Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

hymexazol (ISO); 3-hydroxy-5-methylisoxazole

EC Number: 233-000-6
CAS Number: 10004-44-1

CLH-O-0000001412-86-229/F

Adopted

14 September 2018

14 September 2018

CLH-O-0000001412-86-229/F

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

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

Chemical name: **hymexazol (ISO); 3-hydroxy-5-methylisoxazole**

EC Number: **233-000-6**

CAS Number: **10004-44-1**

The proposal was submitted by **Finland** and received by RAC on **5 July 2017.**

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

PROCESS FOR ADOPTION OF THE OPINION

Finland has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **17 October 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **1 December 2017**.

ADOPTION OF THE OPINION OF RAC

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

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

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **14 September 2018** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	613-115-00-1	hymexazol (ISO); 3-hydroxy-5-methylisoxazole	233-000-6	10004-44-1	Acute Tox. 4* Eye Dam. 1 Aquatic Chronic 3	H302 H318 H412	GHS05 GHS07 Dgr	H302 H318 H412			
Dossier submitters proposal	613-115-00-1	hymexazol (ISO); 3-hydroxy-5-methylisoxazole	233-000-6	10004-44-1	**Retain** Eye Dam. 1 **Add** Repr. 2 Skin Sens. 1B **Modify** Acute Tox. 4 Aquatic Chronic 2	**Retain** H302 H318 **Add** H361d H317 **Modify** H411	**Retain** GHS05 GHS07 Dgr **Add** GHS08 GHS09	**Retain** H302 H318 **Add** H361d H317 **Modify** H411			
RAC opinion	613-115-00-1	hymexazol (ISO); 3-hydroxy-5-methylisoxazole	233-000-6	10004-44-1	**Retain** Eye Dam. 1 **Add** Repr. 2 Skin Sens. 1 **Modify** Acute Tox. 4 Aquatic Chronic 2	**Retain** H302 H318 **Add** H361d H317 **Modify** H411	**Retain** GHS05 GHS07 Dgr **Add** GHS08 GHS09	**Retain** H302 H318 **Add** H361d H317 **Modify** H411		oral: ATE = 1600 mg/kg bw	
Resulting Annex VI entry if agreed by COM	613-115-00-1	hymexazol (ISO); 3-hydroxy-5-methylisoxazole	233-000-6	10004-44-1	Repr. 2 Acute Tox. 4 Eye Dam. 1 Skin Sens. 1 Aquatic Chronic 2	H361d H302 H318 H317 H411	GHS08 GHS07 GHS05 GHS09 Dgr	H361d H302 H318 H317 H411		oral: ATE = 1600 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Hymexazol is an active substance, a fungicide under Directive 91/414/EEC. It has an existing CLP Annex VI entry. This proposal aims at modifying the existing classification based on new data on developmental toxicity. In addition, the assessment of the available data (in the DAR) supports the confirmation of the existing Acute Tox. 4; H302 classification as well as the proposal for adding the Skin Sens 1 classification.

The classification proposal is based on the Draft Assessment Report (DAR; Finland 2007) and Additional report (Finland, 2009), Addendums (Finland, 2010), Peer Review Report on hymexazol (EFSA, 2010) and Conclusion on pesticide peer review (EFSA, 2010), and the two additional developmental toxicity studies (IIA 5.6.2/06, IIA 5.6.2/07) which are provided in section 13 of the IUCLID file and were not evaluated in the Pesticide review programme.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute oral, dermal and inhalation toxicity

Acute oral toxicity:

Summary of the Dossier Submitter's proposal

According to the dossier submitter (DS), in the oral studies performed on rats and mice, moderate toxicity was observed in both species. In rats there were no clear differences between sexes (LD_{50} for males was 1600 mg/kg bw and for females 1700 mg/kg bw). In mice however, males were more sensitive than females (LD_{50} for males 1700 mg/kg bw and for females 2300 mg/kg bw). In both species, administration of high oral doses caused mortality. Post mortem examination of the rats that died during the study revealed brown fluid in the bladder; post mortem examinations of mice did not reveal any macroscopic abnormalities. Other symptoms of toxicity recorded in both species included pilo-erection, pallor of the extremities, abnormal body carriage (hunched posture), abnormal gait (waddling) and lethargy. Also decreased respiratory rate, ptosis, prostration, ataxia and body tremors were observed in both species.

Guideline Route, Species GLP	Species, Strain Sex No of animals	Dose levels Frequency of application	Results	Remarks	References
OECD 401 Oral, rat GLP	5/sex in each dosage group	Dose levels: 0.4, 1.26, 1.6 and 2.0g/kg bw Single dose	LD_{50} combined = 1700 mg/kg bw (1600–1900 mg/kg bw) LD_{50} male: 1600 mg/kg bw (1400–1900 mg/kg bw) LD_{50} female: 1700 mg/kg bw (1600–2000 mg/kg bw)	The mortality rates for males were 0, 0, 2 and 5 and for females 0, 0, 1 and 4 at the dose levels of 0.4, 1.26, 1.6 and 2.0 g/kg bw, respectively. One male rat dosed at 2.0 g/kg bw died within 2 hours of treatment; the remainder of deaths occurred on days 2 and 3.	Baldrick 1992a. Key study

OECD 401 Oral, mouse GLP	CD-1 mice 5/sex in each dosage group	Dose levels: 1.26, 1.6, 2.0 and 3.2 g/kg bw Single dose	LD_{50} male: 1700 mg/kg bw (1500–2100 mg/kg bw) LD_{50} female: 2300 mg/kg bw (1900–3100 mg/kg bw)	The mortality rates for males were 0, 3, 3 and 5 and for females 0, 0, 1 and 5 at the dose levels of 1.26, 1.6, 2.0 and 3.2 g/kg bw, respectively. Most of the deaths occurred during 2 days after dosing.	Baldrick, 1992b Key study

The lowest oral LD_{50} values for hymexazol were 1600 mg/kg bw (male rat) and 1700 mg/kg bw (male mouse and female rat). According to the CLP (Regulation (EC) No 1272/2008), hymexazol should be classified as Acute Tox. 4; H302, because the LD_{50} is within the limits 300 < ATE ≤ 2000 (oral, mg/kg bw). The minimum classification Acute Tox. 4* is thus considered confirmed.

Comments received during public consultation

The proposed classification as Acute Tox. 4; H302 was supported by 2 MSCAs. A third MSCA suggested to add an ATE.

Assessment and comparison with the classification criteria

The current classification of hymexazol for acute oral toxicity is Acute Tox 4*; H302. RAC agrees with the DS that hymexazol should be **classified as Acute Tox. 4; H302** since the LD_{50} values in male/female rats and male mice are within the limits of 300 ≤ ATE < 2000 mg/kg bw.

The proposed ATE value for Acute Tox. 4 is 1600 mg/kg bw. This value can be used in the formulas for the classification of mixtures. The LD_{50} values provided (cf. Table 9 of the CLH report) are calculated by statistical methods, and the "range" provided likely refers to the 95% confidence interval (since e.g. 1900 mg/kg has not been tested). **Therefore, the lowest LD_{50} of 1600 mg/kg from male rats should also be the ATE**.

<u>Acute dermal toxicity</u>:

Summary of the Dossier Submitter's proposal

According to the DS, in a key study on rabbit using hymexazol technical (purity 99.1 %) was prepared as a stiff paste at a concentration of 79 % (w/v) in distilled water and administered at a volume of 2.53 ml/kg bw. Five male and 5 female rabbits, 11 to 12 weeks of age, were treated at 2.0 g/kg bw. Test material was held in contact with the shaved skin for 24 hours. After 24 hours the skin was washed with warm water and blotted dry.

All rabbits survived until study termination. There were no signs of systemic reaction to treatment. One male animal showed a minor weight loss and a female rabbit markedly reduced body weight gain after one week but satisfactory body weight gains were recorded at the end of observation period. There was no evidence of irritation reactions or other dermal changes in any animal throughout the observation period. Terminal autopsy revealed no macroscopic abnormalities. The dermal LD_{50} was higher than 2 g/kg bw in male and female rabbits, warranting no classification.

Comments received during public consultation

None.

Assessment and comparison with the classification criteria

RAC agrees with the DS that **no classification for acute dermal toxicity is required** as the LD_{50} was higher than 2 g/kg in both males and females rabbits.

Acute inhalation toxicity:

Summary of the Dossier Submitter's proposal

In an acute inhalation study (Hardy et al. 1989), similar to OECD 403 in design but with treatment performed at two dose levels only, the highest dose tested, 0.65 mg/L (droplets) did not cause acute toxic effects (LC_{50}>0.65 mg/L). The DS considered the data not conclusive for classification, as the highest dose tested is well below the maximum limits for classification. The limits for dusts and mists under the CLP for Cat 3 are 0.5 < ATE ≤ 1.0 (mg/L) and for Cat 4 1.0 < ATE ≤ 5.0 (mg/L). There are no studies available performed with hymexazol concentrations that would fall between the limits for Cat 4. There are, however, studies considered supplementary, one performed with a preparation containing 70% hymexazol and another with a 30% hymexazol preparation. The LC_{50} values for these preparations were greater than 4.53 and 4.68 mg/L, respectively. The DS proposed no classification.

Comments received during public consultation

None.

Assessment and comparison with the classification criteria

No mortality was observed at the highest concentration tested of 0.65 mg/L (droplets)). As this concentration was the highest practically attainable, RAC considers the LC_{50} of >0.65 mg/L not to warrant classification. '**No classification' for inhalation toxicity is therefore appropriate**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

According to the DS, two studies were performed with hymexazol, a Guinea Pig Maximisation Test (GPMT) and a Buehler assay.

Guideline Route, Species GLP	**Species, Strain Sex No of animals**	**Dose levels Frequency of application**	**Results**	**Remarks**	**Reference**
OECD 406, GPMT	Std:Hartley guinea pigs 20 males	First induction (intradermal): 1,5 % w/v (The induction was given both with and without Freund´s Complete Adjuvant).	Sensitising	Positive skin reactions (score 1) observed in 10/20 hymexazol	Miyazaki, 2005 Key study

Guinea pig GLP		Second induction (topical application): 35 % w/v Challenge (topical application): 5 % w/v		treated animals at 24 and 48 h after the challenge.	
OECD 406 (Buehler modified) Guinea pig Stated to have been conducted according to GLP	Dunkin /Hartley guinea pig, 10 animals	Induction (topical): 60 % w/w Challenge (topical): 60 % w/w 60 % w/w for the induction was stated to have been the maximum practical concentration that could be prepared and did not give rise to irritating effects. The reason for a choice of a vehicle was not given.	Not sensitizing The study was not considred acceptable because of a lower animal number than indicated necessary in the guideline. Also, contrary to the guideline, the concentration used in the induction exposure was not sufficient to cause mild irritation.	No signs of toxicity were recorded.	Parcell, 1993

The GPMT test by Miyazaki (2005), is regarded as the key study. Seven days after the first, intradermal induction, the second induction (topical application) was given on the same area of the skin. The induction site of the skin was treated with 10 % sodium lauryl sulphate in white petrolatum the day before the second induction. Animals were challenged 21 days after the first induction treatment. The challenge sites were examined for skin reactions at 24 and 48 hours after the termination of the challenge treatment.

Positive skin reactions (discrete or patchy erythema, score 1) were observed in 10 of 20 animals at 24 and 48 hours after the challenge in the test substance treated group (sensitisation rate 50 %). The negative control group showed no skin reactions at any application areas of the animals.

