CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name: Hydrogen Peroxide solution …%

EC Number: 231-765-0

CAS Number: 7722-84-1

Index Number: 008-003-00-9

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CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Hydrogen peroxide
Other names (usual name, trade name, abbreviation)	Belox CLARMARIN® H2O2 HYPROX® Hydrogen Peroxide Hydrogen Peroxide, Solution 35 ÷ 49,9 % Hydrogen Peroxide, Solution 50 ÷ 60 % Hydrogen peroxide Interox NANOPURE® OXTERIL® PERALKALI® PERSYNT® PERTRONIC® PROPULSE™ Wasserstoffperoxid eau oxygenée eau oxygénée perossido di idrogeno peroxyde d hydrogène
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	231-765-0
EC name (if available and appropriate)	Hydrogen peroxide
CAS number (if available)	7722-84-1
Other identity code (if available)	-
Molecular formula	H_2O_2
Structural formula	
SMILES notation (if available)	OO
Molecular weight or molecular weight range	34.0147 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable (the structure of the substance does not demonstrate stereo-isomerism)
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable (The substance is not an UVCB substance)

Degree of purity (%) (if relevant for the entry in Annex VI)	Hydrogen peroxide is sold as an aqueous solutions, where the hydrogen peroxide concentrations varies typically between 35 and 70 % (w/w). On a calculated dry weight basis the minimum purity of hydrogen peroxide is estimated close to 99.5% (by wt). The sum of hydrogen peroxide and water is close to 100 %.

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)
Hydrogen peroxide, CAS 7722-84-1	-	Ox. Liq. 1; H271 Acute Tox. 4; H302 Acute Tox. 4; H314 Skin Corr. 1A; H332	Ox. Liq. 1; H271 Acute Tox. 4; H302 Acute Tox. 4; H314 Skin Corr. 1A; H332 STOT SE 3; H335 Met. Corr. 1; H290 Flam. Liq. 2; H225 Aquatic Chronic 2; H411 Aquatic Chronic 3; H412

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling

Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: For substance with an existing entry in Annex VI of CLP

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	008-003-00-9	hydrogen peroxide solution ...%	231-765-0	7722-84-1	Ox. Liq. 1 Acute Tox. 4 * Acute Tox. 4 * Skin Corr. 1A	H271 H332 H302 H314	GHS03 GHS05 GHS07 Dgr	H271 H332 H302 H314		Ox. Liq. 1; H271: C ≥70 %**** Ox. Liq. 2; H272: 50 % ≤ C < 70 % **** * Skin Corr. 1A; H314: C ≥ 70 % Skin Corr. 1B; H314: 50 % ≤ C < 70 % Skin Irrit. 2; H315: 35 % ≤ C < 50 % Eye Dam. 1; H318: 8 % ≤ C < 50 % Eye Irrit. 2; H319: 5 % ≤ C< 8 % STOT SE 3; H335; C ≥ 35 %	B
Dossier submitter's proposal	008-003-00-9	hydrogen peroxide solution ...%	231-765-0	7722-84-1	**Retain** Ox. Liq. 1 **Modify** Acute Tox. 4 Acute Tox. 3 **Add** Aquatic Chronic 3	**Retain** H271 **Modify** H332 H301 **Add** H412	**Retain** GHS03 **Modify** GHS06	**Retain** H271 **Modify** H332 H301 **Add** H412		**Add** inhalation: ATE = 11 mg/l (vapour) oral: ATE = 100 mg/kg bw **Modify** Ox. Liq. 1; H271: C ≥ 60 % Ox. Liq. 2;	

										H272: 20 % ≤ C < 60 % Ox. Liq. 3; H272: 8 % ≤ C < 20 %	
Resulting Annex VI entry if agreed by RAC and COM	008-003-00-9	hydrogen peroxide solution ...%	231-765-0	7722-84-1	Ox. Liq. 1 Acute Tox. 4 Acute Tox. 3 Skin Corr. 1A Aquatic Chronic 3	H271 H332 H301 H314 H412	GHS03 GHS05 GHS06 Dgr	H271 H332 H301 H314 H412		Ox. Liq. 1; H271: C ≥ 60 % Ox. Liq. 2; H272: 20 % ≤ C < 60 % Ox. Liq. 3; H272: 8 % ≤ C < 20 % inhalation: ATE = 11 mg/l (vapour) oral: ATE = 100 mg/kg bw Skin Corr. 1A; H314: C ≥ 70 % Skin Corr. 1B; H314: 50 % ≤ C < 70 % Skin Irrit. 2; H315: 35 % ≤ C < 50 % Eye Dam. 1; H318: 8 % ≤ C < 50 % Eye Irrit. 2; H319: 5 % ≤ C< 8 % STOT SE 3; H335; C ≥ 35 %	B

Table 7: Reason for not proposing harmonised classification and status under consultation

Hazard class	Reason for no classification	Within the scope of consultation
Explosives	Data inconclusive	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Data conclusive but not sufficient for classification	Yes
Flammable solids	Hazard class not applicable	No
Self-reactive substances	Hazard class not considered due to a proposal on another hazard class	Yes
Pyrophoric liquids	Data conclusive but not sufficient for classification	Yes
Pyrophoric solids	Hazard class not applicable	No
Self-heating substances	Hazard class not assessed in this proposal	No
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Harmonised classification proposed	Yes
Oxidising solids	Hazard class not applicable	No
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Data conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	Harmonised classification proposed	Yes
Acute toxicity via dermal route	Hazard class not assessed in this proposal	No
Acute toxicity via inhalation route	Harmonised classification proposed	Yes
Skin corrosion/irritation	Hazard class not assessed in this proposal	No
Serious eye damage/eye irritation	Hazard class not assessed in this proposal	No
Respiratory sensitisation	Hazard class not assessed in this proposal	No
Skin sensitisation	Hazard class not assessed in this proposal	No
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	Hazard class not assessed in this proposal	No
Reproductive toxicity	Hazard class not assessed in this proposal	No
Specific target organ toxicity-single exposure	Hazard class not assessed in this proposal	No
Specific target organ toxicity-repeated exposure	Hazard class not assessed in this proposal	No
Aspiration hazard	Hazard class not assessed in this proposal	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Hazard class not assessed in this proposal	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The harmonized classification, as presented in the Table in Annex VI of the CLP Regulation (Index number 008-003-00-9), is the translation of the harmonised classification made for the substance under Directive 67/548/EEC (Dangerous substances directive, DSD). Hydrogen peroxide was first introduced to Annex I of the Directive 67/548/EEC in the first adaptation (1976) to technical progress (ATP) with classification O; R8 and C; R34 and NOTA B. Concentration limits were as follows:

C ≥ 20 %: C; R34

C > 60 %: O; R8

The classification was modified in the twelfth ATP (1991) by adding classification Xi; R 36/38 to concentrations 5 % ≤ C < 20 %, and refining concentration limits regarding O; R8 to C ≥ 60 %.

The classification was further modified in the twenty-ninth ATP (2004) to R5, O; R8, C; R35, Xn; R20/22 with following concentration limits:

C ≥ 70 %: C; R20/22-35

50 % ≤ C < 70 %: C; R20/22-34

35 % ≤ C < 50 %: Xn; R22-37/38-41

8 % ≤ C < 35 %: Xn; R22-41

5 % ≤ C< 8 %: Xi; R36

Footnote:

C ≥ 70 %: R5, O; R8

50 % ≤ C < 70 %: O; R8

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level.

5 IDENTIFIED USES

According to the information available on ECHA's dissemination website (https://echa.europa.eu/information-on-chemicals/registered-substances/-/disreg/substance/100.028.878), hydrogen peroxide is used by consumers and/or professionals in e.g., cosmetics and personal care products, washing and cleaning products, bleaching products, biocides (e.g. disinfectants, pest control products), pH regulators and water treatment products, textile treatment products and dyes and water treatment chemicals, and laboratory chemicals.

In industrial sites, hydrogen peroxide is used e.g. in metal finish industry, bleaching of fibrous and non-fibrous materials, production of chemically modified foodstuff (oxidizing agent), laboratories (oxidizing agent), synthesis of chemicals, electronic industry.

As a biocidal active substance, hydrogen peroxide has been approved in 2015 as disinfectant in product types 1 to 5 (Human hygiene, Disinfectants and algaecides not intended for direct application to humans or animals, Veterinary hygiene, Food and feed area, Drinking water) and as preservative in produt type 6 (Preservatives for products during storage). The application for renewal in these product types is expected to come in July 2025. For product types 11 (Preservatives for liquid-cooling and processing systems) and 12 (Slimicides) notifications with new participants have been made and the application deadlines are in spring 2026.

6 DATA SOURCES

The Competent Authority Assessment Report of hydrogen peroxide under Regulation (EU) No 528/2012 was used as the main source (Competent Authority Assessment Report (CAR) Finland, 2017): https://echa.europa.eu/fi/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/1315/PT01

In addition, data from the REACH registration dossier, EU risk assessment report on hydrogen peroxide (European Commission, 2003) as well as from open literature were used.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid	Registration dossier, ECHA's website, 2022	Visual inspection (35 % w/w in aqueous solution)
Melting/freezing point	-0.43°C	Registration dossier, ECHA's website, 2022	Calculated
Boiling point	150.2°C	Registration dossier, ECHA's website, 2022	Interpolation of vapour pressure composition curves
Relative density	1.4425 g/cm3	Registration dossier, ECHA's website, 2022	Estimated by extrapolation from densities of aqueous solutions
Vapour pressure	299 Pa at 25°C	Registration dossier, ECHA's website, 2022	Calculated
Surface tension	80.4 mN/m at 20°C	Registration dossier, ECHA's website, 2022	Extrapolated from experimental values
Water solubility	Miscible	Registration dossier, ECHA's website, 2022	Expert knowledge
Partition coefficient n-octanol/water	log Kow = -1.57 (Log Pow)	Registration dossier, ECHA's website, 2022	Calculated (QSAR)
Flash point	-	Registration dossier, ECHA's website, 2022	Not necessary
Flammability	Not flammable	Registration dossier, ECHA's website, 2022	Expert statement
Explosive properties	Not explosive	Registration dossier, ECHA's website, 2022	EU Method A.14 (87 % w/w in aqueous solution)
Self-ignition temperature	Not flammable	Registration dossier, ECHA's website, 2022	Expert statement

Property	Value	Reference	Comment (e.g. measured or estimated)
Oxidising properties	Oxidising	Registration dossier, ECHA's website, 2022	UN Manual of Tests and Criteria: Test O.2
Granulometry	-	Registration dossier, ECHA's website, 2022	Not necessary
Stability in organic solvents and identity of relevant degradation products	Mixtures or solutions of hydrogen peroxide with organic compounds can exhibit explosive properties. Additionally, other products may be formed in the hydrogen peroxide-organic compound-water system that are more dangerous than the original mixture.	Registration dossier, ECHA's website, 2022	Expert statement
Dissociation constant	pKa = 11.62-11.65 at 20 °C	Registration dossier, ECHA's website, 2022	Literature
Viscosity	1.249 mPa · s at 20 °C	Registration dossier, ECHA's website, 2022	Oswalt viscometer method

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 9: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
UN gap test Test 1 (a)	Hydrogen peroxide 90% (w/w): No total fragmentation or a hole punched through the witness plate Hydrogen peroxide 93% (w/w): Total fragmentation in three tests of four H_2O_2 90%: "-" H_2O_2 93%: "+"	The interior surfaces of the steel tubes were pre-treated with a flurocarbon resin	Anonymous (1998)
UN gap test Test 2 (a)	Hydrogen peroxide 95% (w/w): No total fragmentation or a hole punched through the witness plate Hydrogen peroxide 98% (w/w): No total fragmentation or a hole punched through the witness plate H_2O_2 95%: "-" H_2O_2 98%: "-"	The interior surfaces of the steel tubes were pre-treated with a flurocarbon resin	Anonymous (1998)
EC A.14	Hydrogen peroxide 87 % (w/w):	Comparable to the	Anonymous (1988)

Method	Results	Remarks	Reference
Thermal sensitivity GLP: No	An explosion occurred in the test with the nozzle of 1 mm diameter, but the steel disk was not rend to pieces Test 1 (b): "-" Test 2 (b)): "-"	UN Koenen test (Test 1 (b), Test 2 (b)) Diameters tested: 2 mm, 1,5 mm, 1 mm	
UN Time/pressure test Test 1 (c) Test 2 (c)	Hydrogen peroxide 49.5 % (w/w): Gauge pressure 2070 kPa was achieved, pressure rise time > 30 ms Test 1 (c): "+" Test 2 (c): "-"		Anonymous (2023a)
UN Time/pressure test Test 1 (c) Test 2 (c)	Hydrogen peroxide 59.2 % (w/w): Gauge pressure 2070 kPa was achieved, pressure rise time > 30 ms Test 1 (c): "+" Test 2 (c): "–"		Anonymous (2023b)
UN Time/pressure test Test 1 (c) Test 2 (c)	Hydrogen peroxide 69.7 % (w/w): Gauge pressure 2070 kPa was achieved, pressure rise time < 30 ms		(Anonymous 2023c)

8.1.1 Short summary and overall relevance of the information provided on explosive properties

The UN gap test, Test 1 (a), was used to measure the ability of the aqeous solution of hydrogen peroxide, under confinement in a steel tube, to propagate a detonation. Hydrogen peroxide 90% (w/w) does not propagate a detonation as the tube was not fragmented completely or a hole was not punched through the witness plate. Hydrogen peroxide 93% (w/w) caused a total fragmentation of the tube (in three tests of four) and propagates a detonation.

The UN gap test, Test 2 (a), was used to measure the sensitivity of the aqeous solution of hydrogen peroxide, under confinement in a steel tube, to detonative shock. Hydrogen peroxide 95% and 98% (w/w) are not sensitive to detonative shock as the tube was not fragmented completely or the witness plate was not holed.

The thermal sensitivity of an aqueous solution of 87% (w/w) hydrogen peroxide was tested in the steel tube test according to the EU Method A.14. Plates with orifices 2 mm, 1,5 mm, 1 mm were used. An explosion occurred in the test with the nozzle of 1 mm diameter, but the steel disk was not rend to pieces. The test result is considered positive if an explosion occurs in either tests with a 6.0 mm diameter orifice plate or a 2.0 mm diameter orifice plate. A test resulting in the fragmentation of the tube into three or more pieces, which in some cases may be connected to each other by narrow strips of metal, is evaluated as giving an explosion. The conclusion is that hydrogen peroxide 87% (w/w) does not present a danger of explosion when submitted to the effect of a flame. The method A.14 is basically the same as the UN Koenen test. In the Koenen test plates with the orifices of 12.0 - 8.0 - 5.0 - 3.0 - 2.0 -1.5 and finally 1.0 mm are used until, the "explosion" in obtained. According to the Koenen test the substance does not show effect on heating under confinement if the limiting diameter is less than 1.0 mm (Test 1 (b)) and the substance does not show violent effect on heating under confinement if the limiting diameter is less than 2.0 mm (Test 2 (b)).

The UN time/pressure test was used to determine the effects of igniting the sample under confinement in accordance with Test 1 (c) and Test 2 (c). All tested samples, 49.5 %, 59.2 % and 69.7 % hydrogen peroxide solutions, showed the ability to deflagrate as the maximum pressure

reached with all samples was greater than 2070 kPa (Test 1 (c)). Only 69.7 % hydrogen peroxide solution showed the ability to deflagrate rapidly as the time for a pressure rise from 690 kPa to 2070 kPa was less than 30 ms (Test 2 (c)).

8.1.2 Comparison with the CLP criteria

The acceptance procedure is used to determine whether or not a substance is a candidate for the class of explosives or is an unstable explosive. The test methods used for deciding on provisional acceptance into the class of explosives are grouped into four series (CLP Annex I, Figure 2.1.2).

The following test are used in Test Series 1 and Test Series 2:

a) UN Gap test (the gap is zero in Test 1 (a) and greater than zero in Test 2 (a))

b) Koenen test

c) Time/pressure test

Test Series 1: If a '+' is obtained in any of the three types of tests (Test 1 (a), Test 1 (b), Test 1 (c)), the substance is an explosive and further testing is needed. Otherwise the substance is rejected from this class.

Test Series 2: If a '+' is obtained in any of the three types of test (Test 2 (a), Test 2 (b), Test 2 (c)), the substance is an explosive and further testing is needed. Otherwise the substance is rejected from this class.

Based on the UN time/pressure result, an aqueous solution with a hydrogen peroxide concentration of at least 69.7 % (w/w) is classified as an explosive and further investigation should be conducted, e.g. about the thermal stability and the sensitivity to mechanical stimuli (Test Series 3).

Substances which fail Test Series 2 but pass Test Series 3 shall be subjected to the procedure for assignment to the appropriate division of explosives. However, the substance may leave the class of explosives after Test Series 6 of the assignment procedure due to proper packaging.

It is important to note that the time/pressure test may not be suitable for hydrogen peroxide. Hydrogen peroxide may react with the by-products from the detonator used within the experiment, and this reaction causes the pressure rise that leads to the test being considered a positive result in the time pressure test. Also for this reason further investigation should be conducted.

8.1.3 Conclusion on classification and labelling for explosive properties

An aqueous solution with a hydrogen peroxide concentration of no more than 59.2 % (w/w) is not classified as an explosive. Further investigation should be conducted on solutions with higher hydrogen peroxide concentrations. Harmonised classification is not proposed.

8.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable.

8.3 Oxidising gases

Hazard class not applicable.

8.4 Gases under pressure

Hazard class not applicable.

8.5 Flammable liquids

Table 10: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
EN ISO 3679 (rapid equilibrium, closed cup method)	90% of hydrogen peroxide has been determined to have no flash point below 95.0°C		Anonymous (2023d)

8.5.1 Short summary and overall relevance of the provided information on flammable liquids

The flash point was determined by following the standard EN ISO 3679 (rapid equilibrium, closed cup method). The test item was determined to have no flash point below 95.0 °C.

8.5.2 Comparison with the CLP criteria

Flammable liquid means a liquid having a flash point of not more than 60°C. Inorganic oxidising liquids are not flammable and therefore do not have to be subjected to the classification procedures for the hazard classes flammable liquids or pyrophoric liquids (Guidance on the Application of the CLP Criteria, Version 6.0 - Jan 2024).

In the test performed, 90% of hydrogen peroxide did not have a flash point below 95°C and it supports that hydrogen peroxide as an inorganic oxidising liquid is not flammable.

8.5.3 Conclusion on classification and labelling for flammable liquids

Hydrogen peroxide is not classified as flammable liquid. Harmonised classification is not proposed.

8.6 Flammable solids

Hazard class not applicable.

8.7 Self-reactive substances

Hazard class is not considered for classification due to a proposal for oxidising liquid (CLP Annex I, 2.8.2.1(b)).

8.8 Pyrophoric liquids

Experince shows that hydrogen peroxide does not ignite spontaneously on coming into contact with air at normal temperatures. Data conclusive but not sufficient for classification (CLP Annex I, 2.9.4.1).

8.9 Pyrophoric solids

Hazard class not applicable.

8.10 Self-heating substances

Hazard class not assessed in this proposal.

