CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Dodemorph acetate

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

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

Index Number: **-**

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Dodemorph (but not dodemorph acetate) was included in Annex I of Directive 67/548/EEC and inserted in Annex VI of Regulation (EC) No 1272/2008 (CLP; ATP001). In 2007, a DAR was prepared for inclusion of dodemorph in Annex I to Directive 91/414/EEC; this was amendend in 2008 (Final addendum to DAR on dodemorph, July 2008). This resulted in a different proposal for classification of dodemorph when compared to the classification in Annex VI of CLP. Because dodemorph acetate is the actual substance of the manufactured material used in the plant protection products (and not dodemorph), it seems wise to also include dodemorph acetate in Annex VI of Regulation (EC) No 1272/2008, especially since the current classification of dodemorph is probably based on studies with dodemorph acetate. Therefore, this proposal for the harmonised classification and labeling of dodemorph acetate is prepared. Only the endpoints resulting in classification according to the DAR are discussed in this dossier.

Table 1: Substance identity

Substance name:	***Dodemorph acetate***
EC number:	***250-778-2 (EINECS)***
CAS number:	***31717-87-0***
Annex VI Index number:	**-**
Degree of purity:	***The minimum content of pure dodemorph acetate, the substance used in all toxicological studies, is 950 g/kg in the manufactured material, relative to the dry material. Dodemorph acetate is due to the irritant nature however never produced as pure dry material, but as a solution, avoiding any potential exposure to worker. The specification for the technical concentrate is > 544 g/kg.***
Impurities:	***The substance does not contain any impourities considered relevant for the classification and labelling of the substance.***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation for dodemorph acetate	-	-
Current proposal for consideration by RAC for dodemorph acetate	Skin corr Cat 1; H314 Skin sens Cat. 1A; H317 Repro Cat. 2; H361d Aquatic Chronic 1; H410 M factor: Chronic M-factor of 1	C; R34 Xi; R43 Repro Cat.3; Xn; R63 N; R51-53
Resulting harmonised classification for dodemorph acetate (future entry in Annex VI, CLP Regulation)	Skin corr Cat 1; H314 Skin sens Cat. 1A; H317 Repro Cat. 2; H361d Aquatic Chronic 1; H410 M factor: Chronic M-factor of 1	C; R34 Xi; R43 Repro Cat 3; Xn; R63 N; R51-53

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Classification for human health and environmental hazards.

The 2nd ATP has implemented the 3rd revised edition of GHS in which classification and assignment of M-factors can also be based on chronic aquatic toxicity. The 2nd ATP will come into force on 1 December 2012. Harmonised classifications using criteria of the 2nd ATP will not be mandatory before 1 December 2012. Based on the criteria of the 2nd ATP, a classification and an M-factor based on the chronic aquatic toxicity is proposed in addition to a classification and an M-factor based on the acute aquatic toxicity.

According to Directive 67/548/EEC and Directive 1999/45/EC as amended by Directive 2006/8, no distinction between acute and chronic SCLs can be made since only acute aquatic toxicity data are allowed for deriving classifications and SCLs. Therefore, only one set of SCL was considered for classification of dodemorph acetate according to DSD criteria. However, based on the available data it is concluded that no SCLs are required for environmental classification.

Also, sub-classification for the sensitizing properties will be performed.

Table 3: Proposed classification for dodemorph acetate according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	none	Not classified	conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	none	Not classified	conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	none	Not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	none	Not classified	conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	none	Not classified	conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	none	Not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	none	Not classified	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	none	Not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	none	Not classified	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	none	Not classified	conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Not classified	none	Not classified	conclusive but not sufficient for classification
	Acute toxicity - dermal	Not classified	none	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	none	Not classified	Data lacking
3.2.	Skin corrosion / irritation	Skin corr Cat. 1	none	Not classified	
3.3.	Serious eye damage / eye irritation	Not classified[3)]	none	Not classified	
3.4.	Respiratory sensitisation	Not classified	none	Not classified	Data lacking
3.4.	Skin sensitisation	Skin sens Cat. 1A	none	Not classified	

3.5.	Germ cell mutagenicity	Not classified	none	Not classified	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	none	Not classified	conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Repro Cat. 2	none	Not classified	
3.8.	Specific target organ toxicity –single exposure	Not classified	none	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	none	Not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	none	Not classified	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic chronic 1	M factor 1	Not classified	
5.1.	Hazardous to the ozone layer	Not classified	none	Not classified	conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification
[3)] Classification for eye damage is implicit, due to the corrosive properties of the substance

Labelling: Signal word: Danger
Hazard statements: H314; H317; H361d; H410; EUH071
Precautionary statements:

Proposed notes assigned to an entry:

Table 4: Proposed classification for dodemorph acetate according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	Not classified	none	Not classified	conclusive but not sufficient for classification
Oxidising properties	Not classified	none	Not classified	conclusive but not sufficient for classification
Flammability	Not classified	none	Not classified	conclusive but not sufficient for classification
Other physico-chemical properties *[Add rows when relevant]*	Not classified	none	Not classified	conclusive but not sufficient for classification
Thermal stability	Not classified	none	Not classified	conclusive but not sufficient for classification
Acute toxicity	Not classified	none	Not classified	conclusive but not sufficient for classification
Acute toxicity – irreversible damage after single exposure	Not classified	none	Not classified	conclusive but not sufficient for classification
Repeated dose toxicity	Not classified	none	Not classified	conclusive but not sufficient for classification
Irritation / Corrosion	C; R34	none	Not classified	
Sensitisation	Xi; R43	none	Not classified	
Carcinogenicity	Not classified	none	Not classified	conclusive but not sufficient for classification
Mutagenicity – Genetic toxicity	Not classified	none	Not classified	conclusive but not sufficient for classification
Toxicity to reproduction – fertility	Not classified	none	Not classified	conclusive but not sufficient for classification
Toxicity to reproduction – development	Xn; R63	none	Not classified	
Toxicity to reproduction – breastfed babies. Effects on or via lactation	Not classified	none	Not classified	conclusive but not sufficient for classification
Environment	N; R51-53	none	Not classified	

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Indication of danger: C; Xi; Xn, N
R-phrases: R34; R43; R63; R51-53
S-phrases: S(1/2); S26; S28; S36/37/39; S45; S61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Dodemorph, but not dodemorph acetate, was included at the latest in the 25th ATP (30-12-1998). Although no exact info is available, this is probably based on data from dodemorph actetate. In 2007, a DAR was prepared by the Netherlands, in the context of the possible inclusion of dodemorph in Annex I of Council Directive 91/414/EEC (addenda to the DAR were prepared in 2008, final addendum in July 2008). Nevertheless, dodemorph acetate (and not dodemorph) is the substance as present in plant protection products. At the meeting of experts (PRAPeR 49, June 2008) it was agreed that the observed effects in the toxicological studies that are performed with dodemorph acetate can all be attributed to the dodemorph moiety of dodemorph acetate. The substance was not recently discussed by TCC&L.

2.2 Short summary of the scientific justification for the CLH proposal

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

No harmonised classification exists for dodemorph acetate.

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

No harmonised classification exists for dodemorph acetate.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Not relevant for this dossier

2.4.2 Current self-classification and labelling based on DSD criteria

Not relevant for this dossier

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Dodemorph is an active substance in the meaning of Directive 91/414/EEC and according to article 36 of CLP such substances are normally subject to harmonised classification. However, dodemorph acetate is the form in which the active substance is actually placed on the market. On this basis dodemorph acetate also meets the definition of an active substance and is therefore also subject to harmonised classification.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

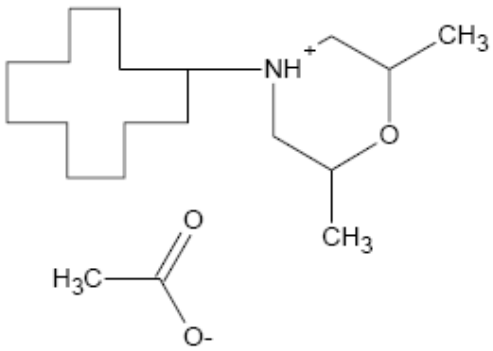
1.1 Name and other identifiers of the substance

Table 5: Substance identity

	Dodemorph acetate
EC number:	250-778-2
EC name:	Dodemorph acetate
CAS number (EC inventory):	
CAS number:	31717-87-0
CAS name:	Morpholine, 4-cyclododecyl-2,6-dimethyl-,acetate (1:1)
IUPAC name:	4-cyclododecyl-2,6-dimethylmorpholin-4-ium acetate
CLP Annex VI Index number:	-
Molecular formula:	$C_{18}H_{35}NO.C_2H_4O_2$ or $C_{20}H_{39}NO_3$
Molecular weight range:	341.5

Structural formula:

Dodemorph acetate:



1.2 Composition of the substance

The minimum content is 950 g/kg dodemorph acetate in the manufactured material (technical a.i.), on a dry weight basis. Dodemorph acetate is used in all toxicological studies. Nevertheless, dodemorph acetate is never produced as dry material due to the irritant nature, but as a solution (TK), avoiding any potential exposure to worker. The specification for the technical concentrate (TK) is >544g/kg.

It is noted that dodemorph acetate has a salt like structure which in aqueous environments consists of dodemorph-H+ and Ac- at pH < 6.5 or dodemorph and Ac- at pH > 10.5. At in-between pH values (pH 6.5-10.5) both forms of dodemorph exist.

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
4-cyclododecyl-2,6-dimethylmorpholin-4-ium acetate			All studies are performed with dodemorph acetate. The minimum content of pure active substance (dodemorph acetate) is 950 g/kg in the manufactured material, relative to the dry material. . Dodemorph acetate is never produced as dry material due to the irritant nature, but as a solution (not further specified), avoiding any potential exposure to worker. The specification for the technical concentrate is > 544 g/kg. The active substance is a mixture of cis and trans isomers ranging from a ratio of minimally 50:50 cis:trans and maximally 60:40 cis:trans.

Current Annex VI entry of dodemorph acetate:

-

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential information			The substance does not contain any impurities relevant for classification and labeling

Current Annex VI entry: -

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
Confidential information				

Current Annex VI entry: -

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	non-homogenous solid to a viscous liquid (at 40°C) with a yellow acid aromatic or lemon piquant odour	DAR January 2005 + addendum July 2008	
Melting/freezing point	35 – 48°C	DAR January 2005 + addendum July 2008	
Boiling point	no boiling point could be determined, as probably at 158°C the loss of the acetate as acetic acid is taking place	DAR January 2005 + addendum July 2008	
Relative density	-	DAR January 2005 + addendum July 2008	
Vapour pressure	3.62 x 10^{-3} Pa at 25 °C	DAR January 2005 + addendum July 2008	
Surface tension	-		
Water solubility	2.29 mg/L at pH 9 to 736 mg/L at pH 5 at 25 °C	DAR January 2005 + addendum July 2008	
Partition coefficient n-octanol/water	4.6	DAR January 2005 + addendum July 2008	
Flash point	73.8 °C	DAR January 2005 + addendum July 2008	
Flammability	autoflammable	DAR January 2005 + addendum July 2008	
Explosive properties	no	DAR January 2005 + addendum July 2008	
Self-ignition temperature	264 °C	DAR January 2005 + addendum July 2008	
Oxidising properties	No	DAR January 2005 + addendum July 2008	
Granulometry	-		
Stability in organic solvents and identity of relevant	-	2008	

degradation products			
Dissociation constant	-		
Viscosity	-		

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this type of report.

2.2 Identified uses

Dodemorph acetate is used as a fungicide. The plant protection product is sprayed to the horticultural crops after dilution in water (aqueous dispersion) with an interval between applications of 7-10 days.

Summary of intended uses (a.s. given as dodemorph acetate)

Crop and/or situation (a)	Member State or Country	Product Name	F, G or I (b)	Pests or group of pests controlled (c)	Formulation		Application				Application per treatment			PHI (days)	Remarks (m)
					type (d-f)	conc of as (i)	method kind (f-h)	growth stage & season (j)	number min-max (k)	interval between applications (min-max)	kg as/hL; min-max	water L/ha; min-max	kg as/ha; min-max		
roses	Northern and Southern Europe	Mehltaumittel®	G	powdery mildew, *Sphaeroteca pannosa*	EC	385 g/L	spray (n)	not stated	1-10	7-10	0.1	2000	2	-	

a. For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (e.g. fumigation of a structure)
b. Outdoor or field use (F), glasshouse application (G) or indoor application (I)
c. E.g. biting and sucking insects, soil born insects, foliar fungi, weeds

d. E.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)

e. GCPF codes - GIFAP Technical monograph No2, 1989
f. All abbreviations used must be explained
g. Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench

h. Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
i. Concentration in g as/kg of g as/L
j. Growth stage at last treatment (BBCH monograph, Growth stages of plants, 1997, Blackwell, ISBN 3-8263-3152-4)
k. The minimum and maximum number of applications possible under practical conditions must be provided.
l. PHI - minimum pre-harvest interval
m. Remarks may include: extent of use / economic importance / restrictions
n. Roses are mostly sprayed manually using handheld knapsack equipment and spray lances.
o. The number of applications should not exceed 2 x 5 sprays per season (total of 10 sprays).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks
Flammability	Autoflammable auto-ignition temperature 264 °C	
Flash point	73.8 °C	
Explosive properties	No explosive properties: test substance does not explode under the effect of a flame, is not sensitive to shock or friction (EEC A.14).	
Oxidising properties	no oxidizing properties (EEC A.17 (burning pile)	

3.1.1 Summary and discussion of physico-chemical properties

Dodemorph acetate is autoflammable at 264°C and has a flashpoint of 73.8 °C. It is not explosive and has no oxidising properties.

