Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Bis(α,α-dimethylbenzyl) peroxide

EC Number: **201-279-3**

CAS Number: **80-43-3**

Index Number: **617-006-00-X**

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Note on confidential information

Please be aware that this report is intended to be made publicly available. Therefore it should not contain any confidential information. Such information should be provided in a separate confidential Annex to this report, clearly marked as such.

CONTENTS

1 PHYSICAL HAZARDS

Not evaluated for this dossier.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not evaluated for this dossier.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

Not evaluated for this dossier.

3.2 Acute toxicity - dermal route

Not evaluated for this dossier.

3.3 Acute toxicity - inhalation route

Not evaluated for this dossier.

3.4 Skin corrosion/irritation

3.4.1 Animal data

3.4.1.1 Acute dermal irritation test in the rabbit

Study reference:

Life Science Research Limited, P. B. Rees, 1993, LSR Report No: 92/AKL213/0905

Detailed study summary and results:

Test type

The study is said to be designed to meet the requirements of the OECD guideline 404 (1981). The study is also said to be conducted in general conformance with the OECD GLP Principles with the following exception: "the test substance characterisation and stability data, while available, were not developed in accordance with the standard". The laboratory states that this should not affect the study results.

Test substance

- Dicumyl peroxide; EC number 201-279-3 and CAS number 80-43-3

- Degree of purity: 99.61 %. A certificate of analysis for the batch of test material used in this study was included in an annex.
- Impurities were not mentioned.
- Batch number: 041920580858.
- Physicochemical properties that could indicate potential for skin irritation/corrosion: being a peroxide it is expected that it has oxidising properties that could indicate irritant/corrosive properties. In the CLH guidance document the following is stated:

  Substances with oxidising properties can give rise to highly exothermic reactions in contact with other substances and human tissue. High temperatures thus generated may damage/destroy biological materials. This applies, for example, to organic peroxides, which can be assumed to be skin irritants, unless evidence suggests otherwise (Guidance on IR/CSA Section R.7.2.3.1).

  For a hydro peroxide classification as Skin Corrosive Category 1B should be considered, whereas Skin Irritation Category 2 should be considered for peroxides. Appropriate evidence must be provided in order to consider non-classification of substances with oxidising properties.

Test animals

- Species/strain/sex: Young male albino rabbits of an outbred New Zealand White strain (supplied by Rosemead Rabbits, Essex England).
- No. of animals per sex per dose: three animals were tested, one in an initial test and two in a confirmatory test.
- Age and weight at the study initiation: the rabbits were approximately 3.5 - 4 months old and weighed 2.9 - 3.12 kg at the start of the study.

Administration/exposure

- Duration of test/exposure period: 4 hours
- Total dose: a single dose of 0.5 g was directly applied to the skin. This dose is in accordance with the OECD guideline.
- Post exposure observation period: assessment of skin irritation responses at the control and treated test sites were made 1, 24, 48 and 72 hours after removal of the bandages.
- Control group and treatment: Each animal had two test sites on the dorsum. The right test site was the control and was treated in the same way as the left test site except for the application of the test material. It was covered in the same way but there was no control substance.
- Vehicle: there was no vehicle. The test substance was applied as such, as a crystalline powder directly to the skin. **This is not in line with the current OECD 404 guideline.** In the guideline it is stated that "When testing solids (which may be pulverised, if considered necessary), the test

chemical should be moistened with the smallest amount of water (or, where necessary, of another suitable vehicle) sufficient to ensure good skin contact".

- Time points at which grading/scoring took place: 1, 24, 48 and 72 hours.
- Grading scale: Draize (1959) as described in the OECD 404 guideline.
- Preparation of the test site: On the day before dosing, the dorsum between the limb girdles was clipped. Each animal was examined for abnormalities or irritation of the dermal test site before allocation to study. On the day of dosing two test sites (6 x 6 cm) were marked on either side of the clipped area of dorsum, moistened by direct application of approximately 0.2 ml of distilled water per test site. A single dose (0.5 g) of the test substance was applied directly to the skin and covered by an unmedicated gauze patch (3 x 2 cm) which was held in place on the left test site by strips of waterproof plaster. The right test site, the control site, was covered by a similar semi-occlusive dressing but otherwise remained untreated. Pads of cotton wool and elasticated bandage were used to protect the patches and ensure good contact between the skin and the test material during the four-hour exposure period. The elasticated bandage was held in place by thin strips of waterproof plaster at both edges.
- Removal of test substance: the dressings were removed after four hours exposure and the treatment sites were gently washed with warm water and dried with paper towels to remove excess test material adhering to the skin.
- Statistical methods: none.

Results and discussion

Table 1: Irritant/corrosive response data

Animal	Type of response	Score after removal of dressings							
		1 hour		24 hours		48 hours		72 hours	
		Test site	Control site	Test site	Control site	Test site	Control site	Test site	Control site
Initial test animal	Erythema	0	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0
Confirmatory test animal 1	Erythema	1	0	1	0	1	0	0	0
	Oedema	0	0	1	0	0	0	0	0
Confirmatory test animal 2	Erythema	1	0	1	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0

Table 2: Mean values for erythema and oedema 24, 48 and 72 hours after treatment.

	Erythema		Oedema	
	Test	Control	Test	Control
Initial test animal	0.0	0.0	0.0	0.0
Confirmatory test animal 1	0.7	0.0	0.3	0.0
Confirmatory test animal 1	0.3	0.0	0.0	0.0

Very slight erythema, grade 1, was seen at the test site of two rabbits 24 hours following bandage removal, continuing in one rabbit at the 48-hour examination. Very slight oedema, grade 1, was evident at one test site at the 24-hour observation. The test site of both these rabbits was overly normal at the 72-hour examination. No dermal effects were observed at the test site of the remaining rabbit at any time during the 72-hour observation period. The control sites did not show any response to the control procedure.