In the Buehler study by Parcell (1993), hymexazol technical (purity 99.3 %) was applied in a volume of 0.5 ml in a patch of surgical gauze on the shaved skin of 10 albino Guinea pigs of Dunkin/Hartley strain.

In the challenge phase, hymexazol technical at 60 % w/w in Alembicol D was administered topically two weeks after the third induction application on the shaved skin of test animals and controls for test animals. Positive control animals and their controls were challenged with formalin 15 % v/v in distilled water. The length of exposure was 6 hours and it was performed on the other side of an animal than the induction. The challenge sites were evaluated 24, 48 and 72 hours after removal of the patches.

No dermal reactions were observed in any of the test or control animals receiving hymexazol as a challenge. Slight to well-defined dermal reactions were seen in all 10 animals receiving formalin. There were no dermal reactions in the corresponding controls. However, the study is not considered acceptable as it was performed with 10 animals only. A minimum of 20 test substance treated animals and at least 10 control animals are required for a proper Buehler test.

In a GPMT, with intradermal induction of a 1.5 % mixture in water, hymexazol produced positive skin sensitising reactions in 10/20 animals (score 1), thus the sensitisation rate is 50 % and hymexazol should be classified for skin sensitisation. According to the new criteria in the 2nd ATP of CLP (Regulation (EC) No 286/2011), the results fulfil the criteria for subcategory 1B (≥ 30 % responding at > 1 % intradermal induction dose). The subcategory 1A was excluded since the

response was not > 60% at concentration < 1%. The DS concluded that hymexazol should therefore be classified as Skin Sens. 1B; H317.

Comments received during public consultation

There were support from 4 MSCAs for classification Skin Sens., however 2 of the 4 MSCAs suggested no sub-categorisation as no potency could be deduced from the assay. In its response, the DS concurred with this view and no longer proposed sub-categorisation.

Assessment and comparison with the classification criteria

RAC agrees that the negative result of the Buehler test should be disregarded because of too low animal numbers and concentration of the test substance. The only other study, a GPMT, showed 50% positive skin reactions in hymexazol treated animals (1.5% w/v first intradermal induction) at 24h and 48h after the challenge. These findings are consistent with a Skin Sens. 1B categorisation (≥ 30% responding at > 1% intradermal induction dose). Lower induction concentrations possibly meeting the criteria for 1A (≥ 30% responding at ≤0.1% intradermal induction dose, or ≥ 60% responding at intradermal induction dose between 0.1-1%) were however not tested. Although the latter criterion for 1A can be excluded based on a 50% response at 1.5% induction, the former cannot.

Therefore RAC recommends **Skin Sens. 1, without sub-categorisation**.

RAC evaluation of reproductive toxicity

Fertility:

Summary of the Dossier Submitter's proposal

A preliminary and a two generation reproduction study were available; the details of which are summarised below.

Method Guideline GLP	**Species Strain Sex No/group**	**Exposure period**	**Doses tested/ Route**	**NOAELs/LOAELs**	**Reference**
Preliminary reproduction toxicity study Non-guideline GLP	Rat CD 6 ♂ + 6 ♀ per group	15 days before mating – day 4 post partum	0, 1250, 2500, 5000 or 10000 ppm Dietary (corresponding to 0, 102, 195, 396 or 795 mg/kg bw/day for males and 0, 111, 244, 438 or 902 mg/kg bw/day for females)	**Adults:** The NOEL for maternal toxicity was 5000 ppm (corresponding 396 mg/kg bw/day for males and 438 mg/kg/day for females) for decreased body weights, food consumption. **Offspring:** No LOEL for pup toxicity. **Reproduction:** The NOEL for reproduction was 1250 ppm (corresponding to 102 mg/kg bw/day for males and 111 mg/kg bw/day for females) for reduced litter size and post-implantation survival index at ≥ 2500 ppm (corresponding to 195 mg/kg/day for males and 244 mg/kg/day for females), decreased implantation sites at ≥ 5000 ppm, prolonged gestation length at 5000 ppm and disturbed oestrous cycle at 10000 ppm.	Willoughby, 1990b Supportive

Two-generation reproduction study OECD 416 GLP	Rat CD 24 ♂ + 24 ♀ per group	F_0 and F_1: 14 weeks prior to mating, 2 weeks of mating, gestation and lactation	0, 100, 500, 2500 ppm Dietary (approximately 0, 6.3, 31 or 159 mg/kg bw/day for F_0 males and 0, 7.5, 37.5 or 192 mg/kg bw/day for F_0 females)	**Adults:** The NOAEL for parental toxicity was 2500 ppm (corresponding to approximately 159 mg/kg bw/day for males and 192 mg/kg/day for females). No LOAEL. **Offspring:** NOAEL for pup toxicity was 2500 ppm. No LOAEL **Reproduction:** The NOAEL for reproduction was 500 ppm (corresponding to 31 mg/kg/day for F_0 males and 38 mg/kg/day for F_0 females) for slightly extended gestation length (F_0 and F_1) and reduced litter size at birth due to increased post-implantation loss (F_0 and F_1) at 2500 ppm.	Willoughby, 1992 Key study

According to the DS, based on the results of this range-finding study by Willoughby (1990b), clear effects on reproduction were seen at and above 5000 ppm (corresponding to 396 and 438 mg/kg bw/day for males and females, respectively) without indication of systemic toxicity except for reduced body weights in males at 10000 ppm. At 5000 ppm, females had increased number of resorptions, reduced litter size and prolonged gestation length, and at 10000 ppm, oestrous cycle was disturbed and all females had total resorptions. Litter size was slightly reduced also at 2500 ppm (corresponding to 195 and 244 mg/kg bw/day for males and females, respectively) but at 1250 ppm (corresponding to 102 and 111 mg/kg bw/day for males and females, respectively) there was no indication of adverse effects. The study is acceptable as supporting information.

Oestrous cycles

There was some evidence that a dietary level of 10000 ppm could disturb oestrous cycles, but mating performance and fertility were unaffected by treatment. Two females at 10000 ppm showed irregular or slightly extended oestrous cycles and two animals became acyclic during the smearing period. Three females at 1250 ppm and two females at 5000 ppm became acyclic during the pairing period, after initially demonstrating regular oestrous cycles, and showed prolonged pre-coital interval suggestive of pseudo-pregnancy. Only one female at 1250 ppm failed to become pregnant. Despite the effects seen in oestrous cycles at 10000 ppm, all females mated and only one female was non-pregnant, and this was one that had shown a regular oestrous cycle.

The main significant observations in preliminary reproduction toxicity study (Willoughby, 1990b) were resorptions, changes in oestrous cycles and prolonged gestation length.

Parameter	**0 ppm**	**1250 ppm**	**2500 ppm**	**5000 ppm**	**10 000 ppm**
Number of females / Number of pregnant females	6/6	6/6	6/6	6/6	6/5
Oestrous cycles - regular - irregular - acyclic/pseudopregnant	6	6	6	6	2 **2** **2**
Mean number of implantation sites	17.0	16.8	16.0	13.3[a]	13.0
Females with total resorptions	0	0	0	1	5
Gestation index (%)	100	100	100	83	0

Number of animals with gestation length of					
- 22.0 days	2	2	0	0	0
- 22.5 days	2	1	2	0	0
- 23.0 days	2	0	4	2	0
- 23.5 days	0	2	0	2	0
- 25.0 days	0	0	0	1	0
Litter size at Day 1 pp[b]	14.7	15.0	12.7	4.6	-
Live birth index	98	100	95	67	-
Number of alive					
- on Day 1 pp	14.3	15.0	12.0	5.0	-
- on Day 2 pp	14.3	15.0	11.8	5.0	-
- on Day 4 pp	14.0	15.0	11.5	4.5	-
Post-implantation survival index[c]	86	89	79	29	0

[b]*Post partum*

[c]Percent of live pups per implantation sites

* p <0.05 ** p<0.01, *** p<0.001

In the two-generation reproduction study in rat (Willoughby, 1992) the NOAEL for reproduction was 500 ppm (corresponding to 31 and 38 mg/kg bw/day for F0 males and females, respectively) based on slight prolongation of gestation, increased post-implantation loss and reduced litter size at 2500 ppm (corresponding to 159 and 192 mg/kg bw/day for F0 males and females, respectively). Prolonged gestation and increased resorptions were observed in both generations. These effects seem not to be secondary to maternal toxicity (NOEAL for maternal toxicity 2500 ppm). The NOAEL for offspring toxicity was 2500 ppm based on no adverse effects observed during the development of the offspring after birth.

The slight maternal body weight changes during gestation in F0 females and during lactation in F0 and F1 generations, increased pup weights and slightly enhanced development of pups were not considered as signs of toxicity. Based on the large variation in mean body weight gain during lactation period among F1 females, it was considered that the difference observed in F0 generation between females at 2500 ppm and controls was not biologically significant. The decrease in maternal body weight gain during gestation was not associated with increased gestation length or increased resorptions. Decrease in body weight gain during gestation was slight (13%) and observed only in F0 generation at 2500 ppm and not in F1 generation.

No significant changes with dose-response relationship in F0 males.

No significant changes with dose-response relationship were observed in F1 males.

Litter data F1 offspring

Parameter	**0 ppm**	**100 ppm**	**500 ppm**	**2500 ppm**
F_1 offspring				
Number of implantation sites	15.9	14.5	15.9	14.8
Number of pups at Day 1	15.0	13.5	13.9	**11.0*** **
Number of live pups				
- Day 1	14.5	13.4	13.6	**10.7****
- Day 4	12.4	12.8	13.1	10.4
- Day 4[a]	7.4	7.7	7.9	6.9
- Day 7-25	7.3-7.2	7.7	7.9-7.8	6.8
Post-implantation survival index (%)[a]	91	91	87	**74***
Live birth index (%)	97	99	98	98
Viability index (%), Day 4	81	96	96	97

[a]Percent of live pups per number of implantations

* p <0.05 ** p<0.01, *** p<0.001

Litter data F2 offspring

Parameter	**0 ppm**	**100 ppm**	**500 ppm**	**2500 ppm**
F_2 offspring				
Number of implantation sites	14.1	16.0	15.5	15.0
Number of pups at Day 1	13.6	14.2	13.9	**11.3***
Number of live pups				
- Day 1	13.6	14.2	13.9	**11.3***
- Day 4	13.6	13.8	13.7	**11.0***
- Day 4[a]	7.6	8.0	7.9	7.2
- Day 7-25	7.5	7.9	7.8	7.5-7.3
Post-implantation survival index (%)[a]	93	87	89	**74****
Live birth index (%)	100	100	100	100
Viability index (%), Day 4	100	97	98	97

[a]Percent of live pups per number of implantation sites
* p <0.05 ** p<0.01, *** p<0.001

The DS conclusion for fertility is that, in the two-generation rat study, slightly prolonged gestation length (by approximately half a day in both F0 and F1 generations) and increased number of resorptions with consequent reduction in mean litter size were observed at the highest dose of 2500 ppm (corresponding to 159 mg/kg bw/day) which was a non-maternally toxic dose. The gestation index was reduced in F0 but not in F1 females at this dose, and parturition was unaffected in either generation. Oestrus cycle, mating performance, conception rates and fertility indices were not affected except for the F1 generation where at 500 ppm a low conception rate and fertility index were observed, but these were not dose-dependent effects. Live birth, viability or lactation indices were unaffected in either generation. The level of concern is low for the prolonged gestation length in the absence of other adverse effects on fertility. The mechanism(s) behind the observed effects (prolonged gestation time, increased number of resorptions) were not identified, also open literature search for mechanistic toxicity studies on hymexazol was unsuccessful. In summary, the magnitude of the effects on the litter size and on the gestation length does not justify for classification, therefore no classification is proposed for fertility.

