.37; 11.3%oc) Loamy sand (pH 4.83; 0.7%oc)				distribution coefficient) since calculated desorption values were negative	
Phototransformation in air, (estimation method) QSAR Model	The overall half-life in air for OH radicals is 3.191 h or 0.266 days (based on a 12-h day).				(22)

5.2.1 ADSORPTION/DESORPTION

Adsorption of chloralose onto soils has been studied in five soils at 1:1 soil-solution ratio, using the batch equilibrium method.

Chloralose is slightly adsorbed onto soil. The amount of chloralose adsorbed to soil at equilibrium time (32 h) ranged from 21.43% (Motivahiyal loamy sand) to 31.78% (Vansda clay loam/clay).

Based on KaOC values, which ranged from 5.49 (Jageshwar sand/loamy sand) to 120.00 (Vansda clay loam/clay) and according to SSLRC mobility classification (Soil Survey and Land Research Council, UK), chloralose can be considered very mobile in sand/loamy sand soil, mobile in sandy clay loam, clay and loamy sand soils and moderately mobile in clay loam/clay soil.

Due to its low adsorption onto soils and being readily soluble in water, chloralose is expected to move from soil into water.

5.2.2 VOLATILISATION

The low vapour pressure and low Henry's law constant of chloralose, indicates that it is non volatile and it is not expected to move from water and soil to air.

The photo-oxidative degradation of chloralose in air was estimated by a structural activity relationship (QSAR) method using the Atmospheric Oxidation Program v1.91 (AOPWIN). The half-life in air for the OH radicals is estimated to be $t_{1/2}$ (OH) = 3.191 h (1.5×10^6 OH radicals/cm^3).

If present in the air chloralose is expected to be quickly degraded by photo-oxidation.

5.2.3 DISTRIBUTION MODELLING

Not performed.

5.3 AQUATIC BIOACCUMULATION

5.3.1 AQUATIC BIOACCUMULATION

5.3.1.1 BIOACCUMULATION ESTIMATION

The log K_{ow} was measured using the EC Method A.8, the Shake Flask Method. The substance surface tension is 50.076 mN/m (<60 mN/m) and should be regarded as being surface-active. Considering that the Shake Flask Method is not suitable for surface active substances the measured log K_{ow} value is not valid.

Additionally there is no indication that chloralose has the potential to bioaccumulate.

Taking into account that this endpoint doesn't affect the classification proposal no further data was deemed necessary for this purpose.

5.3.1.2 MEASURED BIOACCUMULATION DATA

Measurements of aquatic bioaccumulation of chloralose were not performed due to its physical and chemical properties and its limited exposure to the aquatic environment.

5.3.2 SUMMARY AND DISCUSSION OF AQUATIC BIOACCUMULATION

It's not possible to conclude on the substance aquatic bioaccumulation since data isn't available on measured bioaccumulation neither on estimated. However the classification proposal is not affected.

5.4 AQUATIC TOXICITY

Table 23: Summary of relevant information on aquatic toxicity

Method	Results			Remarks	Reference
Acute toxicity to fish (Rainbow trout *Oncorhynchus mykiss* 96h-LC_{50}) EC Method C.1	LC_0 (mg/L)	LC_{50} (mg/L)	LC_{100} (mg/L)	Semi-static test (renewal of test solutions after 24h) Test vessel volume: 50 L Number animals/vessel: 10 Number vessels/concentration: 1 No test concentrations measured Lethal concentrations based on nominal values Stability of test substance through 24h monitored with a 100 mg/L solution	(23)
	0.6	2.4	9.6		
Acute toxicity to fish (Rainbow trout *Oncorhynchus mykiss* 96h-LC_{50}) EC Method C.1	LC_0 (mg/L)	LC_{50} (mg/L)	LC_{100} (mg/L)	Semi-static test (renewal of test solutions after 24h) Test vessel volume: 50 L Number animals/vessel: 10 Number vessels/concentration: 1 Test concentrations measured at t = 0h Lethal concentrations based on nominal values Stability of test substance through 24h monitored with 0.1 and 50 mg/L solutions (from a range-finding study)	(24)
	0.78	5.01	40.00		