There might have been more irritation if the test substance had been moistened with water in order to ensure good skin contact, as it should have been according to the OECD 404 guideline. However, even with more skin contact it would probably not have reached a level of irritation that would have warranted classification for irritation, in other words a mean score of irritation above 2.3. Considering that the eye irritation test (see below) is performed according to guideline and does not warrant classification it is not probable that the addition of liquid to the test substance would have changed the results of the skin irritation test much.

3.4.2 Human data

No human data

3.5 Serious eye damage/eye irritation

3.5.1 Animal data

3.5.1.1 Acute eye irritation study in rabbits

Study reference:

LPT Laboratory of Pharmacology and Toxicology GmbH. Acute eye irritation/corrosion test of dicumyl peroxide in rabbits. 2010.

Detailed study summary and results:

Test type

The study is said to be designed according to EC method B.5. (2004/73/EC) and OECD guideline 405 (2002).

The study is also said to be performed in compliance with Good Laboratory Practice Regulations in the EC enacted in Germany in the Chemikaliengesetz [Chemicals Act], current edition, and according to "OECD Principles of Good Laboratory Practice", Document Nos. 1 and 13 ENV/MC/CHEM (98) 17, ENV/JM/MONO (2002) 9, respectively. The following regulations were also considered: USFDA GLP Regulations – 21 Code of the Federal Regulations, Part 58, current edition and the Japanese Guidelines for Non-clinical Studies of Drugs Manual 1995; Guidelines for Toxicity Studies of Drugs. Japanese Ministry of Health and Welfare.

Test substance

- Dicumyl peroxide; EC number 201-279-3 and CAS number 80-43-3.
- Degree of purity was not stated. **This is not in accordance with the guideline.** The laboratory states that they only compared information received from the registrant with the information on the batch of test substance, and that "no further identification was performed by LPT for this study". They also write that no certificate of analysis was available to LPT. However, on ECHAs dissemination site the degree of purity is stated to be 99.5% based on CoA.
- Impurities were not mentioned. **This is not in accordance with the guideline.**
- Batch number was not indicated. However, the receipt number was stated to be 44229.
- Physicochemical properties that could indicate potential for eye damage/eye irritation (e.g. pH value, oxidising properties): being a peroxide it is expected that it has oxidising properties that could indicate irritant/corrosive properties. In the CLH guidance document R7a, Figure R.7.2–5 Testing and assessment strategy for evaluating the serious eye damage/eye irritation potential of substances, peroxides are considered likely to be both skin and eye irritants. In addition in the Appendix R7.2-1 it is stated: "Other substances may cause eye injuries that start as mild but progress to be more severe at a later period e.g. substances that react with cellular constituents via alkylation or oxidative attack on macromolecules. An example of these types of substances are e.g. peroxides, mustards and bleaches (Scott et al., 2010)".
- Is the substance skin corrosive or skin irritant? The substance is a peroxide and has a classification for skin irritation, however, as the study above indicates there does not seem to be grounds to classify the substance as an irritant based on a skin irritation study in the registration.

Test animals

- Rabbit, Himalayan, only male. According to the OECD guideline, the albino rabbit is the species of choice. The laboratory has not given any reason to use another rabbit breed than the albino. **This is not in accordance with the guideline.** The Himalayan breed however has red eyes, as do the albino rabbits.
- Three animals
- Age: 6,5 - 7,5 months. Weight: 2,4 - 3,0 kg.

Administration/exposure

- Duration of test/exposure period: At least 20 adaptation days, 1 test day and follow-up period of 72 hours.
- Total dose: 100 mg mortared test item was administered into the conjunctival sac of the right eye in each of three animals. 100 mg is the maximum recommended in the guideline. The lids were held together for about one second in order to prevent loss of the material. The left eye, remained untreated and served as control. The test was initially performed on one animal. As no corrosive or severe irritant effects were observed in this animal 2 further animals were tested 24 hours after the start of the initial test. 1 hour after instillation the eyes were rinsed with 20 mL 0.9 % NaCl solution,

according to the guideline.

Post exposure observation period: the eyes were examined 1, 24, 48 and 72 hours after the administration. According to the guideline, the test can be terminated as soon as the effects seem to be reversed. In this test, the effects were gone at 72 hours after application of the test substance.

- Control group and treatment: the left eye was left untreated and served as control.
- Vehicle: no vehicle was used. The test substance was mortared and applied to the eye as such, according to the OECD guideline.
- Time points at which grading/scoring took place: 24, 48, 72 hours.
- Tool used to assess scores: the eyes were examined ophthalmoscopically with a slit lamp prior to the administration and in the observations after administration. 24 hours after administration fluorescein was applied to the eyes before being examined to see possible lesions.
- No special preparation of the test eyes was done except that they were examined ophthalmoscopically before administration.
- Grading scale: the grading scale was the same as the one specified in the OECD guideline.
- Removal of test substance (e.g. water or solvent): 1 hour after instillation the eyes were rinsed with 20 mL 0.9 % NaCl solution.
- There is no information on the application of anaesthesia. **This is not in accordance with the latest guideline.** However, since this study was performed before the last update of the guideline this may not have been a requirement at the time.

Results and discussion

The following results were seen:

Table 3: Irritant/corrosive response data

Time after administration	Cornea	Iris	Conjunctivae	
	Opacity		Redness	Chemosis
	Animal no. : 1 / 2 / 3			
Before dosing	0 / 0 / 0	0 / 0 / 0	0 / 0 / 0	0 / 0 / 0
1 hour	0 / 0 / 0	0 / 0 / 0	1 / 1 / 1	0 / 0 / 0
24 hours	0 / 0 / 1	0 / 0 / 0	0 / 1 / 1	0 / 0 / 0
48 hours	0 / 0 / 1	0 / 0 / 0	0 / 0 / 0	0 / 0 / 0
72 hours	0 / 0 / 0	0 / 0 / 0	0 / 0 / 0	0 / 0 / 0

Table 4: Mean values for effect seen 24, 48 and 72 hours after treatment.