Comments received during public consultation

Two MSCAs proposed Repr. 2 for fertility based on an extension of the gestation length in F0 and F1 females at 2500 ppm, as well as a reduction in litter size due to an increase in post-implantation loss in F0 and F1 females at 2500 ppm. These signs were observed at a dose that did not induce maternal toxicity. In response, the DS presented some additional data on gestation length (see Additional key elements below).

Assessment and comparison with the classification criteria

In the two-generation study the observations to the reduced litter size in both F1 and F2 offspring, there was a statistically significant reduction in the post-implantation survival index at 2500 ppm at a non-maternal toxic dose (74% for both generations, 91% and 93% for controls, respectively) and therefore also a reduced litter size at birth. These developmental effects will be considered under developmental toxicity.

As regards fertility effects, RAC agrees with the DS that the biological significance of a slightly prolonged gestation is of low concern, as other fertility parameters were not affected. With the explanations provided by the DS for the effects on oestrus cycle, gestation index and gestation length, RAC agrees that the effects seen at the high dose of 2500 ppm in the two-generation study are not of sufficient concern to warrant classification for fertility. RAC however notes that this dose of 2500 ppm is a non-maternally toxic dose, and that therefore a more appropriate top

dose could have been selected, in view of the results of the preliminary reproduction toxicity study (no maternal effects at 5000 ppm, and at 10000 ppm only changes in weight parameters and few clinical symptoms). Therefore RAC agree to **no classification for fertility**.

Developmental toxicity:

Summary of the Dossier Submitter's proposal

Three preliminary / Range finding studies and 3 main teratology key studies are submitted together with one pilot toxicity study. The details of which are summarised below.

Method Guideline GLP	**Species Strain Sex No/group**	**Exposure period**	**Doses tested/ Route**	**NOAELs/LOAELs**	**Reference**
Preliminary teratology study Range-finding study Non-guideline GLP	Rat CD 6 pregnant ♀ per group	GD 6-15	0, 500, 1000, 2000 or 3000 mg/kg bw/day Oral gavage	**Maternal:** The NOEL for maternal toxicity was 500 mg/kg bw/day (clinical signs, decreased body weights, mortality at ≥ 1000 mg/kg bw/day) **Embryotoxicity/teratogenicity**: The NOEL was < 500 mg/kg bw/day (decreased foetal weight at 500 mg/kg bw/day, and resorptions and malformations (limb, tail and liver) at 1000 mg/kg bw/day)	Willoughby 1990c /01 Supportive study
Teratology study Essentially in compliance with OECD 414 GLP	Rat CD 24 ♀ per group	GD 6-15	0, 20, 100 or 500 mg/kg bw/day Oral gavage	**Maternal:** The NOAEL for maternal toxicity was 500 mg/kg bw/day (no LOAEL) **Embryotoxicity/teratogenicity**: The NOAEL was 100 mg/kg bw/day for decreased foetal weights, increased incidences of skeletal variations at 500 mg/kg bw/day.	Willoughby 1990d /02 Key study
Pilot toxicity study Non-guideline GLP	Rabbit New Zealand White 2 non-pregnant ♀ per group	2-13 doses were administered	500, 750 or 1000 mg/kg bw/day	The NOEL was < 500 mg/kg bw/day (for transient body weight loss). At ≥ 750 mg/kg bw/day, clinical signs and morbidity/mortality were observed.	Jones & Brennan 1993 /03 Additional information
Preliminary teratology study Range-finding study Non-guideline GLP	Rabbit New Zealand White 5-7 pregnant ♀ per group	Days 7-19 *post coitum*	0, 400, 500 or 600 mg/kg bw/day Oral gavage	**Maternal**: No NOEL. Decreased body weight (days 7-9) at ≥ 400 mg/kg bw/day, marked clinical sign at ≥ 500 mg/kg bw/day **Embryotoxicity/teratogenicity:** NOEL was 400 mg/kg bw/day for slightly increased pre-and post-implantation losses, reduced number of implantations and number of live foetuses and litter weight.	Jones 1993a /04 Supportive study

Teratology study OECD 414 GLP	Rabbit New Zealand White 18 ♀ per group	Days 7-19 *post coitum*	0, 50, 150 or 450 mg/kg bw/day Oral gavage	**Maternal**: NOAEL was 150 mg/kg bw/day (mortality, clinical signs, body weight loss, reduced food consumption at 450 mg/kg bw/day) **Embryotoxicity/teratogenicity:** NOAEL was 50 mg/kg bw/day based on increased post-implantation loss and a single finding of an incomplete inferior vena cava in one foetus at 150 mg/kg bw/day. Malformations affecting heart, great vessels and face were observed at ≥ 150 mg/kg, including 3 foetuses with incomplete inferior vena cava at 450 mg/kg bw/day. There was no NOEL for variant sternebrae.	Jones 1993b /05 Key study
Range-finding study Non-guideline GLP	Rabbit New Zealand White 4 pregnant ♀ per group (7 dams at 600 mg/kg of which 3 sacrificed on day 11 after a single dose)	Days 11-19 *post coitum*	0, 300, 450 or 600 mg/kg bw/day Oral gavage	The study was designated to identify the MTD in pregnant rabbits, not to establish NOAEL. **Maternal:** All dams at 600 and two dams at 450 mg/kg bw/day were killed prematurely due to severe clinical sigs and/or persistent inappetence or body weight loss. Clinical signs at all doses. No effects on maternal organ weights or macroscopic findings in surviving dams at necropsy. The MTD was 300 mg/kg bw/day. **Embryotoxicity/Teratogenicity** No clear effect on embryo-foetal growth and survival. Post-implantation loss 14-100% at all doses. No skeletal or visceral examinations were carried out on the foetuses.	2015a Additional information
Teratology study Modified OECD 414 GLP	Rabbit New Zealand White 11 (necropsy on day 20) or 22 (necropsy on day 29) pregnant ♀ per group	Days 7-19 *post coitum*	0 or 350 mg/kg bw/day Oral gavage	The study was designated to clarify the relationship between maternal toxicity and the occurrence of incomplete inferior vena cava in the foetuses. **Maternal**: At 350 mg/kg bw/day rapid onset of clinical signs, reduced maternal food consumption, decreased body (rectal) temperature, increased skin temperature, and changes in some haematology and blood chemistry parameters **Embryotoxicity/Teratogenicity** No clear effects on embryo-foetal growth and survival and no treatment-related abnormalities in Day 20 and Day 29 foetuses. No skeletal examinations were carried out.	2015b Key study

In the first preliminary range finding study by Willoughby (1990c), the NOEL for maternal toxicity was 500 mg/kg bw/day (based on clinical signs, decreased maternal body weight, mortality at ≥ 1000 mg/kg bw/day) and the NOEL for resorptions was 500 mg/kg bw/day. There was no NOEL for foetal toxicity because of decreased foetal weights at 500 mg/kg bw/day. Liver and digit

malformations were observed at maternally toxic dose of 1000 mg/kg bw/day. The higher doses were in the range of acute toxicity doses and therefore this study is acceptable for supportive information only.

Main observations of the preliminary teratogenicity study in rats, Willoughby 1990c.

Parameter	Control	500 mg/kg bw/day	1000 mg/kg bw/day	2000 mg/kg bw/day	3000 mg/kg bw/day
Number of pregnant animals	6	6	6	6[a]	6[b]
Killed/dead animals	0	0	3	2	2
Pregnant animals at termination	6	6	3	4	0
Maternal body weights (g)					
-day 7	261	253	254	242 (5)	-
-day 10	279	273	278	263 (4)	-
-day 15	318	312	309 (3)[c]	291 (4) [↓8%]	-
-day 20	393	381	363 (3) [↓8%]	310 (4) [↓21%]	-
Food consumption (g/rat/day)					
-days 6-8	29	27	26	17	-
-days 9-11, 12-15, 16-17, 18-19	28, 31, 34, 35	29, 32, 35, 35	31, 30[d], 36, 35	27, 31, 29, 31	-
Water consumption (ml/rat/day)					
-days 6-8, 9-11	38, 39	40, 44	46, 47	65, 74	-
-days 12-15, 16-17, 18-19	44, 48, 44	46, 49, 47	55[d], 52, 54	50, 43, 41	-
Corpora lutea	15.7	15.3	14.7	16	15.7
Implantations	14.8	15.2	14.7	15	15.0
Viable young	14.2	14.7	10.3	15	-
- Males	7.5	6.3	5.0	9	-
- Females	6.7	8.3	5.3	6	-
Resorptions	0.67	0.50	4.33	0	15.0
- Early	0.67	0.33	4.33	0	15.0
- Late	0	0.17	0	0	0
Preimplantation loss	5.3	5.2	0	6.3	6.3
Post-implantation loss	4.5	3.3	29.5	0	100
Foetal weight (g)	3.63	3.23 [↓11%]	2.41 [↓34%]	3.35	-
Placental weight (g)	0.47	0.46	0.36	0.52	-
External examination of foetuses					
No. of foetuses/litters examined					
-small foetus	85 / 6	88 / 6	31 / 3	15 / 1	-
-reduced fore limbs	1.2 (1)[e]	30.7 (6)	93.5 (3)	13.3. (1)	-
-digits absent in fore limbs	0	0	3.2 (1)	0	-
-reduced hind limbs	0	0	6.5 (2)	0	-
-digits absent in hind limbs	0	0	3.2 (1)	0	-
-shortened tail	0	0	3.2 (1)	0	-
-small placenta	0	0	38.7 (1)	0	-
-large placenta	0	1.1 (1)	3.2 (1)	0	-
	0	2.3 (1)	0	0	-
Internal examination of foetuses					
No. of foetuses/litters examined					
-thickened liver	46 / 6	44 / 6	16 / 3	8 /1	-
-pale contents in GI-tract	0	0	25.0 (2)[e]	0	-
-bilateral hydronephrosis	0	0	6.3 (1)	0	-
-bilateral hydroureter	2.4 (1)	2.3 (1)	18.8 (1)	0	-
	2.4 (1)	4.5 (2)	18.8 (1)	0	-

In the key rat developmental toxicity study by Willoughby (1990d), the NOAEL for maternal toxicity was 500 mg/kg bw/day (no LOAEL) and the NOAEL for embryo/foetal toxicity was 100 mg/kg bw/day (decreased foetal body weights (9.3%), increased skeletal variations at 500 mg/kg bw/day). At non-maternotoxic dose level of 500 mg/kg bw/day, hymexazol did not induce malformations. RAC however notes that a more appropriate top dose could have been selected in view of the results of the preliminary range finding study, in which 500 mg/kg bw/day was

already assessed to be a non-maternotoxic dose. The potential of hymexazol to induce malformations at higher, not markedly maternally toxic doses can therefore not be excluded. In the preliminary study malformations were observed at 1000 mg/kg bw/day, but the dose was toxic to dams. The study is acceptable to conclude that hymexazol is not teratogenic up to doses of 500 mg/kg bw/day and that the NOAEL for foetal toxicity was 100 mg/kg bw/day in the rat.

