Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone

EC Number: 404-360-3
CAS Number: 119313-12-1

CLH-O-0000001412-86-124/F

Adopted

16 September 2016

16 September 2016

CLH-O-0000001412-86-124/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: **2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone**

EC Number: **404-360-3**

CAS Number: **119313-12-1**

The proposal was submitted by **BASF SE** and received by RAC on **17 September 2015.**

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

PROCESS FOR ADOPTION OF THE OPINION

BASF SE 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 **27 October 2015**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **11 December 2015**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Marja Pronk**

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 **16 September 2016** 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	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	606-047-00-9	2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone	404-360-3	119313-12-1	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410	-	-	-
Dossier submitters proposal	606-047-00-9	2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone	404-360-3	119313-12-1	**Add** Repr. 2	**Add** H361d	**Add** GHS08	**Add** H361d	-	-	-
RAC opinion	606-047-00-9	2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone	404-360-3	119313-12-1	**Add** Repr. 1B	**Add** H360D	**Add** GHS08	**Add** H360D	**-**	**-**	**-**
Resulting Annex VI entry if agreed by COM	606-047-00-9	2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone	404-360-3	119313-12-1	Repr. 1B Aquatic Acute 1 Aquatic Chronic 1	H360D H400 H410	GHS08 GHS09 Dgr	H360D H410	-	-	-

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

2-Benzyl-2-dimethylamino-4'-morpholinobutyrophenone is used as a photosensitive agent in printing inks, pigmented coatings and photopolymers for imaging applications. The substance is a racemic mixture with purity between 98 and 99.9%.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sexual function and fertility

In a GLP-compliant one-generation study according to OECD test guideline (TG) 415 (BASF SE, 2011), 20 rats/sex/group were given 2-benzyl-2-dimethylamino-4'- morpholinobutyrophenone by gavage at dose levels of 0 (vehicle (propylene glycol) only), 30, 100, or 300 mg/kg bw/day. Reproductive indices were not affected, and in females no treatment-related effects were found on reproductive organs. In males in the high dose group, an absolute and relative testes weight increase was observed (107% and 113%, respectively), as well as an absolute and relative prostate weight decrease (80% and 85%, respectively) and an absolute, but not relative, seminal vesicles weight decrease (87%). However, no correlation to histopathology was observed for these weight changes.

The CLH report further refers to three subacute oral toxicity studies in rats (all by gavage) in which some parameters related to fertility were investigated. In a 14-day range-finding study with 2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone at dose levels ranging from 100-3000 mg/kg bw/day, the gonad weights were not affected (Hazleton, 1989a). In the subsequent main study of 28-day duration, dose levels ranging from 10-500 mg/kg bw/day did not reveal macroscopic findings on reproductive organs, and therefore their weights were not determined and histopathology was not performed (Hazleton, 1989). In a 28-day range-finding study to the one-generation study, with dose levels of 100 and 500/250 mg/kg bw/day (500 mg/kg bw were not tolerated by the rats and reduced to 250 mg/kg bw after 9 days), no treatment-related effects were observed on histopathology of testes and epididymides, sperm motility and spermatogenesis (NOTOX, 2009).

In the absence of adverse effects observed on sexual function and fertility, the DS concluded that no classification for this endpoint is warranted.

Developmental toxicity

The one-generation study was also used to evaluate the occurrence of developmental effects. Mid- and high-dose females (100 and 300 mg/kg bw/day) had a significantly increased number of stillborn pups (that was still within historical control range at the mid dose). At the high dose only, a decreased live birth index was seen in females indicating an adverse effect of the test compound on reproductive performance at this dose level. Pup mortality was statistically significantly increased and pup body weights were statistically significantly reduced in the high-dose group (300 mg/kg bw/day). The respective findings were seen at doses where the dams prenatally and postnatally showed a reduction of body weight (in one dam) or body weight gain, and/or their food intake was affected. Other maternal effects reported in the mid and high dose

groups were enlargement of the liver and adrenal glands, green/brown discoloration of the liver and kidneys, and red discoloration of the glandular stomach. These effects increased in a dose-dependent manner, with additional histopathology findings in liver and adrenal glands at the high dose only. According to the DS, the findings of systemic toxicity observed in the one-generation study were consistent with those of the subacute studies. These studies show that 2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone causes a strong increase in liver weight up to a certain dose level. If that dose level is exceeded, the most prominent effect quickly becomes reduced food consumption and body weight loss. The actual dose level of this threshold appears to be influenced by vehicle and/or rat strain. Whereas the dose level of 500 mg/kg bw was tolerated without effects on body weight in one 28-day study with Sprague-Dawley rats and CMC as vehicle (Hazleton, 1989), it resulted in a strong decrease in food consumption and body weight loss within 9 days of dosing in another 28-day study with Wistar rats and propylene glycol as vehicle (NOTOX, 2009).

