Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling at EU level of

4-cyclododecyl-2,6-dimethylmorpholin-4-ium acetate;

Dodemorph acetate (ISO)

EC Number: 250-778-2

CAS Number: 31717-87-0

CLH-O-0000002169-72-02/F

Adopted

13 September 2013

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CLH-O-0000002169-72-02/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:

Chemicals name: **4-cyclododecyl-2,6-dimethylmorpholin-4-ium acetate; Dodemorph acetate (ISO)**

EC Number: **250-778-2**

CAS Number: **31717-87-0**

The proposal was submitted by **the Netherlands** and received by RAC on **14 August 2012.**

In this opinion, all classifications are given firstly in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS) and secondly, according to the notation of 67/548/EEC, the Dangerous Substances Directive (DSD).

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands 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 **18 December 2012**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **10 February 2013**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Andrew Smith**

Co-rapporteur, appointed by RAC: **José Luis Tadeo**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **13 September 2013** by **consensus**.

OPINION OF THE RAC

RAC adopted the opinion that Dodemorph acetate (ISO) should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
Current Annex VI entry	No current Annex VI entry									
Dossier Submitter's proposal	607-712-00-6	dodemorph acetate (ISO); 4-cyclododecyl-2,6-dimethylmorpholin-4-ium acetate	250-778-2	31717-87-0	Repr. 2 Skin Corr. 1 Skin Sens. 1A Aquatic Chronic 1	H361d H314 H317 H410	GHS08 GHS05 GHS07 GHS09 Dgr	H361d H314 H317 H410	EUH071	M = 1
RAC opinion			250-778-2	31717-87-0	Repr. 2 STOT RE 2 Skin Corr. 1C Skin Sens. 1A Aquatic Chronic 1	H361d H373 (liver) H314 H317 H410	GHS08 GHS07 GHS05 GHS09 Dgr	H361d H373 (liver) H314 H317 H410	EUH071	M = 1
Resulting Annex VI entry if agreed by COM			250-778-2	31717-87-0	Repr. 2 STOT RE 2 Skin Corr. 1C Skin Sens. 1A Aquatic Chronic 1	H361d H373 (liver) H314 H317 H410	GHS08 GHS07 GHS05 GHS09 Dgr	H361d H373 (liver) H314 H317 H410	EUH071	M = 1

Classification and labelling in accordance with the DSD

	Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits
Current Annex VI entry	No current Annex VI entry						
Dossier Submitters proposal	607-319-00-0	dodemorph acetate (ISO); 4-cyclododecyl-2,6-dimethylmorpholin-4-ium acetate	250-778-2	31717-87-0	Repr. Cat. 3; R63 C; R34 R43 N; R51-53	Xn; C; N R: 34-43-63-51/53 S: (1/2-)26-28-36/37/39-45-61	
RAC opinion			250-778-2	31717-87-0	Repr. Cat. 3; R63 C; R34 R43 N; R51-53	Xn; C; N R: 34-43-63-51/53 S: (1/2-)26-28-36/37/39-45-61	C; R34: C ≥ 10 % Xi; R36/37/38: 5 % ≤ C < 10 %
Resulting Annex VI entry if agreed by COM			250-778-2	31717-87-0	Repr. Cat. 3; R63 C; R34 R43 N; R51-53	Xn; C; N R: 34-43-63-51/53 S: (1/2-)26-28-36/37/39-45-61	C; R34: C ≥ 10 % Xi; R36/37/38: 5 % ≤ C < 10 %

SCIENTIFIC GROUNDS FOR THE OPINION

RAC general comment

All hazard classes for which data were provided were assessed by RAC.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Liquid substances having a flash point ≥ 21 °C, but ≤ 55 °C (DSD) or ≤ 60 °C (CLP) should be classified as flammable. The flashpoint of dodemorph acetate is 73.8 °C and therefore outside the range for classification. There is no indication of explosive or oxidising properties.

Assessment and comparison with the classification criteria

RAC agreed with the assessment and summary provided by the Dossier Submitter

HUMAN HEALTH HAZARD ASSESSMENT

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The Dossier Submitter presented data showing that both the oral and dermal LD_{50} values in rats and rabbits, respectively, are greater than 2000 mg/kg/day. No inhalation toxicity study is available. However, based on the corrosive properties of dodemorph acetate, according to CLP, the substance should be labeled with EUH071: Corrosive to the respiratory tract.

Comments received during public consultation

In addition to EUH071 (CLP), it was suggested by one MSCA that the precautionary statement P260 "Do not breathe dust/fume/gas/mist/valour/spray" (CLP) should be applied given the corrosive nature of this substance and the possibility of inhalation (e.g. during spraying).

Assessment and comparison with the classification criteria

The LD_{50} values for oral and dermal toxicity are above the classification range. No inhalation toxicity study was available and no classification proposed. However, in the opinion of the RAC, observations on the corrosive nature of dodemorph acetate to skin (see below) justify supplemental labelling with EUH071 "Corrosive to the respiratory tract" (CLP).

In relation to the suggestion to add P260, "Do not breathe dust/fume/gas/mist/valour/spray" (CLP), it should be noted that precautionary statements are not included as part of the harmonised classification in Annex VI of the CLP Regulation

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

In the acute oral toxicity studies, some toxic effects were observed. However, all these effects are considered to result in lethality at increasing doses. In the acute dermal toxicity study, no systemic toxic effects were observed.