Main observations of the rat developmental toxicity study, Willoughby 1990d.

Parameter	**Control**	**20 mg/kg bw/day**	**100 mg/kg bw/day**	**500 mg/kg bw/day**
Number of mated females	24	24	24	24
Number of pregnant females	24	24	23	21
Maternal body weights (g)				
- Day 7	267	265	269	263
- Day 10	286	285	287	286
- Day 15	327	326	328	327
- Day 20	410	405	411	406
Food consumption (g/rat/day)	29 – 34	29 - 34	30 - 35	30 - 34
Water consumption (ml/rat/day)	42 – 51	38 - 50	39 - 52	41 - 53
Necropsy findings on Day 20				
Number of animals examined	24	24	24	24
-Staining on head	0	0	1	1
-Unilateral hydroureter	0	0	0	1
-Bursal cyst on ovary	0	1	0	0
-Gas in caecum	0	0	1	0
Corpora lutea	16.5	16.5	16.7	16.6
Implantations	15.5	15.0	15.7	15.0
Viable young	15.1	14.0	14.3	14.7
- Males	7.6	6.3	6.9	6.9
- Females	7.5	7.7	7.4	7.8
Resorptions	0.46	0.96	1.30	0.24
- Early	0.46	0.92	1.30	0.24
- Late	0	0.04	0	0
Preimplantation loss	6.0	9.1	6.0	10.0
Post-implantation loss	2.9	6.4	8.3	1.6
Foetal weight (g)	3.67	3.69	3.76	**3.33****
Placental weight (g)	0.49	0.51	0.51	0.52

Foetal malformations and variations, Willoughby (1990d).

Parameter	**Control**	**20 mg/kg bw/day**	**100 mg/kg bw/day**	**500 mg/kg bw/day**
Selected external findings in foetuses[a]				
No. of foetuses/litters examined	362 / 24	337 / 24	330 / 23	309 / 21
-Small foetus	0.6 (2)	1.5 (2)	0.3 (1)	5.8 (7)
-Large foetus	8.0 (7)	7.4 (10)	12.4 (11)	0.6 (1)
-Limb(s) – haemorrhage	0.8 (1)	0	0	0.3 (1)
-Tail – agenesis[b]	0	0	0	0.3 (1)
-Small placenta	0.6 (2)	0.6 (2)	0.6 (2)	1.0 (2)
-Large placenta	0	0.3 (1)	0.6 (2)	4.9 (4)
Internal examination of foetuses (abdomen and thorax)[a]				
No. of foetuses/litters examined	183 /24	168 / 24	166 / 23	153 / 21
-Abdomen – blood	0	0	0	0.7 (1)
-Bilateral hydronephrosis	0.5 (1)	0.6 (1)	1.8 (3)	0
-Unilateral hydroureter	1.1 (2)	0.6 (1)	0	0
-Bilateral hydroureter	1.1 (2)	0.6 (1)	1.8 (3)	0

Selected internal findings in foetuses (free-hand serial)[a]				
No. of foetuses/litters examined	179 / 24	169 / 24	164 /23	156 /21
Head				
-Palate – haemorrhage	0	0	0	0.6 (1)
-Tongue/mouth/trachea - blood	1.7 (3)	1.2 (2)	1.8 (3)	1.3 (2)
-Dilatation of lateral ventricles	0.6 (1)	0	0.6 (1)	1.3 (2)
-Brain – haemorrhages	2.2 (3)	0.6 (1)	0.6 (1)	1.3 (2)
-Internal hydrocephaly	0	0	0.6 (1)	0
-Haemorrhagic fluid in ventricle	0	0	0	0.6 (1)
Thorax and abdomen				
-Space between organs and body	0	0	0	2.6 (3)
-Small conal septal defect	0	0	0	0.6 (1)
-Abdominal haemorrhage	1.1 (2)	1.2 (2)	0.6 (1)	3.2 (5)
-Unilateral hydronephrosis	0	0	0.6 (1)	3.8 (4)
-Bilateral hydronephrosis	0	0	0	1.9 (2)
-Unilateral hydroureter	5.6 (7)	3.0 (4)	4.3 (4)	3.8 (4)
-Bilateral hydroureter	3.4 (4)	1.8 (3)	1.8 (3)	7.7 (8)
Subcutaneous haemorrhage(s)				
Nasal	0	0	0.6 (1)	1.9 (3)
Cranial	1.1 (2)	1.2 (2)	2.4 (4)	4.5 (5)
Jaw	3.9 (3)	5.3 (5)	6.1 (6)	8.3 (7)
Submandibular	1.1 (2)	0.6 (1)	1.2 (2)	10.3 (8)
Fore-/hind-limb(s)	11.7 (9)	7.7 (9)	11.6 (11)	21.2 (12)
Abdominal	0	1.8 (2)	1.2 (2)	3.8 (5)
Generalized oedema[c]	2.8 (2)	1.2 (2)	3.7 (5)	1.9 (1)
Intramuscular haemorrhages[d]	3.9 (4)	0	2.4 (4)	3.2 (3)
Multiple malformations[e]	0	0	0.6 (1)	0
Selected skeletal findings				
-Large anterior fontanels	0.5 (1)	0	1.8 (2)	6.5 (7)
-Incomplete ossification of				
-supraoccipital bone	12.0 (11)	8.9 (10)	9.0 (11)	28.1 (14)
-interparietal bone	20.2 (15)	15.5 (16)	23.5 (14)	36.6 (16)
-1 sternebrae	24.6 (18)	23.2 (17)	21.7 (15)	0.7 (1)
-4 sternebrae	3.3 (5)	4.2 (5)	4.8 (7)	19.6 (15)
-thoracic vertebral centra	15.3 (15)	8.9 (12)	15.1 (12)	36.6 (19)
-lumbar vertebral centra	0.5 (1)	0.6 (1)	0	4.6 (5)
-caudal vertebrae	0	0.6 (1)	1.8 (2)	15.7 (9)
-metacarpals/metatarsals	0.5 (1)	2.4 (2)	1.2 (2)	12.4 (8)
-Ribs 14/14	10.4 (9)	6.0 (7)	10.8 (9)	**22.2 (16)***
-Additional cervical rib(s)	2.2 (2)	0	1.2 (2)	6.5 (7)
-Ossification of ventral arch of 1st cervical vertebra	15.3 (15)	18.5 (14)	13.3 (11)	0
-1st thoracic vertebral centrum unossified	0	1.2 (2)	0.6 (1)	7.2 (6)
-Metacarpals/metatarsal 4/4	21.3 (19)	14.3 (16)	31.9 (17)	8.5 (6)

In the range-finding pilot study in non-pregnant rabbits by Jones & Brennan (1993), the NOAEL for non-pregnant females rabbits seems to be approximately 500 mg/kg bw/dag. The study is acceptable as supplemental information.

In the range-finding rabbit developmental toxicity study by Jones (1993a), the NOEL for dams was less than 400 mg/kg bw/day and the NOEL for intrauterine development was 400 mg/kg bw/day. The study is acceptable as supplemental data.

In the key rabbit developmental toxicity study (Jones, 1993b), the NOAEL for dams was 150 mg/kg bw/day based on decreased body weight gain during early gestation and clinical signs at 450 mg/kg bw/day. Increased post-implantation loss, reduced number of live young, reduced litter weight, increased number of foetuses with malformations and variations were observed at

450 mg/kg bw/day. A single finding of an incomplete inferior vena cava in one foetus was observed at 150 mg/kg bw/day without maternal toxicity. At a maternally toxic dose of 450 mg/kg bw/day two more foetuses in two different litters had incomplete inferior vena cava. In addition, one dam having less significant maternal toxicity at 450 mg/kg bw/day had a foetus with incomplete inferior vena cava. In total, there were four foetuses with incomplete inferior vena cava.

Main observations in developmental toxicity study in rabbits, Jones (1993b).

Parameters	**0 mg/kg bw/day**	**50 mg/kg bw/day**	**150 mg/kg bw/day**	**450 mg/kg bw/day**
Number of dosed dams	18	18	18	18
Died	0	0	0	1
Killed	0	0	0	2[a]
Aborted	0	2	2	0
Non-pregnant	2	2	3	1
With dams with live young at Day 29	16	14	13	14
Clinical signs				
Number of dams examined	18	18	18	15
Post-dosing:				
Unsteadiness	0	0	0	11
Slumped posture	0	0	0	6
Increased respiration	0	0	0	7
Salivation	0	0	0	7
Daily examination:				
Off feed/reduced faecal output (days 7-9)	0	1	1	9
Orange-stained tray paper	0	0	8	13
Mean weight change (g) relative to Day 7				
- Gestation day 9	29	44	8	**-148****
- Gestation day 11	76	92	50	**3***
- Gestation day 15	189	216	144	158
- Gestation day 20	282	289	252	291
- Gestation day 29	404	413	397	458
Mean body weight change (g) during days				
- Days 0-7	244	255	268	275
- Days 7-9	29	44	8	**-148****
- Days 9-20	253	244	244	**439****
- Days 20-29	122	124	145	167
Litter data				
Corpora lutea	11.6	11.1	13.4	11.4
Implants	9.8	9.4	9.8	9.3
Preimplantation loss (%)	16.4	15.1	25.0	19.6
Resorptions	1.0	1.2	1.3	1.6
-Early resorptions	0.6	0.6	1.2	0.6
-Later resorptions	0.4	0.6	0.2	0.9
Post-implantation loss (%)	10.8	11.7	13.3 (↑23%)	18.4 (↑70%)
Live young	8.8	8.2	8.5	7.7 (↓12.5%)
Litter weight (g)	373.1	347.7	357.9	315.2(↓15.5%)
Mean foetal weight (g)	44.7	43.8	43.8	41.6 (↓6.9%)
Gravid uterine weight (g)	538.6	515.9	507.9 (↓6%)	476.2 (↓11.6%)
Sex ratio (% males)	58.7	44.8	47.1	44.4

* $p \leq 0.05$; ** $p \leq 0.01$

[a]Includes one female showing evidence of abortion

Malformations and variations observed in the developmental toxicity study by Jones (1993b).

Parameters	**0 mg/kg bw/day**	**50 mg/kg bw/day**	**150 mg/kg bw/day**	**450 mg/kg bw/day**
Number of foetuses examined **Number of litters examined**	140 16	115 14	110 13	108 14
Foetuses (litters) with malformations	2 (2)	3 (2)	4 (4)	6 (4)
- Cebocephaly (with IVS) - Multiple cranial malformations			 1 (1)[b]	1 (1)[a]
- Interventricular septal defect (IVS) - Incomplete inferior vena cava - Malformed cervicothoracic arteries		 1 (1)[f]	 1 (1)[d]	1 (1)[c] 2 (2)[e]
- Umbilical hernia				1 (1)[g]
- Bilateral forelimb oligodactyly - Right forelimb brachydactyly - Cervicothoracic/thoracic scoliosis - Irregular and incomplete ossification	 1(1)[i] 1 (1)[j]	 2 (1)[m]	1 (1)[h] 1 (1)[k]	 1 (1)[l]
Mean % foetuses per litter affected	1.1	2.8	3.6	4.6

* $p \leq 0.05$; ** $p \leq 0.01$

[a]Foetus with fused nasals and premaxillae, single nare, absent upper incisors, **double outlet right ventricle**; **dorsally displaced pulmonary trunk**; **interventricular septal defect**; **incomplete inferior vena cava** with persistent left posterior cardinal vein; displaced left adrenal; additional sternebral centre anterior to 1st; reduced ossification 2nd right cervical vertebral arch

[b]Foetus with **facial cleft**, single nare, bilateral anophthalmia, protruding tongue; absent upper incisors, premaxillae and nasals with markedly reduced and fused maxillae; flattened cranium with markedly reduced frontals, parietals and interparietal; atelectatic lungs; fused 1st to 2nd sternebrae and additional sternebral centre between 5th and 6th.

