Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

bis(α,α-dimethylbenzyl) peroxide

EC Number: 201-279-3
CAS Number: 80-43-3

CLH-O-0000001412-86-217/F

Adopted

8 June 2018

8 June 2018

CLH-O-0000001412-86-217/F

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

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

Chemical name: **bis(α,α-dimethylbenzyl) peroxide**

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

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

The proposal was submitted by **Norway** and received by RAC on **28 April 2017.**

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

PROCESS FOR ADOPTION OF THE OPINION

Norway has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **20 June 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **4 August 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Peter Hammer Sørensen**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **8 June 2018** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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	617-006-00-X	bis(α,α-dimethylbenzyl) peroxide	201-279-3	80-43-3	Org. Perox. F Skin Irrit. 2 Eye Irrit. 2 Aquatic Chronic 2	H242 H315 H319 H411	GHS02 GHS09 GHS07 Wng	H242 H315 H319 H411			
Dossier submitters proposal	617-006-00-X	bis(α,α-dimethylbenzyl) peroxide	201-279-3	80-43-3	**Add** Repr. 2 **Remove** Skin Irrit. 2 Eye Irrit. 2	**Add** H361d **Remove** H315 H319	**Add** GHS08 **Remove** GHS07	**Add** H361d **Remove** H315 H319			
RAC opinion	617-006-00-X	bis(α,α-dimethylbenzyl) peroxide	201-279-3	80-43-3	**Add** Repr. 1B **Retain** Skin Irrit. 2 Eye Irrit. 2	**Add** H360D **Retain** H315 H319	**Add** GHS08 **Retain** GHS07	**Add** H360D **Retain** H315 H319			
Resulting Annex VI entry if agreed by COM	617-006-00-X	bis(α,α-dimethylbenzyl) peroxide	201-279-3	80-43-3	Org. Perox. F Repr. 1B Skin Irrit. 2 Eye Irrit. 2 Aquatic Chronic 2	H242 H360D H315 H319 H411	GHS02 GHS07 GHS08 GHS09 Dgr	H242 H360D H315 H319 H411			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The current proposal for harmonised classification for bis(α,α-dimethylbenzyl) peroxide is intended to cover new data on developmental toxicity that has become available. However, in the process of evaluating the substance it was discovered that the current harmonised classifications for skin and eye irritation were not supported by the available data in the current REACH registration dossier. These classifications date back to before the CLP Regulation came into force and the grounds at that time, for giving this substance a harmonised classification as an irritant, have not been found. Although skin and eye irritation are not prioritised endpoints, while proposing a classification for reproduction toxicity, the dossier submitter (DS) proposed to remove the former classifications at the same time.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS included a study on New Zealand White rabbits to assess the skin irritation potential of bis(α,α-dimethylbenzyl) peroxide. The study was mainly performed according to GLP and OECD TG 404. There was however one deviation from the guideline: the laboratory had not used a vehicle with the test substance, which was a crystalline powder. It may be that the substance did not show its true irritating potential when applied in dry form, as in the OECD TG 404, it is stated that the smallest amount of liquid necessary in order to ensure good skin contact should be used.

The effects of the test substance on the skin were very slight, grade one for both erythema and oedema, and were seen in only two out of three rabbits. The mean scores at 24, 48 and 72 hours were below one for both effects and in all three rabbits and were reversed at 72 hours, see table 9 on the background document. As the study results were below the classification criteria for skin irritation, the DS proposed to remove the existing classification.

Comments received during public consultation

Four MSCAs supported the DS proposal to remove the existing classification. Two MSCAs noted that no vehicle was used in the study and that the purpose of the vehicle was to optimise the contact between the solid substance and the skin. These two MSCAs considered there was a reasonable possibility of an increased skin reaction if the substance had been applied with a vehicle, and thus they questioned whether this study should be considered sufficiently robust to declassify the substance. One of these MSCAs disagreed with the DS proposal to declassify because in their opinion the study suffered from a serious deficiency.

Assessment and comparison with the classification criteria

RAC agrees that the skin irritation study was deficient since the laboratory did not use a vehicle in administrating the test substance, which is lipophilic with a Log K_{OW} of 5.6 and was

administered in a crystalline state, raising doubts as to whether without a vehicle, it was made sufficiently bioavailable in the test.