Although no data are available on inhalation toxicity, based on the corrosive nature of dodemorph acetate to skin it could be assumed that dodemorph acetate is a respiratory tract irritant. The Dossier Submitter noted that this hazard was covered under CLP by the proposal for EUH071:

"Corrosive to the respiratory tract". However, under DSD, the Dossier Submitter argued that Xi; R37 (irritating to the respiratory system) could not be applied to any non-corrosive mixtures because it is unknown at which dilution this substance will induce respiratory tract irritation only.

Assessment and comparison with the classification criteria

RAC agreed in general with the assessment and summary provided by the Dossier Submitter but provided the following comments about classification for respiratory tract irritation.

CLP: The potential of dodemorph acetate to be a respiratory irritant both as a substance and a mixture is covered by the proposal to add EUH071: "Corrosive to the respiratory tract".

DSD: It would be appropriate to account for the possibility that dodemorph acetate is also a respiratory irritant. This would ensure some degree of consistency with labelling of mixtures under CLP. Specifically, given the classification with C; R34, there is the option available to apply R37 at concentrations ranging from 5% up to 10%, aligning with the generic limits for R36 and R38. See for example, the entries in Annex VI for methacrylic acid (EC: 201-204-4) and 4-ethyl-2-methyl-2-isopentyl-1,3-oxazolidine (EC: 410-470-2). Accordingly, RAC is of the opinion that R37 should be applied with a specific concentration limit of 5%.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

In an OECD 404 test, involving a 4-hour, semi-occlusive exposure to the skin of 1 rabbit, dodemorph acetate was found to be corrosive producing well defined erythema from 24 hours and a dark necrotic area from day 2 to the end of the observation period on day 7. This fulfills the criteria for classification as Skin Corr. 1: H314 (CLP) and C; R34 (DSD). There are no data available that allow differentiation between the skin corrosion subcategories 1A/1B/1C.

Comments received during public consultation

In responding to a request from one Member State for further clarification, the Dossier Submitter noted that there were no studies of skin corrosion following exposure periods below 4 hours and that it is therefore unknown whether or not such shorter exposure periods would produce a corrosive response. Therefore, the Dossier Submitter proposed Skin Corr. 1 (i.e. not 1C) as the most appropriate classification, so as to avoid giving the impression that an exposure less than 4 hours might be non-corrosive.

Another Member State suggested that Skin Corr 1B: H314 would be more appropriate, this being more consistent in their view with C; R34. This would be consistent with Note 2 to Table 1.1 of Annex VII to the CLP Regulation.

Assessment and comparison with the classification criteria

The observation of a dark necrotic area of exposed skin in a single rabbit from day 2 to the end of the observation period on day 7 indicates a corrosive response and justifies classification of dodemorph acetate as a corrosive substance. As the exposure period in this study was 4 hours, in the view of the RAC, the criteria for classification with Skin Corr. 1C (CLP) and C; R34 (DSD) are met.

There is a possibility that shorter exposure times could also produce a corrosive response. If an exposure period of 1 hour or less were to produce a corrosive response, a higher classification would be justified. However, as no data are available to show whether such a shorter exposure time could produce a corrosive effect, RAC concludes that there are insufficient grounds to justify a higher classification.

RAC evaluation of serious eye damage/eye irritation

Summary of the Dossier Submitter's proposal

Application of dodemorph acetate to the eye of rabbits causes severe ocular lesions which occur within 72 hours after exposure and which persist for at least 24 hours (corneal opacity equal to or greater than 3 and/or iris lesion greater than 1.5). It therefore fulfills the DSD criteria for classification as Xi; R41 (risk of serious damage to eyes).

Application of dodemorph acetate to the eye of rabbits causes tissue damage in the eye, or serious physical decay of vision, which is not fully reversible within 21 days of application. It therefore fulfills the CLP criteria for classification as Eye Dam. 1; H318 (Causes serious eye damage).

Dossier Submitter did not propose a specific classification for eye irritation potential as this is not appropriate for substances classified as corrosive to the skin (CLP and DSD).

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

RAC agreed with the Dossier Submitter that the results of the eye irritation study show that dodemorph acetate can irritate the eyes. However, a classification for this endpoint is not required given that it is to be classified as corrosive to skin.

RAC evaluation of respiratory sensitisation

Assessment and comparison with the classification criteria

No data were available for this endpoint; no assessment was made.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

In a guinea pig maximisation test (GPMT) test, dodemorph acetate was concluded to be a sensitising agent (>30% showed a more positive response than the control animals at the 48h reading). Therefore, according to the DSD classification criteria, dodemorph acetate should be classified as Xi; R43. According to the CLP criteria, dodemorph acetate should be classified as Skin Sens. 1A; H317.

Comments received during public consultation

One MSCA was in favour of sub category 1A for this endpoint since >60% of guinea pigs responded with a positive reaction in the GPMT after induction with an intradermal injection of 1% dodemorph acetate.

Assessment and comparison with the classification criteria

RAC agreed with the proposal for the Dossier Submitter and the comment provided by one MSCA during the public consultation, i.e. classification as Skin Sens. 1A; H317(CLP) and Xi; R43 (DSD) is justified clearly by the positive result of the GPMT, as presented in the CLH report.

RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity (CLP) – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

Sub-acute and semi-chronic oral studies in the rat and the dog were available. In these studies the main targets for dodemorph acetate were body weight (gain) and the liver. Reductions in body weight (gain), often accompanied to a lesser extent by a reduction in food consumption, was

observed in 3 out of 5 oral studies. The liver appears to be the main target organ. An increase in relative liver weight was observed in 4 out of 5 oral studies. In 3 semi-chronic studies, one in the rat and two in the dog histological changes indicative of liver damage were found. In the semi-chronic dog studies the, increased blood levels of alanine aminotransferase ALAT and alkaline phosphatase (AP) indicate that dodemorph acetate induced hepatocellular damage and were consistent with cholestasis. The 1-year study in the dog provided the lowest NOAEL (10 mg/kg bw/day), based on histological changes in the liver (bile duct hyperplasia, peribiliary fibrosis) observed at 25 mg/kg bw/day and higher. In addition, gastric erosion was observed at doses of 25 mg/kg bw/day and higher, which can probably be attributed to the corrosive nature of dodemorph acetate.

The Dossier Submitter concluded that dodemorph acetate did not fulfil the criteria for oral repeated dose toxicity classification, especially as the LOAEL in the 90-day rat study was clearly above the (classification) cut off value. Further to this, in a comment provided in writing during the period of discussion by RAC, the Dossier Submitter argued that the liver toxicity seen in dogs provided only a "borderline case" for classification. The marked degenerative effect in the 90-day study was observed in only 1/6 dogs at a dose relevant for classification and a comparable effect was not seen in the longer study of 1-year duration (except in 1 female dog killed for ethical reasons at 62.5 mg/kg bw/day). Further, although bile duct effects were seen at relevant doses, the Dossier Submitter noted that they were of "slight to mild severity" and therefore concluded that they were not sufficiently severe to support classification.

The Dossier Submitter also commented that the local gastric irritation did not warrant classification with STOT RE. This was an effect mainly determined by concentration and not by dose, that was only observed after exposure via capsule but not after exposure via diet. The relevance of this route (capsule) for this type of effect was considered limited.

Dermal application for 21 days of dodemorph acetate at doses up to and including 60 mg/kg bw/day did not result in systemic effects and at 60 mg/kg bw/day, only local effects (erythema, oedema, scab formation) were observed. The NOAEL for local dermal effects was 12 mg/kg bw/day.

For dermal repeated dose toxicity, the LOAEL was lower than the cut-off value for classification. However, all observed effects were due to the corrosive properties of dodemorph acetate.

Comments received during public consultation

There were no comments relating specifically to this endpoint.

Assessment and comparison with the classification criteria

The liver and the gastro-intestinal tract are the key target organs following repeated exposure to dodemorph acetate. The relevant findings from the available repeated dose toxicity studies are summarised in the following table.

Main results from the repeated dose toxicity studies

Study design	**Doses (doses relevant for classification in bold and underlined)**	**Severe effects at doses relevant for classification**	**Other significant adverse effects at doses relevant for classification**
Studies involving oral exposure			
Rat, 28 days, diet	0, **50** & **100** mg/kg bw/day (approx)	None	None
Rat, 42+ days, diet one-generation range finding	0, **70/63**, 140/123 & 271/238 mg/kg bw/day for males/females)	None	None

study, diet exposure from at least 42 days before mating up to day 21 after birth of pups.			
Rat, 90 days, diet	0, **<u>20</u>**, **<u>40</u>** & **80** mg/kg bw/day (approx)	None	None
Rat, 90 days, diet (OECD 408)	0, **<u>20/23</u>**, **79/94**, & 229/259 mg/kg bw/day for males/females (approx)	None	None
Rat, 70+ days, diet two-generation study; OECD 416	0, **<u>21</u>**, **<u>64</u>** & 194 mg/kg bw/day	None	None
Rat, 2-year, diet OECD 453	0, **<u>16/21</u>**, 55/73 & 166/222 mg/kg bw/day for males/females (approx)	None	None
Mouse, 18-month, diet OECD 451	0, 45/55, 152/184 & 455/545 mg/kg bw/day for males/females (approx)	None	None
Dog, 28 days gavage	0, **<u>40</u>**, **<u>80</u>** & 160 mg/kg bw/day	None	Vomiting & salivation at 80 mg/kg bw/day
Dog, 90 days diet	0, **<u>32/33</u>**, **<u>79/79</u>** & 187/194 mg/kg bw/day for males/females (approx)	At 79 mg/kg bw/day: marked degenerative changes in the liver (1/6 dogs).	At 32/33 mg/kg bw/day: moderate fatty degeneration in the liver (1/6 dogs). At 79 mg/kg bw/day: clinical chemistry indicative of liver damage (increased ALAT & AP), increased absolute and relative liver weights, pale liver, moderate degenerative changes (5/6 dogs), moderate fatty degeneration (2/6 dogs).
Dog, 1 year capsule OECD 452	0, **<u>10</u>**, **<u>25</u>** & 62.5 mg/kg bw/day	25 mg/kg bw/day: slight (3/8 dogs) or mild (1/8 dog) bile duct hyperplasia, associated with slight peribiliary fibrosis (4/8 dogs). Local effects:	25 mg/kg bw/day: vomiting and salivation.

		microscopic and macroscopic gastric lesions (including gastric erosion) in some dogs, associated with corrosive nature of test substance.	
Studies involving dermal exposure			
Rabbit, 21 days	0, **2.4**, **12** and **60** mg/kg bw/day (aq. Solutions)	No systemic effects Local effects at 60 mg/kg bw/day (2% solution): progressive development of erythema, oedema and scab formation	None

The findings in rats and mice do not support classification. However, the lesions observed in the liver of dogs following 90 days and/or 1 year exposure to dodemorph acetate provide evidence for classification.