The DS concluded that pups of the high dose group were born with a lower body weight that was related to a slightly reduced ability to survive parturition and the first four days after birth, and that these effects were observed in dams suffering from liver toxicity and (adaptive) stress. According to the DS there is insufficient evidence that the developmental toxicity occurs independently of maternal toxicity. Therefore, the DS proposed 2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone to be classified as Repr. 2 for developmental toxicity.

Comments received during public consultation

One manufacturer and two MSCAs agreed with classification in Category 2 for developmental toxicity. One MSCA questioned the involvement of maternal toxicity in the increase in stillborn pups, as the corrected maternal body weight cannot be calculated due to the lack of uterine and foetal weights. In particular, the MSCA they asked whether there was a link between liver toxicity and pup mortality for individual dams. The DS explained that there was no obvious trend, and this was also difficult to determine as there was no extreme variability in either pup mortality or histopathological findings.

Assessment and comparison with the classification criteria

Sexual function and fertility

RAC agrees with the DS that classification for fertility is not warranted, given that the only relevant findings, i.e. changes in the weights of male reproductive organs in the one-generation study, were relatively small and not accompanied by histopathological or functional changes.

Developmental toxicity

An increase in both pre- and postnatal pup mortality as well as a decrease in pup weight were observed in the one-generation study (see for relevant results Tables 1 and 2).

Table 1: Female delivery data and pup mortality

	Historical data	**0 mg/kg bw/day**	**30 mg/kg bw/day**	**100 mg/kg bw/day**	**300 mg/kg bw/day**
Number of litters		18	19	17	17
Total number of pups		194	190	190	162

Pups delivered per dam (mean)	9.3-12.8	10.8	10.1	11.2	9.5
Females with stillborn pups, (N (%))		0 (0)	1 (5.3)	5 (29)*	8 (47)*
Stillborn pups, (N (%))	(0-4.5)	0 (0)	2 (1.0)	6 (3.2)*	9 (5.6)*
Live birth index (%)	95-100	100	99	97	94
Pups dead day 0 (N (%))		0 (0)	1 (0.5)	0 (0)	4 (2.6)
Pups dead day 1-4 (N (%))		0 (0)	3 (1.6)	3 (1.6)	18 (12)*
Pups dead day 5-21 (N (%))		0 (0)	0 (0)	1 (0.5)	1 (0.7)
Pups surviving days 0 to 4 (viability index; N (%))	(94-100)	194 (100)	185 (98)	181 (98)	131* (86)
Pups surviving days 4 to 21 (Lactation index; N (%))	(94-100)	134 (100)	139 (100)	129 (99)	103 (99)

*p<=0.05

Table 2: Overview of pup weight and maternal body weight

	Historical data	**0 mg/kg bw/day**	**30 mg/kg bw/day**	**100 mg/kg bw/day**	**300 mg/kg bw/day**
Pups delivered per dam (mean)	9.3-12.8	10.8	10.1	11.2	9.5
Live pups/litter day 1	9.3-12.8	10.8	9.8	10.7	7.8
Mean pup weight day 1 (g)	5.8 – 6.9	6.3	6.3	5.9	5.5*
Mean pup weight day 21 (g)	41.3 – 53.7	46.6	46.3	44.3	36.9*
Maternal bw gestation day 0 (g)		218.10	217.80	224.90	209.20
Maternal bw gestation day 20 (g)		315.80	311.70	314.50	292.7*

Maternal bw lactation day 0 (g)		248.8	246.4	252.4	232.0*
Maternal bw lactation day 21 (g)	226.7 – 307.7	273	274.5	278.6	260.3*
Maternal bw gain during gestation (g)		97.7	93.9	89.6	83.5
Maternal bw gain during lactation (g)		24.2	28.1	26.3	28.3

*p<=0.05

As can be seen from Table 1, both the number of stillborn pups and the number of females with stillborn pups were significantly increased in the mid- and high dose groups. However, at the mid dose, this increase was within the historical control range. Post-natal pup mortality was increased in the high dose group during the first four days, but not between PND 5-21. It should be noted that the pups were culled on day 4 to reach a maximum of 8 pups/litter, which may have reduced pup mortality after day 4.