8.11 Substances which in contact with water emit flammable gases

The chemical structure of hydrogen peroxide does not contain metals or metalloids. Hydrogen peroxide is miscible with water and is produced as aqueous solutions. Data conclusive but not sufficient for classification (CLP Annex I, 2.12.4.1)

8.12 Oxidising liquids

Table 11: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
UN Test O.2	H_2O_2 50.0 %: Packing Group II/Category 2 H_2O_2 42.5 %, 45.0 %, 47.3 %: Packing Group III/Category 3 H_2O_2 40.0 %, 37.6 % and 34.4 %: Not an oxidising liquid		Anonymous (2001)
UN Test O.2	H_2O_2 69.7 %: Packing Group I/Category 1		Anonymous (2016)
UN Test O.2	H_2O_2 60 %: Packing Group II/Category 2		Anonymous (2018)
UN Test O.2	H_2O_2 63 %: Packing Group II/Category 2		Anonymous (2017)

8.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Samples with different concentrations of H_2O_2 were tested for their oxidizing properties according to UN Test O.2.

In the study of seven samples with H_2O_2 concentrations of 50.0 %, 47.3 %, 45.0 %, 42.5%, 40.0 %, 37.6 % and 34.4 %, only the sample with H_2O_2 concentration of 50.0 % exhibited the mean

pressure rise time less than that of 40 % aqueous sodium chlorate solution meaning that the sample meets the Packing Group II/Category 2 criteria. The samples with H_2O_2 concentrations of 47.3 %, 45.0 % and 42.5% exhibited the mean pressure rise time less than that of 65 % aqueous nitric acid and therefore meet thePacking Group III/Category 3 criteria. The threshold value of 50% perchloric acid for Packing Group I/Category 1 was not considered relevant and was not determined in the study.

Samples with H_2O_2 concentrations of 69.7 %, 63 % and 60% were tested in separate studies. The mean pressure rise time of H_2O_2 concentration of 69.7 % was less than that of 50 % aqueous perchloric acid and meet thePacking Group I/Category 1 criteria. The mean pressure rise time of H2O2 concentrations of 63 % and 60% was less than that of 40 % aqueous sodium chlorate and meet the Packing Group II/Category 2 criteria.

8.12.2 Comparison with the CLP criteria

An oxidising liquid shall be classified in one of the three categories for this class using test O.2 in Part III, sub-section 34.4.2 of the M4 UN RTDG, Manual of Tests and Criteria in accordance with Table 2.13.1. The criteria are as follows:

- Category 1: Any substance or mixture which, in the 1:1 mixture, by mass, of substance (or mixture) and cellulose tested, spontaneously ignites; or the mean pressure rise time of a 1:1 mixture, by mass, of substance (or mixture) and cellulose is less than that of a 1:1 mixture, by mass, of 50 % perchloric acid and cellulose.
- Category 2: Any substance or mixture which, in the 1:1 mixture, by mass, of substance (or mixture) and cellulose tested, exhibits a mean pressure rise time less than or equal to the mean pressure rise time of a 1:1 mixture, by mass, of 40 % aqueous sodium chlorate solution and cellulose; and the criteria for Category 1 are not met.
- Category 3: Any substance or mixture which, in the 1:1 mixture, by mass, of substance (or mixture) and cellulose tested, exhibits a mean pressure rise time less than or equal to the mean pressure rise time of a 1:1 mixture, by mass, of 65 % aqueous nitric acid and cellulose; and the criteria for Category 1 and 2 are not met.

Based on the test results of four different studies the classification should be the following:

- Ox. Liq. 3: 42,5 % < C <50 %
- Ox. Liq. 2: 50 % ≤ C < 70
- Ox. Liq. 1: C ≥ 70 %.

Is is notable that the UN Test O.2 may give erroneous results because hydrogen peroxide may decompose in the test rather than react. The O.2 test is based on the reaction between the oxidizing liquid and a flammable solid. This reaction is induced by a glowing wire and causes a pressure rise. The pressure rise rate is a measure for the oxidizing capacity. Because in this test hydrogen peroxide will also decompose by itself, the test results may not be reliable for such formulations.

In accordance with the UN RTDG Model Regulations, aqueous solutions of hydrogen peroxide should be classified as follows:

- Oxidising liquid, Packing group III, with not less than 8 % but not more than 20 % hydrogen peroxide (UN 2984)
- Oxidising liquid, Packing group II, with not less than 20 % but not more than 60 % hydrogen peroxide (UN 2014)
- Oxidising liquid, Packing group I, with more than 60 % hydrogen peroxide (UN 2015).

Packing Groups I, II and III of he UN RTDG Model Regulations correspond directly to Categories 1, 2 and 3 of the CLP, respectively.

The cut-off values (8% - 20% - 60%) were established at the time on the basis of inland transport regulations and sea regulations. In the 1959 Dangerous Goods List, there was only one entry, with the description "Hydrogen peroxide upon concentration", which later appeared in the 1964 list assigned to UN 1468 without specification of concentrations. This number was later replaced (1965 decision) by UN 2014 and 2015, in which the reference to hydrogen peroxide concentrations appears for the first time. Later it was recommended aligning the UN classification with that used for shipping, which at that time proposed three entries: one for concentrations between 8 and 40%; one for concentrations between 40 and 60%; and one for concentrations above 60%. A combination of these values resulted in the first UN 2014 and 2015. Later (in 1980) the concentrations for UN 2014 were modified and a new UN (UN 2984) was introduced for concentrations between 8 and 20%.

In practice the physical hazards of a substance may differ from those shown by tests. Such experience must be taken into account for the purpose of classification (CLP Article 12(a)). In the event of divergence between test results and known experience in the handling and use of substances or mixtures which shows them to be oxidising, judgments based on known experience shall take precedence over test results (CLP Annex I 2.13.4.3). The longstanding experience from the application of the UN RTDG Model Regulations can be considered “known experience”.

The conclusion is that the same classification should be followed in CLP than in the UN RTDG Model Regulations.

8.12.3 Conclusion on classification and labelling for oxidising liquids

Harmonised classification is proposed as follows: Oxidising liquid, Category 3, H272: 8 % ≤ C < 20%; Oxidising liquid, Category 2, H272: 20 % ≤ C < 60%; Oxidising liquid, Category 1, H271: C ≥ 60 %.

8.13 Oxidising solids

Hazard class not applicable.

8.14 Organic peroxides

Hazard class not applicable.

8.15 Corrosive to metals

Table 12: Summary table of studies on the hazard class corrosive to metals

Method	Results	Remarks	Reference
UN Test C.1	Hydrogen peroxide 69,7 % (w/w) Steel: Maximum mass loss 1.32 x 10^{-3} %, no pitting Aluminium: Maximum mass loss 2.56 x 10^{-2} %, no pitting	GLP: Yes Metal plates used: S235JR grade steel 7075-T6 grade aluminium Exposure time: 28 days	Anonymous (2024)

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

The Classification of Corrosion to Metals of hydrogen peroxide 69,7 % (w/w) has been determined using a procedure designed to be compatible with UN Test C.1. Exposure time was 28 days and the test item was replaced entirely on the 14th day of the test. A preliminary test with hydrogen peroxide concentration monitoring had been performed to set the replacement day. The test is considered

positive if for any specimen the mass loss on the metal specimen is more than 51.5 % or the deepest intrusion exceeds 480 µm. The result was negative for steel and negative for aluminium.

8.15.2 Comparison with the CLP criteria

Hydrogen peroxide 69,7 % (w/w) does not require classification for corrosion to metals in accordance with the criteria set out in Annex I of CLP as the UN Test C.1 was shown to be negative for both mild steel and aluminium.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Data conclusive but not sufficient for classification of an aqueous solution of less than 70% (w/w) hydrogen peroxide as corrosive to metals. Harmonised classification is not proposed.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Absorption and distribution

Biological membranes are highly permeable to H_2O_2; the permeability constants for peroxisomal membranes and for erythrocyte plasma membranes are comparable to water in a variety of membranes. Hydrogen peroxide is expected to be readily taken up by the cells constituting the absorption surfaces, but at the same time it is effectively metabolised, and it is uncertain to what extent the unchanged substance may enter the blood circulation. Hydrogen peroxide is reactive and it degrades rapidly in contact with organic material. The rapid degradation upon contact with skin explains the absence of systemic effects from exposure to hydrogen peroxide. Hydrogen peroxide is a normal metabolite in the cell, but there is uncertainty about the true levels of the substance in biological systems. There appears to be a steady state level appears between its generation and degradation. Hydrogen peroxide passes readily across biological membranes and it can react with organic substrates and can diffuse at considerable distances in the cell (European Commission, 2003).

Metabolism and elimination

There are two main hydrogen peroxide metabolising enzymes, catalase and glutathione peroxidase, which control H_2O_2 concentration at different levels and in different parts of the cell as well as in the blood. At low physiological levels hydrogen peroxide is mainly decomposed by GSH peroxidase whereas the contribution of catalase increases with the increase of hydrogen peroxide concentration. Red blood cells remove hydrogen peroxide efficiently from the blood due to a very high catalase activity whereas in the serum catalase activity is low. Both animal studies and human case reports indicate that at high uptake rates hydrogen peroxide passes the absorption surface entering the adjacent tissues and blood vessels where it is degraded liberating oxygen bubbles, thus causing a hazard of mechanical pressure injury. The hazard of oxygen embolisation is particularly high if the substance is administered into closed body cavities where the liberated oxygen cannot freely escape. Regarding hydrogen peroxide inhalation or skin contact at rates that would correspond to occupational exposures, there are no data on the systemic fate of the substance. Due to the high degradation capacity for hydrogen peroxide in blood it is however unlikely that the endogenous steady state level of the substance is affected. Hydrogen peroxide may also undergo iron-catalyzed reactions resulting in the formation of hydroxyl radicals. The cellular toxicity of hydrogen peroxide appears to depend largely on the generation of hydroxyl radicals. Genetically determined traits such as acatalasaemia and erythrocyte glucose-6-phosphate dehydrogenase deficiency render humans more susceptible to peroxide toxicity (European Commission, 2003).

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Table 13: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Remarks	Reference
Acute oral toxicity study Similar to OECD 401 GLP Reliability 1 **Key Study**	Rat, Crl:CD BR, males and females 5 animals per sex and per dose No control animals	Hydrogen peroxide, 70 % (w/w) aqueous solution	Males: 500, 1000 and 1500 mg/kg bw Females 500, 750 and 1000 mg/kg bw Single dose, administration by gavage	Males: 1026 mg/kg bw Females: 694 mg/kg bw (corresponding in appr. 490 mg/kg bw as 100% H_2O_2)	Mortality: Males: 0/5 died at 500 mg/kg, 2/5 died at 1000 mg/kg, 5/5 died at 1500 mg/kg dose group Females: 1/5 died at 500 mg/kg, 2/5 died at 750 mg/kg and 5/5 at 1000 mg/kg group Clinical signs: In all dose groups, lethargy, immobility, irregular respiration, hunched posture. Other signs included low posture, low carriage, red ocular discharge, ruffled fur. Pathology: Discoloration of mucosa of tongue	Anonymous 1996
Acute oral toxicity study Similar to OECD 401, USA EPA GLP Reliability 1 **Key Study**	Rat, Sprague-Dawley, males and females 10 animals per sex and per dose No control animals	Hydrogen peroxide, 35 % (w/w) aqueous solution	Males: 630, 794, 1000, 1260, 1588 and 2000 mg/kg bw Females: 794, 1000, 1260 and 1588 mg/kg bw Single dose, administration by gavage	Males: 1193 mg/kg bw (corresponding in appr. 420 mg/kg bw as 100% H_2O_2) Females: 1270 mg/kg bw	Mortality: Males: 1/10 died at 630 mg/kg, 5/10 died at 794 mg/kg, 5/10 died at 1000 mg/kg, 2/10 died at 1260 mg/kg, 6/10 died at 1588 and 10/10 died at 2000 mg/kg dose group Females: 1/10 died at 784 mg/kg, 1/10 died at 1000 mg/kg, 5/10 died at 1260 mg/kg, 8/10 died at 1588 mg/kg dose group.	Anonymous 1983

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Remarks	Reference
					Most deaths occurred withing 24 hours of dosing. Clinical signs: tremors, decreased locomotion, recumbency, prostration, oral, ocular and nasal discharges. Pathology: Most rats that died had hemorrhagic stomachs which were white in color, and intestines filled with blood. Many had white tongues. Other findings noted with less frequency included blood-filled bladders, livers containign white foci, and blood in the stomach. All surviving animals appeared normal at necropsy.	
Acute oral toxicity study Similar to OECD 401 Non GLP Reliability 2 Additional data Study referenced in the EU Risk assessment 2003 and REACH registration dossier; not included in biocide assessment reports	Rat, Wistar, males and females 10 animals per sex and per dose 10 control animals per sex	Hydrogen peroxide, 60 % (w/w) aqueous solution	Males 0.351 , 0.535, 0.734, 1.019 and 1.296 ml/kg Females 0.213, 0.323, 0.426, 0.659, 0.879, 1.236 and 1.647 ml/kg Single dose, administration by gavage	Males: 872 mg/kg bw Females: 801 mg/kg bw	Mortality: Males: 0/10 died at 0.351. 3/10 died at 0.535, 4/10 died at 0.734, 9/10 died at 1.019, 10/10 died at 1.296 ml/kg dose. Females: 0/10 died at0.213, 3/10 died at 0.323, 2/10 died at 0.426, 4/10 died at 0.659, 7/10 died at 0.879, 8/10 died at 1.236, 10/10 died at 1.647 ml/kg. Administration of test solution resulted in immediate spasm followed by death. Clinical signs: surviving animals showed reduction in spontaneous movement, and	Anonymous 1981

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Remarks	Reference
					ataxia, but they recovered within 2-4 days. Pathology: Stomachs and intestinal canals of dead animals were highly congested and most of the mucous membrane of the glandular stomach was white.	
Acute oral toxicity study non-GLP Reliability 3 Only the summary of the study is available in English, and translated from Japanese Additional data	Rat, Wistar-JCL, males and females 10 animals per sex and per dose No control animals	Hydrogen peroxide, 9.6 % (w/w) aqueous solution	Males: 847, 1016, 1220, 1464, 1756, 2108, 2529 mg/kg Females: 886, 1063, 1276, 1531, 1837, 2205, 2646 mg/kg Single dose, administration by gavage	Males: 1518 mg/kg Females: 1617 mg/kg	Mortality: Males: 0/10 died at 847, 1/10 died at 1016, 2/10 died at 1220, 5/10 died at 1464, 7/10 died at 1756, 8/10 died at 2108 and 10/10 died at 2529 mg/kg Females: 0/10 died at 886, 1/10 died at 1063, 4/10 died at 1276, 5/10 died at 1531, 5/10 died at 1837, 7/10 died at 2108 and 10/10 died at 2646 mg/kg Clinical signs: dosage-related inhibition of autonomic behaviour was seen immediately after administration. Deaths were seen within 1-24 hours and were dose-dependent. Surviving animals recovered within 4-24 hours. Pathology: In dead animals dilation of the capillaries of the stomach and intestines was seen even at the lowest dose; the degree of dilation was dosage-dependent.	Ito et al., 1976

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Remarks	Reference
					No changes were seen in other organs (chest and abdominal organs were dissected).	
Acute oral toxicity study OECD 401 Limít test GLP Additional data Reliability 1	Rat, Sprague-Dawley, male and female 5 males and 5 females No control animals	Hydrogen peroxide, 50 % (w/w) aqueous solution	225 mg/kg Single dose, administration by gavage	Limit test, no LD_{50} determined	Mortality: 1/5 males and 0/5 females died at 225 mg/kg Clinical signs: decreased locomotion, rales, abdominogenital staining, hypersensitivity to touch, lacrimation, chromorhinorrhea, chromodacryorrhea. All signs resolved within 72 hours. Pathology: the male which died had an ulcerated and hemorrhagic stomach. All other rats appeared normal at necropsy.	Anonymous 1986
Acute oral toxicity study OECD 401 Limít test GLP Additional data Reliability 1	Rat, Sprague-Dawley, male and female 5 males and 5 females No control animals	Hydrogen peroxide, 10 % (w/w) aqueous solution	5000 mg/kg Single dose, administration by gavage	Limit test, no LD_{50} determined, lethal dose > 5000 mg/kg	Mortality: 0/5 males and 1/5 females died. Clinical signs: Decreased or blackened feces, decreased locomotion, hypersensitivity to touch, hematuria, lacrimation, recumbency, cyanosis, ataxia, chromorhinorrhea, nasal discharge, and abdominal staining. Pathology: in the dead female rat which died the only necropsy finding was hemorragic, blood filled stomach and intestines and reddened lungs.	Anonymous 1990a

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Remarks	Reference
Acute oral toxicity test Non-GLP Limited documentation, only a short summary available, original study report not available. Study referenced in the EU Risk assessment 2003 and REACH registration dossier; not included in biocide assessment reports Additional data Reliability 4	Rat, strain unknown Six males per dose	Hydrogen peroxide, 70%	50, 75, 100 mg/kg bw	75 mg/kg	Mortality: 0/6 died at 50 mg/kg, 3/6 died at 75 mg/kg, 6/6 died at 100 mg/kg Pathology: Autopsy revealed severely edematous mucosal linings in the stomach and bleaching of internal surfaces.	Anonymous 1979

Table 14: Summary table of human data on acute oral toxicity

Type of data/report	Test substance, route of exposure, relevant information about the study (as applicable)	Observations	Reference
Case report	Case: 16-month old boy Exposure: accidental ingestion of 3% hydrogen peroxide. The boy was found playing with an empty bottle which had contained 230 g of hydrogen peroxide. The container had a cracked lid that allowed the contents to be sucked. The estimated dose of hydrogen peroxide ingested was 7 g, therefore about 600 mg/kg/bw for a boy of 11.6 kg.	Symptoms: white foam emerged from the child's mouth and nose. He then walked to bed and was found dead 10 hours later. Post mortem examination: frothy blood in the right ventricle of the heart and the portal venous system. The gastric mucosa was red and the brain oedematous. Histopathological examination showed oedema in the lungs, and diffuse interstitial emphysema was evident. Gas emboli were found within the pulmonary vasculature and gastric and intestinal lymphatics. Clear vacuoles were also found within the walls of the gastrointestinal tract, in the spleen, kidney and myocardium.	Cina et al., 1994 (Case included in the study by King et al., 2023)

Type of data/report	Test substance, route of exposure, relevant information about the study (as applicable)	Observations	Reference
Case report	Case: 21-year old male Exposure: accidental ingestion of approximately one mouthful of 3% hydrogen peroxide	Symptoms: vomiting, pain in mouth, throat and epigastrium, tachycardia, mild hypertension Clinical examination: dysphonia with mild erythema and oedema of the oropharynx and uvula; Abdominal and respiratory examinations unremarkable; blood and biochemical investigations were normal. CT scan demonstrated pneumatosis and mucosal thickening throughout the stomach and proximal duodenum as well as extensive portal venous gas. The patient was intubated owing to concerns for developing airway involvement and gastrointestinal perforation. Medication of intravenous antibiotics and reflux was commenced. Follow up: The patient was discharged after 3 days and made a full recovery.	Arnfield et al., 2016
Case report	Case: 2-year old boy Exposure: accidental ingestion of 4 to 6 oz (113 to 170 g) of 35 % hydrogen peroxide. The estimated dose of hydrogen peroxide was about 50 g and about 3800 mg/kg bw for a boy of 13 kg.	Symptoms: The boy rapidly became unresponsive and cyanotic, with stiffening of his left arm. Clinical examination: On arrival at the hospital a chest radiograph showed gas in the right ventricle, mediastinum and portal venous system. He remained paralyzed. Oesophagogastroduodenoscopy showed severe haemorrhagic gastritis without perforation. The eosophagus and duodenum appeared normal. He died on day 4. Autopsy showed marked diffuse cerebral oedema with cerebellar and uncal tonsillar notching.	Christensen et al. 1992 (Case included in the study by King et al., 2023)
Case report	Case: 2-year old girl Exposure: accidental ingestion of 35 % hydrogen peroxide, unknown quantity (two sips).	Symptoms: Immediately after ingestion the girl started foaming at the mouth, and frank hematemesis as well as epigastric pain was observed. Parents described symptoms as confusion, stridor, apnea, cyanosis and cardiorespiratory arrest. Upon arrival at emergency department of a hospital in 45 minutes after ingestion, even as cardiopulmonary resuscitation was started, she had died. Post mortem examination: Gross examination of the mucosal surfaces of the esophagus, stomach and duodenum disclosed congestion with thickened white areas. The distal esophagus, gastric fundus and duodenum showed extensive areas of necrosis and erosions. Congestion of the visceral pleura was observed in the	Indorato et al., 2014 (Case included in the study by King et al., 2023)