Generally, severe or "significant" adverse effects (i.e. changes that clearly indicate functional disturbance, as defined in Section 3.9.2.2 of the CLP guidance) in 90-day repeat dose studies trigger classification with STOT RE if they are seen at a dose of 100 mg/kg bw/day or lower. The observation of treatment-related, marked degenerative liver changes in one of six dogs given 79 mg/kg dodemorph acetate per day for 90 days is compatible with the criteria for STOT RE 2. The observation of similar, but more moderate findings in all the remaining dogs at this dose and some limited evidence of liver toxicity in one dog at the lower dose of about 30 mg/kg bw/day further support the case for STOT RE 2. Similarly, the bile duct hyperplasia, associated with marked peribiliary fibrosis (a non-reversible toxicity), seen in dogs at 25 mg/kg bw/day in the 1-year dog study, is viewed as supporting evidence.

Although there were no comparable findings in the repeated dose toxicity studies with rats or mice, RAC concludes that this level of liver toxicity seen in dogs is sufficient to justify classification with STOT RE 2. There is no evidence to suggest that the findings have no or limited relevance to humans and, in addition, the effective doses are sufficiently low to meet the CLP criteria.

Repeated exposure to dodemorph acetate in capsule form produced macroscopic and microscopic lesions in the gastro-intestinal tract in some dogs at 25 mg/kg bw/day. As explained by the Dossier Submitter, these lesions were a consequence of the concentrated capsular form in which the corrosive test substance was administered in this study, the dose not being the key determinant. Given that dodemorph acetate is to be classified as a corrosive substance, RAC agreed with the Dossier Submitter that no further classification was supported by these findings.

RAC concluded that classification of dodemorph acetate with STOT RE 2; H373 (May cause damage to the liver through prolonged or repeated oral exposure) is warranted.

Due to lower thresholds for repeated dose toxicity classification than in CLP, no classification is justified under the DSD (below 50 mg/kg bw/day in a 90-day study and 12.5 mg/kg bw/day for a one-year study).

Repeated topical dosing of rabbits with dodemorph acetate for 21-days produced no systemic effects. The local lesions observed were consistent with potential corrosivity of the neat substance to the skin. As this hazard is already covered by the classification Skin Corr. 1C, no further classification is needed.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Dodemorph acetate did not induce gene mutations in either bacterial cells or mammalian cells. A negative result was also found in a test for unscheduled DNA synthesis with rat hepatocytes. An in vitro test in Chinese hamster cells for induction of chromosome aberrations and a DNA repair test in *E. coli* bacteria were not considered suitable for evaluation. No acceptable in vitro chromosome aberration test was available. However, an in vivo mouse micronucleus test was negative. Based on all available data, the Dossier Submitter concluded that dodemorph acetate is not genotoxic.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

Based on all available data, RAC concluded that dodemorph acetate is not genotoxic and that no classification is justified.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

According to the dossier submitter, in chronic toxicity studies in rats and mice no tumourigenic potential of dodemorph acetate was identified.

There were occasional increases in the incidence of specific tumour types in rats and mice, but these were either un-related to dose or were at the top dose and within the historical ranges provided by Charles River Laboratories.

Comments received during public consultation

One Member State indicated specifically that they agreed with no classification for carcinogenicity based on the data presented. However, since liver tumours had been seen in treated rats and mice, they requested a more detailed description of the numbers of tumours seen and the historical control data used to disregard these tumours. In response, the Dossier Submitter agreed that this data would be helpful but as they only had access to the DAR, could not provide any details beyond those already given in the CLH report.

Assessment and comparison with the classification criteria

Very little data has been made available for RAC to make an assessment of this endpoint. However, the key findings appear to be in rats, as summarised in Table 20 of the CLH report. The following extract from that table (see below) shows only those malignant tumour findings for which it could be argued there is a relationship to dose. Other findings were sporadic and not related to dose. The results at the 2-year final necropsy are expressed for males/females as a % (number of lesions divided by the number of tissues examined).

	0 ppm (M/F) (%)	**300 ppm (M/F) (%)**	**1000 ppm (M/F) (%)**	**3000 ppm (M/F) (%)**
Ovary adenocarcinoma	0/0	0/0	0/0	0/4.2
Uterus Sarcoma (not otherwise specified)	0/0	0/0	0/0	0/4.0

Uterus Squamous carcinoma	0/0	0/0	0/0	0/2.0

Toxicity at the top dose was not excessive. Unfortunately, it is not absolutely clear how many animals were analysed in each tissue group – nevertheless, the assumption that can be made from the title of the table in the CLH report is that the values are indicative of "group incidences".

According to the Dossier Submitter, these low but increased tumour rates for the ovary and uterus were within historical controls for species and strain tested.

With regard- to the liver, group incidences of 0/0, 0/0, 5.0/0, 0/0 in control, low, mid and high groups, respectively, at the 12 month interim sacrifice and no tumour findings at the 2-year final necropsy in rats convinced the RAC that classification was not appropriate.

There are no further potential carcinogenicity of dodemorph acetate from the shorter term studies provided in the CLH report, including for genotoxicity/mutagenicity. This provided further reassurance that the findings in the rat ovary and uterus, summarised above, were not indicative of a treatment-related carcinogenic response.

Overall, although the level of reporting is poor, RAC was in agreement with the position taken by the Dossier Submitter and concluded that the available data did not indicate a clear tumourigenic response to dodemorph acetate in rats or mice. No classification is therefore justified for this endpoint

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility and reproductive function

A rat 1-generation range-finding study and a rat 2-generation study (OECD 416) were available. In the former, there was a dose-related decrease in mean litter size (15.3, 15.0, 13.6, 11.7 at 0/0, 70/63, 140/123 and 271/238 mg/kg bw/day, in males/females, respectively). At the top dose, in F1 pups there was also a reduced viability index, a decreased body weight on day 1 and decreased body weight gain from day 4-21. The parental animals themselves exhibited dose-dependent reductions in food consumption and body weight gain, which were statistically significant at the top dose. There was no effect on mating or fertility. The most relevant findings are summarised in the Table below.