Pup weight was significantly reduced in the high dose group only (Table 2). In this group, the absolute weight of brain, thymus, and spleen were reduced to 95, 73, and 70% of the weights of the control group respectively. The relative weights were 121, 90, and 87%, which indicates that the weight reduction of these organs was at least partly secondary to the general weight reduction of the pups in the high dose group.

RAC agrees with the DS that the increase in pup mortality and decrease in pup weight should be considered treatment-related effects on development. The DS however only considered the high dose effects relevant for classification, arguing that the increase in stillborn pups at the mid dose level was within the historical control range, just like the reduced livebirth index that was probably due to the relatively large litter size at this dose level. RAC disagrees with this assessment as the number of stillborn pups shows a clear dose-response relationship and the difference at the mid dose group was significant as compared to the concurrent control group, which has preference over historical control data. Stillbirth also occurred in multiple dams, which makes it less likely to be incidental. Besides, the litter size at the mid dose was only a bit larger than that of controls (11.2 vs 10.8) and well within historical control range. Further, also the average weight of the pups was reduced in the mid dose group, albeit not statistically significantly.

The main reason for the DS to propose a classification in Category 2 instead of 1B was the presence of maternal toxicity, consisting mainly of liver toxicity, (adaptive) stress reaction, and reductions in body weight/body weight gain and food consumption, some of which may have contributed to the developmental effects. This warrants a closer look at the maternal effects observed in the one-generation study.

Maternal body weight / body weight gain / food consumption

The maternal body weight gain of the high dose group was ~15% lower compared to the controls at the end of the gestation period. As can be seen from Table 18a in the CLH report, the lowest body weight gain was observed in four dams with small litters (not counting three dams that were not pregnant). There was no clear link between body weight gain of the individual dams and the number of stillborn pups. In the absence of information on corrected maternal body

weights (no data on uterine and foetal weights available), it cannot be determined whether the reduction in maternal body weight was secondary to the developmental effects or the developmental effects are secondary to the reduced maternal body weights.

The mean difference in body weight between high dose animals and controls was 4% at gestation day 0 and increased to 7% at gestation day 20. During lactation, the body weight of the high dose group was 5-8% lower than of the control group, without a clear trend in time.

No signification changes in body weight or body weight gain were noted in the exposed males, nor in the females during the premating period. For the mid dose group, where a small increase in stillbirth was observed, no statistically significant effects on maternal body weight or body weight gain during gestation and lactation was noted (body weight gain was reduced, in the same order of magnitude as the reduced pup weights at the mid dose).

In a 28-day range-finding study to the one-generation study (NOTOX, 2009) marked body weight loss was seen at 500 mg/kg bw/day, but not at 250 mg/kg bw/day, which is only slightly below the high dose of 300 mg/kg bw/day in the one-generation study. Another 28-day study, in another rat strain and with another vehicle (Hazleton 1989), reported no decrease in body weight at 500 mg/kg bw/day.

Food consumption was only affected during the lactation period, and only in high dose females (up to 20% lower food consumption as compared to controls).

Studies that evaluated the effect of maternal feed restriction on reproductive parameters found no effect of maternal body weight on the occurrence of stillborn pups or on pup viability (Carney *et al.* 2004, Chernoff *et al.* 2009). However, a decrease in body weight of the pups was observed at 30% and 50% feed reduction, corresponding to a maternal body weight reduction of 10-20% and 17-32% respectively. The body weight reduction in pups was 10-20% at 30% feed restriction and 12-47% at 50% feed restriction. It should be noted that the body weight reduction in the dams in the one-generation study was at most 8% during gestation and lactation, which is less than the level at which a reduction in pup weight was reported in the feed restriction studies. Additionally, the reduction in pup weight during lactation was 13-24% in the high dose group, which is more than can be adequately explained by the reduction in maternal body weight of 5-8% in the same period.

Based on these considerations, it is the opinion of RAC that the reductions in maternal body weight, body weight gain, and food consumption were at least partly caused by the smaller litters and increased pup mortality in the high dose group. It is considered unlikely that the observed increase in stillborn pups and postnatal mortality was secondary to these maternal effects.