In addition RAC noted that according to ECHA Guidance on the Application of the CLP Criteria (which cross refers to ECHA Guidance on Information Requirements & Chemical Safety Assessment, Chapter R7, section R.7.2.6.2 "Testing and assessment strategy for skin corrosion/irritation"), if a substance is a peroxide it can be considered as a skin irritant Cat. 2[1]. Given the uncertainty of the available test data, the mentioned 'evidence to the contrary' is lacking.

RAC recommended not to remove the current classification based on lack of proper data and in conclusion, agreed in line with the guidance to **retain the current classification of Skin Irrit. 2; H315.**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS included a study on Himalayan rabbits to assess the eye irritation potential of bis(α,α-dimethylbenzyl) peroxide. The study was conducted in 2010 according to OECD TG 405. There were some deviations however, such as a lack of information on the presence of impurities. There was also a lack of information on the application of anaesthesia, which is a requirement in the most recent version of the guideline, but was to be applied on a case-by-case basis in an earlier version used at the time of the study. The laboratory used Himalayan rabbits, which were not albino rabbits and according to the guideline a justification must be given if the albino rabbit is not used. Such a justification was not given in the study report.

There was a small degree of opacity seen in the cornea of animal no. 3 at 24 and 48 hours. Grade 1 opacity was 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 was 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 the control did not show any pathological changes. No other effects were reported in the report, see table 12 in the background document.

As the values from the study results were below those in the classification criteria for eye irritation, the DS proposed to remove the existing classification.

Comments received during public consultation

Four MSCAs supported the DS proposal to remove the existing classification.

[1] **Figure R.7.2-2 line 1b** Consider classifying as: corrosive (Skin Corrosive Cat. 1B) if the substance is a hydroperoxide, or irritating (Skin Irritant Cat. 2) if the substance is a peroxide **OR** Provide evidence for the contrary.

Assessment and comparison with the classification criteria

RAC agrees with the DS that the test substance had only a slight eye irritant effect in rabbits and the effects were reversible at 72 hours after administration. However, considering the lipophilicity and the very low water solubility, the effects seen could be related to a physical/mechanical irritation of the particles in the eye. To adequately observe the irritation effects of this lipophilic substance, an appropriate vehicle should have been used.

In conclusion RAC agreed to **retain the current classification as Eye Irrit. 2; H319**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS did not include studies on adverse effects on sexual function and fertility.