[c]Foetus with dilated ascending aorta/aortic arch and narrow pulmonary trunk/ductus arteriosus

[d]Foetus with persistent left posterior cardinal vein

[e]Both with persistent left posterior cardinal vein and with displaced left adrenal

[f]Narrow left carotid artery with additional narrow artery connecting left and right carotids; variation in origin of arteries arising from aortic arch

[g]Foetus had also left cervical rib

[h]Small, with also absent intermediate lung lobe, abnormal lobation of liver; sutural bone; shortened 1st right rib; reduced ossification odontoid process, midcaudal vertebrae and phalanges, forelimbs; unossified astragali

[i]Foetus had also malrotated hind limbs and abnormal liver lobation

[j]Small foetus with cortical vertebral irregularities including scoliosis due to 5th hemivertebra, misshapen vertebral tubercle, unossified odontoid process, reduced 1st misshapen 2nd bilateral vertebral arches, misshapen 2nd and 6th centra; absent intermediate lung lobe; ossification irregularity right parietal; absent 1st right rib; connected 1st to 2nd left costal cartilage

[k]Scoliosis due to 1st thoracic hemivertebra with absent centrum and left rib; partially fused 4th to 5th ribs with misshapen 4th thoracic centrum; asymmetric costal cartilages

[l]Scoliosis due to fused 9th and 10th bilateral vertebral arches, hemicentric 10th vertebra and 11th hemivertebra; shortened and thickened 10th, absent 11th right ribs

[m]One foetus with irregular and incomplete ossification 1st to 12th bilateral ribs; incomplete ossification cranial centres, ilia, left radius, bilateral scapula, ulna femur and tibia; subcutaneous oedema cervical region. Other foetus with irregular and incomplete ossification 3rd to 12th bilateral ribs; incomplete ossification cranial centres

Summary of visceral and skeletal variations observed in the developmental toxicity study by Jones (1993b).

Parameters	**0 mg/kg bw/day**	**50 mg/kg bw/day**	**150 mg/kg bw/day**	**450 mg/kg bw/day**
Number of foetuses examined **Number of litters examined**	140 16	115 14	110 13	108 14
Foetuses (litters) with gross/ visceral variations (malformed foetuses excluded) Includes foetuses with skeletal variations	17 (8) 3	7 (5) 2	9 (5) 2	19 (8) 6

Parameters	0 mg/kg bw/day	50 mg/kg bw/day	150 mg/kg bw/day	450 mg/kg bw/day
- Dilated ascending aorta/aortic arch	1 (1)[a]			
-Variation in origin of arteries arising from aortic arch	2 (2)	1 (1)[b]		1 (1)
- Preductal narrowing aortic arch, minimal				1 (1)
- Abnormal lobation of liver	7 (3)[c]	3 (1)[d]	2 (2)[e]	11 (4)[f]
- Pale subcapsular area in liver lobe(s)	2 (1)	1 (1)	2 (1)	4 (3)[g]
Foetuses (litters) with skeletal variations only (foetuses with malformations and visceral variations excluded)	19 (12)	14 (7)	12 (8)	23 (9)
- One additional thoracolumbar vertebra				3 (2)[h]
- Right and/or left cervical rib	3 (3)[i]	2 (2)[j]	3 (2)	6 (4)
- Shortened 1st right rib				1 (1)¤
- Extra (13th) rib(s)	29.2	18.2	36.2 (↑ 24%)	**57.3* (↑ 96%)**
- Variant sternebrae(e)	11.1	21.3 (↑ 92%)	20.1 (↑ 81%)	29.1 (↑ 162%)
Mean % foetuses per litter affected				
Visceral anomalies	13.7	6.3	9.1	24.1 (↑ 76%)
Skeletal anomalies	13.2	11.1	10.2	24.5 (↑ 86%)

* $p \leq 0.05$; ** $p \leq 0.01$

¤ premature birth

[a]Foetus had also narrow pulmonary trunk/ductus arteriosus

[b]Foetus had also narrow left carotic artery and other findings

[c]One foetus had also swollen liver with pale subscapsular areas, bilobed gall bladder and some skeletal findings

[d]One foetus had also absent gall bladder and connected 3rd to 5th sternebrae and the other connected 3rd to 5th sternebrae

[e]One with ossification irregularities right parietal

[f]One with sutural bone(s), two with bifurcated 6th sternebrae

[g]One with hepatocyte necrosis and left cervical rib, other with bilateral cervical ribs and absent intermediate lung lobe

[h]One with hemicentric 4th thoracic vertebra with reduced 4th right and misaligned 5th thoracic vertebral arches; reduced ossification 4th right rib, one with additional centre of ossification ventral to 2nd cervical centrum; shortened 1st bilateral rib; one additional thoracolumbar vertebra

[i]One with 7th lumbar hemivertebra with fused 7th (reduced) to 8th lumbar centra; one additional thoracolumbar vertebra.

[j]One with sutural bone(s)

A new dose range finding toxicity study in the pregnant rabbit by oral gavage administration (2015a) was primarily conducted to identify the MTD when hymexazol was administered to pregnant rabbits. The MTD for dams was 300 mg/kg bw/day based on clinical signs and decreased maternal body weight at 450 mg/kg bw/day. No skeletal or visceral examinations (i.e. cardiovascular abnormalities of the heart and blood vessels) were carried out on the foetuses. The study is acceptable as supplemental data.

A key study of maternal effects and embryo-foetal development effects in the rabbit by oral gavage administration (2015b) was particularly aimed at studying maternal toxicity and determine a possible relationship between maternal toxicity and the occurrence of incomplete inferior vena cava in the foetuses. Maternal effects were identified at 350 mg/kg bw/day on endpoints not routinely required for guideline prenatal developmental studies. No clear effects on embryo-foetal growth and survival or treatment-related abnormalities were recorded i.e. cardiovascular abnormalities of the heart and all major blood vessels including the inferior vena cava. The study authors suggest that there was a number of effects which may be expected to alter maternal blood flow and tissue perfusion/oxygenation leading to hypoxia. According to the authors, the findings in this study strongly suggest either that the occurrence of 3 cases of incomplete inferior vena cava at 450 mg/kg bw/day in the original prenatal developmental toxicity study was a spontaneous, non-treatment-related event, or that their occurrence was due to the severity of maternal toxicity at 450 mg/kg bw/day. The DS is of the opinion that although some changes in the maternal non-routine parameters (i.e. body temperature, skin temperature, and haematological and clinical chemistry parameters) were statistically significant, the biological

significance and adversity of these changes is not clear. In addition, the magnitude of the effects on the non-routine parameters are low. At the moment a causal link between the finding incomplete inferior vena cava seen in the previous study on rabbits and hypoxia is only speculative.

Based on the results from the new rabbit studies, it is not possible to conclude that the finding incomplete inferior vena cava in the older prenatal developmental toxicity study on rabbits (Jones, 1993b) was due to maternal toxicity. The two dams without clinical signs and the dam with less significant clinical signs had foetuses with this abnormality.

Also, the incidence of incomplete inferior vena cava (1.83 %) was above the historical control value (range from 0 to 0.46 % over the years 1990-1995) thus questioning the spontaneous nature of the abnormality.

Since it cannot be ruled out that the effects are treatment-related and also relevant for humans, based on the results obtained in rabbit developmental toxicity study (increased incidence of incomplete inferior vena cava) and in rat developmental toxicity study (decreased foetal body weights and increased skeletal variations), the DS proposed hymexazol to be classified as Repr. 2, H361d (Suspected of damaging the unborn child).

Comments received during public consultation

Three MSCA supported classification for developmental toxicity. One of the three proposed Repr. 1B instead of category 2 given that exposure to hymexazol leads to a decrease of the foetuses weight, and to an increase in post-implantation loss as well as severe effects such as subcutaneous haemorrhages in rat or heart and great vessels malformation in rabbit. As two species were affected, dose-dependency was demonstrated for several effects and it cannot be ruled out that the underlying mechanisms is the same in humans, they proposed that perhaps a classification as Repr. 1B; H360D would be more appropriate.

One Company-Manufacturer disagreed with the proposed classification for developmental toxicity by pointing to the two new studies in rabbits (2015a,b) in which the developmental abnormality of inferior vena cava was not reproduced at the dose level of 350 mg/kg bw/day in the main study, even though some deterioration of health conditions were detected on the maternal rabbit of this group and to the steep dose-response curve for maternal toxicity in rabbits (minor and transient effects at 300 mg/kg bw/day, marked adverse clinical signs and some deaths at 450 mg/kg bw/day). According to the Company-Manufacturer, the absence of incomplete formation of inferior vena cava in the new study, strongly suggests that the occurrence of incomplete vena cava in one of 110 foetus at 150 mg/kg bw/day in the original PNDT study was a spontaneous non-treatment related event. In addition to that, it is expected that the maternal rabbits in the 450 mg/kg bw/day groups in the original developmental study had suffered adverse health that could not be revealed without clinical pathological examinations. This suggests that the occurrence of incomplete vena cava of three of 108 foetus at 450 mg/kg bw/ day was secondary to maternal toxicity of hymexazol.

In its detailed response to the comments received, the DS presented further historical control data on pre- and post-implantation loss in rats and rabbits and on some selected internal findings in rabbit (thorax and abdomen, subcutaneous haemorrhages) that were not included in the CLH report. These are presented in the section below.

Assessment and comparison with the classification criteria

RAC has identified 3 crucial endpoints that are relevant for classification of the substance as toxic to the development. The one endpoint is the incidences of incomplete inferior vena cava and the

other is the incidences of anomalies together with subcutaneous haemorrhages and the last is post/pre implantation loss.

The incidences of incomplete inferior vena cava were observed only in rabbits (Jones, 1993b) with one foetus (1/110; 0.9%) at 150 mg/kg bw/d (dose not maternally toxic) and 3 foetuses (3/108; 2.8%) at 450 mg/kg bw/d. There was evidence of maternal toxicity in the 450 mg/kg bw/d, however, one dam having less significant maternal toxicity had a foetus with incomplete inferior vena cava. The malformations of inferior vena cava are regarded as relevant for humans. The total incidence of 1.83% (4/218) was above historical control which ranges from 0-0.46% over the years 1990-1995.

Two studies from 2015 were submitted: a dose range finding toxicity study in pregnant New Zealand White rabbits by oral gavage administration (2015a) and a study of maternal effects and embryo-foetal development effects in the New Zealand White rabbit by oral gavage administration (2015b). No clear treatment related abnormalities were recorded i.e. cardiovascular abnormalities of the heart and all major blood vessels including the inferior vena cava. However, the absence of these findings and the additional investigations in these studies do not fully explain the findings in the key rabbit teratology study by Jones (1993b). The purity of hymexazol in the two studies by Jones (1993a,b) was 99.3%. No information about the purity in the two new submitted studies (2015a,b) was given but is expected to be in the same range.