3.1.2 Comparison with criteria

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. This does not fulfil the criteria for classification. There is no indication of explosive or oxidising properties.

3.1.3 Conclusions on classification and labelling

Dodemorph acetate should not be classified for flammability, explosivity and oxidising properties, conclusive but not sufficient for classification.

4 HUMAN HEALTH HAZARD ASSESSMENT

All toxicological studies have been performed with dodemorph acetate. Dodemorph acetate has a salt like structure which in aqueous environments consists of dodemorph-H+ and Ac- at pH < 6.5 or dodemorph and Ac- at pH > 10.5. At in-between pH values (pH 6.5-10.5) both forms of dodemorph exist At the meeting of experts (PRAPeR 49, June 2008) it was agreed that the observed effects can all be attributed to the dodemorph moiety of dodemorph acetate. The values obtained in the different investigations are presented in the conclusion as dodemorph acetate.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

The summaries below were copied from the DAR 2007 Volume 3 B.6.1 and the addendum of April 2008 volume 3 B6.

4.1.1 Non-human information

Absorption

After oral administration of a single low dose of ^{14}C-labelled dodemorph acetate (10 mg/kg bw) in rats, peak plasma concentrations were reached after 6-8 h, indicating a moderate rate of absorption. Data indicate that at higher doses (1000 mg/kg bw) there is a slower rate of absorption and/or an increased rate of biliary excretion of dodemorph acetate. It is likely that following oral administration part of the radioactivity that is recovered from feces has been absorbed from the gastro-intestinal tract and subsequently excreted in the bile (with possible enterohepatic cycling). However, the fraction of the radioactivity recovered from feces that can be considered to have been orally absorbed cannot be established. Hence the estimate of the percentage oral absorption, solely based on the amounts of radioactivity recovered in urine, expired air and the body, is considered to be 40%.

Distribution

At termination at 96h post oral dosing in rats, in organs, tissues and carcass less than 4% of radioactivity was recovered. Apart from carcass, the highest levels were found in the liver (up to 0.8%).

Metabolism

In the laboratory animal (and human) dodemorph acetate may readily dissociate in dodemorph and acetate. The data indicate that dodemorph acetate is extensively metabolized, either by microflora in the gut or in the body. The data indicate that degradation of the morpholine ring occurs. Analysis of urine and feces samples demonstrated that 6 very polar metabolites were formed. Since identification of the metabolites reported was not possible, no definite metabolic pathway of dodemorph acetate can be deduced.

Excretion

Excretion of radiolabel is rapid following the low dose, with the majority being excreted within 24h after dosing. Following the high dose excretion is somewhat slower, with the majority of radiolabel being excreted within 96-120h. The excretion pattern showed a sex difference, independent of the dose. In males the ratio of urinary: fecal excretion was higher than in females. Males excreted 32-36% of the dose via urine and 49-50% via feces, whereas females excreted 25-28% via urine and 61-66% via feces. This may indicate that in comparison to males, in females less of the administered dose is absorbed and/or more of the compound is excreted through the bile. In both males and females about 7-13 % of radiolabel is excreted in expired air.

Data from a dermal absorption study with male rats indicate that there may be considerable biliary excretion. After dermal application of a low concentration (0.0963 mg/0.1mL) of dodemorph acetate 51, 31 and 9% of the absorbed radioactivity was excreted in urine, feces and expired air, respectively. After dermal application of a high concentration (38.5mg/0.1mL) of dodemorph acetate 41, 37 and 8% of the absorbed radioactivity was excreted in urine, feces and expired air, respectively. However, the study is considered to have some uncertainties: low recovery of

radioactivity, and the apparent kinetic differences between rat and human skin *in vitro* (see human information).

4.1.2 Human information

An *in vitro* dermal absorption study with human and rat skin is available. Dodemorph acetate was tested as concentrate (38.5 mg) or as dilution (0.0963 mg in 0.1 ml). The preparations were applied to the skin samples at a volume of 10 µl/cm^2. Rat skin was more permeable for dodemorph acetate than human skin. Based on the results of this study, absorption values of 2.7% for the concentrate and 20% for the dilution were derived.

4.1.3 Summary and discussion on toxicokinetics

Animal studies with dodemorph acetate indicate a moderate rate of absorption. At higher doses (1000 mg/kg bw) there is a slower rate of absorption and/or an increased rate of biliary excretion of dodemorph acetate. The estimate of the percentage oral absorption, solely based on the amounts of radioactivity recovered in urine, expired air and the body, is considered to be 40%.Based on the *in vitro* dermal absorption study absorption values are 2.7% for the concentrate and 20% for the dilution.

The data indicate that dodemorph acetate is extensively metabolized, either by microflora in the gut or in the body, which includes degradation of the morpholine ring. Several very polar metabolites are formed, but they are not identified.

Excretion of radiolabel is rapid following the low dose, with the majority being excreted within 24h after dosing. Following the high dose excretion is somewhat slower, with the majority of radiolabel being excreted within 96-120h. In males the ratio of urinary: fecal excretion was higher than in females.

4.2 Acute toxicity

Table 11: Summary table of relevant acute toxicity studies (results given as dodemorph acetate)

Method	Results	Remarks	References
FIFRA guideline 81-1	Oral LD_{50}: 5500 mg/kg bw	Rats	DAR 2007 volume 3 B.6.2.1
FIFRA guideline 81-1	Oral LD_{50}: > 2000 mg/kg bw	Rats. Supplementary study	DAR 2007 volume 3 B.6.2.1
FIFRA guideline 81-2	Dermal LD_{50}: > 2000 mg/kg bw	Rabbit	DAR 2007 volume 3 B.6.2.1
Acute LC50	Inhalation LC_{50} : not determined	Rats. Study not performed	DAR 2007 volume 3 B.6.2.1

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

A limit test (FIFRA guideline 81-1) with Sprague Dawley rats was performed with a dose level of 5000 mg dodemorph acetate/kg bw (5m/5f). Due to solubility problems the actual dose level was probably lower (about 1800 mg/kg bw). Therefore, during the following range finding and main tests the test substance was stirred continuously to ensure that an even suspension was administered to the animals. In the range finding tests, dose levels were 100, 500, 1000, 1500 and 2000 mg/kg bw (1m/1f per group) and 3000 and 4000 mg/kg bw (1m/1f per group). In the main study the dose levels were 4200, 4600 and 5000 mg/kg bw (5m/5f per group).
In the limit test, 4 m/4 f died between 4h and two days after exposure. Clinical signs were unusual locomotion, lethargy, tachypnoea, piloerection and catalepsy. At necropsy haemorrhaging in stomach and small intestine and discolouration of liver and kidneys were observed. In the range finding studies no mortality occurred and only lethargy was observed in animals exposed to 3000 and 4000 mg/kg bw.

In the main study 1m/2f died within one day at 5000 mg/kg bw; 2m/1f died within one day at 4600 mg/kg bw; 1f died within 4 h at 4200 mg/kg bw. Lethargy, tachypnoea, catalepsy, and nostril discharge were observed at all dose levels in the main test. Prostration was observed at 5000 mg/kg bw. Most animals that died lost weight. Weight gain was observed in the surviving animals but total gain was lower than observed in the range finding study (dose levels 100 – 2000 mg/kg bw). Haemorrhaging in stomach and small intestine and discolouration of liver, thymus and kidneys were found. Further dark red foci scattered throughout the thymus were found at 5000 mg/kg bw and red fluid in abdominal cavity at 4600 mg/kg bw.

The oral LD 50 value for dodemorph acetate is > 5000 mg/kg bw (95% confidence limits: 4800 – 6300 mg/kg bw).

In a second oral toxicity study with Sprague Dawley rats (FIFRA guideline 81-1), dose levels were 1600, 2000, 2500, 3200, 4000, 5000 and 6400 mm^3 dodemorph acetate/kg bw (10m/10f per group), given by gavage as a 20 or 30% aqueous emulsion (v/v) with gum tragacanth.

Mortality rate was 0, 1, 1, 1, 6, 7 and 8 for males and 0, 0, 5, 7, 9, 10, and 10 for females at 1600, 2000, 2500, 3200, 4000, 5000, and 6400 mm^3/kg, respectively. The highest mortality rate was observed within 48 hours after exposure. About half of the male animals died at a dose level of 4000 mm^3/kg bw (equivalent to ≈ 3944 mg/kg bw) and half of the female animals at 2500 mm^3/kg bw (equivalent to ≈ 2465 mg/kg bw).
At 1600 mm^3/kg only slight dyspnoea and apathy were observed. Clinical signs at the higher dose levels were dyspnoea, diarrhoea and titubation (unsteady gait). Necropsy findings were haemorrhagic gastritis and serosanguineous incrustation of nostrils.
LD50 values were not calculated.

4.2.1.2 Acute toxicity: inhalation

An acute inhalation study was not presented in the DAR. In the PRAPeR Expert Meeting 49, it was concluded that based on the physical chemical properties of the substance (high viscosity) and its corrosivity a valid inhalation study could not be carried out and was also not necessary.

4.2.1.3 Acute toxicity: dermal

A limit test was performed with a dose level of 2000 mg dodemorph acetate/kg bw (5m/5f per group). The semi-solid substance was applied two layers thick onto about 10% of the body surface, and covered with gauze patches. After the exposure period of 24h the skin was rinsed with 0.9% sodium chloride.

There was no mortality, nor were signs of toxicity observed. In all animals the skin site was found to be necrotic from day 1 throughout the 14 days observation period.

The dermal LD50 value for dodemorph acetate is > 2000 mg/kg bw.

4.2.1.4 Acute toxicity: other routes

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4.2.2 Human information

No data are available

4.2.3 Summary and discussion of acute toxicity

An acute inhalation exposure study could not be performed, but a study is not needed based on the physico-chemical and corrosive properties of the substance.

4.2.4 Comparison with criteria

The oral and dermal LD50 values are above the cut of limits of the DSD and CLP criteria (LD50 ≤ 2000 mg/kg bw; dermal LD50 ≤ 2000 mg/kg bw).

4.2.5 Conclusions on classification and labelling

Dodemorph acetate does not need to be classified for acute oral or dermal toxicity. An acute inhalation exposure study was not performed, based on the physico-chemical and corrosive properties of the substance. Additional classification for acute inhalation is therefore not considered

necessary for dodemorph acetate. However, based on the corrosive properties of dodemorph acetate, according to CLP, the substance should be labeled with EUH071: Corrosive to the respiratory tract (see 4.4.3).

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

In the oral toxicity studies, some toxic effects were observed. However, all these effects are considered to be resulting in lethality at increasing doses.

In the dermal toxicity study, no systemic toxic effects were observed.

An acute inhalation exposure study was not performed, based on the physico-chemical and corrosive properties of the substance.

4.3.2 Comparison with criteria

According to the CLP Regulation, substances should be classified for STOT SE when:

- They produce significant toxicity in animals (relevant for humans) or humans following single exposure: Cat 1
- They have the potential to be harmful to animals (relevant for humans) or humans following single exposure: Cat 2
- They have transient narcotic effects or cause transient respiratory tract irritation: Cat 3

Dodemorph acetate does not fulfil these criteria.

4.3.3 Conclusions on classification and labelling

The acute oral and dermal toxicity studies do not require classification for STOT SE. An acute inhalation exposure study could not be performed, but a study is not needed based on the physico-chemical and corrosive properties of the substance. There is no need to classify dodemorph acetate for STOT SE according to the CLP Regulation.

4.4 Irritation

4.4.1 Skin irritation

Table 12: Summary table of relevant skin irritation studies

Method	Results	Remarks	References
OECD 404	Severe oedema 24 hours after patch removal. Slight oedema at 48h, very slight oedema at 72h. No oedema 7 days after patch removal. Moderate to severe erythema from 24 up to 72h. Well-defined erythema at 7 days. Necrosis from day 2 to day 7.		DAR 2007 volume 3 B.6.2.2

4.4.1.1 Non-human information

A skin irritation study was performed in accordance with OECD 404.

The intact, clipped dorsal trunk of one female rabbit was moistened with water after which one water moistened gauze pad containing a single dose of 0.5 g dodemorph acetate (purity 98.2%), and one water moistened control patch were held for 4-hours under semi-occlusive bandage. Observations were performed 1, 24, 48, 72h and 7 days after patch removal.