One developmental toxicity study in Wistar rats was performed according to OECD TG 414. No information on the GLP status was included in the CLH dossier. The study findings are summarised in the table below.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Developmental toxicity study (OECD guideline 414) Rats: 24 sperm-positive Wistar rats/treatment group Reliability score: 1	Bis(α,α-dimethylbenzyl) peroxide Purity: 99.0% Oral: gavage 0, 50, 150, 450 mg/kg bw/day vehicle: sunflower oil Exposure: Days 5-19 of gestation (daily)	Maternal and developmental NOAEL: 150 mg/kg bw/day Maternal and developmental LOAEL: 450 mg/kg bw/day **Maternal toxicity:** Mortality: **Control, 50 and 150 mg/kg** bw/day dose groups: No mortality **450 mg/kg** bw/day dose group: one dam died on gestation day 20 (the day of scheduled necropsy) Clinical symptoms: **control group**: alopecia in one female **50 mg/kg** bw/day dose group: no clinical symptoms. **150 mg/kg** bw/day dose group: salivation (4/21 dams). **450 mg/kg** bw/day dose group: No clinical signs in 7/17 dams. Salivation (8/17 dams); piloerection (3/17 dams); alopecia (3/17); reduced activity, vaginal bleeding, pale, cold, hypo tonicity and red colouration around red eye (deceased dam). Necropsy findings: **0, 50 and 150 mg/kg** bw/day dose groups: no necropsy findings. **450 mg/kg** bw/day dose group: No necropsy findings in 11/17 dams. In the remaining dams: enlarged adrenals (4/17 dams); blood in uterus (3/17); enlarged spleen (2/17); uterus filled up with blood (1/17); stomach distended filled up with darker content (1/17); pale liver and pale kidneys (1/17). See confidential	Study report 788.410.4505, Toxi-Coop Zrt. (2014) (not published)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		annex for individual data. Food consumption: **50 mg/kg** bw/day dose group: a statistically significant temporary decrease in food intake was recorded. **150 and 450 mg/kg** bw/day dose groups: a statistically significant dose related decrease in the food consumption was recorded in the whole treatment period. Body weight: **50 mg/kg** bw/day dose group: a transient decrease in body weight gain. **150 and 450 mg/kg** bw/day dose groups: lower mean body weight, lower corrected body weight, transient weight loss day 5-8 of gestation, and markedly reduced body weight gain and corrected body weight were observed. See annex I and confidential annex for more details. All treatment groups had positive weight gain at the end of treatment period compared with the start weight. **Foetal toxicity:** **50 and 150 mg/kg** bw/day dose groups: no statistically significant effect on the intrauterine development of embryos and foetuses. **450 mg/kg** bw/day dose group: statistically significant increase in the post-implantation loss (17%, 15/17 litters) 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). 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. Foetal weight: **50 and 150 mg/kg** bw/day dose groups: no statistically significant decrease in the pup's body weight compared with control group. **450 mg/kg** bw/day dose group: increase in percentage of foetuses with decreased body weight (11/17 litters; 31 cases) compared with control group (5/11; 6 cases). External malformations: **50 and 150 mg/kg** bw/day dose groups: no external malformations were observed. **450 mg/kg** bw/day dose group: mal-rotated fore- and hind limbs in six foetuses (5/17 litters; 6 cases; statistically significant) and hydrops fetalis in one	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		foetus. Visceral variations: Hydroureter (bilateral) in 4 pups **50 mg/kg** bw/day dose group: hydroureter (bilateral) in two cases. **150 mg/kg** bw/day dose group: no visceral variations. **450 mg/kg** bw/day dose group: hydroureter (bilateral) in two cases (in two litters). Visceral malformations: four malformations in three pups **control group**: one pup with an absent brain tissue and one with situs intersus totalis **50 mg/kg** bw/day dose group: no visceral malformations. **150 mg/kg** bw/day dose group: one pup with absent lung lobes and with situs intersus totalis. **450 mg/kg** bw/day dose group: no visceral malformations. Skeletal variations: **50 mg/kg** bw/day dose group: incomplete ossified sternum (2 cases; 2/20 litters), incomplete ossification marked of skull bones (2 cases; 2/20 litters), one case of not ossified supraoccipital, thoracic or lumbar centra (3 cases; 3/20 litters) and 7 cases (3/20 litters) of wavy ribs. **150 mg/kg** bw/day dose group: incomplete ossified sternum (8 cases; 5/21 litters), one case incomplete ossification (more than three bones), 4 cases (1/21 litters) of incomplete ossification marked of skull bones, one case of not ossified supraoccipital, thoracic or lumbar centra (2 cases; 2/21 litters) and 16 cases (7/21 litters) of wavy ribs. **450 mg/kg** bw/day dose group: incomplete ossification of skull bones (10 cases; 8/17 litters), incomplete ossified sternum (10 cases; 9/17 litters), metacarpal/metatarsal (4 cases; 4/17 litters), thoracic or lumbar centra (4 cases; 4/17 litters) and wavy (24 cases; 11/17 litters) and marked wavy ribs (6 cases; 5/17 litters). Skeletal malformations: **50 mg/kg** bw/day dose group: no skeletal malformations. **150 mg/kg** bw/day dose group: short and/or bent scapula (3 cases; 2/21 litters). **450 mg/kg** bw/day dose group: short and/or bent scapula (12 cases, 9/17 litters), clavicular (2 cases; 2/17 litters), humerus (9 cases; 7/17 litters), radius (8 cases; 7/11 litters) and ulna (5 cases; 5/17 litters).	

One developmental toxicity study in rats had been performed with exposure to bis(α,α-dimethylbenzyl) peroxide, and the maternal and foetal toxicity findings are presented in the table above.

The table below shows the groups and number of animals/foetuses included and analysed in the study.

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

Animals treated with bis(α,α-dimethylbenzyl) peroxide exhibited increased signs of toxicity from the low dose (transient decrease in food consumption and body weight gain) to the high dose, where adverse clinical symptoms some necropsy findings, weight loss, markedly reduced body weight gain, and reduced food consumption were observed. These effects were marked in dams of the highest treatment group (450 mg/kg bw/day) and this dose is considered a LOAEL for maternal effects.

Mortality, clinical symptoms, necropsy

One dam died in 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. However, there were no pathological examination data of the foetuses from the deceased dam: usually examination of foetuses from deceased dams is conducted when the death occurs on the day of scheduled necropsy. The death was considered by the performing laboratory to be treatment related, although it was stated in the study report that the dam "died due to unclear reason". No mortality was observed in the 50 and 150 mg/kg bw/day dose groups.