Type of data/report	Test substance, route of exposure, relevant information about the study (as applicable)	Observations	Reference
		lungs, focally raised by emphysematous microbubbles in interlobar fissures. Diffuse alveolar damage with evidence of necrosis and heavy inflammation of the bronchial basal lamina was the main histological finding in lung parenchyma: marked mixed inflammatory exudate filling alveolar spaces. Large foci of interstitial pneumonia characterized by lymphocytic infiltration, also arranged in a nodular and peribronchial pattern, with necrosis of the bronchial epithelium basal lamina, were observed. Erosion and chronic non-specific inflammation of the tracheal mucosa was also detected. The cause of death could be attributed to respiratory distress syndrome due to hydrogen peroxide ingestion, which caused severe chemical aspiration pneumonia, pulmonary emphysema, esophagitis and gastritis. The time interval witnessed may have been too short with respect to the heavy inflammatory infiltrates detected in the lung. It was concluded that such a discrepancy might have been due to inaccurate reporting by the witness.	
Case report	Case: 3-year old girl Exposure: accidental ingestion of nearly 10 ml of 20% hydrogen peroxide	Symptoms: The previously healthy girl presented to the emergency department with vomiting, lethargy and respiratory distress. She was intubated because of impending respiratory failure. She also had generalized tonic-clonic seizures. Clinical examination: Results from brain computed tomograhpy and magnetic resonance imaging showed diffuse brain edema. She was hospitalized in the pediatric intensive care unit with a Glasgow coma score of 3. Seizures were controlled with intravenous midazolam and phenytoin, and dexamethasone and mannitol were given for brain edema. Her condition was further complicated which required dopamine and dobutamine infusion. Her neurologic status did not improve despite three episodes of hyperbaric oxygen treatment. She was declared brain dead on the sixth hospital day.	Ikiz et al., 2013 (Case included in the study by King et al., 2023)
Case series	Data from a single Poison Center in US Poison center records were searched from 1999 to 2010 to find patients with hydrogen peroxide	In the search 11 cases of portal gas embolism were found. Ages ranged from 4 to 89 years. All but one ingestion was accidental in nature. Soon after ingestion the patients experienced symptoms which included	French et al., 2010

Type of data/report	Test substance, route of exposure, relevant information about the study (as applicable)	Observations	Reference
	exposure and hyperbaric oxygen treatment. Cases were reviewed for the concentration of hydrogen peroxide, symptoms, CT scan findings of portal gas embolism, hyperbaric oxygen treatment, and outcome. In 10 cases 35% hydrogen peroxide was ingested and in 1 case 12% hydrogen peroxide was ingested. The amount of ingestion was described as a sip, 1-2 mouthful, 1 tablespoon, ½-1 cup or 30 ml depending on the patient.	abdominal pain, vomiting, hematemesis, nausea, burns in mouth and throat. All abdominal CT scans demonstrated portal venous gas embolism in all of the cases. Patients were given a single round of hyperbaric oxygen treatment within 2-6 hours after exposure. Hyperbaric oxygen treatment was successful in completely resolving all portal venous gas bubbles in nine patients (80%) and nearly resolving them in two others. Ten patients were able to be discharged home within 1 day, and one patient had a 3.5-day length of stay.	
Retrospective analysis of a structured database	Data from US National Poison Data System (NPDS) of the American Association of Poison Control Centers. The NPDS database contains all cases collected by US poison centers, with all centers using a standard format. The NPDS database was queried for ingestions from 2001-2011 coded as a peroxide product with a concentration greater than 10%. If not explicitly stated in the poison center chart, volume of ingestion was estimated at 15 ml for an adult swallow and 5 ml for a pediatric swallow.	The chart for each poison center was obtained and abstracted in a standardized fashion; 1,054 cases were initially considered and 294 cases met inclusion criteria (high concentration exposure, signs or symptoms of toxicity ranging from emesis to critical illness or death). The primary outcome of possible embolic event was defined as seizure, altered mental status, respiratory distress, hypoxia, hemodynamic instability, ECG changes, radiographic evidence of cerebrovascular accident, focal neurologic deficit on examination, pulmonary embolism, cardiac emboli, elevated troponin level, physician bedside diagnosis, or rapid improvement after hyperbaric oxygen therapy. Both descriptive statistics and logistic regression models were used to analyze the data. In the 10-year study period, 41 of 294 (13.9%; 95% confidence interval 10.2% to 18.4%) symptomatic peroxide ingestion cases demonstrated evidence of embolic events. Final outcomes were severe with 20 of 294 patients (6.8%; 95% CI 4.2% to 10.3%). Death occurred in 5 of 294 patients, and 15 of 294 patients exhibiting continued disability when the poison center chart was closed. Improved outcomes were demonstrated after early hyperbaric oxygen therapy. Endoscopy revealed grade 3 or 4	Hatten et al., 2017 (Cases included in the study by King et al., 2023)

Type of data/report	Test substance, route of exposure, relevant information about the study (as applicable)	Observations	Reference
		lesions in only 5 cases. Conclusions: Symptomatic high-concentration peroxide exposures had a high incidence of associated embolic events in this cohort. Patients with evidence of embolic events had a high rate of death.	
Systematic review based on literature and cases from US Poison Center Data System	The study objectives were to define the time of onset of embolic phenomena after hydrogen peroxide exposure and to describe the proportion of patients who received hyperbaric oxygen therapy. Cases from a systematic literature search were combined with those from a prior study that used data from the American Association of Poison Centers National Poison Data System (Hatten et al. 2017). Literature searches were performed in PubMed, EMBASE, CINAHL Complete, Web of Science, Scopus, and Google Scholar from database conception to May 29, 2019. Articles were included if they were prospective studies, retrospective studies, case series, or case reports describing human patients exhibiting evidence of oxygen embolization after hydrogen peroxide exposure. Cases were merged if the date of publication, demographics, timing, concentration, and embolic phenomena were identical between the National Poison Data System report and an independent journal article. All deaths were reviewed	A total of 766 records were identified in the literature search. Three-hundred and eighty-three duplicate records were identified and removed. Of the 383 remaining records, 156 met inclusion criteria; 88 were excluded based on predetermined criteria yielding 68 records with 85 unique cases. Fortyone cases were extracted from the 2017 National Poison Data System study (Hatten et al. 2017) resulting in a total of 126 cases for analysis. Case descriptions: The 126 cases were analyzed and 213 discrete clinical events were documented, excluding deaths. There were 108 high-concentration exposures, 10 low-concentration exposures, and 8 were unknown. Thirty-five cases were intentional ingestions but not for self-harm, and 84 were unintentional or accidental. Only 4 cases were for self-harm, and there were 23 pediatric cases. There were 99 air-gas emboli reported in 78 patients. Time to symptom onset ranged from immediate to 72 h after hydrogen peroxide exposure. Over 90% of embolic symptoms occurred within 10 h of ingestion. Hyperbaric oxygen therapy: A total of 54/126 cases received hyperbaric oxygen therapy. Of those 54 cases, 31 had primary portal venous gas while the remaining 23 had air-gas emboli. Of the 23 air-gas emboli cases treated with hyperbaric oxygen therapy, 13 made full recoveries while 10 had residual symptoms or died. Mean time from air-gas emboli symptom onset to hyperbaric oxygen therapy in the full recovery group was 9 h compared to 18.2 h in the partial recovery/death group. Portal venous gas: There were 63 total reported cases of portal venous gas. Forty-nine of these cases were primary portal venous gas, 13 were secondary findings in patients with air-gas emboli and one case was secondary to non-air-gas emboli symptoms. Twenty-seven of 49 patients with portal venous gas (55%) as the primary finding had gastrointestinal	King et al., 2023

Type of data/report	Test substance, route of exposure, relevant information about the study (as applicable)	Observations	Reference
	separately for timing and reason for death. Concentrations of ingestions were split into high-concentration (>10% v/v) and low-concentration (≤ 10% v/v).	bleeding. Thirty of the 63 cases received hyperbaric oxygen therapy for portal venous gas without any documented air-gas emboli. Seventeen deaths occurred in the combined cohort, 12 from the literature search and 5 from National Poison Data System data. Concentration was documented in 15/17 cases. Of these, 13 were associated with high-concentration exposures. Low-concentration deaths involved high volume ingestions (170 ml and 240 ml). All deaths with reported time to symptom onset had symptoms within 1 h of exposure. Nine out of the 16 death cases (56.3%) had multiple air-gas emboli after ingestion: 12 had neurologic symptoms, 4 had myocardial infarctions, and 8 suffered from cardiovascular collapse/hemodynamic instability.	

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Animal studies

All the data that was considered under the DSD classification process and in the EU risk assessment report (European Commission, 2003) is documented in the present CLH proposal, and according to our knowledge no new animal studies have been reported. According to our knowledge there are no new animal studies since the classification for acute oral toxicity was agreed by the TC C&L group and finally included in the 29th ATP to the Directive 67/548/EEC with the Commission directive 2004/73/EC.

A total of seven animal studies were identified for acute oral toxicity. Two valid guideline-conform studies on the acute oral toxicity of hydrogen peroxide were performed with solutions of 35% w/w (Anonymous 1983) and 70% w/w (Anonymous 1996). Oral LD_{50} values obtained with 35% solutions were 1193 mg/kg in male rats and 1270 mg/kg in female rats. Oral LD_{50} values of 1026 mg/kg in male rats and 694 mg/kg in female rats were obtained with 70% solutions.

Additional, less reliable studies on the acute oral toxicity of hydrogen peroxide were mentioned in the EU risk assessment report for hydrogen peroxide (European Commission, 2003). An oral LD_{50} value of 75 mg/kg was established for a 70% solution administered to rats of unknown strain (FMC 1979). The oral LD_{50} values in male and female Wistar rats receiving a single dose of 60% solution were 872 mg/ kg and 801 mg/kg, respectively (Anonymous 1981). In a limit test, Sprague-Dawley rats received a single oral dose of 10% solution of 5000 mg/kg (FMC 1990). In another study, a 9.6% solution was administered to Wistar JCL rats at a series of concentrations. The oral LD_{50} value was 1518 mg/kg in male rats and 1617 mg/kg in female rats (Ito et al., 1976). There are no animal studies conducted with lower than 9.6% concentrations.

In table 15 summary of LD_{50} values are listed. A a theoretical value was calculated for 100% concentration of hydrogen peroxide.

Table 15 Summary of the LD50 values

Concentration of H_2O_2 solution used in test (%)	Species, strain	LD_{50} (mg/kg bw)	LD_{50} (mg/kg bw) calculated for 100% theoretical concentration of H_2O_2	Reference
9.6	Rat, Wistar	1517 (male) 1617 (female)	145 (males)	Ito et al., 1976
10	Rat, Sprague-Dawley	> 5000	n/a	Anonymous 1990a
35	Rat, Sprague-Dawley	1193 (males) 1270 (females)	420 (males)	Anonymous 1983
50	Rat, Sprague-Dawley	> 225	n/a	Anonymous 1986
60	Rat, Wistar	801 (females) 872 (males)	480 (females)	Anonymous 1981
70	Rat, Crl:CD BR	694 (females) 1026 (males)	490 (females)	Anonymous 1996
70	Rat, unknown strain	75 (males)	50 (males)	Anonymous 1979

Observation in humans

There are human case reports of poisoning, where intake of different doses of hydrogen peroxide has been either unintentional or intentional. Fatalities has been described after accidental ingestion of hydrogen peroxide solutions with most exposures in children. In many cases hydrogen peroxide has been mistaken for water. Hydrogen peroxide solutions have also been ingested voluntarily for the purpose of hyper-oxygenation therapy to treat various diseases such as cancer, AIDS, dementia and coronary heart disease (Watts et al., 2004).

Some severe cases have been reported with less than 10% concentrations. Most fatal cases have occurred after ingestion of high concentration (> 10 -35%) hydrogen peroxide. However there are cases where high-volume ingestion of low concentration (3-5%) has had death as an outcome, especially with children.

Harm appears to occur from direct caustic injury to tissue, embolic obstruction of arterial blood flow, lipid peroxidation, or perforation of a hollow organ (French et al., 2010). Ingestion of concentrated hydrogen peroxide has been associated with venous and arterial gas embolic events, hemorrhagic gastritis, gastrointestinal bleeding, shock, and death.

Hydrogen peroxide easily crosses biological membranes, resulting in extensive penetration of blood vessels and tissues. The enzyme catalase decomposes hydrogen peroxide into water and oxygen. The amount of oxygen liberated depends on hydrogen peroxide concentration. For example, 1 mL of 3% volume per volume (v/v) hydrogen peroxide releases 10 ml oxygen, whereas 1 ml of 35% (v/v) hydrogen peroxide releases 100 ml oxygen (King et al., 2023, Mullins and Beltran, 1998). It was suggested that not all of the hydrogen peroxide might immediately dissociate into oxygen and water. Some may remain as hydrogen peroxide until passing through the pulmonary vasculature: then it

may decompose into oxygen and water in the arterial system. Alternatively, gas emboli may travel through the venous system into the right heart and pass across a patent foramen ovale into arterial circulation. (Ashdown et al., 1998).

Though embolic phenomena from hydrogen peroxide exposure are infrequent, they have the potential to cause significant morbidity and mortality (King et al., 2023).

10.1.2 Comparison with the CLP criteria

Based on the two key animal studies (Anonymous 1996, Anonymous 1983) the lowest LD_{50} value was 694 mg/kg bw when tested with 70% solution and 1193 mg/kg bw when tested with 35% solution. The obtained LD_{50} values correspond to 490 and 420 mg/kg bw of 100 % hydrogen peroxide. A less reliable study with 60% solution gave a LD_{50} in the similar range (480 mg/kg bw as 100% hydrogen peroxide). Considerably lower LD_{50} values were obtained in two less reliable studies. The lowest tested concentration with 9.6 % solution gave an LD_{50} value of 1500 mg/kg bw which corresponds to 145 mg/kg bw as 100 % hydrogen peroxide. A study with 70% solution gave an LD_{50} of 75 mg/kg bw which corresponds to 50 mg/kg bw as 100 % hydrogen peroxide, however the full study report is not available. Overall, the obtained LD_{50} values are in the range of 50 to 420 mg/kg which means that hydrogen peroxide could be classified to either Acute oral toxicity category 3 or 4 depending which animal studies are considered as most reliable.

Human poisoning cases should be taken into account in determining the final classification. Since hydrogen peroxide is colorless and odorless, it has been mistaken for water especially with children and elderly people. Although hydrogen peroxide is generally considered benign in low concentrations, case reports have described serious toxicity following high concentration exposures with some cases with fatal outcomes. However in most cases the exact dose ingested is not known.

The classification for acute oral toxicity was agreed by the TC C&L group and finally included in the 29th ATP to the Directive 67/548/EEC by the Commission directive 2004/73/EC. Hydrogen peroxide was classified as Xn; R22 (Harmful by inhalation) with a specific concentration limit of C ≥ 8 % instead of the general concentration limit of 25 %. The basis for the set SCL is not clear. In the REACH dossier the registrant speculates that the caustic effects on eyes and the classification as eye irritant category 2 of solutions with C ≥ 8% *may be seen as a suitable proxy setting a limit concentration for potential adverse local effects in the gastrointestinal tract and thus acute toxicity*.

No direct translation from the DSD classification to a CLP classification can be made because of the different cut off limits in the provisions, and thefore a minimum classification of Acute tox 4* was given under the CLP Regulation. In CLP Regulation there are no concentration limits for acute toxicity classification. If the minimum classification of Acute tox category 4 was confirmed based on the lowest LD50 value of 420 mg/kg from the most reliable rat studies, it would mean that the lowest concentration of hydrogen peroxide solution classified for acute oral toxicity category 4 would be 21 %. This concentration is considerably higher than the SCL of ≥ 8 % under the DSD. Therefore if ATE of 420 mg/kg was used the level of safety would be drastically lowered under the CLP Regulation. As pointed out above, there are human poisoning cases with concentrations of 3 % solutions reported, with high-volume of low concentrations, and with concentrations ≥ 10 - 35 % being more severe.

In a total weight of evidence consideration of the data from animal studies and human data, and taking into account the harmonised classification with an SCL of 8 % under the DSD, hydrogen peroxide should be classified as Acute oral toxicity category 3. This classification would ascertain that the same level of safety would be retained under CLP Regulation as was under the DSD. The LD_{50} values of 50 to 145 mg/kg bw derived from the less reliable animal studies are in the range of category 3 (50 < category 3 ≤ 300 mg/kg bw). A question remains regarding derivation of the ATE value. Due to the uncertainty in the animal studies with the lowest LD_{50} values it is suggested to use the converted acute toxicity point estimate of 100 in category 3. This would ensure that hydrogen peroxide concentrations at or above 8% would be classified in category 4.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Classification as Acute oral toxicity category 3; H301 for 100% hydrogen peroxide is proposed and the minimum classification of category 4* removed. An ATE value of 100 mg/kg based on the converted acute point estimate for category 3 is suggested.

10.2 Acute toxicity - dermal route

Not assessed in this dossier.