	Cornea	Iris	Conjunctivae	
	Opacity		Redness	Chemosis
Animal 1	0	0	0	0
Animal 2	0	0	0.3	0

Animal 3	0.7	0	0.3	0

There was a small degree of opacity seen in the cornea of the third animal at 24 and 48 hours. Grade 1 opacity is described as "scattered or diffuse areas of opacity (other than slight dulling of normal lustre), details of iris clearly visible". There was also some redness of the conjunctivae in all three animals at 1 hour and in two animals at 24 hours. Grade 1 redness is described as "some blood vessels hyperaemic (injected)". A fluorescein test was performed at 24 hours after administration and revealed corneal staining in animal no. 3 (up to 25 % of the surface).
At 72 hours, all effects were reversed in all three animals. The untreated eye that served as control did not show any pathological changes. No other effects were reported in the report.

The current harmonised classification for this substance is as an eye irritant. However, to classify a substance as irritating to the eyes there must be a positive response in at least two out of three animals and a grading of ≥ 1 for corneal opacity, and/or ≥ 1 for iritis, and/or ≥ 2 for conjunctival redness and/or ≥ 2 for chemosis. These grades must be a mean of the grades at 24, 48 and 72 hours after instillation of the substance in the eye. As the effects of the test substance in this study gave grades that were well below any of the grades demanded in the criteria there are no grounds to classify this substance as an eye irritant. This is despite the fact that the substance is a peroxide and that it therefore could be expected to be an irritant.

3.5.2 Human data

No human data

3.5.3 Other data

No other data

3.6 Respiratory sensitisation

Not evaluated for this dossier.

3.7 Skin sensitisation

Not evaluated for this dossier.

3.8 Germ cell mutagenicity

Not evaluated for this dossier.

3.9 Carcinogenicity

Not evaluated for this dossier.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Prenatal developmental toxicity study in rats by oral administration

Study reference:

- Study No: 788.410.4505 20 June 2014, Toxi-Coop Zrt., Hungary (Author: Kornelia Kolep Csete)
- BSL Bioservices study no. 151230: Re-evaluation of rat foetal skeletons from Toxi-Coop Zrt. Study No. 788.410.4505 with dicumyl peroxide. Study completion date 12. Oct 2015.

Detailed study summary and results:

Test type

Prenatal developmental toxicity study said to be performed according to OECD Test guideline 414 (Prenatal Developmental Toxicity Study). This was a GLP compliant study.

Test substance

- Name: Dicumyl peroxide
- Chemical Name: bis (α,α-dimethylbenzyl) peroxide
- Composition: bis (α,α-dimethylbenzyl) peroxide, technically pure
- Purity: technically pure
- Assay (peroxide content): 99.00 %
- Active oxygen content: 5.86%
- Storage: Stored in original package at room temperature (max.: 30 °C)
- Batch: 113040311
- Expiry date: August 20, 2014
- Dissolved in sunflower oil (Pharma Produkt Kft, Batch: 1305-4630)

Test animals

- Species / Strain: Hsd. Brl. Han: WIST Rats. Historical control data for these animals are available and some of them are included in the confidential annex.
- Source: Toxi-Coop Zrt. 1103 Budapest, Cserkesz u. 90.
- Number of animals: 120 females, 80 males, 24 sperm positive females per group.
- Age of females at arrival: 6-9 weeks
- Body weight of females at arrival: 100-160 g
- Age of males at arrival: 9-10 weeks
- Body weight of males at arrival: 250-310 g
- Age at start of mating: females 16-18 weeks, males 21-22 weeks
- Body weight of females at mating: 192-274 g

- Acclimatisation time: 64 days for females, 82 days for males

Administration/exposure

Daily gavage 7 days per week from gestational day 5 to 19, at dose levels of 0, 50, 150 and 450 mg/kg bw/day (Table 5), 2 mL/rat/day for all groups. The dose levels were selected in agreement with the registrant based on the results from a dose range finding study (Study number: 788, 410. 4504), and MSDS data. However, the report does not include any information on the range-finding study.

Dicumyl peroxide was stable in the sunflower oil formulations at room temperature for up to 24 hours and at 5 ± 3 °C for up to five days (Validation Report Study no.: 788.l 02.4503).

Table 5. Groups and number of specimens included and analysed

Dose (mg/kg bw/day)	0	50	150	450
Number of females	24	24	24	23 (1 mortality on day 20)
Number of females with pregnant uteri, necropsied	23	20	21	17
Number of foetuses necropsied for skeletal examination	133	109	114	76
Numbers of foetuses re-evaluated for skeletal findings by BSL Bioservice (see details below)	24	23	31	26

Description of test design:

The females were paired to males in the mornings for two to four hours (1 male: 1-3 females) until each group consisted of 24 sperm positive females. Vaginal plugs were checked, and vaginal smears were examined for presence of sperm and for oestrous cycle, and the day of plug and semen was regarded as day 0 of pregnancy. Sperm positive females were separated and caged in groups of 1 to 3 animals, however individual caging was avoided if possible.

The sperm positive females were allocated such that the average body weights in the groups were as similar as possible. If possible, females paired with the same male were allocated to different groups on the same mating day.

Clinical observations were performed after dosing at least once a day. Individual observation included the check of behaviour and general condition. Duration and severity of clinical signs were recorded.

Observations for signs of morbidity and mortality were made twice daily.