Method	Results			Remarks	Reference
Acute toxicity to invertebrates (Acute toxicity to *Daphnia magna.* 24 and 48-h EC_{50}. Immobility and behavioural symptoms) EC Method C.2	EC_0 (mg/L) (48h) 0.006	EC_{50} (mg/L) (24h) 0.058 (48h) 0.027	EC_{100} (mg/L) (48h) 0.096	Renewal of test solution: No Test vessel volume: 500 mL Number of animals/vessel: 5 Number vessels/ concentration: 4 No test concentrations measured. Lethal concentrations based on nominal values. Stability of test substance throughout the study monitored with a 100 mg/L solution.	(25)
Acute toxicity to invertebrates (Acute toxicity to *Daphnia magna.* 24 and 48h EC_{50}. Immobility and behavioural symptoms) EC Method C.2	EC_0 (mg/L) (48h) 0.06	EC_{50} (mg/L) (24h) 0.80 (48h) 0.36	EC_{100} (mg/L) (48h) 1.50	Renewal of test solution: No Test vessel volume: 300 mL Number of animals/vessel: 5 Number vessels/ concentration: 4 Test concentrations measured at t = 0h. Lethal concentrations based on nominal values. Stability of test substance throughout the study monitored with 0.001 and 10 mg/L solutions (from a range-finding study) Positive control study (potassium dichromate) with results in accordance with the guideline.	(26)
Growth Inhibition Test on Algae (*Selenastrum capricornutum.* Percentage reduction in growth (E_bC_{50}). Percentage reduction in growth rate (E_rC_{50}). No observed effect concentration (NOEC)) EC Method C.3	NOE_rC (mg/L) (72h) 0.02	E_bC_{50}[1] (mg/L) (72h) 0.13	E_rC_{50}[2] (mg/L) (72h) 0.52	Volume of test flasks: 250 mL Culturing apparatus: conical flasks Light quality: 6000-10000 lux Test flasks agitated continuously at 100 rpm Number of vessels/ concentration: 3 (6 vessels for the control group) No test concentrations measured. Growth and growth rate inhibition based on nominal values. Stability of test substance throughout the study monitored with a 100 mg/L solution.	(27)
Growth Inhibition Test	NOE_rC (mg/L)	E_bC_{50}[1] (mg/L)	E_rC_{50}[2] (mg/L)	Volume of test flasks: 250 mL Culturing apparatus: conical flasks	(28)

Method	Results			Remarks	Reference
on Algae (*Pseudokirchneriella subcapitata*. Percentage reduction in growth (E_bC_{50}). Percentage reduction in growth rate (E_rC_{50}). No observed effect concentration (NOEC)) EC Method C.3	(72h) 0.13	(72h) 1.00	(72h) 4.90	Light quality: 8350 - 8410 lux Test flasks agitated continuously at 100 rpm Number of vessels/ concentration: 3 (6 vessels for the control group) Lowest and highest test concentrations measured at t = 0h. Growth and growth rate inhibition based on nominal values. Stability of test substance throughout the study monitored with 0.01 and 6.25 mg/L solutions (from a range-finding study)	

[1] calculated from the area under the growth curve; [2] calculated from the growth rate.

5.4.1 FISH

5.4.1.1 SHORT-TERM TOXICITY TO FISH

Two studies performed to assess the acute toxicity (96h) LC_{50} of chloralose in rainbow trout, *Oncorhynchus mykiss*, following EC Method C.1 (1992).

In the first study referred and after performing a range-finding study, fifty fish were divided into five groups, each group comprising ten fish and exposed to chloralose at the nominal test concentrations 0.6, 1.2, 2.4, 4.8 and 9.6 mg/L water and observed for a period of 96 hours. A concurrent control (without chloralose) with ten fish was also observed for the same period. A semi-static toxicity test procedure was followed with renewal of test solution at every 24 h. Water quality parameters viz., temperature, pH, dissolved oxygen and total hardness were measured during the study and found to be within the guideline limits.

Mortality and clinical symptoms such as loss of equilibrium, swimming at surface, erratic swimming, dark pigmentation, light pigmentation, lying on bottom, rapid respiration and sluggishness were observed at 3, 6, 24, 48, 72 and 96 h. No clinical symptoms were observed in the control group. The 96h-EC_0 was found to be 0.6 mg/L. The 96h-LC_{50} of chloralose was determined as 2.40 mg/L, with 95% fiducial limits between 1.61 and 3.57 mg/L.

In the second study and after performing a range-finding study, seven groups of ten fish each were exposed to chloralose nominal test concentrations of 0.00 (negative control), 0.78, 1.71, 3.76, 8.26, 18.18 and 40.00 mg/L water and observed for 96 h. A semi-static procedure, with renewal at every 24h, was also adopted. Water quality parameters were found to be within the guideline limits.

The concentration of chloralose was measured in the range-finding study for the nominal concentrations of 0.10 mg/L and 50 mg/L at 0h and 24h. The measured concentrations remained in the 80-120% range.