Main results from 1-generation reproductive toxicity study (range-finding) in the rat

	Dose (ppm)	**0**	**0**	**600**	**600**	**1200**	**1200**	**2400**	**2400**
	Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
F0 animals	Body weight gain as % change from control -premating -gestation	N/A	N/A	 -4	 -9 +1	 -3	 -11 -11	 -18*	 -21* -27*
	Food consumption as % change from control -premating during gestation during lactation	N/A	N/A	 -1	 -4 -1 -2	 -1	 -6 -7 -9	 -8*	 -12* -14* -36*
F1 pups	Litter size	15.3		15.0		13.6		11.7*	

	Dose (ppm)	0	0	600	600	1200	1200	2400	2400
	Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
	viability index as % survivors, day 0-4	95		96		95		61*	
	Body weight day 1	6.1	5.8	6.1	5.9	6.2	6.0	5.7	5.1*
	Grams body weight gain day 4-21 (% of control)	41.9	39.6	39.7 (-5)	38.7 (-2)	38.3 (-9)	36.7 (-7)	30.0 (-28)	29.6 (-27)

* significantly different; N/A: not applicable

In the 2-generation study, there was a reduction in food consumption and body weight gain of parental (F0 and F1) animals at the highest dose (approx. 194 mg/kg bw/day). Additionally, there was an increased incidence in minimal hypertrophy of periacinar hepatocytes (F0 and F1 females), a reduction in absolute kidney weights (males and females) and a reduced absolute epididymides weight. However, these findings were considered to be related to the decreased body weight, and not directly related to dodemorph acetate toxicity.

Compared to the gestation duration in control animals (22 days), slight but statistically significant reductions in gestation duration were observed for the F1a (21.6 days) and F1b (21.4 days) litters in the top dose group and for the F1b litters (21.6 days) of the mid dose group. These data were outside the historical control range (21.7-22.5 days). No effects were observed on gestation duration for the F2 generation. It should be noted that day 0 of gestation was defined by the day on which sperm was detected after a male and female were mated for a period of about 16 hours. The birth of the litter was generally evaluated in the mornings in connection with the clinical observation. According to the CLH report, the method of establishing both the start of gestation and the birth of the pups lacks accuracy. In view of the small size of the effect, the lack of an effect in the gestation duration of the F2 generation and the lack of accuracy in establishing the gestation duration, these effects are considered not to be toxicologically relevant.

In pups at the high dose, there was a reduced viability index and a reduced body weight at day 1 (approximately -10% in the F1a and F1b generations and -6 to -7% in F2). There was a reduced body weight gain during lactation and a decreased incidence of pups with normal physical development landmarks (pinna [earflap] unfolding, auditory canal opening and eye opening) at high doses. There was also significantly reduced body weight gain from day 4-21 (approx. -7% in both F1a and F2) and significantly decreased pinna unfolding also in the mid dose groups. Main findings are summarised in the following table.

Main results from 2-generation reproductive toxicity study in the rat

	Dose (ppm)	0	0	200	200	600	600	1800	1800
	Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
F0 parents	Body weight gain as % change from control -premating (F1a) -gestation (F1a)	N/A	N/A	 -1	 -2 +4	 -1	 -2 +4	 -13*	 -18* -9
	Food consumption as % change from control -premating (F1a) -gestation (F1a) -lactation (F1a)	N/A	N/A	 0	 0 +4 -4	 +1	 0 +4 -4	 -6	 -10* -7* -14*
F1a,b pups	Viability index F1a F1b		 98 95		 97 94		 98 95		 87* 92
	lactation index F1a		 96		 99		 99		 100

	Dose (ppm)	0	0	200	200	600	600	1800	1800
	Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
	F1b	99		98		99		96*	
	Physical development [A] F1a Pinna unfolding auditory canal opening eye opening F1b Pinna unfolding auditory canal opening eye opening	 98.2 100 95.1 95.5 96.7 93.2		 93.1 96.9 99.5 82.1 94.4 90.6		 89.6 95.8 91.8 79.7* 98.4 87.4		 68.4* 79.3* 71.4* 53.0* 73.6* 62.5*	
	Body weight (g) day 1 F1a F1b	6.6 6.5	6.2 6.1	6.6 6.5	6.3 6.1	6.5 6.3	6.2 6.1	5.9* 5.8*	5.7* 5.5*
	Body weight gain (g) F1a day 1-4 day 4-21 F1b day 1-4 day 4-21	 3.1 45.7 2.7 43.3	 2.9 43.1 2.6 41.3	 2.8 44.2 2.7 41.9	 2.9 42.2 2.5 39.4	 2.6 42.5* 2.3 41.5	 2.5 40.2* 2.2 39.7	 2.1* 34.4* 2.0* 34.5*	 2.1* 33.3* 1.8* 32.8*
F1 parents	Body weight gain as % change from control -premating -gestation	N/A	N/A	 0	 +1 0	 -2	 -4 -7	 -7*	 -6* -19*
	Food consumption as % change from control -premating -gestation -lactation	N/A	N/A	 -1	 0 +2 -3	 0	 -1 +2 -3	 -6*	 -7* -7* -25*
F2 pups	viability index	96		94		98		79*	
	Body weight day 1	6.5	6.1	6.4	6.1	6.8	6.5	6.1*	5.7*
	Body weight gain day 1-4 day 4-21	 2.9 43.7	 2.8 41.9	 2.9 42.9	 2.7 40.8	 2.8 40.6*	 2.7 38.3*	 1.7* 32.4*	 1.7* 30.8*
	Physical development [A] Pinna unfolding auditory canal opening eye opening	 92.7 99.5 93.8		 92.3 100 97.4		 96.9 97.6 97.2		 73.3* 73.5* 78.7*	

* significantly different; dr = dose related; N/A: not applicable

[A] Developmental stage, % of pups reaching criteria. Pinna unfolding at day 4, auditory canal opening at day 13, eye opening on day 15.