Maternal histopathological effects

Other maternal effects in the one-generation study consisted of gross lesions (discolorations) in the liver, kidneys, and glandular stomach (see Tables 23-25 in the CLH report) at both the mid and high dose. The dose-dependent discoloration of the kidneys was without histopathological correlate. The red focal discolorations in the glandular stomach correlated with mucosal hyperemia, most likely caused by local irritation. They occurred in 5/20 mid dose dams and in 3/20 high dose dams (so not dose-related), but also in 2/20 control dams. The findings were only of minimal to slight severity, and they were considered adaptive by the DS. RAC noted that in males the glandular stomach findings showed a dose-response relationship and were more pronounced than in females, without however affecting body weight or food consumption. RAC considered it very unlikely that the slight irritative effect in females has caused the developmental effects (in particular the pre-natal and postnatal mortality), also noting that at the high dose only 3 dams had this effect, whereas 8 dams had stillborn pups.

The absolute and relative liver weights were increased in a dose-related fashion in the mid 114 and 112%, respectively) and high dose females (144 and 150%, respectively), with histopathological correlate in the high dose only. Given the magnitude of the effect, RAC considered the increases in liver weight in the high dose females to be adverse, but noted that the histopathological findings in this group were limited to minimal to slight hepatocellular hypertrophy only. Hence, the enlargement of the liver is likely an adaptive response rather than true liver toxicity. According to the DS there is no clear link between liver toxicity and pup mortality for the individual dams.

The absolute and relative adrenal weights were also dose-relatedly increased in the mid and high dose females, again with a histopathological correlate in the high dose only, consisting of cortical cells with condensed eosinophilic cytoplasm devoid of lipid vacuoles in the zona fasciculata. The DS suggested that this effect may represent an ACTH-induced depletion related to stress and therefore a secondary, adaptive effect.

Conclusion

2-Benzyl-2-dimethylamino-4'-morpholinobutyrophenone caused developmental effects in a one-generation study in rats. These consisted of increases in the number of stillborn pups (statistically significant and dose-related at the mid and high dose, but outside the historical control range only for the high dose) and in postnatal mortality (statistically significant, at the high dose), and in decreases in pup body weight (dose-related at the mid and high dose, but statistically significant only at the high dose). These are considered severe effects, in particular the first two which, when combined, indicate a rather strong effect (approximately 20% mortality at the high dose). At the high dose the developmental effects were observed in the presence of several maternal effects.

In the absence of evidence in humans, category 1A is not applicable.

Given that the developmental effects were observed in the presence of other toxic effects, classification may not be appropriate if the developmental effects can be considered as secondary non-specific consequence of these other toxic effects. When looking individually at the maternal effects at the high dose, neither the reduction in maternal body weight (gain) nor the liver toxicity are likely to be directly causative for the developmental effects observed given their relatively small magnitude and nature. It was suggested that some non-specific mechanisms related to stress in the dams may have played a role at the high dose. RAC however noted that no stress or other significant maternal effects were observed at the mid dose, whereas developmental effects were also observed at that dose in a dose-related way. RAC therefore considers the developmental effects observed not to be a secondary non-specific consequence of maternal toxicity, and classification in either category 1B or 2 is warranted. According to the criteria, category 2 could be more appropriate than category 1B if the quality of the study makes the evidence less convincing. That, however, is not the case here, as the one-generation study in which the effects were seen is of good quality study. Looking further at the severity of the effects observed, RAC considers in particular stillbirth and postnatal mortality to be severe effects, relevant for humans. Given additionally the statistical significance of these effects and the dose-response relation found for stillbirth, classification in category 1B is considered more appropriate than category 2. Hence, RAC recommends 2-benzyl-2-dimethylamino-4'-morpholinobutyrophenone to be classified as **Repr. 1B (H360D; May damage the unborn child)**.

Additional references

Carney E.W., Zablotny C.L., Marty M.S., Crissman J.W., Anderson P., Woolhiser M., Holsapple M. (2004). The effects of feed restriction during in utero and postnatal development in rats. Toxicol. Sci. Nov;82(1):237-49.

Chernoff N., Gage M.I., Stoker T.E., Cooper R.L., Gilbert M.E., Rogers E.H. (2009). Reproductive effects of maternal and pre-weaning undernutrition in rat offspring: Age at puberty, onset of female reproductive senescence and intergenerational pup growth and viability. Reproductive Toxicology 28 (4): 489–494.

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).