Severe oedema was noted 24 hours after patch removal. This reduced to slight oedema at 48h and very slight oedema at 72h. No oedema was present 7 days after removal of the patch. Moderate to severe erythema was noted from 24 up to 72h. Well-defined erythema was still present at 7 days. A dark necrotic area was evident from day 2 to day 7 at which point it was decided to terminate the study.

4.4.1.2 Human information

No data are available

4.4.1.3 Summary and discussion of skin irritation

Based on the observed necrotic area, dodemorph acetate is considered corrosive to the skin.

4.4.1.4 Comparison with criteria

When applied to healthy intact animal skin for 4 hours, dodemorph acetate induces full thickness destruction of skin tissue. It therefore fulfils the DSD criteria for C; R34
Dodemorph acetate produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in 1 tested animal after exposure to a 4 hour duration. It therefore fulfils the CLP criteria for classification as Skin Corr. 1 (H314). There are no data available that allow differentiation between the skin corrosion subcategories 1A/1B/1C.

4.4.1.5 Conclusions on classification and labelling

According to the criteria of DSD, dodemorph acetate should be classified with C; R34 because of the observed necrosis in the skin irritation study. According to the CLP criteria, dodemorph acetate should be classified with Skin Corr. 1; H314.

4.4.2 Eye irritation

Table 13: Summary table of relevant eye irritation studies

Method	Results	Remarks	References
FIFRA guideline 81-4	*severe eye irritation*	*rabbits*	DAR 2007 volume 3 B.6.2.2

4.4.2.1 Non-human information

An eye irritation study was performed in accordance with FIFRA guideline 81-4**.**

An amount of 0.1 ml of undiluted dodemorph acetate (purity 99.6%) was placed in the conjunctival sac of the right eye of 3 rabbits (sex unknown). After an exposure period of 24 h, the eye was rinsed with deionised water in order to remove the test compound. Observations were performed 1, 24, 48, 72 hours, 7, 14 and 21 days after instillation.

Table 14: Eye irritation scores according to Draize scheme

	1h	24h	48h	72h	7d	14d	21d
Cornea	2/2/2	3/2/3	3/2/3	3/2/3	3/3/3	3/3/2	4/4/3
Iris[1]	-/-/-	2/-/-	2/2/2	2/2/2	-/-/-	2/2/1	-/-/1
Redness	3/3/3	3/3/3	3/3/3	3/3/3	2/2/1	3/3/2	2/3/2
Chemosis	4/4/4	4/4/4	4/4/4	4/4/4	3/4/2	3/4/1	2/2/1
Discharge	3/3/3	3/3/3	3/3/3	3/3/3	3/3/2	3/3/1	3/3/1

[1] iris obscured by corneal opacity

4.4.2.2 Human information

No data are available

4.4.2.3 Summary and discussion of eye irritation

Dodemorph acetate produced severe eye irritation in rabbits, which persisted throughout the 21 days observation period.

4.4.2.4 Comparison with criteria

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 (cornea opacity equal to or greater than 3 and/or iris lesion greater than 1.5). It therefore fulfills the DSD criteria, for classification as 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 damage 1; H318 (Causes serious eye damage).

4.4.2.5 Conclusions on classification and labelling

According to the EC classification criteria dodemorph acetate should be classified as R41 (risk of serious damage to eyes). However, since the compound is already classified as R34 being corrosive,

the risk of severe damage to the eyes is considered implicit. According to the criteria of Regulation (EC) No 1272/2008, the results of the eye irritation study require classification as Eye damage 1; H318. However, since dodemorph acetate is already classified as Skin Corr. 1 (H314), serious damage to eyes is implicit.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

No data are available

4.4.3.2 Human information

No data are available

4.4.3.3 Summary and discussion of respiratory tract irritation

4.4.3.4 Comparison with criteria

Dodemorph acetate is a corrosive substance. According to the CLP criteria, if a substance is classified for inhalation toxicity and data are available that indicates that the mechanism of toxicity is corrosivity or the substance is classified for skin corrosivity and the substance is not tested for acute inhalation toxicity and can be inhaled, the substance or mixture shall also be labelled as EUH071: ‘corrosive to the respiratory tract (CLP Annex II 1.2.6). Dodemorph acetate has not been tested for acute inhalation toxicity but was corrosive to the skin. This fulfils the first criterion for labelling with EUH071. Although dodemorph acetate is a substance with high viscosity, it is used as spray. Therefore, it is possible that aerosols are inhaled. The second criterion for labelling with EUH071 is therefore also fulfilled.

Classification with R37 could be considered in analogy with EUH071. However, as the DSD criteria require positive results in appropriate animal test and there are no animal tests with inhalatory exposure, the criteria are not fulfilled.

Further, R37 could be considered as an SCL at concentration at which a classification for corrosivity is not required but classifications for skin and eye irritation are required. However, such an SCL is not proposed as there is no direct evidence for the induction of respiratory tract irritation after inhalation. Although it is clear that undiluted dodemorph acetate will cause respiratory tract irritation (or likely even stronger effects), it is unknown at which dilution this substance will induce respiratory tract irritation only.

4.4.3.5 Conclusions on classification and labelling

According to Regulation (EC) No 1272/2008, labelling with EUH071 is proposed for dodemorph acetate (see Annex I 3.1.2.3.3 and footnote 1 to table 3.1.3, Annex II 1.2.6, CLP Guidance 3.1.4.2). Classification with R37 is not proposed based on absence of data.

4.5 Corrosivity

4.5.1 Non-human information

A skin irritation study was performed in accordance with OECD 404. The results of this study are also relevant for the endpoint corrosivity. For details please refer to Section 4.4.1 (skin irritation) of this document.

4.5.2 Human information

No data are available.

4.5.3 Summary and discussion of corrosivity

Based on the observed necrotic area, dodemorph acetate is considered corrosive.

4.5.4 Comparison with criteria

According to the DSD criteria, a substance should be classified as C; R34 if, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to 4 hours exposure.
According to the CLP criteria, a substance should be classified as corrosive Cat 1A, B or C when it produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least 1 tested animal after an exposure of ≤ 3 minutes, >3 minutes - ≤ 1 hour or >1 hour - ≤ 4 hour, respectively. When there are no data available that allow differentiation between the skin corrosion subcategories 1A/1B/1C the substance should be assigned skin corrosive Category 1. After an exposure period of 4 hours, dodemorph acetate induces necrosis. However, there are no data on the effects after an exposure shorter than 4 hours.

4.5.5 Conclusions on classification and labelling

According to the criteria of DSD, dodemorph acetate should be classified with C; R34 because of the observed necrosis in the skin irritation study. According to the CLP criteria, dodemorph acetate should be classified with Skin Corr1; H314.

4.6 Sensitisation

4.6.1 Skin sensititsation

Table 15: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	References
OECD 406	positive	guinea pigs	DAR 2007 volume 3 B.6.2.2

4.6.1.1 Non-human information

A maximisation study was performed in accordance with OECD 406. Female guinea pigs (5 controls, 10 test animals were treated with dodemorph acetate (purity 94%). A 1% concentration was selected for the intra dermal injections on day 1 and a 20% concentration was selected for the topical induction exposure on day 8 (48 h exposure). The control animals were treated with the vehicle corn oil. On day 21 all animals were challenged with a 5% (non-irritating) concentration (24h exposure). The treated sites were assessed for challenge reactions 24 and 48h after patch removal.

Skin reactions were observed in both the control and treated group. At the 48 h readings 5 animals of the test group showed higher readings (score 2) than those in the control group. Therefore, at least 50% of the animals showed a sensitising reaction. In view of the increased incidence and intensity of the responses in the treated group as compared to the control group, it is considered that dodemorph acetate induces sensitisation.

Table 16: Irritation scores GPMT

Challenge reading	Control group	Test group
24h reading	1/0/0/0/2 (40%)	1/0/2/2/0/0/0/2/2/2 (60%)
48h reading	0/0/0/0/1 (20%)	0/2/1/2/1/1/2 s/1/2/2 (90%)

s = eschar formation

4.6.1.2 Human information

No information available.

4.6.1.3 Summary and discussion of skin sensitisation

In a GPMT test with dodemorph acetate >30% of the test animals showed a more positive response than the control animals at the 48h reading.

4.6.1.4 Comparison with criteria

According to the DSD criteria, a substance shall be classified as sensitising when there are positive results from appropriate animal tests (a response of at least 30% of the animals in an adjuvant-type test).

According to the CLP criteria, a substance should be classified as sensitising when there are positive results from appropriate animal tests (a response of at least 30% of the animals in an adjuvant-type test).

These criteria are fulfilled in the study with dodemorph acetate. As a response in more than 60% of the animals after 48 hours was observed above the control (90% minus 20%) after intradermal induction with 1%, the criteria for sub category 1A are fulfilled.

4.6.1.5 Conclusions on classification and labelling

In a 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 EC classification criteria, dodemorph acetate should be classified as sensitizing: R43. According to the criteria of EC 1272/2008, dodemorph acetate should be classified as Skin Sens 1A; H317.

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

No information available.

4.6.2.2 Human information

No information available.

4.6.2.3 Summary and discussion of respiratory sensitisation

4.6.2.4 Comparison with criteria

4.6.2.5 Conclusions on classification and labelling

No classification proposed due to a lack of data.

4.7 Repeated dose toxicity

Table 17: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	References
21 days dermal study, no guideline	NOAEL (local): 12 mg/kg bw/day (~ dodemorph: 10 mg/kg bw/day). NOAEL (systemic) 60 mg/kg bw/day (~ dodemorph: 49 mg/kg bw/day).	Rabbit, 0, 2.4, 12 and 60 mg/kg bw/day	DAR 2007 volume 3 B.6.3
28 days diet study, no guideline	No toxicologically relevant effects were observed	Rats, 0, 50 and 100 mg/kg bw/day	DAR 2007 volume 3 B.6.3
28 days gavage study, no guideline	NOAEL: 40 mg/kg bw/day (~ dodemorph: 33 mg/kg bw/day). LOAEL: 80 mg/kg bw based on vomiting and salivation	Dog, 0, 40, 80 and 160 mg/kg bw/day	DAR 2007 volume 3 B.6.3
90 days diet study, no guideline	mild increase in relative liver weight in females top dose. NOAEL: 80 mg/kg bw	Rat, 0, 20, 40 and 80 mg/kg bw/day	DAR 2007 volume 3 B.6.3
90 days diet study, OECD 408	increased agitated, aggressive and nervous behaviour. Decreased food intake, increased relative liver weight (female), with minimal centrilobular hypertrophy. NOAEL: 79 mg/kg bw	Rat, 0, 20, 79 and 229 mg/kg bw/day for males and 0, 23, 94 and 259 mg/kg bw/day for females	DAR 2007 volume 3 B.6.3
90 days diet study, no guideline	vomiting and salivation, reduced body weight and food intake. Increased liver weight, increased levels of enzymes indicative of liver damage, pale liver, degenerative changes and fatty degeneration. NOAEL: 32 mg/kg bw	Dog, 0, 32, 79 and 187 mg/kg bw/day for males and 0, 33, 79 and 194 mg/kg bw/day for females	DAR 2007 volume 3 B.6.3
1 year oral study, OECD 452	vomiting, salivation and effects on fecal excretion, decreased body weight and food intake, bile duct hyperplasia, associated with marked peribiliary fibrosis, gastric lesions NOAEL: 10 mg/kg bw	Dog, 0, 10, 25 or 62.5 mg/kg bw/day	DAR 2007 volume 3 B.6.3
18 months diet study, OECD 451	Decreased body weight and food intake, increased relative liver weight, accompanied by minimal histological changes in the liver at the high dose NOAEL: 45 mg/kg bw	Mouse, 0, 45, 152 and 455 mg/kg bw/day for males and 0, 55, 184 and 545 mg/kg bw/day for females	DAR 2007 volume 3 B.6.5
2 year diet study, OECD 453	Decreased food intake, increased relative organ weights, not accompanied by macroscopic or microscopic changes (except for liver). NOAEL: 45 mg/kg bw	Rats, 16, 55 and 166 mg/kg bw/day for males and 0, 21, 73 and 222 mg/kg bw/day for females)	DAR addendum 2008 volume 3 B.6

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

In a 28 day study (pre-guideline), dietary treatment of rats with dodemorph acetate at doses of 500 and 1000 ppm, (equivalent to about 50 and 100 mg/kg bw/day) did not cause toxicologically relevant effects.

In a 28 day study (pre-guideline), beagle dogs (3/sex/dose) received daily oral (gavage) administrations (distributed in 2 single gifts) of dodemorph acetate (in 1% aqueous methylcellulose-gel 300 P) at doses of 0, 40, 80 and 160 mg/kg bw/day. In animals of the 80 and 160 mg/kg bw/day groups vomiting and salivation were observed, with increased frequency and severity at the highest dose. At the high dose, intervals of slight sedation and soft stools were observed. Food consumption was decreased and the animals lost weight (not specified for sex) in the highest dose group, in particular during the first 2 weeks of treatment. A statistically significant increase in absolute and relative liver weight (data of males and females combined) was observed at 160 mg/kg bw/day. No other treatment-related effects were observed.