Some incidences of skeletal and visceral abnormalities were increased in the key rat teratogenicity study by Willoughby (1990d): there was an increase in thorax/abdomen anomalies and in subcutaneous haemorrhages at the highest dose group in the absence of maternal toxicity (namely nasal, cranial, jaw, submandibular, abdominal and limbs). Some of the incidences of subcutaneous haemorrhages were increased in a dose-dependent way, but not all (see table below). These findings were not statistically different from concurrent control. All incidences were above the mean values of laboratory background control data of this rat strain but most of the values were within the background control ranges.

***Table**: Foetal findings from Willoughby (1990d).*

Parameter	**Control**	**20 mg/kg bw/day**	**100 mg/kg bw/day**	**500 mg/kg bw/day**	**Laboratory background control data[b]**	
					Mean	**Study ranges**
Selected internal findings in foetuses (free-hand serial)[a] No. of foetuses / litters examined	179 / 24	169 / 24	164 / 23	156 / 21		
<u>Thorax and abdomen</u>						
Space between organs and body	0	0	0	2.6 (3)	2.32	0.0-8.9
Small conal septal defect	0	0	0	0.6 (1)		-
Abdominal haemorrhage	1.1 (2)	1.2 (2)	0.6 (1)	3.2 (5)	2.22	0.0-5.5
Unilateral hydronephrosis	0	0	0.6 (1)	3.8 (4)	1.12	0.0-4.2
Bilateral hydronephrosis	0	0	0	1.9 (2)	0.6	0.0-7.3
Unilateral hydroureter	5.6 (7)	3.0 (4)	4.3 (4)	3.8 (4)	9.17	2.8-19.5

Bilateral hydroureter	3.4 (4)	1.8 (3)	1.8 (3)	7.7 (8)	4.88	0.0-21.9
Subcutaneous haemorrhage(s)						
Nasal	0	0	0.6 (1)	1.9 (3)	1.04	0.0 - 4.3
Cranial	1.1 (2)	1.2 (2)	2.4 (4)	4.5 (5)	3.26	0.0 - 14.1
Jaw	3.9 (3)	5.3 (5)	6.1 (6)	8.3 (7)	4.88	0.0-15.4
Submandibular	1.1 (2)	0.6 (1)	1.2 (2)	10.3 (8)	2.04	0.0-7.1
Fore-/hind-limb(s)	11.7 (9)	7.7 (9)	11.6 (11)	21.2 (12)	19.11	0.0-38.3
Abdominal	0	1.8 (2)	1.2 (2)	3.8 (5)	1.35	0.0-8.3

[a]Incidence (%) (No. of litters)

[b]30 studies (4013 foetuses)

Regarding post-implantation loss, a statistically significant reduction in the post-implantation survival index (74% for both generations, 91% and 93% for controls, respectively) was observed in the rat two-generation study at a non-maternal toxic dose of 2500 ppm (in females corresponding to 244 mg/kg bw), and consequently also a reduced litter size at birth. In the key teratogenicity study in rat by Willoughby (1990d) a dose-dependent increase in post-implantation loss was seen at the low and middle dose group (2.9, 6.4, 8.3 and 1.6 % at dose levels of 0, 20, 100, 500 mg/kg bw/day, respectively). In contrast to the two-generation study, there was no effect on post-implantation survival up to and including the highest dose of 500 mg/kg bw/day. As on a mg/kg bw/day basis this dose is 2x higher than in the two-generation study, this reduces somewhat the concern for the findings in the latter study. RAC notes that in the key teratogenicity study there was a slight increase in pre-implantation loss at the highest dose (6.0, 9.1, 6.0 and 10 % at dose levels of 0, 20, 100, 500 mg/kg bw/day, respectively). There was also an increase in pre-implantation loss at the lowest dose. But the dosing started on day 6 p.c., so probably after the time of implantation. The foetal weight was slightly (9.3%) albeit statistically significant reduced at the highest dose level group (500 mg/kg bw/day) when compared to concurrent control. At the highest dose no maternal toxicity was observed. Effects on implantation were not statistically significant from concurrent control. Incidences of post-implantation loss at the low and middle dose group were above the mean value of laboratory background controls but were within the range of the control values from 36 studies in this rat strain (pre-implantation loss: mean 9.0, range 4.9-27.2 %; post-implantation loss: mean 5.52, range 1.90-10.90 %).

In a key rabbit teratogenicity study in rabbit by Jones (1993b), a dose-dependent increase of post-implantation loss was seen of 8.3%, 23% and 70% at dose levels of 50, 150 and at 450 mg/kg bw/day compared to control group (10.8, 11.7, 13.3, 18.4 at dose levels of 0, 50, 150 and at 450 mg/kg bw/day groups, respectively). Higher post-implantation loss at the highest dose level was a consequence of slight increase in late embryonic deaths. The litter size was slightly reduced (12.5 %). There was an increase of pre-implantation loss of 52 % and 20 % at the middle and high dose groups (16.4, 15.1, 25.0, 19.6 at dose levels of 0, 50, 150 and 450 mg/kg bw/day groups, respectively). Numbers of corpora lutea were slightly higher at the middle dose than controls, which may account in part for the slightly increased pre-implantation loss (number of implants was similar in all groups). The dosing started on day 7 p.c., so probably after the time of implantation. The highest dose level (450 mg/kg bw/day) was maternally toxic but the middle dose level (150 mg/kg bw/day) was not. Therefore, it is not possible to conclude that effect on post-implantation loss was secondary to maternal toxicity. However, the differences in late embryonic deaths and pre- and post-implantations losses were not statistically

significant when compared to concurrent controls. The incidences of post-implantation loss at the mid and high dose levels were above the mean value of historical controls. At the mid dose level it was within the range of historical control data and at the highest dose level it was slightly above the range values from historical control data (Froxfield historical control data: pre-implantation loss: mean 17.5, range 13.2-22.7 %; post-implantation loss: mean 12.6, range 9.5 to 16.2 %, seven studies, over the years 1990-1992).

The overall conclusion by RAC for developmental toxicity, taking into account the indications of increase in post-implantation loss in rats and rabbits, the significant but relatively low incidence of incomplete inferior vena cava in rabbits and the additional concerns for the subcutaneous haemorrhages, is classification in category 2, although some effects can be considered more supportive than leading to classification (e.g. because of absence of dose-relationship or statistical significance). RAC considers that in line with the proposal of the Dossier Submitter, **Repr. 2; H361d for developmental toxicity** is warranted.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Hymexazol is an active substance in the meaning of Directive 91/414/EEC and is currently classified as Aquatic Chronic 3 (H412) in Annex VI to the CLP Regulation.

The available acute ecotoxicity data supports that hymexazol is not acutely toxic to the environment according to EC 1272/2008 (CLP) as the lowest IC_{50} value is 9.4 mg/L (>1 mg/L) for the aquatic plant *Lemna gibba*.

The DS proposed to amend the existing harmonized entry for chronic hazard. Hymexazol is not rapidly degradable in the environment and has a low potential for bioaccumulation in aquatic organisms (Log Kow < 4). Chronic toxicity data is available for algae, the aquatic plant *Lemna*, the aquatic invertebrate *Daphnia magna*, but not for fish. The available prolonged acute fish test (OECD 215) is not considered adequate for the chronic classification. Therefore chronic classification was assessed using two approaches as specified by Section 4.1.2.3 of the CLP regulation (2nd ATP). Based on the most sensitive chronic toxicity data, i.e., an EC_{10} value of 0.4 mg/l (≤ 1 mg/l) an Aquatic Chronic 2 classification (H411) is warranted, whereas the combination of acute aquatic toxicity data, 96-h LC_{50} value for fish of >100 mg/L and the environmental fate data, log Kow < 4 gives no aquatic chronic classification for hymexazol. The most stringent outcome shall be chosen and therefore hymexazol shall be classified as Aquatic Chronic Category 2, H411 according to Regulation EC 1272/2008.

The measured water solubility of hymexazol is 65.1 g/L at 20°C (unbuffered water), 58.2 g/L at 20°C (pH 3) and 67.8 g/L at 20°C (pH 9). The dissociation constant of pKa=5.92 at 20°C has been determined for hymexazol. Data indicate that the vapour pressure for hymexazol is low at 1.82×10^{-1} Pa at 25°C. The Henry's Law Constant of 1.4×10^{-4} $Pa.m^3/mol$ indicates that hymexazol is not volatile from water. The adsorption of hymexazol to soil is dependent on the soil pH. In neutral and acidic soil types the substance is moderately mobile with KOCs in the range 99 to 124 mL/g. In alkaline soil the substance is mobile to very mobile with KOCs in the range 12 to 27 mL/g. Hymexazol is the primary constituent with a typical concentration of 98.5%. There are two main impurities (flagged as confidential) which are reported not to affect the classification of hymexazol.

Degradation

Stability

Hydrolysis of hymexazol was tested according to US EPA Subdivision N, guideline 161-1 and following GLP principles. The test was not conducted to tiered approach as recommended in EU guideline however, the data available was considered sufficient, pH 5 used (rather than pH 4). Hymexazol is considered stable to hydrolysis (i.e. DT_{50} > 1 year) in aqueous sterile buffers at pH 5, 7 and 9 when incubated at temperatures of 25, 37, and 50°C. Hymexazol is also stable to hydrolysis at a temperature of 37°C in buffer at pH 1.95 and at a temperature of 70°C in buffer at pH 7 and 9. Only at pH value of 5 and at a temperature of 70°C hymexazol is hydrolysed with a DT_{50} value of 35 days.

The photodegradation of [^{14}C]-hymexazol in aqueous buffer solution, was tested according to US EPA Subdivision N, guideline 161-2 and following GLP principles. The substance is stable to photolysis (DT_{50} value > 1 year) in aqueous sterile buffer solutions at pH values of 5, 7 and 9 at a temperature of 25°C.

The photodegradation of [^{14}C]-hymexazol on soil, was tested according to US EPA Subdivision N, guideline 161-2 (1982) and following GLP principles. Hymexazol degraded when exposed to an artificial light source, on a soil surface. The DT_{50} value was determined to be 2.3 days, compared to 37.5 days for the dark controls. Chemical analysis showed that the only component extracted from the soil was unchanged hymexazol and no degradation products were observed. Photodegradation may be a relevant degradation pathway of hymexazol in soil surface.

A computer estimation of the photochemical oxidative degradation rate using the Atkinson equation has been conducted with the atmospheric oxidation program AOPWIN version 1.86. The hydroxyl reaction half-life was estimated to be 0.641 hours based on a 12 hour day. Hymexazol does not contain alkene or alkyne groups so no ozone reactions were estimated.

Biodegradation screening tests

In a valid ready biodegradability test minimal amounts of hymexazol were degraded. Ready biodegradation was tested following the OECD guideline No. 301C (modified MITI-test) (1989). The report submitted was an English translation of the original report in Japanese. The oxygen uptake of a stirred solution of hymexazol (100 mg/L) containing activated sewage sludge was monitored over a period of 28 days, in the dark at a temperature of 25°C. The BOD and TOC levels of solutions containing hymexazol and activated sludge remained unchanged over the incubation period and thus the ultimate degradation was 0%. Analysis of the test solutions by HPLC at the end of the incubation period confirmed that minimal amounts of the test substance had degraded (removal of test substance 0%, 2% and 2% in three replicate bottles). The DS noted that the translated report did not discuss the toxicity control, where the reference substance was apparently readily degraded. Lack of microbial toxicity was, however, confirmed by the activated sludge respiration inhibition test according to OECD TG 209 where an 3h-EC50 of 217 mg/L was reported (Bealing et al. 2002). The DS concluded that the substance is not readily biodegradable under the conditions of the modified MITI test.