No treatment-related effects of dodemorph acetate on reproductive function were observed in either of these studies.

Developmental toxicity

In a rat developmental study with dodemorph acetate, doses of 30-300 mg /kg bw/day, no developmental effects were observed at the highest dose that did not induce maternal toxicity (30 mg/kg bw/day). However, at the top dose, statistically significant increased litter incidences of misshapen sternebrae, unossified sternebrae and incomplete ossification of the lumbar arch were found. These incidences were at the upper end or above the historical control range. At this dose, maternal toxicity was evident from impairments in body weight and body weight gain, decreased food consumption, increased liver weight (>10%), a marked increase in triglycerides and slight

deviations in serum electrolytes and increased platelets. The main findings are summarised in the following table.

Main results from the developmental toxicity study in rats

	Dose (mg/kg bw per day)	**0**	**30**	**100**	**300**	**HCD[2]: mean (range)**
Maternal findings	Corrected body weight gain in grams (% of control) [1]	33.7	31.6 (-6)	26.0 (-23)	20.4* (-31)	-
	Food consumption GD 6-13 as % of control	N/A	91 %	79%	53%*	-
	Clinical chemistry - triglycerides (mmol/L)	5.01	6.15	7.37	11.06*	-
	Relative liver weight as % change from control	N/A	0.5	1	17*	-
Foetal findings	Skeletal variations (litter incidences in %)					
	- misshapen sternebra	64	82	76	90*	67 (25-92)
	- unossified sternebra	24	23	19	52*	30 (17-46)
	- incomplete ossification of lumbar arch	0	5	0	21*	1 (0-4)

* statistically significant;
\- no data; N/A: not applicable
[1] Corrected body weight gain = terminal body weight minus uterine weight minus day 6 body weight.
[2] Historic control data were included in the study report and consisted of 9 gavage studies and 1 inhalation study in Wistar rats from the same supplier, performed in the period of January 2000 up to June 2001.

In a rabbit developmental study, no maternal toxicity was observed. The NOAEL for maternal toxicity was 120 mg/kg bw/day (the highest dose tested). In the rabbit foetuses, a slight increase in incidence of malformations (cleft palate in 1 foetus and open eye in 4 foetuses, only in one litter) was observed at the highest dose level, and the percentage of animals with irregularly shaped sternebrae was increased. Although in this study the open eye was observed only in one litter, open eye was also observed in 7 out of 16 foetuses from a single litter in the range-finding study in the rabbit at a higher dose level, indicating that the effect is dose-related. In the range-finding study, anasarca was also observed in 4 out of 16 foetuses from a single litter. No historical control data were provided. The main findings are summarised below.

Results from a developmental toxicity study in rabbits.

Dose (mg/kg bw per day)	**0**	**10**	**40**	**120**
Maternal toxicity	No maternal toxicity			
Post implantation loss, in %	6.2	13.0	5.1	18.4
No. of dams with all resorptions	0	0	0	1
No. of early resorptions[a]	0.2	0.3	0.2	0.7
External malformations **- cleft palate (no. of foetuses)** **- open eye (no. of foetuses)**	 0 0	 0 0	 0 0	 1 4
Skeletal variations/retardations **- sternebra irregular shape (foetuses/litter)**	 1.6	 5.0	 6.0	 10.9*

* statistically significant
[a]unit not specified in CLH report (probably mean foetal incidence by litter)

The Dossier Submitter noted that in the repeated dose toxicity studies, only a slight increase in relative liver weight was observed at 80 mg/kg bw/day in a 90 day study in rats, while in a 28 day study in rats, no effects were observed up to 160 mg/kg bw/day.

The Dossier Submitter proposed the classification Repr. 2; H361d (CLP) and, consistent with this, Repr. Cat. 3; R63 (DSD) based on:

- in the 2 generation study, reduced body weight gain of the pups was observed at a dose without maternal toxicity as well as decreased pinna unfolding in 1 generation.
- in a developmental study with rabbits, an increase in early resorptions and post implantation loss, and in four foetuses from one litter, an increase in the incidence of open eye was observed at the top dose tested (no maternal toxicity).

The Dossier Submitter justified their proposal for a Category 2 classification (CLP), rather than Category 1B, by the argument that these findings were not seen consistently in all litters or were not of particularly high severity***.***

Comments received during public consultation

Three MSCA expressed specifically their support for classification as Repr. 2 (H361d).

Assessment and comparison with the classification criteria

Fertility and reproductive function

RAC agreed with the assessment provided by the Dossier Submitter; no classification is justified.