In a 90 day study (pre-guideline), Sprague Dawley rats (20/sex/dose) received dodemorph acetate in the diet at 0, 400, 800 or 1600 ppm (equivalent to 0, 20, 40 and 80 mg/kg bw/day). Dietary treatment of female rats with dodemorph acetate caused a mild increase in relative liver weight (12%) at 1600 ppm. However, since these effects were small and not accompanied by histopathological effects, it is not considered in the establishment of the NOAEL. Therefore, the NOAEL in this study is 1600 ppm, equivalent to 80 mg/kg bw/day, i.e. the highest dose tested.

In a 90 day study according to OECD 408, Sprague Dawley rats (10/sex/dose) received dodemorph acetate in the diet at 0, 300, 1200 or 3600 ppm (equal to 0, 20, 79 and 229 mg/kg bw/day for males and 0, 23, 94 and 259 mg/kg bw/day for females). Males of the 3600 ppm group displayed increased agitated, aggressive and nervous behaviour. These signs were observed toward the end of the study. The weekly detailed clinical observations revealed no treatment-related effects. In the functional observational battery and motor activity test no treatment-related clinical effects were observed. Body weight (females) and body weight gain (males and females) were significantly reduced in animals of the high dose group. Food consumption was significantly decreased in males (7-10%) and females (10-27%) of the high dose group. Females of the 3600 ppm group had an increased relative liver weight (17%). Histopathological examination revealed minimal centrilobular hypertrophy in some of the females in this group. In males of the high dose group a 16% reduction (not statistically significant) in relative prostate weight was observed. Since no histopathological effects in the prostate were found, and this finding was not observed in other studies, the reduction in relative prostate weight was considered not toxicologically relevant. The NOAEL was 1200 ppm, equal to 79 mg/kg bw/day.

In a 90 day study (no guideline), Beagle dogs (3/sex/dose) received dodemorph acetate in the diet at 0, 1000, 2500 or 6250 ppm (equal to 0, 32, 79 and 187 mg/kg bw/day for males and 0, 33, 79 and 194 mg/kg bw/day for females). Food was provided in 2 daily portions. In animals of the 2500 ppm group vomiting and salivation were observed, in general only after the first feeding of the day. Similar effects, with increased severity, were observed in the 6250 ppm group. At this dose, these effects were observed after both daily feedings. From the 3rd week onwards vomiting subsided markedly, but could still be observed occasionally. Due to the vomiting test substance may be lost. At 2500 and 6250 ppm, intervals of sedation were observed. At 6250 ppm the animals lost weight. Food consumption was dose-dependently reduced (not specified for sex). Clinical chemistry showed increased levels of enzymes indicative of liver damage at 2500 and 6250 ppm. Absolute and relative liver weights (not specified for sex) were dose-dependently increased at the mid and high dose. At these doses macroscopy demonstrated pale liver. Histological examination revealed degenerative changes and fatty degeneration. The incidence and severity increased with dose (degenerative changes: moderate in 5/6 animals of the mid dose and 1/6 animals of the high dose, marked in 1/6 animals of the mid dose and 5/6 animals of the high dose; fatty degeneration:

moderate in 1/6 animals of the low dose, 2/6 animals of the mid dose and 2/6 animals of the high dose, marked in 3/6 animals of the high dose). NOAEL is 1000 ppm, equal to 32 mg/kg bw/day.

In a 1 year study according to OECD 452, Beagle dogs (4/sex/dose) received oral (capsule) administrations of dodemorph acetate at 0, 10, 25 or 62.5 mg/kg bw/day. One female of the high dose group was killed on day 43 for ethical reasons. Necropsy demonstrated a poor general condition and pale liver. In this animal blood levels of liver enzymes were increased, consistent with the degenerative changes in the liver observed microscopically (capsular fibrosis, bile duct hyperplasia, peribiliary fibrosis of portal tracts, small foci of hepatocellular necrosis in subcapsular areas, sinusoidal dilatation of liver parenchyma and vacuolar degeneration mainly in centrilobulat hepatocytes, iron pigment in macrophages entrapped in the capsular fibrosis). In the other animals, dose dependent increases in the incidence and severity of vomiting, salivation and effects on fecal excretion were observed at the mid and high dose groups. Vomiting was already observed in the first week of treatment. Due to the vomiting test substance may be lost. The vomiting and salivation observed in the low dose animals only occurred sporadically and was not considered to be toxicologically significant. In the high-dose males body weight, body weight gain and food consumption were decreased as compared to controls. Occasional haematological changes were considered incidental. At 13, 26 and 52 weeks, clinical chemistry showed dose-dependently increased levels of enzymes indicative of liver damage at 62.5 mg/kg bw/day; the increase at 25 mg/kg bw/d was only slight and not consistent over time (see Table 6.3.3-4b). Absolute and relative liver weights tended to be increased at all dose levels, although there was no clear dose-response relationship. At the mid and high doses microscopical examination demonstrated bile duct hyperplasia, associated with marked peribiliary fibrosis. At these doses macroscopical and microscopical examination revealed gastric lesions in some animals. The NOAEL is 10 mg/kg bw/day.

In an 18 month study according to OECD 451, Crl:CD-1 (ICR) BR VAF/plus mice (50/sex/dose) were exposed to dodemorph acetate in the diet at 0, 300, 1000 or 3000 ppm (equal to 0, 45, 152 and 455 mg/kg bw/day in males and 0, 55, 184 and 545 mg/kg bw /day in females). Body weight gain was reduced at the mid and high dose. Slight reductions in food consumption were observed in the high dose group. Relative liver weight was significantly increased in males of the mid-dose group, and males and females of the high-dose groups. These liver weight effects were accompanied by minimal histological changes in the liver at the high dose. Occasionally statistically significant increases in other organ weights relative to body weights were observed. The changes in organ weights were not accompanied by macroscopic or microscopic changes, and the organ weights relative to brain weight were not affected. Therefore, these changes are considered secondary to the decreased body weights in these animals, and considered not a direct effect of treatment. Females of all treatment groups had a lower relative spleen weight than the control animals. However, these effects were not dose dependent, were not found in males, and may be related to a high spleen weight in the control females. NOAEL in this study is 300 ppm, equal to 45 mg/kg bw/day.

In a 2 year study according to OECD 453, Sprague Dawley rats (50/sex/dose) were exposed to dodemorph acetate in the diet at 0, 300, 1000 or 3000 ppm (equal to 0, 16, 55 and 166 mg/kg bw/day in males and 0, 21, 73 and 222 mg/kg bw /day in females). Small but statistically significant decreases in food consumption were observed in the high dose animals throughout the duration of the study. The sporadic changes observed in haematological, clinical chemistry and urinalysis were considered incidental and not treatment-related. At the interim and terminal kill, in animals of the high dose groups statistically significant increases in organ weights relative to body weights (males: liver (11%) and testes (11%); females: adrenals (16%), kidneys (15%), liver (26%) and lung (20%) were observed. The changes in organ weights were not accompanied by macroscopic or microscopic changes (except for liver), were not consistent when comparing 12 and 24 month data

or only occurred in one sex. Moreover, the organ weights relative to brain weight were not affected. Therefore, these changes are considered secondary to the decreased body weights in these animals (males -8%; females -20%), and considered not a direct effect of treatment. In the liver of the 3000 ppm group increased incidences of foci of cellular alterations and regenerative hyperplasia were observed. Hyperplasia was also observed in the females of the 1000 ppm group at the interim kill. In the lungs dose dependent increases in incidences of white-grey foci, macrophage infiltration and granulomatous inflammation of the lung were observed. According to the study author, these effects, and the presence of fluid and plant material in the lungs of both control and treated animals are attributable to the inadvertent inhalation of foreign material, and are therefore considered not related to oral exposure to dodemorph acetate. The NOAEL is 1000 ppm, equal to 55 mg/kg bw/day.

4.7.1.2 Repeated dose toxicity: inhalation

No data available.

4.7.1.3 Repeated dose toxicity: dermal

In a 21 days (pre-guideline) study, New Zealand White rabbits (3/sex/dose), with intact or scarified skins, received daily dermal administrations of 3 g/kg bw of a 1% aqueous methylcellulose solution containing 0, 0.08, 0.4 or 2.0% dodemorph acetate for 21 days. These doses are equal to 0, 2.4, 12 and 60 mg/kg bw/day. The test solution was spread over a 15 x 16 cm^2 area (i.e. 240 cm^2), and covered with a rubber flap. Based on an average body weight of 2.7 kg, the local dodemorph acetate doses were 0, 0.027, 0.135 and 0.675 mg/cm^2. Animals of the 2% group showed progressive development of local erythema and oedema and scab formation. Erythema and oedema were first observed after 4 days (intact skin) or 2 days (scarified skin) of treatment, reaching maximum Draize score by day 17 (intact skin) or day 15 (scarified skin). The local skin effects in animals with scarified skin fully recovered during a 21-day recovery period.
No treatment-related systemic effects were observed.

Further dermal repeated dose studies were not performed since:

1. in terms of animal welfare it would be deemed outwith Inveresk severity limits to repeatedly apply a test item known to cause burns.

2. any results gained from such a study would be difficult to interpret i.e. it would be very difficult to determine which were systemic toxicity effects and which were due to distress inflicted on the animal by the corrosive material.

4.7.1.4 Repeated dose toxicity: other routes

4.7.1.5 Human information

4.7.1.6 Other relevant information

4.7.1.7 Summary and discussion of repeated dose toxicity

Subacute and semichronic oral studies in the rat and the dog were available. In these studies the main targets for dodemorph acetate were body weight and 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 semichronic 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 ALAT and AP indicate that dodemorph acetate induced hepatocellular damage and 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, in the dog study 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.

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. At a dermal dose of 60 mg/kg bw/day local effects (erythema, oedema, scab formation) were observed. The NOAEL for local dermal effects was 12 mg/kg bw/day

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

See paragraph 4.7.1.7

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

According to DSD, the cut off values for classification as causing danger of serious damage to health by prolonged exposure are:

<50 mg/kg bw/day in a 90 day oral study (or 150 mg/kg bw/day in a 28 day oral study)

<100 mg/kg bw/day in a 90 day dermal study (or 300 mg/kg bw/day in a 28 day dermal study)

Dodemorph acetate does not fulfil the criteria for classification for oral repeated dose toxicity as the LOAEL in the 90-day studies was clearly above the cut-off value.

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

Inhalation studies with dodemorph acetate are not available.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

Dodemorph acetate does not fulfil the criteria for classification for oral repeated dose toxicity.

For dermal repeated dose toxicity, the LOAEL is lower than the cut off value for classification. However, all observed effects are due to the corrosive properties of dodemorph acetate. Since dodemorph acetate is already classified for these effects (C; R34), further classification for repeated dermal toxicity is considered not necessary.

Based on the physical chemical properties of the substance (high viscosity) and its corrosivity a valid inhalation study could not be carried out and was also not necessary. No classification for repeated inhalation toxicity is proposed.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

See paragraph 4.7.1.7

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

According to CLP, the cut off values for classification for STOT RE are:

<100 mg/kg bw/day in a 90 day oral study

<200 mg/kg bw/day in a 90 day dermal study

Dodemorph acetate does not fulfil the criteria for classification for oral repeated dose toxicity because the effects at the LOAEL that was just below the cut off value were limited (bile duct hyperplasia without consistent enzymatic changes or significant changes in organ weight).

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

Inhalation studies with dodemorph acetate are not available.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Dodemorph acetate does not fulfil the criteria for classification for oral repeated dose toxicity.

For dermal repeated dose toxicity, the LOAEL is lower than the cut off value for classification. However, all observed effects are due to the corrosive properties of dodemorph acetate. Since dodemorph acetate is already classified for these effects (Skin Corr1C; H314), further classification for STOT RE is considered not necessary.

Based on the physical chemical properties of the substance (high viscosity) and its corrosivity a valid inhalation study could not be carried out and was also not necessary. No classification for repeated inhalation toxicity is proposed.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 18: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	References
Ames test, resembles OECD 471	negative	With and without S9	DAR 2007 volume 3 B.6.4.
HGPRT, resembles OECD 476	negative	With and without S9	DAR 2007 volume 3 B.6.4.
Chromosome aberration in vitro, resembles OECD 473	Results not suitable	With and without S9 Not acceptable	DAR 2007 volume 3 B.6.4.
DNA repair assay, in vitro	Results not suitable	With and without S9 Not acceptable	DAR 2007 volume 3 B.6.4.
UDS, in vitro	Negative		DAR 2007 volume 3 B.6.4.
Micronucleus test in vivo, resembles OECD 474	Negative		DAR 2007 volume 3 B.6.4.