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. Salivation was judged to be treatment-related however, it was not considered an adverse effect. In the 450 mg/kg bw/day dose group, 1/17 dams had vaginal bleeding, 3/17 had piloerection and 1/17 was hypotonic. These were considered adverse clinical signs and an effect of the test item.

Necropsy findings in the high dose group were: 4/17 dams had enlarged adrenals and bloody uterine content (blood in the uterus (2/17 dams), blood in uterine horn (1/17 dam) and uterus filled with blood (1/17 dam)). One dam had an enlarged spleen. These findings were considered to be treatment related. There were no necropsy findings in the remaining 11/17 dams examined in the high dose group.

Overall, a majority of the examined dams did not have adverse clinical symptoms, and only 4/17 dams (23%) had both adverse clinical signs and necropsy findings, while 5/17 dams (29%) had no adverse clinical signs and no necropsy findings. Another 5 dams (29%) had salivation and/or alopecia as only clinical signs and no necropsy findings.

Food consumption

Evaluation of food consumption data showed that there was a test substance treatment related decrease in the average food intake in the 150 and 450 mg/kg bw/day dose groups and a temporary decrease in the average food intake in the 50 mg/kg bw/day dose group. The food consumption reduction in the 150 mg/kg bw/day dose group, although statistically significant, was judged not to be adverse and was considered biologically non-relevant since the lower food consumption only resulted in a small reduction of body weight (less than 10% lower than the control group). When the individual food consumption data for the dams in the high dose group were compared with the data for observed clinical signs and its adversity, there was no clear correlation between lower food intake and adverse clinical symptoms.

Body weight

Evaluation of the body weight parameters showed a dose-dependent decrease in all recorded body weight parameters, for the 150 and 450 mg/kg bw/day dose groups. The decrease in the body weight parameters were considered to be related to the test item. Further, a transient reduced body weight gain was noticed in the 50 mg/kg bw/day dose group and it was considered to be a non-adverse effect. In the 150 mg/kg bw/day group the body weight reduction at the end of treatment was less than 10% lower than the control group, however body weight gain was reduced by 15%. The dams of the high dose group had a body weight at the end of treatment that was 17% lower than the control dams, however the body weight gain was about half of the gain seen in the control group. At the end of the treatment period, all dams in all treatment groups gained some weight compared with the start weight.

The body weight parameters of the dams with adverse clinical signs did not differ with statistical significance from the dams without such signs. Thus, reduction both in food intake and body weight gain alone could not explain the observed clinical signs and necropsy findings.

Toxicity in pups

In the 450 mg/kg bw/day dose group, examination of the dams showed a statistically significant increase in post-implantation loss (17%, 15/17 litters) compared with the control group (7%, 14/23 litters). There were 32 cases of post-implantation loss. Ten of these cases occurred in five dams without clinical or necropsy findings.

A statistically significant decrease in the number of viable foetuses was observed in the high dose group and this was considered treatment related. Furthermore, a statistically significant increase in total intrauterine mortality was observed. There were 65 cases of intrauterine mortality (29% vs 14% in the control groups). Five dams with no clinical signs or necropsy findings had 20 cases (20/65) of the total intrauterine mortality; i.e., ~1/3 of the total intrauterine mortality was found in dams without any adverse clinical symptoms or necropsy findings.

This suggests that post-implantation loss and increased intrauterine mortality was not related to maternal clinical symptoms nor necropsy findings in the dams and thus raises a concern for the developmental effects of bis(α,α-dimethylbenzyl) peroxide.

Furthermore, there was an increase in the percentage of foetuses with body weight retardation in the 450 mg/kg bw/day dose group (11/17 litters; 31 cases) compared with the control group (5/23; 6 cases). These observations could not be explained by maternal toxicity, since several dams without adverse clinical signs, necropsy findings, or drastically reduced body weight or food intake, had foetuses with decreased body weight. There was no difference in the incidence of pups with decreased body weight in the 50 (5/20 litters; 5 cases) and 150 (7/21 litters; 8 cases) mg/kg bw/day dose groups compared with the control group (5/23 litters; 6 cases).

External examination of the pups in the 450 mg/kg bw/day dose group showed malrotated fore- and hind limbs in six foetuses (5/17 litters; 6 cases, statistically significant) and hydrops fetalis in one foetus. This was considered to be treatment related. Of the six cases with malrotated fore- and hind limbs, none of them were from the 3/17 dams with adverse clinical symptoms, and 3/6 cases were from two dams with no clinical and necropsy findings.