10.3 Acute toxicity - inhalation route

Table 16: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Remarks	Reference
Acute inhalation toxicity study US-EPA Vol 50 Compliant to 92/69/EEC B.2 Similar to OECD 403 GLP Reliability 1 Key study	Rat, Sprague-Dawley CD. males and females 5 males and 5 females No control animals	Vapour, generated from 50 % hydrogen peroxide The mean analytical exposure concentration was determined by colorimetric analysis. Measurement of any aerosol was determined, no aerosol was measured (particle size distribution measurements showed particulate level of 0.020 mg/m^3 which was comparable to background air levels)	Animals were exposed to a nominal concentration of 7.7 mg/L (50%), the analytical concentration being 0.17 mg/L which was considered as maximum attainable exposure level of vapour. The difference between the nominal and measured exposure level was probably attributable to the water content of the test substance (more volatile than H_2O_2) and the reactivity of any vaporized H_2O_2. Animals received a single 4-hour exposure (whole body). Observations for abnormal signs were conducted at 15 minute	Not determined LC_{50} > 170 mg/m^3 (0.17 mg/L, maximum attainable vapour)	Mortality: None of the animals died during or after the exposure to hydrogen peroxide. Clinical signs: Animals showed decreased activity and eye closure during exposure. Nasal discharge, excessive salivation and anogenital staining were observed on day 1 postexposure. During 14 day postexposure period dried red nasal discharge and dried red material on fur was observed. Pathology: No treatment-related gross internal lesions noted in any animal. Lung weights were comparable to historical values.	Anonymous 1990b

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Remarks	Reference
			intervals during first hour of exposure and hourly for the remainder of the exposure, and once daily during postexposure period for 14 days.			
Acute inhalation toxicity study Non-Guideline Non-GLP Additional data Reliability 3 -Methology not well reported - rat strain unknown - Tests 4 to 8-hour exposures - Exposure to “saturated vapour” - no details given on individual animals	Rat, strain unknown Test 1: 6 rats Test 2: 10 rats per dose	Vapour, generated from 90 % hydrogen peroxide	Test 1: 4000 mg/m^3 or 2880 ppm (nominal concentration; actual concentration in the chamber most likely lower) Test 2: 338 - 427 mg/m^3 (243 to 307 ppm) 4- or 8-hour whole-body exposure Test 1: Nominal chamber concentration of 4000 mg/m3 (not determined by analysis but calculated concentration from the difference in weight of the bubbler before and after dispersion and the total volume of air passed through the liquid), in three separate 8-hour runs 6 rats were exposed for 8 hours, and then killed at various times up to 14 days for pathological	LC_{50} not determined	Mortality: No deaths in either test. No signs of intoxication. Clinical signs: Not given for test 1. In test 2, observations included scratching and licking. After 30 minutes the animals became quiet and slept most of the time. Pathology: In test 1, trachea and lungs from all animals exhibited severe congestion. Animals that were killed during first 3 days following exposure showed small localised areas of pulmonary edema without hemorrhage. In animals killed between 4 and 14 days the majority of the lungs exhibited many areas of alveolar emphysema in addition to the severe congestion. There were no	Comstock CC et al. 1954 (study report) Oberst FW et al. 1954 (publication)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Remarks	Reference
			examination. Test 2: Concentration determined by chemical analysis with titanium sulfate reagent, 10 rats were exposed in each of three runs. One run extended over 4-hour period and the other two over 8-hour period. Concentrations in three runs varied from 338 to 427 mg/m^3. After exposure animals were submitted for pathological examination at various times up to 14 days.		signs of necrosis in pulmonary mucosa. All other organs appeared normal. In test 2 the pathological observations were similar to test 1.	
Acute inhalation toxicity study Non-guideline study Non-GLP Additional data The goal of the study was to examine the effects of air pollutants ozone-hydrogen peroxide mixtures on toxicity, spread of ozone tolerance, and cross tolerance to either	Mouse (Swiss, male) and rat (Wistar, male) 10 mice/group 5 rats/group	Vapour, generated from 90 % hydrogen peroxide or its mixture with ozone The measurements of hydrogen peroxide concentrations in the air mixtures was based on the development of a stable yellow color with titanium sulfate reagent.	Only the results of the control animals exposed to hydrogen peroxide only are described here with data extracted from the tables of the study. The concentrations of hydrogen peroxide in three separate study set ups were 1) 16.1 ppm, 2) 37.4 ppm and 227 ppm, 3) 78.1 ppm (110 mg/m^3),	Not determined	Mortality: There was no mortality in either in mouse (0/10) or rat (0/5) at concentration of 16.1 ppm. (Data extracted from Table I in Svirbely et al.) In the second set up, at 37.4 ppm 0/10 mice died: at 227 ppm 5/25 died in 24 hours and 22/25 died within 2 weeks. (Data extracted from Table III in Svirbely et al.) In the third set up, at 78.1 ppm	Svirbely et al., 1961

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Remarks	Reference
component. Reliability 3 -Data from an academic publication; no study report available nor data on individual animals. - aim of the study was not to examine acute toxicity of hydrone peroxide; mixtures of hydrogen peroxide and ozone were tested			113 ppm (160 mg/m^3), 194 ppm and 226 ppm (321 mg/m^3). Results on rats are given only in one (1) set up. In the tests 4-hour whole-body exposure was used. Postexposure period was 2 weeks. The animals were killed subsequently with intraperitoneal injections of nembutal before histopathological examination.		0/10 mice died either within 24 hours or in 2 weeks; at 113 ppm 1/10 mouse died within 24 hours and 4/10 died within 2 weeks; at 194 ppm 3/10 died in 24 hours and 6/10 died in 2 weeks, at 226 ppm 1/10 died in 24 hours and 5/10 in 2 weeks. (Data extracted from Table IV in Svirbely et al.) It was concluded that mouse was more susceptible to hydrogen peroxide vapour toxicity than the rat. A single prior exposure to hydrogen peroxide afforded a moderate degree of protection against otherwise lethal doses of H2O2. Clinical signs or pathology was not described for the control animals exposed to hydrogen peroxide only.	
Acute inhalation toxicity study Non-guideline study Non-GLP Additional data Reliability: 4	Rat, unknown strain 874 animals	"Vapour" of hydrogen peroxide (no further details given)	4-hour whole-body exposure, exposure chamber (volume 1.5 m^3) Combined inhalation and skin application According to the study description	LC_{50} given as 2000 (1690-2360) mg/m^3 but methodology not described	Mortality: No details given. Primary cause of death was described to be a gas embolus.	Kondrashov et al., 1977

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Remarks	Reference
- Translation from a Russian study -poor documentation, only summary information available -no methods reported			a method was used to determine the concentration of hydrogen peroxide vapours in the air of the exposure chambers, but no details given.			
Acute inhalation toxicity study Non-guideline study Non-GLP Not 4-hour exposure Additional data Reliability 2 -Expression of exposure atmosphere described as both aerosol or "saturated vapour"	Mouse (white), unknown strain 10 males/group	Aerosol generated from 90% hydrogen peroxide Aerosol: Mass median particle size approximately 3.5 microns as determined by cascade impaction.	Not 4-hour exposure Exposure duration 5, 10 or 15 minutes Study 1: 3600-5200 mg/m^3 for 5 minutes. Study 2: 9400-19000 mg/m^3 for 5-15 minutes. Animals were observed during exposure and then daily until sacrified (2 days- 8 weeks).	LC_{50} not determined	Mortality: No deaths occurred at concentrations up to 5200 mg/m^3. At 9400 mg/m^3 with 5 minutes exposure 1/10 died after 6 days. At higher concentrations with 10-15 min exposure mortality: at 13200 mg/m^3 with 10 min exposure 5/10; at 13400 mg/m^3 with 10 min exposure 1/10; at 19000 mg/m^3 with 10 min exposure 1/10; at 11800 mg/m^3 with 15 min exposure 5/10; at 16700 mg/m^3 with 15 min exposure 9/10. The survival time was reduced in the majority of animals to less than an hour. Clinical signs: Animals exposed to lower	Punte et al., 1953

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Remarks	Reference
					concentrations showed mild nasal irritation, blinking of the eyes, slight gasping, and loss of muscular coordination. The symptoms generally disappeared within 30 min after removal from the exposure atmosphere. Animals exposed to higher concentrations exhibited similar symptoms but they were more severe. Animals that died went through a short convulsant period before death. Pathology: At low exposure concentrations there was evidence of lung congestion at necropsy. Four of 20 animals showed some necrosis of bronchial epithelial cells. At higher concentratiosn animals which died showed pulmonary congestion in most instances. Surviving animals showed necrosis of bronchial	

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Remarks	Reference
					epithelium. Survivors of exceptionally high concentrations (9-19 mg/l) may develop corneal damage several weeks after exposure.	
Acute inhalation toxicity study Non-guideline study Study protocol adapted from Zwarf et al. 1992 (Reg. Tox. and Pharmacol., 15, 278-290, 1992; the method is a substitution to the LC_{50}, and based on exposure of couples of animals (1 male , 1 female) to a given concentration for a given time. GLP Not 4-hour exposure Reliability 1 Additional data	Mouse, Swiss Groups of 4 males, 19 groups (only males were used because no difference in sex was expected)	Aerosol generated from 70% hydrogen Test material was administered onto a stream of pressurized air with a stainless steel Lechler nebulizer. Samples of the test atmosphere were analyzed with potentiometric titration. The actual concentration was calculated from the amount of hydrogen peroxide measured and the volume of the air sample. Stability of aerosols was monitored on-line (opacity monitor). Aerosol: particle size not given	Mean actual concentrations ranged from 880 to 4960 mg/m^3 Exposure duration 7.5 to 120 minutes. Nose-only exposure Exposures were as follows: Exposure to 2200, 3430, or 4960 mg/m^3 for 7.5 min; Exposure to 880, 1530, 1720, 3590 mg/m^3 for 15 min; Exposure to 960, 1370, 2050 or 3220 mg/m^3 for 30 min; Exposure to 910, 1370, 2170 or 3130 mg/m^3 for 60 min; Exposure to 920, 1450 or 2000 mg/m^3: for 120 min. 14-day observation period afer exposure	LC_{50} not determined	Mortality: No mortality at any concentration exposed for 7.5 to 60 minutes except at the highest concentration of 3130 mg/m^3 for 60 min where 4/4 animals died. Death occurred in animals exposed for 120 min as follows: 3/4 died at 920 mg/m^3, 1/4 died at 1450 mg/m^3, 2/4 died at 2000 mg/m^3. Clinical signs: Surviving animals showed swelling of the skin on the head, ptosis or closed eyelids, piloerection, noisy breathing. Pathology: Macroscopic findings in dead animals included swelling and/or discolouration of the skin of the head, the tongue, neck, fore paws, and the nose, subcutaneous emphysema and haemorrhages,	Anonymous 1995

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Remarks	Reference
					red lymph nodes, diffuse red lungs. These were attributed to the bleaching and corrosive nature of the test substance.	

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

Acute inhalation toxicity studies have been performed with aerosols (mice) and vapours (rats and mice). All the data that was considered under the DSD classification process and in the EU risk assessment report (European Commission, 2003) is documented in the present CLH proposal, and according to our knowledge no new animal studies have been reported.

There are four studies conducted using vapour in exposures of rats and mice. In a study considered as a key study (Anonymous 1990b) in the evaluation of active substance according to the Regulation EU No 528/2012 on biocidal products and in the REACH registration dossier, rats were exposed to vapour generated from 50% hydrogen peroxide solution. The analytical concentration in the whole body 4- hour exposure was 0.17 mg/l for 49.3% hydrogen peroxide, which was considered the maximum attainable vapour concentration. There were no mortalities and the clinical signs included decreased activity and eye closure, and nasal discharge and transient weight loss. No exact LC_{50} value could be determined and therefore it was > 0.17 mg/l.

Other studies conducted using vapour are considered as additional data only due to considerations on reliability. Rats exposed to 338-427 mg/m^3 for 4-8 hours showed few symptoms other than scratching and licking themselves, and none of the animals died (Comstock et al., 1954). Pathological examination revealed congestion in the trachea and lungs, and the lungs exhibited many areas of alveolar emphysema. Another poorly reported study which concerned a whole-body (shaved skin) exposure of rats to hydrogen peroxide vapour for 4 hours, gave an LC_{50} value of 2 mg/l and noted that the primary cause of death in the animals was gas embolism (Kondrashov et al., 1977).

The study on the effects of air pollutants ozone-hydrogen peroxide mixtures on toxicity and on cross tolerance to either found that the mouse was more susceptible to hydrogen peroxide vapour toxicity than the rat. Data on control animals exposed to hydrogen peroxide only up to 0,3 mg/l for 4 hours showed that within 2 weeks at least half of the animals died. A single prior exposure to hydrogen peroxide afforded a moderate degree of protection against otherwise lethal doses of hydrogen peroxide. (Svirbely et al., 1961).

Concerning hydrogen peroxide aerosols, 2-hour exposures to levels ranging from 920 to 2,000 mg/m^3 (aerosol of 70% hydrogen peroxide) were lethal to at least some mice; macroscopic findings in the dead animals (swelling and/or discolouration of the skin of the head, the tongue, neck, forepaws, and the nose, subcutaneous emphysema and haemorrhages, red lymph nodes, diffuse red lungs) were attributed to the bleaching and corrosive nature of the test substance (Anonymous 1995). Punte et al. (1953) reported in a mouse study that at concentrations from 3,600 to 5,200 mg/m3 there were no deaths, but congestion of the lungs and necrosis of bronchial epithelium were found. At 9,400 mg/m3 the lethality range was reached with death occurring 6 days following exposure. At

12,000-19,000 mg/m3 for 10-15 min, the survival time was reduced in the majority of mice to less than an hour. The symptoms of the animals during exposure to low concentrations consisted of a mild nasal irritation, blinking of the eyes, slight gasping, and loss of muscular coordination. These symptoms generally disappeared within 30 min. Pulmonary congestion was noted, and surviving animals showed necrosis of bronchial epithelium. Gross opacities were present in the eyes of 4 mice exposed to the highest concentration (19,000 mg/m^3) and killed after 8 weeks.

To our knowledge there are no new studies reported after the classification for acute inhalation toxicity was agreed by the TC C&L group in the early 2000 and finally included in the 29th ATP to the Directive 67/548/EEC. It was agreed then to classify hydrogen peroxide as Xn; R20 (Harmful by inhalation) with specific concentration limit of C ≥ 50. The EU risk assessment report (European Commission, 2003) states that "*Due to the corrosive nature of the substance after inhalation exposures to highly concentrated aerosols (70% H2O2 as "droplets"), lethality occurs at quite low air concentrations (0,92-2 mg/l). The lethal event can be attributed to the substance corrosivity rather than its systemic toxicity. Since exposure to significant concentrations of hydrogen peroxide was not observed in the risk assessment and the predominant human exposures were related to vapors only, vapour experiments were preferred in the hazard assessment. Based on vapour inhalation studies in mice and rats the substance was considered to be harmful by inhalation*."

Finland as the DS argued that aerosols should not be taken as basis for classification because deaths were due to secondary effects, caused by the highly concentrated corrosive solutions. Several studies (Anonymous 1990b, Comstock et al., 1954, Svirbely et al., 1961) were available using vapour with comparable exposure concentrations, rendering more realistic scenarios. However the Svirbely et al., 1961 study on mice was considered problematic due to the undelying methology in the study and furthermore rat was considered the model species for acute inhalation studies. Since there were two rat studies using 50% and 90% solutions for vapour generation they were considered suitable for classification.

To our knowledge there are no fatal human poisoning cases reported either from consumer use or from industrial use. There is some data on long term occupational exposure to hydrogen peroxide vapour. Symptoms of respiratory irritation have been reported among manufacturing plant personnel exposed to hydrogen peroxide. A single case of long-term inhalation exposure to hydrogen peroxide with progressive dyspnoea and bilateral diffuse nodular infiltrates of the lungs was seen. The patient improved progressively without treatment after withdrawal from the occupational exposure (Kaelin et al., 1988). Another health monitoring study involving a small group of workers (N = 6) exposed during about 10 months to relatively high levels (2-3 mg/m3 8-hour TWA and up to 11 mg/m3 STE) of hydrogen peroxide vapour in aseptic packaging (Riihimäki et al., 2002) indicated that half of the group had developed sustained airway irritation and inflammation, increased susceptibility to respiratory infections, and other symptoms, which were cleared after the exposures were strongly reduced. In one study on human volunteers exposed to low concentrations of hydrogen peroxide (Ernstgård et al., 2012) subtle acute respiratory effects were found after 2 hours of inhalation exposure to 2.2 ppm levels. The non-effect level was 0.5 ppm.

10.3.2 Comparison with the CLP criteria

Animal data on inhalation toxicity is limited and there are no new studies after the classification for acute inhalation toxicity was agreed by the TC C&L group in the early 2000 and finally included in the 29th ATP to the Directive 67/548/EEC. It was agreed then to classify hydrogen peroxide as Xn; R20 (Harmful by inhalation) with specific concentration limit C ≥ 50%.

No direct translation to a CLP classification can be made because of different cut off limits in the different legislations, and thefore a minimum classification of Acute Tox 4* was given according to CLP Regulation. If following the logic of the classification given under the DSD, hydrogen peroxide is classified for acute inhalation toxicity at concentration at or above 50 %, therefore the ATE according to the CLP regulation should be at least 10 mg/l for vapours. Since the relevant animal studies (Anonymous 1990b, Comstock et al., 1954) did not provide LC_{50} values therefore the ATE value should be based on the hazard category. According to criteria in CLP, ATE of 10 mg/l is at the

upper limit of category 3 and at the lower limit of category 4. Since ATE-value of smaller than 10 would mean that hydrogen peroxide concentrations less than 50% would be classified as category 4 and thus would not follow the logic under the Directive, it is suggested to confirm classification as category 4.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Classification as Acute inhalation toxicity category 4; H332 for 100% hydrogen peroxide is proposed and the asterisk denoting minimum classification removed. An ATP value of 11 mg/l based on the converted point estimate for category 4 for vapours is suggested.

10.4 Skin corrosion/irritation

Not assessed in this dossier.

10.5 Serious eye damage/eye irritation

Not assessed in this dossier.

10.6 Respiratory sensitisation

Not assessed in this dossier.

10.7 Skin sensitisation

Not assessed in this dossier.