4.9.1 Non-human information

4.9.1.1 In vitro data

An Ames test according to OECD 471 (unknown purity) was performed with dodemorph acetate, solved in ethanol. Under the test conditions, dodemorph acetate (purity unknown) did not induce point mutations in S. typhimurium (strains 98, 100, 1535, 1537 and 1538).

A HGPRT locus gene mutation test in Chinese Hamster Ovary cells (resembling OECD 476) was performed with dodemorph acetate, solved in DMSO. Cytotoxicity (>50%) in the main test was observed from 0.0464 mg/mL for cells in absence of S9, and at 0.464 mg/mL for cells in presence of S9. A first test was performed with 5 flasks per dose level up to a dose level of 0.215 mg/mL. In this test a borderline increase of mutation rate (15.10-6) was observed at a low dose level in presence of S9 (0.0215 mg/mL). The test was repeated in presence and absence of S9, with an extra dose level. No increased mutation rates were observed in this test.

A chromosome aberration test in Chinese Hamster Ovary cells according to OECD 473 (unknown purity) was performed with dodemorph acetate, solved in ethanol. Complete cytotoxicity in the main test was observed from 0.11 µL/mL for cells in absence or presence of S9. The slides of these test groups could not be used for cytogenetic analysis because no metaphase cells were observed on the slides. No increased incidence in chromosome aberrations was observed in this test for the remaining dose levels of 0.01 and 0.04 µL/mL. The study is considered not acceptable, due to too many deviations from the guideline (unknown purity, only 2 analysable doses, no additional experiment performed).

A DNA repair assay in E. coli was conducted with dodemorph acetate. In the first test the assay without metabolic activation could not be evaluated because of complete toxicity of the positive control group; in the repeat trial (with an acceptable result in the positive control), the survival indexes were all near or above the survival index for the negative control group. The first test with metabolic activation was negative (only one single observation of lower survival index was observed at 0.01 µL only and this was considered an incidental finding). The repeat test was not valid because of the failure of the positive control to induce a differential survival in the test.

A UDS assay in hepatocytes from male F344 rat (resembling OECD 482) was performed with dodemorph acetate, solved in DMSO. Three replicate cultures were used per dose and 50 cells were

scored/replicate. The highest dose level (50 µg/mL) showed a low survival rate (22%) and there were insufficient cells for analysis. The survival rate at 30 µg/mL was 84% and in all lower dose levels 100%. Under the test conditions, dodemorph acetate (purity unknown) did not induce significant changes in the nuclear labelling of primary rat hepatocytes.

4.9.1.2 In vivo data

A mouse micronucleus test resembling OECD 474 was performed with dodemorph acetate.
Single oral exposures of 0, 250, 500, 1000 mg/kg bw were used. Animals of the high dose showed marked toxicity and were in poor general state (irregular respiration, piloerection, apathy, atony, spastic gait and squatting posture). The mid dose group showed irregular respiration and slight excitation, the low dose group only piloerection. No pathological changes at necropsy were observed in any dose group. No significant changes in NCE/PCE ratio were observed.
Animals were sacrificed at 24 h after exposure, and only in the high dose group also after 16 and 48 h. Only 1000 polychromatic cells /animal (instead of 2000) were investigated. However, 10 analysable animals (5m/5f) were used where only 5 are prescribed in OECD 474. No toxicity was observed in the target organ. However, marked signs of systemic toxicity were observed in animals. Therefore, the study is considered acceptable. Under the test conditions, dodemorph acetate did not induce micronuclei in mouse bone marrow cells.

4.9.2 Human information

No data are available.

4.9.3 Other relevant information

4.9.4 Summary and discussion of mutagenicity

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 a mouse micronucleus test was negative. Based on all available data it is concluded that dodemorph acetate, and dodemorph are not genotoxic.

4.9.5 Comparison with criteria

If there is evidence from in vitro or in vivo studies (or evidence in humans) that a substance (may) induce heritable mutations in humans, they should be classified for mutagenicity (both according to DSD and CLP). Dodemorph acetate do not fulfil these criteria.

4.9.6 Conclusions on classification and labelling

Based on all available data it is concluded that dodemorph acetate is not genotoxic. No classification is therefore proposed.

4.10 Carcinogenicity

Table 19: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	References
OECD 451	not carcinogenic	Mouse	DAR 2007 volume 3 B.6.5
OECD 453	not carcinogenic	Rat	DAR addendum 2008 volume 3 B.6

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

In an 18 month study according to OECD 451, Crl:CD-1 (ICR) BR VAF/plus mice (50/sex/dose) were exposed to dodemorph acetate in the diet at 0, 300, 1000 or 3000 ppm (equal to 0, 45, 152 and 455 mg/kg bw/day in males and 0, 55, 184 and 545 mg/kg bw /day in females).

A number of neoplastic lesions were observed in control and treated animals. These were of a type and incidence that are commonly observed in mice of this strain, age and sex. Statistical analysis showed no dose-related trend. Moreover, the incidences of neoplastic lesions were within the range of historical control data of Charles River Laboratories. Therefore they were considered of a spontaneous nature and not treatment-related.

In a 2 year study according to OECD 453, Sprague Dawley rats (50/sex/dose) were exposed to dodemorph acetate in the diet at 0, 300, 1000 or 3000 ppm (equal to 0, 16, 55 and 166 mg/kg bw/day in males and 0, 21, 73 and 222 mg/kg bw /day in females). In the liver of the 3000 ppm group increased incidences of foci of cellular alterations and regenerative hyperplasia were observed. Hyperplasia was also observed in the females of the 1000 ppm group at the interim kill.

The occasional increased incidences in neoplastic lesions in the high dose group fell within the historical range of Charles River Laboratories, and were considered spontaneous and not related to dodemorph acetate treatment. A number of neoplastic lesions were observed in control and treated animals (see table 20). These were of a type and incidence that are commonly observed in rats of this strain. The incidences of neoplastic lesions were within the range of historical control data of Charles River Laboratories. There was no indication of a treatment-related increase in incidences in number of neoplasms.

Table 20 Group incidences of neoplastic lesions in 2-year oral study in the rat (%)

Dose (ppm)	*0*	*0*	*300*	*300*	*1000*	*1000*	*3000*	*3000*	*dr*
Sex	*m*	*f*	*m*	*f*	*m*	*f*	*m*	*f*	
12 month interim necropsy									
Liver									
- adenoma	*-*	*-*	*-*	*-*	*5.0*	*-*	*-*	*-*	
mammary gland									
- fibroadenoma	*-*	*10.0*	*-*	*-*	*-*	*5.0*	*-*	*15.8*	
thyroid gland									
-follicular cell adenoma	*-*	*-*	*5.0*	*-*	*-*	*-*	*-*	*-*	
pituitary gland									
- adenoma	*5.0*	*-*	*10.0*	*-*	*5.0*	*-*	*-*	*-*	
adrenal gland									
- pheochromocytoma/benign	*5.0*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	
2-year final necropsy									
adrenal gland									
- pheochromocytoma/benign	*-*	*2.0*	*5.9*	*-*	*20.0*	*-*	*4.1*	*-*	
- pheochromocytoma/malignant	*2*	*2.0*	*5.9*	*3.5*	*-*	*-*	*4.1*	*2.0*	
brain									
- granular cell tumor	*-*	*-*	*-*	*-*	*6.3*	*-*	*-*	*2.0*	
duodenum									
-polyp	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*2.3*	
kidney									
- adenoma	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*2.0*	
ovary									
- adenocarcinoma	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*4.2*	
skin									
- basilioma/benign	*-*	*-*	*-*	*-*	*-*	*-*	*4.0*	*-*	
- fibrosarcoma	*-*	*-*	*-*	*-*	*-*	*-*	*2.0*	*-*	
- keratoacanthoma	*-*	*-*	*-*	*-*	*-*	*-*	*2.0*	*-*	
mesenteric lymph node									
- lymphangioma	*2.0*	*-*	*-*	*-*	*-*	*-*	*-*	*2.3*	
pancreas									
- adenoma/islet cell	*4.1*	*4.2*	*11.1*	*-*	*6.6*	*-*	*8.0*	*-*	
pituitary gland									
- adenoma	*42.0*	*67.4*	*58.8*	*85.4*	*64.3*	*80.1*	*48.0*	*80.0*	
thyroid gland									
- adenoma/follicular cell	*4.0*	*2.1*	*-*	*-*	*6.3*	*-*	*2.1*	*4.6*	
uterus									
- sarcoma NOS		*-*		*-*		*-*		*4.0*	
-Schwannoma		*-*		*-*		*-*		*2.0*	
- squamous carcinoma		*-*		*-*		*-*		*2.0*	

Percentages given are based on number of lesions divided by the number of tissues examined.

(For further adverse effects observed in these studies, see paragraph 4.7; repeated dose toxicity)

4.10.1.2 Carcinogenicity: inhalation

No data available

4.10.1.3 Carcinogenicity: dermal

No data available

4.10.2 Human information

No data available

4.10.3 Other relevant information

4.10.4 Summary and discussion of carcinogenicity

In the chronic toxicity studies no tumorigenic potential of dodemorph acetate was identified.

4.10.5 Comparison with criteria

Dodemorph acetate does not fulfil the criteria of DSD and CLP for classification as carcinogenic, since in 2 carcinogenicity studies no tumorigenic potential of dodemorph acetate was identified.

4.10.6 Conclusions on classification and labelling

The available data indicate that dodemorph acetate is not carcinogenic. Therefore, no classification is proposed.

4.11 Toxicity for reproduction

Table 21: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	References
One generation, no guideline (range finding study, main study see below)	Parental: reduction in food consumption and body weight gain. Decreased blood cholesterol, increased blood creatinine. No effects on fertility. Offspring: decreased body weight at birth, decreased body weight gain, decreased litter size and reduced viability index	Rat	DAR 2007 volume 3 B.6.6
OECD 416	Parental: reduction in body weight gain, food consumption and blood cholesterol and histological changes in the liver. No effects on fertility. Offspring: decreased body weight at birth, decreased body weight gain, decreased viability and lactation indices.	Rat	DAR 2007 volume 3 B.6.6
OECD 414 (2001)	Maternal: salivation; bw (corrected)↓ Dev: Decreased body weight; skeletal variations	Rat	DAR 2007 volume 3 B.6.6
Developmental toxicity study, no guideline (range finding study, main study see below)	Maternal: top doses: severe toxicity, resulting in 100% implantation loss. Low dose group: reduced body weight and food intake, increased ALT, GGT and cholesterol. Increased post implantation loss, due to late resorptions. Offspring: anasarca, open eye	Rabbit	DAR 2007 volume 3 B.6.6
OECD 414 (1981)	No maternal toxicity. Dev: Early resorptions; post implantation loss; open eye malformation	Rabbit	DAR 2007 volume 3 B.6.6

All studies are performed with dodemorph acetate.

4.11.1 Effects on fertility

4.11.1.1 Non-human information

In a range-finding one-generation reproductive toxicity test in rats (no GLP, no guideline), dodemorph acetate (purity 98.7%) was administered orally in the diet at 0, 600, 1200 and 2400 ppm (equal to 0, 70, 140 and 271 mg/kg bw/day for males and 0, 63, 123 and 238 mg/kg bw/day for females, i.e. compound intake during gestation) to animals of the F0 generation (10/sex/dose). Treatment started at least 42 days before mating and continued until day 21 after birth of the pups. Animals were checked daily for clinical signs and mortality. Food and drinking water consumption and body weight were measured weekly. Females were weighed on days 0, 7, 14 and 20 of gestation. Females and pups were weighed on days 4, 7, 14 and 21 after giving birth. Male and

female reproduction parameters, and pup parameters were determined. Blood of parental animals was sampled for haematology (differential blood smears and reticulocytes were not evaluated) and clinical chemistry.

Table 22 Results from 1-generation reproductive toxicity study (range-finding) in the rat

	Dose (ppm)	0	0	600	600	1200	1200	2400	2400	dr
	Sex	m	f	m	f	m	f	m	f	
F0 animals	Mortality	no toxicologically relevant effects								
	Clinical signs	no toxicologically relevant effects								
	Body weight gain as % change from control -premating -gestation			-4	-9 +1	-3	-11 -11	-18*	-21* -27*	 dr dr
	Food consumption as % change from control -premating during gestation during lactation			-1	-4 -1 -2	-1	-6 -7 -9	-8*	-12* -14* -36*	 dr dr dr
	haematology	no toxicologically relevant effects								
	clinical chemistry - cholesterol - creatinine			-7 +5	-2 +7	-17* +10*	0 +7	-26* +10*	-15* +12*	 dr dr
	Mating, fertility, gestation	no toxicologically relevant effects								
	gestation duration	no toxicologically relevant effects								
F1 pups	Litter size	15.3		15.0		13.6		11.7*		dr
	viability index as % survivors, day 0-4	95		96		95		61*		
	Lactation index as % survivors, day 4-21	99		99		99			95	
	Sex ratio	no toxicologically relevant effects								
	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)	dr
	Pathology									
	- macroscopy	no toxicologically relevant effects								

* significantly different; dr = dose related

Dietary treatment with dodemorph acetate at 1200 and 2400 ppm induced dose-dependent reductions in food consumption and body weight gain in parental males and females. In addition a decrease in blood cholesterol and an increase in blood creatinine were observed, which may be related to the decreased food consumption and body weight. In the 2400 ppm group a decrease in litter size was observed. In the pups of the 2400 ppm group a reduced viability index, a decreased body weight on day 1 and a decreased body weight gain from day 4-21 was observed.