There was a high incidence of foetuses with skeletal malformations in the 450 mg/kg bw/day dose group: short and/or bent scapula (12 cases, 9/17 litters), clavicular (2 cases; 2/17 litters), humerus (9 cases; 7/17 litters), radius (8 cases; 7/11 litters) and ulna (5 cases; 5/17 litters). In the 150 mg/kg bw/day dose group, short and/or bent scapula (3 cases; 2/21 litters) were recorded. This high incidence of malformations, without marked maternal toxicity, is sufficient to raise a concern about the developmental effects of bis(α,α-dimethylbenzyl) peroxide.

In the 450 mg/kg bw/day dose group, there was a statistically significant increase in the incidence of skeletal variations such as incomplete ossification of skull bones, incomplete ossified sternum, metacarpal/metatarsal, and wavy and marked wavy ribs, and these incidences occurred without adverse maternal toxicity. Similarly, in the 150 mg/kg bw/day dose group, some variations were observed without clear correlation to maternal clinical signs.

Maternal toxicity is apparent in the present study, but there is no clear connection between maternal toxicity and foetal malformations, not even in the high dose group. This indicates that the developing foetuses are more sensitive than the dams to exposure to the test substance. The evaluation of the presented data supports the conclusion that the observed developmental effects following the exposure to bis(α,α-dimethylbenzyl) peroxide are not secondary non-specific consequences of maternal toxicity.

The DS proposed to classify bis(α,α-dimethylbenzyl) peroxide as Repr. Cat. 2; H361d.

Comments received during public consultation

Three MSCAs agreed with the DS proposal for classification in Repr. Cat. 2 for development.

There was disagreement from the company-manufacturer, based on limited evidence only seen from the high dose group and no clear dose-response relationship was observed. In addition, the company-manufacturer pointed out that, marked maternal toxicity was apparent in the high dose group and this may account for the foetal toxicity. A testing proposal related to a PND study in the rabbit as a second species is ongoing. The study may be available within 1 year, therefore the company argued that the assessment for teratogenicity should be discussed when the data are available.

Assessment and comparison with the classification criteria

Reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring. For bis(α,α-dimethylbenzyl) peroxide findings concerned developmental toxicity, which was described in the CLP Annex 1: 3.7.1.4 "Adverse effects on development of the offspring".

Only one prenatal developmental toxicity study in rats was available, performed according to OECD TG 414.

The table below shows the maternal toxicity findings.

Effects	Control	50 mg/kg bw/d	150 mg/kg bw/d	450 mg/kg bw/d
Mortality	0	0	0	1
Salivation	0	0	4/21	8/17
Piloerection	0	0	0	3/17
alopecia	0	0	0	3/17
Clinical signs: (Reduced activity, vaginal bleeding, pale, cold, hypotonicity, red colouration around eye)	0	0	0	10/17
Necropsy finding Enlarged adrenals Blood in uterus Enlarged spleen Uterus filled with blood Stomach distended fill up Pale liver Pale kidney	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	6/17 4/17 3/17 2/17 1/17 1/17 1/17
Food consumption	None	A statistically sign. temporary decrease was recorded.	Statistically sign. decrease was recorded	Statistically sign. decrease was recorded
Body weight Start weight (g) Weight day 11 (g) Weight day 20 (g) Body weight gain (g)	 236 ± 20.7 267.3 ± 21.5 338.7 ± 27.6 102.7 ± 14.7	 236.8 ± 14.9 265.3 ± 16.3 335.8 ± 20.7 99 ± 13.1	 233.1 ± 10.7 **254. 8 ± 13.1*** **321.2 ± 14.5**** **88 ± 12.8****	 234.1 ± 11.0 **246.3 ± 15.2**** **283.6 ± 24.5**** **49.5 ± 20****

** p<0.01

Body weight: The DS argued that the observed body weight parameters of the dams with adverse clinical signs did not differ statistically significantly from the dams without such signs. Thus, reduction in food intake and body weight gain alone could not explain the observed clinical signs and necropsy findings. In addition, no corrected body weight values were given, and the dams at high does had an average of 9.0 pups per litter which was statistically significantly lower than in the control group (11.6/litter). Furthermore, the high dose pups had a lower mean foetal weight, 2.9 vs 3.3 g in the control group. The lower number of pups and their lower weight may explain the lower uncorrected body weight.

RAC identifies the following observations as relevant for the assessment of the developmental toxicity/ teratogenicity classification.