Developmental toxicity

RAC agreed with the Dossier Submitter that the following findings support the proposal to classify dodemorph acetate for developmental toxicity:

- *Reduction of pup body weight gain during lactation and delay in physical development landmarks*.
  The effects were observed in F1a, F1b and F2 generations at the high dose that also produced maternal toxicity (14% decrease in food consumption during lactation). At mid-dose, without maternal toxicity, a significant effect on pup body weight development (day 4-21) in F1a and F2 generations and an effect on pinna unfolding in F1b and F2 generations were also observed. These effects are therefore not considered to be secondary to maternal toxicity.
  However, these effects are not considered as severe and they may be reversible after cessation of exposure. Therefore RAC considers that classification as Repr. 2 is more appropriate than 1B on the basis of these findings.

- *Increase in incidence of malformations at the top dose in the rabbit developmental toxicity study*.
  Cleft palate (1 foetus from one litter) and open eyes (4 foetuses from one litter) were reported.However, no historical control data were provided in the CLH report. Published historical control data (HCD) on the Himalayan rabbit (Viertel, 2003) reports 4 foetuses with cleft palates (0.052%) from 4 different litters (0.35%) and 7 foetuses with open eyes (0.091%) from 7 litters (0.62%). Although these HCD should be used with care as they relate to a different laboratory, it indicates that cleft palate is a rare malformation in the rabbit but a single incidence cannot be attributed with certainty to the treatment.
  Open eyes is also a rare malformation. The observation of 4 incidences in one dose group therefore seems very unusual. As a single litter is affected, this finding is nevertheless in line with the spontaneous isolated incidence reported in the literature. However, open eyes were also reported in the range-finding rabbit study in a similar pattern: a relatively high foetal incidence (7 foetuses affected) but originating from a single litter. Due to the repetition of this finding in the two studies, it is therefore considered unlikely to be of spontaneous origin and this malformation is attributed to treatment.

No maternal toxicity was observed in this study and the effect cannot therefore be secondary to maternal toxicity.
Overall, considering the uncertainty raised by the grouping of cases in single litters and the low litter incidence, RAC considers that Repr. 2 is however more appropriate than 1B on the basis of these findings.

The following effects are also reported and provide supportive evidence for classification as Repr. 2.

In the rabbit developmental toxicity study, in the absence of maternal toxicity:

- an increasing incidence of irregular shaped sternebrae with dose (1.6, 5.0, 6.0 and 10.9 foetuses/litter in the control, low, mid and high dose groups, respectively) that reached statistical significance at the high dose. This abnormality was reported as a variation in the CLH report but provides supportive evidence for classification as Repr. 2.
- increased incidences of early resorptions and post implantation loss observed at the top dose were not statistically significant and the increase in early resorptions was limited. In the absence of historical control data, interpretation of the significance of these findings is difficult. It is noted that effects on post-implantation loss were also noted in the rabbit range-finding developmental study but in the presence of substantial maternal toxicity and an effect secondary to maternal toxicity cannot be excluded. Due to uncertainties in significance of this observation in the main study and the potential link to maternal toxicity in the range-finding study, it provides supportive evidence for classification as Repr. 2.

In the one and two generation rat studies:

- decreased pup body weight at post-natal day (PND) 1 and decreased viability at PND 4 in the one-generation and the two-generation rat studies. The effects were seen in the presence of maternal toxicity as evidenced by decreases in food consumption as well as in maternal body weight gain during the whole exposure period and it cannot be excluded that these foetal effects were a secondary non-specific consequence of maternal toxicity.

RAC also noted the following effects induced by dodemorph acetate, although they were not considered sufficient *per se* to justify classification for development:

- skeletal findings in the rat developmental study. Only unossified sternebrae and incomplete ossification of sternebrae were above the upper limit of historical control data and could be attributed to treatment. Both are generally considered as variations (Solecki, 2001) and as changes in the ossification state that do not involve the normal structure of the bone. In the absence of an adverse effect on foetal body weight, they are not considered to be secondary to general foetal developmental delay but they are of insufficient severity to trigger classification. It is however noted that sternebrae variations are also reported in the rabbit developmental study as discussed above.

In conclusion, although RAC also noted that adverse findings on development were seen in both rats and rabbits, RAC agreed with the Dossier Submitter that this profile best fitted the criteria for Repr. 2; H361d. The findings could not be dismissed as being of no relevance to humans and, as such, classification was necessary. However, as a clear teratogenic effect had not been observed and the level of foetotoxicity seen was not severe, a Category 1B classification seemed inappropriate.

On a similar basis, a classification Repr. Cat. 3; R63 is recommended under DSD.

RAC evaluation of aspiration toxicity

Not evaluated.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter proposed to harmonise the classification for dodemorph acetate as Aquatic Chronic 1, H410 (M=1) according to CLP, and N, R51/53 according to DSD.

Degradation

Degradation was studied in two hydrolysis tests, a photolysis test and an aerobic water/sediment study. A ready biodegradation study was not available.

A hydrolysis study with ^{14}C-dodemorph acetate showed its rapid dissociation into dodemorph and acetate whereas dodemorph was found to be hydrolytically stable at pH 5, 7 and 9 at 24-25ºC (DT_{50}> 32 days). In a second study performed with ^{14}C-dodemorph acetate, according to OECD 111, DT_{50} > 5 days was determined at pH 4, 7, and 9 at 22 ºC.

Regarding photolysis, dodemorph acetate dissociated into dodemorph and acetate in water. The dodemorph formed was photolysed with a DT_{50} equivalent to 3.6 and 1.6 (natural sunlight days) at pH 7 and 9, respectively.