In a 2-generation reproduction toxicity study according to OECD guideline 416, dodemorph acetate (purity 98.7%) was administered orally in the diet at 0, 200, 600 or 1800 ppm (equal to 0, 21, 64 and 194 mg/kg bw/day) to rats (25/sex/dose) of the F0 generation. Treatment started at least 70 days before mating. The F0 animals were mated to produce two litters (F1a and F1b). At day 4 after birth litters were culled, where possible, to 4 males and 4 females. Selected animals from the F1a group were used to produce the F2 generation.
The animals were checked daily for clinical signs. Generally, food consumption and body weights were determined weekly. However, body weights of females were determined on days 0, 7, 14 and 20 of gestation and on days 4, 7, 14 and 21 after giving birth. Food consumption of females was determined for days 0-7, 7-14 and 14-20 of gestation and days 1-4, 4-7 and 7-14 post-partum. Male and female reproduction parameters were determined. Haematology and clinical chemistry were performed on 12 animals/sex/dose from the F0 and F1 parental animals. Litters were examined for number of pups delivered, sex ratio. Pups were checked for viability index (% survival from days 1-

4), lactation index (% survival from days 4-21), body weights, body weight changes, developmental landmarks and behaviour (grip reflex, acoustic startle, pupillary reflex). All pups were examined macroscopically at necropsy. In addition, selected pups were examined microscopically for organ and skeletal findings. For haematology and clinical chemistry, blood from F0 and F1 parental animals was sampled towards the end of the treatment period. At termination the parental animals were killed, necropsied, selected organs were weighed, and the reproductive organs and liver and kidney were histologically examined.

Table 23 Results from 2-generation reproductive toxicity in the rat

	Dose (ppm)	0	0	200	200	600	600	1800	1800	dr
	Sex	m	f	m	f	m	f	m	f	
F0 parents	Mortality	no toxicologically relevant effects								
	Clinical signs	no toxicologically relevant effects								
	Body weight gain as % change from control -premating (F1a) -gestation (F1a)			-1	-2 +4	-1	-2 +4	-13*	-18* -9	
	Food consumption as % change from control -premating (F1a) -gestation (F1a) -lactation (F1a)			0	0 +4 -4	+1	0 +4 -4	-6	-10* -7* -14*	
	haematology	no toxicologically relevant effects								
	clinical chemistry as % change from control - cholesterol			-9	-5	-14	+11	-25*	-11	dr[m]
	Mating, fertility	no toxicologically relevant effects								
	Gestation duration	no toxicologically relevant effects								
	relative organ weight - liver								+8	
	- macroscopy	no toxicologically relevant effects								
	microscopy - minimal hypertrophy periacinar hepatocytes	0/25	0/25	0/25	0/25	0/25	0/25	0/25	13/25*	
F1a,b pups	Litter size	no toxicologically relevant effects								
	Viability index F1a F1b	98 95		97 94		98 95		87* 92		
	lactation index F1a F1b	96 99		99 98		99 99		100 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*		 dr dr dr
	Sex ratio	no toxicologically relevant effects								
	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*	 dr dr dr dr
	macroscopy	no toxicologically relevant effects								
F1 parents	Mortality	no toxicologically relevant effects								
	Clinical signs	no toxicologically relevant effects								
	Body weight gain as % change from control -premating -gestation			0	+1 0	-2	-4 -7	-7*	-6* -19*	dr dr

	Dose (ppm)	0	0	200	200	600	600	1800	1800	dr
	Sex	m	f	m	f	m	f	m	f	
	Food consumption as % change from control -premating -gestation -lactation			-1	0 +2 -3	0	-1 +2 -3	-6*	-7* -7* -25*	
	clinical chemistry as % change from control - cholesterol			-4	-7	-8	-11	-31*	-16	dr
	Mating, fertility, gestation	no toxicologically relevant effects								
	Relative organ weights	no toxicologically relevant effects								
	macroscopy	no toxicologically relevant effects								
	microscopy - minimal hypertrophy periacinar hepatocytes	0/25	0/25	0/25	0/25	0/25	0/25	0/25	9/25*	
F2 pups	Litter size	no toxicologically relevant effects								
	Sex ratio	no toxicologically relevant effects								
	viability index	96		94		98		79*		
	lactation index	99		99		99		95		
	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*	dr
	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*		
	behavioural tests	no toxicologically relevant effects								
	- macroscopy	no toxicologically relevant effects								

* significantly different; dr = dose related

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

At dietary concentrations of 1800 ppm, in the F0 and F1 parental animals a reduction in food consumption and body weight gain was observed, accompanied by a reduction in blood cholesterol levels. At this dose in the F0 and F1 females an increased incidence in minimal hypertrophy of periacinar hepatocytes was observed. In the high dose group a reduction in absolute kidney weights in males and females and a reduced absolute epididymes weight was observed. However, these findings were considered to be related to the decreased body weight, and not directly related to dodemorph acetate treatment.

As 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 litters (21.4 days) in the 1800 ppm group and the F1b litters (21.6 days) of the 600 ppm 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 placed together for a period of about 16 hours. The birth of the litter was generally evaluated in the mornings in connection with the clinical observation. Apparently, 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 toxicologically relevant.

Based on the effects observed at 1800 ppm the NOAEL for parental toxicity was 600 ppm, equal to 64 mg/kg bw/day (equivalent to dodemorph: 52 mg/kg bw/day).

No treatment-related effects of dodemorph acetate on reproductive function were observed at doses up to and including 1800 ppm, equal to 194 mg/kg bw/day (equivalent to dodemorph: 159 mg/kg bw/day).

4.11.1.2 Human information

No information available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

In a developmental toxicity study according to OECD guideline 414, groups of 25 pregnant rats were given dodemorph acetate (purity 98.8%) at a dose of 30, 100 or 300 mg/kg bw per day by gavage on days 6 through 19 of gestation. Controls were treated with vehicle (olive oil). Body weight and food consumption were recorded every second or third day, and the dams were examined daily for clinical signs of toxicity. On gestation day 20 blood was taken from the retro orbital venous plexus of all females (non fasted). Immediately thereafter they were sacrificed and the foetuses were removed from the uterus and dams were examined macroscopically. Blood samples were examined for haematological parameters and clinical chemistry parameters. Apart from uterus and ovaries also the liver was removed; unopened uterus and liver were weighed. Foetuses were removed, weighed, sexed, and observed for gross malformations, then preserved for examination of soft tissue and/or skeletal abnormalities.

Table 24 Results from a developmental toxicity study in rats

	Dose (mg/kg bw per day)	0	30	100	300	dr
Maternal effects	Mortality	no mortality				
	Clinical signs - salivation	0/25	0/25	14/25	25/25	dr
	Pregnant animals	25	22	21	21	
	Body weight at GD20 (g)	284	276	280	269*	
	Corrected body weight gain in grams (% of control) [1]	33.7	31.6 (-6)	26.0 (-23)	20.4* (-31)	dr
	Food consumption GD 6-13 as % of control		91 %	79%	53%*	dr
	Haematology - MCV (FL) - MCHC (mmol/L) - platelet (giga/L)	 56.7 20.01 757	 56.6 20.11 767	 56.1 20.37 760	 55.3* 20.67* 860*	 dr
	Clinical chemistry - Na (mmol/L) - Cl (mmol/L) - Ca (mmol/L) - total Bilirubin (µmol/L) - triglycerides (mmol/L)	 140.3 101.1 2.71 1.37 5.01	 140.2 100.4 2.77 1.22 6.15	 139.7 100.5 2.73 0.93 7.37	 138.0* 98.1* 2.79* 0.37* 11.06*	 dr dr
	Organ weights as % change from control - liver (a/r)		 -2/0.5	 -1/1	 11/17*	
	Abortions	no abortions				
	Gravid uterine weight	no toxicologically relevant effects				
	Corpora lutea	no toxicologically relevant effects				
	Pathology	no toxicologically relevant effects				
Litter response	Live foetuses	no toxicologically relevant effects				
	Foetal weight as % of control		100	103	94	
	Pre implantation loss	no toxicologically relevant effects				
	Post implantation loss	no toxicologically relevant effects				
	resorptions	no toxicologically relevant effects				
Foetus examination	No. of foetuses	no toxicologically relevant effects				
	No. of dead foetuses	no dead foetuses				
	Sex ratio (f/m)	no toxicologically relevant effects				
	Malformations	no toxicologically relevant effects				
	Variations a] Visceral deviations [2] litter incidences + (%)					

	Dose (mg/kg bw per day)	0	30	100	300	dr
	- total visceral variations	1 (4)	4 (18)	7* (33)	5 (24)	
	- dilated renal pelvis	1 (4)	4 (18)	7* (33)	5 (24)	
	b] Skeletal deviations [3] litter incidences + (%)					
	- total skeletal variations	25 (100)	22 (100)	21 (100)	21 (100)	
	- misshapen sternebra	16 (64)	18 (82)	16 (76)	19* (90%)	
	- unossified sternebra	6 (24)	5 (23)	4 (19)	11* (52)	
	- incomplete oss. of lumbar arch	0 (0)	1 (5)	0 (0)	4* (21)	

* statistically significant
dr dose related
(a/r) absolute/relative
1 Corrected body weight gain = terminal body weight minus uterine weight minus day 6 body weight.
2 Mean historical control values and ranges of litter incidence (as percentage of total number of litters) for visceral variations: total visceral variations (22; 0-38), dilated renal pelvis (21; 0-38). 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.
3 Mean historical control values and ranges of litter incidence (as percentage of total number of litters) for skeletal variations: misshapen sternebrae (67; 25-92), unossified sternebrae (30; 17-46); incomplete ossification of lumbar arch (1; 0-4).

Transient salivation was observed in mid and high dosed females only for a few minutes after the gavage dose. No salivation was seen when treatment had ceased. Food consumption was dose relatedly decreased in the mid and high dose group, in particularly during GD 6-13. At initiation of treatment (day 6-8) a lower weight gain of dams was observed in the low dose group, but loss of weight was observed in the mid (-1 g) and high dose (-8 g) groups. However, only in the high dose group significantly lower maternal bodyweights were observed up to the end of the study. The corrected body weight gain calculated for the entire exposure period was dose relatedly decreased in the mid and high dose groups (23 and 39% below the control, respectively). Blood parameters showed increased values for MCHC, platelet counts, calcium and triglycerides and decreased values for MCV, Na and Cl in the high dose dams. In the mid dose dams increased values for MCHC and triglycerides were observed. Decreased values were also found for bilirubin in mid and high dose dams. However, according to the study authors turbid lipid serum samples strongly interfere the method of bilirubin analysis and thus the decreases are not considered test substance related. Absolute and relative liver weights were increased in the high dose group (11 and 17% respectively above control values).

There were no effects on gestational parameters. Effects on the foetuses were only observed in the high dose group. In this group the mean foetal body weights were slightly lower and there were slight but statistically significant increases in the litter incidences of some soft tissue and/or skeletal variations. In the mid-dose group a statistically significant increase in litter incidence of total visceral variations and dilated renal pelvis were observed. However these effects were not dose-dependent, not statistically significant at the high dose and within the range of the historical control data. Therefore the visceral variations are not considered compound related.

At the high dose statistically significant increased litter incidences of misshapen sternebrae, unossified sternebra and incomplete ossification of lumbar arch were found. Since these incidences were also at the upper end of, or outside the historical control range they are considered treatment-related.

The NOAEL of dodemorph acetate for maternal toxicity is 30 mg/kg bw per day(equivalent to dodemorph: 25 mg/kg bw/day), based on reduced food consumption, signs of salivation and increased blood values in dams of the 100 mg/kg bw/day dose group. Overt maternal toxicity was observed in the high dose group (300 mg/kg bw per day) which was substantiated by transient

salivation, reduced food consumption, impairments in body weight and bw gain, slight changes in serum electrolytes, increased number of platelets and a marked increase in triglycerides and increased liver weights.

The NOAEL of dodemorph acetate for embryo/foetotoxicity is 100 mg/kg bw (equivalent to dodemorph: 82 mg/kg bw/day), based on a slight decrease in foetal body weights and slight but significant increases in a few skeletal variations (delayed or incomplete ossification process of sternebra and lumbar arch, and increased incidences of misshapen sternebrae) in the high dose group. There were no indications for teratogenicity.