Biodegradation water/simulation tests

Three studies on aerobic degradation in water/sediment systems were summarised in the CLH report. These included a main study (Muttzall 1994) in which dissipation rates were determined and two other studies (Hall and Lowrie (2004) and Hanstveit and van der Leur-Muttzall (1998)), which were aimed to identify metabolites detected in the main study.

In the main study (Muttzall 1994), the aerobic transformation of radiolabelled hymexazol was investigated in water/sediment study at a temperature of 20°C, according to BBA Guidelines,

Part IV, Section 5-1 (1990) and in compliance with GLP principals. Total recovery ranged from 80.2% to 103.5% and 92.9% to 110.1% of applied radioactivity (% AR) for the clay loam and sandy loam water/sediment systems. The applied radioactivity rapidly dissipated from the water layer, after 28 days the amount remaining comprised 4.0 to 5.8% AR. One significant unknown metabolite was observed mostly in water layers at a maximum level of 14% to 15% AR after 14 days, and rapidly declined to 3 to 4% AR after 28 days. Another minor unknown metabolite was observed in both water and sediment layers at maximum level of 6% to 9% AR. Trace amount of 5-methyl-2-(3H) oxazolone and crotonic acid were also observed at levels < 1% AR (except on a single occasion in one water/sediment system where 6% AR of 5-methyl-2-(3H) oxazolone was detected). CO_2 production (= evaporated) amounted to 25.8 and 52.2% AR after 28 days and to 52.2 and 74.0% AR at test end (= 105 days). Non-Extractable Residues (NER) amounted to 39.3 and 21.5% AR after 105 days. Assuming first-order kinetics, dissipation DT_{50} values were calculated for the water phase of 2.3 and 3.0 days at 20 °C, and for the combined water/sediment systems of 2.4 and 3.1 days at 20°C.

The study by Hanstveit and van der Leur-Muttzall (1998) aimed to identify the unknown metabolites observed in the main study mentioned above. As stated in the DAR, the study was not considered reliable by the notifier. The DS considered this study not relevant for the CLP classification, and therefore it was not described.

Another study was carried out by Hall and Lowrie (2004) to identify the unknown metabolites observed in main study in compliance with GLP and according to EC Directive 91/414 guidelines. The test was conducted using a water/sediment system consisting of sandy loam sediment and associated water at a temperature of 20°C. The significant unknown metabolite observed in the main study (Muttzall 1994) was identified as dissolved/entrapped CO_2. The other minor metabolite was determined to comprise of at least two components (and possibly 3 or more) but these were not characterised. Additionally, a significant amount of 5-methyl-2(3H)-oxazolone was found in the study but not in the main water/sediment studies. No DT_{50} values were calculated based on this study.

Conclusion from the water/sediment simulation tests

Mineralization based on dissolved and evaporated CO_2 amounted to 33 and 61% AR after 28 days in the main study, not fulfilling 70% ultimate degradation in 28 days. The dissipation half-lives in the combined water/sediment systems (2 and3 days at 20 °C) indicate that the rapid degradability criterion could be fulfilled based on primary degradation. This is based on the consideration that a degradation half-life of <16 days corresponds to >70% degradation in 28 days. However, in addition to the primary degradation rate it should also be demonstrated that the degradation products formed do not fulfil the criteria for classification as hazardous to the aquatic environment. In the case of Hymexazol, the rapid degradability criterion is not fulfilled on the basis of primary degradation as some of the degradation products were not identified and therefore could not be characterized for their hazards to the aquatic environment. The DS concluded that hymexazol is not rapidly degradable.

<u>Biodegradation soil simulation tests</u>

Aerobic degradation of hymexazol in soil was studied in four different simulation studies.

Study 1: Ballantine (1993a) studied degradation of radiolabelled hymexazol in one sandy loam soil under aerobic conditions in the dark at 25°C for 50 days. Total recovery ranged 86.1 to 106.6% AR. Up to four minor unidentified metabolites were formed that did not exceed a combined total of 1.1% AR at any one time. CO_2 production after 28 days was 58.5% AR and after 50 days ca. 65% AR. NER levels increased to ca 40% after two weeks but subsequently declined to 28% AR at test end. Subsequent extraction of NER under increasingly harsh

conditions reduced NER levels to 13.5% AR after 14 days and 13.4% AR after 50 days that were tightly incorporated into the soil structure. For dissipation, a DT_{50} value of 7.9 days at a temperature of 25°C was obtained which corresponds to a DT_{50} value of 12.4 days normalized to 20°C. Mineralisation half-life was not determined.

Study 2: Goodyear (1998) studied degradation of radiolabelled hymexazol in three soils, i.e.two sandy loam soils and one loamy sand soil,) under aerobic conditions at 20°C for 120 days. Total recovery ranged 84.9 to 99.6% AR. No metabolites of ≥ 10% AR were formed. Some minor unidentified components were observed at levels up to a combined maximum of 2.4% AR. NER levels increased to a maximum of ca 30% over the period 28 to 90 days after treatment and then subsequently declined. CO_2 was produced throughout the incubation period and was 32.6%, 50.7%, and 15.5% AR after 28 days in the three soils, reaching a level of ca 60% after 90 days. Dissipation rates corresponded to DT_{50} values 15.1, 15.4 and 31.5 days in the three soils at 20 °C.

Study 3: Goodyear (1998) studied degradation of radiolabelled hymexazol in one loamy sand soil at 10°C; same test setup as that used in Study 2 at 20°C. The dissipation rate of hymexazol was dependant on temperature. At the lower temperature of 10°C the DT_{50} value was extended to 101 days compared to 31.5 days at a temperature of 20°C (in the corresponding soil). Mineralisation half-life was not determined. Carbon dioxide production was 4.9% AR after 28 days and 27.1% AR after 120 days.

Study 4: Bashir (1994a) studied degradation of hymexazol in one loamy sand soil at 25 °C same test setup as that used in Study 1. The initial aerobic part of the soil simulation test, dissipation of hymexazol proceeded at a rate corresponding to the DT_{50} value of 11.4 days at 25 °C. Mineralisation half-life was not determined. Carbon dioxide production was 12.0% AR at the end of the aerobic part of the study (10.2 days).
The DS stated that anaerobic simulation test data is available in the DAR but only the aerobic part of the test was included in the CLH report as anaerobic degradation is not relevant for CLP classification in this case. Field studies in soil are also available in the DAR but these address dissipation and not degradation and are therefore not relevant for CLP classification.

DS conclusion on degradation

Hymexazol is not rapidly degradable according to the CLP regulation**.** This conclusion is based on the results on ready biodegradability and hydrolysis. The results of the water/sediment support the conclusion that hymexazol is not rapidly degradable.

Bioaccumulation

Based on experimental data, hymexazol has a measured log Kow of 0.48 in one test (Shake flask method; pH not reported) and 1.01 (pH 5), < 0.3 (pH 7) and < 0.3 (pH 9) at 25°C in another test (HPLC method).

An experimental aquatic biocentration study in carp *(Cyprinus carpio)* following OECD 305C (non-GLP) is available. Carp were exposed to hymexazol at nominal concentrations for eight weeks under flow-through conditions. Overall mean measured concentrations of hymexazol in the 0.2 and 2 mg/L treatments were 0.182 an 1.87 mg/L, representing recoveries of 91 and 94% of the respective nominals. Concentrations of hymexazol in whole-fish tissues from both treatment rates were below the limit of detection (1.1 mg/kg). Because of this, biocentration factors were not obtained. Deviations from guidelines were reported (i.e. no depurations phase, insufficient frequency of fish sampling during exposure/uptake phase). However, the DS states that the deviations are unlikely to have affected the outcome of the test. In conclusion, Hymexazol does not have a potential to bioaccumulate in aquatic organisms.

Aquatic toxicity

Valid aquatic acute toxicity data are available for fish, invertebrate, algae and aquatic plants with aquatic plants being the most sensitive trophic level. Valid aquatic chronic toxicity data are available for aquatic invertebrates, algae and aquatic plants while data for fish is lacking. The ecotoxicological test results are summarized in the following table (key data are highlighted in bold).

Test Guideline	Test Organism	Exposure		End point	Result (mg a.s./L)	Remark	Reference
		Design	Duration				
Short-term toxicity to fish							
US EPA FIFRA 72-1 OECD 203 GLP	*Lepomis macrochirus*	Semi-static	96 hours	LC_{50}	> 100	Limit test Based on nominal concentrations	Doc IIA, 8.2.1/01
US EPA FIFRA 72-1 OECD 203 GLP	*Oncorhynchus mykiss*	Semi-static	96 hours	LC_{50}	> 100	Limit test Based on nominal concentrations	Doc IIA, 8.2.1/02
OECD 215 Prolonged acute toxicity to fish GLP	*Oncorhynchus mykiss*	Flow-through	28 days	LC_{50} EC_{50} NOEC	> 100 > 100 ≥ 100	Based on nominal concentrations Based on wet weight and for length	Doc IIA, 8.2.2.1/01
Short-term toxicity to aquatic invertebrates							
US EPA FIFRA 72-2 OECD 202 (I) GLP	*Daphnia magna*	Static	**48 hours**	**EC_{50}**	**28** (95% CI: 24 – 33)	Based on mean measured concentrations	Doc IIA, 8.2.4/01
Long-term toxicity to aquatic invertebrates							
OECD 211 US EPA OPPTS 850.1300 GLP	*Daphnia magna*	Semi-static	**21 days**	NOEC NOEC NOEC **EC_{10}**	15 3.2 0.8 **0.4**	Mortality Body length Reproduction Reproduction Based on nominal concentrations	Doc IIA, 8.2.5/01
Toxicity to algae and aquatic plants							
OECD 201 GLP	*Selenastrum capricornutum* *Scenedesmus subspicatus*	Static	72 hours	E_bC_{50} E_rC_{50} NOEC	32 32 10 Same effect levels to both test species	Based on mean measured concentrations Changes in the test media pH values exceeding 1.5 units between 0 and 72 hours.	Doc IIA, 8.2.6/01

Test Guideline	Test Organism	Exposure		End point	Result (mg a.s./L)	Remark	Reference
		Design	Duration				
US EPA subdivision J, Sections 122-2 and 123-2 OECD 201 EU Part C.3 GLP	*Selenastrum capricornutum*	Static	72 hours	E_bC_{50} E_rC_{50} NOEC	37 46 (95% CI: 44 -48) 29	Based on mean measured concentrations The study passed the validity test despite the fact that the light intensity was below the guideline recommendations.	Doc IIA, 8.2.6/02
US EPA FIFRA Subdivision J, Series 123-2 GLP	*Lemma gibba G3*	Static	14 days	IC_{50} NOEC	9.4 (95% CI: 8.8-9.8) 3.1	Exposure levels declined over the course of the test, particularly at the lowest concentrations. Based on the levels measured at the start of the incubation.	Doc IIA, 8.2.8/01

Hymexazol is not volatile and is stable to hydrolysis and photolysis.