The body weight of males was not measured. The body weights of females were measured at least once in the pre-mating period. Body weight of sperm positive females was measured on gestation days 0, 3, 5, 8, 11,

14, 17 and 20 (accuracy of 1 g). Corrected body weight was calculated for the 20th day of pregnancy (body weight on day 20 minus the weight of the gravid uterus).
Examination for sign of implantation: On gestation day 13, the sperm positive females were checked for the presence of vaginal bleeding indicating implantation.
Necropsy: All sperm positive females were decapitated under isoflurane anaesthesia on day 20 of gestation. Uterus with cervix and the left ovary was removed and weighed. The right ovary was placed into a Petri dish after removal. Gross pathology of dams' viscera was performed after removing the uterus. Organs and tissues with undiagnosed macroscopic findings were fixed in 4 % buffered formalin solution at necropsy for possible histological examination. Control organs were fixed for comparison.
The number of corpora lutea, implantation sites, live foetuses, early and late embryonic death and foetal death were counted. Animals with unambiguous implantation sites, but without foetuses, were considered pregnant.
Foetuses were removed from the uterus and sunk in a Petri-dish filled with water. Spontaneous movement of foetuses was observed as a viability assessment. Euthanasia of the foetuses was performed by hypothermia. The foetuses were washed with tap water and randomly laid on a filter paper with written numbering. Bleeding from the cut umbilical cord was observed as an indication of viability before euthanasia.
Each live foetus and its placenta was weighed individually (foetus’s accuracy 0.01 g, placentas accuracy 0.001 g), and examined. The gender of the foetuses was determined according to the anogenital distance. The foetuses were individually identified and about the half of each litter was subjected to visceral examination and the other half for skeletal examination. The body of those subjected to visceral examination was fixed in Sanomiya mixture. After fixation, the bodies were micro-dissected under a dissecting microscope.

The statistical evaluation was done using parametric one-way analysis of variance, and with the corresponding non-parametric test when appropriate. Duncan's multiple range test was used to test the difference between test groups. Chi-square test was used to test independence between proportions, when appropriate.

<u>Prenatal developmental toxicity study: Re-evaluation of rat foetal skeletons from Toxi-Coop ZRT study No. 788.410.4505 with dicumyl peroxide (BSL Bioservices)</u>

The study was performed at the request of the registrant. The aim of the re-evaluation was to confirm the skeletal findings reported by the original examiners of the Toxi-Coop ZRT study in order to facilitate appropriate reproductive toxicity classification of the test item. 104 pups (24%) were selected for re-evaluation. The sample included all individuals with findings classified as malformations by the original examiners. An evaluation on an individual dam/litter basis to compare effects was not performed in the re-evaluation.

Results and discussion

In total, there were 23, 20, 21 and 17 evaluated pregnant females with live foetuses at termination on gestation day 20 in the 0, 50, 150 and 450 mg/kg bw/day groups, respectively (Table 1).

In dams:

Mortality: One dam died at the 450 mg/kg bw/day dose group on gestation day 20 (the day of scheduled necropsy) with the following adverse clinical symptoms: vaginal bleeding, piloerection, paleness, coldness and hypotonicity. The death was considered by the performing laboratory to be treatment related, although it is also stated in the study report that the dam "died due to unclear reason"[1]. No mortality was observed in the 50 and 150 mg/kg bw/day dose groups.

Clinical signs: No clinical observations were noted for the dams in the 50 mg/kg bw/day dose group.
The only clinical sign in the 150 mg/kg bw/day dose group was salivation, seen in four (4/21) dams.
Salivation was seen in eight dams (8/17 dams) in the 450 mg/kg bw/day dose group. In both dose groups this observation was made mainly immediately after treatment and in one case before treatment. Salivation was judged to be treatment-related however, it was not considered an adverse effect.
In the 450 mg/kg bw/day dose group, piloerection, reduced activity, paleness, vaginal bleeding, hypotonicity and coldness were noted which was attributed to an effect of the test item. Other symptoms like red discoloration around the eye of one dam and alopecia (3/17 dams) were recorded, however; these symptoms were not considered adverse effects.

Necropsy findings: There were no macroscopic findings observed in the dams in the 50 and 150 mg/kg bw/day dose groups.
In the 450 mg/kg bw/day dose group, 11/17 dams had no macroscopic findings. In the remaining dams findings included enlarged adrenals (5/17), enlarged spleen (2/17), blood in the uterus (3/17), blood in uterine horn (1/17), uterus filled with blood (1/17) and blood in vaginal orifice (1/17). These findings were considered adverse and treatment related.

Only 4/17 dams (23 %) had both clinical signs and necropsy findings, while 5/17 dams (29 %) had no clinical signs and no necropsy findings.

For information on the clinical signs and necropsy findings on an individual basis in the high dose group see table in the confidential annex.

Food consumption: A slight, but statistically significant, and non-adverse reduction in the food consumption was seen in the 50 mg/kg bw/day dose group during the treatment period. There was a

[1] Appendix II, full study report

moderate to marked dose related decrease in the food consumption of pregnant females in the 150 and 450 mg/kg bw/day groups in the whole treatment period, which was ascribed to the treatment. The food consumption reduction in the 150 mg/kg bw/day dose group, although statistically significant, was judged to be not adverse and biologically non-relevant since the lower food consumption only resulted in a small decrease in body weight (less than 10% lower than control at the end of treatment).

Table 6. Food consumption (mean and SD)

		Dose groups			
Time, gestational days		**0 mg/kg bw/day; n=23**	**50 mg/kg bw/day; n=20**	**150 mg/kg bw/day; n=21**	**450 mg/kg bw/day; n=18 day 0-17, n=17 day 17-20**
0-3	Mean	17.2	17.3	17.7	18.1
	SD	1.84	2.18	1.41	1.56
3-5	Mean	19.8	20.5	**20.7****	**21.5**U**
	SD	1.19	2.27	**1.15**	**2.33**
5-8	Mean	19.4	**17.2****	**14.0****	**10.9**DN**
	SD	1.37	**2.16**	**1.53**	**1.43**
8-11	Mean	19.4	**17.7****	**14.5****	**11.5**U**
	SD	1.11	**2.49**	**2.47**	**2.24**
11-14	Mean	20.5	**19.1****	**16.6****	**13.9**U**
	SD	1.41	**1.52**	**1.37**	**2.38**
14-17	Mean	20.9	19.7*	**17.6****	**16.7**** U
	SD	1.29	2.57	**1.58**	**2.08**
17-20	Mean	22.0	21.8	**18.9****	**13.9**** DN
	SD	1.77	2.33	**1.58**	**2.67**