In another (range finding) developmental toxicity study (no GLP, no guideline), dodemorph acetate (purity 98.7%) was administered by gavage to rabbits at dose levels of 200, 600 and 900 mg/kg bw per day on 4 does per group, exposed during days 7-19 of gestation and sacrificed on day 20. Controls were treated with vehicle (olive oil). Body weight and food consumption were recorded and the dams were examined daily for clinical signs of toxicity. Blood samples were taken before sacrifice and examined for haematological parameters and clinical chemistry parameters. Apart from uterus and ovaries also liver and kidneys were removed. On gestation day 20 does were sacrificed and examined macroscopically. Foetuses were removed, weighed, sexed, and observed for gross malformations.

Severe toxicity was observed in the high dose group: drastically reduced or no food consumption, massive body weight loss during treatment; 2 dams died intercurrently, 1 doe was sacrificed in moribund state and one doe died after gavage error. All does showed poor general state, some of them blood in bedding, fur smeared with urine, diarrhoea and/or no defecation. Decrease in WBC RBC, Pt and Ht was noted and a prolonged clotting time was observed. Further, increased plasma AST, ALT, GGT activity as well as increased urea, creatinine, bilirubin, cholesterol and triglyceride levels were recorded. Three does showed ulcerations of the stomach mucosa. Absolute and relative liver and kidney weights were markedly increased. In this group 100% post implantation loss was recorded.

The same, but less severe findings in body weight, food consumption, clinical signs of toxicity, haematology, clinical chemistry and organ weights were also noted in the mid dose group. One doe of this dose group died in poor general condition, all 4 dams had ulcerations in the stomach and also 100% post implantation loss was recorded.
In the low dose group body weight and food consumption were reduced, ALT, GGT, and cholesterol were increased, and an increased post implantation loss (57.5%) was observed, especially due to a high number of late resorptions in 2 does which had no viable foetuses. Foetal toxicity was shown by reduced placental and foetal body weights. Anasarca was observed in 4 and open eye in 7 out of 16 foetuses from a single litter at 200 mg/kg bw. These malformations were considered questionable effects due to the premature status of the foetuses (removed from the uterus on day 20 of gestation instead of day 29).
Dodemorph acetate induces mortality, severe maternal toxicity and post implantation losses at 900 and 600 mg/kg bw per day. At 200 mg/kg bw per day, maternal toxicity and embryo/foetotoxicity (teratogenicity) were also observed.

In a third developmental toxicity study, according to former OECD guideline 414, groups of 15 inseminated rabbits were given dodemorph acetate (purity 92.6%) at a dose of 10, 40 or 120 mg/kg bw per day by gavage on days 7 through 19 of gestation. Controls were treated with vehicle (olive oil). Body weight and food consumption were recorded and the dams were examined daily for clinical signs of toxicity. On gestation day 29 does were sacrificed and examined macroscopically. Foetuses were removed, weighed, sexed, and observed for gross malformations, then preserved for examination of soft tissue and/or skeletal abnormalities.

Table 25 Results from a developmental toxicity study in rabbits

	Dose (mg/kg bw per day)	0	10	40	120	dr
Maternal effects	Mortality	no mortality				
	Clinical signs	no clinical signs of toxicity				
	Pregnant animals	15	13	15	14	
	body weight/weight gain	no toxicologically relevant effects				
	Corrected body weight gain (g)[1]	no toxicologically relevant effects				
	Food consumption	no toxicologically relevant effects				
	Abortions	no abortions				
	Gravid uterine weight (g)	369	366	370	306	
	Corpora lutea	no toxicologically relevant effects				
	Pathology	no toxicologically relevant effects				
Litter response	Live foetuses	no toxicologically relevant effects				
	Foetal weight	no toxicologically relevant effects				
	Pre implantation loss	no toxicologically relevant effects				
	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	0.2	0.3	0.2	0.7	
Foetus examination	No. of foetuses	no toxicologically relevant effects				
	No. of dead foetuses	no toxicologically relevant effects				
	Sex ratio (f/m)	no toxicologically relevant effects				
	Malformations					
	a] external - cleft palate (no. of foetuses) - open eye (no. of foetuses)	 0 0	 0 0	 0 0	 1 4	
	b] soft tissue	no toxicologically relevant effects				
	c] skeletal	no toxicologically relevant effects				
	Variations/retardations	no toxicologically relevant effects				
	a] external	no toxicologically relevant effects				
	b] soft tissue	no toxicologically relevant effects				
	c] skeletal - sternebra irregular shape (foetuses/litter)	 1.6	 5.0	 6.0	 10.9*	

* statistically significant
dr dose related
(a/r) absolute/relative
1 Corrected body weight gain = terminal body weight minus uterine weight minus day 7 body weight.

No mortality was recorded during the study, food consumption was not affected and no statistically significant differences were observed for body weight and body weight gain. There were no clinical signs of toxicity and no abnormal findings were recorded at necropsy. The mean gravid uterus weight of the high dose group was clearly but not significantly reduced and reached only about 83% of the control value. Post implantation loss value and early resorptions were increased at high dose, predominantly caused by one doe, which had no viable foetuses at necropsy, but only early resorptions. This doe also showed a slight, not significant increase in post implantation loss. Since similar but more pronounced effects were seen in the preceding preliminary range finding study at higher dose levels (200, 600 and 900 mg/kg bw per day, see above), the increase in early resorptions and post implantation loss is considered substance related.
In the high dose group there were 4 foetuses all from one doe with external malformations, while none were found in the other groups. All 4 foetuses showed open eye; one foetus had in addition cleft palate. The findings were not statistically significant. Because the open eye malformation was also seen in the range finding study in 7 out of 16 foetuses, also from 1 single litter at 200 mg/kg bw per day, it was considered that it was a treatment related malformation. The only skeletal retardation found was an increased incidence for sternebrae with irregular shape (number of affected foetuses per litter) in the high dose group. No effects on does, gestational parameters, or foetuses were observed in the mid and low dose group.

No maternal toxicity was observed. The decrease in mean gravid uterus weight is considered to be the consequence of an increased resorption rate and higher post implantation loss value, and thus a consequence of fetotoxicity rather than maternal toxicity. Thus the NOAEL of dodemorph acetate for maternal toxicity is 120 mg/kg bw/day (equivalent to dodemorph: 98 mg/kg bw/day), i.e. the highest dose tested.
The NOAEL for fetotoxicity is 40 mg/kg bw/day (equivalent to dodemorph: 98 mg/kg bw/day). This was predominantly based on the finding of a slight increase in incidence of a specific malformation (open eye, only in one litter) at the high dose level (120 mg/kg bw per day), a malformation that was also observed in the range finding study at a higher dose level. In addition, at this dose level the percentage of animals with irregularly shaped sternebrae was increased.

4.11.2.2 Human information

No information available

4.11.3 Other relevant information

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4.11.4 Summary and discussion of reproductive toxicity

In a range-finding one-generation reproductive toxicity test in rats with dodemorph acetate litter size and viability and pup weight (day 1) were decreased at a maternal dose of 238 mg/kg bw. However, at this dose also maternal toxicity was observed (decreased body weight and food consumption, altered plasma creatinin and cholesterol). In a 2-generation reproduction toxicity study, maternal toxicity was observed at the top dose (194 mg/kg bw/day). Effects on fertility were not found in these studies. However, both in F1 as in F2 pups of the 2 generation study, fetal body weight was reduced at doses of 64 mg/kg bw and higher. In addition, in one of the 2 groups of F1 pups, pinna unfolding was decreased at 64 mg/kg bw (also observed at both generations in the top dose groups).

In a rat developmental study with doses from 30-300 mg dodemorph acetate/kg bw, no developmental effects were observed at the highest dose that did not induce maternal toxicity (30 mg/kg bw/day). In a developmental study in the rabbit, no maternal toxicity was observed. The NOAEL for maternal toxicity was 120 mg/kg bw/day, i.e. the highest dose tested. (In the repeated dose toxicity studies, only a slight increase in relative liver weight was observed at 80 mg/kg bw 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).
In the fetuses in this study, a slight increase in incidence of malformations (open eye, only in one litter) at the high dose level (120 mg/kg bw per day) was observed, and the percentage of animals with irregularly shaped sternebrae was increased. Although the open eye was observed only in one litter, open eye was also observed in one litter in the range-finding study in the rabbit at a higher dose level, indicating the effect is dose-related. The NOAEL for fetotoxicity was 40 mg/kg bw per day. No historical control data were provided on the incidence of open eye. It is therefore difficult to say whether these effects are substance related or not.

4.11.5 Comparison with criteria

According to the criteria of DSD, substances should be classified as toxic to reproduction category 2 when there are clear results in appropriate animal studies where effects have been observed in the absence of signs of marked maternal toxicity, or clear evidence in animal studies of impaired fertility in the absence of toxic effects.
Substances should be classified as toxic to reproduction category 3 when there are results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of developmental toxicity in the absence of signs of marked maternal toxicity, but where the evidence is insufficient to place the substance in Category 2, or results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of impaired fertility in the absence of toxic effects, but where the evidence is insufficient to place the substance in Category 2.

According to the criteria of CLP, substances should be classified as Category 1B when there is clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects.
Substances should be classified as Category 2 when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1.

No effects on fertility were found in a 1 generation and 2 generation study with dodemorph acetate. Therefore, dodemorph acetate does not need to be classified for fertility.

In the 2 generation study, reduced body weight gain of the pups was observed at a dose without maternal toxicity (in F1a and F2 pups, but not F1b), as well as decreased pinna unfolding in 1 generation. In addition, in a developmental study with rabbits, an increase in early resoptions and post implantation loss and in four foetuses from one litter an increase in incidence of open eye was observed at the top dose tested (no maternal toxicity). Since these effects are not consistently shown, the effects are considered not severe enough to place the substance in Category 2 of DSD or Category 1B of CLP.

Thus, according to DSD, dodemorph acetate fulfills the criteria to be classified as toxic to reproduction category 3 and assigned the risk phrase R63 (“Possible risk of harm to the unborn child”).

According to CLP, dodemorph acetate fulfills the criteria to be classified as Category 2 reproductive toxicant (H361d).

4.11.6 Conclusions on classification and labelling

No effects on fertility were found in a 1 generation and 2 generation study with dodemorph acetate. Therefore, dodemorph acetate does not need to be classified for fertility.

According to the criteria of DSD, dodemorph acetate should be classified as toxic to reproduction category 3 and assigned the risk phrase R63 (“Possible risk of harm to the unborn child.

According to the criteria of CLP, dodemorph acetate should be classified as Category 2 reproductive toxicant (H361d).

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

4.12.1.2 Immunotoxicity

4.12.1.3 Specific investigations: other studies

4.12.1.4 Human information

4.12.2 Summary and discussion

4.12.3 Comparison with criteria

4.12.4 Conclusions on classification and labelling

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental hazard properties assessment for dodemorph acetate is based on the Draft assessment report (DAR), volume 3 annex B9, prepared in the context of the possible inclusion of dodemorph in Annex I of Council Directive 91/414/EEC (January 2007), RMS The Netherlands).

All tables in the present assessment are copied from the DAR.

5.1 Degradation

Table 26: Summary of relevant information on degradation

Method	Results	Remarks	References

Hydrolysis with ^{14}C-dodemorph acetate Hydrolysis with ^{14}C-dodemorph acetate	DT50 > 32 days at pH 5,7 and 9 at 24-25 ºC. DT50 > 5 days at pH 4, 7, and 9 at 22 ºC.	Guideline study EPA 161-1, radio-labelled substance, acceptable. Guideline study OECD 111, radio-labelled substance, acceptable.	DAR 2007 volume 3 B.8.4
Photolysis with ^{14}C dodemorph acetate	DT_{50} of 3.6 and 1.6 days at pH 7 and 9, respectively	Guideline study EPA 161-2, radio-labelled substance, acceptable.	DAR 2007 volume 3 B.8.4
Aerobic water-sediment system (two systems)	DT_{50} sediment 126 and 281 days, DT_{50} system 53 days	Guideline study directive 95/36/EC,CTB G2.1 (1995), SETAC (1995). Water and sediment were analysed, Radio-labelled substance. acceptable.	DAR 2007 volume 3 B.8.4

It should be noted that in aqueous solution dodemorph acetate dissociates rapidly into dodemorph free base and acetate. As acetate is not relevant for the environmental compartment, it has no toxicological effects, the observed effects can be attributed to dodemorph.

5.1.1 Stability

Stability in water.

A hydrolysis study with ^{14}C-dodemorph acetate was performed in aqueous buffered and sterilised solutions at pH 5, 7 and 9. The two peaks, which were observed in the chromatograms represent dodemorph (cis and trans) and not dodemorph acetate. It was concluded that in aqueous solution dodemorph acetate dissociates very rapidly into dodemorph free base and acetate whereas dodemorph has been found to be hydrolytically stable at pH 5, 7, and 9 at 24-25 °C (DT50 > 32 days).

In a second study performed with ^{14}C-dodemorph acetate, according to OECD 111 guideline, in sterilised buffered solutions at pH 4, 7, and 9. DT 50 values of > 5 were determined at 22 ºC for pH 4, 7, and 9.