Dodemorph, the dissociation product of dodemorph acetate, is not rapidly degradable. In a water/sediment study, it dissipated rapidly from a water column but had long half-lives (> 53 days) in the total system. The mineralisation rate was demonstrated to be slow (15.4% and 23.2% after 103 days). Therefore, taking into account CLP section 4.1.2.9.3, dodemorph acetate was considered to be not rapidly degradable.

Bioaccumulation

The bioconcentration factor (BCF) for fish (*Oncorhynchus mykiss*) was determined in a flow-through study conducted according to OECD 305, in which fish were exposed to dodemorph acetate spiked with radiolabelled dodemorph. BCF values determined for dodermorph in fish varied between 580 and 750 l/kg. Based on the results, the Dossier Submitter concluded that dodemorph acetate is highly bioaccumulative.

Since dodemorph acetate dissociates rapidly into dodemorph and acetate in the aqueous compartment, the Dossier Submitter considered the BCF values of dodemorph as valid also in the case of dodemorph acetate.

Aquatic toxicity

All available studies were performed with dodemorph acetate. Dodemorph acetate dissociates rapidly in aqueous solutions into acetate and dodemorph, therefore dodemorph was the compound determined by HPLC analysis.

Reliable acute and chronic toxicity studies in fish (*Oncorhynchus mykiss*), invertebrates (*Daphnia Magna*), and algae (*Pseudokirchneriella subcapitata)* were reported by the Dossier Submitter. The acute LC_{50} for fish was between 1.23-2.65 mg/land the chronic NOEC was 0.10 mg/l. The acute EC_{50} for invertebrates was 1.48 mg/l and the chronic NOEC was 0.08 mg/l.The reported acute E_rC_{50} for algae was 0.91 mg/l and the chronic NOE_rC 0.5 mg/l.

Algae (*Pseudokirchneriella subcapitata*) was the most sensitive species in acute and chronic tests, with an ErC_{50} of 1.1 mg/l and a NOErC of 0.059 mg/l, respectively.

Comments received during public consultation

The environmental hazard classification was supported by four MSCAs, with some minor editorial comments.

Furthermore, updated information regarding the solubility and surface tension of dodemorph was submitted during public consultation. The Dossier Submitter agreed with the new information, but considered that it did not change the proposed classification.

RAC assessment and comparison with criteria

Degradation

Dodemorph acetate dissociates into dodemorph and acetate in the aqueous compartment. Therefore, dodemorph acetate is considered hydrolytically unstable.

Dodemorph, the degradation product of dodemorph acetate, is not rapidly degradable. In a water/sediment study, it dissipated rapidly from water but had long half-lives (> 53 days) in the total system. The mineralisation rate was slow (15.4% and 23.2% after 103 days). Taking into account section 4.1.2.9.3 of the CLP Regulation, dodemorph acetate must be considered as not rapidly degradable.

Based on the available data, RAC agreed that dodemorph acetate is not readily biodegradable according to DSD and not rapidly degradable according to CLP.

Bioaccumulation

In the current CLP criteria (2nd ATP) bioaccumulation is important only if the surrogate approach is applied for assessing long-term hazards. For dodemorph acetate, chronic adequate toxicity data is available for all trophic levels and, therefore, bioaccumulation data was not used for the classification according to CLP. However, under the DSD, bioaccumulation should be used for assessing long-term adverse effects. BCF values determined for dodemorph in fish varied between 580 and750 L/kg and, as they are above the cut-off value of > 100 l/kg, dodemorph showed a high potential for bioaccumulation.

Dodemorph acetate dissociates rapidly into dodemorph and acetate in an aqueous compartment. The BCF values of dodemorph can therefore also be used for dodemorph acetate.

Aquatic toxicity

Under CLP, classification for acute aquatic hazards is based on the most sensitive species. The reported E_rC_{50} (72-h) for algae (*Pseudokirchneriella subcapita)* equals 1.1 mg/l[i] (mean measured concentrations). This value is > 1 mg/l, therefore dodemorph acetate does not classify as category Acute 1 (H400).

Regarding the classification for long-term aquatic hazard, algae (*Pseudokirchneriella subcapita*) is also the most sensitive species with a NOErC of 0.059 mg/l[iii] (mean measured concentrations).

As dodemorph acetate is not rapidly degradable, RAC agreed to classify it as Aquatic Chronic 1, H410, with an M-factor of 1, because the NOEC value is between 0.01 and 0.1 mg/l.

The E_rC_{50} (72-h) for the most sensitive species(*Pseudokirchneriella subcapita*) of 1.1 mg/l, is between 1 mg/l and 10 mg/l, and taking into account that dodemorph acetate is considered not readily degradable, it does not fulfil the criteria of ready degradability (5.2.1.3 of Annex 6 of 2001/59/EC) and the BCF is higher than 100, therefore a DSD classification as N, R51/53 is justified.

RAC agreed with the Dossier Submitter's proposal to classify dodemorph acetate as Aquatic Chronic 1 (H410) with M-Factor 1 according to CLP. Similarly, RAC agreed with the proposed classification according to the DSD: N; R51/53 according to the DSD.

RAC evaluation of hazards to the ozone layer

Not evaluated.

ANNEXES:

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

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

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[i] In the CLH report and in the DAR, the exponential growth during the test (24 h and 48 h) has not been submitted, therefore it is not possible to reduce the test period. Test concentrations were checked at the beginning (50% of nominal) and at the end of the test (25% of nominal). Mean measured test concentrations varied between 14% and 36 %. The results are reliable, although, according to the guideline OECD 201, for volatile, unstable or strongly adsorbing test substances, additional samplings for analysis at 24 hour intervals during the exposure period should have been done in order to better define loss of the test substance.