10.8 Germ cell mutagenicity

Summary tables of mutagenicity/genotoxicity tests *in vitro* and *in vivo*

Table 17: Mutagenicity (gene mutation assays (in vitro), bacteria and yeasts)

Species	Strain	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Salmonella typhimurium	TA97 TA102 TA104 SB1111 SB1106 SB1106p	ames test - plate incorporation assay - preincubation	H_2O_2 doses: 1, 2 or 4 µM/plate doses: 0.3, 0.6 or 1.2 µM/plate	* TA97 + TA102 + TA104 * SB1111 * SB1106 + SB1106p	NT	Abu-Shakra and Zeiger (1990)
Salmonella typhimurium	TA102	ames test - a liquid incubation assay	H_2O_2 concentrations: 0 or 400µM (without Na_2S) concentrations: 0, 40 or 50 µM (with 100 µM Na_2S)	+	NT	Carlsson et al. (1988)
Salmonella typhimurium	TA97 TA102	ames test	H_2O_2 concentrations: no data	*	*	De Flora et al. (1984)
Salmonella typhimurium	TA92 TA97 TA100 TA102 TA104 TA1535 TA1537	ames test - standard plate incorporation assay	H_2O_2 doses: 0, 0.15, 0.30, 0.60, 1.20 or 2.40 µM/plate	- TA92 - TA97 - TA100 + TA102 + TA104 - TA1535 - TA1537	NT	Glatt (1989)
Salmonella typhimurium	TA92 TA94 TA98 TA100 TA1535 TA1537	ames test - preincubation	H_2O_2 doses: 0.2 mg/plate (max)	- TA92 - TA94 - TA98 + TA100 - TA1535 - TA1537	NT	Ishidate et al. (1984)

Species	Strain	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Salmonella typhimurium	TA97 TA102 TA104 SB1111 SB1106 SB1106p	ames test - plate incorporation assay - preincubation	H_2O_2 doses: 1, 2 or 4 µM/plate doses: 0.3, 0.6 or 1.2 µM/plate	* TA97 + TA102 + TA104 * SB1111 * SB1106 + SB1106p	NT	Abu-Shakra and Zeiger (1990)
Salmonella typhimurium	TA97 TA98 TA100 TA102 TA1537 TA1538	ames test - standard plate incorporation assay - preincubation assay - liquid incubation assay	H_2O_2 concentrations: up to 6 mM concentrations: up to 340 µM concentrations: up to 4.5 µM	+ TA97 + TA98 - TA100 + TA102 + TA1537 - TA1538	- TA97 - TA98 - TA100 - TA102 - TA1537 - TA1538	Kensese and Smith (1989)
Salmonella typhimurium	TA98 TA100 TA1535 TA1537 TA1538	ames test	H_2O_2 doses (-S9): 0.0033 - 0.67 mg/plate (TA98, TA1535, TA1538), 0.001 - 0.33 mg/plate (TA100, TA1537) doses (+S9): 0.010 - 3.3 mg/plate (all five TA strains)	- TA98 + TA100 - TA1535 - TA1537 - TA1538	- TA98 + TA100 - TA1535 - TA1537 - TA1538	Prival et al. (1991)
Salmonella typhimurium	BA9 BA13	bacterial forward mutation - l-arabinose forward mutation test (l-arabinose resistant)	H_2O_2 concentrations: 2941, 5882, 11765 or 17647 nM/ml	+	NT	Ruiz-Rubio et al. (1985)

Species	Strain	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Salmonella typhimurium	TA97 TA102 TA104 SB1111 SB1106 SB1106p	ames test - plate incorporation assay - preincubation	H_2O_2 doses: 1, 2 or 4 µM/plate doses: 0.3, 0.6 or 1.2 µM/plate	* TA97 + TA102 + TA104 * SB1111 * SB1106 + SB1106p	NT	Abu-Shakra and Zeiger (1990)
Salmonella typhimurium	TA100	ames test	H_2O_2 doses: 0.5, 1.0, 1.5, 3.0, 4.5 or 7.5 µM/plate	*	NT	Winquist et al. (1984)
Salmonella typhimurium	TA102	ames test - plate incorporation assay	H_2O_2 doses: 0, 50, 75, 100, 150, 175, 200 or 300 µg/plate	+	NT	Wilcox et al. (1990)
Salmonella typhimurium	TA102 TA2638	ames test	H_2O_2 dose: 100 µg/plate	+ TA102 + TA2638	NT	Levin et al. (1982)
Salmonella typhimurium	TA98 TA100 TA1535 TA1537 TA1538	ames test	H_2O_2 doses 1.0-3333.3 µg/plate (-S9 & +S9)	+ TA100 - TA1535 - TA1537 - TA1538	- TA98 + TA100 - TA1535 - TA1537 - TA1538	SRI International (1980)
Salmonella typhimurium	TA98 TA100	ames test	H_2O_2 concentration not given, with or without Cu^{2+} (10^{-5} M)	* TA98 * TA100	NT	Stich et al. (1978)
Escherichia coli	WP2 (uvrA)	ames test	H_2O_2 doses 1.0-3333.3 µg/plate (-S9 & +S9)	-	-	SRI International (1980)

Species	Strain	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Salmonella typhimurium	TA97 TA102 TA104 SB1111 SB1106 SB1106p	ames test - plate incorporation assay - preincubation	H_2O_2 doses: 1, 2 or 4 µM/plate doses: 0.3, 0.6 or 1.2 µM/plate	* TA97 + TA102 + TA104 * SB1111 * SB1106 + SB1106p	NT	Abu-Shakra and Zeiger (1990)
Escherichia coli	WP2 uvrA (pKM101) WP2 (pKM101)	Escherichia coli reverse mutation	H_2O_2 doses: 0, 50, 75, 100, 150, 175, 200 or 300 µg/plate	+	NT	Wilcox et al. (1990)
Escherichia coli	K12 (katG, katE, katF)	bacterial forward mutation assay (catalase deficient strains) - L-arabinose resistance	H_2O_2 dose levels up to 900 nM/plate	+	NT	Abril and Pueyo (1990)
Escherichia coli	DB2	bacterial forward mutation assay - ampicillin-resistance, preincubation	H_2O_2 concentrations: 0, 20/24, 40, 60 or 80 µg/ml	+	NT	Bosworth et al. (1987)
Escherichia coli	WP2	Escherichia coli reverse mutation	H_2O_2 doses (-S9): 0.033 - 3.3 mg/plate doses (+S9): 0.0010 - 30 mg/plate	-	-	Prival et al. (1991)
Bacillus subtilis		bacterial forward mutation - multigene sporulation test	H_2O_2 concentrations: 0.0005, 0.001, 0.002 or 0.003%	+	NT	Sacks and MacGregor (1982)

Species	Strain	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Salmonella typhimurium	TA97 TA102 TA104 SB1111 SB1106 SB1106p	ames test - plate incorporation assay - preincubation	H_2O_2 doses: 1, 2 or 4 µM/plate doses: 0.3, 0.6 or 1.2 µM/plate	* TA97 + TA102 + TA104 * SB1111 * SB1106 + SB1106p	NT	Abu-Shakra and Zeiger (1990)
Saccharo-myces cerevisiae	ade2 (induction of respiratory deficient nt mutations)	Yeast gene mutation	H_2O_2 concentration: 2 mg/ml	*	NT	Thacker and Parker (1976)

Table 18: Mutagenicity (genetic toxicity (in vitro), bacterial DNA damage and repair)

Species	Strain	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Salmonella typhimurium	TA1535/pSK1002	DNA damage and repair	H_2O_2 concentration: 45 µg/ml	+	NT	Nakamura et al. (1987)
Escherichia coli	PQ37	DNA damage and repair assay (SOS chromotest)	H_2O_2 concentrations: 0, 5, 10, 20, 50, 100, 200 or 500 µM	+	NT	Zhou et al. (1991)
Escherichia coli	WP2 WP67 CM871	DNA damage and repair	H_2O_2 concentrations: no data	+ WP2 + WP67 + CM871	+ WP2 + WP67 + CM871	De Flora et al. (1984)
Escherichia coli	WP2	DNA damage and repair - lambda prophage induction	H_2O_2 doses: 0.78 - 100 µg/well	+	NT	Rosman et al. (1991)
Escherichia coli	PQ37	DNA damage and repair (SOS chromotest)	H_2O_2 concentrations: 0, 0.1, 0.3 or 1.0 mM	+	NT	von der Hude et al. (1988)

Table 19: Mutagenicity (mammalian cell gene mutation assays (in vitro))

Species	Strain/ cells	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Chinese hamster	CHO cells clone K1-BH4, Transformat AS52	HGPRT (GPT assay)	H_2O_2 concentrations: 0, 0.2 or 0.4 µM	+	NT	Hsie et al. (1993)
Chinese hamster	V-79 cells	HGPRT	H_2O_2 concentrations: 10, 20, 30 or 40 µM	+	NT	Nassi-Calò et al. (1989)
Chinese hamster	V-79 cells, CHO cells	HGPRT	H_2O_2 concentrations: 0, 10, 20, 40, 60 or 80 µM	*	NT	Speit (1986)
Chinese hamster	V-79 cells	HGPRT	H_2O_2 concentrations: 27.5-585 µM	-	NT	Bradley and Erickson (1981)
Murine leukaemic lympho-blasts	L5178Y-S (LY-S) L5178 (LY-R)	HGPRT	H_2O_2 concentrations: 03 - 5.0 µM	+	NT	Kruszewski Szumiel (1993)
Chinese hamster	V-79 cells	mammalian cell gene mutation - (6-thioguanine resistance)	H_2O_2 concentrations: 353 µM	-	NT	Bradley et al. (1979)
Chinese hamster	V-79 CHC	mammalian cell gene mutation -(8-azaguanine and ouabain resistance)	H_2O_2 concentrations: 0, 0.1, 0.2, 0.3, 0.5 or 1.0 mM	-	NT	Tsuda (1981)

Species	Strain/ cells	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Chinese hamster	V-79 cells	mammalian cell gene mutation -(6-thioguanine resistance (Tgr clones)	H_2O_2 concentrations: 0.5 - 4.0 mM	+	NT	Ziegler-Skylakakis and Andrae (1987)
African green monkey	kidney cells (CV-1)	Mammalian cell gene mutation -(supF locus of the pZ189 plasmid mutations)	H_2O_2 concentrations: 0.5 - 10 mM	+	NT	Moraes et al. (1990)
Mouse	L5178Y lymphoma cells	TK-locus assay	H_2O_2 without S9: 0.0018 – 0.1 µg/ml (15 dose levels) with S9: 2.3 – 30 µg/ml (10 dose levels)	+	-	Procter & Gamble (1986)
Mouse	L5178Y lymphoma cells	TK-locus forward mutation	H_2O_2 concentrations: 18.6, 37.2, 79.5, 199.0 or496 µM	+	NT	Wangenheim and Bolcsfoldi (1988)

Table 20: Mutagenicity (genetic toxicity (in vitro), mammalian cell DNA damage and repair)

Species	Strain/ cells	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Chinese hamster	V79, lung fibroblasts	DNA damage and repair degradation of cccDNA	H_2O_2 concentrations: 5 µl of 2M H2O2	+	NT	Tachon and Giacomoni (1989)
Chinese hamster	V-79 cells	DNA damage and repair	H_2O_2 concentrations: 353 µM	+	NT	Bradley et al. (1979)

Species	Strain/ cells	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Chinese hamster	V79-379A cells	DNA damage and repair - DNA single- and double-strand breaks	H_2O_2 concentrations: 10–1,000 µM	+	NT	Prise et al. (1989)
Chinese hamster	V79 fibroblasts	DNA damage and repair	H_2O_2 concentrations: 200 or 300 µM	+	NT	Mello Filho and Meneghini (1984)
Mouse	lymphoma cells (L5178/ TK+/-)	DNA damage and repair - DNA single- and double-strand breaks	H_2O_2 concentrations: 0, 200, 218, 233 or 251 µM	+	NT	Garberg et al. (1988)
Mouse	mouse-mouse hybridoma cell line HyHEL-10	DNA damage and repair	H_2O_2 concentrations: 0, 5, 15 or 40 µM	+	NT	Cacciuttolo et al. (1993)
Murine	$P388D_1$ murine macrophages	DNA damage and repair	H_2O_2 concentrations: 0–1,000 µM	+	NT	Schraufstatter et al. (1986)
Rat	hepatocytes	DNA damage and repair	H_2O_2 concentrations: 100–100,000 µM	+	NT	Beales and Suter (1989)
Rat	hepatocytes	DNA damage and repair - (DNA single- and double-strand breaks)	H_2O_2 concentrations: 0, 10, 50, 100, 200, 500 or 1,000 µM	*	NT	Olson (1988)
Rat	hepatocytes	unscheduled DNA synthesis	H_2O_2 concentrations: 1,900-3,200 µM	+	NT	Cattley and Smith-Oliver (1988)

Species	Strain/ cells	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Rat	hepatocytes	unscheduled DNA synthesis	H_2O_2 concentrations: first experiment : 0, 10, 30, 100, 300, 1,000 or 3,000 µg/ml Repeat experiment: 0.78125, 1.5625, 3.125, 6.25, 12.5, 25, 50 or 100 µg/ml	- +	NT NT	CEFIC (1997b)
Bovine	lens epithelial cells	DNA damage and repair - DNA single-strand breaks (alkaline and neutral filter elution)	H_2O_2 concentrations: 10-200 µM	*	NT	Kleiman et al. (1990)
Human	mono-nuclear leucocytes	DNA damage and repair - DNA single-strand breaks (nucleoid sedimentation technique)	H_2O_2 concentrations: 12-100 µM	+	NT	Van Rensburg et al. (1992)
Human	diploid fetal lung cells (WI-38 CCL75)	DNA damage and repair	H_2O_2 concentrations: 0, 0.15, 0.6, 2.3, 9.4, 37.5, 150 or 600 µg/ml	+	NT	Coppinger et al. (1983)
Human	peripherial lymphocytes	DNA damage and repair	H_2O_2 concentrations: 0-1,000 µM	+	NT	Schraufstatter et al. (1986)
Human	fibroblasts (strain N1)	DNA damage and repair	H_2O_2, % not given concentrations: 28-300 µM	+	NT	Mello Filho and Meneghini (1984)
Human	SV40 transformed fibroblast cell line (VA13)	DNA damage and repair	H_2O_2 concentration: 2.8 µm	+	NT	Mello Filho and Meneghini (1984)

Table 21: Mutagenicity (genetic toxicity (in vitro) mammalian cell unscheduled DNA synthesis)

Species	Strain/ cells	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Human	diploid fetal lung cells (WI-38 CCL75)	unscheduled DNA synthesis	H_2O_2 concentrations: 0, 0.6, 2.4, 9.0, 36, 150, 600 or 2400 µg/ml	+	NT	Coppinger et al. (1983)

Human	Full thickness skin models, i.e., in vitro reconstructed skin which consists of a 3D structured dermal equivalent with human fibroblasts overlaid by a stratified, well differentiated epidermis derived from normal human keratinocytes	comet assay	0, 25, 50, 100, 200, 400 µg/cm^2 Positive control: MMS 5 µg/cm^2	- Since no statistically significant increase in % Tail DNA was observed in either the epidermis or the dermis after dosing with hydrogen peroxide, a confirmatory test was performed wherein tissues were also co-exposed to aphidicolin (APC), which is known to induce accumulation of DNA strand breaks for certain compounds and was therefore included in the standard procedure for this test to amplify comet formation. The result remained negative even with the addition of APC.	Not applicable	CEFIC Peroxygens, 2022

Table 22: Mutagenicity (genetic toxicity (in vitro), sister chromatid exchange)

Species	Strain/ cells	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Chinese hamster	V-79 cells, CHO cells	SCE	H_2O_2 concentrations: 0, 10, 20, 40, 60 or 80 µM	+	NT	Speit et al. (1982)
Chinese hamster	V-79	SCE	H_2O_2 concentrations: 10-20 µM	+	NT	Tachon (1990)
Chinese hamster	CHO	SCE	H_2O_2 concentrations: 0.31-130 µM (24h exp) 5-100 µM (3h exp)	+	NT	MacRae and Stich (1979)
Chinese hamster	CHO-AUXB1	SCE	H_2O_2 concentrations: 0, 40, 80, 120, 160, 200 or 240 µM	+	NT	Tucker et al. (1989)
Chinese hamster	V-79	SCE	H_2O_2 concentrations: 353 µM	+	NT	Bradley et al. (1979)
Chinese hamster	V79 cells CHO cells	SCE	H_2O_2 concentrations: 0, 10, 20 or 40 µM	+	- (V79) * (CHO)	Mehnert et al. (1984a)
Chinese hamster	CHO Don-6)	SCE	H_2O_2 concentrations: 0, 0.5, 1 or 2 mM (0, 0.017, 0.034 or 0.068 mg/ml)	+	NT	Sasaki et al. (1980)
Chinese hamster	CHO	SCE	H_2O_2 concentrations: 0.5, 1, 10 or 100 mM	+[1)]	NT	Wilmer and Natarajan (1981)

Species	Strain/ cells	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Human	D98/AH 2 cells (a HeLa variant)	SCE	H_2O_2 concentration added or generated as a photoproduct: 1.3 - 2 µg/ml	+	NT	Estervig and Wang (1984)
Human	WBC (whole blood culture, PLC (purified lymphocyte culture)	SCE	H_2O_2 concentrations: 20-2000 µM	- WBC + PLC	- WBC + PLC (reduced)	Mehnert et al. (1984b)

Species	Strain/ cells	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Human	comet assay was used to assess the extent of DNA damage by H2O2 in HepG2 cells	HepG2 cells	25 µM and 50 µM H2O2, incubated for 5 min, 30 min, 40 min, 1h, and 24 h in parallel	DNA damage in HepG2 cells was dose and duration dependent. The DNA damage increased after 1h of incubation with 25 µM and 50 µM H2O2 and later decreased (24 h), likely due to metabolism of H2O2 and DNA repairs mechanisms. The cells were susceptible to DNA damage by low doses of the test substance H2O2.		Benhusein et al., 2010

Species	Strain/ cells	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Rat	Liver	DNA Damage and Repair, Unscheduled DNA Synthesis (OECD 482)	First experiment: 0, 10, 30, 100, 300, 1000, and 3000 µg/mL Repeat experiment: 0.78125, 1.5625, 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL	Positive	NT	CEFIC, 1997

Table 23: Mutagenicity (genetic toxicity (in vitro), cytogenetic assays)

Species	Strain/ cells	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Chinese hamster	CHL (R-8) parental cells	chromosomal aberrations	H_2O_2 concentrations: 56 µg/ml (R-8), 6 µg/ml (parental)	+	NT	Sawada et al. (1988)
Chinese hamster	CHO	chromosomal aberrations	H_2O_2 concentrations: not given	+	NT	Stich et al. (1978)
Chinese hamster	CHO	chromosomal aberrations	H_2O_2, % not given concentrations: without S9: 25.31, 33.75 or 45.00 nl/ml with S9: 10, 50 or 100 µl/ml	+	+	Procter & Gamble (1985)
Chinese hamster	fibroblasts	chromosomal aberrations	H_2O_2 concentrations: 0.25 mg/ml	+	NT	Ishidate et al. (1984)

Species	Strain/ cells	Measured endpoint	Test conditions	Results without activation	Results with activation	Reference
Chinese hamster	CHO	choromosomal aberrations (CA) chromatid translocations (CT) micronuclei (M)	H_2O_2 concentrations: 0-25 µmol	+ CA + CT + M	NT	Stich and Dunn (1986)
Chinese hamster Syrian hamster Mouse	CHO-K1 V-79 and CHC cells BALB/c newborn mouse back skin cells	chromosomal aberrations	H_2O_2 concentrations: 0, 0.1, 0.2, 0.3, 0.5 or 1.0 mM	+	NT	Tsuda (1981)
Mouse (C57BL/6J)	splenocytes	micronucleus	H_2O_2 concentrations: 0, 10 or 20 µM	*	NT	Dreosti et al. (1990)
Chinese hamster	V79 cells	micronucleus	H_2O_2 concentrations: 10–20 µM	+	NT	Tachon (1990)
Human	D98/AH2 cells (variant of HeLa)	chromosome aberrations	H_2O_2 0–3 µg/ml	- (H_2O_2 only) + (H_2O_2 generated as a photo-product)	NT	Estervig and Wang (1984)
Human	embryonic fibroblasts	chromosomal aberrations	H_2O_2 concentrations: 10-1,000 µM	+	NT	Oya et al. (1986)

Table 24: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, species and strain	Type of study Measured endpoint	Exposure data Test conditions	Results	Remarks	Reference
Mouse Swiss HIM/OG1	Host mediated assay with intraperitoneally inoculated *Salmonella typhimurium* strains TA 1530,	Dosing: 0.003, 0.3, 3% H2O2 in milk for one week 0.5 mL 0.3% H2O2 twice by gavage with a 2	- H_2O_2 in milk + pure H_2O_2	a strong positive response for TA1530, a weak one for G46	Keck et al. (1980)