Dodemorph acetate dissociates in dodemorph and acetate. DT50 values are not available, but the process is expected to be fast.

Photolysis in water

Dodemorph is photolysed rapidly in aqueous solutions, the $DT_{50,photolysis}$ was equivalent to 3.6 and 1.6 natural sunlight days at pH 7 and 9, respectively. One unknown minor metabolite at a maximum of 6.7% of applied substance was formed.

Dodemorph acetate dissociates in dodemorph and acetate. The formed dodemorph will be photolysed.

5.1.2 Biodegradation

A ready biodegradation study is not available.

The behaviour of dodemorph in two aerobic water-sediment systems (silty clay loam: a lake near Lelystad (OVP) and silt loam: a pool at Leerdam (SW)), performed with ^{14}C-dodemorph, indicates that residues of dodemorph may remain for a long time. Dodemorph dissipated rapidly from the water layer of both water/sediment systems to less than 10% after 2 – 7 days (for OVP and SW, respectively) and further to < 1% after 14 days (OVP) and 68 days (SW). 15.4% (OVP)- 23.2% (SW) of the applied radioactivity was completely mineralised after 103 days of incubation. The decrease of radioactivity in the water layer was in the first place the result of transfer of activity to the sediment layer and in the second place the result of degradation of dodemorph in the water layer. The total radioactivity in the sediment layer of the OVP increased rapidly to 71% after 1 day, increased further to 87% after 28 days and decreased to 77% after 103 days. After day 1, the amount of the extractable residues and the unextractable residues remained fairly constant. The extractable residues were in the range of 45.5% - 67.2% and the unextractable residues in the range of 14.0% - 30.4%. The radioactivity in the sediment layer for the other system (SW) was 38% after 1 day, increasing to 89% after 14 days and decreasing to 67% after 103 days. The amount of extractable and unextractable activity increased steadily to 55% and 34%, respectively after 14 days and then decreased to 39% and 27%, respectively after 103 days.
All dodemorph which was still present at the end of the study was recovered in the sediment with 42.5 % in the OVP system and 29.9 % in the SW system, respectively. At HPLC analysis only in one peak > 5% (maximum of 7.9%) of applied radioactivity was found in both systems, these were the totals of fractions from the water and sediment layer. The identity of the compounds could not be confirmed, but it is assumed to be cis and/or trans-2,6-dimethylmorpholine.

The following DT_{50} values were determined:

System	**Water DT_{50} [d]**	**Sediment DT_{50} [d]**	**System DT_{50} [d]**
OVP	0.5	281	Value not reliable.
SW	1.5	126	53

5.1.3 Summary and discussion of degradation

Dodemorph acetate dissociates in dodemorph and acetate in the aqueous compartment. Therefore, dodemorph acetate is considered to be hydrolytically unstable. Dodemorph is photolysed in aqueous solution and consequently dodemorph acetate will also be photolysed.

Dodemorph, the degradation product of dodemorph acetate, is not rapidly degradable. In water-sediment systems, it dissipates rapidly from water but has long half-lives (>53 days) in the total system. Dodemorph is susceptible to primary degradation but mineralization is slow (15.4% and 23.2% after 103 days). According to CLP section 4.1.2.9.3, dodemorph acetate must therefore be considered as not rapidly degradable.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Not relevant for this type of dossier.

5.2.2 Volatilisation

Not relevant for this type of dossier.

5.2.3 Distribution modelling

No data available

5.3 Aquatic Bioaccumulation

Table 27: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	References
Fish bioaccumulation study	BCF: 583 – 746 L/kg	Dodemorph acetate spiked with radiolabelled dodemorph, flow-through study, OECD 305 guideline, acceptable	DAR 2007 volume 3 B.9.2.4

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The bioconcentration factor (BCF) of fish (rainbow trout) exposed to dodemorph acetated spiked with radiolabelled dodemorph was determined in a flow-through study. Two concentrations (3 and 30 µg dodemorph acetate/L) were used. Concentrations in water were analysed daily. Edible and non-edible tissue were extracted and analysed for dodemorph. A depuration phase was included in the study. Equilibrium was reached within 2 days. A kinetic BCF as well as BCF based on the ratio of the concentration in the fish and in the water at apparent steady state were calculated for both concentrations. The resulting BCF kinetics values were 746 and 649L/kg wwt for the lower and higher test concentration, respectively. The BCF values for the steady state concentrations were 692 and 583 L/kg wwt for the lower and higher test concentrations, respectively.

5.3.2 Summary and discussion of aquatic bioaccumulation

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

BCF values determined for dodemorph in fish varied between 580 - 750 L/kg.

5.4 Aquatic toxicity

All available studies on the ecotoxicity have been performed with dodemorph acetate. As already mentioned, dodemorph acetate dissociates rapidly in aqueous solutions into acetate and dodemorph. From the HPLC analysis of the applied dodemorph acetate in the aquatic toxicity studies it is clear that the peak in the HPLC analysis represents dodemorph and not dodemorph acetate.

Table 28: Summary of relevant information on aquatic toxicity
This table shows the lowest available toxicity values for the three aquatic trophic levels fish, invertebrates and algae.

Method	Criteria	Results as mg/l dodemorph acetate	Test condition and reliability	References
Acute fish *Oncorhynchus mykiss*	LC_{50}	1.49 – 3.22	OECD 203 guideline, measured concentrations, acceptable	DAR 2007 volume 3 B.9.2
Chronic Fish *Oncorhynchus mykiss*	NOEC growth and survival	0.12	OECD revised version of 1997 guideline, measured concentrations, acceptable.	DAR 2007 volume 3 B.9.2
Acute invertebrate *Daphnia magna*	EC_{50}	1.8	OECD 202 guideline study. Measured concentrations, acceptable.	DAR 2007 volume 3 B.9.2
Chronic invertebrate *Daphnia magna*	NOEC	0.10	OECD 202 and 211 guideline study, measured concentrations, acceptable.	DAR 2007 volume 3 B.9.2
Algae *Pseudokirchneriella subcapitata*	E_rC_{50} NOE_rC	1.1 0.059	OECD 201 guideline study, measured concentrations, acceptable	DAR 2007 volume 3 B.9.2

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

An acute toxicity study with technical dodemorph acetate (purity 93%) was performed with rainbow trout under static conditions according to OECD guideline 203. Five test concentrations were used (range: 1.0 – 10.0 mg dodemorph acetate/L). Test concentrations were analytically monitored. The measured concentrations at the start were approximately 50% of nominal and decreased during the test to 17 – 25% of nominal. Mortality was 100% in the highest test concentration after 72 hours. No mortality was observed in the control and the lower concentrations during the study. The dose-response curve was very steep and LC50 value was considered to lie between 1.49 and 3.22 mg dodemorph acetate/L), based on mean measured concentrations.

5.4.1.2 Long-term toxicity to fish

The survival and growth of rainbow trout was determined in a 28-days flow-through test according to OECD guideline (revised version of 1997). Five test concentrations were used (0.054 – 1.08 mg

dodemorph acetate/L, a control and a solvent control (acetone). Test concentrations were analytically monitored. The mean measured concentrations were between 87 and 138% of the nominal concentrations and analysis of the stock solution showed actual recoveries from 108 to 121%. The mean measured test concentrations ranged from 0.057 – 0.938 mg dodemorph acetate/L. No mortalities occurred, and only two fish in the highest test concentration showed abnormal swimming behaviour. The body lengths of the fish in the three highest test concentrations were significantly reduced compared to control. NOEC was 0.12 mg dodemorph acatete/L corresponding to 0.10 mg dodemorph/L based on the growth of the fish and measured concentrations.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Daphnia magna were exposed to dodemorph acetate (purity of 93%) for 48 hours under static conditions according to OECD guideline 202. Five concentrations were tested (1 – 10 mg dodemorph acetate). Test concentrations were analytically monitored. Mean recovery varied from 34% to 44% of nominal. Immobilisation of 60% and 100% was observed at the two highest test concentration. A steep dose response curve was observed just like in the acute fish study. The EC50 was 1.8 mg dodemorph acetate/L, based on mean measured test concentrations.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Effects of dodemorph acetate (purity 93%) on mobility and reproduction of *Daphnia magna* were determined under semi-static conditions for 21 days according to OECD guideline 202 and 211. Five test concentrations were used (0.054 – 1.08 mg dodemorph acetate/L). The test water was renewed every 48 hours. Fresh and old test waters were analytically monitored. Measured concentrations were all >80% of nominal (range 81% to 96%). Adult mortality increased with increasing concentrations. Reproduction and body length were significantly reduced in the highest treatment. NOEC values were estimated to be 0.10 mg dodemorph acetate/L for survival and body length, 0.45 mg dodemorph acetate for reproduction. The overall NOEC was determined to be 0.10 mg dodemorph acetate/L, based on nominal concentrations.

5.4.3 Algae and aquatic plants

In the available algae study, green algae were exposed to dodemorph acetate (purity 93%) for 72 hours under static conditions according to OECD guideline 201. Seven concentrations were tested (range: 0.10 – 10 mg dodemorph acetate/L). Concentration series without algae were included. Test concentrations were sampled and analysed at the start and end of the study. Test concentrations at the start were approximately 50% of nominal and decreased to less than 25% after 72 hours of exposure. Mean measured test concentrations varied between 14% and 36% (36% in the highest test concentration). In the highest test concentration without algae mean measured concentration was 43% of nominal. Inhibition of growth and reduced biomass were statistically significant in the four highest concentrations (at nominal 1.0 mg/L and higher). An E_rC_{50} of 1.1 mg dodemorph acetate/L, and a NOE_rC of 0.059 mg dodemorph acetate/L were derived. All values are based on mean measured concentrations.

The test substance concentrations decreased significantly during the study. Nevertheless, the study is considered useful since the results are based on mean measured concentrations. Furthermore, flow-through or semi-static studies in which test substance concentrations may be more stable are very difficult to carry out for algae.

5.4.4 Other aquatic organisms (including sediment)

No data available.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

CLP- Acute aquatic hazards

According to the criteria of the CLP Regulation, a substance is classified for aquatic acute toxicity if in an aquatic acute toxicity study, an $L(E)C_{50}$ of ≤ 1 mg/l is obtained for any of the three trophic levels fish, invertebrates and algae/aquatic plants.

The lowest $L(E)C_{50}$ obtained for dodemorph acetate is 1.1 mg/l in algae. Dodemorph acetate therefore does not fulfil the criteria for classification as Aquatic Acute Cat. 1.

CLP - Aquatic chronic hazards

According to the criteria of the 2nd ATP to the CLP Regulation, when NOEC values are available for all trophic levels, a substance is classified for aquatic chronic hazards if a NOEC of ≤ 1 mg/l is obtained in a long-term aquatic toxicity study. The assignment of a hazard category depends on the NOEC value and whether the substance is rapidly degradable or not.

Dodemorph acetate is considered not rapidly degradable (see section 5.1.3). NOEC values for dodemorph acetate are available for all trophic levels. A NOErC of 0.059 mg/l and a NOEC of 0.10 mg/l were obtained in algae and *Daphnia*, respectively. Dodemorph acetate therefore fulfils criteria for classification as Aquatic Chronic Cat.1.

An M-factor of 1 for chronic toxicity is proposed based on NOEC values 0.01 < NOEC ≤ 0.1 mg/l and the fact that dodemorph acetate is not rapidly degradable.

Directive 67/548/EEC

According to the criteria of Directive 67/548/EEC, a substance can be classified for acute or chronic hazards to the environment. If a substance has acute aquatic toxicity of <100 mg/l and is not readily degradable or has a log Kow of ≥3, it is classified for long-term hazards to the environment. Assignment into division depends on the lowest acute aquatic toxicity value.

Dodemorph acetate

The lowest acute aquatic toxicity values for dodemorph acetate are 1.1, 1.8 and 1.49 mg/l in algae, invertebrates and fish, respectively. Dodemorph acetate is not readily degradable (see section 5.1.3). Furthermore, the log Kow value of dodemorph acetate is 4.6. Dodemorph acetate therefore fulfils the criteria for classification with N; R51/53.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Table 29: Classification according to DSD with SCL and CLP with M-factor.

Substance	**Directive 67/548/EEC**		**CLP Regulation**	
	Classification	**SCL**	**Classification**	**M factor**

Dodemorph acetate	N; R51/53	-	Aquatic Chronic category 1 H410: very toxic to aquatic life with long lasting effects.	1

6 OTHER INFORMATION

This proposal for harmonised classification and labelling is based on the data provided for the registration of dodemorph according to Directive 91/414/EEC. The summaries included in this proposal are partly copied from the DAR volume 3, annex B. Some details of the summaries were not included when considered not relevant for a decision on the classification and labelling of this substance. For more details the reader is referred to the DAR Volume 3 and its addendum.

7 REFERENCES

European Commission. Draft Assessment Report dodemorph, prepared by The Netherlands January 2007.

European Commission. Draft Assessment Report dodemorph addendum, prepared by The Netherlands July 2008.

8 ANNEXES