Mortality and clinical symptoms such as loss of equilibrium, lying on bottom and swimming at surface were observed at 3, 6, 24, 48, 72 and 96 h. No mortalities were observed in the concurrent negative control group. The 96h-EC_0 was found to be 0.78 mg/L. The 96h-LC_{50} of chloralose was determined as 5.01 mg/L, with 95% fiducial limits between 2.68 and 9.38 mg/L.

5.4.1.2 LONG-TERM TOXICITY TO FISH

Not performed.

5.4.2 AQUATIC INVERTEBRATES

5.4.2.1 SHORT-TERM TOXICITY TO AQUATIC INVERTEBRATES

Two studies performed to assess the acute immobilisation, 24h and 48h-EC_{50}, caused by chloralose in *Daphnia magna* were submitted. The methods followed were per EC Method C.2 (1992).

In the first study and after performing a range-finding study, five groups of four replicates with 5 daphnids per replicate were exposed to chloralose at the nominal concentrations of 0.006, 0.012, 0.024, 0.048 and 0.096 mg/L. A similar group was used as a negative control (without chloralose). Water quality parameters viz., temperature, pH and dissolved oxygen were measured at 0 and 48h and found to be within the guideline limits. Hardness measured at the beginning of the test was also within the guideline limit.

Immobility and behavioural symptoms such as lethargy, on surface and flared carapace were observed during the exposure period at 0, 24 and 48h. No behavioural symptoms were observed in the control group. The 48h-EC_0 of chloralose was found to be 0.006 mg/L. The 48h-EC_{50} of chloralose was determined as 0.027 mg/L with 95% fiducial limits of 0.020 and 0.036 mg/L.

The concentration was measured for the nominal concentration of 100 mg/L and the substance concentration was between 80-120% of nominal at 0 and 48h.

The substance is soluble in water, non volatile, non degradable and non hydrolysable, therefore it can be assumed that the test substance concentration were between 80-120% of nominal throughout the study.

In the second study and after performing a range-finding study, five groups of four replicates with 5 daphnids per replicate were exposed to chloralose at the nominal concentrations of 0.06, 0.14, 0.31, 0.68 and 1.50 mg/L. A similar group was used as a negative control (without chloralose). Water quality parameters were found to be within the guideline limits.

Immobility and behavioural symptoms such as lethargy, on surface and on bottom were observed during the exposure period at 0, 24 and 48h. No immobilisation was recorded in the concurrent control group. The 48h-EC_0 was found to be 0.06 mg/L. The 48h-EC_{50} of chloralose was determined as 0.36 mg/L with 95% fiducial limits of 0.20 and 0.64 mg/L.

The test system was previously validated using potassium dichromate as a reference substance. The 48h-EC_{50} of potassium dichromate was determined as 0.97 mg/L with 95% fiducial limits of 0.73 and 1.30 mg/L, which is in accordance with the guideline.

In this study concentrations were measured at t = 0h and concentrations were confirmed through the duration of the test by using two nominal concentrations of 0.001 and 10 mg/L from a range-finding study. At 48h the concentrations were >96% of the initial concentration.

The substance is soluble in water, non volatile, non degradable and non hydrolysable, therefore it can be assumed that the test substance concentration were between 80-120% of nominal throughout the study.

5.4.2.2 LONG-TERM TOXICITY TO AQUATIC INVERTEBRATES

Not performed.

5.4.3 ALGAE AND AQUATIC PLANTS

Two studies performed to assess the alga *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) growth inhibition caused by chloralose were submitted. The methods followed were per EC Method C.3 (1992).

In the first study and after performing a range-finding study, culture medium containing 11792 cells/mL of alga was exposed to chloralose at the concentrations of 0.00 (negative control), 0.02, 0.04, 0.08, 0.16, 0.32, 0.64 and 1.28 mg/L. The algal population was observed at 24, 48 and 72h.

The EC_{50} (0 – 72h) value for chloralose was determined as 0.13 mg/L, with 95% fiducial limits between 0.07 and 0.25 mg/L, and 0.52 mg/L, with 95% fiducial limits between 0.18 and 1.46 mg/L, for growth inhibition (E_bC_{50}) and growth rate reduction (E_rC_{50}), respectively. At 0.02 mg/L concentration there was no statistically growth rate reduction at 5% level when compared with the control values. Hence the NOEC was found to be 0.02 mg/L.

The concentration was measured for the nominal concentration of 100 mg/L and 100% of the nominal was maintained at 0 and 72h.

The substance is soluble in water, non volatile, non degradable and non hydrolysable, therefore it can be assumed that the test substance concentration were between 80-120% of nominal throughout the study.