***Significantly different from controls, p < 0.01*

** Significantly different from controls, p < 0.05*

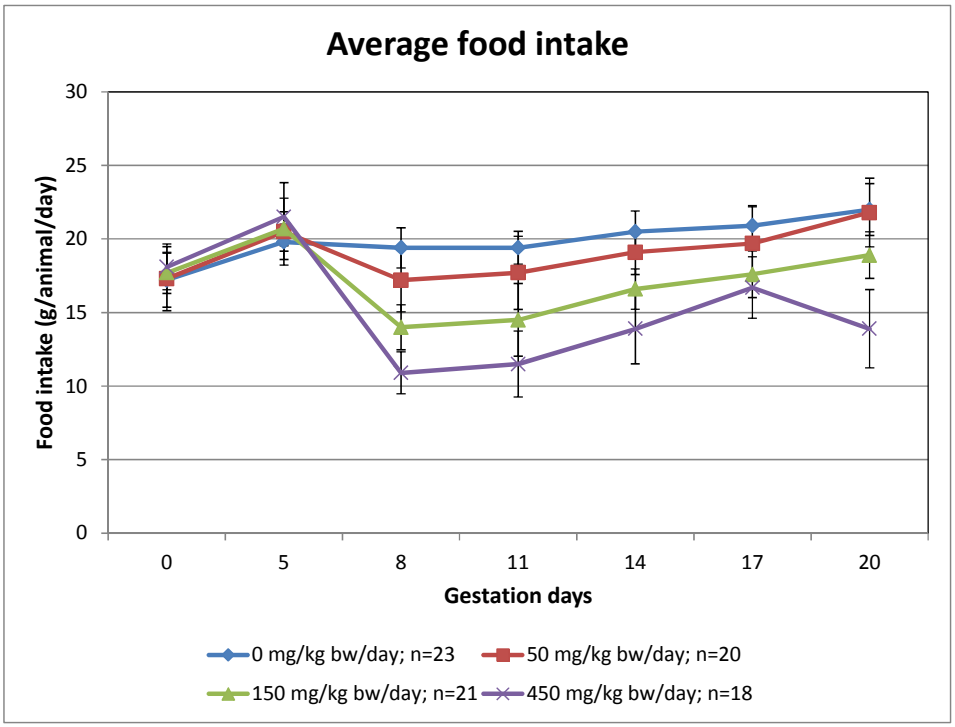

Figure 1. Average food intake

Body weight: A reduced body weight gain of the dams in the low dose group (50 mg/kg bw/day) was seen for the first three days of the treatment. There was a statistically significant and dose related reduced body weight, body weight gain, corrected body weight and corrected body weight gain in the middle and high dose groups compared to the control group. Both dose groups also showed transient decreased body weight day 5-8 of gestation. The high dose group showed in addition lower mean uterus weight. See confidential annex for more details.

Table 6. Weight and body weight gain (mean and SD)

Dosage group	**0 mg/kg bw/day;**	**50 mg/kg bw/day;**	**150 mg/kg bw/day;**	**450 mg/kg bw/day;**
	n=23	**n=20**	**n=21**	**n=18**
Start weight (g)	236 ± 20,7	236,8 ± 14,9	233,1 ± *10,7*	234,1 ± *11,0*
Weight day 11 (g)	267,3 ± 21,5	265,3 ± 16,3	**254, 8 ± 13,1***	**246,3 ± 15,2****
Weight day 20 (g)	338,7 ± 27,6	335,8 ± 20,7	**321,2 ± 14,5****	**283,6 ± 24,5****
Body weight gain (g)	102,7 ± 14,7	99 ± 13,1	**88 ± 12,8****	**49,5 ± 20****

***Significantly different from controls, p < 0.01*

** Significantly different from controls, p < 0.05*

Effects in adult animals from other studies, 28- and 90- day studies:
In the CLP guidance it is stated:

> "***3.7.2.3.1. Use of data from standard repeat dose tests, developmental effects:*** *A detailed assessment of toxicity in pregnant animals cannot be extrapolated from studies with non-pregnant animals. However, information from general toxicity studies might give an indication of the toxicity that can be expected in the dams in a developmental toxicity study."*

In two oral repeated dose toxicity studies (one 28-day oral toxicity study, OECD guideline 407; and one 90-day oral toxicity study, OECD guideline 408), no mortality was observed and the clinical effects (salivation), body weight and food intake reductions were mostly reversible in the recovery period for both sub-acute toxicity studies. In the 28-day-study, rats were exposed to 60, 200 and 600 mg/kg bw/day; thus, higher doses than in the prenatal developmental toxicity study. The NOEL was set at 60 mg/kg bw/day. In the 90-day study the dose levels were 0, 20, 80 or 320 mg/kg bw/day and the NOAEL was set at 80 mg/kg bw/day.

Toxicity in pups:

Pre- and post-implantation loss, intrauterine death:

The intrauterine development of embryos and foetuses was not statistically significant different in the 50 and 150 mg/kg bw/day dose groups compared to the control group. There was no treatment related statistically significant difference in the number of corpora lutea, implantations, early embryonic death and percentage distribution of male and female foetuses among the experimental groups.

In the 450 mg/kg bw/day dose group, there was a statistically significant increase in the post-implantation loss (17%, 15/17 litters) due to late embryonic and foetal death compared to in the control group (7 %, 14/23 litters). By consequence, the number of viable foetuses in the 450 mg/kg bw/day dose group (9.0/litter) was statistically significantly lower than in the control group (11.6/litter).

Pre-implantation loss was also statistically significantly higher in the 450 mg/kg bw/day group (14%, 14/17 litters) than in the control group (7%, 12/23 litters), however this was not higher than the historical control data. It would anyway not have been related to the test substance since pre-implantation loss happens before day 5 of gestation and thus before the first administration of the test substance.