	G46	h interval			
Mouse inbread strain AB Lena Gat.	cytogenetic assay with intraperitoneally inoculated tumour cells (S2 sarcoma, Ehrlich ascites, sarcoma 180)	dosing: 1 mL of 0.01, 0.05, 0.1, 0.5 M H_2O_2 i.p. 48 hours after THE implantation of the tumour cells. chromosomes were studied 48 h after the treatment	increased chromatid aberrations	Local effect; response presumed to depend on the presence or absence of RBs.	Schöneich (1967)
Mouse strain unknown	micronucleus assay of bone marrow polychromatic erythrocytes	Single intraperitoneal injection of ½, 1/5, 1/25, 1/100 LD_{50} dose of H_2O_2	negative	No experimental details given	Liarskii (1983)
Dropsophila melanogaster	dropsophila SLRL test	Single dose of 3% H_2O_2 injected into the male larvae	negative		Di Paolo (1952)
Mouse Sencar	Female pre-screen for carcinogenicity in target tissue (mouse skin) quantity of 8-OH-2'-deoxyguanosine (DNA damage) mutations of codon 61 of c-Ha-ras gene epidermal hyperplasia and dermal cellularity changes	Hydrogen peroxide 70% was applied to skin of 10 female Sencar mice per dose group at dose levels of 10, 100, 200 µmol in 200 µL of ethanol (i.e.0.2-3.2% solution) twice weekly for 4 weeks. Mice treated under the same conditions with DMBA (10 or 100 µmol/animal) or ethanol (200 µL) acted as positive and negative controls, respectively. The animals were killed on days 2 or 4 after the last administration (5 mice on each	negative for all endpoints	At the relatively low concentrations used hydrogen peroxide did not induce local in vivo genotoxicity and mutagenicity in the skin	Society for plastic industry (1997)

		day). The application sites were removed and after fixation and staining, epithelial and dermal thickness and dermal cellularity were determined visually by light microscopy. Non-phenol extraction of fresh frozen tissue was used to isolate DNA from animals killed 2 days after last dosling, and following digestion to nucleosides, 8-OH-2'-deoxyguanosine (8-OH-dG) was quantified by HPLC: mutations in codon 61 of c-Ha-ras gene were determined using DNA isolated from paraffin blocks of whole skin.			
OECD No. 474, GLP, Micronucleus test, Swiss OF1 mice, 5 females/ 5 males/dose	in vivo mammalian somatic cell study: cytogenicity/ erythrocyte micronucleus	0, 500, 1000, 2000 mg/kg body weight, 35% H2O2	Negative	The test substance was administered once by intraperitoneal route using a dose volume of 25 mL/kg, which allowed to test higher doses with less concentrated solutions. The quantitiy of the test substance administered to each animal was adjusted according to the body weight recorded at the time of dosing. The vehicle control animals received the vehicle alone, under the same conditions. The positive control animals received cyclophosphamide, by	Molinier, 1995

				oral routed, at a volume of 10 mL/kg.	
Human volunteers, 30 participants, The aim of this study was to evaluate the genotoxicity of in-office bleaching with 35% hydrogen peroxide in epithelial cells from the gingival and lip tissues.	Clinical evaluation of genotoxicity of in-office bleaching	The 35% hydrogen peroxide gel (Whiteness HP Maxx, FGM) was used during three 15-minute applications over the course of the 45-minute application period Frequency of treatment Two bleaching sessions within a one-week interval were performed on each patient. Post exposure period 1 month	Negative	A lip and cheek retractor was placed in the participant's mouth to avoid the contact of the bleaching gel with the cheek, lips, and tongue. Then the gingival tissue of the teeth to be bleached was isolated from the bleaching agent using a light-polymerized resin dam. In every two teeth, the lightcured gingival barrier was activated for 20 seconds using a LED light-curing unit. At least 1000 cells from each participant were evaluated for micronulei	Rezende, 2016
OECD Guideline for testing of Chemicals No. 486. The study was performed under GLP conditions, Wistar rats	In vivo mammalian cell study: DNA damage and/or repair Type of genotoxicity: DNA damage and/or repair	35 % solution, intravenous infusion, 5 to 6 rats/dose, 25-33 minutes (at a dose rate of 0.2 mL/min, Doses / Concentrations: 0, 25 or 50 mg/kg	Negative	Details on exposure Dosing preparations were made by diluting hydrogen peroxide in water for injection. Dilutions were made using water for injection. The test article preparations were protected from light, stood on ice prior to use and were used within approximately 4 hours of initial formulation. Animals were administered the appropriate concentration dosing solution at a dose rate of 0.2 mL/min, to a total administered dose volue of approximately 25 mL/kg.	Clare, 1997
OECD Guideline for Testing of Chemicals No. 474, GLP, mouse, C57BL/6NCr1BR	In vivo mammalian somatic cell study: cytogenicity / erythrocyte micronucleus	0, 200, 1000, 3000 or 6000 ppm (male: 0, 42.4, 164, 415 or 536 mg/kg bw/day) (female: 0, 48.5, 198, 485 or 774 mg/kg bw/day)	Negative	GI tract was examined for increases in micronucleated PCE and decrease in polychromatic/normochromatic erythrocytes	Sarver, 1995

		for 14 days, 0 males and 10 females, drinking water			
Micronucleus assay, mice	In vivo mammalian somatic cell study: cytogenicity	½, 1/5, 1/25 or 1/100 LD50, intraperitoneal	Negative		Liarskii, 1983
Genotoxic Effect of Two Bleaching Agents on Oral Mucosa	In vivo mammalian somatic cell study: cytogenicity	25 or 38 % hydrogen peroxide, 11 volunteers per dose	Negative	In the first group, the retractor was set in place, the teeth were dried by air stream and the gums were isolated by protective Liquidam gel (Discus Dental) which was illuminated by the polymerization unit (Bluephase; Ivoclar-Vivadent). A 1 to 2 mm-thick layer of bleaching ZOOM2 gel was applied to the labial surfaces of teeth 14-24 and 34-44 using the brush from the original package. Teeth were illuminated by the light source for 15 min. The application of the gel was repeated three times, each application lasting for 15 min. Upon completion of bleaching, the protective gel and retractor were removed and the mouth was rinsed with water. Cell samples from gums and the upper lip lining were collected once again. Participants were scheduled for an appointment in three days, during which the final cell samples from gums and upper lip lining were collected. Samples were obtained in the same manner for the second group treated with Opalesence BOOST gel except that the gums were isolated by protective Opaldam gel (Ultradent Products) and the gel was chemically-, not light-activated. Treatments were performed in the morning at the Department of Endodontics and Restorative Dentistry School of Dental Medicine from February until May.	Klaric, 2013

Dental bleaching	Micronucleus assay	10 or 16% carbamide peroxide	Negative	Thirty-seven patients were divided into two groups and randomly received either a 10% carbamide peroxide (CP) or a 16% carbamide peroxide concentration for 21 days in individual dental trays. Gingival margin cells were collected immediately before the first use (baseline), and then 15 and 45 days after baseline.	Almeida, 2015
Dental bleaching	Micronucleus assay	10% carbamide peroxide	Negative	A tray and 10% carbamide peroxide gel were delivered to each subject (30 smokers (experimental group) and 30 nonsmokers (control group)), Exfoliated oral mucosa was collected before and immediately after the third week of at-home bleaching. The slides were scored for micronuclei. The sample size calculation was based on the frequency of MN per 1000 cells in nonsmokers.	DeGeus, 2015

- negative result; + positive result

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

In vitro

Hydrogen peroxide is consistently positive in various Salmonella typhimurium strains and in E. coli strains. In the Ames test especially the strains sensitive to oxygen radicals), and DNA damage and repair assays have yielded positive results. With mammalian cells, positive results were mostly observed in gene mutation assays, DNA damage and repair assays, UDS assays, SCE assays, and cytogenetic assays for chromosomal aberrations. Positive results have been reported in several mammalian cell mutagenicity assays such as HGPRT and TK-locus mutation assays. In vitro genetic toxicity assays investigating mammalian cell DNA damage and repair show consistently positive results in various assays measuring DNA damage and repair. Genetic toxicity mammalian cell unscheduled DNA synthesis and sister chromatid assays all show positive results in vitro. Likewise, in vitro assays investigating chromosome aberrations are predominantly positive (chromosomal aberration or micronucleus assays).

In vivo

Micronucleus test in mice, intraperitoneal Molinier B, 1995

Materials and Methods

The potential of hydrogen peroxide to induce cytogenetic damage to the bone marrow cells of Swiss OF1 mice was tested in a micronucleus assay in accordance with OECD Guideline No. 474 and under GLP conditions. Preliminary toxicity tests were performed to define the doses to be used in the cytogenetic test. Six groups of 5 male and 5 female mice received the test substance by a single intraperitoneal injection at doses of 250, 500 and 1000 mg/kg. Two groups of 5 males and 5 females received the vehicle (water) alone. One group of 5 males and 5 females was treated with the positive control substance cyclophosphamide administered by a single oral dose of 50 mg/kg. For each animal, bone marrow cell smears were prepared and the micronuclei were counted in 2000 polychromatic erythrocytes. The polychromatic (PE) to normochromatic (NE) erythrocyte ratio was established by scoring 1000 erythrocytes (PE + NE). In the two vehicle control groups, the mean values of micronucleated polychromatic erythrocytes (MPE) were in the range of historical controls.

Results
Cyclophosphamide induced a highly significant increase in the number of MPE and significantly decreased the PE/NE ratio, indicating the cytotoxicity of the control substance. In all groups treated with hydrogen peroxide, the mean values of MPE were similar to those of their respective vehicle controls. A slight statistically significant increase in the MPE number in the low-dose group after 24 hours was considered as biologically insignificant. A statistically significant decrease in the PE/NE ratio in most treated groups after 24 and 48 hours showed that hydrogen peroxide effectively affected the bone marrow cells. It was concluded from the findings that under the experimental conditions hydrogen peroxide did not induce cytogenetic damage in bone marrow cells of mice when administered by the intraperitoneal route.

Conclusion
First clinical signs and death appeared at 1000 mg/kg bw. At 2000 mg/kg bw marked mortality in male and female mice was found. In all groups treated with hydrogen peroxide, the mean of MPE were similar to those of their respective vehicle groups. Considering the result of the PE/NE ratio the test substance was shown to affect the bone marrow.
The frequency of micronucleated polychromatic erythocytes was not increased in mice treated intraperitoneally with 250, 500 and 1000 mg/kg bw hydrogen peroxide.

Mouse bone marrow micronucleus study (OECD 474) Sarver, 1995

Materials and Methods
The genetic toxicity of hydrogen peroxide was tested in a valid mammalian erythrocyte micronucleus assay using male and female C57BL/6NCr1BR mice in accordance with OECD Guideline No. 474 and GLP. The animals received the test substance ad libitum via drinking water at nominal concentrations of 0, 200, 1000, 3000 or 6000 ppm (male: 0, 42.4, 164, 415 or 536 mg/kg bw/day) (female: 0, 48.5, 198, 485 or 774 mg/kg bw/day) for 14 days. Positive control animals received a single intraperitoneal injectional of 20 mg/kg cyclophosphamide on day 13 of the study.

Results
No specific gross findings were attributable to exposure to hydrogen peroxide. Microscopical findings of degenerative and regenerative alterations in the mucosa of the stomach and/or the duodenum were present in the 3000 and 6000 ppm groups and considered to be substance related. No statistically significant increases in the frequency of micronucleated PCEs were observed in the 6000 ppm dose group, neither was any decreased ratio polychromatic/normochromatic erythrocytes noted. Animals receiving cyclophosphamide responded as expected.

Conclusion
Hydrogen peroxide did not show any genotoxic effects at the tested concentrations.

Mouse erythrocyte micronucleus study, intraperitoneal Liarskii, 1983

In a review of hydrogen peroxide toxicology, the authors cite their own micronucleus study with mice using single intraperitoneal injections of hydrogen peroxide. The authors state that the study did not reveal any mutagenic effects on bone marrow reticulocytes. The study cannot be assessed due to lack of all experimental details.

Micronucleus study, human volunteers Rezende et al., 2016

The aim of this study was to evaluate the genotoxicity of in-office bleaching with 35% hydrogen peroxide in epithelial cells from the gingival and lip tissues.

Materials and Methods
Thirty volunteers with central incisors shade A1 or darker were selected for this study. The gingival tissue of the teeth to be bleached was isolated with a light-polymerized resin dam, and the 35% hydrogen peroxide gel was administered during three 15-minute applications over the course of the 45-minute application period. Two bleaching sessions with a one-week interval in between were performed. Exfoliated oral mucosa gingival epithelial cells and upper lip lining were collected at baseline and one month after the in-office dental bleaching. The scraped cells were placed on clean glass slides and smears were prepared. After staining with Giemsa solution, two blinded examiners performed cell and micronuclei counts under a 100x optical microscope.

Results

The frequency of MN was not increased after bleaching with 35% hydrogen peroxide in both study groups (p.0.05).

Conclusions

The in-office bleaching did not induce DNA damage to the gingival and lip tissue during the bleaching period.

Chromosomal aberration study in inoculated tumours Schöneich, 1967

Materials and Methods
The study was not a conventional in vivo study, since tumors (S2 sarcoma, Ehrlich ascites, or sarcoma 180) were inoculated into the peritoneal cavity of the mice. Hydrogen peroxide (1 ml of 0.01-0.5 M hydrogen peroxide) was injected 48 h after the implantation of the tumour. 48 h after treatments of the animals with hydrogen peroxide, the induction of chromosomal aberrations were studied in the inoculated tumors.

Results
Chromatid aberrations were increased with a pronounced variation in response.

Conclusions
Ascites tumour cells had extremely low catalase levels; the variation was presumed to depend on the varied amounts of red blood cells.

Rat liver UDS Clare, 1997

Materials and Methods
The genotoxicity potential of hydrogen peroxide was tested in an Unscheduled DNA Synthesis assay in rat liver according to OECD Guideline No. 486 and under GLP conditions.

Male Wistar rats were administered intravenously the test solutions at concentrations of 1 or 2 mg/mL at a dose rate of 0.2 mL/min to achieve final doses of 25 mg/kg or 50 mg/kg. Animals were sacrificed either after 2 -4 hours or 12 -14 hours. In the 2 -4 hour experiment, dimethylnitrosamine (DMN) was used as the positive control substance and in the 12 -14 hour experiment, the positive control substances was 2 -acetamidfluorene (2 -AAF). Negative control animals were administered water only. After sacrifice, hepatocytes from the liver were sampled and cultures of hepatocytes were treated with [3H]thymidine. Slides were prepared from each animals with fixed hepatocytes and dipped in photographic emulsion to prepare autoradiograms. Slides were examined microscopically and the number of grains present in the nucleus minus the mean number of grains in three equivalent areas of cytoplasm was determined (the net grain count, NNG).

Results
Negative vehicle controls gave a group mean NNG value of less than zero with 0 -0.3 % cells in repair. Group mean NNG values were increased by 2 -AAF and DMN treatment to at least 9.4 and more than 80 % of cells were found to be in repair. In vivo treatment with 25 or 50 mg/kg hydrogen peroxide did not produce a group mean NNG value greatern than zero (-2.1- -2.7 respectively) nor were any more than 0.7 % cells found in repair at either dose or time point.

Conclusion
It was concluded that hydrogen peroxide failed to induce unscheduled DNA synthesis following treatment in vivo, detected under the experimental conditions employed.

Human volunteer study of dermal genotoxicity De Geus et al., 2015

A tray and 10% carbamide peroxide gel were delivered to each subject (30 smokers (experimental group) and 30 nonsmokers (control group)), with verbal instructions for use. All subjects were instructed to wear the tray containing the bleaching agent for at least three hours a day for a period of three weeks. After the daily three-hour period, they were instructed to remove the tray, wash it with water, and brush their teeth as usual.
Exfoliated oral mucosa was collected before and immediately after the third week of at-home bleaching. Before cell collection, the participants rinsed their mouths with tap water for one minute. Subsequently, the cells were scraped with wooden spatulas from the marginal gingiva. The scraped cells were placed on clean glass slides, and smears were prepared. The smear was dried with a jet of air from a triple syringe for one minute at a distance of approximately 30 cm, avoiding excessive dehydration of the cells. The slides were scored for micronuclei. The sample size calculation was based on the frequency of MN per 1000 cells in nonsmokers.

Results
The frequency of MN was significantly higher in the experimental group than in the control group, regardless of the bleaching treatment (p.0.001).

Conclusion

The results of this study indicate that 10% carbamide peroxide gel did not induce DNA damage in gingival tissue during the evaluated period.

Human volunteer study, dermal genotoxicity Klaric et al., 2013

The aim of the study was to analyse genotoxic effect of two hydrogen peroxide-containing bleaching products on oral mucosal cells.

Materials and Methods
The research was conducted on 22 individuals divided into two groups. Group 1 used ZOOM2 and group 2 the Opalescence BOOST bleaching agent. Specimens of the gingival and the upper lip

mucosa were obtained before, immediately after, and 72 h after the bleaching procedure and were analyzed using a micronucleus test.

Results
Seventy-two hours after bleaching treatment with BOOST, samples collected from the oral mucosa exhibited a statistically significant increase of all genotoxicity markers, with large effect sizes (Cohen's d>0.8) observed in the total number of micronuclei (MN), number of cells with 3+ MN, karyolysis and bi-nuclear cells. ZOOM2 treatment showed a significant increase, with medium-to-large effect sizes, in the number of cells with 1 MN, karyolysis, nuclear buds and bi-nuclear cells.

Conclusion
Both bleaching preparations demonstrated potential genotoxic effects on oral mucosal cells. The evidence obtained suggests that bleaching treatment affects the genome of mucosal cells to a certain extent, but it is difficult to assess the clinical significance of these findings. Due to the lack of similar studies, it is not possible to discuss the ultimate genotoxic potential of bleaching preparations, although it is probably negligible compared with daily exposure to other genotoxic factors. The patterns presented here provide a framework for ongoing further research, aimed at quantifying the observed effects and their long-term consequences on cells of the oral mucosa.

Human volunteer study, dermal genotoxicity Almeida et al., 2015

The aim of this study was to evaluate the genotoxic response using a micronucleus (MN) assay, after the application of two concentrations of carbamide peroxide. Thirty-seven patients were divided into two groups and randomly received either a 10% carbamide peroxide (CP) or a 16% carbamide peroxide concentration for 21 days in individual dental trays. Gingival margin cells were collected immediately before the first use (baseline), and then 15 and 45 days after baseline.

Materials and Methods

The patients were selected from a previously conducted double-blind clinical trial aimed at evaluating the efficacy and safety of two CP concentrations (10% and 16%; Whiteness Perfect, FGM Dental Products, Joinville, Brazil) for a home bleaching treatment. Patients with active caries, periodontal disease, previous hypersensitivity, smokers, alcohol drinkers, and pregnant or lactating women were excluded. The examiners and participant were blinded to the concentration of the agent that was being delivered.
Forty patients from the previous clinical trial were invited to participate in this study, 37 of which accepted. The participants included 30 women and 7 men. The average age of the volunteers was 28.14 ± 7.94 years for men and 27.5 ± 6.82 years for women. The researchers and participants were blinded to the CP concentration used by each patient.

The participants were given the trays and three tubes of bleaching gel. They were instructed to dispense the same amount of gel into the trays each day and insert them into their mouth to cover at least the anterior teeth for 2 h daily for 3 weeks. Participants bleached both their maxillary and mandibular arches at the same time. The use of bleaching agents was standardized according to the manufacturer's instructions. All patients received a hands-on practical demonstration and written instructions regarding both the proper use of the bleaching agents and the dietary restrictions during treatment. The participants also received toothbrushes and dentifrice without whitening agents to standardize their oral hygiene regimen.

For the genotoxicity study, gingival margin cells were collected from each patient on three occasions: immediately before the bleaching treatment (baseline), at 15 days and at 45 days after starting the bleaching treatment. Cells were collected from marginal gingiva, from premolar to premolar of both jaws. To determine the MN rate, 1000 cells were counted per slide (for each volunteer), for each period of time, and the number of MN in these cells was recorded.

Results

A slight increase in MN was observed for both groups, in comparison with the baseline, at 15 days. However, no difference was observed between the two groups (10% and 16%), at either 15 or 45 days (p = 0.90).

Conclusions

When bleaching is not prolonged or done very frequently, bleaching agents containing carbamide peroxide alone do not cause mutagenic stress on gingival epithelial cells. However, repetitive exposure to bleaching agents should be avoided, at least in the short term. Future studies should explore whether exposure to these products, in association with other factors, such as tobacco, alcohol and hot beverages, has the potential to cause genetic damage.