In the second study and after performing a range-finding study, exponential growing cultures of algae (10358 cells/mL) were exposed to chloralose at the nominal test concentrations of 0.13, 0.28, 0.61, 1.34, 2.95 and 6.50 mg/L. A control without chloralose was run concurrently. The algal population was observed at 24, 48 and 72 h.

The concentration of chloralose was measured in the range-finding study for the nominal concentrations of 0.01 mg/L and 6.25 mg/L at 0h and 72h (2 vessels/concentration). The measured concentrations remained in the 80-120% range with the exception of the 0.01 mg/L concentration that in the end of the test (72h) was 77% of nominal.

The test system was previously validated using potassium dichromate as a reference substance. The EC_{50} (0–72h) value for potassium dichromate was determined as 0.63 mg/L, with 95% fiducial limits between 0.54 and 0.73 mg/L, and 1.69 mg/L, with 95% fiducial limits between 1.38 and 2.07 mg/L, for growth inhibition (E_bC_{50}) and growth rate reduction (E_rC_{50}), respectively, which matches well with the guideline.

The EC_{50} (0–72h) value for chloralose was determined as 1.00 mg/L, with 95% fiducial limits between 0.85 and 1.17 mg/L, and 4.90 mg/L, with 95% fiducial limits between 3.66 and 6.55 mg/L, for growth inhibition (E_bC_{50}) and growth rate reduction (E_rC_{50}), respectively. The growth rate reduction was taken into consideration to decide the NOEC and LOEC. The NOEC and LOEC of chloralose over the entire 72 h exposure period were found to be 0.13 and 0.28 mg/L, respectively.

However, some deviations from the guideline occurred, namely:

1. pH values ranged from 6.98 – 10.41 which is not in accordance to the guideline. Such difference may be due to an increase in algal blooms which tend to raise pH to very high levels. This deviation, however, is not considered to affect the results of the test.
2. Culture medium was not prepared according to EC method C.3. The algal culture medium used was in line with the one recommended by ATCC (American Type Culture Collection),

for the strain of the alga ATCC No 22662. However, the limits for P, N, chelators and hardness stated on OECD guideline 201 were exceeded (P - 8.9 mg/L; N - 81.9 mg/L; EDTA - 0.003 mg/L). Nevertheless, the above mentioned positive control study with potassium dichromate was carried out with the same medium used in the present study and, on the other hand, algal growth in control group after 72 h was 57.93 times, which meets the EC guideline. The validity of the study is not considered to be affected.

5.4.4 OTHER AQUATIC ORGANISMS (INCLUDING SEDIMENT)

Not performed.

5.5 COMPARISON WITH CRITERIA FOR ENVIRONMENTAL HAZARDS (SECTIONS 5.1 – 5.4)

Considering that the 48h-EC_{50} = 0.027 mg/L value was obtained for *Daphnia magna* is lower than 1 mg/L, chloralose meets the criteria for classification as Aquatic Acute 1 for environmental hazard according to CLP criteria. As this value is within the range of 0.01-0.1 mg/L a M-factor 10 is allocated.

For the long term aquatic hazard the available data is only for one trophic level therefore the surrogate approach should be used.

- Chloralose is not rapidly degradable and the 72h-NOEC value for *Selenastrum capricornutum* is lower than 0.1 mg/L (0.02 mg/L), hence category Chronic 1;
- Surrogate system, the lowest acute aquatic toxicity 48h-EC_{50} = 0.027 mg/L (*Daphnia magna)* is ≤ 1 mg/L and not rapidly degradable, hence category Chronic 1;
- Conclusion: category Chronic 1
- Applying the surrogate system the M factor is based on the acute toxicity data following the most stringent outcome (acute aquatic toxicity to *Daphnia magna*) within the range of 0.01-0.1 mg/L and M-factor 10 is allocated.

5.6 CONCLUSIONS ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS (SECTIONS 5.1 – 5.4)

According to CLP Regulation criteria

Classification: Aquatic Acute 1; H400

Aquatic Chronic 1; H410

Labelling:

Pictogram: GHS09

Signal word: Warning

Hazard statements: H410: Very toxic to aquatic life with long lasting effects

Precautionary statements: P273: Avoid release to the environment

P391: Collect spillage

P501: Dispose of contents/container to … … in accordance with local/regional/national/international regulation (to be specified)

Acute M-factor: 10

Chronic M-factor: 10

6 OTHER INFORMATION

7 REFERENCES

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(28) Alga (Pseudokirchneriella subcapitata) Growth Inhibition Test with Alphachloralose (2005).

8 ANNEXES