Effects	Control	50 mg/kg bw/d	150 mg/kg bw/d	450 mg/kg bw/d
Pre implantation loss	7%	12%	9%	**14%****
Post implantation loss	7% (14/23 litters)	4%	5%	**17%** (15/17 litters)**
Late embryonic death	1%	1%	1%	**12%****
Dead foetuses	0%	0%	0%	**3%****
Total intrauterine mortality	14%	16%	13%	**29%****
External examination				
Foetuses with abnormalities	2.5%	2.3%	3.5%	**26.2%****
Variations	2.5%	2.3%	3.5%	**21.5%****
Malformations	0%	0%	0%	**4.7%****
Visceral examination				
Foetuses with abnormalities	1.3%	2%	1%	2%

Skeletal examination				
Foetuses with abnormalities	19.4%	15%	22.7%	**61.4%****
Variations	17.8%	15%	19.9%	**39.8%****
Malformations	1.6%	0%	2.9%	**21.6%****
Type of skeletal abnormalities, variations				
Skull				
Incomplete ossification, marked (> three bones)	1%	0%	0%	1%
Incomplete ossification, marked (1 bone or more)	2%	2%	4%	**13%****
Supraoccipital not ossified	0%	1%	1%	1%
Hyoid not ossified	1%	0%	0%	1%
Sternebrae				
Three or less ossified	4%	2%	7%	**13%****
Misaligned	1%	0%	0%	0%
Bipartite	0%	0%	0%	1%
Ribs				
Wavy	6%	6%	**14%***	**32%****
Wavy, marked	0%	1%	1%	**8%****
Type of skeletal abnormalities, malformations				
Sternebrae				
Xiphoid split	1%	0%	1%	3%
Vertebrae, thoracic centra				
thoracic bipartite cartilage dumb-bell shaped	2%	0%	0%	0%
Pectoral girdle				
Scapula bent and/or short	0%	0%	3%	**16%****
Clavicula bent and/or short	0%	0%	0%	2%
Forelimbs				
- Humerus bent and/or short	0%	0%	0%	**12%****
Ulna bent and/or short	0%	0%	0%	**8%****
Radius bent and/or short	0%	0%	0%	**11%****
Hind limbs				
Femur short, bent	0%	0%	0%	**5%****
Tibia bent and/or short	0%	0%	0%	3%
Fibula bent and/or short	0%	0%	0%	**4%***

** ($p<0.01$)

A statistically significant decrease in the number of viable foetuses was observed in the high dose group and this was considered treatment related by the DS. Furthermore, a statistically significant increase in total intrauterine mortality was observed (65 cases of intrauterine mortality). Five dams with no clinical signs or necropsy findings had 20 (~1/3) cases of total intrauterine mortality. This suggests that post-implantation loss and increased intrauterine mortality may not be related to maternal clinical symptoms.

Toxic effects of the substance were noted in both the dams and the foetuses of the high dose group at 450 mg/kg bw/d. There was an increase in some clinical signs as well as reduced body weight, body weight gain, reduced food intake and some necropsy findings in the dams of the high dose group compared to the control group.

There was 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.

There was a statistically significant increase in post implantation loss, late embryonic death, foetal death and a statistically significant reduction in number of viable foetuses in the high dose group. However, the DS assessed the relationship between the individual dams with symptoms of maternal toxicity and the individual pups showing skeletal abnormalities or with a high incidence of intrauterine mortality including post implantation losses. Regarding intrauterine mortality, the DS reported that it was not possible to relate the higher incidences to the maternal toxicity. 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/or necropsy findings and the intrauterine mortality. Therefore, these findings cannot be ascribed to maternal toxicity and RAC considers the implantation losses and the total intrauterine mortality to be related to the substance administration.

The observations of skeletal malformations, including the statistically significant higher incidences of effects in the pectoral girdle, the forelimbs and the hind limbs, were specific and could not be explained only by maternal toxicity.

Placing greater weight, both on the increased intrauterine mortality and on the specific effects observed from the skeletal malformations, and with the comparisons of the individual dam/litter data between maternal toxicity and foetal toxicity showing no correlation, then the observed teratogenicity / developmental toxicity was not secondary to the maternal toxicity. Overall RAC considered that the criteria for classification for developmental toxicity were met for a presumed human reproductive toxicant, thus bis(α,α-dimethylbenzyl) peroxide warrants classification as **Repr. 1B; H360D**.

ANNEXES:

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

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