Hyperplasia and DNA damage in mouse skin Society for the Plastic Industry, 1997

Materials and Methods
Hydrogen peroxide 70% was applied to the skin of 10 female Sencar mice per dose group at dose levels of 10, 100, or 200 mmol in 200 ml of ethanol twice weekly for 4 weeks. Mice treated under the same conditions with DMBA (10 or 100 mmol/animal) or ethanol (200 ml) acted as positive and negative controls, respectively. The animals were killed on days 2 or 4 after the last administration (5 mice on each day). The application sites were removed, and after fixation and staining epithelial and dermal thickness, and dermal cellularity were determined visually by light microscopy.Non-phenol extraction of fresh frozen tissue was used to isolate DNA from animals killed 2 days after last dosing, and following digestion to nucleosides, 8-OH-deoxyguanosine was quantified by HPLC. Mutations in codon 61 of Ha-ras gene were determined using DNA isolated from paraffin blocks of whole skin.

Results
Treatment with hydrogen peroxide at all dose levels gave negative responses in all effect endpoints. The positive control, DMBA, induced DNA-damage (increased 8-OH-dG), Ha-ras mutations, epidermal hyperplasia and dermal cellularity changes. Calculation of the concentrations of hydrogen peroxide used gives 0.2, 1.6, or 3.2%.

Conclusions
Limited conclusions can be drawn from this experiment due to the low concentrations used in the study.

Host mediated mutagenicity assay (Swiss mouse and *Salmonella typhimurium* (TA 1530; G 46)) Keck, 1980

Materials and Methods
Doses / Concentrations: 0.003, 0.03 or 3.0 % hydrogen peroxide were administered in milk for 1 wk. 0.5 ml 0.3% hydrogen peroxide twice by gavage to Swiss mice with a 2 h interval; bacteria were injected after the second dose and harvested 4 h later.
The study design was not a complete in vivo study, since the mutagenic effects were analysed in bacteria which were injected into the peritoneal cavity of the exposed mice. The actual aim of the study was to investigate whether milk which was treated with hydrogen peroxide could have mutagenic potential.

Results
Pure hydrogen peroxide, but not the milk treated with hydrogen peroxide, gave a positive result. There was a strong positive response with TA1530 and a weak positive response with G46.

Summary of mutagenicity

While hydrogen peroxide has demonstrated mutagenic effects in several *in vitro* studies showing both mutations and chromosomal aberrations, there are no studies conducted *in vivo* which show positive results. However, the dataset does not contain a *in vivo* mutation assay, such as the OECD TG488 (Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay), which, considering the results of the *in vitro* studies could be indicated.

10.8.2 Comparison with the CLP criteria

Category 1

There is no positive evidence from human epidemiological studies. Hydrogen peroxide does not induce heritable mutations in the germ cells of humans.

The classification in Category 1A is not warranted.

There are no studies with positive results from *in vivo* heritable germ cell mutagenicity tests in mammals or *in vivo* somatic cell mutagenicity tests in mammals, or evidence that the substance has potential to cause mutations to germ cells.

The classification in Category 1B is not warranted.

Category 2

The classification in Category 2 is based on positive evidence obtained from experiments in mammals from *in vitro* experiments and somatic cell mutagenicity tests *in vivo* or other *in vivo* somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.

Hydrogen peroxide is clearly positive in different *in vitro* genotoxicity assays. However, there is no evidence from the available in vivo studies that hydrogen peroxide is mutagenic.

Classification in Category 2 is not warranted.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Data conclusive but not sufficient for classification.

10.9 Carcinogenicity

Not assessed in this dossier.

Not assessed in this dossier.

10.10 Reproductive toxicity

Not assessed in this dossier.

10.11 Specific target organ toxicity-single exposure

Not assessed in this dossier.

10.12 Specific target organ toxicity-repeated exposure

Not assessed in this dossier.

10.13 Aspiration hazard

Not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Guideline studies for degradation are not generally applicable and available for hydrogen peroxide, as it an inorganic substance. The assessment and comparison to the classification criteria is therefore based mainly on non-guideline studies.

According to Section 4.1 of Annex I to CLP, the term 'degradation' is defined as "the decomposition of organic molecules to smaller molecules and eventually to carbon dioxide, water and salts" and therefore the term cannot be used in a context of inorganic substances. Instead, in this section the term 'decomposition' is used.

Generally, hydrogen peroxide is biologically and abiotically decomposable. According to European Commission (2003) "Aerobic bacteria produce catalase enzymes that converts H2O2 to water and oxygen. Catalase is present in most aerobic bacteria and therefore biological degradation starts readily when hydrogen peroxide is in contact with microbial material (no remarkable lag-phase)". Abiotic decomposition is catalysed by transition (Fe, Mn and Cu) and heavy metals, and by reactions with organic substances.

11.1 Rapid degradability of organic substances

Table 25: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
OECD TG 209: Activated Sludge, Respiration Inhibition Test	Half-life: ≤ 2 min on all concentrations (1 – 1000 mg/l)	The test cannot be used for assessing 'ready biodegradability', as the conditions for degradation were more favorable compared to OECD TG 301.	Groeneveld and de Groot, (1999)
Non-guideline study Non-GLP Media: Freshwater (Jacks Lake, Ontario)	Initial concentration: 3.4 µg/L Filter (µm) / Half-life (h) none / **7.8** 64,12,5 / **8.6** 1 / 31 0.45 / >24	Temperature: NA Conditions: Darkness Analysis: semi-quantitative (scopoletin horseradish peroxidase fluorescent decay method; lower and upper LOD 0.34 and 8.5 µg/l)	Cooper and Lean, 1989

Method	Results	Remarks	Reference
Non-guideline study Non-GLP Media: Freshwater (Lake Ontario, Lake Erie)	Initial concentration: 1.5 – 6.0 µg/l. Filter: none — Half-life (h): **9.6 – 21.6** Filter: 0.45 — Half-life (h): no decline after 7h	Temperature: NA Conditions: Darkness Analysis: semi-quantitative (scopoletin horseradish peroxidase fluorescent decay method; lower and upper LOD 0.34 and 8.5 µg/l)	Cooper et al., 1989
Non-guideline study Non-GLP Media: Freshwater (Greifensee, Switzerland)	Initial concentration: 13.6 - 17 µg/L Filter: none — Half-life (h): **≤ 3 - 5** Filter: 0.45 — Half-life (h): 10 - 100	Temperature: NA Conditions: NA Analysis: semi-quantitative (DPD method; LOD 0.34 µg/l)	Sturzenegger, 1989
Non-guideline study Non-GLP Media: Freshwater (River Saone, France)	Initial concentration: 100 – 10000 mg/l Conc (mg/l) / Filter / Half-life (**days**) 100 / none / 20 100 / 0.2 / 15.8 250 / none / 12.4 250 / 0.2 / 9.5 500 / none / 6.9 1000 / none / 8.1 10000 / none / 2.5	Temperature: NA Conditions: Ambient light Analysis: NA	Air Liquide, 1991
Non-guideline study Non-GLP Media: Salty lake (Thao lagoon, France)	Initial concentration: NA Filter: none — Half-life (h): **5.8 – 6.3** Filter: 0.2 — Half-life (h): 53 - 69	Temperature: NA Conditions: NA Analysis: semi-quantitative (Fenton-OHBA; LOD 0.85 µg/l)	Herrmann & Herrmann, 1994
Non-guideline study Non-GLP Media: Seawater (Mediterranean Sea)	Initial concentration: 3 – 5 µg/l.. The concentration decreased continuously at a rate of 0.13 µg/l/hour. According to the authors, it is not clear whether the decomposition rate was linear or followed specific dissipation kinetics. If the rate followed first-order dissipation kinetics, the half-life would have been **5.3 hours,** which is inline with other studies.	Temperature: room temperature Conditions: darkness Analysis: semi-quantitative (N-ethyl-N-(sulfopropyl)aniline and 4-aminoantipyrene formation; LOD 0.4 µg/l)	Johnson *et al.,* (1989)

11.1.1 Ready biodegradability

One guideline test following OECD TG 209 is available, conducted by Groeneveld and de Groot, (1999). The primary objective of the test is to determine respiration inhibition of activated sludge, but biologically mediated decomposition of hydrogen peroxide was also extracted from the data.

Initial concentration of hydrogen peroxide was 1.0, 3.0, 10, 30, 100, 300 and 1000 mg a.s./L. The test solutions were prepared in glass beakers from appropriate amounts of hydrogen peroxide stock solution, isotonic medium, synthetic wastewater, and sludge suspension immediately before incubation.

The test included two controls to verify viability of the activated sludge as well as one flask which was sterilised with autoclaving.

The duration of the test was 120 minutes, of which hydrogen peroxide was measured semi-quantitatively from 1 to 30 minutes after the start of the incubation. Merckoquant® colorimetric test strips were used that provide concentration estimates in the ranges of 0.5, 2, 5, 10 and 25 mg a.s./L. The accuracy and precision of the colorimetric test strips was confirmed by determination of hydrogen peroxide at 1, 10 and 100 mg/L in fortified isotonic medium.

Rapid decomposition of hydrogen peroxide was observed in all concentration levels, with a half life of ≤ 2 minutes. Concentrations below the limit of determination (0.5 mg/L-range) were reached at one minute (1.0 mg/L hydrogen peroxide), 2 - 10 minutes (3.0 - 100 mg/L), and 30 minutes (300 - 1000 mg/L). No decomposition was observed in the sterile control.

The EC_{50} values for the activated sludge respiration inhibition were determined to be 466 mg/L after 0.5 h and >1000 mg/L after 3 h based on nominal concentrations.

The Dossier Submitter consider that the study cannot be used to conclude that hydrogen peroxide is ‘readily biodegradable’ as it is not standardized ready biodegradability test. In addition, the conditions for decomposition are more favourable compared to the OECD TG 301, as the concentration of suspended solids are much higher in OECD TG 209 (1600 mg ss/l compared to the ≤ 30 mg ss/l in OECD TG 301). The results can be used as a supporting information, and it gives an indication of rapid biologically mediated decomposition of hydrogen peroxide.

11.1.2 BOD_5/COD

11.1.3 Hydrolysis

Hydrogen peroxide is not expected to be hydrolysable based on its molecular structure.

11.1.4 Other convincing scientific evidence

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

Hydrogen peroxide is ubiquitously present in the environment and is formed in photochemical, chemical, and biological processes. According to European Commission (2003), the actual concentration of hydrogen peroxide in the environment is a result from a dynamic equilibrium between its production and decomposition. Typical concentrations in freshwater and estuaries are from some micrograms to some tens of micrograms per litre. In seawater, the measured concentrations in the surface range from 0.3 to 10 µg/l (Johnson et al., 1989).

11.1.4.2 Inherent and enhanced ready biodegradability tests

No information available

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Non-guideline studies are available from the literature where the decomposition of hydrogen peroxide has been studied in the laboratory setting using natural water samples. Similar experimental setups were used in these studies. The concentrations were determined at the start and during the incubation.

Decomposition in natural water

Cooper and Lean (1989) studied the diel (24h period) variation in hydrogen peroxide concentrations in a northern temperate oligotrophic lake water (Jack's lake, Ontario, Canada). As a part of the study, a dark decomposition rate of hydrogen peroxide was studied from the samples collected from the lake. Sampled water was filtered through 64, 12, 5, 1 and 0.45 µm mesh size to gain insight on the cause of the decomposition. The hydrogen peroxide concentration was analysed semi-quantitatively using scopoletin horseradish peroxidase fluorescent decay method. Lower and upper limit of detection for the method was 0.34 µg/l and 8.5 µg/l, respectively. Replicate analyses on the same sample were within +/- 5% throughout the range of hydrogen peroxide concentrations. The initial concentration of hydrogen peroxide was approximately 3.4 µg/l. The decomposition followed first order kinetics and the resulting half-lives are presented in the Table 26.

Table 26. The decomposition of hydrogen peroxide in unfiltered and filtered natural water samples (Cooper and Lean (1989)).

Filter	$t_{1/2}$**(h)**
Unfiltered	7.8
64, 12, 5 µm	8.6
1 µm	31
0.45 µm	>24

According to the results, the decomposition of hydrogen peroxide in unfiltered freshwater is rapid. The authors suggest that the biological decomposition was primaliry caused by the 0.2 – 2 µm sized picoplankton. The < 1 µm filtrates contained 5% of the the phytoplankton biomass, and 90% of the bacterial biomass. The study can be used for the classification purposes.

Decomposition rate of hydrogen peroxide was also measured by Cooper *et al.,* (1989) in samples collected from different depths (0 – 16.4 m) from Lake Ontario and Erie, located in the North America. Water samples were incubated in dark and hydrogen peroxide concentrations were determined approximately every 4 h, using the same semi-quantitative method as in Cooper and Lean (1989). The concentrations were ± 10 % of the 1.7 µg/l in a replicate analysis for the same sample. The results from the study are summarized in

Table 27.

Table 27. Decomposition of hydrogen peroxide in unfiltered natural water samples, collected from different depths (Cooper *et al.,* (1989)).

Depth	**H_2O_2 (µg/l)**	***k*** **(h^{-1})**	$t_{1/2}$**(h)**
Lake Ontario			
0	3.809646	0.047	14.7
1	3.877676	0.046	15.1
3	4.421911	0.047	14.7
5	3.945705	0.04	17.3
10	1.496647	0.032	21.6
Lake Erie			

Depth	H_2O_2 (µg/l)	k (h^{-1})	$t_{1/2}$(h)
0	6.020602	0.072	9.6
1	4.251838	0.052	13.3
5	4.660014	0.062	11.1
10	3.673588	0.065	10.2
13	3.605558	0.06	11.6
15	2.449058	0.043	16.3
16,4	1.768764	0.034	20.2

The decomposition followed first-order kinetics and was rapid with a half-lives between 14.7 – 21.6 hours. In the same study, no decomposition was observed over the period of 7 hours in samples which were filtered with 0.45 µm membrane filters.

Similar fast decomposition of hydrogen peroxide has been observed by Sturzenegger (1989), where ≤ 3 – 5 h half-life was obtained in three lake water samples collected from euthropic lake Greifensee located in Swizerland. Initial hydrogen peroxide concentrations in this study were in the range of 13.6 – 17 µg/l. In the samples filtered with 0.45 µm filter, the decomposition half-life was between 10 – 100 h. The hydrogen peroxide concentrations were analysed using DPD method, in which N,N-Diethyl-p-phenylenediamine (DPD) is oxidized by a peroxidase catalyzed reaction and the reaction product can be detected photometrically. The detection limit for the method is 0.34 µg/l.

Herrmann & Herrmann (1994) observed half-lives in the same range (5.8 – 6.3 h) for hydrogen peroxide in the salty lakewater from Thau lagoon. In samples filtered with 0.2 µm filter, half-life of 53 – 69 h was obtained. The initial concentrations of the hydrogen peroxide are not reported in the study. The method used for concentration determinations was semi-quantitative Fenton-OHBA, where fluorescent products (OHBA; isomeric hydrobenzoic acids) of hydroxyl radicals and benzoic acid are detected photometrically. The detection limit for the method is 0.85 µg/l.

Decompositon time of hydrogen peroxide has been studied also in a seawater samples at room temperature as a part of a study investigating vertical advection of hydrogen peroxide (Johnson *et al.*, 1989). Hydrogen peroxide concentrations in the samples were analysed using photometric detection, where the coloured condensation product of N-ethyl-N-(sulfopropyl)aniline and 4-aminoantipyrene is formed in the presence of hydrogen peroxide and peroxidase. The detection limit for the method was 0.4 µg/l.

The initial concentration of hydrogen peroxide in the seawater samples was 3 – 5 µg/l. The concentration decreased continuously at a rate of 0.13 µg l/hour, but it is not clear in the study whether the decrease was linear or did it follow specific dissipation kinetics. Nevertheless, the authors state that in a case of linear decrease, the initial concentration of 3 – 5 µg/l in surface water samples would have reached zero after 23 – 39 hours. If the decomposition rate followed first order kinetics, using the equation $t_{1/2}=\ln(2)/k$, the resulting half-life would be 5.33 hours.

The Dossier Submitter considers that these studies are sufficient to show that at environmentally relevant concentrations, the decomposition of hydrogen peroxide in natural waters is rapid. However, there is evidence that at higher concentrations (≥ 100 mg/l), the decomposition rate may decrease substantially. Air Liquide (1991) obtained half-lives of 20.1 days in natural water (River Saone) at initial concentration of 100 mg/l. At concentrations of 250, 500, 1000 and 10000 mg/l, the half lives decreased to 15.2 ± 2.5, 8.2 ± 2, 8.1 and 2.5 days, respectively. In European Commission (2003) it is indicated, that at higher concentrations "the inhibitive effect of hydrogen peroxide on naturally occurring microbes is beginning to have more influence thus giving longer half-lives".

Decomposition in sediment

According to the low estimated log Koc, hydrogen peroxide is not expected to adsorb into sediment. Therefore, sediment is not relevant compartment in the context of classification. In addition, according to the

European Commission (2003), "it may also be assumed that the adsorbed portion of hydrogen peroxide may still be effectively degraded because normally sediments contain a lot of catalytical abiotic and biotic material capable to degrade hydrogen peroxide".

Decomposition in soil

Natural soil environments usually contain large amounts of catalase positive microorganisms. Catalase enzyme is known to have a high specific activity (i.e. enzyme molecule has a high capability to decompose substrate molecules) and it effectively catalyses a rapid biodegradation of hydrogen peroxide in soil into water and oxygen (Pardieck et al., 1992). According to European Commission (2003) "In soil H2O2 is normally a short-lived substance. Rapid degradation will occur due to high concentration of catalytic material like transition metals, enzymes, easily oxidised/reduced organic substances and living microbes (Spain et al., 1989).

Hydrogen peroxide is used as a source of oxygen (for aerobic microbes) in polluted groundwater sites (enhanced bioremediation). Therefore specific information on degradability in soil is available. The problem in these applications where hydrogen peroxide is introduced directly into the ground is linked to a too rapid degradation. Observed half-lives of hydrogen peroxide in soil vary from 15 hours (soil without microbiological activity and few minerals) to several minutes (soils with 10^8-10^9 cells/g total solids, and in the presence of iron and manganese (Aggarwal et al., 1991; ECETOC 1993; Hinchee and Downey 1988; Pardieck et al., 1992).

In the assessment it is estimated that **the degradation half-life in soil is 12 hours**."

11.1.4.4 Photochemical degradation

According to European Commission (2003) direct photolysis is not expected to be an important degradation process in the aquatic environment. Hydrogen peroxide has absorption bands in the infrared, but is not decomposed by the light of these frequencies. The UV absorption spectrum is a continuous spectrum but the measured molar extinction coefficient values are low. Highest value is $\in$ = 4.2 l/mole.cm (at 280 nm) decreasing continuously to 0.22 l/mole.cm (at 320 nm) and 0.00066 l/mole.cm (at 400 nm)

11.1.5 Conclusion on rapid degradability

The eMSCA considers that the presented studies are sufficient to demonstrate that hydrogen peroxide is 'rapidly degradable' in the enviroment. It is however to be noted that these decomposition rates are determined from studies that are likely performed in room temperature, and the decomposition rates can be overestimated compared to decomposition happening in environmentally relevant temperatures. In European Commission (2003), it is speculated that half-lives of 1 – 3 days would represent adequate estimate of the average annual degradation in surface waters with low microbial density. In the report, a worst-case half-life of 5 days is given for surface waters, which is still much lower than the boundary half-live for > 70 % degradation within 28 days (16 days), given in the Annex I of CLP for rapid degradation.