Furthermore, a statistically significant increase in total intrauterine mortality was observed. The total intrauterine mortality in the high dose group (65 cases) was 29 % of the number of examined corpora lutea, compared to 14% in the control group.

Table 7. Intrauterine mortality, viable foetuses

Groups (mg/kg bw/day) Number of dams		Control 23	50 20	150 21	450 18
Corpora lutea	Sum	310	258	260	231
Preimplantation loss (data compared to no. of corpora lutea)	Sum %	23 7	30 12	23 9	**33**** **14§**
Implantation	Sum	287	228	237	198
Early embryonic death #	Sum %	17 6§	8 4	10 4	3* 2
Late embryonic death #	Sum %	4 1	2 1	2 1	**24**** **12**
Dead fetuses #	Sum %	0 0	0 0	0 0	**6**** **3**
Postimplantation loss#	Sum %	21 7	10 4	12 5	**33**** **17**
Total intrauterine mortality#	Sum %	44 14	40 16	35 13	**66**** **29**
Viable fetuses	Sum	266	218	225	153

* = $p < 0.05$; CH^2

** = $p < 0.01$; CH^2

(data compared to no. of implantations)

§ Within historical control data (see confidential annex for more details)

Examination of placentas: Examination of the placentas revealed dark brownish discoloration (29%) and fibrinoid degeneration (7%) in the 450 mg/kg bw/day group and were considered treatment related. There were no differences in the absolute and relative placental weights between the experimental groups.

Examination of foetuses:

No pathological examination was done on the foetuses from the deceased dam – examination of foetuses from deceased dams is usually conducted when the death occurs on the day of scheduled necropsy so this should have been done. The data below applies for the pups of the live dams.

Body weight: The mean body weight of the foetuses in the control, 50 and 150 mg/kg bw/day groups were identical. The foetal body weight was statistically significantly lower in the 450 mg/kg bw/day dose group compared to the control group.

Table 8. Litter means of fetal weight (g)

	DOSE GROUPS (mg/kg bw/day)			
	Control	50	150	450
n	23	20	21	17
MEAN	3.3	3.3	3.3	2.9**
SD	0.18	0.19	0.19	0.29**

** = $p < 0.01$; CH^2

External examination: There were no statistically significant findings in the control, 50 and 150 mg/kg bw/day dose groups. Whereas there was a statistically significant increase in foetuses with abnormalities, foetuses with variations and foetuses with malformations in the high dose group compared to the control group. External malformations such as malrotated fore and/or hind limbs were seen in 6 of 153 foetuses in the high dose group and was judged to be an effect of the treatment of the dams.

Table 9. Summary of findings in external, visceral and skeletal examination of the foetuses (percentile litter mean and SD).

		Dose groups (mg/kg bw/day			
		Control	**50**	**150**	**450**
Litters examined	**N**	**23**	**20**	**21**	**17**
External examination					
Foetuses examined	N	266	218	225	153
Foetuses with abnormalities	Mean SD	2.5 5.46	2.3 4.12	3.5 5.59	**26.2 ** U** **24.34**
Variations	Mean SD	2.5 5.46	2.3 4.12	3.5 5.59	**21.5 ** U** **24.62**

Malformations	Mean SD	0.0 0.00	0.0 0.00	0.0 0.00	**4.7** DN** **9.03**
Retarded in body weight	Mean SD	2.5 5.46	2.3 4.12	3.5 5.59	**22.2** U** **23.72**
Visceral examination					
Foetuses examined	N	133	109	111	77
Foetuses with abnormalities	Mean SD	1.3 4.47	2.0 8.94	1.0 4.36	2.0 NS 5.78
Variations	Mean SD	0.0 0.00	2.0 8.94	0.0 0.00	2.0 NS 5.78
Malformations	Mean SD	1.3 4.47	0.0 0.00	1.0 4.36	0.0 NS 0.00
Skeletal examination					
Foetuses examined	N	133	109	114	76
Foetuses with abnormalities	Mean SD	19.4 21.32	15.0 24.29	22.7 31.81	**61.4 ** DN** **30.69**
Variations	Mean SD	17.8 19.61	15.0 24.29	19.9 27.56	**39.8 ** DN** **23.91**
Malformations	Mean SD	1.6 5.31	0.0 0.00	2.9 9.56	**21.6** U** **28.75**

** = $p < 0.01$; CH^2

Visceral examination: there were no adverse, or toxicologically significant, effects in any of the groups. There were three foetuses with visceral malformations found, one in the 150 mg/kg bw/day and two in the control group. One of the foetuses in the 150 mg/kg bw/day dose group was found with absent lung lobes and situs inversus totalis (total reversal of organs). One of the foetuses in the control group had also situs inversus totalis and the other one absent brain tissue. There were no visceral malformations in the 50 and 450 mg/kg bw/day groups.

Skeletal examination, variations: There was no treatment related findings of skeletal variations in the 50 and 150 mg/kg bw/day dose groups. There was a statistically significantly higher incidence of skeletal variations in the high dose group compared to the control group. See table below for types of variations seen.

Table 10. Types of skeletal abnormalities, variations.

		Dose groups (mg/kg bw/day			
		Control	**50**	**150**	**450**
Number of Dams	N	23	20	21	17
Number of Foetuses examined	N	133	109	114	76
Number of Foetuses with abnormalities	N %	27 20	15 14	24 21	44 ** 58
Variations	N %	25 19	15 14	21 18	30 ** 39
Malformations	N %	2 2	0 0	3 3	14 ** 18
Foetal variations					

Skull					
Incomplete ossification, marked (> three bones)	N %	1 1	0 0	1 1	2 3
Incomplete ossification, marked (1 bone or more)	N %	3 2	2 2	4 4	10 * 13
Supraoccipital not ossified	N %	0 0	1 1	1 1	1 1
Hyoid not ossified	N %	1 1	0 0	0 0	1 1
Sternabra					
Three or less ossified	N %	5 4	2 2	8 7	10* 13
Misaligned	N %	1 1	0 0	0 0	0 0
Bipartite	N %	0 0	0 0	0 0	1 1
Ribs					
Wavy	N %	8 6	7 6	16* 14	24** 32
Wavy, marked	N %	0 0	1 1	1 1	6** 8

* = p<0,05

** = p<0,01

Skeletal examination, malformations:

There were no treatment related findings of skeletal malformations in the 50 and 150 mg/kg bw/day dose groups. There was a statistically significantly higher incidence of skeletal malformations in the high dose group compared to the control group. See table below for types of malformations seen.