Two acute studies run as limit tests were conducted in fish under semi-static conditions over a period of 96 hours. The reported 96-hour LC50s values were greater than 100 mg/L (nom). A prolonged toxicity test to juvenile fish was conducted under flow-through conditions, resulting in a 28-day LC_{50} value was greater than 100 mg/L (nom). No long-term toxicity test is available for fish.

An acute study with *D. magna* was conducted following OECD TG 201 (I) and according to GLP principles, resulting in a 48 hours EC_{50} of 28 mg/L (mm). A chronic toxicity study of hymexazol to *D. magna* was conducted under semi-static conditions following OECD 211 and according to GLP. The 21-day NOEC for reproduction was 0.8 mg/L (nom) and the EC_{10} was 0.4 mg/L based on reproduction. The EC_{10} value is used for classification purposes instead of the NOEC value.

Toxicity studies on algae (two studies) and aquatic plants (one study) are included in the CLH report. The inhibition of algal growth inhibition studies was assessed on two species of algae. The 72-hour E_rC_{50} of hymexazol to both S*. capricornutum* and *S. subspicatus* was 32 mg/L (mm) and the NOEC was 10 mg/L (mm) for both species. Changes in the test media pH values exceed 1.5 units between 0 and 72 hours. The DS considered the study as acceptable even with the reported variation in the test media. Another algal study was conducted with *S.capricornutum* under static conditions over a 72 hours period. The 72-hour E_rC_{50} was reported to be 46 mg/L (mm) and the 72-hour NOEC was 29 mg/L (mm).

A 14-day acute toxicity test to the duckweed *Lemna gibba G3*, was determined in a static test system at concentrations of 0, 3.1, 6.3, 13, 25, 50 and 100 mg/L. Statistically significant reductions both frond and plant production were observed at concentrations from 5.9 up to 98 mg/L (measured initial concentration), together with an increase in the percentages of dead fronds. Colony break-up and root destruction became apparent after three days of exposure at concentrations equal to and greater than 13 mg/L and fronds also appeared to be smaller than in the controls. Statistically significant, higher percentages of necrotic fronds were observed at

the end of the experiment period in the treatments containing 13, 14, 98 mg/L. The 14-day IC_{50} and NOEC were 9.4 mg/L and NOEC 3.1 mg/L, respectively. The results are based on measured initial concentration.

Based on the available information for aquatic toxicity, the DS concluded that hymexazol is not acutely toxic to the aquatic environment based on the lowest IC_{50} value of 9.4 mg/l (> 1 mg/l), so no classification is warranted. Based on a reliable EC_{10} of 0.4 mg/L for *Daphnia*, a classification as Aquatic Chronic 2 is warranted. However, due to the lack of chronic toxicity data for fish, the DS used the surrogate approach by combining environmental fate and acute toxicity data for fish. Considering that hymexazol is not rapidly degradable and LC_{50}s for fish are all > 100 mg/L, this results in no classification. Therefore, the DS proposes classification as Aquatic Chronic 2.

Comments received during public consultation

Five Member State Competent Authorities (MSCA) provided public comments. Three agreed with the proposed classification with no further comments. One agreed to the classification and indicated minor editorial mistakes in the CLH report. Another MSCA agreed to the proposed classification, but pointed out that the general exposure for Lemna toxicity testing is 7 days according to two test guidelines, OECD 221 and US EPA 850.4400.

One industry organisation agreed with the proposed classification however they suggested the surrogate approach should apply to all trophic levels. The DS responded that when using the surrogate approach, the lowest acute test result from trophic levels where there is no chronic data available are considered.

Assessment and comparison with the classification criteria

Degradation

Hymexazol is hydrolytically stable under relevant environmental conditions (DT_{50} is > 1 year at pH 5, 7, and 9 at 25°C) and is not readily biodegradable. Primary degradation was demonstrated in a water/sediment simulation study with DT_{50s} between 2.4 and 3.1 days at at 20 °C, corresponding to DT_{50} of 5.1 and 6.6 days when normalized to 12 °C (which corresponds to a degradation of > 70% within 28 days). Degradation products were formed but not all were identified, as a consequence the non-classification of these products could not be established. Consequently, this does not support that hymexazol would fulfil the criteria for primary degradation in the environment.

RAC agrees with the DS proposal to consider hymexazol as not rapidly degradable for classification and labelling.

Bioaccumulation

In a bioconcentration study with *C. carpio* hymexazol did not accumulate in fish with residues of hymexazol below measurable levels in whole fish tissues after eight weeks of exposure under flow-through conditions. The study is reported as non-GLP however RAC considers this not to affect the outcome of the test. The low bioaccumulation potential of hymexazol is also supported by experimental LogKow values. Log K_{ow} values ranging <0.3 to 1.01 have been determined for hymexazol by shake flask and HPLC methods. Hymexazol has a pK_a of 5.92, and thus the molecule is increasingly neutrally charged at lower pH values. No pH value has been reported for the shake flask study, therefore it cannot be determined if the log Kow corresponds to the neutral molecule. Only the log K_{ow} of 1.01 has been estimated by HPLC at a pH below the pK_a, i.e. pH 5,

and therefore this is considered to correspond to the neutral molecule. Therefore, RAC agrees with the DS proposal to consider hymexazol as a substance with low potential to bioaccumulate.

Aquatic toxicity

Acute aquatic toxicity

Acute toxicity data are available for three trophic levels. Hymexazol is of low toxicity to fish, aquatic invertebrates and algae with reliable LC_{50}/EC_{50} values above 1 mg/L. The lowest toxicity value is a 14-day IC_{50} of 9.4 mg/L for *Lemna gibba* G3. As noted by a MSCA during public consultation the general exposure for Lemna toxicity testing is 7 days as given in OECD TG 221 and US-EPA 850.4400. The CLP guidance indicates that the lemna test can last up to 14 days. In general, a 7-day exposure period is preferred for the purposes of determining an EC_{50} and a $NOEC/EC_{10}$. Extending test duration could lower test reliability as Lemna growth could unintentionally be inhibited, e.g. due to overcrowding and/or nutrient depletion, and test substance dissipation could lead to lower exposure levels. The CLP guidance does not discuss in detail the most appropriate exposure period for Lemna for the purpose of classification. Considering the prolonged test duration, RAC decided to check if this affected the validity of the test by calculating the doubling time of frond numbers of the control. This was determined to be 2.67 days, which exceeds the validity criterion of OECD TG 221 and US-EPA 850.4400, which state that the doubling time of frond numbers in the control must be less than 2.5 days. Data were not available to assess if the validity criterion was met after 7 days (but frond numbers were determined according to the DAR). RAC further notes that the effects in the Lemna study referred to biomass rather than growth rate and that the reported concentrations referred to initial measured concentrations on day 0. The measured concentration decreased with time, and ranged 42 to 101% of the day 0 concentrations after 7 days, and 12 to 102% after 14 days. As in line with the CLP guidance and the OECD 23 guidance document, geometric mean concentrations should be applied when measured concentrations are not within ± 20% of nominal values. Taken all deviations together, the Lemna study is considered less reliable (Klimisch score of 3). When calculating the geometric mean test concentrations using the methodology described in OECD TG 221 and US-EPA 850.4400 (based on growth rate) and statistical analysis (log-logistic in Graph pad), RAC derived a 14-day IC_{50} of 28.2 mg/L. This value is just above the 48-hour EC_{50} of *Daphnia magna* of 28 mg/L, making Daphnia the most sensitive species to hymexazol. As the lowest acute toxicity value is above 1 mg/L. **Hymexazol does not fulfil the criteria for acute toxicity**, based on Table 4.1.0 (a) and does not warrant classification as Aquatic Acute 1.

Chronic aquatic toxicity

A chronic toxicity study with *Chironomus riparius* was identified by RAC. This study is reported in the DAR however it was not included in the CLH report. The study is summarized below.

Test Guideline	Test Organism	Exposure		End point	Result (mg a.s./L)	Remark	Reference
		Design	Duration				
Toxicity to other aquatic organisms							
OECD Draft (1988) No deviations	*Chironomus riparius*	Static	28 day	EC_{50} NOEC	> 1.6 1.6	Emergence and development Based on nominal concentrations	Doc IIA, 8.2.1/01

Midges (*C. riparius*) were exposed to hymexazol (purity 99.90%, non-radiolabelled mixed with ^{14}C-hymexazol radiolabelled) in water in a static system over a period of 28 days to concentrations of 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 mg/L of overlaying water. Four test vessels were allocated for the assessment of biological effects at each treatment. Emergence, development rate and survival rate were used to determine toxicity endpoints. Initial measured concentrations of the substance ranged from 94 to 106% of nominal concentrations. Hymexazol concentrations in the surface water phase declined: 63 to 78% and 7 to 17% of nominals at days 7 and 28 respectively. Total recovery in overlaying water ranged from 12% to 96% and 17% to 101% in the 0.05 mg/L and 1.6 mg/L treatments, respectively. Total recovery in pore water ranged from 14% to 17% and 15% to 19% in the 0.05 mg/L and 1.6 mg/L treatments, respectively.

The chronic *C. riparius* study is a water-sediment study, and therefore exposure via (ingestion of) sediment cannot be ruled out. However hymexazol, exhibits moderate to high mobility in soil (Kocs in the range 99 to 124 mL/g in neutral and acidic soil types and 12 to 27 mL/g in alkaline soil) with adsorption being pH dependent. As soil pH inreases hymexazol mobility increases. Base on the low sorption potential and test design using water-spiking, exposure to hymexazol is considered to occur primarily via the water. Also, the Chironimous spend their most sensitive larval stage (first instar) free swimming in the water phase and will therefore be exposed to hymexazol via the water in this stage.

The 28-day EC_{50} of hymexazol to the emergence and development of *Chironomus riparius*, based on nominal concentrations applied to the overlying water in a static water/sediment system was greater than 1.6 mg/L. The NOEC was 1.6 mg/L. There were no statistically significant differences between combined controls and any of the hymexazol treatments. The NOEC was recalculated by RAC to take into account the loss of hymexazol during the exposure period. This was done by determining the geometric mean for test concentrations 1.7 mg/L (day 0), 1.3 mg/L (day 7) and 0.28 mg/L (day 28). This resulted in 28-day NOEC of 0.85 mg/L which is considered suitable for classification purposes as supporting information. Exposure to hymexazol is considered to occur primarily via water.

Aquatic chronic toxicity data on hymexazol are available for aquatic invertebrates, algae, aquatic plants and sediment dwelling organisms but not fish. In the absence of adequate long-term toxicity data for fish, the surrogate approach is applied as recommended in CLP guidance section 4.1.3.3 and Table 4.1.0. The substance is considered not rapidly degradable and has a low bioaccumulation potential.

- Classification based on adequate chronic toxicity data. Aquatic invertebrate long-term testing provides a 21-day EC_{10} of 0.4 mg/L. The EC_{10} is ≤ 1 mg/L and the substance is not rapidly degradable. The substance fulfils the criteria for Category chronic 2 classification, based on Table 4.1.0 (b) (i).
- Classification based on surrogate data for fish. Two limit tests and a prolonged acute toxicity test, resulted in 96-h LC50 values of >100 mg/L. The 96-h LC50 is > 100 mg/L and the substance is not rapidly degradable. Based on Table 4.1.0(b)(iii) hymexazol does not warrant classification.
- Overall conclusion: category Chronic 2 applies following the most stringent outcome.

RAC agrees with the DS that hymexazol fulfils the CLP criteria for classification as **Aquatic Chronic 2; H411**.

ANNEXES:

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

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