At very high concentrations (>100 mg/l), the inhibitive effect of hydrogen peroxide on naturally occurring microbes is beginning to have more influence thus giving longer half-lives.

11.2 Environmental transformation of metals or inorganic metals compounds

11.3 Environmental fate and other relevant information

Adsorption

Hydrogen peroxide is highly water soluble and highly polar substance, and therefore no significant adsorption to soil and sediment is expected. KOCWIN v2.00, available in EpiSUITE v4.11, estimates a log Koc of -1.3630 indicating negligible partitioning to the soil and sediment. It is to be noted however, that this value can be used only for indicative purposes, as inorganic compounds are not included in the training data set for the methodology utilized in the software.

Equilibrium partitioning

A Mackay I equilibrium partitioning model calculation at 20℃ is available in the European Commission (2003) (Table 28), using vapor pressure of 3 hPa and measured Henry's law constant H = 7.10^{-4} Pa·m3 /mol at 20℃.

Table 28. Mackay model I equilibrium partitioning

Compartment	Distribution %
Air	$0.3.10^{-3}$
Water	99.98
Soil	0.01
Sediment	0.01

11.4 Bioaccumulation

11.4.1 Estimated bioaccumulation

Hydrogen peroxide is inorganic reactive and short-lived polar substance and therefore no bioaccumulation is expected. A KOWWIN v1.68 estimate of the log Kow is -1.57, which further indicates that the potential of bioaccumulation in aquatic organisms is low. Moreover, hydrogen peroxide is normally found in mammalian cells as a endogenous metabolite, and the enzyme catalase is almost ubiquitously distributed in biotic systems enabling the organisms to convert hydrogen peroxide into water and oxygen. Due to this, eMSCA considers that bioaccumulation endpoint is not relevant for hydrogen peroxide, and the substance should be considered as non-bioaccumulative in the classification.

11.4.2 Measured partition coefficient and bioaccumulation test data

No information available.

11.5 Acute aquatic hazard

Hydrogen peroxide is a clear colourless liquid which is normally handled as an aqueous solution. Hydrogen peroxide in itself (at NTP) is stable. Also pure aqueous solutions in clean inert containers are relatively stable. Stability is at a maximum at pH 3.5-4.5. Commercial solutions must be stabilised with additives to prevent possibly violent decomposition due to catalytic impurities or elevated temperatures and pressure.

Hydrogen peroxide is a special substance since all organisms, excluding anaerobic bacteria, produce hydrogen peroxide as a by-product in their normal metabolism. Organisms have developed ability to decompose hydrogen peroxide (and other reactive oxygen species) since excess hydrogen peroxide has potential to cause oxidative damages to cells. Decomposition and detoxifying of reactive oxygen species is based on the antioxidant enzyme system, of which the catalase and glutathione peroxidise enzymes are able to decompose hydrogen peroxide (European Commission, 2003).

There are a large number of toxicity tests with hydrogen peroxide to aquatic organisms. However, only few of them are both relevant for classification purposes and adequately done. In those tests where the concentration of the solution of the test substance is reported (i.e. 30% or 50% H2O2,), the results are calculated as 100% hydrogen peroxide. The aquatic toxicity results are derived based on hydrogen peroxide content of the test material. Only studies with analytical monitoring and results based on measured concentrations of the test substance are considered valid and reliable for classification purposes. Discarded studies as not reliable for classification purposes can be found from the CAR annexed to this classification proposal and REACH registration dossier.

Table 29: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Fish					
Equivalent to OECD TG 203 96h semi-static Non GLP	*Pimephales promelas* (Fathead minnow)	Hydrogen peroxide 50 % w/w (aqueous solution)	EC50 (mortality) = 16.4 mg/L (mm[1])	Test temperature of 20 ± 1 °C was outside recommended range of 21 to 25 to be used for *Pimephales promelas*	(Anonymous, 1989a) Klimisch 2
Aquatic invertebrates					
Equivalent to OECD TG 202 48h semi-static Non GLP	*Daphnia pulex*	Hydrogen peroxide 50 % w/w (aqueous solution)	EC50 (immobility) = 2.4 mg/L (mm)	No mortality (immobility) was observed at the lowest treatment (1.0 mg/L) but a 100 percent mortality was found at the next treatment level (5.5 mg/L) and at all treatments thereafter.	(Anonymous, 1989b) Klimisch 2

[1] mm = mean measured concentration

11.5.1 Acute (short-term) toxicity to fish

There is one reliable acute 96h semi-static toxicity test available for hydrogen peroxide (50% w/w aqueous solution) for fish (*Pimephales promelas*) (Anonymous, 1989a). The test follows the US EPA TSCA test guideline (40 CFR parts 796, 797, 798) and it is in compliance with the main requirements of the OECD TG 203. The test was performed at temperature of 20 ± 1 °C, which deviates from the recommended temperature range of 21 to 25 °C to be used for *Pimephales promelas.* However, Dossier Submitter considers that this deviation does not invalidate the test. Also the photoperiod used was not specified in the test report but it can be assumed to be 16:8h light:dark ratio following test guideline. Hydrogen peroxide concentrations were analysed from the fresh and aged test media during the renewal of media (at 24h intervals). Test medium samples of all test substance groups were analysed at the beginning and at the end of each test medium renewal interval of 24 hours. The analysis was based on a standard industrial titration with potassium permanganate. Hydrogen peroxide was unstable at test conditions, as the average concentration in the aged media was 42 % of the concentration in fresh media. The variation in the decomposition rate was also great, as the aged medium at minimum represented only 7 % of the initial concentration while at maximum 93 % of the initial hydrogen peroxide was found from the aged medium. Due to variable decomposition at test conditions, the effect data is based on mean measured concentrations.

The mean measured test concentrations of hydrogen peroxide were 0.5, 4.3, 18.5, 33.0, 162.4 and 413.7 mg/L. All fish in the two highest treatments died within 4 hours, and 100% and 40% of fish exposed to 33.0 and 18.5 mg/L were dead at the end of the test period. No adverse effects were found in fish in the control, 0.5 and 4.3 mg/L treatment groups. Therefore, the 96h LC50 value of 16.4 mg/L was determined for hydrogen peroxide based on mean measured concentrations. The validity criteria of OECD 203 can be considered fulfilled. Therefore, the study can be considered valid and reliable for the classification purposes for hydrogen peroxide.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

There is one reliable acute 48h semi-static toxicity test available for hydrogen peroxide (50% w/w aqueous solution) for *Daphnia pulex* (Anonymous, 1989b). The test follows US EPA TSCA test guidelines and it is in compliance with the requirements of OECD TG 202. In the test 1:1 mixture of laboratory grade deionised

water (TritonR distilled) and lake water (Lake Cammack, Alamance County, NC, USA; buffered by addition of sodium bicarbonate and aerated; 0.12 mm filtered) was used as a dilution water. Hydrogen peroxide concentrations were analysed from the fresh and aged test media during the renewal of the media and from the aged media at the end of the test. The analysis was based on a standard titration with potassium permanganate. The concentrations of hydrogen peroxide in the test media were almost stable at the two highest treatments and decreased between 10 and 67% at the other treatments. The mean measured test concentrations were 0.5, 4.6, 22.4, 38.2, 246.9 and 489.5 mg/L.

There was no immobility in the control and the lowest treatment group of 1.0 mg/L, whilst in the remaining treatment groups all daphnids were dead within 24 hours. The 48h EC50 value of 2.4 mg/L was determined for hydrogen peroxide based on mean measured concentrations but it was not possible to give confidence intervals because of the lack of partial mortality. The Trimmed Spearman-Karber method was used. The eCA in the CAR used binomial method to recalculate EC50, since there were no partial mortality. Thus EC50 was calculated as follows: (1 mg/l x 5.5 mg/l)½ = 2.34 mg/l. The validity criteria of OECD TG 202 was fulfilled. The reliability of the EC50 is slightly decreased by the used, non-optimal treatment levels but the effect value can be considered valid and reliable for the classification purposes for hydrogen peroxide.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

According to the biocidal eCA: "*Effects of hydrogen peroxide to primary producers have been investigated in numerous freshwater algal species and, in addition, in two marine diatoms. Most of the data is from the public domain, summarising the findings from experiments conducted with a scientific background rather than as a response to registration requirements. Therefore, the test methods are often not in compliance with standard methods and the set-ups are not optimised for EC_{50} or NOEC value calculation. Despite these technical limitations, one acceptable key study for algae with valid EC_{50} and NOEC values was found for the risk assessment purposes and the data from the other non-key studies (mainly LOEC values) are only used to provide additional information.*"

However, due to the analytical deficiencies and due to the effect concentrations being based on nominal concentrations in that key study for algae (Anonymous, 1997), eCA concluded in the CAR that the results do not represent intrinsic toxicity of hydrogen peroxide to algae. Analytical method was not appropriate to reliably quantify hydrogen peroxide concentrations in the test medium containing algae, because high algae concentrations and debris seemed to interfere the analytical method. Due to analytical deficiencies, the eMSCA considers that this study is not considered valid and reliable for the classification purposes for hydrogen peroxide.

Therefore, Dossier Submitter considers that no reliable data is available for toxicity to algae or other aquatic plants for hydrogen peroxide.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No data available.

11.6 Long-term aquatic hazard

Only studies with analytical monitoring and results based on measured concentrations of the test substance are considered valid and reliable for the classification purposes. Discarded studies as not reliable for the classification purposes can be found from the CAR annexed to this classification proposal and REACH registration dossier.

Table 30: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
Aquatic invertebrates					
Similar to OECD TG 211	*Daphnia magna*	Hydrogen peroxide 35 %	NOEC (reproduction) = 0.63 mg/L	Test media was well water.	(Meinertz et al.,

21d flow-through Non GLP		w/w (aqueous solution)	NOEC (length) < 0.34 mg/L	The differences in mean length of live parental daphnids at the two lowest treatments are not considered biologically meaningful.	2008)

[1] Indicate if the results are based on the measured or on the nominal concentration

11.6.1 Chronic toxicity to fish

No reliable and valid data available for classification purposes.

11.6.2 Chronic toxicity to aquatic invertebrates

There is one reliable 21d flow-though long-term toxicity test (Meinertz et al. 2008) available for hydrogen peroxide (35% w/w aqueous solution) for aquatic invertebrate (*Daphnia magna)*. The test was done in compliance to ASTM E 1193-97 (1997) and similar to OECD TG 211. Daphnids (< 24 h old) were exposed under flow-through conditions for 21 days to nominal concentrations of 0.32, 0.63, 1.25, 2.5 and 5.0 mg/L. A blank dilution water control group was run in parallel. On day 0, ten daphnids were individually set up per test group.

The cumulative number of offspring per test group at the test end was 1516, 1564, 1388, 1000 and 1 in 0, 0.32, 0.63, 1.25 and 2.5 mg/L test group, respectively, showing statistically significant difference between the control and the 1.25 and 2.5 mg/L group. The mean measured test concentration were 0, 0.34, 0.63, 1.27, 2.61 and 5 mg/L (Table 31: Effects of hydrogen peroxide (35%) to survival and reproductive output of *D. magna* (cumulative or mean of 10 replicates each with 1 female per test group). This results in a NOEC value of 0.63 mg/L for hydrogen peroxide for reproductive output.

All adult daphnids of the 2.5 and 5.0 mg/L treatment were dead by day 19 and 9, respectively. No mortality was seen in the control treatment but one dead daphnid was found in each hydrogen peroxide treatment levels of 0.32 mg/L (day 20), 0.63 mg/L (day 11), and 1.25 mg/L (day 15). In addition one daphnid at 1.25 mg/L was found to be missing on day 21 (due to overflow).

The length of alive parental daphnids was significantly greater in the control group than in all hydrogen peroxide treatments, however, the difference was only -3.5% and -6.6% at the 0.32 and 0.63 mg/L treatment level, in contrast to -15.5% at the 1.25 mg/L treatment level. The differences at the two lowest treatment levels were considered not biologically meaningful by the eCA in the CAR. The Dossier Submitter agrees that the effects on length of parental daphnids should not be used for the classification purposes at the two lowest treatment levels of hydrogen peroxide. Further, the reproductive output of the exposed parents at the two lowest concentrations does not differ from that of the control parents.

Meinertz et al. (2008) discussed that hydrogen peroxide will oxidize any organic material and it was not possible to ascertain in the study if the effect on growth and reproduction was the result of detrimental effects to the *Daphnia* itself or the result of a reduced food source caused by oxidation of the food source. However, the Dossier Submitter considers that the NOEC value of 0.63 mg/L obtained can be considered to represent intrinsic chronic toxicity to *D. magna* as it is based on the mean measured values from the flow-through exposure system.

Table 31: Effects of hydrogen peroxide (35%) to survival and reproductive output of *D. magna* (cumulative or mean of 10 replicates each with 1 female per test group)

Test group (nominal / mean measured) [mg/L]	Survival at 21 d (day of death) [%]	Cumulative no. of offspring per test group / female on day 21	Mean & range days to first brood / Mean no. of broods per test group	Mean length (range) [mm]
Blank control / 0.02	100	1516 / 151.6	11 d (8-13 d) / 41	4.616 (4.408-4.791)
0.32 / 0.34	90 (day 20)	1564 / 156.4	12 d (10-16 d) / 40	4.455 (4.143-4.715) *
0.63 / 0.63	90 (day 11)	1388 / 154.2	10 d (9-13 d) / 39	4.391 (4.052-4.662) *
1.25 / 1.27	90 (day 15)[&]	1000 / 111.1 *	10 d (8-13 d) / 40	3.901 (3.675-4.206) *
2.5 / 2.61	0 (100% at day 19)	1 *	16 (--) *	n.a.
5.0 / 5.00	0 (90% at day 2, 100% at day 9)	0	n.a.	n.a.

LOD = 0.02 mg H_2O_2/L;
[&] one additional death occurred on day 21 due to an overflowing test chamber;
* statistically significantly different to blank control, Chi-square test ($P \leq 0.05$)

Instead of using the defined media given in the OECD TG 211, the test was performed in well water. Feeding of the daphnids during the test was well described in the paper and the use of the well water is considered acceptable, because the control validity criteria concerning the parent mortality and the offspring production were fulfilled. Instead of using a total number of living offspring produced per parent animal alive at the end of the, a total number of offspring produced in the test group was reported in Meinertz et al. (2008). No original data of of the offspring produced per parent is extractable. Statistical testing of the treatment effects on survival of the parents was based on a Cox proportional hazards model (with hydrogen peroxide as categorical variable). No NOEC was determined in the paper for hydrogen peroxide on reproductive output using the ANOVA and an appropriate multiple comparison method as suggested in the OECD TG 211. Instead a total number of offspring produced was modelled by using a negative binomial distribution. The hypotheses about the relationship between the total number of offspring and hydrogen peroxide concentration was tested with likelihood-ratio Chi-square tests. However, the Dossier Submitter considers that these deviations from the OECD TG 211 do not invalidate the study and Meinertz et al. (2008) can be considered as valid and reliable for the classification purposes for hydrogen peroxide as a key study.

11.6.3 Chronic toxicity to algae or other aquatic plants

No reliable data is available for toxicity to algae or other aquatic plants for hydrogen peroxide (further information in section 11.5.3).

11.6.4 Chronic toxicity to other aquatic organisms

No data available.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Valid and reliable acute aquatic toxicity data for classification purposes are available for fish and aquatic invertebrates for hydrogen peroxide. The 48h toxicity value of 2.4 mg/l by (Anonymous, 1989b) for aquatic invertebrate (*Daphnia pulex*) is proposed as the lowest and the most reliable acute endpoint.

For acute aquatic hazards, on the basis of this acute aquatic invertebrate endpoint being ≥ 1 mg/l, no classification is warranted for hydrogen peroxide for acute aquatic hazard.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Bioaccumulation

Hydrogen peroxide can be considered non-bioaccumulative. It is normally found in mammalian cells as a endogenous metabolite, and the enzyme catalase is almost ubiquitously distributed in biotic systems enabling the organisms to convert hydrogen peroxide into water and oxygen. Furthermore, a KOWWIN v1.68 estimate of the log Kow is -1.57, which further indicates that the potential of bioaccumulation in aquatic organisms is low.

Rapid degradation

Hydrogen peroxide is biologically and abiotically decomposable. According to the European Commission (2003) "Aerobic bacteria produce catalase enzymes that converts H2O2 to water and oxygen. Catalase is present in most aerobic bacteria and therefore biological degradation starts readily when hydrogen peroxide is in contact with microbial material (no remarkable lag-phase)". Abiotic decomposition is catalysed by transition (Fe, Mn and Cu) and heavy metals, and by reactions with organic substances.

According to the decomposition data presented in the section 11.1.4.3 hydrogen peroxide decomposes rapidly in surface waters at environmentally relevant concentrations. It is however to be noted that these half-lives are determined from studies that are likely performed in room temperature, and the decomposition rates can be overestimated compared to decomposition happening in environmentally relevant temperatures. In the European Commission (2003), it is speculated that half-lives of 1 – 3 days would represent adequate estimate of the average annual degradation in surface waters with low microbial density. In the report, a worst-case half-life of 5 days is given for surface waters, which is still much lower than the boundary half-live for 70 % degradation within 28 days (half-life 16 days), given in the CLP-criteria for rapid degradation. Therefore, in a context of CLP the Dossier Submitter considers that hydrogen peroxide is classified as 'rapidly degradable'.

Toxicity

Valid and reliable long-term aquatic toxicity data for classification purposes is only available for aquatic invertebrate (*Daphnia magna*) for hydrogen peroxide. Long-term aquatic toxicity data available in fish and aquatic algae studies can not be considered valid for the classification purposes due to the analytical deficiencies or lack of analytical monitoring of hydrogen peroxide concentrations. The NOEC value of 0.63 mg/L (reproduction) by Meinertz et al. (2008) is proposed as reliable chronic endpoint.

Since there are adequate chronic toxicity data available for only one trophic level, hydrogen peroxide should be classified according to the Figure 4.1.1 in the CLP based on the most stringent outcome (surrogate method):

(a) according to the criteria given in Table 4.1.0(b)(i) or 4.1.0(b)(ii) (depending on information on rapid degradation), and

(b) (if for the other trophic level(s) adequate acute toxicity data are available) according to the criteria given in Table 4.1.0(b)(iii).

Hydrogen peroxide can be classified according to the criteria set out in CLP in Table 4.1.0(b)(ii). In this case classification of Aquatic Chronic 3 is applicable for hydrogen peroxide based on the lowest NOEC value of 0.63 mg/l for *Daphnia magna* (≤ 1 mg/l).

Since hydrogen peroxide is rapidly degradable and the log Kow ≤ 4 it will not be classified according to the criteria given in Table 4.1.0(b)(iii). Thus, hydrogen peroxide is classified according to the available valid and reliable long-term aquatic toxicity data for aquatic invertebrate.

For long-term aquatic hazards, hydrogen peroxide should be classified according to Regulation EC 1272/2008 as Aquatic Chronic 3 (H412).

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Conclusions on classification and labelling for environmental hazards of hydrogen peroxide.

Hazard Class and Category code(s)	M factor	Hazard Statement
Aquatic Chronic Category 3, H412	-	Harmful to aquatic life with long lasting effects

12 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this dossier.

13 ADDITIONAL LABELLING

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15 ANNEXES