Table 11. Types of skeletal abnormalities, malformations.

		Dose groups (mg/kg bw/day			
		Control	50	150	450
Number of Dams	N	23	20	21	17
Number of Foetuses examined	N	133	109	114	76
Foetal malformations					
Sternebra					
- Xiphoid split	N %	1 1	0 0	1 1	2 3
Vertebrae, thoracic centra					
- thoracic bipartite cartilage dumb-bell shaped	N %	2 2	0 0	0 0	0 0
Pectoral girdle					
- Scapula bent and/or short	N %	0 0	0 0	3 3	12 ** 16
- Clavicula bent and/or	N	0	0	0	2

short	%	0	0	0	3
Forelimbs					
- Humerus bent and/or short	N %	0 0	0 0	0 0	9 ** 12
- Ulna bent and/or short	N %	0 0	0 0	0 0	6 ** 8
- Radius bent and/or short	N %	0 0	0 0	0 0	8** 11
Hindlimbs					
- Femur short, bent	N %	0 0	0 0	0 0	4** 5
- Tibia bent and/or short	N %	0 0	0 0	0 0	2 3
- Fibula bent and/or short	N %	0 0	0 0	0 0	3* 4

* = p<0,05

** = p<0,01

The study report concluded that the test item related changes in the intrauterine parameters, body weight retardation; external and skeletal malformations of foetuses in the 450 mg/kg bw/day dose group were ascribed to maternal toxicity. There was however no assessment of correlation between data of individual dams to individual pups.

The test report concludes with the following:
NOEL maternal toxicity: 50 mg/kg bw/day
NOAEL maternal toxicity: 150 mg/kg bw/day
NOAEL for developmental toxicity: 150 mg/kg bw/day

Results of the re-evaluation study (BSL Bioservices)
There were some small differences in findings in the two examinations. At re-examination some structures that had not been noted at the original examination were found to be abnormal and there were occasional cases where structures found to be abnormal at the first examination was not confirmed or considered to show a different severity grading at the re-examination. There were numerous small discrepancies in the ossification data, however the re-examinators concluded nevertheless that the result of the re-examination was that the skeletal findings critical to the result of the study were considered reliable and the conclusions reached at the original study was acceptable.

The re-evaluation did not assess effects in the dams. The conclusion did however note that although there was clear maternal toxicity in the study it was recommended that further analyses should be performed on an individual dam/litter basis to see if there were links between maternal effects in a particular dam and findings in the foetuses derived from the same dam.

Overall, the re-evaluation did not provide any new information relevant to this CLH-dossier.

Non-guideline supporting study:

The registrant has included a non-guideline embryotoxicity study in white leghorn chicken embryos in the registrations. Dicumyl peroxide was administered to three-day old chick embryos in the inner shell membrane of air chamber at the following doses: 0.38, 0.75, 1.5 and 3.0 µmole/egg. 30 eggs/dose. Treatment time was 14 days. This study shows a high frequency of malformations (defects of the right eye and right wing, twisting and stunting of the back, and defects of the coelomic wall) observed in chicken embryos exposed to dicumyl peroxide. The NOAEC was 0.38 µmole/egg. "Number of early deaths (after 2d) were only about 20% (7 and 6 from 30 eggs at 1.5 and 3.0 µmole/egg, respectively). At a concentration of 3.0 µmole/egg 16 late deaths showed malformation at day 14 (53% of initial). 8 and 7 late deaths showed malformation at 0.75 and 1.5 µmole/egg, respectively. The authors discussed that the low rate of early deaths may be related to the fact that the chemical was not lethal at the doses tested or that high lethal concentrations did not reach the embryo during the 2 days, but lower teratogenic doses did."[2]

Discussion and Conclusion

Maternal toxicity correlated to foetal toxicity

There is a toxic effect of the substance in both the dams and the foetuses of the high dose group. There is an increase in some clinical signs as well as reduced body weight gain, reduced food intake and some necropsy findings in the dams of the high dose group compared to the control. These parameters are affected in a dose-related manner.

There is also a clear effect of the test substance on the foetuses of the high dose group, manifested as increased intrauterine mortality, lower foetal weight and an increase in the incidence of variations and malformations in the pups in the high dose group compared to the control group.

However, the report refers to maternal toxicity as the main cause of the findings in the high dose group. The dossier submitter cannot see that there is basis for this statement in the data. Neither the laboratory performing the original study, nor the consultant performing the re-evaluation of the skeletal findings, assessed the relationship between the *individual* dams with symptoms of maternal toxicity and the *individual* pups with presence of skeletal abnormalities, or other signs of developmental effects such as post-implantation loss or intrauterine mortality. The consultant performing the re-evaluation did however point out that this should be done.

[2] ECHA registration site: https://echa.europa.eu/registration-dossier/-/registered-dossier/14703/7/9/4

The dossier submitter has therefore looked into the full study report in order to see whether there seems to be a link between the toxicity seen in the individual dams and the toxicity seen in the respective litters. Such a link has not been found.

There seems to be no direct individual correspondence between maternal weight, food intake and the presence of malformations. The incidence and percentage of skeletal malformations increased with statistically significance in the 450 mg/kg bw/day group, while it was at the control level in the 50 and 150 mg/kg bw/day groups. Maternal toxicity is apparent in the high dose group in the present study, however there is not a clear connection between maternal toxicity and foetal malformation. An attempt to correlate the number of malformations to uterine weight, corrected weight gain or similar observations related to maternal toxicity did not reveal any correlation coefficient statistically significantly different from zero. Non-parametric methods did not show any stronger correlations. There cannot be seen correlation between malformations and clinical signs; nor is there correlation between number of/percentage malformed foetuses and pathological findings. There is no correlation between malformations and any maternal weight parameter.

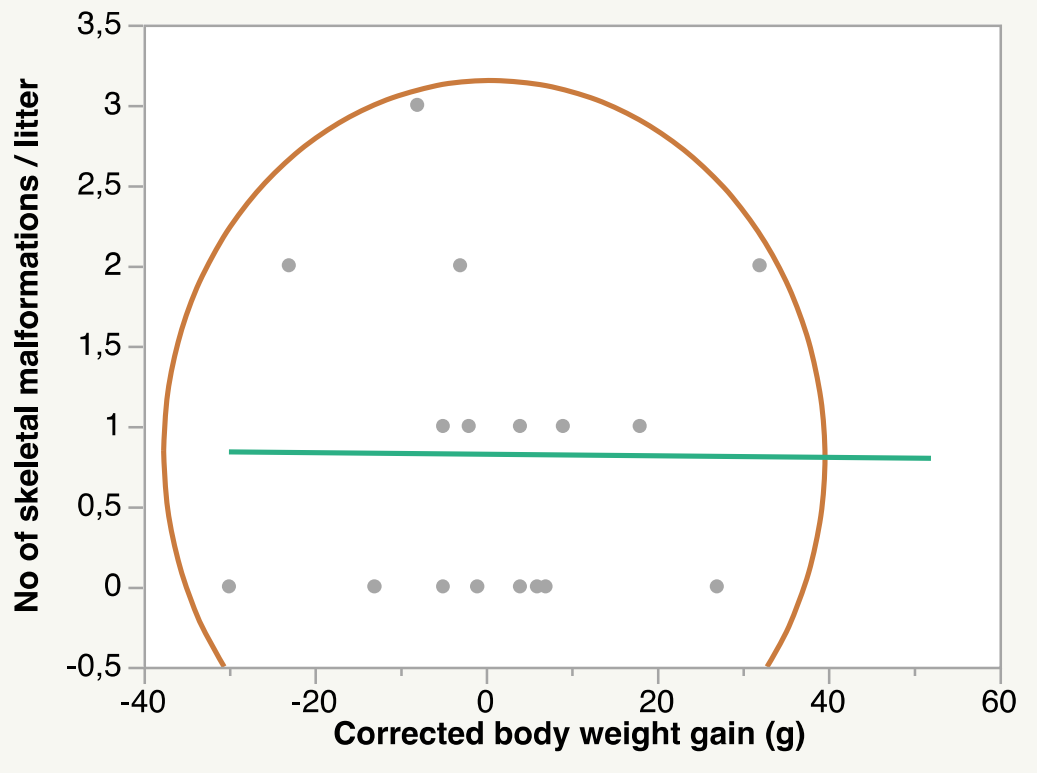

Figure 2 Correlation between the dam's body weight and malformations, high dose group. The 95% density ellipse is not different from a circle, and the regression line has a slope of zero, both indicating no relationship between the observations.

If we hypothesise that the malformations are caused by maternal toxicity, there should be a high correlation between the number of malformations in each litter and maternal weight gain. Figure 2 illustrates that there

is no such correlation. Similarly, no other correlation can be found between signs of maternal toxicity and malformations in the 450 mg/kg bw group.

Nor was the observed maternal stress/toxicity in the high dose group severe enough to support the idea that dicumyl peroxide is extremely toxic. The main signs of toxicity in the dams was reduced body weight gain and salivation. The one case of mortality can probably not be ascribed to the test substance, as other studies with far higher doses did not cause any mortalities. 5/17 dams had no clinical signs nor necropsy findings. Another 5 dams had salivation and/or alopecia as only clinical signs and no necropsy findings. The lack of severe maternal toxicity is therefore also an argument against the assumption that the developmental effects seen in the pups were only due to maternal toxicity.

Intrauterine mortality

In addition, it has not been possible to relate the higher incidence of intra-uterine mortality to the maternal toxicity. There was a statistically significant increase in post-implantation loss, late embryonic death, foetal death, total intra-uterine mortality, and a statistically significant reduced number of viable foetuses in the high-dose group. When the findings were studied on an individual basis it was seen that there was no clear correlation between the dams with clinical signs of toxicity and necropsy findings and the intra-uterine mortality. These findings can therefore not be ascribed to maternal toxicity.

See figure 2 in the confidential annex showing individual data for the dams and corresponding litter/foetal data.

Conclusions: From maternal toxicity results, at the high dose group (450 mg/kg bw/day), only one dam died and of unclear reasons; 5/17 dams had no clinical signs nor necropsy findings. Another 5 dams had salivation and/or alopecia as only clinical signs and no necropsy findings. Treatment of pregnant female rats with dicumyl peroxide at the dose level of 450 mg/kg bw/day resulted in increased post-implantation loss, late embryonic death, foetal death and total intra-uterine mortality, increased incidences of body weight retarded foetuses, mal-rotated fore- and hind-limbs and increased incidence of skeletal malformations in the absence of adverse maternal clinical signs. Dicumyl peroxide is embryotoxic and leads to malformations and a higher incidence of intra-uterine mortality, and the data does not support the statement that this is attributed to maternal toxic effects. The dicumyl peroxide developmental toxicity effects could not be directly connected to maternal toxicity when the results were evaluated on an individual basis.

3.10.2 Human data

No human data

3.10.3 Other data (e.g. studies on mechanism of action)

No other data

3.11 Specific target organ toxicity – single exposure

Not evaluated for this dossier.

3.12 Specific target organ toxicity – repeated exposure

Not evaluated for this dossier.

3.13 Aspiration hazard

Not evaluated for this dossier.

4 ENVIRONMENTAL HAZARDS

Not evaluated for this dossier.